

Induce triploidy by heat shock in Eurasian perch, *Perca fluviatilis*

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Abstract

In Eurasian perch (*Perca fluviatilis*), females grow significantly faster than males. Moreover, gonadal development has a significant negative impact on somatic growth and fillet yield. In order to induce sterility, triploidy induction was attempted by subjecting fertilised eggs to heat shocks. Different combinations of temperature (28, 30, 34, 35 and 36 °C), duration (2, 5, 10 and 25 min) and time of shock initiation (TI = 3, 5 and 7 min post-fertilisation) were tested. Flow cytometry analysis was used to assess ploidy level of control and heat-shocked larvae. Low intensity (28–30 °C) and long duration (10 and 25 min) shocks lead to significantly higher survival ($44 \pm 26\%$) and triploidisation ($71 \pm 26\%$) rates than high intensity (34–36 °C) and short duration (2 and 5 min) shocks ($17 \pm 19\%$ and $21 \pm 26\%$, respectively). The most effective conditions for efficient triploidy induction were low intensity shock of 30 °C, applied 5 min post-fertilisation for 25 min. This treatment led to the production of all-triploid populations (100%) with up to 43% survival rate.

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Résumé

Induction de la triploïdie chez la perche Eurasiennne, *Perca fluviatilis* par application de chocs thermiques. La perche eurasiennne, *Perca fluviatilis*, présente un dimorphisme sexuel de croissance en faveur des femelles. Cependant, le développement important des gonades (IGS = 20%) réduit significativement cet avantage notamment en ce qui concerne le rendement du filetage. L'objectif de cette étude est de réduire ce développement gonadique en produisant des perches triploïdes stériles par application de différents chocs thermiques à des oeufs fécondés. Différentes combinaisons de températures (28, 30, 34, 35 et 36 °C), de durée (10 et 20 min) et de moment d'initiation du choc thermique (TI = 3, 5 et 7 min après fécondation) ont été testées. Le taux de ploïdie des lots témoins et des triploïdes a été vérifié par cytométrie de flux. Les chocs de faible intensité (28–30 °C) et de longue durée (10 et 25 min) donnent des taux de survie ($44 \pm 26\%$) et de triploïdie ($71 \pm 26\%$) significativement plus élevés que les chocs de forte intensité (34 à 36 °C) et de courte durée (2 et 5 min, $17 \pm 19\%$ de survie et $21 \pm 26\%$ de taux de triploïdie). Un choc thermique de faible intensité (30 °C) appliqués sur les oeufs 5 min après fécondation pendant 25 min permet d'obtenir 100% d'individus triploïdes pour 43% de taux de survie.

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1. Introduction

Current interest in polyploidy induction is almost entirely due to its potential application in fish farming, for the production of triploid and tetraploid fish (Purdom, 1993; Pandian and Koteeswaran, 1998). Induced triploidy is used to produce partially or completely sterile fish, whose three

chromosome sets impair the meiotic division involved in germ cell formation (Thorgaard and Allen, 1987; Chourrou, 1988; Levanduski et al., 1990). The interest in sterility lies in the possibility that this may lead to increased growth: the energy, which will normally be directed for gonadal growth (gonad formation) and reproduction is used for somatic growth (Pandian and Koteeswaran, 1998). The introduction of sterile exotic fish as for example grass carp (*Ctenopharyngodon idella*) in order to control the growth of macrophytes is also preferred to avoid the expansion of the species in the environment (Allen and Wattendorf, 1986).

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Triploidy induction refers to the production of individuals with three sets of chromosomes. Triploidy can be induced in fish by inhibiting the second meiotic division and the extrusion of the second polar body by shocking eggs shortly after fertilisation (Chourrout, 1988; Malison et al., 1993). Retention of the second polar body has been successfully induced to produce triploid individuals in a number of freshwater species such as rainbow trout, *Oncorhynchus mykiss* (Chourrout, 1980, 1984, 1986), brown trout, *Salmo trutta* (Quillet et al., 1991), common carp, *Cyprinus carpio* (Gervai et al., 1980), yellow perch, *Perca flavescens* (Malison et al., 1993; Malison and Garcia-Abiado, 1996) as well as marine species such as flatfish (Purdom, 1972, 1976) and sea bass *Dicentrarchus labrax* (Felip et al., 1997; see also reviews by Pandian and Koteeswaran, 1998).

