Aquacult Int (2013) 21:783–799
DOI 10.1007/s10499-012-9578-5

Synchronization of ovulation in brook char (*Salvelinus fontinalis*, Mitchill 1814) using emulsified D-Arg^{6}Pro^{9}NEt sGnRHa

Viktor W. Svinger · Tomas Policar · Christoph Steinbach · Simona Polakova · Antonin Jankovych · Jan Kouril

Received: 12 July 2011 / Accepted: 31 July 2012 / Published online: 14 August 2012

Abstract Effects of physiological saline (PS)-dissolved or Freund′s incomplete adjuvant (FIA)-emulsified sGnRHa on the induction and advancement of ovulation in brook char were assessed. Two-year-old females were randomly divided into 5 groups. Groups A and B received intraperitoneal injection of FIA-emulsified sGnRHa (sGnRHa-FIA) at dosages of 50 and 25 μg kg^{-1} body weight (BW), respectively. Females in group C were treated with a double injection (DI) of PS-dissolved sGnRHa (sGnRHa-PS) at 25 μg kg^{-1} BW spaced 3 days apart. Fish in group D received a 25 μg kg^{-1} BW single injection (SI) of sGnRHa-PS. Group E was established as a control group. After stripping, ovarian plasma pH level was measured, and an egg sample was taken from each female to record egg weight and diameter and survival to the eyed stage. Females in the GnRHa-treated groups ovulated significantly earlier than did females in the control group ($P < 0.01$). No significant differences were found among GnRHa-treated groups in ovulation dynamics and mean time to ovulation. Ovarian fluid pH was significantly higher in groups A, B, and C compared to control group E ($P < 0.05$). Significantly lower egg weight was found only in group B ($P < 0.01$), although all advanced groups tended to have lower egg weight than the control group. Egg diameters paralleled egg weight. Survival to the eyed stage was significantly higher in GnRHa-advanced groups compared to the control. A negative relationship was found between egg weight end eyed eggs percent ($R^2 = 0.26$). No pre-spawning or post-spawning mortality was observed during a 6-month period. Neither sGnRHa-PS nor sGnRHa-FIA are associated with negative influences on the health of females. The sGnRHa-FIA injections proved to exhibit the same efficacy as the DI protocol with sGnRHa-PS. Although no statistical differences were found in ovulation dynamics, we do not recommend the use of SI with sGnRHa-PS in brook char.

V. W. Svinger (✉) · T. Policar · C. Steinbach · A. Jankovych · J. Kouril
Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, Zátiší 728/II., 389 25 Vodňany, Czech Republic
e-mail: svinger@frov.jcu.cz

S. Polakova
Faculty of Science, University of South Bohemia in Ceske Budejovice, Branisovska 31, 37005 Ceske Budejovice, Czech Republic

Keywords Brook char · Freund′s incomplete adjuvant · GnRHa · Synchronization of ovulation

Abbreviations

BW	Body weight
FIA	Freund′s incomplete adjuvant
FCR	Food conversion ratio
sGnRHa-FIA	Salmon gonadotropin-releasing hormone analog diluted in physiological saline and homogenized in FIA
sGnRHa-PS	Salmon gonadotropin-releasing hormone analog diluted in physiological saline
LH	Luteinizing hormone
MIS	Maturation-inducing steroid
MPF	Maturation-promoting factor
pGSI	Pseudo-gonadosomatic index
PS	Physiological saline
SGR	Specific growth rate

Introduction

Synthetic analogs of gonadotropin-releasing hormone neuropeptide (GnRHa) are effective in stimulating secretion of luteinizing hormone (LH) from the pituitary (Zohar et al. 2010). Luteinizing hormone stimulates ovarian production of maturation-inducing steroids (MIS), for example 17α,20β-DP and maturation-promoting factors (MPF), followed by ovulation (Nagahama and Yamashita 2008). The potential for using GnRHa to control reproduction has been well known for more than 3 decades. Its successful experimental use in salmonids has been widely reported for purposes such as limiting handling, stress reduction, shortening the spawning period, and better control of production at hatcheries (Breton et al. 1990; Goren et al. 1995; Gillet et al. 1996, Park et al. 2007). The simplest method of GnRHa delivery is with injection of GnRHa peptide dissolved in physiological saline. Since the GnRHa is rapidly degraded by endo-enzymatic processes (Goren et al. 1990; Zohar et al. 1990a), a single injection does not provide the prolonged and controlled LH secretion from the pituitary necessary for gonadotropin control of gametogenesis in salmonid species (Breton et al. 1983). Consequently, a double injection (DI) is needed, increasing the frequency of handling of the broodstock. To avoid this, sustained release preparations have been developed, including cholesterol pellets, ethylene–vinyl acetate implants, and biodegradable polyesters of lactic acid and glycolic acid (Mylonas and Zohar 2001). The high cost of these preparations, however, limits their practicality for brook char fry producers, since the mean size of cultivated broodstock often does not exceed 1.0–1.5 kg, which makes implantation difficult and dramatically raise the cost of treatment.

Adjuvants are used to initiate and augment the inflammatory reaction required for induction of an optimal innate and adaptive immune response to vaccines, as well as to ensure long-lived immunity (Safari et al. 2011). Adjuvants can also allow a lower dose and thereby increase the potency of antivenoms (Pratanaphon et al. 1997) and reduce vaccine costs (Singh and O'Hagan 1999). Freund′s incomplete adjuvant (FIA) mechanism of action is promotion of the formation of depots of antigen at the site of immunization (Guy 2007).

In salmonid species, the evidence for the efficacy of FIA as a carrier for GnRHa is restricted to rainbow trout (*Oncorhynchus mykiss*) (Arabaci et al. 2004; Vazirzadeh et al. 2008) and chum salmon (*Oncorhynchus keta*) (Park et al. 2007). There is, however, a wide range of sources describing the use of FIA in salmonid aquaculture for vaccination (Poppe and Breck 1997). Although the use of water-in-oil emulsion with GnRHa in FIA (GnRHa-FIA) appears promising, post-spawning broodstock mortality has been found in rainbow trout (Arabaci et al. 2004).

Egg quality from hormone-treated broodstock is still questionable. Salmonids are, in general, a low fecundity species. Reduction in egg quality through hormonal manipulation would dramatically increase the costs of treatment. A number of studies describe a negative effect of hormone intervention on egg quality in salmonid fishes (Fitzpatrick et al. 1984; Mylonas et al. 1992; Taranger et al. 1992; Olito et al. 2001; Mikolajczyk et al. 2005; Bonnet et al. 2007; Noori et al. 2010). Others have reported that egg quality following hormonal treatment remained unaffected (Hunter et al. 1978, 1981; Donaldson et al. 1981a, 1981b; Sower et al. 1984; Zohar et al. 1990a, b; Slater et al. 1995; Arabaci et al. 2004; Park et al. 2007; Vazirzadeh et al. 2008). Egg quality is influenced by many common exogenous factors, for example stress, water temperature, age of broodstock, so may not be the result of hormone treatment alone. Several markers can be used to determine the quality of egg batches (Craik and Harvey 1984; Lahnsteiner et al. 1999a, b; Lahnsteiner 2007). Ovarian fluid pH and egg weight and size are parameters easily detectable in the field (Kallert 2009) and potentially useable for determination of reproductive quality (Lahnsteiner et al. 1999b; Aegerter and Jalabert 2004; Mansour et al. 2008). We used these parameters as the main indicators of egg quality.

