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RESEARCH PAPER

Optimization of Thermal Shock for Poliplody Induction in Rainbow Trout (*Oncorhynchus mykiss*) under Photoperiodic Control of Spawning

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Abstract

Natural fish stocks could be at risk due to interbreeding or competition with farmed fish, which often differ genetically from their counterparts in wild. Farming of sterile or all-female triploids could be a powerfull preventative measure for preservation of natural gene resources or biodiversity of native species. Although, techniques for ploidy are well described in many fish species, there is no report whether the previous techniques applicable in out-off-season production induced by artificial photoperiod that is widely used in salmonid aquaculture. In order to optimize and refund ploidization protocol for off-season production of diploid gynogenomes and triploids three experiments were conducted to determine the optimal UV inactivation of sperm and heat shock treatment of eggs in rainbow trout (*Oncorhynchus mykiss*). High survival at yolk sac absorption stage (82.3–84.5%) and high yields (approx. 100%) were achieved from the range of treatments applied. There were significant differences in survival rates among treatments and the best optimisation was defined as the shock timing at 20 minutes post-fertilisation for 10 min duration at the shock temperature of 26 °C.

Keywords: Rainbow trout, heat shock, UV, photoperiod, spawning.

Gökkuşağı Alabalığı (*Oncorhynchus mykiss*)'nın Fotoperiyodik Kontrollü Yumurtlamasında Poliploidi için Sıcaklık Şoku Uygulamasının Optimizasyonu

Özet

Doğal balık stokları kendilerinden genetik olarak farklılıklar gösteren çiftlik balıkları ile soyiçi çiftleşme ya da rekabetten kaynaklı risk altındadırlar. Kısır veya tümü dişi triploid bireylerin yetiştirilmesi doğal gen kaynaklarının veya yerel türlerin biyolojik çeşitliliğinin korunması açısından önleyici bir tedbir olabilir. Ploidi için teknikler iyi açıklanmasına rağmen, bunların salmonidlerde yaygın olarak kullanılan yapay fotoperiyod ile uyarılan mevsim dışı üretimde uygulanabilirliği ile ilgili bir bilgi bulunmamaktadır. Diploid gino-genomların ve triploidlerin mevsim dışı üretimdeki ploidizasyon protokolünü optimize etmek ve yeniden belirlemek için, gökkuşağı alabalığı'nda (*Onchorhynchus mykiss*) en uygun UV ile sperm inaktivasyonunun ve yumurtalara sıcaklık şoku uygulamasının belirlenmesi amacıyla üç deneme kurulmuştur. Uygulanan işlem aralığında besin keseli yavruda yüksek yaşama oranı (%82.3-84.5) ve yüksek verim (yaklaşık %100) elde edilmiştir. Uygulanan işlemler arasında yaşama oranı açısından önemli farklılıklar bulunmakla birlikte, en iyi optimizasyon döllenmeden 20 dk sonra 10 dk süreyle uygulanan 26 °C'lik sıcaklık şokuyla belirlenmiştir.

Anahtar Kelimeler: Gökkuşağı Alabalığı, sıcaklık şoku, UV, fotoperiyot, yumurtlama.

Introduction

Rainbow trout (*Oncorhynchus mykiss*) is one of the most important fish in aquaculture and its culture has become widespread worldwide with international threat on the integrity of wild populations (Bellinger *et al*., 2014). In addition, early maturation is a major problem in rainbow trout farming, since the species shows sex-specific differences in growth, feed conversion and flesh quality, which have been reduced by sexual maturation. These facts have pointed out environmentally-friendly and better aquaculture management is inevitable in all phases of the operations. The production of all-female or sterile populations by using gynogenesis and triploidy has utility in alleviating the common problems in the industry (Palti *et al*., 1997). Diploid gynogenetic females allow the maintenance in the embryos of an extra chromosome set of maternal origin and may be used as males directly after hormonal sex-reversal

without progeny testing, are the simplest and quickest way to initiate large-scale production of sex reversed females (Quillet and Gaignon, 1990). It is also a method to make the rapid production of inbred lines for breeding programs and research feasible, which are important to improve desired phenotyping characters in fish farming (Grimholt *et al*., 2009). The triploids particularly have been considered a possible strategy for large-scale commercial aquaculture operations both to maximize food production and to minimize ecological impacts (e.g genetic contamination, invasion of non-native species, Benfey, 2015).

