Early Growth and Gonadal Development of Triploid Rainbow Trout, *Salmo gairdneri*

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3배체 무지개송어의 초기성장과 생식소 발달에 관하여

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ABSTRACT

Experiments were carried out to examine early life history, growth and gonadal development of triploid rainbow trout to evaluate their suitability for commercial aquaculture. A high degree (96.5%) of triploid fish was induced when the fertilized eggs were treated with heat shock. Mean embryonic development time of triploids was slightly shorter than diploids and survival rate of triploid at swim-up was 80.2% relative to control. Erythrocytes and their nuclei of triploid were larger in both axes than those of diploid. Triploid rainbow trout showed improved growth relative of diploid after 1 year of age. Histological analysis of the gonads revealed sterility in triploid fish.

요 지

3배체 무지개 송어의 산업적 유용성을 검토하기 위하여 3배체 유도율, 부화 소요시간, 생존율, 세포의 크기, 성장 및 생식소 형성 등을 조사하였다. 그 결과 96.5%의 3배체가 유도되었으며 부화까지 소요시간은 3배체가 2배체에 비해 약간 빨랐고 생존율은 대조군의 80.2%에 달하였다. 세포의 크기는 장, 단축 모두 대체로 1.2~1.3배 정도 3배체가 컸으며 성장은 초기에는 2배체가 약간 빨랐으나 부화후 1년이 지나면 3배체가 대조군보다 성장율이 증가되었다. 조직학적 분석결과 3배체의 경우 성숙기에 도달하여도 생식소가 성숙되지 않았다.

INTRODUCTION

Induced polyploidy refers to the production of individuals with extra sets of chromosomes. The use of induced triploidy as genetic technique in aquaculture has attracted considerable attention in recent years (Thorgaard, 1986). Triploids, organisms with

three sets of chromosomes may show enhanced growth and altered cell physiology (Meyer, 1985). The induction of triploidy in fish and its application to fish culture has recently been reviewed (Purdom, 1983 ; Thorgaard, 1983 ; 1986).

The benefit of triploids to fish culture lies in their potential sterility, as a consequence of having an uneven number of chromosome sets. Triploid fishes are expected to avoid the deleterious side-effects of maturation, such as reduced appetite, reduced food efficiency, deterioration of flesh quality, and high post spawning motality (Benfey and Sutterlin, 1984). Furthermore, triploids have higher heterozygosity than diploids (Allendorf and Leary, 1984). This has been shown to be associated with higher developmental stability as measured by fluctuating asymmetry (Leary et al., 1985).

The rainbow trout (*Salmo gairdneri*) is an important commercial aquaculture species. Induced triploidy as a means of suppressing gonad development may have great commercial advantages in rainbow trout culture because major problems in the culture of this species on a large scale are detrimental effects of sexual maturation on growth, survival and marketability of fish (Bye and Lincoln, 1981 ; Johnstone et al., 1979 ; Kim et al., 1986).

Relative growth of triploid rainbow trout compared to diploid varies according to various experimental conditions. Thorgaard and Gall (1979) reported that triploid rainbow trout showed improved growth relative to diploids after maturation. But, Wright et al. (1982) found twenty month old triploid rainbow trout still showed retarded growth and only female triploids appeared to benefit from the increased growth potential, because gonadal development is completely arrested in female (Thorgaard, 1985). If it is in fact the case, their benefit to fish farming may be reduced.

Recently, triploid rainbow trout have been produced on a large scale practice by the application of temperature shock in Korea (Kim et al., 1986). The purpose of this research was to examine early life history, growth and gonadal development of triploid rainbow trout to evaluate their profitability for commercial aquaculture.

MATERIALS AND METHODS

Triploidy was induced by heat shocking shortly after the fertilization of eggs (Kim et al., 1986). Ploidy levels at 4-month-old fish were determined by chromosome numbers and cell and nuclear sizes (Kim et al., 1982 ; Park and Chung, 1985). The total lengths of diploid and triploid individuals were measured at 3 intervals over a 12-month period from hatching. The gonads were excised and fixed Bouin's solution. Every fixed sample was sectioned in 4μm thickness and stained with Harris's hematoxylin and eosin-phroxin for histological study of the gonads (Park and Kim, 1984). At the period of maturation (about 2-year-old fish), 20 diploid and 20 triploid fish were killed and their trunk morphology was observed.