The brook char (*Salvelinus fontinalis*, Mitchill 1814) was introduced into the Czech Republic in the late nineteenth century, and its importance in Czech salmonid aquaculture is currently increasing. It has proven to be a suitable species for recirculation aquaculture systems because of its aggressive food intake, satisfactory growth rates, relative tolerance to a changing environment, and popularity among consumers. Demand for its interspecific hybrid with arctic char (*Salvelinus alpinus*), the so-called Alsatian char, is rising in Czech neighborhoods, especially in Germany. Increase in its production requires greater amounts of high-quality eggs. As in other salmonids, the spawning period of brook char can extend over more than 4 weeks. It is sensitive to fungal diseases such as saprolegniosis throughout the spawning period, which results in extensive loss of broodstock. Hormone treatments in artificial reproduction of brook char could be an effective tool to minimize these problems and to better control egg production.

The goal of this study was to investigate the efficacy of sGnRHa-FIA treatment on induction and synchronization of ovulation. Since this is the first investigation of GnRHa treatment in artificial reproduction of brook char, the efficacy of single and double injection (DI) of sGnRHa-PS was also evaluated. Additionally, the influence of all treatments on egg quality was assessed. Survival rate to the eyed stage was monitored in egg samples. Broodstock mortality and health was observed during a 6-month post-spawning period.

Materials and methods

Experimental design

At the beginning of October 2010, sexually maturing 2-year-old first spawning brook char females (350 ± 24 g) were transported from a commercial farm to the experimental

facility of the Faculty of Fisheries and Protection of Waters in Vodnany (South Bohemia, Loc: 49°N, 14°E) approximately 5 weeks prior to the peak of the natural spawning period. Females were randomly selected and divided into 5 groups ($n = 10$) each placed into a 0.8-m^3 raceway supplied with running water from the Blanice River for a 7-day acclimatization period. The oxygen level was 11.8 ± 0.5 mg l^{-1}. Mean water temperature decreased during the experiment from 9.6 °C initially to 5.5 °C and subsequently rose to 9 °C at the end of October. Fish were kept under a natural photoperiod regime for our geographic location. The experimental groups were intraperitoneally injected as follows:

Group A: sGnRHa-FIA at 50 μg kg^{-1} BW
Group B: sGnRHa-FIA at 25 μg kg^{-1} BW
Group C: DI of sGnRHa at 25 μg kg^{-1} BW with 3-day interval
Group D: SI of sGnRHa at 25 μg kg^{-1} BW
Group E: injected with physiological saline only

GnRHa preparation

Salmon D-Arg^{6}Pro^{9}NEt-GnRHa (Bachem AG, Germany) was used. To prepare GnRHa-FIA treatments, the GnRHa was dissolved in 0.9 % NaCl physiological saline (PS) and mixed with Freund′s incomplete adjuvant (FIA, Sigma Aldrich) 1:1v/v using an Ika T-10 homogenizer. Each female received a total volume of 0.5 ml GnRHa-FIA preparation. GnRHa-PS treatments were prepared by dilution of sGnRHa in 0.9 % NaCl to the required concentration. Concentration of sGnRHa in solution was 25 μg ml^{-1}. Females treated with GnRHa-PS injections (Group C and D) received 1 ml of solution per 1 kg BW.

Egg quality

Immediately after stripping, ovarian fluid was collected and pH was measured using a multifunctional inoLAB 720 pH meter (WTW, 823 62 Weilheim, Germany). Pseudogonadosomatic index (pGSI) was calculated as: pGSI = weight of stripped eggs × 100 × fish weight^{-1}. The diameter ($n = 35$) and absolute weight ($n = 35$) to the nearest 0.01 g (KERN 572-33) of non-hardened eggs randomly selected from each female were determined. A small sieve and fine filter paper was used to remove remaining ovarian fluid to ensure accurate measurements. Egg diameter was measured using Quick PHOTO CAMERA 2.2 software (Olympus, Hamburg, Germany) from photographs taken with a binocular microscope Olympus BX51 fitted with an Olympus E-510 digital camera.

Egg sampling, fertilization and incubation

A sample of 200 ± 10 eggs was taken from each female and fertilized with an equal volume of a sperm mixture from 4 to 5 males. Milt was collected at the time of fertilization using a 1-ml syringe for each male. Sperm was mixed in a small dish during the process of fertilization and examined for motility before use. Eggs were incubated in separate small incubators (see Kallert 2009, p. 22) equipped with independently controllable inflow. Incubators were placed in a recirculation system with a self-cooling system maintaining the water temperature at 6.1 ± 0.4 °C throughout the incubation period. Oxygen content during incubation was maintained at 11.4 mg l^{-1}, and pH level was in the range of 7.80 ± 0.02. Other water parameters such as Cl^-, Fe, NH_3, NH_4, N–NO_2, and N–NO_3 were well below limits that could negatively impact egg development. Dead white eggs

were removed and counted from the incubators using glass tube when eyed stage of living eggs was reached. Survival to the eyed stage was calculated in all samples as the percent of eyed eggs in the total number of eggs (survival to the eyed stage = number of dead eggs × 100 × total number of eggs^{-1}).

Post-spawning mortality observation

When the second injection was administered to group C, fish in all groups were monitored every 3 days for ovulation by manual stripping. Each stripped female was identified by a fin cut and removed from the group into a 54-m^3 concrete pond to observe post-spawning mortality for a 6-month period. The concrete pond was supplied with running water from the Blanice River. Mean water temperature was initially 8.9 ± 1.1 °C, decreasing during the winter to 0.5 ± 0.8 °C, and subsequently rising gradually to 12 ± 1.5 °C in April 2011 when observation was terminated. The mean oxygen content throughout this period was 12 ± 1.3 mg l^{-1}. Approximately 3–4 days after stripping, females began to accept food and were fed with a commercial diet for trout (Biomar, EFICO Enviro 920, 3 mm) (1.0–1.5 % of total biomass). Food conversion ratio (FCR) was calculated as: FCR = Food consumed × $(W_T - W_t)^{-1}$. Specific growth rate (SGR, % day^{-1}) was: SGR = [(ln W_T − ln W_t) × T^{-1}] × 100, where W_T and W_t represent fish weight at the start and the end of the observation period (T = 160–180 days). At the end of the observation period (April 14, 2011), fish were counted and killed and dissected to evaluate health status.

Statistical analysis

All data were analyzed by Statistica 9 Cz (StatSoft, Tulsa, USA). Ovulation dynamics and percent of ovulated females were analyzed using survival analysis (Z test). Differences in mean time to ovulation were analyzed using nonparametric multicomparison Kruskal–Wallis test. One-way ANOVA was used to characterize differences in pH levels of ovarian fluid and egg weight. Differences in egg diameter were assessed by hierarchical (nested) ANOVA with individual females nested in treatment. Linear regression analysis was applied to correlate egg weight and survival to the eyed stage. Nonlinear and linear regression model was used to correlate ovarian fluid pH and survival to the eyed stage. Differences in percent of eyed eggs were assessed using one-way ANOVA or Student′s t test after arcsin transformation. If significant differences were found by ANOVA, Tukey′s HSD test was applied for detailed multicomparison assay. The Kolmogorov–Smirnov test was used to prove normal distribution of the data following homoscedasticity verification by Chochran–Bartlett′s test. Broodstock mortality was compared by χ^2 test. A significance level (α) of 0.05 was applied to all tests except where indicated. Data are presented as mean ± SEM.