A variety of treatments have proven to be effective in inducing polar body retention, including thermal (cold or heat), chemical (colchicine or cytochalasin B) or hydrostatic pressure shocks (Thorgaard, 1986; Ihssen et al., 1990; Malison et al., 1993). Even if pressure shock led generally to high survival and/or triploidisation rate, temperature shocks are preferred for the achievement of 100% triploid fish and for situations in which a large volume of eggs need to be treated, because of the cost of the pressure shock system (Thorgaard, 1986; Thorgaard and Allen, 1987; Ihssen et al., 1990; Malison et al., 1993). Chemical inductors are rarely used for the triploid induction of fish because they lead to mosaicism (Ihssen et al., 1990; Pandian and Koteeswaran, 1998). Triploid fish can also be produced by crossing tetraploid with diploid breeders (Chourrout and Nakayama, 1987; Malison et al., 1993).

Eurasian perch, *Perca fluviatilis*, exhibit sexual growth dimorphism in which females grow faster (20–30%) and reach a larger ultimate size than males (Mélard et al., 1996; Fontaine et al., 1998). This sexually related dimorphic growth is correlated with the onset of vitellogenesis (Craig, 2000). As triploids are partially or completely sterile (Pandian and Koteeswaran, 1998), production of triploid female perch should prevent sexual maturation from occurring and avoids the negative effects of gonadal development (mean gonadosomatic index: 20%) on growth, survival, and flesh quality. In warm water culture, Eurasian perch do not mature so the need for triploidisation lies mainly in the production of Eurasian perch in temperate waters (from 4 to 25 °C depending on season). Although yellow perch (*P. flavescens*) has been extensively studied, its Eurasian relative, *P. fluviatilis*, has, up to now, received very little attention.

As the intensity and duration of the shock applied on eggs depend on the species being cultured (Thorgaard and Allen, 1987; Pandian and Koteeswaran, 1998), the objective of this study was to assess the effectiveness of different combinations of three treatment variables of importance in triploidy induction: the delay between fertilisation and initialisation of the shock (TI), the duration of the shock, and the temperature of the shock that would maximise retention of the second polar body and survival in Eurasian perch.

2. Material and methods

2.1. Broodstock management

Reproductively mature Eurasian perch (*P. fluviatilis*) were reared in the Aquaculture Research Station of the University of Liège, in Tihange (Belgium). Breeders were held in 1.6 m³ tank, flowed with non-regulated (from 4 to 25 °C depending on season) Meuse river water (O₂ > 6 ppm). Perch breeders mature spontaneously under increasing water temperature in breeding tanks. In early April, they were screened daily and females emitting oocytes under gentle abdominal pressure were selected. In order to ensure maximum egg quality and to prevent spontaneous egg laying in the tank (perch spawn in the early morning), fish were examined just after sunrise.

2.2. Fertilisation, heat shock and incubation

Before each manipulation, fish were anaesthetised in 40 mg l⁻¹ of quinaldine sulphate. Semen was collected in a syringe by stripping mature males and was kept on ice until fertilisation. Egg ribbons from each mature female were manually extracted by stripping and were divided into small batches in order to test each parameter with one egg ribbon. After dry mixing with semen, water at 16–17 °C was added to induce fertilisation. Eggs were rinsed two or three times and the ribbon was cut into portions of approximately 500 eggs. Heat shocks were applied by immersing eggs into a thermostatic water bath maintained at the experimental temperature (± 0.1 °C). After shock administration, eggs were transferred into the hatchery, with water maintained at 16–17 °C (O₂ > 6 ppm). Survival is assessed on embryos at 6 d post-fertilisation as the ratio of eyed embryos to fertilised eggs.

Two series of experiments were carried out. In the first experiment we tested high intensities (34, 35 and 36 °C) and short duration (2 and 5 min) heat shocks (high intensity-short duration, HI-SD) applied 3 or 5 min post-fertilisation. In the second experiment, we tested low intensities (28 and 30 °C) and long duration (10 and 25 min) heat shocks (low intensity-long duration, LI-LD) applied 5 or 7 min after fertilisation. HI-SD were tested with three different females and LI-LD were tested with two to six different females. For each experiment, a control group was kept at 16–17 °C.

