Effect of Salinity on Hatching in Monogenean Ectoparasites of Seawater-Cultured Tilapia

KARL W. MUELLER[1], WADE O. WATANABE[1], and WILLIAM D. HEAD[2]
[1]*Caribbean Marine Research Center*
805 46th Place East
Vero Beach, Florida 32963 USA
[2]*Caribbean Marine Research Center*
1501 Northpoint Parkway, Suite 102
West Palm Beach, Florida 33407 USA

ABSTRACT

A laboratory experiment was conducted to determine the effect of salinity on egg development and hatching in *Neobenedenia melleni*, a marine monogenean ectoparasite of Florida red tilapia, *Oreochromis urolepis hornorum* x *O. mossambicus*, cultured in full-strength seawater. Eggs of *N. melleni* were incubated in salinities ranging from fresh water to seawater (0, 6, 12, 18, 24, 30, and 37 ppt) for 48, 72 and 96 hours. Varying degrees of post-treatment development and hatching occurred when natural seawater conditions (37 ppt) were restored. The most effective treatments in reducing monogenean hatching were prolonged exposures (>72 hours) to freshwater (0% hatching success) and 96-hour exposure to 6, 12, and 18 ppt (5.5-11.9% hatching success).

INTRODUCTION

Studies conducted in the Bahamas have shown the feasibility of culturing Florida red tilapia, *O. urolepis hornorum x O. mossambicus*: Behrends *et al.*, 1982) in full-strength seawater (Watanabe *et al.*, 1988: Ernst *et al.*, 1989). It was noted, however, that seawater-cultured tilapia were susceptible to a marine monogenean (Ernst *et al.*, 1989), later identified as *Neobenedenia melleni* (MacCallum, 1927). Lack of an effective treatment sometimes resulted in high mortalities (Watanabe, in press).

A number of chemical treatments have been proposed to control monogenean infections in cultured fish (Martinez, 1981; Schmahl and Mehlhorn, 1985; Gallet de St. Aurin *et al.*, 1990); however, chemical treatments might not be practical because of high costs and restrictions by the U.S. Food and Drug Administration. Freshwater dips also are effective in removing adult monogeneans from cultured fish (Hoshina, 1968; Kaneko II *et al.*, 1988), but this type of treatment could be impractical on a large scale or in areas where freshwater is limited.

Watanabe (in press) found that treatment with brachish water (18 ppt for 72 hours) alleviated symptoms of *N. mellini* parasitosis in seawater-cultured Florida red tilapia. These symptoms, however, reappeared after 24-84 days, suggesting that the egg stages were resistant to treatment. The purpose of this study was to

determine the effects of salinity on egg developement and hatching in *N. melleni*, as a basis for improving methods of controlling parasitosis in cultured fish.

MATERIALS AND METHODS

The study was conducted at the Caribbean Marine Research Center (CMRC, Lee Stocking Island, Exuma Cays, Bahamas), from January to March, 1991. Eggs of *N. melleni*, collected from adults parasitizing Florida red tilapia reared in seawater pools, were incubated at different salinities (0, 6, 12, 18, 24, 30, and 37 ppt) for 48, 72 and 96 hours. For each experiment, adult monogeneans (3 to 5 mm) were removed from infected fish and immediately placed in covered petri dishes containing seawater, in which egg-laying occurred within one hour.

Clusters of 15 to 40 eggs were transferred to 50 ml clear glass incubator jars containing water of prescribed salinity at 25+- •C. Each treatment was replicated five times. Folllowing incubation, water was siphoned from the incubator jars and replaced with full-strength seawater. Eggs were incubated for an additional 13 days, with seawater exchanged daily. Eggs were monitored dialy for post-treatment development and hatching. Final hatching success was expressed as the percentage of empty egg cases to the total number of eggs incubated.

Multiple comparisons were made among treatments with the Mann-Whitney test (Zar, 1984) and the Fligner-Policello test, a modified Mann-Whitney test that is robust for small, unequal sample sizes (Figner and Policello, 1981; Day and Quinn, 1989).