RESULTS

The 57 karyological examinations performed on 4-month-old fish revealed triploid chromosome complements except for only 2 diploid fish (Fig. 1).

FIGURE LENGENDS

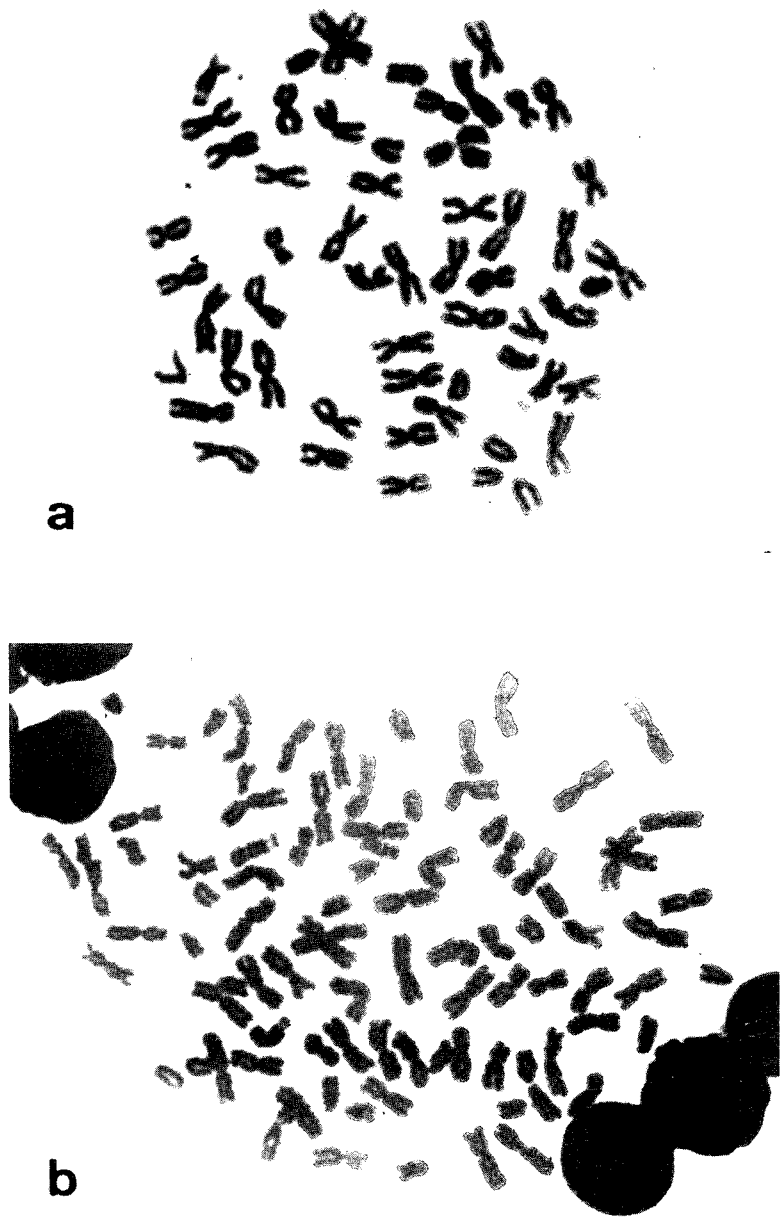

Fig. 1. Metaphases of diploid (a) and artificial triploid (b) rainbow trout.

Mean embryonic development time of triploids was slightly shorter than diploid controls. Triploids showed relatively low survival rate during incubation and yolk absorption as compared to diploids (80.2% of the control). But, triploid fish did not differ from diploids in body configuration (Fig. 2).

