FINAL REPORT

Effect of Netting Materials on Fouling and Parasite Egg Loading on Offshore Net Pens in Hawaii

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Introduction

Parasitic skin flukes, primarily Capsalid Monogeneans, *Benedenia sp.* and *Neobenedenia sp.* are a scourge to the marine aquaculture industry worldwide (Figure 1). These parasites infect a wide range of hosts in tropical and temperate waters, causing immediate irritation and stress to the animal and, if levels are left unchecked, can lead to bacterial infections and eventual death. The life cycle and physiology of these two genera lend themselves to exponential growth in fish tanks and seacages. A series of topical chemical treatments can often remedy the problem in land-based containment systems, but topical treatments are less effective in offshore net pens.

Figure 1: *Benedenia seriolae* infection on Japanese yellowtail

Neobenedenia, the parasite of concern in Hawaiian offshore mariculture, reproduces by releasing (up to) several hundred eggs daily (Whittington and Horton, 1996). These eggs (Figure 2) are composed of a rugged shell and a long tendril. The tendrils allow the eggs to attach directly to netting or to fouling on the netting. Bathing of contained fish in a chemical compound such as Hydrogen peroxide only removes the infective stage of the parasite from the skin of the fish, leaving unhatched eggs attached to the netting unharmed. These eggs will tend to hatch and re-infect the fish within the net pen. Preventing reinfection requires a significant reduction in the ability of parasite eggs to efficiently colonize the netting material. The virulence of *Neobenedenia* infection can be greatly reduced by identifying netting materials which inhibit the efficacy of parasite egg colonization.

Figure 2: Adult *Neobenedenia* with eggs

Several attributes make a netting material more or less resistant to parasite egg colonization. First, is the material porous or contain areas of overlap, such as in a braided rope? The small crevices formed by the strands of a braided rope give egg attachment mechanisms greater purchase. Second, is the material easy to clean? Smooth surface netting materials tend to facilitate easier removal of eggs and fouling with active cleaning methods such as pressure washing. Third, is there an antifoulant compound coated on the netting, or is it made of a compound that has antifoulant properties that will inhibit egg attachment or health?

Scope of Work

To determine the effect of various netting materials on *Neobenedenia* egg colonization, it was proposed to compare the amount of *Neobenedenia* eggs found on three types of net material, after the net material was allowed to foul for several weeks on an offshore net pen. Specifically, the three types of netting compared were; Kikko Net (a semi-rigid, polyester monofilament), a copper alloy mesh (CAM) and a standard braided nylon net. Small panels of each net type were attached to a net pen used for commercial *Seriola* culture in Kona, Hawaii from April 18, 2012 to June 21, 2012. No antifoulant had been used on any of the materials tested.

Materials and Methods

Netting Materials

A. Kikko

Kikko Net is a semi-rigid, polyester monofilament mesh manufactured in Japan. It is used in commercial aquaculture in Asia as well as in Hawaii. Its smooth surface seems to decrease the speed at which fouling organisms take hold, and it is relatively easy to clean. The Kikko netting used in this trial had a mesh size of 67mm on the vertical (Figure 3a).

B. Copper Alloy

The copper alloy mesh is manufactured in Chile. It is used in salmon farming in South America. The anti-microbial attributes of copper make this netting material resistant to organic fouling. The netting used in this trial had a mesh size of 50mm on the diagonal (Figure 3b).

C. Nylon

The nylon netting used is a (three stranded) Rachel Braid 210/120 mesh. This type of material is used extensively in aquaculture applications. Nylon netting is inexpensive and easy to handle. The nylon netting used in this trial had a mesh size of 52mm on the diagonal (Figure 3c).

