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Efficacy of oral praziquantel treatment against the skin fluke infection of cultured chub mackerel, *Scomber japonicus*

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ABSTRACT

Outbreaks of skin flukes and associated mortalities have been observed in cultured chub mackerel, *Scomber japonicus*. Although freshwater bathing effectively removes the parasites, large post-treatment mortalities are occasionally observed especially under high water temperature conditions due to the stress from handling and confinement. Since outbreaks of the skin fluke infections often occur in high temperature seasons, development of an effective and safe control method is essential for the advancement of mackerel aquaculture. The present study aims to: 1) identify the skin fluke of cultured chub mackerel and 2) develop an alternative control measure using oral drug administration. The skin fluke was identified as *Neobenedenia girellae* by morphology and molecular data targeting ITS region of rDNA. Two trials of oral administration of praziquantel (PZQ), a common anthelmintic, were conducted and parasite intensity was compared before and after the treatment and between treated and untreated control fish. Fish rejected PZQ-coated commercial pellets, but oral administration was successfully achieved using frozen krill as a basal diet. The three-day administration with a dose of 150 mg·kg^{-1} BW·day^{-1} resulted in over 80% reduction in worm intensity. However, some proportion of the skin flukes survived the drug treatment. The resistance to PZQ does not relate to worm's developmental stage. Freshwater bathing was more effective and eradicated the parasite, but some post-treatment mortality of host fish was observed. Moreover, the parasite intensity drastically increased after the freshwater bathing, possibly due to stress and loss of mucus during the bathing. The study indicates the PZQ oral treatment is effective to control *N. girellae* in chub mackerel aquaculture when the drug is properly administrated with an appropriate feeding technique.

1. Introduction

Mackerel is one of the most important fishery resources around the world. Chub mackerel, *Scomber japonicus* have been highly popular and common seafood in Japan due to its low price and high yield. However, Japanese mackerel stock has significantly declined over the last few decades, from over 1.6 million tons annual catch to less than 500 thousand tons between 1977 and 2009 (data from The Ministry of Agriculture, Forestry and Fisheries of Japan, http://www.jfa.maff.go.jp). To meet the demands and to protect wild stocks, aquaculture of mackerel has recently been started in Japan. Since the establishment of the complete culture cycle of *S. japonicus* in 2002 (Murata et al., 2005), the mackerel culture receives growing attention and further development of the industry is expected. As in any other aquaculture, infectious diseases are one of the potential problems in the advancement of mackerel culture. Although not much has been studied about diseases of cultured mackerel, several parasites are recognised as potential threats. A brain myxozoan *Myxobolus acanthogobii* associated with scoliosis has been reported in cultured mackerel (Yokoyama et al., 2005). Another brain myxozoan, *Kudoa yasunagai*, has also been found in cultured mackerel though its pathology is still unclear (Shirakashi, personal observation). Among them, the most common and important parasite is a skin fluke (Okamoto et al., 2005; Yamamoto et al., 2006).

Capsalid skin flukes are considered as a serious and chronic problem in aquaculture (Ogawa, 2004; Ogawa and Yokoyama, 1998; Ogawa et al., 1995). The culturing conditions, high host density and parasite eggs entangling to the net, lead to epidemics of skin flukes in fish farms. Heavily infected fish often suffer from skin lesions and blindness resulting in growth reduction, secondary bacterial and viral infections and mortalities (Ogawa, 2004). Apparently, chub mackerel is highly susceptible to the skin fluke (Okamoto et al., 2005; Yamamoto et al., 2006). Mortalities associated with heavy skin fluke infection have been observed when water temperature is greater than 24 °C (Yamamoto et al., 2006). To date, the control of skin fluke infections of cultured mackerel is limited to freshwater. These treatments are commonly practiced for treating skin fluke infestations. However, they are extremely labour-intensive and time consuming. Moreover, bathing and handling can cause great stress to the fish and post-treatment mortalities often occur under high water temperature conditions (Stephens et al., 2003; Yamamoto et al., 2006). This is especially problematic for mackerel as they are

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relatively sensitive and highly susceptible to handling. For a successful mackerel aquaculture, frequent bathing treatments are required during the high water temperature season in order to control the skin fluke infestation. However, this is associated with negative consequences. In fact, we experienced a loss of more than 1000 adult mackerel after 6 min freshwater bathing in a summer month (water temperature 28.5 °C, Yamamoto personal observation). Therefore, the development of alternative control measures that are both effective and safe for the fish is vitally needed.

Praziquantel (PZQ) is an anthelmintic commonly used for human and animal parasites such as schistosomes. The drug is also used for treating fish parasites and approved in Japan for aquaculture usage against *Benedenia seriolae*, a skin fluke of seriola fish. Oral PZQ administration, normally mixed with commercial food pellets, is shown to be effective against several fish parasites, including common skin fluke *B. seriolae* and *Neobenedenia girellae* (Hirazawa et al., 2004; Williams et al., 2007). However, PZQ is extremely bitter and has low palatability for fish, which makes oral administration difficult (Sitja-Bobadilla et al., 2006; Williams et al., 2007; Yamamoto et al., 2006). Therefore, feeding technique must be re-evaluated for the practical use of PZQ in aquaculture. In addition, efficacy of PZQ may differ between parasite species and between host fish (Poynton et al., 1997), thus evaluation of the drug should be conducted using the exact target host/parasite system. The aims of the present study was to identify the skin fluke observed in cultured chub mackerel using morphological and molecular data and to evaluate the efficacy of PZQ against the parasite using an improved oral administration method.