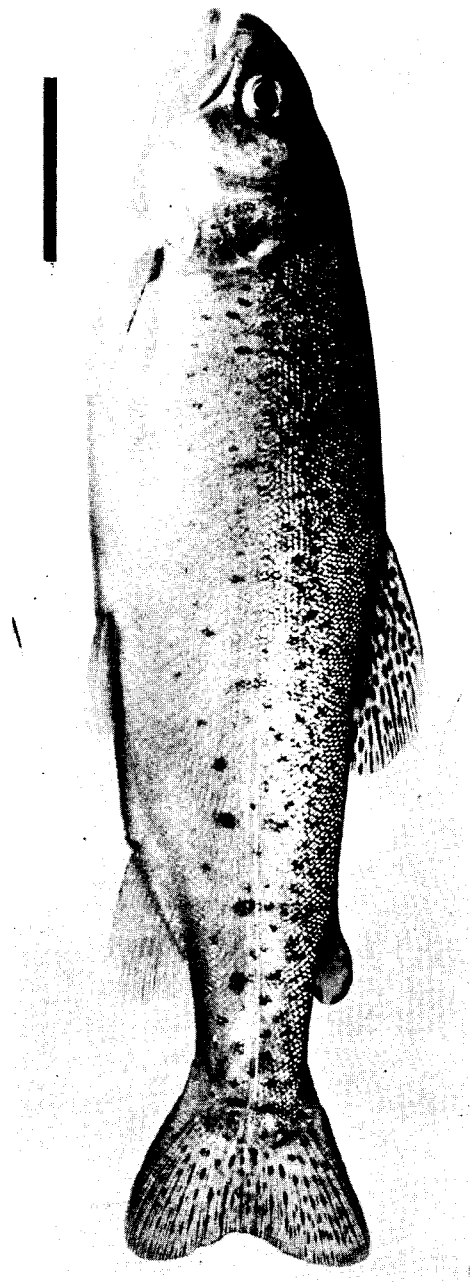

Fig. 2. External morphology of triploid rainbow trout. Bar indicates 50 mm.

Table 1 and figure 3 shows the average cell and nuclear volumes of erythrocytes from the fish classified as diploid and triploid. Erythrocytes and their nuclei of triploid were lager in both axes than those of diploid. In the diploid fish, the mean of erythrocytic size was 14.92×9.27 μm, and that of nuclear size 7.24×4.82 μm, whereas the corresponding size to the triploid fish were 18.32×10.77 μm and 9.38×6.02 μm respectively. The means of the surfase areas of erythrocytes and their nuclei were 108.12 μm^2 and 28.06 μm^2 in the diploid fish, and those of triploid were 154.16 μm^2 and 48.86 μm^2 respectively. The ratio of the area of the triploid to diploid was 1.43 for cells and 1.73 for nuclei. The volume of triploid cells was 1.66 fold the diploid ones, while the volume of the nuclei was 2.19 fold the diploid nuclei volume (Table 1).

Diploid showed a higher initial growth rate. However, the growth rate of triploids soon started to increase and showed the similar growth rate to that of the diploid fish up to the age of about 11 months. At the 12 months of age, triploids were significantly larger than diploids (Table 2).

Histological analysis of the gonads showed that triploidy did not alter sex ratio significantly (male/female = 1.2). Diploid ovaries were packed with oocytes, mostly at perivitellogenic state with some upto endogenous perivitellogenesis. But triploid ovaries did not show these developments. The triploid females were identified by the presence of oogonia and ovarian cavity (Fig. 4). Testes of the triploid fish were similar to those of the diploid controls in early growth stage. Diploid testes in the advanced stage of spermiogenesis contained large areas containing spermatids and spermatozoa, whereas triploid testes contained few cells which were developed beyond the spermatocyte stage and no spermatozoa were observed (Fig. 5).

Triploid testes and ovaries, though well developed, did not have spermstozoa and matured oocytes typical of diploid ones and internal space of the trunk of triploid was smaller than diploid controls (Fig. 6).

Table 1. Comparison of erythrocytic size between diploid and triploid in rainbow trout

		Diploid*	Triploid*	Ratio of triploid to diploid
Erythrocyte :	Major axis (μm)	14.92± 0.69	18.23± 0.74	1.22
	Minor axis (μm)	9.27± 0.32	10.77± 0.25	1.16
	Surface area (μm^2)	108.12± 5.29	154.16± 8.45	1.43
	Volume (μm^3)	668.71±46.76	1109.11±82.04	1.66
Nucleus :	Major axis (μm)	7.24± 0.45	9.38± 0.67	1.30
	Minor axis (μm)	4.82± 0.18	6.02± 0.34	1.25
	Surface area (μm^2)	28.09± 1.19	48.86± 5.56	1.73
	Volume (μm^3)	88.06± 8.43	192.74±32.31	2.19

* Values are means ± standard deviation

Fig. 3. Microphotographs of diploid (a) and artificial triploid (b) erythrocytes of rainbow trout Bars indicate 20 μm.