In order to achieve sustainability and environmental protection the teqniques must be able to produce similar yields and high-quality products for year-round harvest of each market-sized fish. There are reliable methods for UV inactivation, thermal induction of diploidization and triploidy in rainbow trout (Palti *et al*., 1997). However, there is limited research available on practical application to off-season production, which is highly desirable to enable the year-round supply of fry in rainbow trout aquaculture. Photoperiod has been regarded as the most important environmental factor controlling the timing of spawning in salmonids (Taranger *et al*., 2003). Although photoperiod manipulation has the advantage of being cheap and simple to install on commercial farms (Bromage *et al*., 1992), there are some contradictions among the studies about gamete quality regarding to photoperiodical control of reproduction. While the manipulation was previously reported with no adverse effect on gamete quality and fertilization, Bonnet *et al*., (2007a) demonstrated alterations characterized by embryonic mortalities and morphological abnormalities. Moreover, the time of stripping, over-ripening, and rearing temperature of broodstock have significant effects on gamete quality and progeny survival (Bromage *et al*., 1992). Similarly, significant correlation was reported between gynogenesis efficacy and gamete quality in salmonids (Palti *et al*., 1997; Grimholt *et al*., 2009, Taylor *et al*., 2011). A critical further point of induced gynogenesis is the application of the appropriate UV dose to achieve the complete DNA sperm inactivation. Furthermore, optimizing thermal shock protocols is also complicated by many variables including temperature, time of shock following fertilization, duration, preshock incubation water temperature, species or strain differences and all of these factors can alter the effectiveness of the given protocols in the literature. The aims of this study were, therefore, to determine (1) the optimal UV treatment, (2) shock treatment timing, (3) shock intensity and shock duration, and (4) the effect of photoperiod-induced manipulation of spawning on diploid gynogenetic and triploid induction.

Materials and Methods

The study performed consecutively during the out-of season spawning (June–July-August) from 2013 to 2014. All experiments were conducted under flow-through hatchery conditions at a private fish farm (Fethiye, Turkey).

Fish

Broodstock were maintained under a photoperiod regime of 16h light/8h darkness from January, thereafter short day (8h light/16h darkness) from April in a light-proofed building. Light was supplied by eight fluorescent lamps (50 W) fixed 3 m above the water's surface. The fish were held in a raceway (10x10x2 m) supplied with spring water resulting in annual temperature range of 8-12 º C (Figure 1). All fish were fed with commercial feed twice daily.

Gamete Collection

Before each handling, fish were anesthetized in 0.05% 2-phenoxy-ethanol. Females (4 - 6 kg) were checked for ovulation 2 times a week. Eggs of batch were collected from a female in sterile plastics by manual stripping then divided into equal groups. At

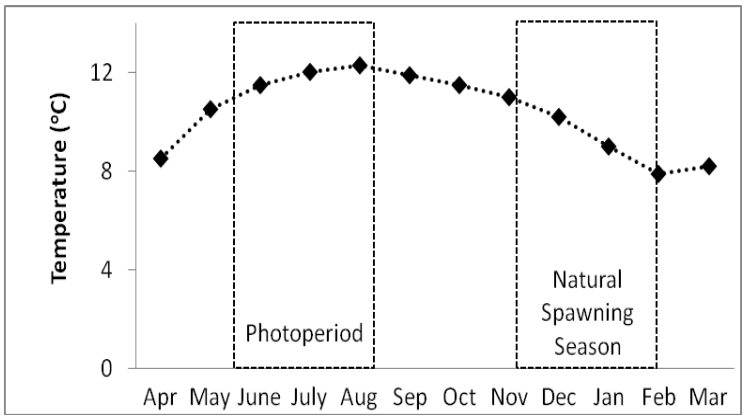

Figure 1. The water temperature in the broodstock raceway (Photoperiod: 8L:16D).

each egg collection day, sperm samples were collected from 2-3 mature males (3 - 4 kg) in 50 ml dry and sterile plastic tube in order to fertilize eggs with a sperm pool. Prior to fertilisation, eggs and milt were protected from contact with water to prevent water hardening and sperm activation, and kept cool until treatment. Sperm motility was verified for each portion of sperm sample according to Altunok *et al*., (2004), where the motility was estimated using a scale of six scores from M0 (no motility) to M5 (80-100%) under a light microscope. The semen of good initial motility (minimum M4) was used in the experiments.

Sperm Irradiation

Induction of gynogenesis was carried out by fertilizing the eggs with sperm irradiated with UV light. The pooled sperm kept on ice was diluted 1/5 with % 0.7 NaCl solution (Altunok *et al*., 2004). The sperm solution (4 ml) was put into a petri dish (80 mm diameter). Irradiation was performed under a 254 nm UV lamp (15 W) and continually stirred with a magnet (60-80 rpm) according to previous studies (Refstie, 1983). The lamp was warmed minimum up to 15 min before the onset of the irradiation. The spermatozoa was irradiated using different UV dose rates (240, 730, 912, 1095, 1596 or 2394 J/m^2) and durations (2 or 3 min, Table 1). Each of the UV dose was triplicated with the sperm pool to account for possible variability between the fish.

