C-2.2.3 Studies on the Effects of Acidification to Fishes

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Abstract : Effect of exposure with low pH and Al on early developmental stage of Koi was observed from fertilized egg for 40 days. Low concentration of Al, 50 ppb in rearing water might bring significant large mortality at pH5.5 for 4 days and at pH6.0, not so low pH, for 19 days. An aftereffect was not observed in the growth and survival rate of acid exposured survivors of Koi or pond smelt. Spowing of medaka stopped under the water pH below 3.9,at that pH some death happened but normal spowing continued under the pH above 4.1,at that pH no death happened. Spowning and ovulation of Ayu were disturbed below pH 5.2. There was a difference between males and females of Medaka in resistance against acid exposure and the females showed higher ability of osmoregulation than the males. 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 female fish, 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.The effects on the oocyte were observed in adult female sockeye salmon which were exposed to acidic condition for more than 1 week before the timing of ovulation. These results suggest that acid stress affects the endocrinological mechanisms of final maturation in fish gametocytes.

Key Words Acidification,Salmon,Carp, Gametogenesis, embryo development, sex steroids

1.Introduction

Acid rain of about same pH as in Europe and north America is observed over Japan but damage of lakes and river due to acid rain is not yet reported. It is predicted that exhaust volume of SOx and NO2,causing substance of acid rain,in China and Korean peninsula become three times of 1986 year's volume up to 2010 year. So,there is a threat of damage appearing on japanese lakes and rivers after 10 or 20 years. In these circumstances,studies of effects of acidification on fish characteristic to japan and studies on uncultivated field of acid rain research by Europe and north America are expected.

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 there productive 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.

2.Research Objective

To clarify the short and medium term effect of acidification and aluminium exposure on the most sensitive stage,early developmental stage of fishes ,the aftereffect on survival rate and growth of survivors from acid exposure, the effect of acidification on spowing of ayu, the mechanism of sexal difference of resistance to acid exposure in medaka and to examine the effect of acidification on salmonid matured fish just before the spowing were the research objectives.

3.Research Method

3.1. Effect of low pH and aluminium on early deevelopmental stages of fishes

Fertilized egg, hatched out fish and 5 day fish after hatch of topmouth gudgeon(Psuedorasbora parva), silver crucian carp(Carassius gibeliolangsdorfi) and koi(Cyprinus carpio) were admitted in the tanks adjusted pH to 7.0,5.5 and 5.2 adding sulfuric acid to artificial fresh water with water hardness 25 ppm CaCO3(Tabata 1969), with Al concentration 0, 100, 200 ppb using AlCl3. Survival was observed for 5-8 days at water temperature 20 ℃. In the case of pond smelt(Hypomesus olidus), mortality was observed for 7 days at 15 ℃ rearing with similar pH, with Al 0, 100 ppb. To clarify longer term exposure effect, fertilized koi eggs were admitted in the tanks for 40 days at 20 ℃, with pH 7.0, 6.0, 5.5, with Al 0, 50 ppb supplying natural diet fullly.

3.2 Growth and survival of survivors from acid exposure

To examine the growth and survival of pond smelt and koi returned to normal water after acid water exposure ,otolith staining method with arizarin complexson for mixed rearing of acid exposured and control fish was considered and decided. Following this method, 67 % survived pond smelt and 40 % survived koi were compared growth and survival respectively with control fish.

3.3. Effect of acidification on Spowing of freshwater fish

Experimental fishes were ayu(Plecoglossus altivelis),captured at the mouth of Shin-shinano river and grown at Ueda experimental station and medaka(Oryzias latipes). Ayu were admitted in the tanks with suitable physical condition for spowing, water current and gravels, controled with pH controller to pH7.0 or 5.2 with addtion sulfuric acid to well water. Pairs of medaka were admitted in the circulating tanks respectively controlled light condition to 14L-10D,water temperature at 26 ℃, pH7.0, examining for spowing , for 1 month and pairs spowning every day admitted in the tanks controlled pH 7.0, 4.3, 4.1, 3.9, 3.7, emamining spowning, fertilization rate and survival.