Results

Synchronization and advancement of the ovulation

First ovulations occurred on day 9 post first injection in all sGnRHa-treated groups. Ovulation rate in groups A, B, C, and D was 30, 60, 40, and 30 %, respectively (Fig. 1). On day 12, the percent of ovulated females increased to 70, 80, 100, and 50 % in groups A, B,

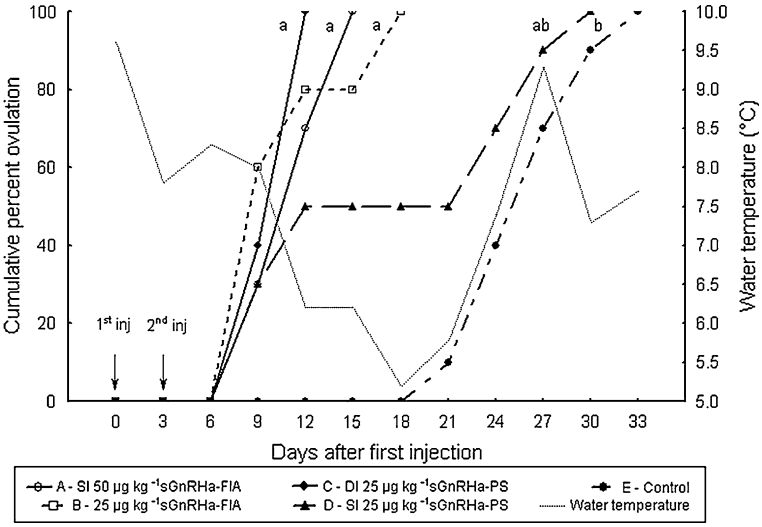

Fig. 1 Ovulation dynamic in brook char after four hormone treatments (**a** sGnRHa-FIA 50 µg kg^{-1}; **b** sGnRHa-FIA 25 µg kg^{-1}; **c** sGnRHa-PS DI 25 µg kg^{-1}; **d** sGnRHa-PS SI 25 µg kg^{-1}; **e** Control). Arrows indicate first and second hormone injection on day 0 and 3, respectively. *Curves* with the different *letters* are significantly different (survival analysis, $P < 0.05$)

C, and D, respectively, with 100 % of the fish in group C stripped within 3 days (Fig. 1). On day 15, 100 % of fish in group B were ovulated, and on day 18 100 % of fish in group A were ovulated. Ovulation was arrested from day 12 to day 21 in group D. Fish in this group were observed to be once again ovulating on day 24, once more reaching 100 % ovulation on day 30. In the control group E, first ovulation was recorded on day 21. The ovulation rate in this group increased gradually, and 100 % of females were stripped by day 33 of the experiment. The advancement of ovulation in hormone-treated groups compared to the control was shown to be highly significant with survival analysis ($\chi^2 = 23.5$; $df = 4$; $P = 0.0001$). Mean time to ovulation in groups A, B, and C was 12 ± 2.4; 11.4 ± 3.7, and 10.8 ± 1.5 days, respectively, and differed significantly from the control group E (26.7 ± 3.7 days) using Kruskal–Wallis multicomparison test ($n = 50$; $df = 4$; $P < 0.01$) (Fig. 2). Mean time to ovulation in group D was an intermediate value, 18.3 ± 8.8 days, and no statistical differences were found between group D and groups A, B, C, or E (Fig. 2).

Egg quality

The highest ovarian fluid pH levels were found in groups with the most advanced ovulation, A, B, and C (8.46 ± 0.07, 8.38 ± 0.09, and 8.38 ± 0.17, respectively). Significant differences were found only between group A and groups D and control group E (8.29 ± 0.09 and 8.25 ± 0.13, respectively) using Tukey′s HSD test ($P = 0.025$ and $P = 0.003$ for group D and E, respectively) (Table 1).

Generally, egg weight tended to be lower in the hormone-treated groups. However, significantly lighter eggs than in group E (25.2 ± 1.8 mg) were detected in group B (20.3 ± 2.9 mg) ($P < 0.01$). In addition, egg weight in group A (21.7 ± 1.7 mg) compared to group E appeared to be close to the level of significance ($P = 0.054$), suggesting

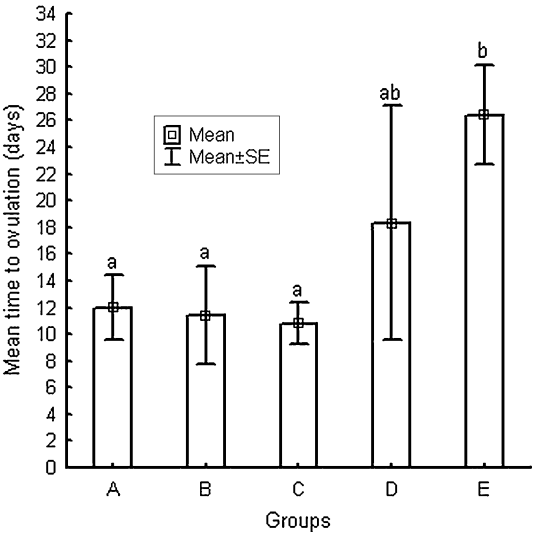

Fig. 2 Mean time to ovulation dependent on hormone treatment (**A** sGnRHa-FIA 50 μg kg^{-1}; **B** sGnRHa-FIA 25 μg kg^{-1}; **C** sGnRHa-PS DI 25 μg kg^{-1}; **D** sGnRHa-PS SI 25 μg kg^{-1}; **E** Control). Means (± SE, $n = 10$) with the different *letters* are significantly different (Kruskal–Wallis test)

Table 1 pGSI, ovarian fluid pH, egg weight, egg diameter, and eyed egg values of brook char females treated with GnRHa treatments

Treatment	pGSI (%) Mean ± SE $n = 10$	Ovarian fluid pH Mean ± SE $n = 10$	Egg weight (mg) Mean ± SE $n = 10$	Egg diameter (mm) Mean ± SE $n = 350$	Eyed eggs (%) Mean ± SE $n = 10$
A	8.8 ± 2.3^{a}	8.46 ± 0.07^{c}	21.7 ± 1.7^{ab}	3.65 ± 0.24^{b}	27 ± 22^{b}
B	7.3 ± 2.9^{a}	8.38 ± 0.09^{bc}	20.3 ± 2.9^{a}	3.61 ± 0.18^{a}	39 ± 24^{b}
C	6.5 ± 3.4^{a}	8.38 ± 0.17^{bc}	22.5 ± 3.0^{ab}	3.72 ± 0.25^{c}	30 ± 18^{b}
D	8.6 ± 2.2^{a}	8.29 ± 0.09^{ab}	22.7 ± 2.9^{ab}	3.73 ± 0.26^{c}	22 ± 24^{ab}
E	9.1 ± 2.8^{a}	8.25 ± 0.13^{ab}	25.2 ± 1.8^{bc}	3.81 ± 0.17^{d}	5 ± 5^{a}

Values with the different letters are significantly different (one-way ANOVA followed by Tukey′s HSD multiple comparison, $P < 0.05$)

that if more cases were obtained, statistical differences may be demonstrated also in this group. There were, however, no egg weight differences found between groups C and D (22.4 ± 2.9 mg and 22.7 ± 2.9 mg, respectively) in comparison with any of the other groups. No significant differences were found in pGSI level among the treatments (Table 1).

Egg diameter was found to correspond to the trends in egg weight (Table 1). Comparison of the data by hierarchical (nested) ANOVA detected significant differences among both individual females and treatment groups ($P < 0.01$ for both). The analysis of egg diameter with Tukey′s HSD test showed significant differences among all treatments (MSE = 0.0165; $df = 1632$; $P < 0.01$), except between groups C and D (Table 1), with the smallest eggs being found in groups A and B (3.65 ± 0.24 mm and 3.61 ± 0.18 mm, respectively) and largest eggs in the control group E (3.81 ± 0.17 mm).

