Accumulation and distribution of skin fluke Neobenedenia girellae eggs on a culture cage

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ABSTRACT

Skin fluke causes chronic problems in marine finfish aquaculture. Their eggs possess a long filamentous appendage that allows them to easily attach to culture cages. Fish farmers periodically change and clean cage nets to remove eggs from the culture sites. However, there is very little information about the accumulation rate and distribution of skin fluke eggs on culture cages. We conducted an experiment at a culture site to assess the accumulation of eggs of the Neobenedenia girellae skin fluke at different culture cage depths. In all three trials, we observed the highest egg accumulation in shallow areas, and the egg number decreased with the depth; however, the second highest accumulation was observed at the deepest depth. These distribution patterns likely reflect the behavior of fish within the cage. The highest accumulation rate was 161.2 eggs/cm²/day, with an estimated daily accumulation of more than 10 million eggs on the entire cage (excluding the cage floor). Such information is useful for optimizing and managing cage net changing and also for the development of a new method of removing/killing parasite eggs.

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1. Introduction

Skin fluke infections have long been a major problem in the aquaculture industry. Benedenia seriola has been a chronic problem in yellowtail culture since the establishment of the cage culture system in the 1950s (Harada, 1966; Ogawa and Yokoyama, 1998; Ogawa et al., 1995). During the 1990s, Neobenedenia girellae, a temperate species, was introduced to Japanese water from China, and it has been causing even greater problems due to its low host specificity and fast proliferation (Ogawa et al., 1995). Skin flukes have a simple direct life cycle that is suitable for multiplication in aquaculture environments. Adult flukes live on fish body surfaces and produce eggs that are easily attached to cage nets by a long filamentous appendage. Free-swimming larvae, oncomiracidia, hatch on the cage nets and are able to easily find their next hosts within the cages.

The eggs on the cage nets are the major source of infection. Fish farmers try to remove the eggs with periodical net changes and cleanings. However, these procedures are extremely time and labor intensive. In addition, all cages in the area need to be cleaned at the same time for effective control of the parasite in a culture site. The accumulation rate of skin fluke eggs on culture cages is not well known; thus, the optimal time intervals for net changes and cleanings remain unclear. In addition, only limited information is available about the egg distribution pattern within culture cages. In the present study, we conducted a quantitative assessment of N. girellae eggs on a culture cage to determine the egg accumulation rate and their vertical distribution pattern.

2. Materials and methods

Accumulations of N. girellae eggs were assessed in an experimental culture cage for greater amberjack Seriola dumerili. The cage measured 3 × 3 × 3 m and harbored approximately 250 fish (average length of approximately 20 cm). We placed pieces of blue nylon net (30 cm long × 20 cm wide, 2.5 mm opening) at different depths of the inner side of the cage to assess egg attachment (Fig. 1). Each net was divided into 24 equal sections by 5 cm grid lines. Four nets were firmly attached onto every side of the cage at 60 cm vertical intervals. The upper edge of the top net was at the water surface and the lower edge of the bottom net was at the lowest part of the cage. The nets were left on the cage for 2 or 3 days and then retrieved to assess N. girellae egg accumulation. An electro-magnetic current meter (INFINITY-EM AEM-USB, JFE Advantech Co. Ltd.) was placed at 40 cm depth beside the cage to record the velocity and direction of the water current every 10 min. The experiment was repeated 3 times during September and October 2014 (Trial 1, 26–29 Sept.; Trial 2, 14–16 Oct.; Trial 3, 16–18 Oct.). Trials 1, 2 and 3 were conducted after 8, 14 and 16 days of freshwater bath treatment of all fish in the experimental cage, respectively. The average surface water temperatures at each trial were 25.6 ± 0.4 °C, 23.3 ± 0.4 °C and 23.4 ± 0.2 °C, respectively.

After retrieving the nets, six randomly selected 5 cm² sections were cut from each net, and the attached eggs in each section were counted. The nets were rinsed with tap water to remove excess debris, and the
water was filtered through a 30-μm micromesh to trap the detached eggs. A piece of net was placed in a Petri dish, and the eggs were counted under a dissecting scope. In many cases, groups of eggs were entangled, forming egg clusters. In such cases, the egg clusters were removed from the net and placed on a glass slide; the eggs were separated using a fine forceps and were flattened under a cover glass for easier counting. The remaining eggs in the rinsed water and the Petri dish were also counted.

The comparison of the egg numbers between the trials, depths and cage direction was based on Wilcoxon/Kruskal–Wallis tests with JMP Statistical software (SAS Institute).

3. Results and discussion

Large numbers of eggs accumulated on the egg collection nets during the two to three day experimental periods. The overall egg numbers increased for Trials 1, 2 and 3 (7,532, 100,956 and 165,668 eggs, respectively) most likely due to parasite proliferation. The average numbers of attached eggs at different depths are shown in Fig. 2. In all trials, the highest accumulation was detected near the water surface (0–20 cm depth) comprising 48.5–82.4% of all of the eggs counted (Wilcoxon, p < 0.05). A higher distribution of N. girellae eggs near the surface has previously been reported. Murase et al. (2011) monitored skin fluke eggs (N. girellae and B. seriolae) attached to stainless steel wires placed in a culture cage and found greater egg accumulation at a depth of 0–1 m compared to 1–2 m. This is consistent with our past observation that the highest infection of N. girellae occurs near the surface (Shirakashi et al., 2013). Although the specific gravity of skin fluke eggs has not been evaluated, they are heavier than the seawater because the eggs readily sink when egg clusters are placed in seawater. Therefore, the eggs likely accumulate on the bottom if no other factors are involved. The higher egg density in shallow areas may reflect the host behavior and their distribution within culture cages. Cultured fish are most active during feeding, which takes place at the surface. The eggs on adult flukes might detach during the active movement of the host, and they may drift away to the cage net with the water current generated by the fish. Also, the fish might have distributed in shallower area during the experiment. The vertical distribution of cultured fish differs between fish species and size. Moreover, various environmental factors such as water temperature and light affect fish distribution in cages (Miura et al., 2014; Oppedal et al., 2001). The observed distribution pattern of N. girellae eggs may reflect the fish distribution under specific experimental conditions and can differ under other conditions and/or fish species.