RESULTS

Hatching success of *N. melleni* eggs (Table 1) exposed to full strength seawater (37 ppt) ranged from 96.1 to 97.4% and did not differ significantly ($P > 0.05$) from that of eggs exposed to 30 ppt (range = 94.8 - 99.4%). Hatching success after exposure to 24 ppt (range = 69.9-80.8%) was significantly lower ($P < 0.05$) lower than at 30 or 37 ppt. At 18 ppt and below, hatching success was significantly ($P < 0.05$) lower than at 24 ppt and varied considerably with duration of treatment. With all treatment durations, hatching success did not differ significantly ($P > 0.05$) among 6, 12, and 18 ppt treatments. Hatching success remained at moderate levels after 48 hours (range = 26.0 – 32.5%) and 72 hours (range=23.6-34.0%), but declined to relatively low levels after 96 hours (range=5.5-11.9%).

In fresh water (0 ppt), hatching success after 48 hours was 13.3% (Table 1). Hatching success at 0 ppt was significantly ($P < 0.05$) lower than at 6 ppt, but did not differ significantly ($P > 0.05$) from rates at 12 and 18 ppt. This inconsistency is attributed to the large variation in percent hatching found among replicates within the 12 and 18 ppt treatments. No hatching occurred after exposure to 0

Table 1. Hatching success (mean% + SEM) of N. melleni eggs in seawater (37 ppt) after exposure to different salinities for 48, 72, or 96 hours. Eggs were collected from adult N. melleni at 37 ppt. Means are based on four or five replicate determinations.

	Treatment Salinity (ppt)						
Exposure	**0**	**6**	**12**	**18**	**24**	**30**	**37**
48 hour	13.3±1.8^{a}	26.0±7.9^{b}	32.5±10.7^{a,b}	30.9±10.7^{a,b}	80.8±2.7^{c}	99.4±0.6^{d}	96.1±1.7^{d}
72 hour	0	26.1±3.0^{a}	23.6±3.2^{a}	34.0±5.0^{a}	78.0±5.0^{b}	93.0±1.7^{c}	97.4±1.9^{c}
96 hour	0	5.5±2.3^{a}	11.9±1.1^{a}	5.8±2.7^{a}	69.9±5.4^{b}	94.8±0.9^{c}	97.4±1.9^{c}

For all treatment durations, significant differences among salinities were observed ($p<0.001$, Kruskal-Wallis). Means in the same row with common superscripts are not significantly different ($P>0.05$, Mann-Whitney for the 48-h trial; Fligner-Policello for the 72 and 96-h trials).

ppt for 72 or 96 hours. Less than 5% of the eggs developed eyespots after exposure to 0 ppt for 72 hours and no development was observed with the exposure.

DISCUSSION

The results of our study indicate that the eggs of *N. melleni* are extremely resistant to hyposaline conditions. Eggs exposed to fresh water (0 ppt) for 48 hours had a hatching success of 13.3% in 37 ppt water. A minimum freshwater exposure of 72 hours was required to eliminate hatching.

Recent studies have shown that adult *N. melleni* are killed within 48 hours of exposure to salinities as high as 15 ppt (E. Ellis, pers comm., CMRC, Lee Stocking Island, Bahamas). Results of the present study clearly show that eggs of *N. melleni* are far more resistant to reduced salinities than adults. Eggs exposed for 48 hours to a relatively low salinity of 12 ppt had a hatching success of 32.5% Thus, while short-term (96 hours) treatment with brackish water could alleviate symptoms of parasitosis in seawater-cultured tilapia by killing adult *N. melleni* (Watanabe, in press), autoinfection is likely to occur because of survivability of the eggs.

Results of this study revealed that hatching success of *N. melleni* eggs was impaired by exposure to salinities as high as 24 ppt and was reduced to very low levels (5.8%) following exposure to 18 ppt for 96 hours. Thus, longer durations (96 hours) of exposure to low-salinity (<18 ppt) could be effective in preventing hatching and could represent a more practical approach to disease management than treatment with fresh water. Additional studies are required to determine the minimum duration of exposure of *N. melleni* eggs to brackish water (i.e., 18 ppt) to eliminate hatching.

Because prevention id the key to effective disease management, we recommend that land-based commercial growers of tilapia filter influent seawater to remove *N. melleni*. Considering the size of *N. melleni* eggs (120 µm), this can be achieved by using a buried intake or seawater well (Watanabe, 1991). The parasite load of fish stocks should be monitored periodically. If necessary, fish can be treated by reducing salinity long enough to kill adult *N. melleni* and to prevent development and hatching of eggs.

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