Aquaculture Research, 2002, 33, 299–309

Effects of temperature and salinity on the reproductive success of Arctic charr, *Salvelinus alpinus* (L.): egg composition, milt characteristics and fry survival

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Abstract

The effects of temperature and salinity on the reproductive success of Arctic charr, *Salvelinus alpinus* (L.), were examined by holding broodstock under the following conditions from mid-May until the end of September: fresh water at ambient temperature (NFW; 8–16 °C); salt water (25–30‰) at ambient temperature (NSW; 4–10 °C); fresh water cooled to saltwater temperature (CFW; 4–10 °C); or salt water heated to freshwater temperature (HSW; 8–16 °C). The relative fecundity of females was similar among groups ($P > 0.05$; 2685 ± 706 eggs), but females reared in NSW produced significantly larger eggs than those raised in NFW. The highest spermatozoa concentrations were found in milt from males reared in SW and the highest milt glucose concentration was from males kept under coldwater conditions (CFW, NSW). Eggs from NSW and HSW females contained more proteins than eggs produced by NFW females. Eggs from NSW females also contained 40% more lipids than was observed in the other groups, and total energy content was 27% higher in eggs from NSW females than in eggs from NFW females. When FW was cooled (CFW), females produced eggs with protein contents similar to those in NSW, but the lipid contents remained 30% lower. Finally, the best survival at the eyed stage and at hatch was observed in families produced by NSW broodstock. Intermediate values were observed in families from NFW or CFW while the highest mortality occurred in families from the HSW group. All these results suggest that, under the experimental conditions used in the present study, coastal seawater conditions offered the most favourable summer rearing conditions with respect to the reproductive success of Arctic charr.

Keywords: Arctic charr, reproduction, salinity, egg, milt, fry survival

Introduction

Problems relating to egg quality are a major constraint in aquaculture development (Brooks, Tyler & Sumpter 1997), and there are numerous factors that may influence reproductive success in teleosts (Bromage, Jones, Randall, Thrush, Davies, Springate, Duston & Barker 1992; Brooks *et al.* 1997). In cultured salmonids, age at maturation, weight, feeding conditions, and energetic status during ovarian development may all influence fecundity, egg size, and the biochemical composition of the eggs at spawning (Watanabe 1985; Tyler & Sumpter 1996; Adams & Huntingford 1997), but the environmental conditions under which the broodstock are held (e.g. temperature, pH, or salinity) may also have significant effects on egg quality (e.g. Brooks *et al.* 1997). For example, exposure of broodstock to environmental stress may affect not only reproduction but also egg and fry survival (Campbell, Pottinger & Sumpter 1992). In Arctic charr, *Salvelinus alpinus* (L.), reproductive success has been shown to be dependent on temperature:

holding broodstock in water warmer than 12 °C during gonadal development will delay ovulation by 3–4 weeks compared with females held at 4 °C (Jobling, Johnsen, Pettersen & Henderson 1995), and ovulation is completely inhibited if females are maintained at 11 °C over the long term (Gillet 1991; Gillet & Breton 1992).

In south-eastern Canada, Arctic charr have been identified as an interesting alternate species for developing aquaculture (Delabbio 1995); however, this development is done in areas naturally occupied by brook charr, *Salvelinus fontinalis* (Mitchill), where summer and early fall freshwater temperatures can be much warmer than 12 °C. On the other hand, temperature conditions found in coastal salt water can be much colder and so represent an interesting option for Arctic charr culture. Although the production of Arctic charr in marine conditions is thought to have great potential, there is little scientific information available to aid the development of marine aquaculture (Heasman & Black 1998). Salinity conditions may influence the reproductive success both in natural and cultured populations of salmonids. In nature, anadromous populations of Arctic charr show later sexual maturation and an increased fecundity compared with non-migrating populations (Tallman, Saurette & Thera 1996). It has also been shown that in some populations, sexually mature animals will not migrate to sea water the year they will reproduce or will be the first ones to migrate back to fresh water if they do migrate (Johnson 1980; Dempson & Green 1985). In captivity, maintaining sexually mature animals in sea water can result in osmotic imbalance, abnormal seminal plasma, inhibition of ovulation or milt production and a decrease in fecundity and egg viability (Arctic charr: Staurnes, Sigholt, Gulseth & Eliassen 1994; other salmonid species: Clarke, Griffioen & Solmie 1977; Morisawa, Hirano & Suzuki 1979; Lundqvist, Borg & Berglund 1989; Haffray, Fostier, Normant, Fauré, Loir, Jalabert, Maisse & Le Gac 1995). On the other hand, the seasonal hypo-osmoregulatory ability of charr (Arnesen, Halvorsen & Nilssen 1992; Staurnes 1993; Schmitz 1995) could offer culture opportunities.