Artificial Fertilisation

Within each experiment, eggs were divided into equal groups (500 eggs) and fertilised in beakers (200 ml) with irradiated or normal sperm (0.5 ml/group), both of which were diluted with the immobilizing solution. After mixing gently for 30s water activation was initiated using hatchery water at 11-12 ºC for 2-3 minutes water hardening. Activation time was set as times zero in the development of the eggs. After rinsing the fertilized eggs, hatchery water was filled into the beakers and the eggs left undisturbed until required for thermal shocking. Following the fertilization and treatments the eggs were transferred to vertical-flow tray incubators (in separate hatching boxes) supplied by the hatchery water, dead eggs were removed regularly and survival was monitored at eyed stage (15 DAF) and after hatching (21 DAF) at yolk sac larvae (YSL).

Heat Shock Treatments

Gynogenetic diploidy and triploidy was induced by restoring second polar body by immersion of the eggs to heated water bath at 26 ºC. The shock treatment variables were time of initiation (5, 10 or 20 min post-fertilization, mpf) and duration (10 or 20 min). The 3 x 2 factorial treatments were duplicated with eggs from a single female and further triplicated with different females (Table 1). A total of sixteen groups per female were set as two controls, two haploid controls, twelve of diploid gynogenetics. Haploids were fertilized eggs with irradiated sperm but unshocked to assess the effectiveness of UV inactivation as haploid embryos show abnormal haploid syndrome and died at hatch (Chourrout and Quillet, 1982; Refstie, 1983). Normal diploid controls fertilized with fresh sperm. Similar experimental design was set for triploidy.

Statistical Analysis

Survival in treatment groups was expressed in percentages of developing eggs relative to their corresponding controls after adjustment of the latter to 100%. The data at eyeing stage and YSL are analysed, within treatments, by x^2 tests. Differences are accepted as significant when $P<0.05$. For the significant differences in proportion of sexes between controls and gynogenetics or triploids the χ^2-test was

Table 1. Experimental design for UV and heat shock optimization

	Experiments					
	I	II	III	IV	V	VI
UV irradiation						
Distance from light source (cm)	5	5	10	15	15	40
Duration (min)	2	3	2	2	3	3
Average number of eggs	500±43					
Photoperiod water temperature (preshock temperature)	11 – 12 Cº					
Thermal shock variables						
Time of initiation (min)	5, 10, 20 mpf					
Duration (min)	10, 20					
Shock temperature	26 Cº					
Postshock water temperature (Incubation temperature)	11-12 Cº					
Number of females per treatment	3	3	3	3	3	3
Number of replicates per female	2	2	2	2	2	2

set at the P<0.05 level. Statistical analysis was conducted using SPSS (20.0; SPSS Inc., Chicago).

Results

The percentage of eyed eggs and yolk-sac larvae in controls were used to characterize the gamet quality. The parameters used to evaluate egg quality showed significant differences among females. Overall survival from fertilization to eyeing, control groups exhibited variable embryonic survival during out-of-season spawning (Table 2). This difference further increased until hatching and throughout the yolk–sac absorption. The results verified the consistent potency of the egg and milt with respect to gamet quality.

Optimum UV Irradiation

Although, UV irradiation of the sperms worked for all of the experimental dosages, the best dose applied from 15 cm for 2 minutes showed higher survival rate (42.8%) at yolk-sac larvae and lower haploids (1.9%) than other UV dosages. The efficiency of UV dosages was also verified with mortality rates (100%) of the haploids and most of the embryos did not hatch or died during the yolk sac stage except the UV treatment (40 cm distance for 3 min), which resulted in 18% survival. The result showed that the dosage was not suitable from the distance with given exposure time for fully inactivation of all sperms as also evidenced later by presence of male individuals in this experimental group. Therefore, UV light exposure from 15 cm distance for 2 min was adjusted to produce gynogenetic progeny

Heat Shock Optimisation

The effectiveness of heat shock in promoting diploid gynogenesis was expressed by surviving yolk-sac larvae relative to diploid controls and total gynogenetic yield. The highest diploid gynogenomes were observed when the shock started 20 mpf ($P < 0.05$) than those obtained with the heat shocks applied at earlier times. Optimal duration for applying shock was found to be 10 minutes (short shock). This treatment group produced a much improved gynogen yield (99%<). Eggs inseminated with UV-irradiated sperm, but not heat shocked, produced 0.3% of abnormal haploids, which indicated that UV irradiation for this group. Eggs from all treatments

