

# Chromosome Manipulations

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- 1) induction sterility
  - 2) Transgenesis
  - 3) Sex reversal
    - 3-1) Feminization
    - 3-2) Masculinization
  - 4) Hybridization
  - 5) Gynogenesis
  - 6) Androgenesis
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# Chromosome Manipulations

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مشکلات ایجاد بلوغ  
جنسی در دوره پرورش

◆ کاهش شدت تغذیه

◆ کاهش رشد

◆ افزایش حساسیت ابتلا به بیماریها

◆ تغییر در کیفیت گوشت

◆ تغییرات ظاهری و کاهش بازار پسندی

◆ تولید ماهی با اندازه متفاوت

◆ رها سازی در منابع آبهای طبیعی

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# Chromosome Manipulations

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1) induction sterility

- 1. Surgical**
  - 2. Irradiation**
  - 3. Hormonal**
  - 4. Hybridization**
  - 5. Polyploidy**
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# Triploid Hybrids

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- Triploids where one diploid set of chromosomes comes from one species and one haploid set comes from another.
    - e.g. grass carp x common carp or rainbow trout x brook trout;
  - Often show increased survival;
  - Sterility is more sure;
  - Can often reproduce the growth advantage of triploids without the mortality or deformity rates sometimes seen in triploids (e.g. coho x chinook salmon triploids)
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# Chromosome Manipulations

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## 1) induction sterility

### **Polyploidy**

Polyploidy has been thoroughly studied in fish and shellfish. The polyploid state refers to individuals with extra sets of chromosomes.

The normal and most common chromosome complement is two sets (diploid).

Triploidy refers to individuals with three sets of chromosomes and tetraploidy refers to individuals with four sets.

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# Methods to Induce Triploid

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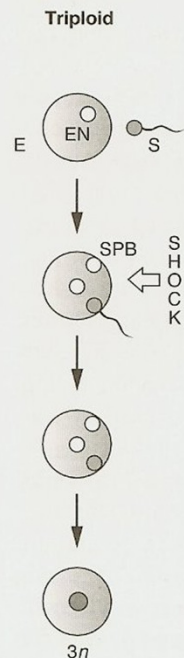
- Direct Method

- Indirect Method

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# Polyloid Induction in Fish

## Direct Method



**Triploidy** is induced by allowing normal fertilization and then forcing retention of the second polar body (Chourrout, 1980, 1984; Lou and Purdom, 1984).

**Fig. 7.10** The timing of the shocks that are needed to create triploids, tetraploids, gynogens and androgens. See the text for the abbreviations and explanations. E, egg; S, sperm; EN, egg nucleus. After Tave (1990b)

# Direct Method

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## Physical shocks

Thermal shock

Cold Shock

Heat Shock

Hydrostatic Pressure

## Chemical treatments

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جدول ضمیمه ۲ - مروری بر شوکهای دمایی استفاده شده توسط محققین مختلف

| مدت اعمال شوک | زمان اعمال شوک<br>پس از لقاح | دمای شوک<br>(°C) | گونه مورد تحقیق     | ماخذ                 | ردیف |
|---------------|------------------------------|------------------|---------------------|----------------------|------|
| ۱-۱۰ min      | ۱ min                        | ۳۵               | کیور معمولی         | Stanly (1975)        | ۱    |
| ۵-۳۰ min      | ۲۵ min                       | ۲۴، ۲۶، ۲۸       | قزل آرای رنگین کمان | Chourout, D. (1982)  | ۲    |
| ۴h            | ۰/۵-۱۰ min                   | -۰/۵             | ماهی آزاد کوهو      | Refstie (1982)       | ۳    |
| ۱۰ min        | ۴۰ min                       | ۲۸               | قزل آرای رنگین کمان | Thompson, D. (1984)  | ۴    |
| ۱۰ min        | ۳۰ min                       | ۲۸               | قزل آرای رنگین کمان | Purdom, C. (1985)    | ۵    |
| ۶۰ min        | ۵-۱۵ min                     | ۰-۴              | کیور معمولی         | Linhart, o. (1986)   | ۶    |
| ۲-۲۰ min      | ۲۵ min                       | ۲۶               | قزل آرای رنگین کمان | Chourrout, D. (1986) | ۷    |
| ۳۰ Sec-۳۰ min | ۱-۵ min                      | ۳۵-۴۰            | کیور معمولی         | Hollebeq (1986)      | ۸    |
| ۲۵ min        | ۵ min                        | ۰-۴              | لای ماهی            | Linhart (1986)       | ۹    |
| ۱۰ min        | -                            | ۲۹               | قزل آرای رنگین کمان | Disney (1987)        | ۱۰   |