3.4. Effect of acidification on osmoregulation of medaka

Medaka were transfered to acid water pH4.1 and concentration of Na ion in blood and of Na+,K+-ATPase in gill were measured. And to clarify the mechanism of endocrinological regulation on osmoregulation in acidic condition, medaka was administered adrenal cortex hormone,cortisol and transfered to the water pH 3.8 and effects on concentration of Na ion in blood and of Na+,K+-ATPase in gill were examined. Addtionally to clarify the mechanism of difference between male and female in acid water adaptation, estradiol ,male sex hormone for vertebrate involving fishes, was administered.

3.5. Experiment 5

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 bythe 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 , 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-17b(E2),11-ketotestosterone(11-KT),17 α 20 β -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). c2-test, Student's t-test and Duncan's multiple range test were used for statistical analyses.

3.6. Experiment 6

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.

3.7. Experiment 7

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.

3.8. Experiment 8

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 analyses3.

4. Results and Discussion

4.1. Effect of low pH and aluminium on early developmental stages of fishes

In the rearing water with water hardness 25 ppm $CaCO_3$, exposure with pH5.2 without Al bring no effect on mortality of pond smelt but exposure with same pH with 100 ppb Al high mortality and exposure with pH 5.5 with Al over 100 ppb bring destructive effect on fertilized egg and hatched out fish of topmouth gudgeon and silver crucian carp. In the case of koi , exposure with pH 5.5 without Al bring destructive effect on hatched out fish. Effect of exposure with low pH and Al on early developmental stage of Koi was observed from fertilized egg for 40 days. It was made clear that low concentration of Al, 50 ppb in rearing water might bring significant large mortalityat pH5.5 for 4 days and at pH6.0, not so low pH, for 19 days.

4.2 Growth and survival of survivors from acid exposure

Experimental fishes showed the same growth and survival as control fishes respectively, indicating that the pond smelt and koi after acid exposure become normal in normal waters.

4.3. Effect of acidification on Spowing of freshwater fish

Spowning and ovulation of Ayu were disturbed below pH 5.2 and those of medaka was stopped below pH 3.9,at this pH some fish died but normal spowning was continued above pH 4.1,at this pH no fish died.

4.4. Effect of acidification on osmoregulation of medaka

When medaka were transfered to acid water pH4.1, concentration of Na ion in blood which become an index of osmoregulation dropped for a while but recovered to original level in male after 1 week(Yada and Itou 1996a). In the case of female, concentration of plasma sodium was not effected when transfered to pH4.1 water, indicating female has higher ability in osmoregulation than male. Retaining of plasma sodium and activation of gill Na+,K+-ATPase in acid water were observed concentration dependingly in medaka administered cortisol, indicating cortisol having important role in activation of sodium retaining system. Estradiol administration to male showed the effect for sodium-retaining, unchanging the actibity of gill Na+,K+-ATPase but no effect to female. Sexal difference in acidic water tolerance observed in medaka might be brought indirectly by more secretion of estradiol in female in the relation of prolactin(Yada and Itou 1996a).

Acidification of environmental water results in a large increase in net sodium loss in freshwater fish. In the studies with medaka and tilapia(Yada and Itou 1996b), there are at least two mechanisms to maintain plasma sodium levels in acidic water; an activation of gill Na+,K+-ATPase to increase sodium influx, and a decrease in membrane permeability to minimize sodium efflux. Cortisol showed sodium-retaining effect with the activation of gill Na+,K+-ATPase. Prolactin also has the effect but ATPase activity unchanged. Those two seem to play important roles in the regulation of the two mechanisms in sodium-retaining in acidic water.

Numbers of cortisol and prolactin producing cells and these two hormone secretion reported to increase according to acidification and these two hormone were thought to be important candidate hormone which regulate adaptation to acidic water(Wood 1989). Our results showed directly that these hormone have effects to retain plasma sodium in acidic water.

4.5. 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. 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. 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. However, DHP contents in ovulated eggs were lower in acidified females, 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. 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. This result suggests that the critical timing of acid stress affecting oogenesis may exist about 1 week before ovulation. 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.

4.6. The effects of acidification on male fish

In Experiment 5, 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. 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. In experiment 4, more precise changes in plasma androgen levels were observed. 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 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.

5. References

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