2.3. Assessment of ploidy

The ploidy of embryos (6 d post-fertilisation) was assessed by flow cytometry, which measures the fluorescence of a specific fluorophore bound to target molecules within the cell, in this case nuclear DNA. Embryos were first digested in trypsin citric buffer solution. After 10 min of incubation, a trypsin inhibitor, a nucleus stabiliser (spermidine) and RNase were added to the first solution. After 10 min of incubation, the resulting nuclear suspension was stained with propidium iodide (Cycle Test[®], Becton Dickinson). Analysis was performed with a Becton Dickinson FACSTAR PLUS[®]. Unshocked diploid embryos were used as a diploid standard

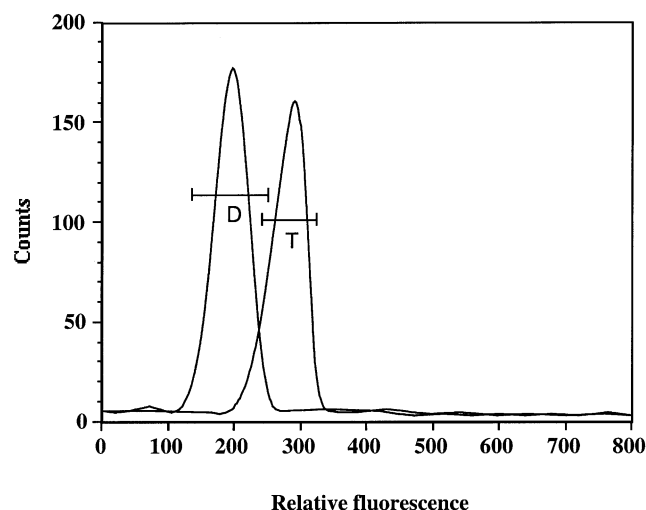


Fig. 1. Flow cytometry diagram (program: CELLQuest FACStation 3.1) showing the differences between diploid (D) and triploid (T) nuclear fluorescence intensity of Eurasian perch (6 d post-fertilisation), after staining with propidium iodide.

for the calibration of the cytometer, with a relative fluorescence set at “200” value, which allows clear separation between triploid and diploid nuclei. A minimum of 20 embryos per batch were analysed except when the number of survivors was lower (in 10 batches the number of embryos analysed was 11–18).

2.4. Statistical analysis of data

The data of experiment 1 were analysed using Kruskal-Wallis test. The data of experiment 2 were analysed using Mann-Whitney test. A one-way analysis of variance (ANOVA) was used to compare survival, triploidisation rates and yield of triploids between HI-SD ($n = 36$) and LI-LD ($n = 22$) shocks. $P < 0.05$ was the level of significance for all tests.

3. Results

Flow cytometry analysis permits us to determine rapidly and accurately the ploidy status in Eurasian perch larval (Fig. 1).

Table 1

Effect of time of initiation (expressed in min after fertilisation), temperature and shock duration (min) on survival (%) and triploidy induction of Eurasian perch eggs exposed to HI-SD heat shock. N of tests = number of females. Values are means \pm standard error (S.E.)

	T (°C)	Duration (min)	Time of initiation (min)							
			3			5				
			N of tests	Survival (%)	Triploids (%)	Yield of triploids (%)	N of tests	Survival (%)	Triploids (%)	Yield of triploids (%)
Control	17	–	3	64 \pm 18	0	0	3	64 \pm 18	0	0
Heat-shocked group	34	2	3	39 \pm 12	7 \pm 3	3 \pm 2	3	37 \pm 13	0	0
			5	3	11 \pm 2 ^a	55 \pm 17	6 \pm 3	3	7 \pm 3 ^a	24 \pm 15
	35	2	3	37 \pm 7	7 \pm 3	3 \pm 1	3	24 \pm 9	43 \pm 12	9 \pm 2
			5	3	3 \pm 3 ^a	14 \pm 14	1 \pm 1	3	0 ^a	0
	36	2	3	14 \pm 7	44 \pm 16	8 \pm 6	3	31 \pm 18	55 \pm 9	20 \pm 14
			5	3	0 ^a	0	0	3	0 ^a	0

^a Survival at 5 min duration were significantly ($P < 0.05$) lower than at 2 min duration.

