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Diurnal pattern of skin fluke infection in cultured amberjack, *Seriola dumerili*, at different water depths

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ABSTRACT

Skin fluke infection is a major parasitic problem in the aquaculture industry. Present control measures such as bath treatments are effective for removing flukes from fish but require extensive labour and time. Moreover, bath treatments cause great stress to the fish, and occasionally lead to post-treatment mortality. Our ultimate goal is to develop a culture technique that prevents or lowers the chance of fish encountering larval skin flukes, thus reducing infection. However, little is known about when and where these infections occur on fish farms. We conducted a field experiment at a culture site to determine the diurnal pattern of skin fluke infection at different water depths. Juvenile amberjack, *Seriola dumerili*, were placed in enclosed mesh cages and exposed to skin flukes at depths of 0, 2 or 4 m for 4 h during different times of the day. A *Neobenedenia girellae* infection occurred predominantly at the surface during the mid-day. The infection rate declined considerably during the night. The parasite burden was significantly decreased with water depth, and up to an 80% and 95% reduction in intensities was observed when fish were exposed at a depth of 2 and 4 m, respectively. These results suggest that skin fluke infection can be significantly reduced by systematic management strategy and modification of culture techniques.

1. Introduction

A skin fluke infection is one of the most common and chronic parasitic problems in finfish aquaculture (Ogawa, 2004; Ogawa and Yokoyama, 1998). Infected fish suffer from skin lesions, blindness, decreased growth and secondary bacterial and viral infections that can lead to mass mortality (Ogawa, 2004). In Japan, the skin fluke, *Benedenia seriolae*, which infects yellowtail, *Seriola quinqueradiata*, has been considered a serious problem since the early era of net cage aquaculture in the 1950s (Harada, 1966). Since then, skin flukes have become a significant issue in aquaculture around the world. Furthermore, the introduction of *Neobenedenia girellae* into the Japanese waters from China in the early 1990 through the importation of amberjack, *Seriola dumerili*, has caused an even greater problem (Ogawa et al., 1995). *N. girellae*, possibly synonymous with *Neobenedenia melleni* (Whittington and Horton, 1996), has low host specificity and infects various marine fish. Amberjack is particularly susceptible to *N. girellae* and its outbreak during the high water temperature season causes significant impacts on fish farms (Ohno et al., 2008).

Current control of *B. seriolae* and *N. girellae* is limited to bath treatments with freshwater and hydrogen peroxide (1 kg formulation per 1 m^2) or oral administration of praziquantel (150 mg/kg body weight per day for 3 days). Although these treatments effectively remove the flukes, they are associated with shortcomings. Bath treatments require extensive labour, are time consuming and cause a significant amount of stress to the fish (Stephens et al., 2003). Treated fish suffer from mucus loss and there is a high risk of post-treatment mortality, particularly during the high water temperature season (Yamamoto et al., 2006). Praziquantel treatment is more expensive and its low palatability makes administration of an effective dose very difficult (Williams et al., 2007; Yamamoto et al., 2011). Moreover, the use of praziquantel in Japanese aquaculture is only approved for *B. seriolae*. Considering the impact of skin flukes on the aquaculture industry and the limitation of current control measures, development of alternative methods for solving the skin fluke problem is urgently needed.

Our aim was to develop a culture method that reduces infection rates at fish farms and mitigates skin fluke-related costs. The first and essential step is to understand the infection process under culture conditions. Several laboratory studies have revealed the general biology of *B. seriolae* and *N. girellae*, including egg production rhythms (Hirazawa et al., 2010; Lackenby et al., 2007; Mooney et al., 2008), hatching patterns (Kearn et al., 1992) and behaviour of oncomiracidia (Shirakashi et al., 2010). However, information about their infection biology under aquaculture conditions is limited. Recent anecdotal observations have shown that skin fluke infections decrease significantly or are virtually eliminated in yellowtail farms that use submersible net cages. This led to our hypothesis that infection occurs in a limited area of the water column. Thus, in the present

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study, we conducted a field experiment at a culture site to determine the diurnal pattern of skin fluke infection at different depths of water.

2. Materials and methods

2.1. Experimental fish

We obtained approximately 2 month old artificially produced juvenile amberjack, *S. dumerili*, from the Kinki University hatchery. The fish were reared from eggs in land-based tanks and had never been in open water until the experiment. For individual identification, we marked 200 experimental fish with a passive integrated transponder tag by abdominal injection. Tagged fish were kept in 500 L polycarbonate tanks with flow-through sand-filtered sea water and fed 1–3 times a day with *ad libitum* amount of commercial dry pellets.

2.2. Natural infection experiment

We conducted three experimental trials between July and September 2012 at the Kinki University culture site in Wakayama prefecture, Japan. A heavy *N. girellae* infection was observed among amberjack and other fish cultured in the area during the experimental period. The experiment was designed to determine the diurnal pattern of skin fluke infection at different water depths. We placed 10 randomly selected tagged fish in each of three cylindrical nylon-mesh cages (diameter 34 cm, height 16 cm, mesh opening 4 mm) and naturally exposed the fish to skin flukes at the culture site. The three experimental cages were vertically connected with lines and immersed in the water column so that the top surface of each cage was at depth of 0, 2 or 4 m (Fig. 1). The experimental cages were placed approximately 30 cm away from a net pen (length × width × depth; 3.6 × 3.6 × 4.0 m), in which approximately 100 1 year old yellowtail, *S. quinqueradiata* were cultured. This yellowtail cage was considered the primary source of the parasite. There was only one yellowtail cage in close proximity, but the number of cages for culturing various fish, such as red sea bream, *Pagrus major*, and Japanese mackerel, *Scomber japonicus*, were in the same area (minimum distance approximately 10 m from the experimental cages). The fish were exposed for 4 h at different times during the day: 0:00–4:00, 4:00–8:00, 8:00–12:00, 12:00–16:00, 16:00–20:00 and 20:00–24:00. All experimental fish were treated with a freshwater bath 1 h prior to the experiment to eradicate any previous infection. The average fork lengths of the experimental fish used for trials 1, 2 and 3 were 11.67 ± 0.55, 12.20 ± 0.64 and 15.40 ± 1.06 cm, respectively.