# Direct Method

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- Triploidy successful depends largely on three factors

|                      | Shock intensity  | Time of shock initiation    | Duration of treatment |
|----------------------|------------------|-----------------------------|-----------------------|
| Heat shock           | 26-28 C          | 20 -40 min<br>(300-600 CTM) | 10 -20 min            |
| Hydrostatic Pressure | 7000 – 10000 psi | (300-600 CTM)               | 3-10 min<br>(5)       |

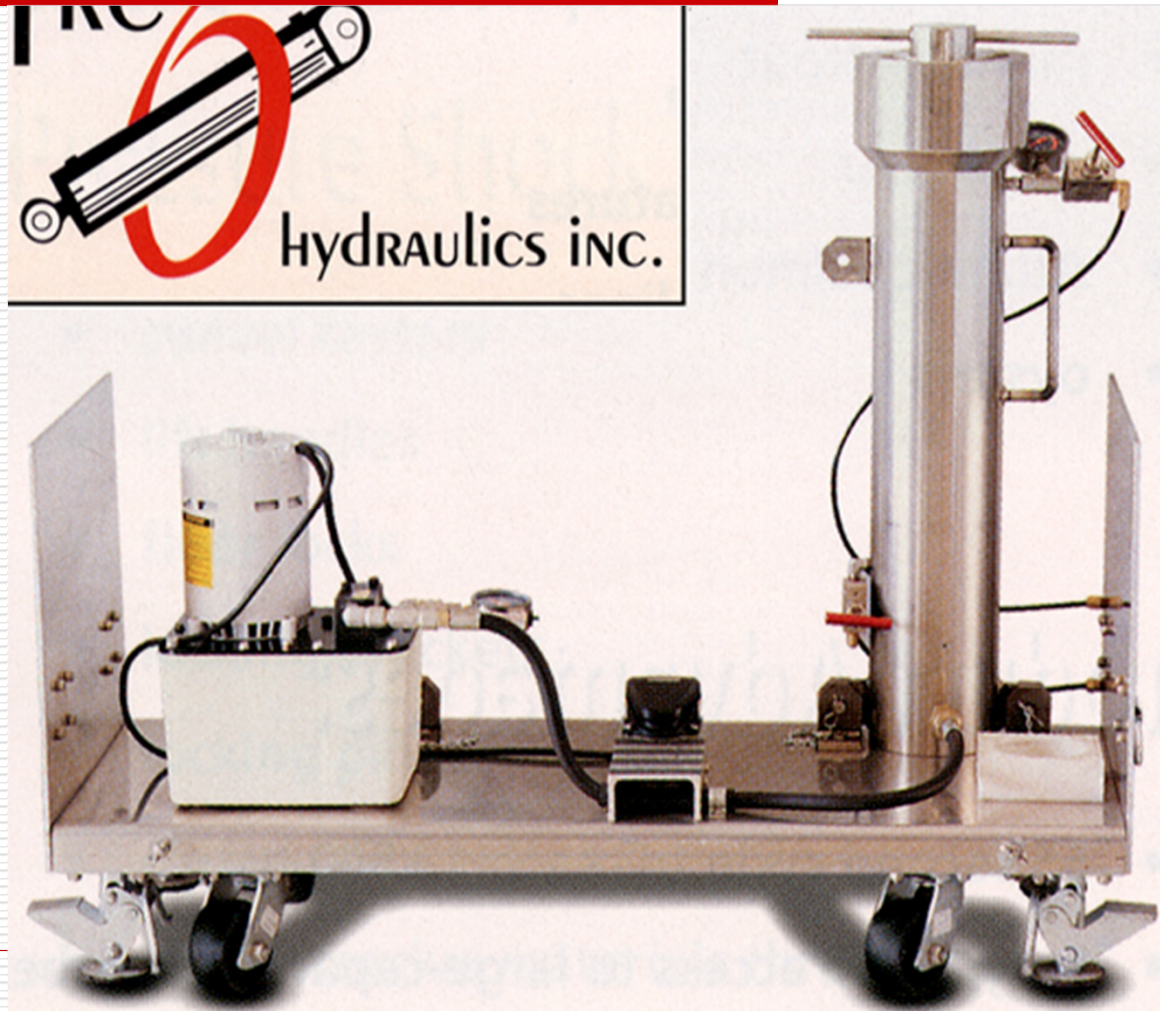
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# Direct Method



