EFFECTS OF LOW pH EXPOSURE OF ADULT SALMONIDS ON GAMETOGENESIS AND EMBRYO DEVELOPMENT

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Abstract. Mature male and female rainbow trout were treated with low pH (Av. 4.5) sulfuric acid water for 1 or 2 weeks. Percentage of eyed embryos in eggs from control, 1-week-treated and 2-week-treated females were 100%, 80% and 0%, respectively. Low pH exposure of male trout induced 11% deformation in embryos fertilized with their sperm. In order to clarify the physiological mechanism of the effects of low pH exposure, sex hormone levels were compared. In female fish, plasma sex hormones levels showed no difference among the groups, but egg contents of 17α20β-dihydroxy-4-pregnen-3-one, which induces oocyte maturation, were significantly lower in low pH exposed groups. Acidified male fish showed higher plasma levels of the spermiation inducing hormone, 11-ketotestosterone. Effects on eggs were manifested under pH lower than 5. Under continual rearing of land-locked sockeye salmon in acid water, the effects on the oocyte were observed in those adult females which were exposed to acidic condition for more than 1 week before the timing of ovulation. These results suggest that low pH affects oocyte maturational events occurring just 1 week before ovulation, and that acid stress affects the endocrinological mechanisms of final maturation in fish gametocytes. Thus, acidification of the aquatic environment may reduce the reproductive activity of fish at rather low acidity levels.

Key words: low pH, acid exposure, salmonid fish, gametogenesis, embryo development, sex steroids

1. Introduction

A highly acidic environment directly kills embryos, larvae and juveniles of salmonid fishes (Rambough, 1983; Ikuta *et al.*, 1993). However, some fish populations may sustain damage when the acidic environment affects the reproductive activities of mature fish in the early stages of the acidifying process or as a result of exposure of adults to low pH episodes (e.g. during snow-melt). Vuorinen *et al.* (1992a) reported that the breeding season of perch, which is an acid tolerant teleost species, was delayed in acidic lakes. Brook trout avoid acidic environments in the selection of spawning site (Johnson and Webster, 1977). These observations and others suggest that acidification of the environment is a negative factor affecting the reproductive process in fish.

Tam and Payson (1985), Mount *et al.* (1988) and Tam *et al.* (1990) reported that acidification inhibited vitellogenesis and ovulation of the oogonia in salmonids, resulting in the delay of spawning and atresia of the ovary. Weiner *et al.* (1986) reported that development, hatching, and yolk-sac absorption were reduced in eggs stripped from acid-exposed female rainbow trout. These results suggest that acidification of adult fish affects not only the maturational process of the gonads but can also indirectly affect the survival of their progeny. In the present study, we therefore exposed male and female salmonid fish to low pH at the final maturational stage, and analyzed changes in concentration of sex hormones to examine endocrinological mechanisms that may effect gametogenesis and later development of progeny.

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2. Materials and methods

2.1. EXPERIMENT 1

Mature male and female rainbow trout *Oncorhynchus mukiss* (3^+ years old) were treated with low pH (fluctuated 3.0-6.0, average 4.5), generated by the addition of sulfuric acid with aeration to reduce [CO_2], for 0 (control), 1 and 2 weeks. Each group (n=5) was reared in a 300 l round tank using running spring water (9.4±0.4°C, pH 7.2±0.2, alkalinity 53 $CaCO_3$ ppm, Ca 5.12 ppm, Mg 2.06 ppm, Na 11.14 ppm, K 2.61 ppm). The same rearing conditions were used for the following experiments. At the beginning of the experiment, no female fish ovulated and all male fish were ripe. At the end of the acid exposure, eggs and sperm were taken from ripe female and male fish in the control and acidified groups. Gametes were pooled in each group, and then interbred among the groups. The fertilized eggs were incubated under normal conditions. Unfertilized eggs were checked and removed after 24 hr. Survival of embryos was observed at various developmental stages. Plasma and gonads were sampled at the beginning and end of the acidification to measure internal pH and concentration of sex hormones, *i.e.* testosterone (T), estradiol-17β (E2), 11-ketotestosterone (11-KT), 17α20β-dihydroxy-4-pregnen-3-one (DHP). Hormones were extracted from eggs according to the method described by Feist *et al.* (1990), and measured by radioimmunoassay systems as described by Ikuta *et al.* (1987). χ^2-test, Student's t-test and Duncan's multiple range test were used for statistical analyses.