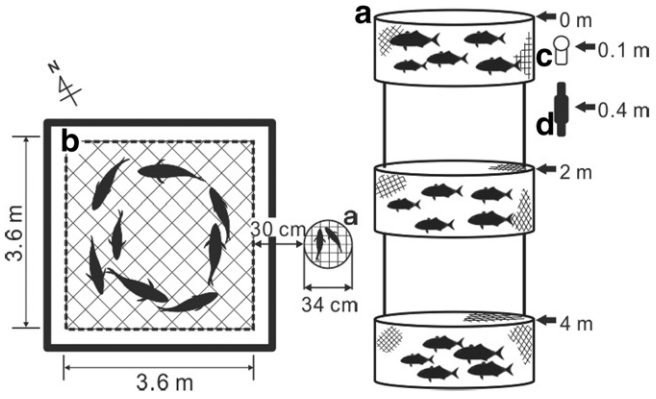

Fig. 1. Schematic diagrams of the experimental setup. The left diagram shows the size and the location of (a) the experimental cages and (b) the primary source of skin flukes, a cage with infected yellowtail. The right diagram shows the depths of (a) the vertically connected experimental cages, (c) light sensor, and (d) current metre.

The light intensity at a depth of 0.1 and 2 m was monitored every 10 min using an *in situ* sensor (MDS-MkV/L; JFE Advantech, co., Ltd, Tokyo, Japan) to evaluate the possible effects of environmental factors on skin fluke infection. Light intensity at 4 m was estimated from the following equation $E_u(0)e^{-K_u}$ where $E_\mu(0)$ is the light intensity at the surface and K_μ is the extinction coefficient calculated from the equation $\frac{1}{2}\log_e\frac{E_u(0)}{E_u(2)}$ where $E_\mu(2)$ is the light intensity at a depth of 2 m. Additionally, water current (direction and velocity) and temperature at a depth of 0.4 m just beside the experimental cages were monitored every 10 min by an electromagnetic current metre (INFINITY-EM, JFE Advantech) during trials 2 and 3. We were unable to monitor the water current during trial 1 due to the lack of equipment. The average water temperatures at the surface during trials 1, 2 and 3 were 28.95 ± 0.35 °C, 29.5 ± 0.26 °C, and 29.33 ± 0.28 °C, respectively. The sunrise, sunset and high tide times were 5:06, 19:05 and 23:12 during trial 1, 5:15, 18:56 and 20:58/9:10 during trial 2, and 5:33, 18:27 and 18:23/6:15 during trial 3, respectively. We also attempted to assess the temporal change in oncomiracidial density at different water depths by collecting 2 L seawater from 0, 2 and 4 m every 4 h when we changed the experimental fish. The water was immediately filtered through a 20 μm nylon mesh, and the residues were examined for oncomiracidia under a dissecting scope.

After a 4 h exposure, the fish were kept in the 500 L tanks in the laboratory for 3–4 days to allow the flukes to grow to approximately 3 mm to make worm counting and identification more accurate. Inflow was filtered through a 64 μm nylon mesh and 50 μm cartridge filter to minimize the risk of infections during this grow-out period. In addition, we added three uninfected fish into the tanks as a control to investigate potential horizontal transmission. Fish were maintained in the same manner as described earlier. Fluke from each fish were collected by bathing an individual fish in approximately 2 L of dechlorinated tap water for 7 min. Dislodged worms were collected by a 64 μm nylon mesh and were fixed in 70% ethanol. We enumerated the total number of worms from each fish under a dissecting microscope and identified the species using the morphological characteristics described by Kinami et al. (2005). We repeated the entire experiment three times using the same group of fish.

2.3. Statistical analyses

Normality of the data was assessed using the Shapiro–Wilk test. We used Spearman's rank correlation analysis to determine the relationship between mean fluke intensity and water depth. Kruskal–Wallis and Steel–Dwass multiple tests were employed to detect the difference in mean worm intensity between different time periods and between the trials as well as to detect differences in light intensity between water depths. All analyses were performed on JMP ver. 9 (SAS Institute, Cary, NC. USA).

3. Results

The fish acquired a considerable number of skin flukes during the 4 h exposure at the culture site. The highest number of flukes retrieved was 1009 from a fish exposed at 0 m from 12:00 to 16:00 during trial 3. The overall average intensities for trials 1, 2 and 3 were 9.22 ± 11.30, 29.12 ± 43.77 and 78.19 ± 174.07 worms, respectively, and the intensity and prevalence of infection significantly increased as the season progressed (Table 1, Kruskal–Wallis and Steel–Dwass, $p < 0.0001$). The majority of skin flukes were identified as *N. girellae*. Only 4.36% and 0.85% *B. seriolae* were observed in trial 1 and 2, respectively, and none were observed in trial 3.

Both water depth and time of day influenced infection rate. A significant negative correlation was observed between worm intensity and water depth (Spearman's rho = −0.43, −0.38 and −0.28 for trials 1, 2 and 3, respectively, $p < 0.0001$). Although the number