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Early Life History of Triploid Rainbow Trout (*Salmo gairdneri* Richardson)

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ABSTRACT

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A study of the early life history of triploid rainbow trout (*Salmo gairdneri* Richardson) showed that the mean embryonic development time was significantly shorter in triploid fish ($381^{\circ}\text{C} \times \text{days}$) than in the control group ($384^{\circ}\text{C} \times \text{days}$; $P < 0.01$). Hatching speed (slope of the hatching curves) was identical in both groups. Triploid survival rates from fertilization to eyed stage, hatching and swim-up were 84.7, 95.0 and 97.9% respectively, relative to the controls, resulting in a total reduction of survival rate associated with triploidy of 21.3% at start feeding. Absorption of water by the yolk reserves was similar for both genotypes, but at swim-up, triploids were significantly lighter (95% of the controls) than their diploid full-sibs ($P < 0.01$). Starvation resulted in an increase in water content and a decrease in wet weight that were significantly greater for triploids ($P < 0.01$) until all animals died 3 weeks after swim-up. As soon as feeding started, differences between diploids and triploids increased rapidly. Thus, at day 106, triploid wet weights represented 91% of the control. A 10-day delay in start feeding did not modify this scheme.

INTRODUCTION

Sexual maturation in salmonids generally produces a detrimental effect on fish culture by the mobilization of energy for gonadal growth, low somatic growth rate, depreciation of the flesh quality and high mortalities at sexual maturity. Among the different techniques studied to overcome these problems (see review by Stanley, 1981), induction of triploidy appears effective as it produces infertile animals with a reduction of gonadal development (Stanley, 1981; Donaldson and Hunter, 1982; Chevassus, 1983; Purdom, 1983; Thorgaard, 1983; Lincoln and Scott, 1984; Solar et al., 1984; Chevassus et al., 1985). Application of a heat shock a few minutes after fertilization was the first suc-

cessful method to produce triploid rainbow trout populations (Chourrout, 1980; Chourrout and Quillet, 1982; Lincoln and Scott, 1983). Lower survival rates and smaller size of triploids at the end of early development have already been described (Chevassus, 1983; Solar et al., 1984). In this study we investigated the first 4 months of life of triploids in order to describe more accurately events occurring during incubation, yolk resorption and initiation of feeding, with particular emphasis on duration of embryonic development, survival and growth.

MATERIALS AND METHODS

Fertilization — triploidization

Breeders from the CP 80 rainbow trout strain were kept in the experimental station of Gournay (INRA). Artificial fertilization was performed in April 1983. The eggs were stripped from eight 3–4-year-old females by abdominal pressure and each spawn was kept separate. Milt was collected from fifteen 3-year-old males and pooled. Fertilization was carried out according to Billard (1974) using a saline buffer. The heat shock (26.5°C) was applied for a duration of 20 min as described in Chourrout (1980) and Chourrout and Quillet (1982) but the activation period was reduced to 16 min at 14°C ; incubation temperature was 10°C . On day 24 the eggs reached the eyed stage. Dead embryos were removed and their number recorded. The fertilization rate was calculated as the ratio of eggs at the eyed stage over the initial number of ova. In the same period two spawns (females 2 and 3) with the lowest fertilization rates were discarded.

Hatching and survival

The experimental hatching set was designed as follows: 6 spawns $\times$ 2 genotypes (diploid controls and triploids) $\times$ 2 replicates. Each replicate contained 150 eyed eggs set in small plastic boxes with nylon mesh bottoms. Water temperature was maintained at $9.5 \pm 0.5^{\circ}\text{C}$. Counting of hatched larvae was done 2 to 4 times a day and dead embryos were removed. The percentage hatching rate was calculated as the ratio of hatched larvae over the total number of eyed eggs. Probit transformations (Finney, 1962) were applied to perform regression of hatching percentages as a function of time. The hatching speed was estimated as the slope of the regression line whereas the time when 50% of the larvae hatched provided an estimation for mean embryonic development time ($^{\circ}\text{C} \times \text{days}$). The three parameters were analysed by a two-way ANOVA. Percentages were transformed as follows: $p' = \arcsin \sqrt{p}$.