2.2. EXPERIMENT 2

To clarify the critical acidity level affecting oogenesis, mature female rainbow trout (3^+) before ovulation were reared in pH 7.2 (control), 6.5, 5.5 and 4.5 for 2 weeks. Acidity was precisely maintained by automatic pH controllers. At the end of acid exposure, eggs were stripped from ovulated fish, and fertilized with sperm pooled from 3 control males. Development of embryos from each group was individually observed under normal condition.

2.3. EXPERIMENT 3

To clarify the timing of which acid stress affects oogenesis, mature female land-locked sockeye salmon *O. nerka* (3^+) before ovulation were reared for 1 month in pH 7.2 (control) and 5.0 sulfuric acid water generated by a pH controller. Ovulation of all fish was checked every day, and eggs were stripped from ovulated fish. Plasma and ovarian fluid were also sampled to measure internal pH. The eggs were immediately fertilized with sperm pooled from 3 control males, and individually incubated under normal condition. Changes in eyeing rates of embryo were compared between the groups.

2.4. EXPERIMENT 4

To examine the endocrinological effects of low pH on spermatogenesis, changes in plasma levels of androgens (T and 11-KT) were measured in mature male rainbow trout (3^+). The male fish were reared at pH 7.2 (control), 5.0 and 4.0, and blood and sperm were sampled at 1, 3, 7 and 14 days to measure changes in the internal pH, haematocrit, spermatocrit and hormone levels. The same methods as Experiment 1 were used for hormone analyses.

3. Results and Discussion

3.1. THE EFFECTS OF ACIDIFICATION ON FEMALE FISH

Percentages of eyed embryos were significantly reduced by the acid exposure of their maternal fish in Experiment 1 (Table I). While the percentages were almost 100% in the eggs from control females, percentages were reduced to 80 and 0% in the eggs from 1-week and 2-week-acid exposed groups, respectively. Since the inhibition of development was seen only regarding the eyeing rates, acid exposure of females might affect the early development of their embryos. These results suggest that low pH affects gametogenesis in gonads at the final maturational stage.

TABLE I

Survival and teratism rates of progeny from acidified and control mature rainbow trout

Acid treatment of parental fish Males Females	No fertilized eggs	% eyeing	% hatching	% teratism	% emergence	Cumulative % emergence
1. Control (5) x Control (3)	549	98.2	99.6	1.9	97.8	95.6
2. 1-week (5) x Control (3)	595	98.3	98.6	4.3[b]	90.6[a]	87.9[a]
3. 2-week (4) x Control (3)	493	98.6	99.6	11.0[a]	88.4[a]	86.8[a]
4. Control (5) x 1-week (4)	1046	82.8[a]	99.1	2.2	96.7	79.3[a]
5. 1-week (5) x 1 week (4)	1100	80.8[a]	97.8	1.5	95.2[b]	75.2[a]
6. Control (5) x 2 week (2)	403	0.50[a]	50.0	0	100	0.25[a]
7. 2-week (4) x 2-week (2)	357	0.28[a]	0	-	-	0[a]

Number of fish used for fertilization are shown in parentheses. a; $p < 0.01$, b; $p < 0.05$ (χ^2-test)

There was no significant difference in pH of plasma, yolk and ovarian fluid among the groups in every experiments, suggesting that internal pH is precisely maintained by the acid-base regulation function as reported by Tang *et al.* (1987). Thus, low pH probably affects oocytes indirectly through physiological mechanisms. Weiner *et al.* (1986) reported similar results, but no differences were found in plasma sex hormones levels of acid-exposed rainbow trout. We also found no significant difference in the plasma levels of T , E2 and DHP, and the egg contents of T and E2 (data not shown). However, DHP contents in ovulated eggs were lower in acidified females (Figure 1), whereas the contents were elevated in ovulated eggs of control fish in proportion to the increase in plasma DHP levels. DHP is well-known as an oocyte maturation hormone which induces germinal vesicle breakdown leading to meiosis (Nagahama, 1987). Therefore, acid stress possibly affects this process in females.