Significantly higher survival to the eyed stage was seen in groups A, B, and C (27 ± 22 %, 39 ± 24 %, and 30 ± 18 %, respectively) compared to 5 ± 5 % in the control group, E ($P < 0.05$, 0.01, and 0.01 for groups A, B, and C, respectively, with

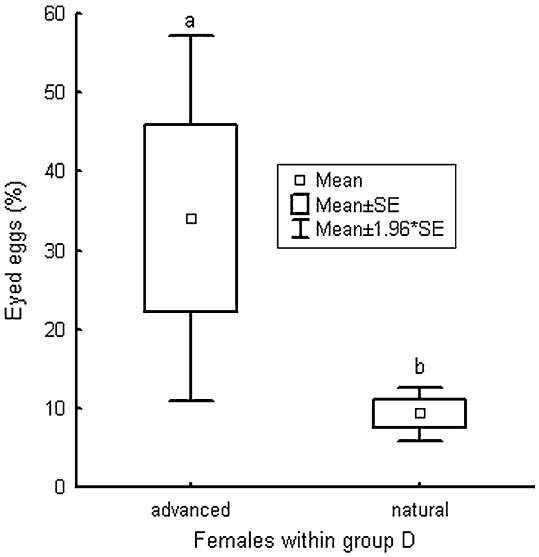

Fig. 3 Differences in survival to the eyed stage between eggs obtained from hormonally advanced females (advanced) and naturally ovulating females (natural) in group D sGnRHa-PS SI 25 μg kg^{-1}. Means (± SE, $n = 5$) with the different *letters* are significantly different (Student's t test)

Tukey's HSD test). Percent of eyed eggs in group D was 22 ± 24 % and did not differ from either control group E or other treatment groups (Table 1). Analysis with Student's *t* test showed that the 50 % of fish in group D that ovulated in advance (days 9 and 12) (Fig. 1) exhibited a significantly higher percent of eyed eggs (41 ± 24 %) than did those that ovulated beginning on day 24 (9 ± 4 %) ($P = 0.035$) (Fig. 3). This discrepancy was not observed in the other groups. In addition, the females in the advanced ovulation groups tended to produce lighter and smaller eggs, had higher pH levels of ovarian fluid (Table 1), and higher survival rates to the eyed stage. However, after plotting the data from all females into a single linear regression model (Fig. 4), we found a low negative relationship between egg weight and survival to the eyed stage ($R^2 = 0.26$). Those eggs that weighed less than 23 mg exhibited slightly higher survival to the eyed stage than eggs weighing more (Fig. 4.). The relationship between ovarian fluid pH and the percent of eyed eggs was low ($R^2 = 0.12$), as analyzed using a nonlinear regression model (Fig. 5). Detailed regression analysis within individual groups revealed that some females producing eggs at the extreme small end of the range exhibited higher ovarian fluid pH with decreased survival to the eyed stage, for example group A (Tables 2, 3). In addition, in some groups, neither the non-hardened egg weight nor the ovarian fluid pH showed an association with the percent of eyed eggs (Tables 2, 3).

Post-spawning mortality of broodstock

No mortality was recorded throughout the 6-month period in any of the groups. In counting the fish in April 2011, however, we found fewer than expected. The percent of remaining fish in groups A, B, C, D, and E was 90, 100, 90, 90, and 80 %, respectively. All surviving females were in excellent condition, and mean final weight (720 ± 47 g) did not differ among the groups. No differences were detected in either FCR or SGR levels at the end of the monitoring period. Specific growth rate was approximately 0.47 % day^{-1}, and FCR levels were 1.1–1.2 in all groups. Fish willingly accepted food even when water temperature decreased to below 1 °C and the holding pond was fully covered in ice. Normally developing ovaries were found in each dissected fish, and no visible abnormalities

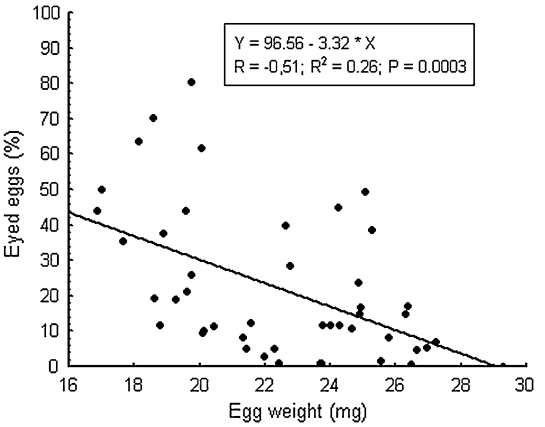

Fig. 4 Relationship between egg weight and survival to the eyed stage from all experimental females (linear regression model)

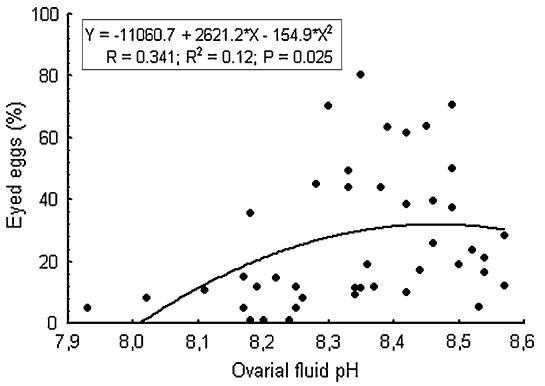

Fig. 5 Relationship between ovarian fluid pH and survival to the eyed stage from all experimental females (nonlinear regression model)

Table 2 Simple regression models describing the relationship between eyed egg percent and ovarian fluid pH

Treatment	*R*	*P*	R^2	Equation
A	0.22	0.55	0.05	$y = -799.6 + 97.96x$
B	−0.03	0.94	0.001	$y = 103.14 - 7.08x$
C	−0.61	0.08	0.37	$y = 976.1 - 111.9x$
D	0.32	0.4	0.1	$y = -537 + 67.21x$
E	−0.42	0.25	0.18	$y = 147.4 + 17.31x$

(intra-abdominal lesions, bruises, hematomas, or granulomas) were observed in either body wall or organ epithelium.

Discussion

Synchronization and advancement of the ovulation

In our study, all sGnRHa (D-Arg^6,Pro^9,NEt-GnRHa) treatments significantly advanced and synchronized ovulation of brook char compared to the control. These results are consistent with results of other authors using different or the same synthetic GnRHas in salmonids

Table 3 Simple regression models describing the relationship between eyed egg percent and weight of non-hardened eggs

Treatment	R	P	R^2	Equation
A	0.90	0.0008	0.82	$y = -162.73 + 9.1x$
B	0.53	0.22	0.28	$y = 117.43 + 3.7x$
C	−0.21	0.58	0.05	$y = 59.18 + 1.15x$
D	−0.61	0.08	0.37	$y = 126.51 + 4.65x$
E	−0.007	0.99	0.0	

such as (D-Ala^6,Pro^9,NEt-GnRHa) (Erdahl and McClain 1987; Taranger et al. 1992; Mylonas et al. 1992; Haraldsson et al. 1993), (D-Arg^6,Pro^9,NEt-GnRHa) (Jansen 1993) and ([D-Nal(2)^{6}aza-Gly^{10}]-GnRHa) (Mikolajczyk et al. 2008). Both sGnRHa-FIA (25 or 50 μg) and sGnRHa-PS double injection of 25 μg were highly effective in accelerating and synchronizing ovulation in brook char. An ovulation rate of 100 % was recorded within 3 days in those females treated with DI of 25 μg. Fish in both 25 μg and 50 μg sGnRHa-FIA treated groups showed nearly the same ovulation pattern as those treated with the DI protocol. These results are similar to previous experiments conducted in rainbow trout (*Oncorhynchus mykiss*) using FIA as a carrier of ([D-Ser(tBu)^6,Pro^9-NEt]-GnRHa) and (D-Arg^6,Pro^9,NEt-GnRHa) (Arabaci et al. 2004; Vazirzadeh et al. 2008).