Growth

During yolk absorption, weight measurements were collected weekly using the same experimental design as for hatching rates. Fry were gently dried on filter paper and individually weighed to the nearest 10^{-3} g. Mean dry weights were obtained from 30 individuals in each replicate sample, which were then frozen for 48 h and oven-dried at 90°C for 48 h. Dry matter was measured to the nearest 10^{-5} g and water content was obtained by difference.

At swim-up, survival rates were estimated and another experimental design was set up to follow further performances. Fry issuing from the two females with extreme-sized eggs were kept (females 1 and 8) in order to detect any possible effect of the egg size. For each female three groups of fry were composed as follows:

A0: the animals did not receive any food

A1: the fry were fed at the beginning of swim-up (day 64)

A2: the fish received food by the end of swim-up (day 74)

Fry were fed ad libitum automatically with $600\text{-}\mu\text{m}$ calibrated pellets (Tess). Wet weights, dry weights and water content were measured as described earlier, while instantaneous growth rates (IGR) were calculated according to Gall and Gross (1978).

RESULTS

Embryonic development and hatching

Mean embryonic development times of triploids ($381^{\circ}\text{C}\times\text{days}$) were significantly shorter ($P<0.01$) than those of the control group ($384^{\circ}\text{C}\times\text{days}$) as shown in Table 1. Moreover, a similar statistical difference ($P<0.01$) was recorded between the eggs of the different females. However, diploids and triploids did not exhibit discrepancies as far as hatching rates and hatching speeds were concerned.

Survival

Percentage survival at the eyed stage, hatching and swim-up are given in Fig. 1. Triploids showed a particularly low survival rate at the eyed stage as compared to controls (84.7% of the control, $P<0.01$). At hatching and swim-up, mean survival rates of triploids relative to the control were respectively 95.0 and 97.9% (no statistical difference). The detrimental effect of triploidy on survival rate from fertilization to start feeding reached 21.3% relative to the control. A strong correlation ($P<0.005$) was recorded between the performance of triploids and their diploid sibs at all stages, as well as a significant maternal effect ($P<0.01$).

TABLE 1

Initial mean weight of eggs and hatching parameters (hatching rates, mean embryonic development durations and hatching speeds)

Females	Initial mean weight of eggs (mg)	Genotype	Hatching rate (%)	Mean embryonic development duration ($^{\circ}\text{C} \times \text{day}$)	Hatching speed (slope)
1	106	2n	100.0 ± 0.0	386.4 ± 1.7	194.0 ± 1.4
		3n	97.3 ± 2.8	381.3 ± 1.0	204.0 ± 15.6
4	105	2n	100.0 ± 0.0	391.1 ± 0.0	146.5 ± 12.0
		3n	95.7 ± 0.5	388.3 ± 0.4	152.5 ± 34.7
5	98	2n	99.4 ± 0.9	385.2 ± 0.3	192.0 ± 21.2
		3n	99.0 ± 1.4	382.0 ± 0.6	208.5 ± 0.7
6	88	2n	98.7 ± 0.0	381.2 ± 0.1	127.0 ± 11.3
		3n	97.7 ± 0.5	378.4 ± 0.0	127.0 ± 18.4
7	78	2n	98.0 ± 0.0	384.6 ± 1.0	112.5 ± 9.2
		3n	99.0 ± 0.4	381.3 ± 1.1	126.0 ± 17.0
8	82	2n	100.0 ± 0.0	378.4 ± 0.1	220.5 ± 24.7
		3n	98.7 ± 0.9	374.1 ± 0.6	180.0 ± 1.4

Yolk resorption

Values for wet weight, dry weight and water content are given in Figs. 2, 3 and 4 respectively, and represent the means of the two females. The decrease in wet weight observed on day 40 (Table 2) was due to the loss of chorion and perivitelline fluid after hatching. Later on, the water content increased rapidly (Fig. 4), resulting in positive IGR values (Table 2). The initial difference in egg weight between females was still observed with wet and dry weights of fry (significant maternal effect ($P < 0.01$) for both parameters). Triploids, initially identical to their controls (Fig. 2), became significantly lighter by the end of yolk resorption ($P < 0.01$ at day 64 for wet weight; $P < 0.01$ at day 59 for dry weight).

