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Levels of Chorionase Activity During Embryonic Development of *Salmo salar* Under Acid Conditions

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Acid precipitation is regarded as the cause of the loss of biota from many lakes in northwestern Europe and eastern North America (BEAMISH et al. 1975; JOHANSSON et al. 1973). Exposure of fish eggs to low (4.0-6.0) pH causes mortality or delayed hatching (KWAIN 1975; MENENDEZ 1976; CARRICK 1979; PETERSON et al. 1980). For Salmo salar eggs held at pH 4.5 from the "eyed" stage there was a delay in hatching, but continuous exposure to pH 4.5 from fertilization did not delay hatching (PETERSON et al. 1980). The suggested cause of the delay was inhibition of the hatching enzyme, chorionase, on shorter exposures to acid conditions whereas exposure from fertilization allowed adaptive processes to compensate.

In an earlier study (HAYA & WAIWOOD 1981) we found the mean level of chorionase activity in salmon eggs held at pH 4.5 for varying time periods after development of eye pigmentation to be significantly lower than in eggs held at pH 6.5. The present study was designed to follow the time course of levels of chorionase activity from eye development through to hatching, in order to determine whether continuous exposure to acid conditions would result in levels of chorionase activity similar to those found at ambient pH.

MATERIALS AND METHODS

Salmon, Salmo salar, eggs were stripped from a two-sea-winter female (North American Salmon Research Centre stock), fertilized and hardened in Chamcook Lake water at the North American Salmon Research Centre before transportation to the Biological Station. Within 4 h of fertilization the eggs were transferred to the experimental containers. Approximately 1500 eggs were placed in each box, two boxes at ambient pH and two at pH 4.5 (nominal). Details of the holding facilities were as described by DAYE (1980). Water for control conditions was from Chamcook Lake (PETERSON et al. 1980) at ambient temperature and pH, whilst low pH was maintained by using sulphuric acid with an automatic titrator. Ambient temperature and pH values over the period of the experiment (December 1980-April 1981) are shown in Fig. 1.

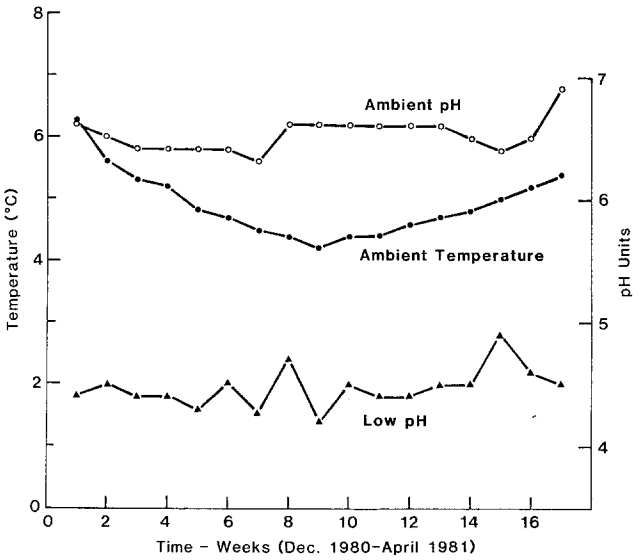

Fig. 1. Ambient temperature and pH values over the time period of the experiment (December 1980 - April 1981). Weekly averages were calculated for ease of plotting.

Within 4 wk of the start of the experiment there was a heavy fungal infection in the low pH boxes which resulted in high mortality. All infected eggs were removed and mortality records were kept on a daily basis for the rest of the experiment. Initially it was planned to sample immediately after eye pigmentation developed but the large mortality at pH 4.5 precluded this; therefore sampling was started as soon as the first embryo hatched (392 degree days, March 2 1981). Hatching of sampled eggs was carried out mechanically in the appropriate incubation water (1 mL/egg). Extracts were either filtered through glass wool or centrifuged (10,000 rpm, Sorvall SS3, 15 min) to remove debris, then divided into aliquots and frozen at -20°C until analyzed. Numbers of alevins hatched were not recorded. Initial samples were of 10 eggs but, as hatching proceeded, this was dropped to 5 in order to sample over as long a time period as possible. Levels of chorionase activity were measured during 390-560 degree days of development.