A SI of sGnRHa-PS is economically the most advantageous. However, a SI of sGnRHa-PS at 25 μg in our study evoked synchronization of ovulation in only 50 % of the females. Subsequently, ovulation in this group was arrested for 12 days and recurred concomitant with the first natural ovulations in the control group beginning day 24. This corresponds with other experiments in salmonids and/or coregonids, in which a single GnRHa injection of ([D-Ser(tBu)^6,Pro^9-NEt]-GnRHa) (Arabaci et al. 2004), ([D-Nal(2)^{6}aza-Gly^{10}]-GnRHa) (Mikolajczyk et al. 2005), (D-Tle^6,Pro^9,NEt-GnRHa) (Svinger et al. 2010) and (D-Ala^6,Pro^9,NEt-GnRHa) (Noori et al. 2010) was only partially successful in inducing ovulation. This failure of SI in some individuals may be due to rapid clearance of effective GnRHa from the circulation profile (Zohar et al. 1990b), leading to insufficient LH plasma levels (Breton et al. 1990). Ovulation in such individuals is interrupted and continues some time later in response to natural secretion of native GnRH peptide from the hypothalamus.

Our results suggest that the SI of 25 μg is insufficient to induce a high ovulation rate compared to other assessed treatments in brook char. However, Taranger et al. (1992) report that even 1 μg kg^{-1} BW of GnRHa was effective in Atlantic salmon (*Salmo salar*) if given close to natural ovulation time. Similar results were obtained by other authors (Crim and Glebe 1984; Crim et al. 1986; Fitzpatrick et al. 1984). In our experiment, hormone treatments were administered approximately 4 weeks prior to natural ovulation time at 9–9.5 °C water temperature, which is approximately 3.5–4.0 °C above normal spawning temperature of brook char in our region. There is strong evidence that elevated temperatures impede peri-ovulatory physiological readiness of females via an inhibitory effect of dopamine on LH release from pituitary in Arctic char (*Salvelinus alpinus*) (Gillet et al. 1996; Gillet and Breton 2009). Impairment of ovarian steroidogenic activity at elevated temperatures has been reported in Atlantic salmon (King and Pankhurst 2004a, b; King et al. 2003; Watts et al. 2004; Vikingstad et al. 2008; Pankhurst and King 2010) and rainbow trout (Pankhurst et al. 1996; Pankhurst and Thomas 1998). Whether these mechanisms were, at least partially, in effect during the first and second injection periods, at 9 °C, remains unclear. If so, they seemed to have been fully counteracted by GnRHa-FIA treatment and the double injection, while the single injection of 25 μg did not have such a strong counteraction. It has to be noted that higher effectiveness of DI applied in

group C could be caused by the higher total dose. Nevertheless, the theory of low counteraction ability of single injection seems to be more probable since double injection of 12.5 μg sGnRHa-PS and 12.5 μg of sGnRHa-FIA was much more effective than single injection of 50 μg sGnRHa-PS at 8.5 °C in an additional experiment carried out 1 year later (in 2011) in the same strain of brook char (Svinger, unpublished results). Similar phenomenon was observed in Northern whitefish (*Coregonus peled*), where double injection of 5 μg mGnRHa was far more effective compared to single injection of 25 μg (Svinger et al. 2010), and Breton et al. (1990) reported 100 % ovulation in rainbow trout treated with GnRHa-sustained release form, despite dosages of 12.5–50.0 μg, whereas 100 % ovulation rate was never achieved in those females treated with acute injections at dose of 20 μg. These suggest that hormone delivery method (sustained release or multiple injections versus single injection) is more important than the dose of hormone used.

Exposure of salmonids to colder water appears to accelerate steroidogenic activity (Vikingstad et al. 2008) and suppress dopamine inhibitory tone on LH secretion (Gillet et al. 1996; Gillet and Breton 2009) allowing onset of natural ovulation time. This is supported by the fact that females in the control group began to ovulate some days after a sharp drop in water temperature to 5.5–6.0 °C (Fig. 1). Thus, we cannot exclude the possibility that if SI is given later at lower water temperatures, the physiological system of the fish will respond more favorably. Thorough research to determine temperature levels that affect or modulate final stages of the reproductive cycle in brook char is necessary.

Egg quality

The percent of eyed eggs was low in all groups. This was probably due to utilization of first spawners (Kallert 2009) and, in addition, extremely high late summer temperatures. This heat resulted in elevated water temperatures (19–20 °C) at the production farm from which the char was obtained. High temperature during vitellogenesis in salmonids is commonly known to result in reduced egg quality in the ensuing spawning season (Gillet 1991; Taranger and Hansen 1993; Pankhurst et al. 1996).

However, the most GnRHa-accelerated groups showed significantly higher survival to the eyed stage than the control group. The 50 % of the females in the SI group that ovulated in advance produced a significantly higher percent of eyed eggs than did the fish that released eggs simultaneously with the control group. This is in direct contradiction with other reports where the early stripped females of brown trout (*Salmo trutta*) (Mylonas et al. 1992), Atlantic salmon (Crim and Glebe 1984), coho salmon (*Oncorhynchus kisutch*) (Fitzpatrick et al. 1984), and chum salmon (Park et al. 2007) produced lower fertilization and survival rates than did later stripped individuals. On the other hand, a number of studies have reported that hormone treatment did not result in alteration of egg quality (Jalabert et al. 1978; Donaldson et al. 1981a; Hunter et al. 1981; Billard et al. 1984; Arabaci et al. 2004; Vazirzadeh et al. 2008), or that eggs obtained from hormone-treated females had higher survival rates than those from spontaneously ovulating females (Sower et al. 1984). Mylonas et al. (1992) explain these discrepancies as slight asynchrony between the process of meiotic maturation regulated by the maturation-inducing steroids and the process of ovulation regulated by prostaglandins. Gillet et al. (1996) found the percent of eyed eggs in Arctic char to be negatively correlated with the plasma LH level, which could be the major factor in the asynchrony causing reduction in egg quality. This was also suggested by Billard et al. (1984). In addition, Gillet and colleagues report that plasma LH level is not the only factor involved in the control of egg quality, citing variable responsiveness of females to GnRH injection and the difference in maturation stage of

females at the time of treatment. Since plasma LH levels were not measured in our study, we are unable to say whether any of the hormone treatments elicited such phenomenon in brook char or whether it influenced egg quality, since eggs of treated females exhibited higher survival rates.

Nevertheless, if each brood fish at the time of hormone treatment is at a slightly different physiological and maturation stage, there should be accompanying measurable parameters by which the sexual product quality is being influenced. Both egg size and ovarian fluid pH levels are usable and easily detectable parameters for egg quality assessment. Decreased pH of ovarian fluid caused by water inflow into the coelomic cavity, ovarian secretion changes, or injection of intracellular content of broken eggs into ovarian fluid (Lahnsteiner et al. 1999b; Dietrich et al. 2007; Wojtczak et al. 2007) reduces sperm motility and promotes premature egg hydration resulting in lower egg fertilization (Aegerter and Jalabert 2004).