In experiment 1, HI-SD heat shocks were tested. Survival rates ranged between 0% and 39% (Table 1) and were not significantly ($P > 0.05$) different between the three temperatures tested (34, 35, 36 °C). There was a significant ($P < 0.05$) negative effect of treatment duration on survival in all experimental batches (survival rate was better for a 2 min duration shock at the three temperatures tested). The triploidisation rates ranged between 0% and 55% (Table 1) and were not significantly ($P > 0.05$) different between the three temperatures tested. A temperature of 36 °C, applied for 5 min, 3 or 5 min post-fertilisation is lethal (0% survival). The highest triploidisation rate (55 \pm 17%) was obtained at 34 °C, when heat shock was applied 3 min post-fertilisation for 5 min duration, and the best yield of triploids (20 \pm 14%), expressed as the product of the survival of the eggs after the treatment and the frequency of triploids in the survivors (Purdom, 1993), was obtained with a shock at 36 °C applied 5 min post-fertilisation during 2 min.

In experiment 2, LI-LD heat shocks were tested. Survival rates ranged between 26.3% and 61.5% (Table 2) and were not significantly ($P > 0.05$) different between the two temperatures tested (28 and 30 °C). The mean triploidisation rates ranged between 36% and 100% and were significantly ($P < 0.05$) higher at 30 °C (94 \pm 3%) than at 28 °C (44 \pm 5%). The mean yields of triploids ranged between 12.0% and 44% and were significantly ($P < 0.05$) higher at 30 °C (37 \pm 5%) than at 28 °C (19 \pm 6%). There was no significant ($P > 0.05$) effect of time of application (5 or 7 min post-fertilisation) and heat shock duration (10 or 25 min) on survival, triploidisation rates and yield of triploids. The best triploidisation rate (100 \pm 0%) with a yield of triploids of 43 \pm 34% was obtained at 30 °C, when heat shock was applied 5 min post-fertilisation for 25 min duration. Nevertheless, the highest yield of triploids (44 \pm 16%) was obtained at 30 °C, when heat shock was applied 7 min post-fertilisation for 25 min duration.

Comparing the two categories of shocks (one-way ANOVA, Fig. 2), LI-LD shocks gave significantly higher survival (40 \pm 5%, $P < 0.05$, $F = 16.53$, residual = 56), higher triploidisation rates (71 \pm 6%, $P < 0.05$, $F = 48.47$, residual = 56) and higher yield of triploids (29 \pm 4%, $P < 0.05$, $F =$

Table 2

Effect of time of initiation (expressed in min after fertilisation), temperature and shock duration (min) on survival and triploidy induction of Eurasian perch eggs exposed to LI-LD heat shock. *N* of tests = number of females. Values are means \pm S.E.

	<i>T</i> (°C)	Duration (min)	Time of initiation (min)							
			5				7			
			<i>N</i> of tests	Survival (%)	Triploids (%)	Yield of triploids (%)	<i>N</i> of tests	Survival (%)	Triploids (%)	Yield of triploids (%)
Control	17	–	6	70 \pm 12	0	0	6	70 \pm 12	0	0
Heat-shocked group	28	10	3	37 \pm 19	37 \pm 8	14 \pm 9	2	62 \pm 25	38 \pm 2	24 \pm 11
		25	3	26 \pm 8	44 \pm 7	12 \pm 5	2	46 \pm 34	62 \pm 12	32 \pm 26
	30	10	4	36 \pm 3	88 \pm 6 ^a	30 \pm 2 ^a	4	38 \pm 7	98 \pm 2 ^a	37 \pm 6 ^a
		25	2	43 \pm 34	100 ^a	43 \pm 34 ^a	2	47 \pm 17	93 \pm 0 ^a	44 \pm 16 ^a

^a Triploids and yields of triploids significantly higher ($P < 0.05$) at 30 °C.