Table 2. Comparison of mean length of 4-, 8-, and 12-month-old diploid and triploid rainbow trout

Month	Total length (cm)	
	diploid*	triploid*
4	8.70±0.77	8.03±0.55
8	13.08±2.60	12.43±1.21
12	17.47±3.68	18.51±1.26

* Values are means ± standard deviation

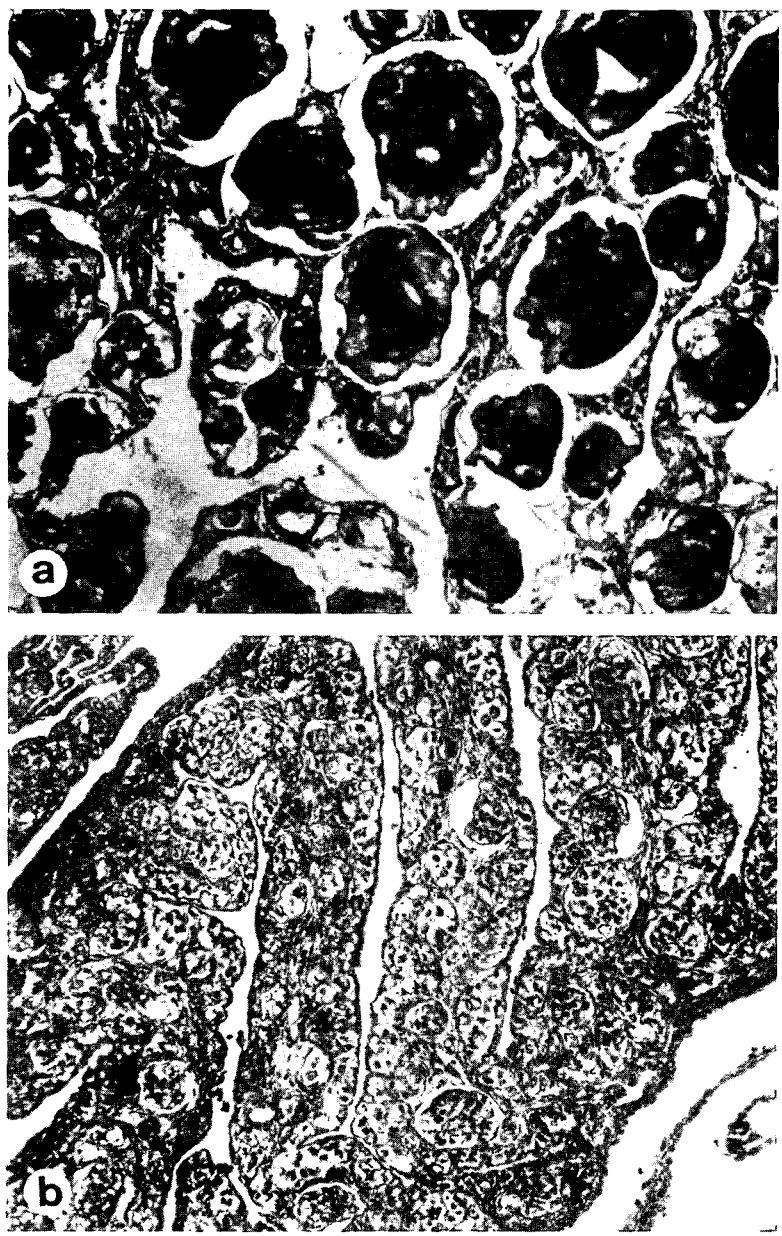

Fig. 4. Transverse sections of diploid (a) and artificial triploid (b) ovaries.