Table 2: Survivals (%) assessed in the experiments were expressed in percentages relative to control (100%) and as mean of the replicates ± SEM. Superscripts denote significant differences between treatments (P<0.05)

Experiment	Treatments	UV Source (cm)	Timing (mpf)	Duration (min)	T (°C)	Eyeing (%)	YSL (%)
UV Irradiation	Controls					75.6±14.5	62.4±14.1
	Gyn 1	5		2		41.7±6.0^{a}	15.9±4.3^{a}
	Hap 1					37.2	0.5
	Gyn 2	5		3		56.7±1.1^{b}	14.5±4.5^{ac}
	Hap 2					58.9	0.9
	Gyn 3	10		2		39.9±13.8^{a}	12.1±1.5^{ac}
	Hap 3				11	47.6	6.4
	Gyn 4	15		2		68.8±1.3^{c}	42.8±6.5^{b}
	Hap 4					21.7	1.9
	Gyn 5	15		3		70.3±4.7^{c}	11.0±0.2^{c}
	Hap 5					81.7	1.3
	Gyn 6	40		3		25.7±6.5^{d}	7.4±0.3^{d}
	Hap 6					31.3	18.0
Diploid Gynogens	Controls					88.6±5.8	81.3±8.6
	Haploids					58.9±17.9	0.3±1.0
	Gyn 1		5	10		47.5±13^{a}	17.5±7.2^{a}
	Gyn 2		10	10	12	57.1±11.4^{b}	33.7±3.4^{b}
	Gyn 3		20	10		98.3±8.4^{c}	82.3±5.9^{c}
	Gyn 4		5	20		70.4±8.3^{d}	27.9±9.0^{d}
	Gyn 5		10	20		65.8±9.2^{d}	46.1±3.1^{e}
	Gyn 6		20	20		60.5±14.4^{b}	61.3±6.8^{f}
Triploids	Controls					93.9±0.4	78.5±5.1
	Trip 1		5	10		81.5±15.4^{a}	48.3±30.2^{a}
	Trip 2		10	10		85.0±9.1^{a}	64.7±10.1^{b}
	Trip 3		20	10	12	84.2±3.2^{a}	84.5±2.1^{c}
	Trip 4		5	20		69.4±10.5^{b}	49.0±21.0^{a}
	Trip 5		10	20		70.3±10.1^{b}	68.7±10.7^{b}
	Trip 6		20	20		78.3±4.5^{a}	78.9±4.2^{d}

showed highly variable survival to YSL (Table 2) and a significant effect of heat-shock timing on offspring viability was observed in both short and long shock durations.

In agreement with what was observed in induction of gynogenetic diploidy, a short heat shock at 20 mpf generated the highest survival (84.5%) of triploid YSL with relative to the corresponding controls. Survival in the heat shock treatment at 5 mpf for both durations was lower than all other treatments and the differences were statistically significant ($P<0.05$). Survival of control groups in compare to gynogentic and triploid groups remained high throughout the all experiments ($P<0.05$), except the viable embriyos heat shocked at 20 mpf for short treatment at eyeing stage ($P>0.05$). Repeated trials with the combination of timing and duration indicated that the tested shock durations usually provided similar survivals in triploidy whereas gynogenetic diploidy showed significant differences with timing and duration.

Confirmation of Gynogenesis and Triploidy

Randomly selected fish from all experiments were reared up to 5 month of age. Gynogenomes were confirmed by examination of gonads. Femaleness and survival for UV treatments are shown in a comparative Figure 2. Mean female ratio in heat-shock experiment was 99.7% whereas 0.3 % were asexual in comparison to femaleness of controls (50.9%), ($p<0.05$). Triploidy was confirmed by measurement of erythrocytes and triploid yields were ranging of 93-97%.