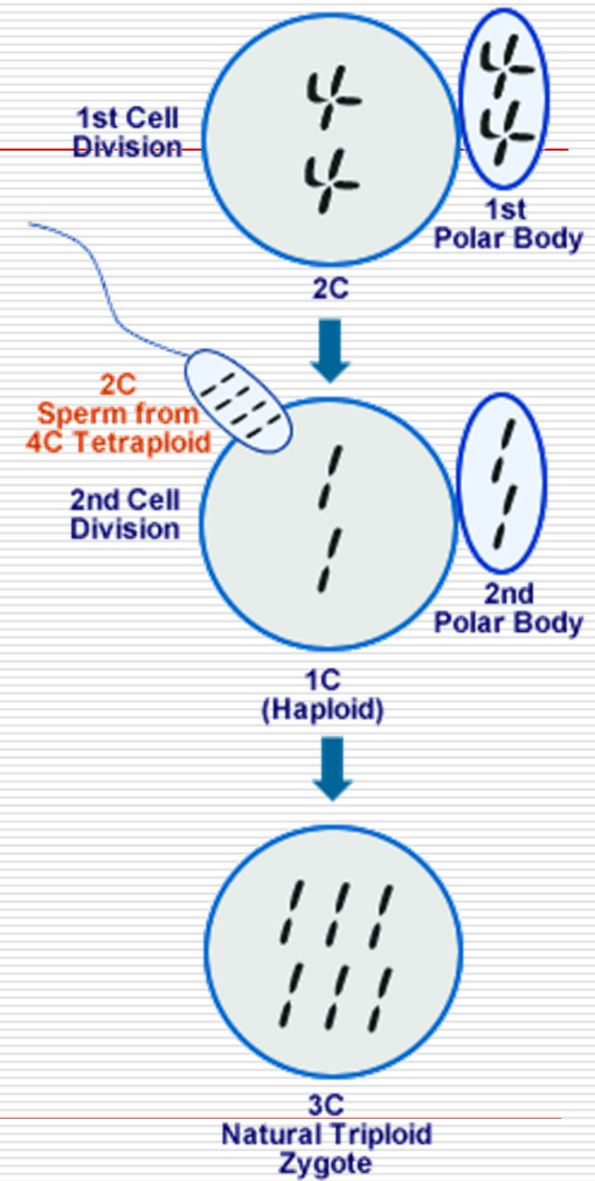
# Direct Method

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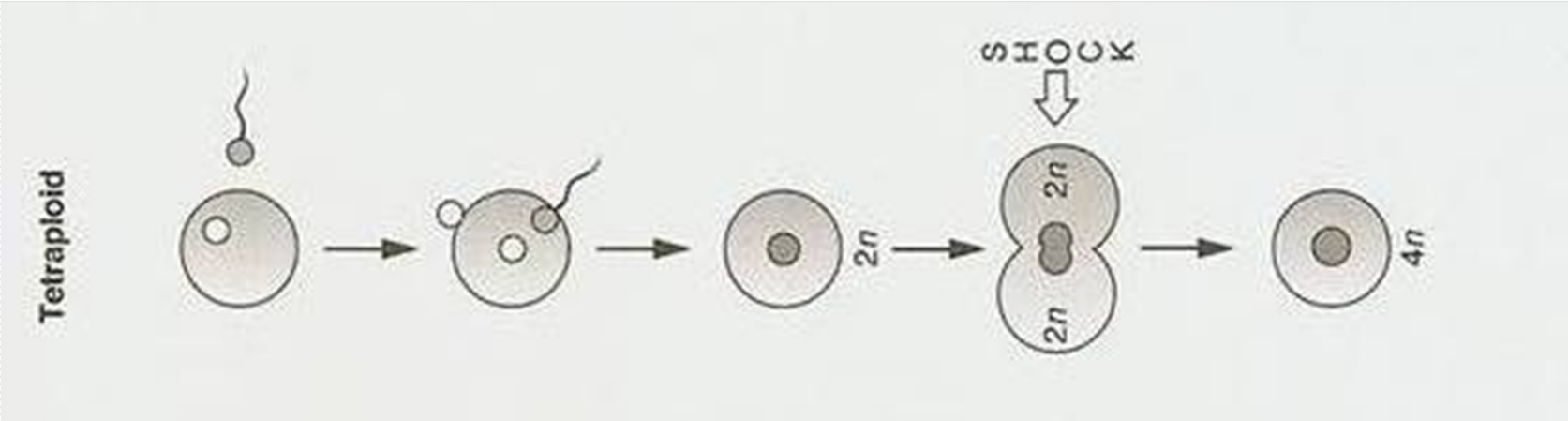


# Indirect Method

$$4n * 2n \longrightarrow \text{All } 3n$$



# Induction Tetraploid



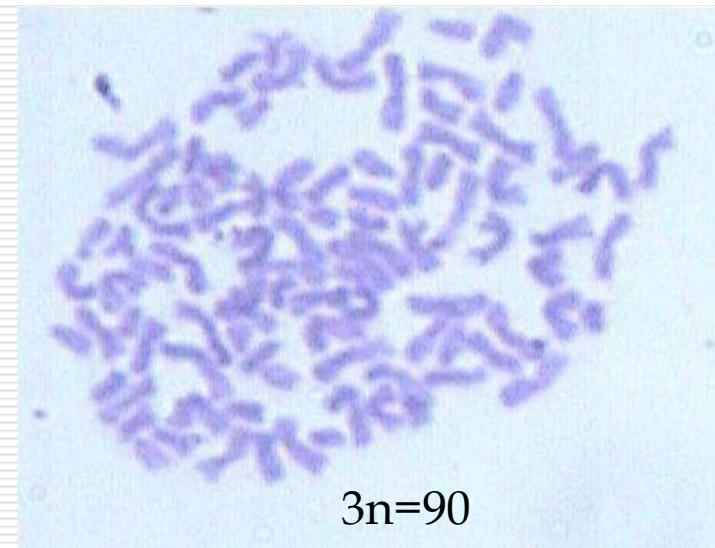
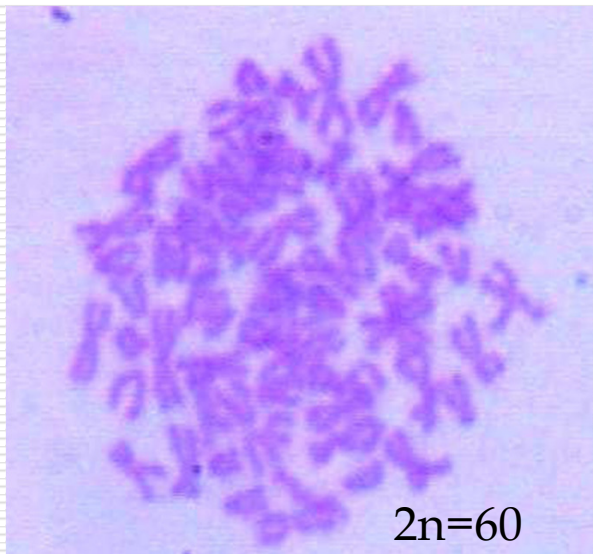
# Ploidy Determination

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**Karyotyping**, the actual visualization and enumeration of chromosomes, is, of course, the most accurate method for determining ploidy level.