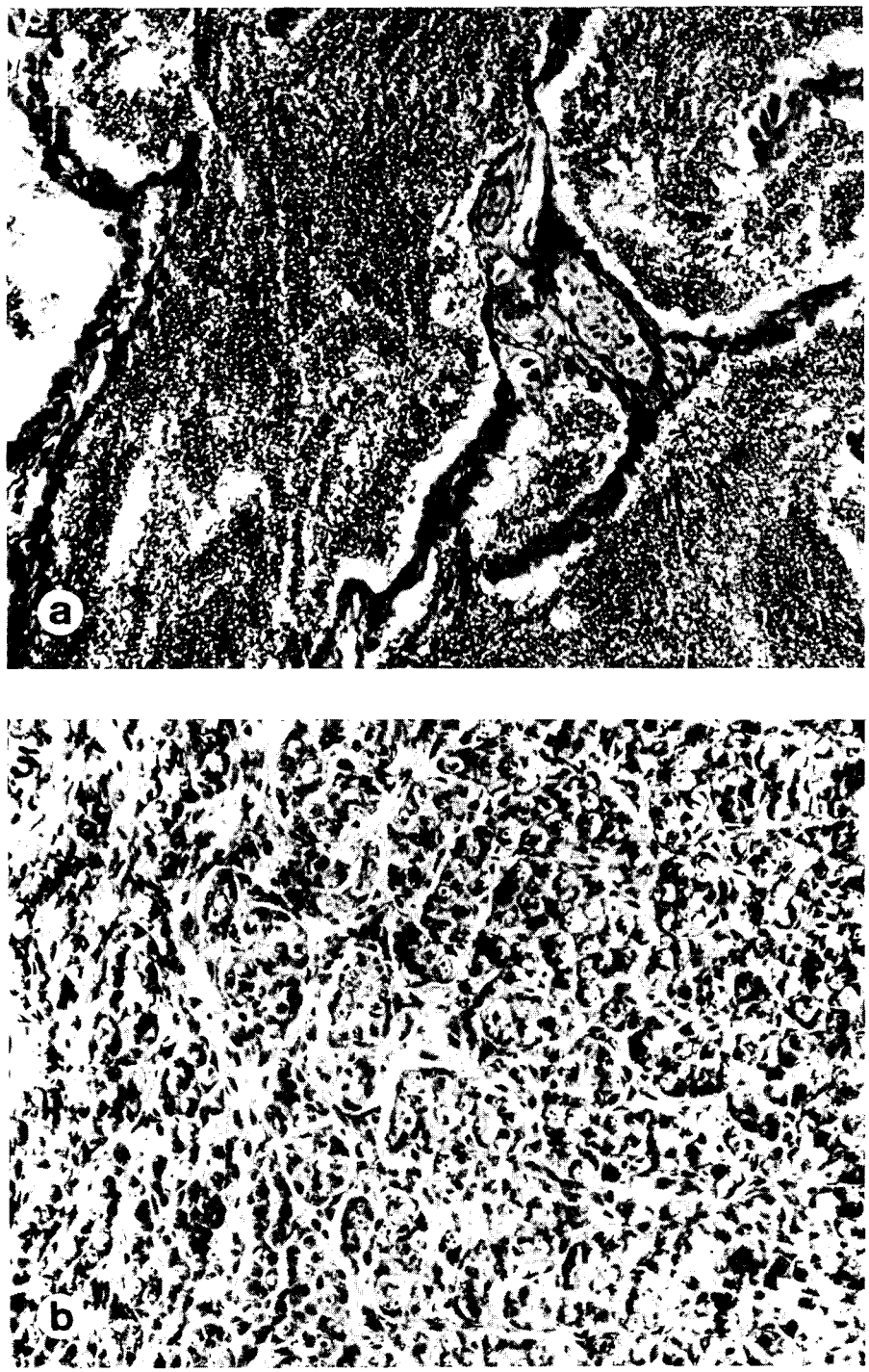

Fig. 5. Transverse sections of diploid (a) and artificial triploid (b) testes.