Discussion

The major aim of the present study was to improve ploidization technique for all-year-round and reliable mass production of diploid gynogenetic and triploid progeny in rainbow trout, which was photoperiodically induced to spawn at ambient temperatures in summer. The study demonstrated that the optimal heat shock treatment for retention of the second polar body of the eggs in rainbow trout induced off-season spawning is not identical to that which has been often considered the optimum for those obtained in natural spawning season. It was also suggested that the time of initiation of the thermal shock is a highly critical variable during artificial photoperiod spawning, at least when initiated between 5 and 20 mpf. Variations of fish egg quality, which is highly variable among adults, can be correlated with significant effects of breeding conditions (Bonnet *et al*., 2007a). In the present study, similar to previous studies (Davies and Bromage, 2002), it was estimated that the egg sizes were smaller in photoperiod spawning than the eggs obtained in natural period (personal observations). However, Estay *et al*., (1994) determined that the egg size was not correlated with survival of fry in rainbow trout. The egg size and survival was also not correlated for ploidy (Taylor *et al*., 2011). According to these reports, it was expected in our study that the out-off-season eggs could result in embrio of similar quality to those under natural spawning season. On the other hand, Bonnet *et al*., (2007b) reported that photoperiodic manipulation of ovulation had a negative impact on egg quality. In present work, there were some variations in survival of control groups among females (41.2 - 95.0%) possibly due to gamet quality or parental effects. Similarly, survival of embrio (54.7 - 98.2%) and hatching rates (3.9 - 39.1%) varied widely among females that had been fertilized with the same irradiated sperm. It was also observed in all of the experimental and control groups mean durations of hatching were shortened (approx. 21 day at 12 °C) when compared to the eggs incubated in natural spawning season in the fish farm (35 days at 9 °C) and in other previous studies (36 to 25 days for 8.5 to 15 ºC, respectively), (Quillet *et al*., 1988; Yanik *et al*., 2002). Quillet *et al*., (1988) considered that the gynogenesis does not appear changing incubation duration but induces larger variability of this

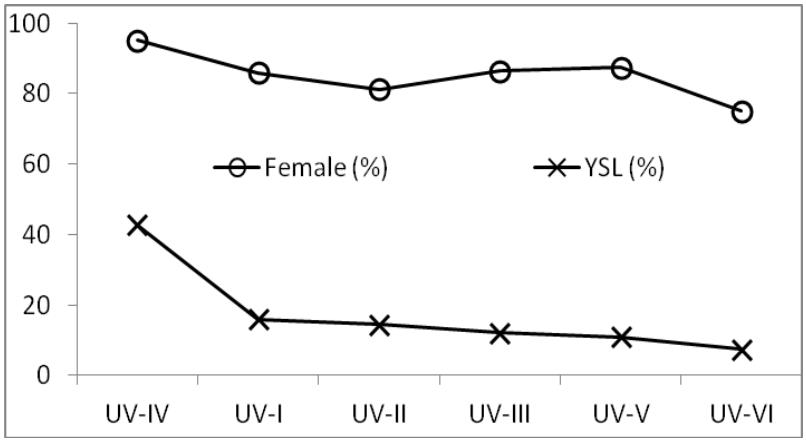

Figure 2. Sex ratio and survival of groups regarding to UV treatments. Survival is expressed relative to that of the control group (set at 100%).

parameter.

Temperature has a modifying role particularly in reproductive processes with corresponding effects on fertility, embryogenesis and larval survival. Moreover, exposure of the broodstock to increased water temperatures (13-14 ºC) can significantly reduce survival of eggs to the eyed stage (Taranger and Hansen, 1993). In another study, rainbow trout eggs of normal appearance and similar fecundities showed significantly lower fertilization rates than its control (Davies and Bromage, 2002). Most of the data relating to egg quality and success of hatching for trout are complicated by the use of different experimental conditions, treatment of broodfish and other variables (e.g. parental effects, incubation temperature), Bromage *et al*., 1992), make it difficult to compare previously published available data. However, gametes quality and survival rates (76% at YSL) for all normal diploid controls in the present study were comparable with those reported for eying (70-90%) and hatching rates (60-84%) observed in experimental conditions or commercial hatcheries previously (Lincoln and Scott, 1984; Thorgaard *et al*., 1990; Bromage *et al*., 1992; Bonnet *et al*., 2007b). In another study, Bonnet *et al*. (2007b) reported a decrease in survival of 37% at YSL in control group for rainbow trout. Low gamete quality is important detrimental factor in genome manipulation also in other fish species, where a negative correlation was found to exist between the quality of spawn and the occurrence of ploidy (Kucharczyk *et al*., 2014; Nowosad *et al*., 2015).

Sperm irradiation is important factor for induction of gynogenesis and therefore, it was tested for optimisation. The simple procedure described in literatures for UV inactivation of rainbow trout sperm was maintained in the study. Several authors have reported the wide range of effective UV irradiation dose, which were terminated 6-40 cm distance from UV source at various times (2-20 min), (Chourrout, 1982; Levanduski *et al*., 1990; Samonte-Padilla *et al*., 2011). Most of the haploid controls inseminated with irradiated sperm but not heat shocked developed abnormal embryos and low hatching survival, which was the characteristic haploid syndrome. Most of them mainly died before hatching or first feding except the UV treatment with the lowest dosage (group Hap6, Table 2). The fry hatched in this haploid group probably underwent spontaneous diploidy (Chourrout *et al*. 1980) or the distance/duration combination might be quite possibly inefficient. Over exposure to UV may kill the sperm (Samonte-Padilla *et al*. 2011), which might account for lowest gynogenetic survival observed with decrased UV distances (5-10 cm) in our study. Generally, it was obtained the control groups developed normal embryos and low mortality after the eyeing stage but gamete quality is another factor influencing UV inactivation and gynogenesis (Goryczko *et al*., 1991, Ocalewicz *et al*., 2010).