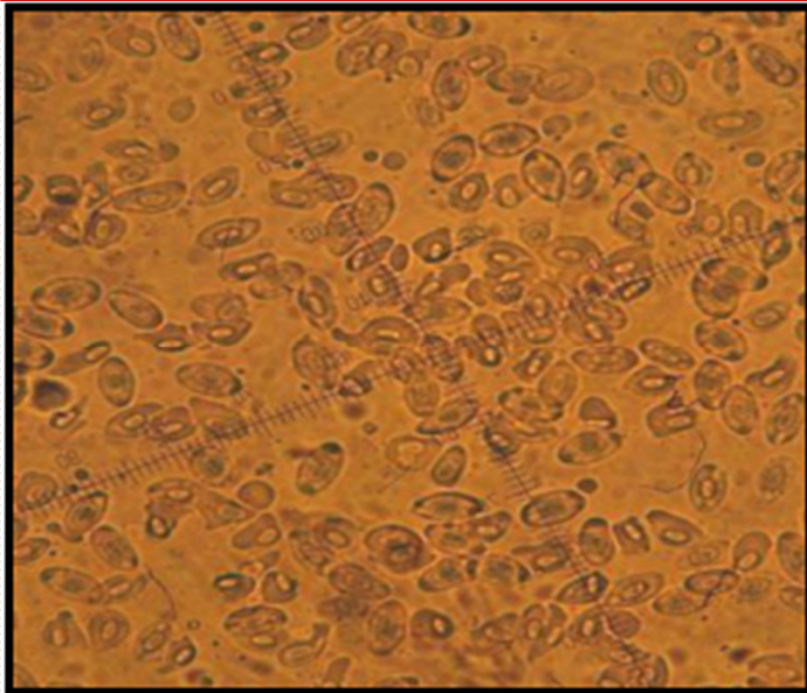
**cell-size measurement** with a blood smears.

**Flow cytometry**



# Ploidy Determination

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Erythrocytes of diploid (left) and triploid (right) brown trouts ( $\times 40$  magnification, each unit  $2.5 \mu\text{m}$ )

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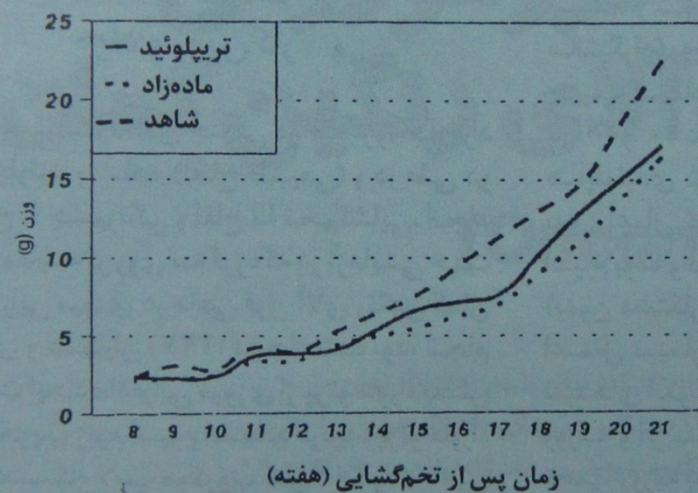