Starvation and feeding experiments

In the case of complete starvation (group A0) the above-mentioned difference in wet (Fig. 2) and dry weights (Fig. 3) of triploids remained unchanged until the death of the animals, the latter occurring 3 weeks after swim-up. The exhaustion of the body reserves was marked by an increase of water content (Fig. 4) which finally reached 88% of the body weight. In both diploids and

Fig. 1. Comparative survival rates at different periods (values represent means $\pm$ standard deviations). Open columns, diploid; shaded columns, triploid.

triploids, the time of 100% mortality occurred 3 days later for the progenies of female 1 (large eggs) than in those of female 8 (small eggs).

In fed groups, the growth patterns depended on the date of start feeding. In early fed groups (group A1), IGR values were moderate during the first 10 days while group A2 reacted in the same way as group A0; a loss of weight, as shown in Fig. 2, was recorded as well as an increase in water content to 82.8% instead of 81.2% for group A1. During the next 10-day period, both groups exhibited high IGR values (Table 2). Finally, during the last period, IGR values decreased in both groups though much more drastically in group A1 than in group A2, the latter compensating partly its delay in growth.

By the end of the experiment (day 106), whenever feeding had started, tri-

TABLE 2

Evolution of instantaneous growth rates (IGR) during yolk absorption (a) and feeding experiments (b) (values are expressed in percent of body weight per day)

		Days					
(a)	Genotype	31	40	46	52	59	64
	2n	−0.57	2.19	2.75	2.53	0.84	
	3n	−0.57	2.52	2.36	2.53	0.49	
		Days					
(b)	Genotype	64	74	85	95	106	
A0	2n	−1.19	−1.76	100% death at day 83			
	3n	−1.11	−1.93				
A1	2n	5.00	8.16	3.72	3.55		
	3n	4.84	7.46	3.83	3.65		
A2	2n	−0.94	7.63	5.44	4.82		
	3n	−0.80	6.98	5.53	4.61		

A0, starvation; A1, fry fed at day 64; A2, fry fed at day 74.

ploid wet weights were significantly lower (91% relative to the control, $P < 0.01$) while water contents for both genotypes were equivalent (Fig. 4).

DISCUSSION

Embryonic development and hatching

A significant reduction of embryonic development time was recorded for all females. This phenomenon has already been described by Quillet (1986) and to an even larger extent in Quillet et al. (in press) (up to 13°C×days instead of 3°C×days in our case). Although an “accelerating” effect of the heat shock itself cannot be excluded, it seems insufficient, as far as °C×days are concerned, to explain such a difference. Swarup (1959), working on sticklebacks, showed that triploids had a similar body size to diploids but were composed of fewer cells. These observations were confirmed by several recent works on other fish species (Gervai et al., 1980 on carp; Thorgaard et al., 1982 and Benfey et al., 1984 on salmonids). Beatty and Fischberg (1951), working on polyploid blastocysts of mice, also described a decrease in cell number proportional to the increase in the degree of ploidy. Assuming that the rate of mitotic division is not too strongly affected by polyploidy, as suggested by data on embryonic

Fig. 2. Evolution of wet weights during yolk absorption (▼, diploid; ▽, triploid). Evolution of wet weights during feeding experiments (▲, diploid and △, triploid, group A0; ■, diploid and □, triploid, group A1; ●, diploid and ○, triploid, group A2). Values represent means $\pm$ standard deviations. * $P < 0.05$; ** $P < 0.01$. S.F., start feeding.