The incubation temperature was reported to have no significant effect on postfertilization timing for heat-induced second polar body retention (Palti *et al*., 1997). It is now considered that the pretreatment incubation temperature is also important factor for the optimum heat treatment for ploidy induction (Benfey, 2015). In the present study, survival at hatching for all gynogenomes and triploids was significantly affected by the shock timing at an incubation temperature of 12 °C. The shock timing was demonstrated the most critical variable for polar body retention in salmonids since even small variations resulted in significant changes in survivals and yields (Refstie, 1983; Johnstone, 1985; Quillet and Gaignon, 1990). The present results indicated that gynogenetic progenies was generated with reasonable losses of eggs and larvae (Table 2). The survival might be slightly higher than what has been recorded (75% of the control) by previous authors (Palti *et al*., 1997; Chourrout and Quillet 1982) at similar heat shock temperatures, but it is very higher than what is observed in rainbow trout (55% of control at hatching) with similar treatments (Quillet *et al*., 1988). In other salmonids, gynogenotes were also obtained with high survivals (62-79% of control) at hatching or start feeding (Levanduski, 1990). Very precocious treatments (within the first 10 mpf) usually resulting lower (32-57% of control) survivals in salmonids (Quillet and Gaignon, 1990; Smoker *et al*., 1995) and other fish species (Kucharczyk *et al*., 2014). Similarly, our findings identified the heat-shock timing of 20 min after egg activation was effective in production during photoperiodic control of spawning. The ovarian development obtained in gynogenetic progenies at age of 4-5 month, exhibited strong evidence of gynogenomic origin of surviving fish. Additionaly, triploidy results (with good survival 84.5% of control at YSL) pointed the same shock treatment for manipulation during photoperiodic control of reproduction. It is also very similar with the results of previous authors (72-89% of controls) in rainbow trout and salmonids with the same types of treatments (Lincoln and Scott, 1983; Solar *et al*., 1984; Johnstone, 1985; Chevassus *et al*., 1985; Quillet and Gaignon, 1990), but lower than findings of Chourrout and Quillet, (1982) and Quillet *et al*., (1988). In fact, triploidisation resulted in significant difference between diploid controls and triploids may be concluded as an evidence of correlation between photoperiod and triploid survival rates due to the poor egg quality increases the sensitivity to the triploidization treatment. When triploids were produced towards the end of spawning season and poor egg quality increased the mortality in salmonids (Taylor *et al*., 2011). High temperature may also result in reduced egg size, fertility, hydration and survival as well as inhibition of spermiation in salmonids (Pankhurst and King, 2010). The findings of previous studies indicated that temperature changes the quality and size of eggs also in other fish species (Kupren *et al*., 2011; Kucharczyk *et al*., 2014). The adverse effects including reduced embryo survival

rate could be found in the fish, which might be very sensitive to rapid temperature changes, even within the temperature ranges optimal for the species (Nowosad *et al*., 2014). It is also, therefore, possible to be affected by other factors such as water temperature at stripping or incubation. Diaz *et al*., (1995) demonstrated that a tendency for decreased survival (63%) at higher water temperatures (12-14°C at stripping and incubation), compared to a higher percentage of triploids (85.9%) at lower water temperatures of 6-8°C. Recently, a marked increase in deformities was reported with increasing incubation temperature and triploidy (Fraser *et al*., 2015).

In the present study, it is also reasonable to conclude that the duration of the thermal shock appear to be more important for induction of high percentages of off-season diploid gynogenomes than triploidy induction. Only the difference between short and long shock durations was significant within triploid groups heat shocked at 20 mpf. The general similarity of survival regarding to effect of the shock duration was described by previous authors (Refstie, 1983; Levanduski, 1990; Smoker *et al.*, 1995), who revealed that it is also crucial for the retention of the second polar body in salmonids under ambient photoperiod. In both of gynogenomes and triploids, heat shocking for 10 min gave a higher average hatching rates than heat shocking for 20 min. This optimum has been commonly used for rainbow trout by (Refstie, 1983; Solar *et al*., 1984; Lincoln and Scott, 1984;) whereas substantially different from the thermal shock duration of 20 min used for other salmonids (Johnstone, 1985; Quillet and Gaignon, 1990; Levanduski *et al*. 1990; Smoker *et al*. 1995)

