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Tolerance of Developing Salmonid Eggs and Fry to Nitrate Exposure

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This paper reports on tests which show significant effects on early salmonid life stages of nitrates at levels commonly found in groundwaters in geographical areas that are influenced by fertilizer application. It has long been known, from fish cultural experience, that in certain site specific locations, chronic problems can be expected with salmonid egg development and early fry mortality. However, fingerlings which survive usually grow normally. A complete explanation is lacking although several environmental factors have been proposed to account for this phenomenon. One, which has so far received little attention, is that nitrate levels in the ground and surface waters of many areas have been increasing significantly over historical background levels. Ammonia, urea, and other potential sources of nitrate can enter natural waters from a variety of sources, such as domestic or industrial sewage, animal feedlots, or seepage and return flows from agricultural lands. The latter may be the largest contributor, since billions of tons of nitrate fertilizers are applied to agricultural crops on a worldwide basis each year. In addition, intensive forest management techniques include the aerial application of nitrate fertilizer to increase the yield of wood products, while range management practices call for use of nitrates to increase forage production. The nitrate that is not taken up by plants ultimately appears in ground or surface waters.

Only a recommended standard, rather than a mandatory one has been set by the U.S. Environmental Protection Agency (EPA) since nitrate has long been considered to be almost nontoxic to fish (EPA 1976). For example, WESTIN (1974) reported a 96-h TLm of 5,800 mg/L nitrate to chinook salmon fingerlings (Oncorhynchus tshawtscha) and 6,000 mg/L for rainbow trout fingerlings (Salmo gairdneri). However, there is essentially no fish toxicity information available on the effect of nitrate on earlier life stages, such as egg development, hatching success, or early fry survival (KOCH et al. 1978). If the present wholesale land application of nitrogenous materials ultimately increases the nitrate level in surface waters, and results in egg or early fry mortality; this would have potentially adverse impacts on survival of fish populations in streams and lakes through impaired reproductive success. Nitrate toxicity can also be an important consideration in hatchery production, particularly in water reuse hatcheries, which generate nitrate, and in assessing the potential environmental impact of proposed water and land use projects such as sewage treatment plants, or proposed forest, range, and agricultural practices.

With this background in mind, a series of tests were designed to develop guidelines for allowable nitrate exposure in waters of low total hardness needed to protect the egg and early fry stages of chinook (Oncorhynchus tshawytscha) and coho salmon (O. kisutch); anadromous (steelhead) and nonanadromous rainbow trout (Salmo gairdneri), and Lahontan cutthroat trout (S. clarki), a threatened species.

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METHODS

Fertilized (green) eggs were obtained, water hardened in the (sodium) nitrate concentration to be tested, and transported to the laboratory during the shock resistant stage. Chinook and coho salmon, and steelhead and rainbow trout toxicity testing was done at the National Fisheries Research Center, Seattle. The Lahontan cutthroat trout bioassays were carried out at the Bioresources Center of the Desert Research Institute, University of Nevada System, Reno, Nevada. Bioassay water quality characteristics were similar at the two institutions and are summarized in Table 1.

TABLE 1

Average water quality characteristics used for nitrate toxicity bioassay at the National Fisheries Research Center (NFRC) Seattle, and the Desert Research Institute (DRI), Reno.

Constituent	NFRC	DRI
Total hardness (mg/L as $CaCO_3$)	25	39
pH	6.2	7.6
Chlorine	*	*
Calcium (mg/L)	8	12
Chloride (mg/L)	2.3	5
Magnesium (mg/L)	1.2	2
Ammonia-nitrogen (mg/L)	0.01	*
Nitrate-nitrogen (mg/L)	0.01	.07
Nitrite-nitrogen (mg/L)	*	*
Dissolved oxygen (mg/L)	8-10	6-9
Temperature (^{o}C)	10	13

* not detectable

Flow-through systems were used in each case with four nitrate concentrations tested in duplicate on rainbow and steelhead trout and chinook and coho salmon, and five tested on Lahontan cutthroat trout. Standard bioassay conditions were used (EPA 1975).