In a preliminary study, Atse (1999) compared rearing in fresh water (FW) and salt water (SW) under natural temperature conditions and showed that raising Arctic charr in sea water during summer can stimulate gonad growth in both males and females as well as improve egg survival in the first developmental stages. However, as natural temperature conditions were different between salt and fresh water, one cannot say if this improvement was due to temperature or salinity effects. The objective of the present study was to discriminate between the effects of these two key environmental factors (salinity and temperature) on the reproductive success of Arctic charr.

Materials and methods

Experiments were conducted with Arctic charr of the Fraser strain (derived from an anadromous population from the Fraser River in Labrador, Nfdl). The fish used in our study were all produced in our laboratory facilities (Station aquicole de Pointe-au-Père, Qué., 48°31′N, 68°29′W) and reared under similar conditions from egg incubation until sexual maturation. Young adult males and females (2+ or 3+) were randomly chosen from stocking tanks for this experiment and will subsequently be referred to as 'broodstock.' From mid-May to the end of September, broodstock fish were held under one of the following experimental conditions (Fig. 1): fresh water at ambient temperature (8–16 °C; NFW); salt water from the St. Lawrence estuary (25–30‰) at ambient temperature (4–10 °C; NSW); fresh water cooled to mimic temperatures of NSW (4–10 °C; CFW); or salt water heated to mimic NFW (8–16 °C; HSW). Fish were held in 500-L tanks at an average density of 35.5 ± 0.8 kg m^{-3}, with one tank per experimental condition. For NSW and HSW groups, saltwater transfer was performed gradually over a 13-day period, with salinity being increased by 2‰ per day. All groups of fish were exposed to natural photoperiod conditions. On August 6, a problem with the aeration system in the NFW tank unfortunately caused the death of 19 fish. The fish were fed an excess ration of 1% of the wet weight per day (Moore-Clarks, Hi-Energy, 9.0 mm pellets; Moore-Clarks, Vancouver, Canada). One hour after feeding, remaining food was removed from the tanks. Fish displayed strongly reduced appetites starting in mid-August and eventually ceased feeding; at this time, feeding was reduced to twice a week until spawning. At the end of September, NSW and HSW groups were gradually returned to fresh water (salinity decreased by 2‰ per day). For the 4 weeks preceding the beginning of spawning, all fish were held in fresh water under ambient temperature conditions (9.5 °C).

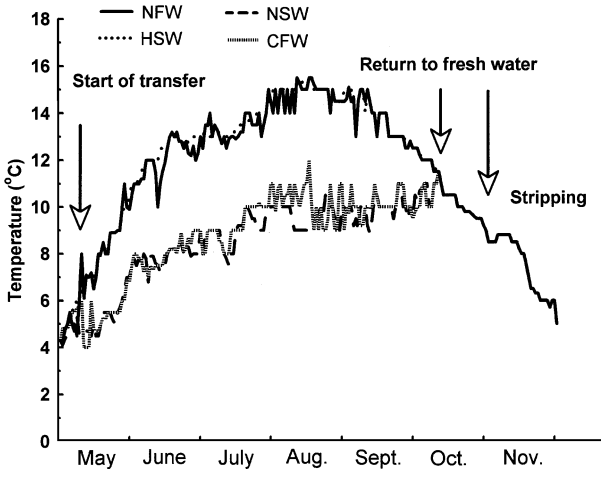

Figure 1 Temperature condition during the study. The experimental period lasted from mid-May to the end of September after which all fish were maintained in fresh water until spawning. NFW: fresh water, natural temperature conditions; NSW: salt water, natural temperature conditions; CFW: fresh water cooled to mimic salt water temperature; HSW: salt water heated to mimic freshwater temperature.

