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The Effect of B-Vitamins on a Swim-up Syndrome in Lake Ontario Lake Trout

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ABSTRACT. *Collect ions of lake trout spawners and eggs were made in Lake Ontario to assess the ameliorative effects of several B-vitamins (thiamine, riboflavin, folic acid, nicotinic acid, pyridoxine hydrochloride) on mortality associated with swim-up syndrome. Vitamins were administered either by water immersion (1 gm/L to eggs from one female) or intraperitoneal injection (30 ppm to eggs from five females) into the yolk sac. Thiamine, but none of the other B-vitamins, was effective both in reversal and prevention of the clinical signs and mortality associated with the syndrome.*

INDEX WORDS: *Thiamine, lake trout, mortality, reproduction, vitamins, Great Lakes, diseases.*

INTRODUCTION

Despite suspicions that contaminants have been a primary cause of the failure of lake trout reproduction in some of the Great Lakes (Zint *et al.* 1995), no compelling evidence exists to support the suspicions (Fitzsimons 1995). Both Mac and Edsall (1991) and Fitzsimons *et al.* (1995) were unable to correlate mortality associated with swim-up syndrome in lake trout with egg concentrations of organic or inorganic contaminants. Other contaminants may be involved with this source of egg mortality (Fitzsimons *et al.* 1995), but this seems unlikely given the comprehensive chemical analysis done by these authors. Their analyses included non-ortho substituted PCBs, dioxins, and furans — substances that show high toxicity to lake trout in laboratory studies (Walker *et al.* 1991, Walker and Peterson 1991). In addition, the period of highest incidence of swim-up syndrome mortality documented by Mac and Edsall (1991) for Lake Michigan (1978-1981) and Fitzsimons *et al.* (1995) for Lake Ontario (1990-1991) occurred after contaminant burdens in the flesh of adult lake trout from these two lakes had declined significantly (Baumann and Whittle 1988).

The continued inability to link swim-up syndrome mortality to contaminant burdens and pathogenic organisms (Mac *et al.* 1985, Fitzsimons *et al.* 1995) led to consideration of an alternate general hypothesis that the syndrome is the result of nutritional factors. This was suggested by the results of Fitzsimons *et al.* (1995) that showed that fertilization and hatching rate were not correlated with syndrome occurrence, and fry still appeared to have adequate amounts of yolk present at the abrupt onset of clinical symptoms and mortality associated with the syndrome. These clinical symptoms, that included anorexia, loss of equilibrium, hyperexcitability, and eventually death, most closely resembled those of a B-vitamin deficiency rather than a vitamin A deficiency where eye and fluid retention anomalies predominate or a vitamin C deficiency where vertebral anomalies predominate (Halver 1972). A trace metal deficiency also seemed unlikely since Takeuchi *et al.* (1981) reported that low tissue mineral levels were associated with low egg fertility and hatchability in rainbow trout, whereas Fitzsimons *et al.* (1995) found that egg fertility and hatchability was high.

The purpose of this study was to determine if B vitamins administered either intra-peritoneally or by waterborne exposure would ameliorate the clinical signs and mortality associated with the swim-up syndrome and to determine if some B-vitamins were more effective than others.

METHODS

Thiamine Immersion

Swim-up fry affected with the syndrome, that hatched from eggs from one female collected at Grimsby (Lake Ontario), 31 October 1990, were used to assess the effects of a thiamine solution immersion procedure. The thiamine solution was prepared by stirring two pulverized thiamine hydrochloride (50 mg) tablets in 100 mL of dechlorinated tap water for 1 hour at room temperature and then removing the undissolved particulates by filtration. The resulting solution was used to treat three replicate groups of 15 affected fry by immersion in a 250 mL beaker for 2 minutes every second day for 1 month. Three replicate control groups were moved from one part of the raceway to another, on the same day as the thi-

amine treatments, where they remained for 2 minutes before being returned to their original location. After 1 month, all fry were held in plastic strainers in a horizontal flow raceway until mortality was 100% in all groups. Mortality was checked daily and was assumed to have been swim-up syndrome mortality.

Intraperitoneal Injection with Thiamine

To test whether thiamine administered before swim-up was also effective in reducing the syndrome and to determine if this effect was female dependent, sac-fry were injected with thiamine. Solutions were made up in phosphate buffered saline (PBS) using thiamine pyrophosphate (TPP) (Sigma C8754). Nominal dosages (30 ng thiamine/mg wet wt.) were injected into the yolk sac of fry hatched from five females, collected at Stony Island Reef, Lake Ontario, 13 November 1991, that were reared at 4°C as described in Fitzsimons *et al.* (1995). Injection procedures followed those of Metcalfe *et al.* (1988). Three replicates of approximately 25 sac-fry were injected approximately 1 month before swim-up with either TPP or PBS as controls, and were held approximately 1 month after swim-up.