# Growth of Diploid, Triploid and Tetraploid

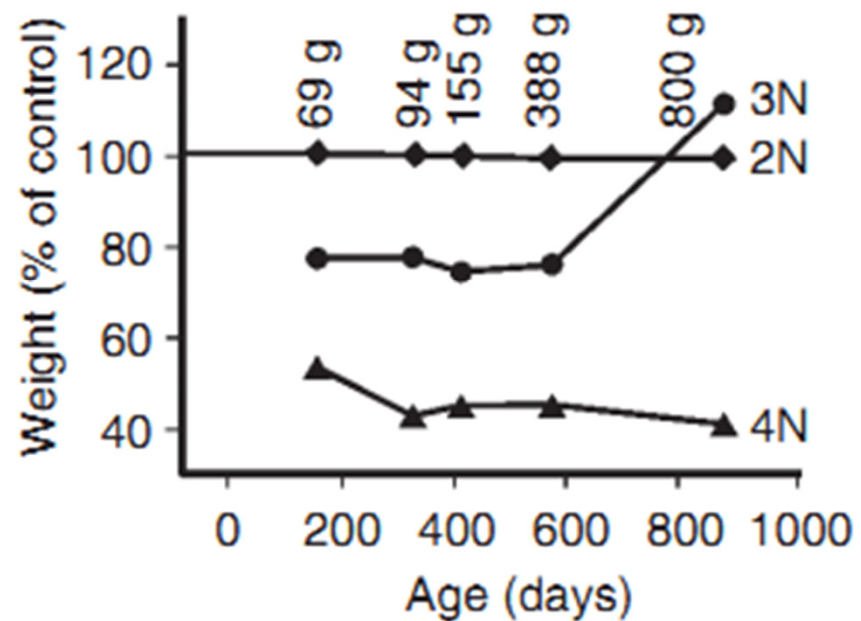
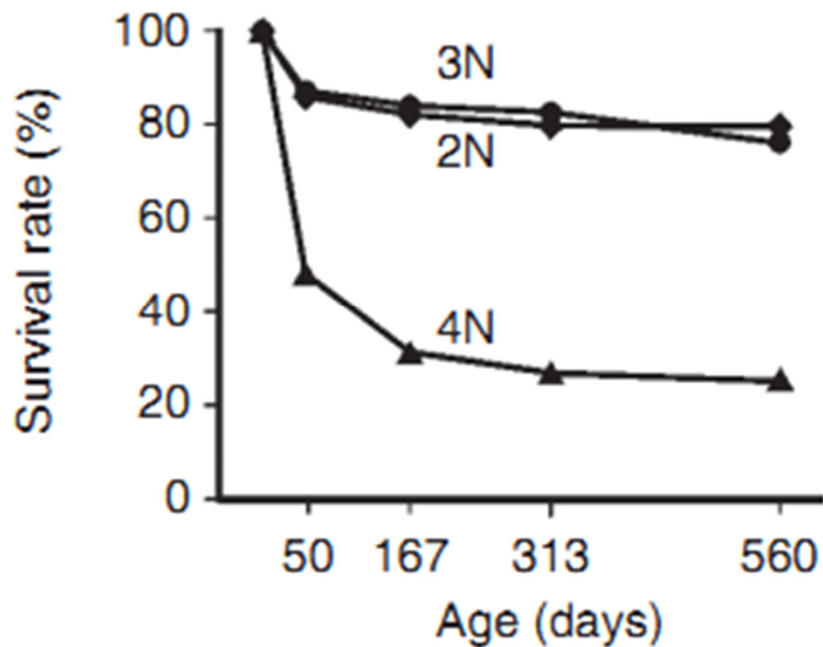
شکل شماره ۲- مقایسه طول کل در طی ۲۱ هفته پرورش پس از تخم‌گذاری در بچه ماهیان تریپلوئید، ماده‌زاد و شاهد.



شکل شماره ۳- مقایسه وزن در طی هفته هشتم تا بیست و یکم پس از تخم‌گذاری در بچه ماهیان تریپلوئید ماده‌زاد و شاهد.