The greater egg accumulation in the shallow area suggests a possibility of developing a new egg removal/killing method. In general, skin fluke eggs are susceptible to desiccation. Ernst et al. (2005) showed that egg hatching of B. seriolae was completely suppressed after being air dried for 3 min. Sharp et al. (2004) reported that exposure of B. seriolae eggs to the air for over 3 h prevented them from hatching. Although largely dependent on conditions, eggs on the cage net can be killed by drying under sunlight for a certain period. If large proportions of eggs are attached to the cage net close to the surface, the upper part of the cage may need to be raised to expose it to the air rather than changing the entire cage. Furthermore, eggs that drift near the surface may be trapped by placing substrates or a device, such as a fibrous brush, onto which the eggs are easily attached (Murase et al., 2011). Whether such methods are effective and practical for fish farms needs further assessment, but trying new ideas is important for the development of alternative methods of reducing skin fluke infection in fish farms.

The egg accumulation rates were significantly reduced in the deeper areas, but the second highest accumulation was observed at the deepest part (240–260 cm). The eggs at the 240–260 cm depth represented 11.2–23.5% of total eggs, and these values were significantly greater than the 80–100 and 160–180 cm depths (Wilcoxon, p < 0.05). This contradicts a previous report by Murase et al. (2011) who found a very small amount of eggs at the bottom. Such a difference might be due to the differences in experimental conditions such as size of fish and experimental cages as well as the egg collection methods. In the present study, we used nets that were directly attached to the culture cage, so our results represent actual egg accumulation onto the culture cage. On the other hand, Murase et al. (2011) used stainless steel wires that were placed away from the cage nets. Fish infected with skin fluke often rub their body against the cage floor, and such behavior is frequently observed in infested cages. It may be that eggs on adult flukes detach during the host’s rubbing behavior causing the relatively large number of eggs to attach to the nets near the cage bottom. We did not assess the egg accumulation on the floor net of the cage, but it is possible that a considerable amount of eggs accumulate on the floor. In that case, entire cage nets need to be replaced to reduce parasite eggs in a culture site.

We did not observe any obvious directional tendency of egg accumulation, except for Trial 2 in which significantly more eggs were found on north side compared to the south and west sides (Wilcoxon, p < 0.02, Fig. 3). This result might reflect the water currents. The data from the current meter suggests that there was higher proportion of northward current during Trial 2 (Fig. 3). This indicates that the water current may affect egg distribution to some extent. However, precise effect of current is unknown as we did not measure the vertical water accumulation.
movement. It may be that fish behavior and their distribution within the cage play a more important role in egg distribution.

Combining all of the trials and all of the depths, the average number of eggs accumulated on a 25 cm² net was 471.6 ± 838.8 eggs per day. The highest egg count was 8058 eggs/25 cm² in two days, which represents the daily accumulation rate of 161.2 eggs in a square cm. It is estimated that a total of approximately 6 million eggs accumulated on the entire cage, excluding the bottom floor, in one day. This number was even greater, at over 10 million eggs, during Trial 3 when the highest egg accumulation was detected. Although the numbers are somewhat overestimated because the mesh opening of the net used in the present study is much smaller than actual cage net, these results emphasize the significant number of eggs that accumulate on culture cages within short periods. The average oviposition rate of *N. girellae* in vitro was reported to be 0.6 eggs/min (Bondad-Reantaso et al., 1995). Our recent study also showed that *N. girellae* is capable of producing an egg every minute, and oviposition continues throughout the day and night (Hirano et al., 2015). Such high fecundity is a characteristic of this species and is the main reason that *N. girellae* causes such a significant problem for fish farms. Furthermore, our data showed that the egg accumulation rate rapidly increases over time. The daily accumulation almost doubled between Trial 2 and 3, which were conducted immediately after the other. This indicated that prompt egg removal (net change) is essential for minimizing the parasite proliferation. In the present study, however, we did not determine the number of flukes on the experimental fish. This makes difficult to predict the timing of egg removal in accordance with the severity of infection. We previously demonstrated that an adult *N. girellae* produces approximately 21 eggs every hour in average and the maximum of over 700 eggs per day. Based on this data we can predict the number of eggs released in the culture area in a given infection condition. It takes 4 to 5 days for *N. girellae* eggs to hatch at 25–30 °C (Bondad-Reantaso et al., 1995; Hirazawa et al., 2010), and it was reported that the next generation of *N. girellae* appears within 13 days at 25–30 °C. Considering this information, farmers may need to perform their egg removal procedures at least once a week during the high water temperature season.

**Fig. 3.** The average numbers of *N. girellae* eggs accumulated on a 5-cm² piece of net on the four cardinal directions of culture cage (top) and the velocities and directions of water current during each experimental period (bottom).
The present study is the first to provide quantitative data on *N. girellae* egg accumulation in a culture cage. The difference in the accumulation rate between the depths may provide important information for the development of a new and effective egg removal method. Although whether the observed pattern is also applicable for actual farming cages needs further investigations, we believe that the accumulation of such basic information is important for developing new methods for the control of parasitic infections in aquaculture sites.

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