In Experiment 2, the effects of acid exposure of maternal fish on development to the eyed stage were apparent in only the pH 4.5 group (Figure 2). Therefore, the critical level of pH which manifests effects on oogenesis may exist between pH 5.5 and 4.5. In Experiment 3, the reduction of eyeing rates was observed when female fish were exposed to pH 5.0 for at least 1 week or more before ovulation (Figure 3). This result suggests that the critical timing of acid stress affecting oogenesis may exist about 1 week before ovulation.

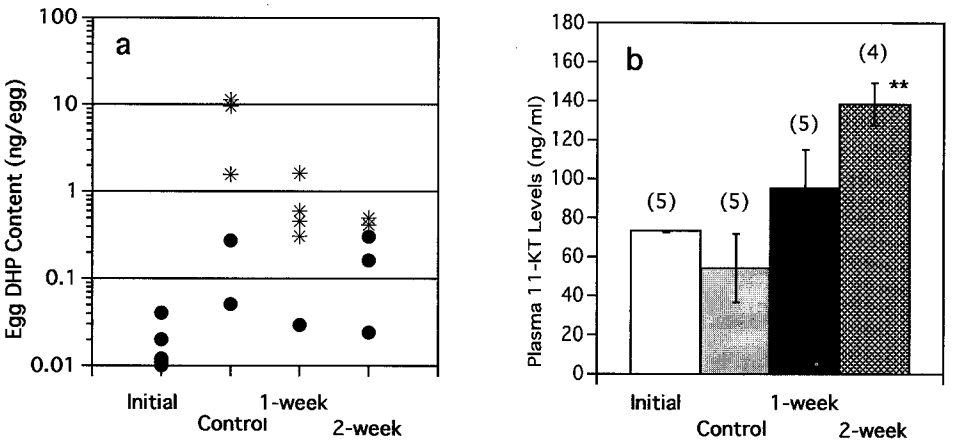

Fig. 1. The contents of DHP in ovulated eggs (✳) and non-ovulated eggs (●) in female rainbow trout (a), and the plasma levels of 11-KT in male rainbow trout (b), in initial control, control, 1-week-acidification and 2-week-acidification groups. The numbers in parentheses represent the number of ovulated females / the number of fish used (a), and the number of fish sampled (b). Vertical lines represent S.E., and ** indicates $p<0.01$ (ANOVA followed by Duncan's multiple range test) (b).

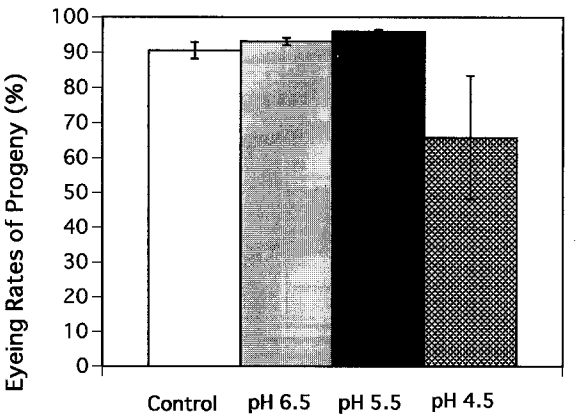

Fig. 2. The eyeing rates of progeny from female rainbow trout in control, pH 6.5-treated, pH 5.5-treated and pH 4.5-treated groups. Vertical lines represent S.E.

Campbell *et al.* (1992) reported that stress reduced the quality of gametes in rainbow trout. It is known that stress induces elevation of an adrenal hormone, cortisol. Since the plasma cortisol levels are also elevated by acid stress (VanDijk *et al.*, 1993), it is necessary to examine the effects of cortisol on gametogenesis under acidic conditions.

3.2. THE EFFECTS OF ACIDIFICATION ON MALE FISH

In Experiment 1, acid exposure of male fish showed no effects on development to the eyed stage, but percentages of swim-up fry (% emergence) tended to decrease in the

progenies of acid-exposed males and control females (Table I). This was because teratism rates of the hatched alevins increased with low pH exposure of parental fish. There was no significant difference in pH of plasma and seminal plasma among the groups. However, low pH stimulated the 11-KT secretion in male fish (Figure 1).