2. Materials and methods

2.1. Fish and parasite

A total of 1600 artificially produced 5 month old juvenile Chub mackerel of average body weight (BW) approximately 45 ± 11 g were used for the study. Fish were reared from eggs in land-based tanks for 4 months and then transferred to the sea cages. Prior to the transfer, half of the fish were vaccinated for red sea bream iridovirus by abdominal injection. The rest was injected with PBS in the same manner. The vaccination was conducted to reduce the risk of mass mortalities by the virus disease and for another study not reported in this paper. Eight days after the vaccination, four hundred vaccinated or non-vaccinated fish were transferred to each of the four net cages on the sea (4×4 m). In the cages, fish were fed once a day with ad lib amount of commercial dry pellet and mortality was checked daily. Fish were naturally infected to the skin flukes in the sea cages. Two to three fish were sampled as needed for monitoring of the skin fluke infection. Fish were taken from the cage by a hook and line and individually placed in a bucket with ice cold freshwater (4–7 °C). The cold water was used as anaesthetic to reduce stress and to make a measurement easier. Fish were placed in the cold freshwater for more than 6 min and skin and fins were wiped by hand to dislodge the parasite. The water was then filtered through a mesh (opening 64 μm) and the worms collected from each fish were counted under a stereomicroscope. Every sampled fish was measured for fork length and body weight.

2.2. Parasite identification

Skin flukes of various sizes that were dislodged during the sampling were used for identification. The worms that were fixed in 70% ethanol or mounted on a glass slide and stained with aceto-carmine were sent to Dr. K. Ogawa (Meguro Parasitological Museum) for morphological identification. Additionally, molecular analyses of three frozen specimens of different size were conducted. Genomic DNA extraction was carried out using the QIAmp DNA Mini Kit (QIAGEN Inc., Germany), following manufacturer's instructions. Internal transcribed spacer (ITS) regions of rRNA genes were PCR amplified using the following primer pair; PD-ITS-450F (forward: 5′-AGGTGAACCTGCAGAAGGATC-3′) and PD-ITS-R (reverse: 5′-TAATGCTTAAATTCAGCGGGT-3′) and with the following cycling program; initial denaturation of 95 °C for 2 min followed by 30 cycles of 95 °C for 50 s, 55 °C for 50 s, 72 °C for 50s and a final extension at 72 °C for 4 min (Hayward et al., 2001). The PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN) and subjected to bi-directional sequencing on a BioRad DNA Engine Dyad PTC-220 Peltier Thermal Cycler using an ABI BigDye™ Terminator v3.1 Cycle Sequencing Kit with AmpliTaq DNA polymerase (FS enzyme) (Applied Biosystems), following the manufacturer's instructions employing the same primers as used in PCR.

The sequence data was edited and aligned with the desired sequences obtained from GenBank and a previously obtained sequence of *N. girellae* (502 bp) sampled from Japanese flounder, *Paralichthys olivaceus,* using Genetix ver. 9.1 (Genetyx Corporation).

2.3. Praziquantel treatment

We have conducted series of preliminary experiments to improve palatability of PZQ treated commercial pellets. The base medicated pellets were prepared by mixing with PZQ formulation powder or soaked in PZQ solution (powder and 95% ethanol) and dried. The pellets were further coated with various feeding stimulants (fish oil, krill extracts, sugar, or commercial fish attractants) and/or coating agents (agar or carboxymethyl cellulose sodium salt). In addition to the dry pellet, frozen krill coated with PZQ formulation was prepared. Equal amounts of the drug were used for each feed. Treated feed was administered to the fish kept in a 500 L polycarbonate tank and their behaviour was monitored. The feed was given until the fish stop to eat and the feed left in the bottom of the tank after 30 min of feeding were recovered to measure the feed intake. When medicated dry pellets were given, Fish showed strong avoidance behaviour and 70–95% reduction of feed intake compared to untreated pellets was observed. Therefore, we were unable to administer the desired dose of PZQ using commercial pellet. On the other hand, the fish showed adequate intake (14–22% reduction) without apparent avoidance toward the medicated frozen krill, thus, the treatment experiment was conducted using frozen krill as a basal feed.

The PZQ feed was prepared by mixing 300 mg kg^{-1} BW of Benesaru (50% praziquantel formulation, Aska Pharmaceutical Co., LTD, Tokyo) and sodium alginate (0.5% weight of total feed, spreading and sticking agent) with 1.3 to 1.6 kg (approx. 8% BW) of semi-defrosted frozen krill. This dose was chosen to follow the manufacturer's recommendation dose for *Benedenia seriolae* (Okabe, 2000). The PZQ treatment trial was conducted after the mean skin fluke intensity of sampled fish reached 10. Two trials were conducted during 15th–19th (trial 1 with vaccinated fish) and 20th–30th (trial 2 with non-vaccinated fish) November, 2010. In each trial, two cages were used and fish in one cage were treated with PZQ and those in another cage were assigned as untreated control. The water temperature during trials 1 and 2 were 20.8–23.0 °C and 18.9–21.7 °C, respectively.

Fish were sampled 1 day (trial 2, $N = 20$) or 4 days (trial 1, $N = 6$) prior to the drug treatment to assess the initial parasite intensity and were treated with PZQ for 3 consecutive days. The medicated feed was given once a day in the morning. Feeding was performed in a way so that the majority of fish in a cage ingest the given feed. The same feed without PZQ (krill and sodium alginate) was given to the control fish. To compare the efficacy of PZQ to the traditional treatment, control fish were subjected to freshwater bathing. The bathing treatment was conducted on the day after the end of the drug administration for the treatment group. Fish in a control cage were first chased into a small nylon cage and transferred to a lined cage filled with 1500 l of freshwater. In this manner, no dip netting was required and handling was minimised. Following 5 minutes bathing, fish were returned back