In our study, females in the most GnRHa accelerated groups (A, B, and C) produced smaller and lighter eggs and had higher ovarian fluid pH levels. The significance was most noted in egg diameters, where the highest number of observations was made ($n = 350$ for each group). These groups exhibited also higher percent of eyed embryos compared to the control group. The most comparable results to these were reported by Mansour et al. (2008), who found that, in Arctic char held at 7 °C, good-quality eggs exhibited lower absolute non-hardened egg weight and egg diameter accompanied by higher ovarian fluid pH in comparison with poor-quality eggs. Similar results were found by Aegerter and Jalabert (2004) in rainbow trout. Mansour et al. (2008) considered poor-quality eggs as over-ripe. We found, in contrast, that there was a strong positive relationship between egg weight and eyed egg percent ($R^2 = 0.81$) in group A. Further, some females of this group exhibited the highest levels of ovarian fluid pH followed by low survival to the eyed stage, suggesting that some non-ripe females in this group may have been stripped. Elevated spawning water temperatures, which occurred at the time of the first ovulation of females in the control group, might have also accelerated the over-ripening process in some individuals in group D and controls, which resulted in low survival rates at the eyed stage.

When data from all females were plotted into simple regression models, only low relationships between non-hardened egg weight, ovarian fluid pH, and survival to the eyed stage ($R^2 = 0.26$ and $R^2 = 0.12$ for egg weight and ovarian fluid pH, respectively) were found. In contrast, Lahnsteiner et al. (1999b) found a much higher regression coefficient ($R^2 = 0.46$) in a simple regression model describing the relationship between ovarian fluid pH and eyed egg percent in lake trout (*Salmo trutta lacustris*). When detailed regression analysis was applied in our study, the highest correlations were found in groups C and E, $R^2 = 0.37$ and $R^2 = 0.18$, respectively. This may be due to more accurate pH measuring or to other factors involved in survival to the eyed stage (Lahnsteiner et al. 1999b; Lahnsteiner 2007). The limited number of observations could also have played a role. Further, most ovarian fluid pH levels were in the range of 8.2–8.4, which could diminish the possibility of detection of distinct relationships, since this is considered to be the most suitable level in salmonid species (Wilcox et al. 1984; Lahnsteiner et al. 1999b; Mansour et al. 2008).

The hormone-treated females were stripped in a short window of time, which markedly reduced the frequency of anesthetizing and handling necessary when monitoring for ovulation, in comparison with group D and control group E. Enhanced stress at the final stage of reproductive development can not only result in disruption of reproductive endocrinology, but can also affect egg size and survival of the progeny in rainbow trout and brown trout (Campbell et al. 1992, 1994). Stress response differences are found among

salmonids and even within different strains within a salmonid species (Fevolden et al. 1991; Pottinger and Pickering 1992; Heath et al. 1993). How, and to what extent, the increased handling influenced measured parameters and survival rates in brook char in this study remains unclear.

Mortality of broodstock

Since we did not find dead fish throughout the 6-month post-treatment period, 100 % survival was expected in all groups. However, after counting the broodstock in April 2011, we observed that numbers were reduced in all groups with the exception of group B (25 μg sGnRHa-FIA), which had 100 % survival rate. These missing fish were most probably eaten by gray herons (*Ardea cinerea*), which often visit our experimental facility before the ponds are covered by ice. Nevertheless, there were no statistical differences found among all groups in terms of post-spawning mortality, which is in agreement with other studies where GnRHa treatments were found to improve survival of the broodstock (Mikolajczyk et al. 2005; Goren et al. 1995). In contrast, GnRHa-FIA treatment has been reported to inflict high post-spawning mortality in rainbow trout (Arabaci et al. 2004). The authors do not present any rationale for the high mortality. In a more recent study, GnRHa-FIA was not associated with post-spawning mortality in rainbow trout 3 months post-injection (Vazirzadeh et al. 2008). These authors suggest that this was due to a lesser amount of FIA used compared to the study by Arabaci et al. (2004). In our current experiment, the ratio of FIA to weight of treated females was even higher than that used by Arabaci et al. (2004); hence we cannot confirm this suggestion.

In salmonid aquaculture, adjuvants are widely used in vaccines against various pathogens, for example *Vibrio salmonicida* and *Aeromonas salmonicida*. Most reported side effects induced by mineral oil adjuvant vaccines are lesions at the injection site, abdominal adhesions, post-vaccination mortality, poor feed uptake and conversion, retarded growth, and downgrading at slaughter (Lillehaug et al. 1992; Press and Lillehaug 1995; Midtlyng et al. 1996; Poppe and Breck 1997). In the current study, no abnormalities were found in any GnRHa-FIA-treated brook char. The fish appeared to be in excellent condition throughout the observation period and possessed active appetite even at low temperatures 0.2–3.5 °C from late November until late February. However, both lower FCR (0.8–0.9) and higher SGR (0.6–0.7 % day^{-1}) levels than in our experiment are usually observed in intensively reared brook char (Reiter 2006). These discrepancies may be unrelated to GnRHa-FIA treatment and may more likely be associated with fish in our study being kept for long periods at very low temperatures and/or at low stocking density (Jørgensen et al. 1993; Jobling et al. 1993), which was only 0.3–0.65 kg m^{-3}. There were no differences found in either FCR or SGR among any of the groups. Based on our results, neither sGnRHa-PS nor sGnRHa-FIA are associated with negative influences on the health of brook char females.

Conclusions

All hormone treatments were effective tools to advance and synchronize ovulation in brook char except the single acute injection, which cannot be recommended for these purposes if administered 3–4 weeks prior to natural spawning period and at water temperature above 8 °C. Both sGnRHa-FIA treatments exhibited strong advancing and synchronizing effects similar to the double acute injection and thereby provided effective single injection

protocols. Handling of broodstock was also minimized by these treatments, which can limit stress. Nevertheless, our results suggest that hormone treatment may have a partial capacity to impact characteristics of ovarian fluid and thereby influence survival of eggs in brook char. Further, although survival rates to the eyed stage were higher in advanced ovulation groups, egg size in these groups was significantly decreased with unknown subsequences for hatching and the progeny. Other studies are necessary for more detailed specification of these changes, which could help to develop more efficient hormone therapies for brook char.

Acknowledgments This study was financially supported by projects: CZ.1.05/2.1.00/01.0024, GAJU 047/2010/Z, NAZV QH71305, Kontakt ME ME 10126 and NAZV QH91310. We thank The Lucidus Consultancy for providing English corrections. We are also grateful to Dr. Tom Hansen, Institute of Marine Research in Bergen, Research Station in Matre (Norway), and to Dr. Dennis Kallert, Veterinary Medical Research Institute in Budapest (Hungary), for support and consultations.