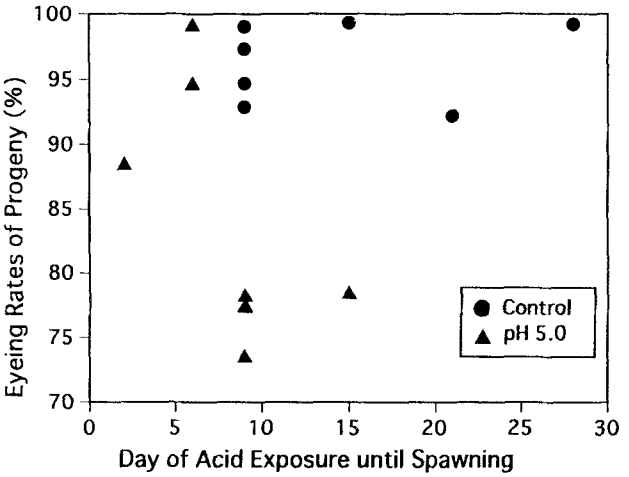

Fig. 3. The eyeing rates of progeny from female land-locked sockeye salmon reared in pH 7.2 as control (●) and pH 5.0 (▲).

In experiment 4, more precise changes in plasma androgen levels were observed (Figure 4). The levels of T and 11-KT showed abnormally high peaks on the 7th day under pH 5.0 conditions. 11-KT is thought to be an inducing factor in the spermatogenesis of male fish (Miura *et al.*, 1991). Therefore, there is a possibility that abnormally high 11-KT levels result in low quality sperm leading to teratism of

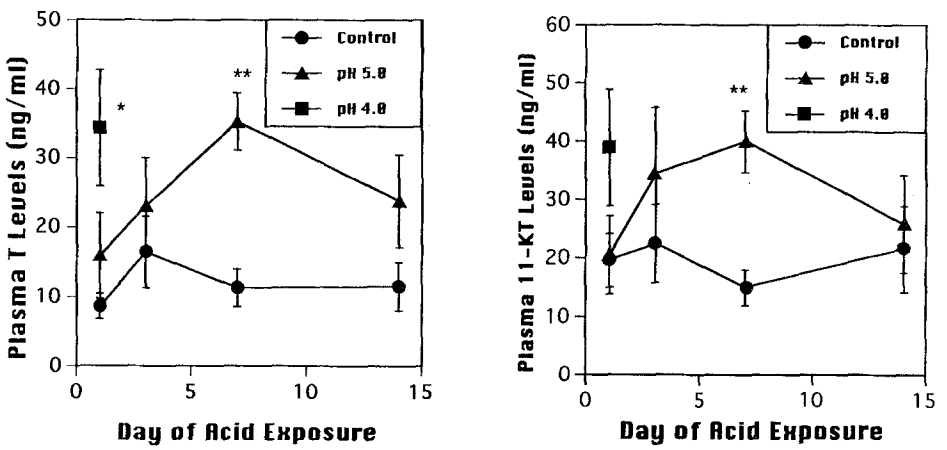

Fig. 4. The changes in plasma levels of T (a) and 11-KT (b) in acid-exposed mature male rainbow trout. All fish died at 2nd day in pH 4.0-treated group. Vertical lines represent S.E. * and ** indicate $p<0.05$ and $P<0.01$ (Student's t-test), respectively.

embryos. Vuorinen and Vuorinen (1992) reported that testes become enlarged in male whitefish due to acidification and addition of aluminum to water. Although it was discussed that this phenomenon was due to the delay of spawning time, elevation of androgen levels might be involved. In this investigation, significant difference was not observed in the spermatocrit, but the haematocrit was higher in acidified males, suggesting that the fish were stressed, but that sperm production was not influenced. The pH of blood plasma and seminal plasma became higher in the pH 5.0-exposed males than in control males.

4. Conclusion

Since low pH exposure of adults reduced the embryonic development of progeny or induced their teratism, we could speculate that acid stress probably damaged the chromosomes at meiosis during the process of gametogenesis through abnormal sex hormone changes. Because relatively low acidity such as pH 5.0 was able to reduce the reproductive activity of salmonid fish, these kinds of physiological effects could damage fish populations in the early stages of acidification before direct effects would be actualized. They may also play important roles in adults exposed to low pH pulses just prior to spawning. However, precise mechanisms of the effects on gametocytes are still unclear; further research is necessary.

Acknowledgments

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