Table 1: Netting Materials

	A (Kikko)	B (Copper Alloy)	C (Nylon)
Material	Polyester monofilament	Copper Alloy	Nylon (Rachel Braid)
Mesh Shape	Hexagon	Square	Square
Mesh Size	67 mm vertical	50 mm diagonal	52 mm diagonal
Gauge / Ply	2.9 mm	4.0 mm diameter	2.0 mm diameter

Figure 3: Netting Mesh

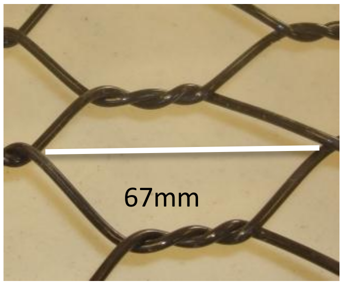


Figure 3a (Kikko)

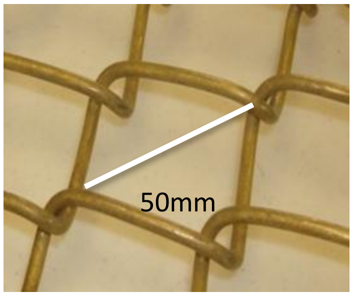


Figure 3b (Copper Alloy Mesh)

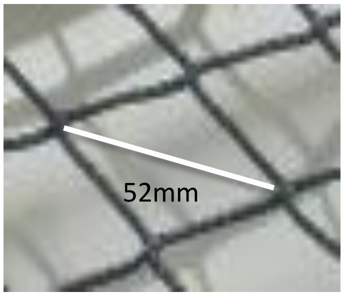


Figure 3c (Nylon)

Netting Deployment

Three panels of each type of material were made for the trial (nine panels total). Each panel consisted of a .25m X .25m frame made from 1.0" PVC. The various netting materials were cut to size and attached to their respective frames with cable ties (Figure 4). For ease of deployment and retrieval, all nine the panels were lashed together and attached as a single unit to one of Blue Ocean Mariculture's 3000m^3 SeaStation™ net pens. The panels were kept on the net pen for 64 days. The SeaStation™ net pens contained marine finfish *(Seriola rivoliana)* at a density of approximately 30kg/m^3. The fish had been stocked in the net pen five months prior to initiation of the trial. SeaStation™ net pens are constructed to allow for air drying of the top half of the cage. Consequently, the net panels were put on the bottom half and never allowed to dry. The net pen received one bathe treatment of 35% hydrogen peroxide during trial period to remove the infective stages of *Neobenedenia* from the fish.

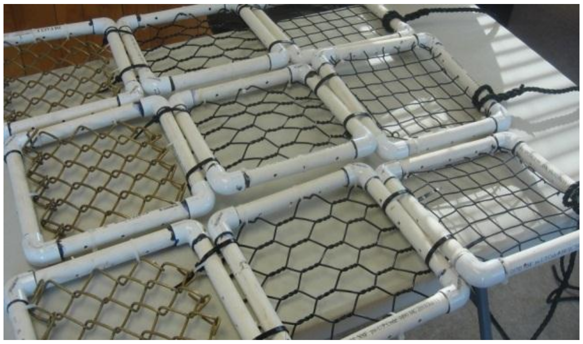


Figure 4: Netting panels prior to deployment

Figure 5: Copper Alloy after 4 weeks in the net pen

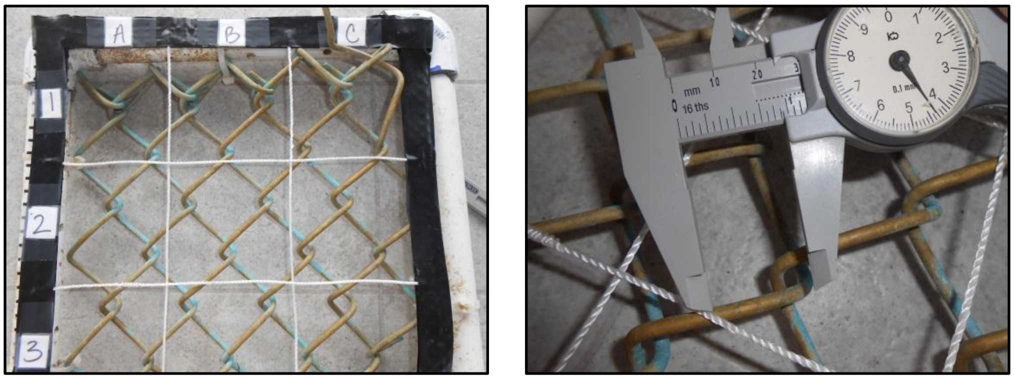
The panels were examined in-situ four weeks into the trial period. Divers noted no structural or extraordinary changes, but it was noted that there was little to no fouling on the copper alloy netting (Figure 5), and some algal growth beginning on the nylon netting. The panels were removed from the SeaStation™ net pen at 64 days. Upon removal from the SeaStation™ the panels were immediately transported to Blue Ocean Mariculture's on-shore facility for analysis. To prevent desiccation and cross contamination during transport, the panels were separated by type and placed into 3mm black plastic bags, filled with seawater.