References

Aegerter S, Jalabert B (2004) Effects of post-ovulatory oocyte ageing and temperature on egg quality and on the occurrence of triploid fry in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 231:59–71

Arabaci M, Diler I, Sari M (2004) Induction and synchronization of ovulation in rainbow trout, *Oncorhynchus mykiss*, by administration of emulsified buserelin (GnRHa) and its effects on egg quality. Aquaculture 237:475–484

Billard R, Reinaud P, Hollebecq MG, Breton B (1984) Advancement and synchronization of spawning in *Salmo gairdneri* and *S. trutta* following administration of LRH-A combined or not with pimozide. Aquaculture 43:57–66

Bonnet E, Fostier A, Bobe J (2007) 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

Breton B, Fostier A, Zohar Y, Le Bail PY, Billard R (1983) Gonadotropine glycoproteique et oestradiol-17β pendant le cycle reproducteur chez la truite fario (*Salmo trutta*) femelle. Gen Comp Endocrinol 49:220–231

Breton B, Weil C, Sambroni E, Zohar Y (1990) Effects of acute versus sustained administration of GnRH on GtH release and ovulation in the rainbow trout, *Oncorhynchus mykiss*. Aquaculture 91:371–383

Campbell PM, Pottinger TG, Sumpter JP (1992) Stress reduces the quality of gametes produced by rainbow trout. Biol Reprod 47:1140–1150

Campbell PM, Pottinger TG, Sumpter JP (1994) Preliminary evidence that chronic confinement stress reduces the quality of gametes produced by brown and rainbow trout. Aquaculture 120:151–169

Craik J, Harvey S (1984) Egg quality in rainbow trout: the relation between egg viability, selected aspects of egg composition, and time of stripping. Aquaculture 40:115–134

Crim LW, Glebe BD (1984) Advancement and synchrony of ovulation in Atlantic salmon with pelleted LH RH analog. Aquaculture 43:47–56

Crim LW, Glebe BD, Scott AP (1986) The influence of LHRH analog on oocyte development and spawning in female Atlantic salmon, *Salmo salar*. Aquaculture 56:139–149

Dietrich GJ, Wojtczak M, Slowinska M, Dobosz S, Kuzminski H, Ciereszko A (2007) Broken eggs decrease pH of rainbow trout (*Oncorhynchus mykiss*) ovarian fluid. Aquaculture 273:748–751

Donaldson EM, Hunter GA, Dye HM (1981a) Induced ovulation in coho salmon (*Oncorhynchus kisutch*). II. Preliminary study of the use of LH-RH and two high potency LH-RH analogues. Aquaculture 26:129–141

Donaldson EM, Hunter GA, Dye HM (1981b) Induced ovulation in coho salmon (*Oncorhynchus kisutch*). III. Preliminary study on the use of the antiestrogen tamoxifen. Aquaculture 26:143–154

Erdahl D, McClain J (1987) Effect of LH-RH analogue treatment on egg maturation (ovulation) in lake trout brood stock. Prog Fish Cult 49:276–279

Fevolden SE, Refstie T, Roeed KH (1991) Selection for high and low cortisol stress response in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 95:53–65

Fitzpatrick MS, Suzumoto BK, Schreck CB, Oberbillig D (1984) Luteinizing hormone-releasing hormone analogue induces precocious ovulation in adult coho salmon (*Oncorhynchus kisutch*). Aquaculture 43:67–73

Gillet C (1991) Egg production in an Arctic char (*Salvelinus alpinus* L.) brood stock: effects of temperature on the timing of spawning and the quality of eggs. Aquat Living Resour 4:109–116

Gillet C, Breton B (2009) LH secretion and ovulation following exposure of Arctic char to different temperature and photoperiod regimes: responsiveness of females to a gonadotropin-releasing hormone analogue and a dopamine antagonist. Gen Comp Endocr 162:210–218

Gillet C, Breton B, Mikolajczyk T (1996) Effects of GnRHa and pimozide treatments on the timing of ovulation and on egg quality in Arctic char (*Salvelinus alpinus*) at 5 and 10 °C. Aquat Living Resour 9:257–263

Goren A, Zohar Y, Fridkin M, Elhanati E, Koch Y (1990) Degradation of gonadotropin-releasing hormones in the gilthead seabream, *Sparus aurata*. I. Cleavage of native salmon GnRH and mammalian LHRH in the Pituitary. Gen Comp Endocr 79:291–305

Goren A, Gustafson H, Doering D (1995) Field trials demonstrate the efficacy and commercial benefit of a GnRHa implant to control ovulation and spermiation in salmonids. In: Goetz FW, Thomas P (eds) Reproductive physiology of fish symposium 95, Austin, Texas: pp 99–101

Guy B (2007) The perfect mix: recent progress in adjuvant research. Nat Rev Microbiol 5(7):505–517

Haraldsson H, Sveinsson T, Skúlason S (1993) Effects of LHRHa treatments upon the timing of ovulation and upon egg and offspring quality in Arctic char, *Salvelinus alpinus* (L.). Aquacult Fish Manag 24:145–150

Heath DD, Bernier NJ, Heath JW, Iwama GK (1993) Genetic, environmental, and interaction effects on growth and stress response of chinook salmon (*Oncorhynchus tshawytscha*) fry. Can J Fish Aquat Sci 50:435–442

Hunter GA, Donaldson EM, Stone ET, Dye HM (1978) Induced ovulation of female Chinook salmon (*Oncorhynchus tshawytscha*) at a production hatchery. Aquaculture 15:99–112

Hunter GA, Donaldson EM, Dye HM (1981) Induced ovulation in coho salmon (*Oncorhynchus kisutch*). I. Further studies on the use of salmon pituitary preparations. Aquaculture 26:117–127

Jalabert B, Goetz FW, Breton B, Fostier A, Donaldson EM (1978) Precocious induction of oocyte maturation and ovulation in coho salmon, *Oncorhynchus kisutch*. J Fish Res Board Can 35:1423–1429

Jansen ME (1993) Induction of ovulation in Arctic char held under unchanging temperature and photoperiod. Prog Fish Cult 55:32–34

Jobling M, Jørgensen EH, Arnesen AM, Ringø E (1993) Feeding, growth and environmental requirements of Arctic char: a review of aquaculture potential. Aquacult Int 1:20–46

Jørgensen EH, Christiansen JS, Jobling M (1993) Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic char (*Salvelinus alpinus*). Aquaculture 110:191–204

Kallert DM (2009) Der Einfluss exogener und endogener Parameter auf den Erbrütungserfolg bei Salmoniden: Eine integrative Untersuchung von Problemen bei der Vermehrung von Bachforelle, Bachsaibling und Seesaibling. Bezirk Oberfranken, Bayreuth 2009, http://www.bezirk-oberfranken.de/fileadmin/5_Natur/fischerei/publikationen/AB_Kallert_Endfassung.pdf

King HR, Pankhurst NW (2004a) Effect of maintenance at elevated temperatures on ovulation and luteinizing hormone releasing hormone analogue responsiveness of female Atlantic salmon (Salmo salar) in Tasmania. Aquaculture 233:583–597

King HR, Pankhurst NW (2004b) Effect of short-term temperature reduction on ovulation and LHRHa responsiveness in female Atlantic salmon (*Salmo salar*) maintained at elevated water temperatures. Aquaculture 238:421–436

King HR, Pankhurst NW, Watts M, Pankhurst PM (2003) Effect of elevated summer temperatures on gonadal steroid production, vitellogenesis and egg quality in female Atlantic salmon. J Fish Biol 63:153–167

Lahnsteiner F (2007) First results on a relation between ovarian fluid and egg proteins of *Salmo trutta* and egg quality. Aquacult Res 38:131–139

Lahnsteiner F, Berger B, Weismann T (1999a) Sperm metabolism of the teleost fishes *Chalcalburnus chalcoides* and *Oncorhynchus mykiss* and its relation to motility and viability. J Exp Zool 284:454–465

Lahnsteiner F, Weismann T, Patzner RA (1999b) Physiological and biochemical parameters for egg quality determination in lake trout, *Salmo trutta lacustris*. Fish Physiol Biochem 20:375–388

Lillehaugh A, Lunder T, Poppe TT (1992) Field testing of adjuvanted furunculosis vaccines in Atlantic salmon, *Salmo salar* L. J Fish Dis 15:485–496

Mansour N, Lahnsteiner F, McNiven MA, Richardson GF (2008) Morphological characterization of Arctic char, *Salvelinus alpinus*, eggs subjected to rapid post-ovulatory aging at 7 °C. Aquaculture 279:204–208