Comparison of Intraperitoneal Injection of Thiamine with Other B-Vitamins

To assess the effect of other B-vitamins on the occurrence of the syndrome, sac-fry were injected with either thiamine (TPP), nicotinic acid (NIC), riboflavin (RIB), folic acid (FOL), or pyridoxine hydrochloride (PYR). Solutions for all vitamins were made up in PBS to deliver a nominal dose of 30 ng/mg. Because FOL and RIB were not totally soluble in PBS at the concentrations used, solutions were allowed to sit overnight before being filtered through Whatman #1 filter paper. Approximately 25 fry were injected in triplicate with one of the vitamins or PBS as controls.

Sac-fry for injection were hatched from eggs collected in nets buried in a spawning shoal at Pt. Weller (Lake Ontario) as described in Fitzsimons (1995). Eggs collected over the spawning period were removed from the shoal 27 October 1992 and incubated in the lab at 4°C until injected 29 March 1993, approximately 2 weeks before swim-up. Sac-fry were held at a mean temperature of 6°C until 14 May 1993, approximately 1 month after swim-up, when the experiment was terminated.

Swim-up Syndrome Observations

Swim-up syndrome mortality had previously been found to be associated with and preceded by a distinct set of clinical symptoms (Fitzsimons *et al.* 1995). These included loss of equilibrium at or just before swim-up—fry lay on their sides on the bottom of the tank, and became hyperexcitable when physically disturbed. Fry showing the above signs were removed and classified as having died of the swim-up syndrome in the injection experiments. Since fry were checked regularly there was sufficient time to remove affected fry before they died, but at that time it was not possible to ascribe a cause of death. Previous work (unpublished observations) indicated that once fry showed the above clinical signs, recovery did not occur and all affected fry eventually died.

Statistics

Either t-tests or one-way ANOVA of arcsine transformed mortality were used to assess treatment effects. Two-way ANOVA was used to assess the effect of female parentage and thiamine on occurrence of the syndrome. I used Student-Newman-Keuls multiple range test for comparison of individual groups. The probability of a Type I error was set at 0.05 for all tests.

RESULTS

Recovery of fry affected with the clinical symptoms of the swim-up syndrome and reduced mortality were evident in fry immersed in a thiamine solution. After 20 days of immersion, mean cumulative mortality in the thiamine group (22.2% ± 2.2 (SE)) was significantly lower ($t = -2.71$, $P = 0.05$) than in controls (53.3% ± 10.2). After 30 days, the difference in mortality was also significant ($t = -10.38$, $P = 0.0004$) — average mortality in the thiamine group was 42.2% ± 9.7, but mortality in the control group was 100%.

Injection of sac-fry with thiamine (TPP, 30 ppm) before swim-up resulted in a significant ($F = 37.98$, $P = 0.004$) improvement in 1-month post swim-up survival relative to controls. This effect was not female dependant ($F = 1.64$, $P = 0.323$). Average mortality in the thiamine treated group (9.4% ± 5.4 ($N = 5$)) was only one-eighth as high as that in the PBS control group (75.8% ± 11.1 ($N = 5$)).

Thiamine was the only B-vitamin that proved effective in reducing swim-up syndrome mortality (Fig. 1). Cumulative average mortality in the thiamine treated group for 32 (5.6% ± 3.7; $q = 5.0$, $P \leq 0.05$) and 42 (18.4% ± 9.8; $q = 6.3$, $P \leq 0.05$) days post-injection was significantly lower than in the PBS injected controls for the 32 (51.9% ± 11.7) and 42 (70.9% ± 9.4) day periods. At no time was cumulative mortality in any of the other groups significantly different from PBS-injected controls.

DISCUSSION

This study clearly showed that thiamine ameliorates the clinical symptoms and mortality associated with the swim-up syndrome. Thiamine not only reversed the clinical signs, but also prevented their development. Fry with clinical signs survived longer than control fry when immersed in a thiamine solution. Similarly sac-fry injected with thiamine prior to the swim-up stage showed a