Starting in mid-October, the evolution of sexual maturation was checked every week (presence of milt in males and ovulation in females). Females were removed from each tank to verify abdominal hardness and development of the genital pore. When sub-samplings indicated that sexual maturation may have been attained by some of the females, all females were anaesthetized (MS 222, 0.1 g L^{-1} neutralized with NaOH), weighed and measured (total length), and stripped if ovulation had occurred. This was repeated every week until the last experimental female had been stripped. Ovulation occurred from November 4 (9.5 °C) to December 2 (6 °C). For each female, the number of eggs was counted using the Von Bayer method (M.L. C.P.Q. 1983) and the relative fecundity index (RFI; number of eggs kg^{-1} whole body weight) was calculated. Egg diameter (20 eggs per female) was measured to the nearest 0.1 mm. An average egg diameter was calculated for each female so only one data point per female was used in the statistical analyses (n = number of females). Males used for fertilization were randomly sampled from among the sexually mature males in the same experimental group as the stripped females. Each male was used only twice and then identified (fingerling tags inserted below the dorsal fin) so it was not used again. Males were weighed, measured, and carefully rinsed (to avoid mixing MS 222 and milt); excess milt was sampled (two aliquots) just after egg fertilization, and males were returned to their tanks. A first milt aliquot (100 μL) was used to measure sperm concentration. After diluting 25 μL of milt in 0.8% NaCl (1:2500 ratio), the number of spermatozoids per mL of milt was calculated based on triplicate counts using a hematocytometer (chamber volume 0.00025 mm^3) at 250× magnification. The second aliquot (the remaining milt) was centrifuged for 10 min at 500 g and the seminal fluid stored at −80 °C until further analysis. Seminal fluid osmolality was measured with an osmometer (Advanced™ Micro-Osmometer Model 3MO plus [Advanced Instruments, Inc., Needham Heights, Massachusetts], 25 μL per assay); glucose concentration was measured by spectrophotometry using a hexokinase enzymatic method (Sigma, 16 UV, 10 μL per assay; Sigma-Aldrich Canada, Oakville, Ontario); sodium concentration was measured by atomic absorption spectrophotometry (Perkin-Elmer, model 460, 10 μL per assay; Perkin-Elmer, Montreal Canada); and chloride concentration was measured with a chloridometer (Chloride Analyzer 925, Corning, 20 μL per assay; Ciba-Corning, Mississauga, Ontario).

Eggs from each female were divided in two groups to be fertilized by two different males that had been held in the same experimental conditions. These two groups of eggs were thereafter considered as two different families. Eggs from each family were incubated separately. Eggs were counted, but only 600–700 eggs $family^{-1}$ were kept for incubation in order to have similar densities between incubators. Eggs were incubated in darkness and at 4.5 °C until 100% hatching. The water temperature was then gradually raised to 8 °C (0.5 °C per day) until first feeding. During egg incubation, dead eggs were removed and counted twice a week. The number of eggs reaching 24-h post-fertilization, the eyed stage, and hatching, and the number of fry surviving until first feeding were counted. The duration of development to the different stages is expressed

in degree-days and was estimated to be the time when 50% of individuals had reached the specific stage.

Eighteen eggs per female at stripping (non-fertilized eggs), 18 fertilized eggs, and 18 fry per family at the eyed stage, at hatching, and at first feeding were randomly sampled and stored at -80 °C prior to analyses for lipid and protein contents. The number of sampled eggs was subtracted from total egg counts so that survival measurements were not biased. Total lipid and protein contents were measured on sub-samples from each female and each family (three different subsamples, each consisting of a pool of three eggs or three fry). An average value was calculated for each family, giving one data point per family for statistical analyses (n = number of families). Total lipids were determined using the method of Frings, Sendley, Dunn & Queen (1972) and proteins following the Biuret method described by Boyer (1993) with bovine serum albumin as a standard. Lipids and proteins are expressed as mg egg^{-1} or mg fry^{-1}. The energy content (joules egg^{-1} or joules fry^{-1}) was calculated by summing the energy in proteins and lipids (Crisp 1984; Smith 1989).