Egg mortality counts were done daily but, to minimize handling loss, dead eggs were not removed from test or control groups until eye pigmentation developed (eyed stage). Malachite green or formalin prophylaxis was deliberately not used and thus occasional Saprolegnia infestations occurred during the sensitive development phase when mortalities could not be removed daily. After the eyed stage, dead eggs were counted and removed daily. The egg mortality due to nonfertilization was negligible, as determined by clearing in acetic acid (Stockard's) solution. After hatching, the fry mortality was determined and removed daily. Morphologically abnormal fry were preserved in Bouins for later examination. Nitrate exposures were terminated approximately 30 days past yolk absorption (first feeding or "swim-up") stage.

RESULTS AND DISCUSSION

As shown in Table 2, nitrate is at least mildly toxic to the early life stages of several salmonid species. However, in the case of coho salmon, both eggs and early fry were resistant to nitrate toxicity. Chinook salmon, rainbow, Lahontan, or steelhead trout egg or fry mortalities during nitrate exposure were significant at concentrations as low as 5 mg/L though somewhat variable (Table 2). One reason for the variability was the Saprolegnia infestations. An attempt was made to segregate egg mortality due to fungus infestation from that due to nitrate exposure alone, but a cause and effect relationship was sometimes difficult to establish.

Egg mortality as a result of nitrate exposure during incubation did not increase in chinook or coho salmon, but did for steelhead and rainbow trout exposed at 5 or 10 mg/L respectively. Results with Lahontan cutthroat trout were in good agreement in that exposure above the 10 mg/L level resulted in increased egg mortality (Table 2).

Early fry mortality was also species related. For chinook salmon, nitrate exposure at 20 mg/L resulted in significantly increased fry and total (egg plus fry) mortality. Rainbow trout fry mortality was increased at 10 mg/L and above. For coho salmon and steelhead trout, nitrate exposure caused no significant dose-related increases in early fry mortality, but early fry mortalities were significantly increased at the highest (30 mg/L) nitrate concentration with Lahontan cutthroat trout. Since substantial egg mortality occurred in the nitrate-exposed steelhead and cutthroat trout, the fry that hatched were probably from the most resistant fraction of the population and, having survived the incubation period, were able to develop normally. Only the Lahontan cutthroat fry exposed to nitrate at concentrations of 30 mg/L experienced additional mortality.

In summary, nitrate at 5-10 mg/L is mildly toxic to developing eggs and the early fry stages of rainbow and steelhead trout. Chinook salmon and Lahontan cutthroat trout were affected only at exposure levels of 20 mg/L or higher. Coho salmon were sufficiently resistant so that nitrate exposure at the tested levels during incubation will probably not be of concern. Thus, as a guideline, nitrate at levels of 10 mg/L (2 mg/L NO_3-N) in surface waters of low total hardness would be expected to limit survival of some salmonid fish populations because of impaired reproductive success. In the case of soft-water reuse hatcheries using biological nitrification, egg incubation in recycled water should be done with caution. Additional work is needed to determine the point in fry development at which tolerance to nitrate begins to increase to adult levels.

For environmental impact assessment purposes, additional work involving a wider range of water chemistry is needed to more clearly delineate conditions and standards for allowable nitrate levels in areas where fish populations are intended to survive through natural reproduction.

TABLE 2

Egg and fry mortality caused by incubation in nitrate containing water from fertilization through 30-days past first feeding; * indicates significant increase (P = 0.05).

Fish Species	Nitrate (mg/L)		Mortality (%)		
	NO_3	NO_3-N	Egg	Fry	Total
Chinook Salmon	0	0	22	2	24
	5	1.1	18	6	23
	10	2.3	7	6	13
	20	4.5	25	10*	35*
Steelhead Trout	0	0	10	5	15
	5	1.1	29*	7	36*
	10	2.3	29*	3	32*
	20	4.5	30*	3	33*
Rainbow Trout	0	0	22	3	24
	5	1.1	15	5	19
	10	2.3	31*	15*	47*
	20	4.5	31*	13*	44*
Coho Salmon	0	0	9	2	11
	5	1.1	10	2	11
	10	2.3	7	1	9
	20	4.5	9	1	10
Cutthroat Trout	0	0	21	5	26
(Lahontan)	3	0.6	11	8	19
	10	2.3	5	8	13
	20	4.5	34*	7	41*
	30	7.6	74*	12*	85*

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