Analysis of proteolytic activity and protein content were carried out in triplicate as described previously (HAYA & WAIWOOD 1981). Proteolytic activity is reported as PU/egg/h where 1 PU is equivalent to an absorbance change of .001 at 280 nm under the assay conditions used. Data were evaluated by tests for variance and multivariate discriminant analysis.

RESULTS

Of the approximately 3000 eggs held at pH 4.5 at least 800 were removed at the time of the fungal infection. After this initial fungal infection, mortality in eggs held at pH 4.5 was greater

(203) than that of control eggs (43). The numbers include unfertilized eggs, deformed embryos prior to hatching, embryos dying during the hatching process and alevins dying prior to the last egg hatched. Thus, the mortality levels on a percentage basis were approximately 9% at pH 4.5 and 1.5% in control pH, the majority of which were fertile eggs (77% in pH 6.5 and 84% at pH 4.5).

During early samplings for chorionase the eggs were noticeably resistant to mechanical hatching regardless of incubation pH, presumably an indication that chorionase action had not yet softened the inner membrane. By 470 degree days some eggs at pH 4.5 were so soft that it required almost no pressure to break open the chorion; however, the embryos had not hatched. At no time were embryos which had partially hatched, i.e. the tail had emerged from the chorion but yolk sac and anterior end had not, used in samples.
There was a large range in the values of chorionase activity, 24-1097 PU/egg/h at pH 6.5 and 20-620 PU/egg/h at pH 4.5 over the total time of the experiment (Fig. 2). The one-way ANOVA showed that the difference between the means (425 PU/egg/h at control pH, 154 PU/egg/h at low pH) was significant ($p < .01$).

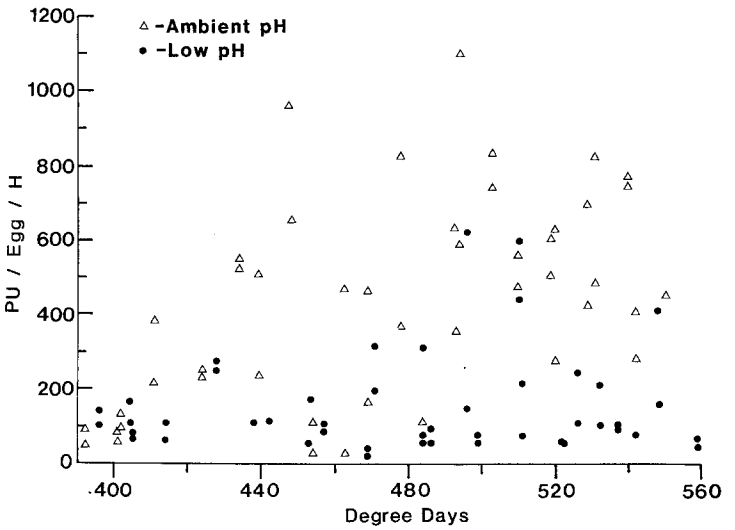

Fig. 2. Chorionase activity (PU/egg/h) of S. salar eggs exposed to low pH (•) and ambient pH (△) from time of fertilization.

Multivariate analysis, using Hotelling's - T^2 statistic, showed a significant difference ($p < .01$) between the two treatments when both degree days and chorionase acitivty were considered.

DISCUSSION

The difference between the mean levels of chorionase activity, 154 PU/egg/h at pH 4.5 and 425 PU/egg/h at control pH, agreed with our previous findings (210 PU/egg/h at pH 4.5 and 500 PU/egg/h at pH 6.5 in a 1979/80 experiment (HAYA & WAIWOOD 1981). Once again the observation of the lack of physical breakdown of the outer layer of the chorion by the alevin, a common observation (MENENDEZ 1976) at low pH, seemed to be the major problem in the hatching process. The ease with which the chorion could be opened in embryos from pH 4.5 sampled later on in the experiment would suggest that the problem does not lie in a lack of action of chorionase but either in a structural change to the outer membrane, or a lack of embryonic movement to achieve breakdown of that membrane.