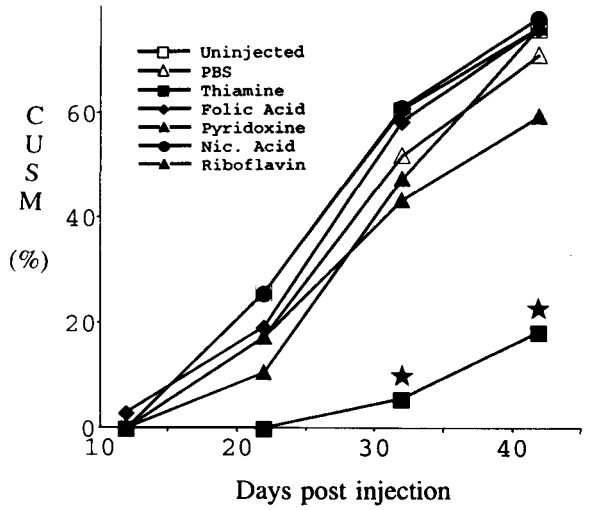

FIG. 1. Relationship between cumulative syndrome mortality (CUSM) and time (days) postinjection with one of five B-vitamins or PBS or uninjected. ★ indicates significantly different ($p < 0.05$) from injected PBS controls.

significant reduction in syndrome mortality up to 1 month after swim-up.

The therapeutic effect of thiamine on this swim-up syndrome, that was characterized by a general lethargy of affected fry, was consistent with the reduced physiological condition noted in thiamine deficient rainbow trout fingerlings (Spannhof *et al.* 1978). Thiamine's effect was also consistent with the known deficiency signs that include anorexia, instability and loss of equilibrium, and hyperexcitability. Despite similarities in the reported deficiency signs of the other B-vitamins tested to those of the syndrome (Halver 1972), thiamine appeared to be the only B-vitamin that was therapeutic for the swim-up syndrome. Injection with nicotinic acid, folic acid, pyridoxine hydrochloride, and riboflavin did not result in a significant reduction in swim-up syndrome mortality.

Thiamine deficiencies have been produced in other salmonids by feeding a thiaminase-containing diet consisting of raw salt-water herring (Wolf 1942, Lehmitz and Spannhof 1977). Thiaminase, a group of enzymes capable of destroying thiamine (Green and Evans 1940, Krampitz and Woolley 1944), occurs in several marine fish species but more importantly is found in rainbow smelt *(Osmerus mordax)* and the alewife *(Alosa pseudoharengus)* (Gnaedinger and Krzeczkowski 1966), two marine invaders of the Great Lakes that are now naturalized and form a major component in the diet of lake trout. The alewife is the primary food source for lake trout in Lake Ontario (Jones *et al.* 1993) and Lake Michigan (Jude *et al.* 1987, Miller and Holey 1992,) where Fitzsimons *et al.* (1995), Mac *et al.* (1985), and Mac (1988) reported the swim-up syndrome to occur. In Lakes Huron and Superior, where in 1980 Mac *et al.* (1985) found the swim-up syndrome to be absent, lake trout fed primarily on smelt (Conner *et al.* 1993, Diana 1990). The commonality of forage fish that contain thiaminase in the diet of lake trout from the Great Lakes with and without the swim-up syndrome does not appear to support the hypothesis of the syndrome being the result of a thiamine deficiency mediated through the diet. Gnaedinger and Krzeczkowski (1966), however, reported that alewives had six times more thiaminase than smelt. When Coble (1965) fed smelt to lake trout, only mild deficiency signs were observed and no mortality occurred. Although not conclusive, the available evidence suggests that the occurrence of the swim-up syndrome in lake trout from Lakes Ontario and Michigan is related to the presence of alewives with their high thiaminase content in the diet. Other factors are probably also involved because swim-up syndrome incidence in Lake Ontario lake trout during the early 1980s when alewives were also important in their diet, was less than 5% (Symula *et al.* 1990; H. Simonin, New York Department of Environmental Conservation, Cortland, NY. pers. comm.)

Whether the swim-up syndrome represents a thiamine deficiency in the eggs or the parents (Morito *et al.* 1986) or both is not known although no unusual mortality of adult lake trout in Lake Ontario has been reported. Determination of the thiamine status of eggs and parents in Lake Ontario will help to clarify the matter. Alternatively, a change in thiamine metabolism may be involved as has been reported to occur in rats dosed with chlorinated hydrocarbons (Yagi 1979). This seems improbable, however, since swim-up syndrome incidence in Lake Ontario lake trout appears to be increasing (Fitzsimons *et al.* 1995) at the same time that levels of chlorinated hydrocarbons in adult lake trout are declining (Baumann and Whittle 1988).

This study represents the first report of a vitamin ameliorating a mortality syndrome in a non-captive salmonid. More importantly it provides the first evidence that an early mortality syndrome in a Great Lakes salmonid, the lake trout, is the result of a vitamin deficiency. Further study is required, however, to characterize the nature and cause of the deficiency.

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