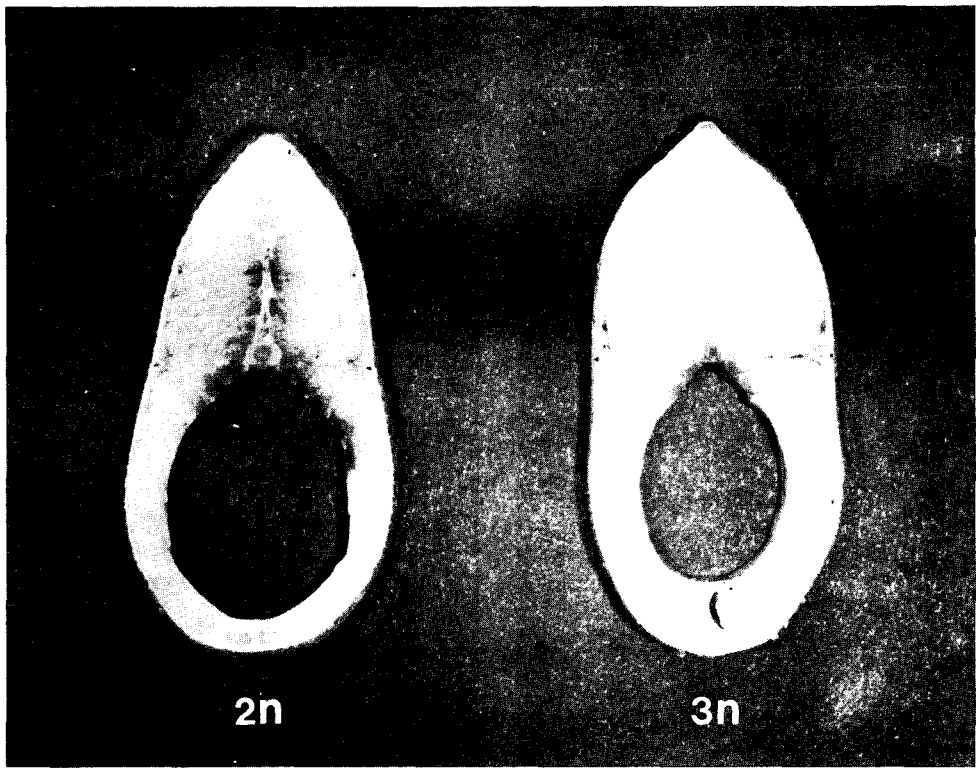

Fig. 6. Trunk morphology of adult male. 2n : diploid, 3n : triploid.

DISCUSSION

The present study revealed that heat shock administered to the fertilized eggs of rainbow trout can inhibit the extrusion of the second polar body and give reasonable rates of triploid induction. This rate is not significantly different from that of other reports (Chourrout and Quillet, 1982 ; Lincoln and Scott, 1983 ; Thorgaard et al., 1981).

Difference of incubation times between triploid and diploid was recorded in our experiment. This phenomenon has been described by other strain of rainbow trout (Happe et al., 1988) and for other fish species (Benfey et al., 1984 ; Gervai et al., 1980). But, the mechanism of this phenomenon is not yet known (Happe et al., 1988). Overall survival rates of triploid at swim-up were about 80% relative to the control. This value is equivalent to other previous results (Chevassus et al., 1985 ; Happe et al., 1988). Solar et al. (1984) suggested that the heat shock could have a more drastic effect than the triploid condition per se on the survival of the eggs.

It is well known that triploid causes an increase in cellular and nuclear size (Sezaki and Kobayashi, 1978). In channel catfish, Wolters et al. (1982) found that

major axis was more increased than minor axis. Similar values were obtained in our study. Wolters et al. (1982) considered that the measurement of the major axis of erythrocyte nuclei alone would suffice to discriminate between diploids and triploids. In the present investigation the average nuclear volume of cells classified as triploid more than doubled that of fish classified as diploid. Thorgaard and Gall (1979) found extensive variations in nuclear volume within an individual fish and among individuals of a given ploidy.

Much of the interest in induced polyploidy in commercially important fish is based on the assumption that triploids would be sterile and may show better growth than normal fish at least after the time of sexual maturation (Solar et al., 1984). The lack of gonadal development in triploid fish observed in this study is consistent with other report of this species (Lincoln and Scott, 1983). The data obtained in the present study suggest that triploid rainbow trout are growing faster than normal fish after 1 year of age. Thorgaard and Gall (1979) suggested that triploid rainbow trout should show improved growth relative to diploids after maturation, as diploid shunt a greater proportion of their energy to gonadal development and spawning activities. Only female triploids appear to benefit from this increased growth potential, because gonadal development is almost completly arrested in females (Thorgaard, 1986). But, Wright et al. (1982) found that twenty month old triploid rainbow trout still showed retarded growth. Lincoln and Bye (1984) found that when diploid and triploid fish were reared together triploid fish showed a slower growth rate, and when reared separately or with a low percentage of diploids, as would happen commercially, they grew equally well.

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