42.28, residual = 56) than HI-SD shocks (survival: 17 \pm 3%, triploidisation rate: 21 \pm 5% and yield of triploids: 4 \pm 1%).

4. Discussion

Our results showed that heat shocks can be used to induce triploidy in Eurasian perch, *P. fluviatilis*, as has been accomplished in *P. flavescens* (Malison et al., 1993; Malison and Garcia-Abiadi, 1996) or on other temperate species as *C. idella*, (Cassani and Caton, 1986), *Salmo salar* (Quillet and Gagnon, 1990), and *D. labrax* (Felip et al., 1997). Testing multiple combinations of temperature, TI and shock duration lead us to a 100% triploidisation rate when heat shock of 30 °C was applied to eggs 5 or 7 min post-fertilisation for a 10 or 25 min duration. These parameters are close to those tested by Malison et al. (1993) and Malison and Garcia-Abiadi (1996) who obtained 100% of triploid yellow perch, *P. flavescens*, using heat shock of 28–30 °C applied at 5 min during 10 or 25 min.

High variability of survival and triploidisation rates among the different batches of eggs submitted to the same

heat shock treatment was observed. Similar variability also reported in other species was attributed to eggs quality (*C. idella*, Cassani and Caton, 1986; *O. mykiss*, Diaz et al., 1993) or to the difference between the rearing temperature of the breeders and the thermal shock temperature (*O. mykiss*, Diaz et al., 1993; *P. flavescens*, Malison et al., 1993; *Clarias macrocephalus*, Na-Nakorn, 1995).

Comparison of LI-LD versus HI-SD shocks underlined the greater efficiency of the first treatment to induce triploidy. With long treatments, temperature gradients within the eggs ribbon are eliminated, which leads to homogeneous exposure of all eggs to the chosen temperature (Malison et al., 1993). As far as survivals are concerned, temperatures of 34–36 °C are at the limit of lethal temperature. At these temperatures, macromolecules denaturation could occur compromising egg viability (Malison et al., 1993).

Shock duration considerably influences survival of triploids in experiment 1. The negative effect of long duration treatment was also observed on *O. mykiss* (Chourrout, 1984), *C. idella* (Cassani and Caton, 1985). Eggs can probably support high temperature for a short time, but longer shock duration could apparently disrupt other cellular processes necessary for survival, as suggested by Cassani and Caton (1986).

Our study demonstrates the feasibility of producing all-triploid perch larvae using LI-LD heat shocks, with a relative high initial survival. The polyploidisation can be used for the production of all-triploid populations in the Eurasian perch, *P. fluviatilis*, having improved characteristic for commercial aquaculture.

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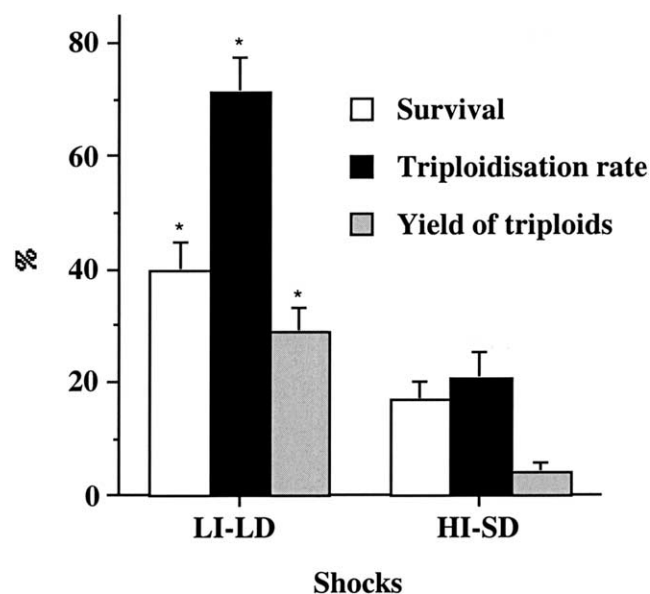


Fig. 2. Comparative efficacy of LI-LD and HI-SD shocks on survival (%), triploidisation rate (%) and yield of triploids (%) of Eurasian perch. Values are means \pm S.E. * LI-LD group significantly higher than HI-SD according to one-way ANOVA ($P < 0.05$).

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