Conclusion

A general review of the results and discussion seems to indicate that optimizing thermal shock protocols is complicated by the large number of variables. Under photoperiod control of spawning, it is expected that gamete quality can alter the effectiveness of a given protocol as it was strongly highlighted in some works (Taylor *et al*., 2011) that egg quality appeared to have a larger effect than ploidy. However, the noticeable percentage of larvae is in total agreement with previous findings with the exception of the triploidy, which showed significant difference between control and triploids at YSL. Moderate temperature shock at 26 °C for 10 min, 20 min after fertilization would be the recommended treatment to induce diploidization and triploidy in off-season production of rainbow trout. Selection programs may improve the progeny quality and survivals for higher pecentages of triploids in commercial off-season production.

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References

Altunok, M., A Mueller-Belecke, G Hoerstgen-Schwark, 2004. Cryopreservation of Tilapia (*Oreochromis niloticus*) sperm. Deutscher Tropentag 2004, October 5-7, 2004, Berlin. Book of Abstracts, ISBN 3-86004-182-7, p. 397.

Benfey TJ, 2015. Effectiveness of triploidy as a management tool for reproductive containment of farmed fish: Atlantic salmon (*Salmo salar*) as a case study. Reviews in Aquaculture 7:1–19.

Bellinger KL, GH Thorgaard and PA Carter, 2014. Domestication is associated with reduced burst swimming performance and increased body size in clonal rainbow trout lines. Aquaculture, 420:154-159.

Bonnet E, A Fostier, J Bobe, 2007a. Characterization of rainbow trout egg quality: a case study using four different breeding protocols, with emphasis on the incidence of embryonic malformations. Theriogenology 67, 786–794.

Bonnet E, J Montfort, D Esquerre, K Hugot, A Fostier *et al*., 2007b. Effect of photoperiod manipulation on rainbow trout (*Oncorhynchus mykiss*) egg quality: A genomic study. Aquaculture, 268: 13-22.

Bromage, N, J Jones, C Randall, M Thrush, B Davies *et al*., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 100:141-166.

Chevassus B, E Quillet and D Chourrout, 1985. La production de truites stériles par voie génétique. Piscic. Fr., 78: 10-19.

Chourrout D, 1982. Gynogenesis caused by ultraviolet irradiation of salmonid sperm. J Exp Zool, 223:175-181.

Chourrout D and E Quillet, 1982. Induced gynogenesis in the rainbow trout: sex and survival of progenies production of all-triploid populations. Theor Appl Genetic, 63: 201-205.

Chourrout D., B Chevassus and F Herioux, 1980. Analysis of a Hertwig effect in the rainbow trout (*Salmo gairdneri Richardson*) after fertilization with gamma-irradiated sperm. Reprod. Nutr. Dév. 20: 719–726.

Davies B and N Bromage, 2002. The effects of fluctuating seasonal and constant water temperatures on the photoperiodic advancement of reproduction in female rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 205:183–200.

Diaz NF, P Iturra, A Veloso, F Estay and N Colihueque, 1993. Physiological factors affecting triploid production in rainbow trout, *Oncorhynchus mykiss.* Aquaculture, 114: 33-40.

Estay F, N Diaz, R Neira and X Fernandez, 1994. Analysis of reproductive performance of rainbow trout in a hatchery in Chile. Prog. Fish-Cult. 56.

Fraser TWK, T Hansen, MS Fleming and PG Fjelldal, 2015. The prevalence of vertebral deformities is increased with higher egg incubation temperatures and triploidy in Atlantic salmon *Salmo salar* L. Journal of Fish Diseases, 38: 75–89. doi: 10.1111/jfd.12206.

Goryczko K, S Dobosz, T Mäkinen and L Tomasik, 1991. UV-irradiation of rainbow trout sperm as a practical method for induced gynogenesis. J Appl Ichthyol,

7:136-146.

Grimholt U, R Johansen and AJ Smith, 2009. A review of the need and possible uses for genetically standardized Atlantic salmon (*Salmo salar*) in research. Laboratory Animals 43: 121–126.

Johnstone R, 1985. Induction of triploidy in Atlantic salmon by heat shock. Aquaculture 49: 133–139.

Kucharczyk D, D Żarski, K Targońska, MJ Łuczyński, A Szczerbowski, J Nowosad, R Kujawa and A Mamcarz, 2014. Induced artificial androgenesis in Common Tench, *Tinca Tinca* (L.), using Common Carp and Common Bream eggs, Italian Journal of Animal Science, 13:1, 2890.doi:10.4081/ijas.2014.2890.