Fig. 3. Evolution of dry weights during yolk absorption (▼, diploid; ▽, triploid). Evolution of dry weights during feeding experiments (▲, diploid and △, triploid, group A0; ■, diploid and □, triploid, group A1; ●, diploid and ○, triploid, group A2). * $P < 0.05$; ** $P < 0.01$. S.F., start feeding.

metabolism of polyploid mice (Eglitis and Wiley, 1981), then reduction of embryonic development time is likely to be observed in triploids. However, one should keep in mind that hatching represents a quite arbitrary end of embryonic development and does not constitute an absolute physiological stage since it is subject to much variation (Vernier, 1969).

Fig. 4. Evolution of water content during yolk absorption ($\blacktriangledown$, diploid; $\triangledown$, triploid). Evolution of water content during feeding experiments ($\blacktriangle$, diploid and $\triangle$, triploid, group A0; $\blacksquare$, diploid and $\square$, triploid, group A1; $\bullet$, diploid and $\circ$, triploid, group A2). Values represent means $\pm$ standard deviations.

Survival

A good correlation was observed between diploid and triploid survival rates; therefore, poor egg quality increases the sensitivity to the triploidization treatment. Accordingly, the use of prime quality spawns is advised for the treatment in order to reduce the depressive effect observed on survival. Overall survival rates of triploids at swim-up were 78.7% relative to the control, which is equivalent to other results recorded on the same species (78% by Chevassus et al., 1985; 73% by Quillet, 1986).

Yolk resorption

Absorption of the yolk involves an increase of the water content which may essentially result from the imbibition of the vitelline reserves considered as more or less dessicated nutriments (Gray, 1926). Evolution of wet weight during this period was consistent with previous work (Gray, 1928) showing that the specific growth rate of the embryo is proportional to the available amount of yolk. Thus, the maximum wet weight of the larva is reached before the embryonic cycle is completed. After this time, the wet weight of the yolk being used up for maintaining the embryo is more important than the wet weight of the larva being produced (Gray, 1928).

Concerning the influence of the egg size for both genotypes, it is noticeable that fry died 3 days later in the spawn with bigger eggs since the quantity of yolk available allowed them a longer respite. This corroborates data on effects of egg size on the early growth of rainbow trout (Yazbeck-Chemayel, 1974; Escaffre, 1986).

Even if initial absorption of the yolk does not seem to be affected by triploidy, since increase of water content is similar for both groups, the pattern of yolk utilization later on is quite different. Indeed, by day 64 (swim-up) wet and dry weights of triploids are significantly lower than in diploids. Moreover, this tendency was observed subsequently in the starved groups. Swarup (1959) measured different organs of triploid sticklebacks and showed that the hind brain, the inner layer of the retina or the pronephric duct were composed of fewer cells than in diploids. Thus, it could be assumed that such morphological modifications could induce physiological differences between the two genotypes. Recent work on metabolism of triploid rainbow trout embryos (Oliva-Teles and Kaushik, 1987) did not reveal discrepancies as far as oxygen consumption, ammonia excretion, energy and protein expenditures were concerned. However, the measurements were stopped 20 days after hatching, a time at which differences were also undetectable in our study.

Early growth on exogeneous food

There was clear evidence of an influence of the time of start feeding on the pattern of early growth. IGR values during the first 10-day period of feeding were lower in animals fed earlier (group A1) than in those fed later on (group A2). Such a result could mean either that the food introduction was too early in the first group and that all the animals were not yet able to feed properly at that time or that the slight starvation caused by the 10-day delay of feeding provoked a compensatory growth in the second group. Similar works on salmonids (Bilton and Robins, 1973; Twongo and MacCrimmon, 1976) showed that a slight delay in food presentation after swim-up did not alter the ability of the fry to feed and promoted a more regular and more efficient growth.

As far as differences between diploids and triploids were concerned, early or delayed start feeding led to the same result by the end of the experiment, triploids being about 9% lighter than the diploid controls. This result is in good agreement with previous works (Chevassus et al., 1985; Quillet, 1986) and shows that it does not seem possible to prevent this "early triploidy depression" through the choice of the start feeding period.

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