Data are expressed as means $\pm$ standard deviations. Normality and homogeneity of variances were checked by Kolmogorov–Smirnov and F_{max} tests ($\alpha = 0.05$) respectively. Data on weight had to be log-transformed prior to statistical analysis to obtain normality. Data on per cent mortality at 24-h post-fertilization were transformed as arcsine$\sqrt{(x)}$ and those on egg lipid content as (x^2) to obtain homogeneity of variances. Salinity and temperature regimes used for the broodstocks' summer rearing conditions were used as factors to analyse data (two-way ANOVA). When the ANOVA indicated significant factor effects and/or interactions between factors, an appropriate a posteriori analysis was performed (one-way ANOVA and/or a posteriori Tukey's test of comparison of means). For those parameters for which transformations failed to give homogeneity of variances, the Games and Howell test for comparisons of means, designed for heterogeneous variances, was applied (Sokal & Rohlf 1995). All analyses were performed with Statistica software.

Results

At the beginning of the experiments, the average weights, lengths, and condition factors of fish in the experimental tanks were similar ($P > 0.05$; Table 1). The same was true for fish that reached sexual maturation (Table 1), and neither temperature nor salinity had a significant effect on the overall condition of males or females ($P > 0.05$). Ovulation occurred from November 4 and lasted through December 2. In the NFW group, the first ovulation occurred 2 weeks later than in the three other groups. No delay in milt production was observed in any of the four experimental groups; all males reached maturation in mid-October, 2 weeks before the females.

The relative fecundity of females was similar among groups ($P > 0.05$; 2685 ± 706 eggs). Females reared in NSW produced bigger eggs ($P < 0.05$) than those raised in NFW; the other two groups showed intermediate values (Fig. 2). In males, salinity was the only factor that showed a significant effect on milt quality (Table 2), and milt from males reared in SW during summer (NSW, HSW) contained 43% more spermatozoids than those kept in FW (NFW, CWF). Seminal fluid osmolality was also significantly higher in males raised in salt water (Table 2). While thermal conditions had a significant effect on seminal glucose, salinity did not (Table 2): males reared in cold water (NSW, CFW) had average seminal fluid glucose concentrations almost twice those observed in males kept under warmer water conditions during summer (NFW, HSW).

Twenty-four hours after fertilization, we observed a mortality three times higher in eggs produced by broodstock fish kept at cold temperatures (NSW, CFW; Table 3). However, this tendency was reversed in the later stages of development. The best survival at the eyed stage (Fig. 3a) and at hatch (Fig. 3b) was observed in families produced by NSW broodstock. At first feeding, the broodstock's

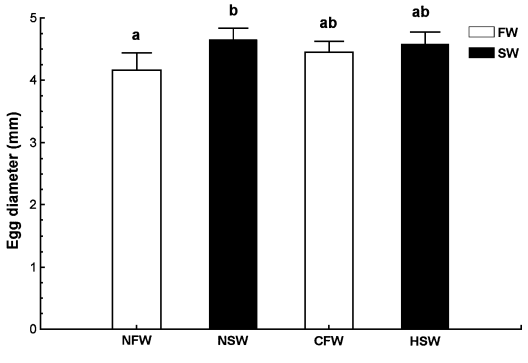

Figure 2 Egg diameter in females held in different environmental conditions.

Table 1 Length, weight, and condition of Arctic charr, *Salvelinus alpinus* (L.), at the beginning of summer and at spawning time (before stripping)

Group	*n*	Sex	Length (cm)	Weight (kg)	K
At group formation (mid-May)					
NFW	28	ND	36.9 ± 5.8	0.59 ± 0.4	1.1 ± 0.3
CFW	30	ND	37.4 ± 4.8	0.64 ± 0.3	1.2 ± 0.3
NSW	28	ND	38.1 ± 6.0	0.64 ± 0.4	1.0 ± 0.4
HSW	27	ND	39.0 ± 6.5	0.71 ± 0.4	1.1 + 0.3
At stripping					
NFW	5	F	41.7 ± 2.6	0.8 ± 0.3	1.1 ± 0.1
	4	M	44.5 ± 3.5	1.0 ± 0.3	1.1 ± 0.2
CFW	9	F	44.2 ± 4.0	1.2 ± 0.4	1.3 ± 0.2
	17	M	42.8 ± 5.7	1.0 ± 0.4	1.2 ± 0.1
NSW	9	F	44.4 ± 4.4	1.1 ± 0.4	1.2 ± 0.2
	12	M	42.8 ± 6.1	1.0 ± 0.5	1.2 ± 0.2
HSW	8	F	44.2 ± 2.8	1.1 ± 0.2	1.2 ± 0.1
	8	M	46.5 ± 6.2	1.2 ± 0.4	1.2 ± 0.1

The animals were subjected to one of four different summer rearing environments: NFW: fresh water, natural seasonal temperature variations; CFW: fresh water cooled to mimic seawater temperature variations; NSW: sea water, natural seasonal temperature variations; HSW: sea water, heated to mimic summer fresh water temperature variations. K: condition factor. Values are presented as mean ± SD; *n* ndicates the number of individuals.