Enumerating Egg Loading

Neobenedenia eggs can be seen quite clearly when using a dissecting microscope (20-100X magnification). By placing the mesh panels under a microscope one can get a true count of egg number. To count the eggs, each .25m X .25m netting panel was divided into nine squares and eggs were counted in five of the nine squares. To determine which five of the nine squares would be counted on each panel, the squares were assigned a code (A1, A2, A3, B1, B2 etc. Figure 6a) and Microsoft Excel's random number generator function was used to randomly pick five squares to be counted. In total, 15 squares of each netting material were counted (five squares on each panel).

Since each netting material had a different mesh size, the total length of material in each square was measured to the nearest millimeter using calipers (Figure 6b). Eggs were counted on both the front and back sides of the netting material to obtain the total actual number of eggs/linear cm. Initially, it was attempted to remove this fouling to identify and weigh it, however, the fouling proved difficult to remove without damaging *Neobenedenia* eggs or other organisms. Therefore, counting the eggs was done directly off the netting squares and the fouling was not weighed.

Figure 6: Net Panel Measurements



6a. Marked squares on panel

6b. Measuring length of netting material

Results

The results are shown in Table 2. An Analysis of Variance Procedure (single-factor ANOVA, Microsoft Excel) was used to determine if there was a statistically significant difference in the total number of eggs per sample square based on netting material. A significant difference was seen ($P<0.05$).

Table 2: Neobenedenia Egg Loading on Net Panels

	Average Linear Netting Material per Square	Number of Eggs per Square (Mean ± SD)	Number of Eggs per cm Netting Material
A. Kikko	17.7cm	27.0 ± 11.2	1.50
B. Copper Alloy	22.2cm	0.1 ± 0.5	0.01
C. Nylon	28mm	91.0 ± 58.6	3.30

Additionally, an ANOVA was performed to learn if there was a significant difference in the number of eggs per linear centimeter of material. There was a significant difference between all three. The Copper Alloy netting material had significantly fewer eggs per square and per centimeter then either the Kikko or Nylon materials. The Kikko had significantly fewer eggs per square and per centimeter than the Nylon.

There was very little fouling seen on the Copper Alloy. A patina had formed while in the water, but there was almost no growth of any type of algae. The Kikko was moderately colonized by pink, encrusting calcareous algae, with some filamentous algae and a few isopods. The Nylon mesh was heavily colonized by several types of isopods, filamentous algae and other unidentified organisms.

Discussion

The Copper Alloy netting material clearly performed better at preventing fouling. This is likely due to primarily to copper's anti-fouling effects and secondarily because there is no place where the mesh is woven together. On any material, the place where the material comes together makes a small nook for organisms to settle. The Kikko material, which had the second lowest egg loading rate, was colonized by calcareous algae, which did not seem to have as many eggs adhering to it. Both the Copper Alloy and Kikko are rounded, smooth surfaces which make it difficult for the *Neobenedenia* eggs to adhere. Kikko does have areas where the material is twisted together, and in most cases, higher concentrations of eggs were seen in these areas. The Nylon material was clearly a good environment for organisms to settle. Algae could easily adhere and grow and a variety of other organisms lived on it was well. Often there appeared to be mucus on the Nylon from some of the colonizing organisms with higher concentrations of *Neobenedenia* eggs. Eggs were also found in the weave of the mesh itself. Based on previous work, by the author, the braided nature of this type of net does not allow for easy cleaning. Pressure washing will remove loose algal filaments, macrorganisms and macroalgae, but some of the filamentous algae as well as *Neobenedenia* eggs will remain even after pressure washing. Based on these results the Copper Alloy material was most effective at minimizing fouling rates and *Neobenedenia* egg colonization, and Nylon netting material was the least effective.

Reference

Whittington, I.A. and M.A. Horton. A revision of *Neobenedenia* Yamaguti (Monogenea: Capsalidae) including a rediscription of *N. mellini* (MacCallum, 927) Yamaguti 1963. Journal of Natural History, 1996, **30**, 1113-1156.