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Preliminary results of temperature shock effects on newly fertilized eggs of gilthead sea bream *Sparus aurata* Linnaeus, 1758

Preliminarni rezultati o utjecaju temperaturnih šokova na oplođena jaja komarče *Sparus aurata* Linnaeus, 1758

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INTRODUCTION

Commercial production of fish could benefit from the use of triploid fish. Triploids are generally sterile and show better growth during sexual maturation than diploids (Purdom, 1983). Triploidy in fish can be induced by physical or chemical treatments of newly fertilized eggs which cause the retention of second polar body during meiosis (Purdom, 1983).

The most frequent treatments of newly inseminated eggs are temperature schocks, either heat (25—40°C) or cold ones (5—10°C). The main problem in triploidy induction is mortality of treated eggs caused by temperature schocks which should be made acceptable by both experimental and commercial production.

In this paper we summarize the preliminary results of temperature shock effects on survival of newly fertilized eggs of gilthead sea bream as potentially beneficial in future attempts of production of gilthead sea bream triploids.

MATERIALS AND METHODS

Ripe eggs were obtained by induced spawning (HCG) from two females ($\overline{W}$ = 800 g, $\overline{l}_{(t)}$ = 35 cm) inseminated with the milt from three males ($\overline{W}$ = 320 g, $l_{(t)}$ = 22 cm). Dry fertilization in water bath, at ambient temperature of the basin for broodstock conditioning (18°C), took 10 min. After fertilization, the rest of the milt was rinsed through a sieve and eggs were put into a glass cylinder with filtered sea water of 18°C.

In each experiment 5000 newly inseminated eggs (quantity determined volumetrically) in glass cylinders with mild aeration were stressed at 4, 11, 28 and 35°C for 10 min duration. Cold shocks, at 4 and 11°C, were delivered in water baths with decreased temperature kept constant by ice. For heat water bath treatments at 28 and 35°C, temperature was maintained by aquarium heaters with thermostate. Control group eggs developed at fertilization temperature (18°C). Duplicate temperature shocks were carried out.

After treatments eggs were transferred to 20—1, slightly aerated glass tanks with no flow-through circulation and incubated at constant temperature of 18°C and salinity of 37.5‰, without gradual adaptation. Filtered sea water was used and streptomycin sulphate (30 mg/1) added to prevent bacterial contamination.

Rate of egg development, egg survival, abnormalities of hatched larvae (spinal deformations, side position of yolk sac, head region deformations) and larval mortality were observed.

Egg and larval survival were analysed statistically by G-test of independence (Sokal and Rohlf, 1969).

RESULTS

Rate of embryonic development at incubation of stressed eggs is given in Table 1. The time from fertilization to the beginning of individual stages is given in hours and minutes. Heat shock treatments not only shortened the time to the first cleavage, but affected proportionally the acceleration of all the developmental stages and finally the time to hatching.

Cold treatment at 11°C prolonged the time to the first cleavage for 15 min and delayed hatching for 65 min relative to controls. At 4°C hatching was delayed for 128 min (Table 1).

Table 1. Time of embryonic development of gilthead sea bream ($t = 18°C$) in eggs stressed by different temperature shocks. Eggs were exposed for 10 min. Incubation was carried out at ambient temperature (18°C) with no previous acclimatization.

	Shock temperature (°C)				
Developmental stages	35	28	18	11	4
First cleavage	1.00	1.05	1.25	1.40	1.55
Second cleavage	1.10	1.15	1.35	1.50	2.05
32-cells	2.00	2.10	2.20	3.20	3.30
Morula	3.05	3.20	3.35	5.10	5.30
Gastrula	5.45	5.50	6.10	6.50	7.10
Embryo	23.15	23.30	23.50	25.10	25.45
Free larva	48.45	49.20	50.10	51.15	52.18

The best temperature shock treatment with regard to egg and larval survival rates was 28°C.

However, it was significantly poorer ($P \leqslant 0.001$) relative to controls (Fig. 1).

Egg mortality was highest for the first 18 hours after treatment. It was particularly marked at temperature shocks of 4, 35 and 11°C. Dead eggs were mainly at early cleavage stages with clearly visible deformations (a large number of blastomeres of different sizes, irregular cleavages). Mortality rate was later decreased but remained constant, so that by the end of yolk sac resorption, all the larvae, hatched from stressed eggs at 4 and 35°C, died.

Hatching was significantly reduced in all the treatments relative to controls. It was highest at shock temperature of 28°C (53%). High percentage of hatched larvae was deformed, particularly those hatched from eggs stressed at 35 and 4°C. The best temperature shock treatment with regard to the number of deformed larvae relative to controls was 28°C (Table 2).