As shown by YAMAGAMI (1972), the level of enzyme activity in the casein assay system is a quantitative measure of the amount of enzyme present; thus, our results indicate that there is less chorionase present in the perivitelline fluid of salmon eggs held in water at pH 4.5. This is true throughout the incubation period; however, since hatching was initiated at 392 degree days in control pH when chorionase activity is predicted to be 113 PU/egg/h, it would seem that in both regimes there is enough enzyme to achieve hatching at some time.

The analysis of chorionase activity was conducted at the optimal pH (7.9) for the enzyme (YAMAGAMI 1973). The casein hydrolysis assay could not be used to see if chorionase from eggs held at pH 4.5 had undergone any changes in pH optimum since casein will not solubilize at pH's lower than 5.0. However, both HAGENMAIER (1974) and YAMAGAMI (1973) present profiles for chorionase activity at pH values lower than 5.0, using the casein technique. Another assay substrate is necessary before the pH optimum can be definitively measured. Our work indicates that salmon chorionase is stable enough to be active at pH 4.5 after prolonged exposure at low pH but, whether this is due to increased synthesis and rapid turnover, altered structure resulting in lower activity or a change in kinetics, we cannot say. DIMICHELE (1981), using Fundulus heteroclitus enzyme preincubated outside the optimal pH range and assayed at pH 8.1 (a similar situation to ours), found a distinct loss of activity. However, in vivo, the Fundulus enzyme is active in conditions which cause a 90% loss of the optimum activity in vitro.

Whether the level of chorionase activity is coordinated with the morphological stage of embryonic development cannot be answered unless the individual eggs were staged for degree of development before sampling plus only eggs of the same stage were used in individual assays. It is acknowledged that chorionase release is aided by embryonic movement (YAMAGAMI 1981) and recent observations (PETERSON, pers. comm.) of Salmo eggs held at pH 4.5 have shown significant reductions in the number of embryonic movements which could be reversed by switching incubation pH. This would agree with our observations of difficulty in emergence

of alevins from the chorion. Possibly this reflects a change in the biochemical function of alevin musculature.

Thus continuous exposure to low pH from the time of fertilization produces a similar inhibitory effect on levels of chorionase activity as did exposure for short time periods after development of eye pigmentation.

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REFERENCES

BEAMISH, R. J., W. L. LOCKHART, J. C. VAN LOON and H. H. HARVEY: Ambvio 4, 98 (1975).

CARRICK, T. R.: J. Fish. Biol. 14, 165 (1979).

DAYE, P. G.: Can. J. Fish. Aquat. Sci. 37, 1035 (1980).

DIMICHELE, L., M. H. TAYLOR and R. SINGLETON, JR.: J. Exp. Zool. 216, 133 (1981).

HAGENMAIER, H. E.: Comp. Biochem. Physiol. 49B, 313 (1974).

HAYA, K. and B. A. WAIWOOD: Bull. Environm. Contam. Toxicol. 27, 7 (1981).

JOHNASSON, N., J. E. KIHLSTROM and A. WAHLBERG: Ambio 2, 42 (1973).

KWAIN, W-H.: J. Fish. Res. Board Can. 32, 493 (1975).

MENENDEZ, R.: J. Fish. Res. Board Can. 33, 118 (1976).

PETERSON, R. H., P. G. DAYE, and J. L. METCALFE: Can. J. Fish. Aquat. Sci. 37, 770 (1980).

YAMAGAMI, K.: Devel. Biol. 29, 343 (1972).

YAMAGAMI, K.: Comp. Biochem. Physiol. 46B, 603 (1973).

YAMAGAMI, K.: Amer. Zool. 21, 459 (1981).

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