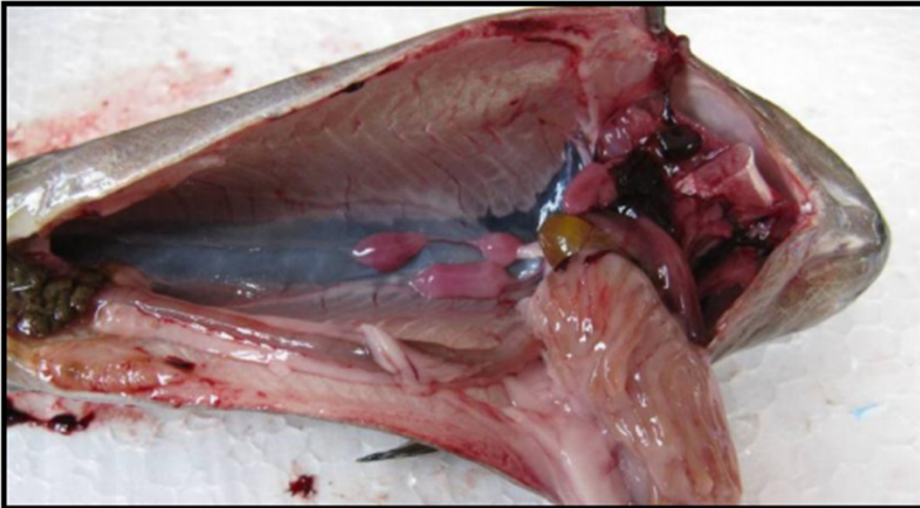
# Growth of Diploid, Triploid and Tetraploid



# Problems with Triploids

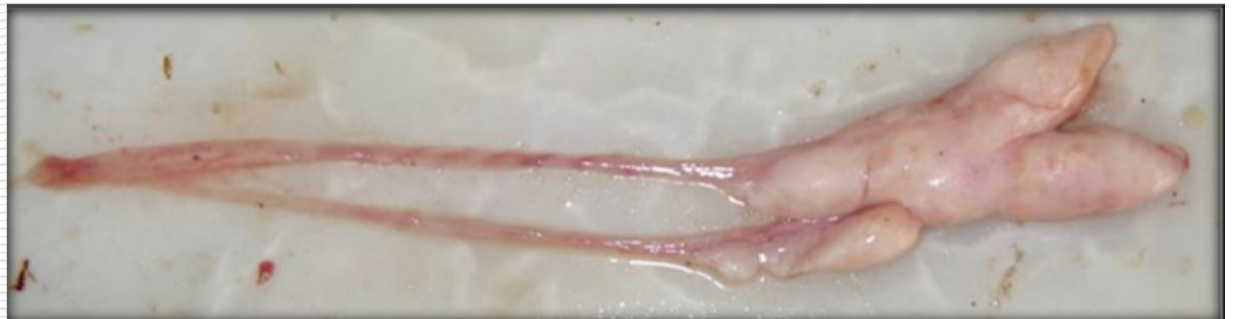
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- ❑ For many species triploids are not allowed by law (e.g. sea bass in Europe);
  - ❑ Although sterile many triploids differentiate and develop gonads to some extent (mosaics) , so growth advantage is not always there;
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Macroscopic appearance of triploid ovarium in the female brown trout on the 19<sup>th</sup> month post-fertilization

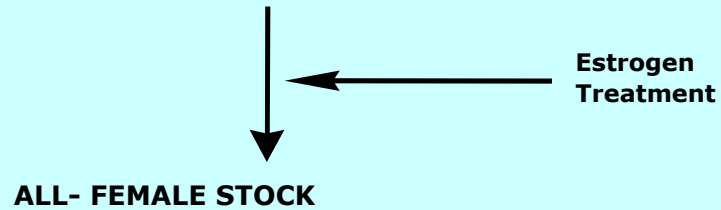
Macroscopic appearance of triploid testis in the male brown trout on the 19<sup>th</sup> month postfertilization



# All female triploid

## A) DIRECT FEMINIZATION. ANY GENETIC SYSTEM

SEXUALLY UNDIFFERENTIATED FISH



## B) INDIRECT FEMINIZATION. FEMALE HOMOGAMETY