Table 2 Sperm counts, osmolality, and glucose concentration of seminal fluid

Group	Sperm concentration ($\times 10^9$ spzm L^{-1})	Osmolality (mosmol L^{-1})	Glucose concentration (mmol L^{-1})
NFW	9.4 ± 4.9	251.2 ± 27.5	2.9 ± 3.7
CFW	9.5 ± 4.8	252.6 ± 23.2	4.7 ± 1.4
NSW	13.3 ± 4.4	276.1 ± 12.2	6.7 ± 2.9
HSW	14.0 ± 3.7	266.9 ± 16.9	3.2 ± 1.6
ANOVA			
A: Temperature	NS	NS	$P = 0.005$
B: Salinity	$P = 0.0135$	$P = 0.0357$	NS
Interaction AXB	NS	NS	NS

Mean ± SD. NFW: fresh water, natural seasonal temperature variations; CFW: fresh water cooled to mimic seawater temperature variations; NSW: sea water, natural seasonal temperature variations; HSW: sea water, heated to mimic summer freshwater temperature variations; NS: not significant.

summer rearing temperature had a significant effect on fry survival: families produced by broodstock raised at colder temperatures (NSW, CFW) had a fry survival rate that was five times higher than families produced by fish kept in warmer waters (NFW, HSW; Table 3).

The parental group had a significant effect on the length of time required for egg development. Eggs from NSW broodstock reached the eyed stage more rapidly than those produced by broodstock kept in CFW or HSW (Fig. 4a); they also hatched before those produced by NFW broodstock (Fig. 4b). First feeding was reached after 627 ± 13 degree-days in all families.

During the incubation period, eggs reached their maximal weight at the eyed stage. Broodstock raised in salt water produced heavier eggs than those raised in fresh water (Table 4) irrespective of temperature conditions ($P > 0.05$) and this difference was maintained throughout embryonic development. On the other hand, temperature conditions had a significant effect on the type of energy reserves

Table 3 Egg mortality 24 h after fertilization and fry survival at first feeding

Broodstock	Egg mortality 24 h after fertilization (%)	Survival at first feeding (%)
NFW	2.1 ± 1.5	6.6 ± 7.6
CFW	8.1 ± 8.1	24.7 ± 20.6
NSW	6.6 ± 8.1	47.9 ± 23.2
HSW	1.8 ± 2.2	6.9 ± 9.8
ANOVA		
A: Temperature	$P = 0.017$	$P < 0.0001$
B: Salinity	NS	NS
Interaction AXB	NS	NS

Mean ± SD. NFW: fresh water, natural seasonal temperature variations; CFW: fresh water cooled to mimic seawater temperature variations; NSW: sea water, natural seasonal temperature variations; HSW: sea water, heated to mimic summer freshwater temperature variations; NS: not significant.

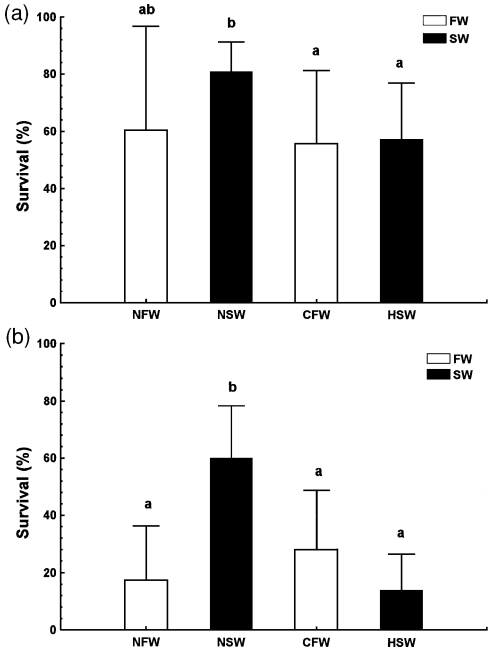

Figure 3 Egg survival at the eyed stage (a) and at hatch (b). All eggs were incubated under similar temperature conditions although the parents were held under different environmental conditions during gonadal development.