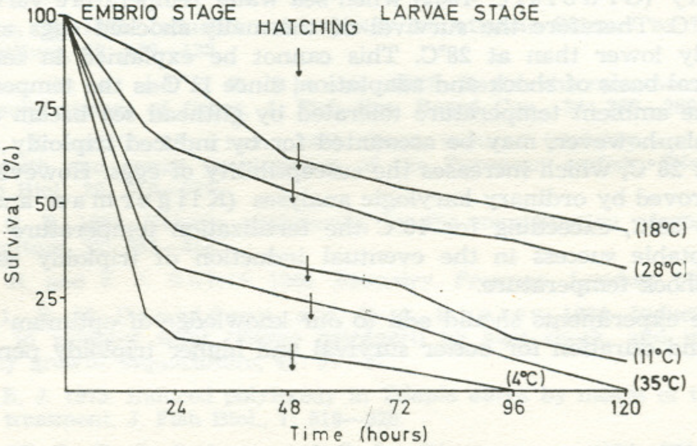

Fig. 1. Survival of gilthead sea bream eggs and larvae after 10-minutes exposure of newly fertilized eggs to different temprature shocks.

Table 2. Percentage of hatched and deformed larvae after 10 min exposure of newly fertilized eggs to temperature shocks

Shock temperature (°C)	Percentage of hatched larvae (%)	Percentage of deformed larvae (%)
35	11	40
28	53	14
18	62.5	8
11	32	22
4	24	34

DISCUSSION

It was observed that gilthead sea bream eggs showed much greater susceptibility to temperature shoch treatments than eggs of fresh water fishes which can endure shoch treatments temperatures which exceed or are lower for 20°C than fertilization temperature. This applies to the rainbow trout *Salmo gairdneri* (Solar et al., 1984), landlocked Atlantic salmon, *Salmo salar* (Johnstone, 1985) and many other salmonid fish which spawn at 4—10°C temperature ranges and eggs showed good survival after heat shocked ad 26—40°C.

Eggs of the warm water fish, such as *Tilapia aurea* (Valenti, 1975), carp, *Cyprinus carpis* (Gervais *et al.*, 1980), European catfish, *Silurus glanis* (Krasznai and Marian, 1986) and channel catfish, *Ictalurus punctatus* (Wolters *et al.*, 1982) are able to endure well thermal shocks at 5—10°C, even though spawn at 20—25°C.

Gilthead sea bream spawn in the Adriatic during November, December and January (Grubišić, 1962) when sea water temperature varies between 17 and 11°C. Therefore the survival of thermally shocked eggs at 11°C was surprisingly lower than at 28°C. This cannot be explained in terms of the physiological basis of shock and adaptation, since 11°C is the temperature very close to the ambient temperature tolerated by gilthead sea bream eggs in the nature. This, however, may be accounted for by induced triploidy eggs in the shocked at 28°C, which increases the susceptibility of eggs. However, triploidy was not proved by ordinary karylogic analyses (Kligerman and Bloom, 1977). The 28°C, exceeding for 10°C the fertilization temperature and providing acceptable success in the eventual induction of triploidy seems to be optimum shock temperature.

Future experiments should add to our knowledge of optimum shock temperature and duration for better survival and higher triploidy perentage.

PRELIMINARNI REZULTATI O UTJECAJU TEMPERATURNIH ŠOKOVA NA OPLOĐENA JAJA KOMARČE *Sparus aurata* Linnaeus, 1758.

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KRATKI SADRŽAJ

U radu su opisani utjecaji toplotnih šokova na netom oplođena jaja komarče, *Sparus aurata* L. Po 5000 netom oplođenih jaja tretirano je desetminutnim toplotnim šokovima od: 4, 11, 28 i 35°C, nakon čega su jaja inkubirana na ambijentalnoj temperaturi od 18°C bez postupne adaptacije. Kontrolna grupa jaja nije podvrgnuta šoku te su se jaja stalno razvijala na 18°C.

Preživljavanje jaja i izvaljenih larvi nakon 10-min. toplotnog šokiranja netom oplođenih jaja je najbolje pri temperaturi šoka od 28°C, ali je ipak značajno slabije u odnosu na kontrolnu grupu.

Izvaljivanje je značajno reducirano u svim tretmanima, a najveće je na temperaturi šoka od 28°C (53%). Postotak deformiranih larvi je visok, posebno u tretmanima od 35 i 4°C. Najmanji broj deformiranih larvi u odnosu na kontrolnu grupu je zabilježen na temperaturi šoka od 28°C.

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