Midtlyng PJ, Reitan LJ, Speilberg L (1996) Experimental studies on the efficacy and side-effects of intraperitoneal vaccination of Atlantic salmon (*Salmo salar* L.) against furunculosis. Fish Shellfish Immunol 6:335–350

Mikolajczyk T, Kuźmiński H, Dobosz S, Goryczko K, Enright WJ (2005) The effects of Gonazon™, a commercially available GnRH analogue, on induction of ovulation and egg duality in cultured European whitefish (*Coregonus lavaretus* L.). Adv Limnol 60:187–194

Mikolajczyk T, Skolowska-Mikolajczyk M, Szczerbik P, Duc M, Goryczko K, Dobosz S, Glogowski J, Epler P, Enright WJ (2008) The effects of the GnRH agonist, azaglynafarelin (Gonazon™), on ovulation and egg viability in the European grayling (*Thymallus thymallus* L.). Aquaculture 281:126–130

Mylonas CC, Zohar Y (2001) Use of GnRHa-delivery systems for the control of reproduction in fish. Rev Fish Biol Fish 10:463–491

Mylonas CC, Hinshaw JM, Sullivan CV (1992) GnRHa-induced ovulation of brown trout (*Salmo trutta*) and its effects on egg quality. Aquaculture 106:379–392

Nagahama Y, Yamashita M (2008) Regulation of oocyte maturation in fish. Dev Growth Differ 50:195–219

Noori A, Amiri BM, Mirvaghefi A, Baker DW (2010) LHRHa-induced ovulation of the endangered Caspian brown trout (*Salmo trutta caspius*) and its effect on egg quality and two sex steroids: testosterone and 17α-hydroxyprogesterone. Aquac Res 41:871–877

Olito C, Loopstra D, Hansen P (2001) Acceleration of sexual maturation in chinook salmon broodstock using luteinizing hormone-releasing hormone analog. N Am J Aquacult 63:208–214

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

Pankhurst NW, Thomas PM (1998) Maintenance at elevated temperature delays the steroidogenic and ovulatory responsiveness of rainbow trout *Oncorhynchus mykiss* to luteinizing hormone releasing hormone analogue. Aquaculture 166:166–177

Pankhurst NW, Purser GJ, Van Der Kraak G, Thomas PM, Forteath GNR (1996) Effect of holding temperature on ovulation, egg fertility, plasma levels of reproductive hormones and in vitro ovarian steroidogenesis in the rainbow trout *Oncorhynchus mykiss*. Aquaculture 146:277–290

Park WD, Lee ChH, Lee ChS, Kim D-J, Kim J-H, Tamaru CS, Sohn YCh (2007) Effects of gonadotropin-releasing hormone analog combined with pimozide on plasma sex steroid hormones, ovulation and egg duality in freshwater-exposed female chum salmon (*Oncorhynchus keta*). Aquaculture 271:488–497

Poppe TT, Breck O (1997) Pathology of Atlantic salmon *Salmo salar* intraperitoneally immunized with oil adjuvanted vaccine. A case report. Dis Aquat Org 29:219–226

Pottinger TG, Pickering AD (1992) The influence of social interaction on the acclimation of rainbow trout, *Oncorhynchus mykiss* (Walbaum) to chronic stress. J Fish Biol 43:121–130

Pratanaphon R, Akesowan S, Khow O, Sriprapat S, Ratanabanangkoon K (1997) Production of highly potent horse antivenom against the Thai cobra (*Naja kaouthia*). Vaccine 15(14):1523–1528

Press CM, Lillehaug A (1995) Vaccination in European salmonid aquaculture: a review of practices and prospects. Brit Vet J 151:45–69

Reiter RH (2006) Leistungs und Qualitätseigenschaften jeweils zweier Herkünfte des Seesaiblings (*Salvelinus alpinus*) und des Bachsaiblings (*Salvelinus fontinalis*) sowie ihrer Kreuzungen. Bayerische Landesanstalt für Landwirtschaft (LfL), Freising-Weihenstephan

Safari D, Dekker HAT, Rijkers G, Snippe H (2011) Codelivery of adjuvants at the primary immunization site is essential for evoking a robust immune response to neoglycoconjugates. Vaccine 29:849–854

Singh M, O'Hagan D (1999) Advances in vaccine adjuvants. Nat Biotechnol 17(11):1075–1081

Slater CH, Schreck CB, Amend DF (1995) GnRHa injection accelerates final maturation and ovulation/spermiation of sockeye salmon (*Oncorhynchus nerka*) in both fresh and salt water. Aquaculture 130: 279–285

Sower SA, Iwamoto RN, Dickhoff WW, Gorbman A (1984) Ovulatory and steroidal responses in coho salmon and steelhead trout following administration of salmon gonadotropin and D-Ala^6, des Gly^{10} gonadotropin-releasing hormone ethylamide (GnRHa). Aquaculture 43:35–46

Svinger VW, Kouril J, Pavlista R (2010) Induced and synchronized ovulation in northern whitefish (*Coregonus peled*) using GnRHa (D-Tle^6,Pro^9-NEt) Lecirelin in different dosages. In: Abstracts of aquaculture Europe 2010 conference, Porto, 5–8 October 2010, pp 1279–1280

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

Taranger GL, Stefansson SO, Hansen T (1992) Advancement and synchronization of ovulation in Atlantic salmon (*Salmo salar* L.) following injections of LHRH analogue. Aquaculture 102:169–175

Vazirzadeh A, Hajimoradloo A, Esmaeili HR, Akhlaghi M (2008) Effects of emulsified versus saline administration of GnRHa on induction of ovulation in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 280:267–269

Vikingstad E, Andersson E, Norberg B, Mayer I, Klenke U, Zohar Y, Stefansson SO, Taranger GL (2008) The combined effects of temperature and GnRHa treatment on the final stages of sexual maturation in Atlantic salmon (*Salmo salar* L.). Fish Physiol Biochem 34:289–298

Watts M, Pankhurst NW, King HR (2004) Maintenance of Atlantic salmon (*Salmo salar*) at elevated temperature inhibits cytochrome P450 aromatase activity in isolated ovarian follicles. Gen Comp Endocrinol 135:381–390

Wilcox KW, Stoss J, Donaldson EM (1984) Broken eggs as a cause of infertility of coho salmon gametes. Aquaculture 40:70–87

Wojtczak M, Dietrich GJ, Slowinska M, Dobosz S, Kuzminski H, Ciereszko A (2007) Ovarian fluid pH enhances motility parameters of rainbow trout (*Oncorhynchus mykiss*) spermatozoa. Aquaculture 270:259–264

Zohar Y, Pagelson G, Gothilf Y, Dickhoff WW, Swanson P, Duguay S, Gombotz W, Kost J Langer R (1990a) Controlled release of gonadotropin releasing hormones for manipulation of spawning in farmed fish. In: VHL Lee (ed) Controlled release of bioactive materials. Proceedings of the 17th international symposium, 22–25 July 1990, Reno, Nevada, pp 51–52

Zohar Y, Goren A, Fridkin M, Elhanati E, Koch Y (1990b) Degradation of gonadotropin-releasing hormones in the gilthead seabream, *Sparus aurata*. II. Cleavage of native salmon GnRH, mammalian LHRH, and their analogs in the pituitary, kidney, and liver. Gen Comp Endocr 79:306–319

Zohar Y, Muñoz-Cueto JA, Elizur A, Kah O (2010) Neuroendocrinology of reproduction in teleost fish. Gen Comp Endocr 165:438–455