present in eggs at spawning. Eggs from NSW and HSW females contained more proteins than eggs produced by NFW females. Eggs from NSW females also contained 40% more lipids than what was observed in the other groups ($P < 0.05$). Finally, total energy content was 27% higher in eggs from NSW

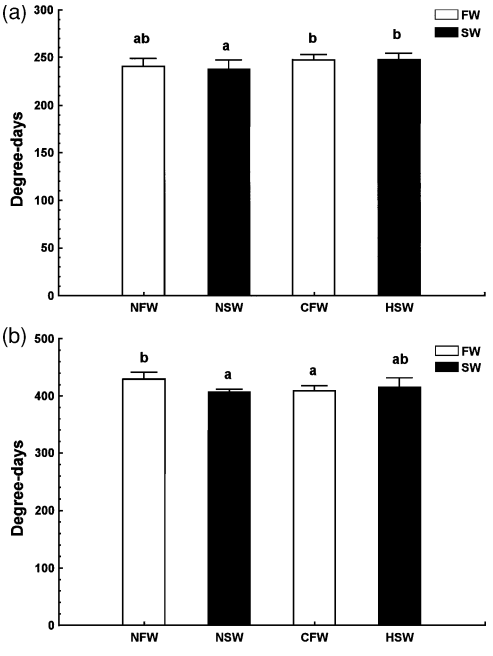

Figure 4 Time of development (degree-days) to reach the eyed stage (a) and hatching (b). All eggs were incubated under similar temperature conditions although the parents were held under different environmental conditions during gonadal development.

females than in eggs from NFW females (Table 5). When FW was cooled (CFW), females produced eggs with protein contents similar to those in NSW, but the lipid contents remained 30% lower. HSW females produced eggs with lipid and energy contents similar to those of NFW females but with higher protein content (Table 5). All these differences related to parental groups were no longer present at the eyed stage ($P > 0.05$).

Energy reserves were used differently among families (Table 5). Proteins continuously decreased from spawning to first feeding in progeny of the CFW and HSW fish. In progeny of the NFW broodstock, decreased protein levels were only observed at hatch while the protein content of eggs decreased from spawning to hatch in progeny of NSW charr. In NSW progeny, the lipid content gradually decreased throughout embryonic development while lipids seemed to have been used only from the eyed stage to first feeding in progeny from the three other groups. The energetic reserves in eggs from NSW, HSW, and CFW decreased from spawning to first feeding; this decrease was also observed but less rapid in eggs from NFW families.

Table 4 Egg and fry wet weight (mg) as a function of the broodstock's summer rearing conditions

	Egg and fry wet weight (mg)			
Broodstock	**Non-fertilized eggs[a]**	**Eyed stage[c]**	**Hatch[b]**	**First feeding[b]**
NFW	42.7 ± 4.2	62.3 ± 4.1	58.3 ± 7.2	58.7 ± 4.7
CFW	46.1 ± 6.4	63.0 ± 6.0	61.7 ± 6.6	54.7 ± 5.8
NSW	52.4 ± 5.4	69.6 ± 8.1	64.7 ± 10.4	61.0 ± 4.5
HSW	52.6 ± 8.5	67.0 ± 8.3	59.4 ± 6.9	63.3 ± 3.3
ANOVA				
A: Temperature	B: Salinity	C: Stage	A × B × C	
NS	$P < 0.001$	$P < 0.001$	NS	

Mean ± SD. NFW: fresh water, natural seasonal temperature variations; CFW: fresh water cooled to mimic seawater temperature variations; NSW: sea water, natural seasonal temperature variations; HSW: sea water, heated to mimic summer freshwater temperature variations; NS: not significant. Different letters indicate significant differences among developmental stages.

Discussion

For the Fraser Arctic charr strain and under the specific environmental conditions used in the present study, NSW was the most favourable environment to ensure reproductive success both in males and females. The temperature regime of coastal water is not the only factor responsible since salinity also seems to play an important role: the manipulation of temperature conditions alone either in fresh or in salt water very often produced intermediate results.