Kupren K, A Mamcarz and D Kucharczyk, 2011. Effect of variable and constant thermal conditions on embryonic and early larval development of fish from the genus Leuciscus (Cyprinidae,Teleostei). Czech J Anim Sci, 56: 70-80.

Levanduski M, J Beck and J Seeb, 1990. Optimal thermal shocks for induced diploid gynogenesis in Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture, 90: 239-250.

Lincoln RF and AP Scott, 1984. Production of all-female triploid rainbow trout. Aquaculture, 30: 375-380.

Nowosad, J., Targońska, K., Chwaluczyk, R., Kaszubowski, R. and Kucharczyk, D. 2014. Effect of temperature on the effectiveness of artificial reproduction of dace [Cyprinidae (*Leuciscus leuciscus* L.)] under laboratory and field conditions. Journal of Thermal Biology, 45: 62-68. doi: 10.1016/j.jtherbio.2014.07.011.

Nowosad, J., Kucharczyk, D., Liszewski, T., Targońska K. and Kujawa, R. 2015. Comparison of temperature shock timing to induced artificial mitotic gynogenesis and androgenesis in common tench. Aquacult Int, 23: 45-53. doi:10.1007/s10499-014-9796-0.

Ocalewicz K, S Dobosz, H Kuźmiński, J Nowosad and K Goryczko, 2010. Chromosome rearrangements and survival of androgenetic rainbow trout (*Oncorhynchus mykiss*). J Appl Genet, 51:309–317.

Pankhurst NW and HR King, 2010. Temperature and salmonid reproduction: implications for aquaculture. J Fish Biol, 76: 69–85.

Palti Y, JJ Li and GH Thorgaard, 1997. Improved efficiency of heat and pressure shocks for producing gynogenetic rainbow trout. Prog Fish Cult, 59: 1-13.

Quillet E and J Gaignon, 1990. Thermal induction of gynogenesis and triploidy in Atlantic salmon (*Salmo salar*) and their potential interest for aquaculture. Aquaculture, 89: 351-364.

Quillet E, B Chevassus and A Devaux, 1988. Timing and duration of hatching in gynogenetic, triploid, tetraploid, and hybrid progenies in rainbow trout. Genet. Sel. Evol, 20:199-210.

Refstie T, 1983. Induction of diploid gynogenesis in Atlantic salmon and rainbow trout using irradiated sperm and heat shock. Can. J. Zool. 61: 2411-2416.

Samonte-Padilla IE, C Eizaguirre, JP Scharsack, TL Lenz and M Milinski, 2011. Induction of diploid gynogenesis in an evolutionary model organism, the three-spined stickleback (*Gasterosteus aculeatus*). BMC Developmental Biology 11:55.

Smoker, WW, PA Crandell and M Matsuoka, 1995. Second polar body retention and gynogenesis induced by thermal shock in pink salmon, *Oncorhynchus gorbuscha* (Walbaum). Aquacult. Res. 26: 213–219. doi: 10.1111/j.1365-2109.1995.tb00904.x

Solar I, EM Donaldson, GA Hunter, 1984. Induction of triploidy in rainbow trout (*Salmo gairdneri* Richardson) by heat shock, and investigation of early growth. Aquaculture, 42: 57–67.

Taranger GL and T Hansen, 1993. Ovulation and egg survival following exposure of Atlantic salmon, *Salmo salar* L., broodstock to different water temperatures. Aquacult. Fish. Manage. 24:151–156.

Taranger GL, E Vikingstad, U Klenke, I Mayer, SO Stefansson *et al*., 2003. Effects of photoperiod, temperature and GnRHa treatment on the reproductive physiology of Atlantic salmon (*Salmo salar L.*) broodstock. Fish Physiol Biochem. 28:403-406.

Taylor JF, AC Preston, D Guy and H Migaud, 2011. Ploidy effects on hatchery survival, deformities, and performance in Atlantic salmon (*Salmo salar*). Aquaculture, 315*:* 61–68.

Thorgaard GH, PD Scheerer, WK Hershberger and JM Myers, 1990. Androgenetic rainbow trout produced using sperm from tetraploid males show improandd survival. Aquaculture, 85: 215-221.

Yanik T, SA Hisar and C Bölükbasi, 2002. Early development and growth of Arctic Charr (*Salvelinus alpinus*) and Rainbow Trout (*Oncorhynchus mykiss*) at a low water temperature. Israeli J. Aquacult–Bamidgeh, 54: 73-78.