In salmonids, bigger eggs are considered to produce bigger fry that can survive longer without external feeding (Hayashizaki, Hirohashi & Ida 1995). However, a previous study done by Srivastava & Brown (1991) showed that energy content of eggs is a better predictor for growth and survival of fry than egg weight or egg diameter in Atlantic salmon, *Salmo salar* (L). Roche-Mayzaud, Mayzaud & Audet (1998) also showed that smaller eggs with higher lipid contents can ensure better fry survival in brook charr. Combining saline and cold water conditions (NSW) led to higher viability of both eggs and fry that was associated with higher lipid and energy contents in non-fertilized eggs. These effects seem partly related to salinity water conditions as NSW and HSW females both produced larger eggs with similar protein content. However, HSW females produced eggs with lower lipid content indicating that warm conditions partly reduced the benefits of seawater rearing.

In salmonids, several biotic factors have significant effects on egg size, including female length (Thorpe, Miles & Keay 1984; Wootton 1992), female age, and feeding regime during gonadal development (Billard 1992; Morita, Yamamoto, Takashima, Matsuishi, Kanno & Nishimura 1999). Also, egg size, egg energy content, and viability were shown to be largely dependent on the females' nutritional status (e.g. Brooks *et al.* 1997). In the present study, the length and age of females were similar among groups. Also, daily rations were larger than the quantity eaten (excess food was removed after 1 h), so in theory all fish had adequate food. Thus, environmental conditions can be assumed to play a major role in the differences observed among groups.

Temperature and salinity conditions for broodstock rearing have already been shown to have significant effects on egg size and egg biochemistry. In accordance to the present study, Roche-Mayzaud *et al.* (1998) showed that total lipid content was 20% higher in the eggs from brook charr breeders held in sea water during the summer than in those produced by parental groups always kept in fresh water. Blaxter (1988) showed that high water temperature results in a decrease in the diameter of teleost eggs in wild conditions, which is similar to the temperature effects observed on egg diameter in our own study. Jobling *et al.* (1995) showed that rearing Arctic charr females under constant warm temperature conditions between mid-June and the end of September did not decrease the lipid content in the eggs but changed the proportions of phospholipids and triacylglycerols. They suggested that this could have been related to the 3–4 week delay in ovulation and a decrease in phospholipid synthesis and deposition in the oocytes. In other teleost

Table 5 Proteins, lipids, and energy content of eggs and fry

		Developmental stage			
	Broodstock	**Non-fertilized eggs**	**Eyed stage**	**Hatch**	**First feeding**
Proteins	NFW	$^{a}9.2 \pm 1.2^{a}$ (5)	7.8 ± 0.9^{a} (10)	4.7 ± 1.1^{b} (10)	5.6 ± 1.3^{b} (7)
(mg egg^{-1})	CFW	$^{ab}11.1 \pm 1.8^{a}$ (11)	8.8 ± 1.0^{b} (18)	6.9 ± 2.1^{c} (18)	4.4 ± 1.3^{d} (14)
or					
(mg fry^{-1})	NSW	$^{b}11.6 \pm 1.4^{a}$ (10)	8.7 ± 0.9^{b} (18)	6.2 ± 1.0^{c} (18)	5.2 ± 1.6^{c} (15)
	HSW	$^{b}11.5 \pm 0.9^{a}$ (10)	8.4 ± 0.9^{b} (16)	6.5 ± 1.4^{c} (16)	3.4 ± 1.3^{d} (10)
Lipids	NFW	$^{a}2.4 \pm 0.5^{a}$ (5)	2.1 ± 0.3^{a} (10)	1.5 ± 0.2^{b} (10)	0.4 ± 0.2^{c} (7)
(mg egg^{-1})	CFW	$^{a}2.3 \pm 0.4^{a}$ (11)	2.1 ± 0.3^{a} (18)	1.8 ± 0.3^{b} (18)	0.8 ± 0.3^{c} (14)
or					
(mg fry^{-1})	NSW	$^{b}3.3 \pm 0.6^{a}$ (10)	2.2 ± 0.3^{b} (18)	2.0 ± 0.5^{b} (18)	0.9 ± 0.5^{c} (15)
	HSW	$^{a}2.4 \pm 0.4^{a}$ (10)	2.2 ± 0.4^{a} (16)	1.6 ± 0.3^{b} (16)	0.7 ± 0.1^{c} (10)
Energy	NFW	$^{a}311 \pm 28^{a}$ (5)	266 ± 24^{a} (10)	170 ± 29^{b} (10)	148 ± 34^{b} (7)
Content					
(j egg^{-1})	CFW	$^{a}350 \pm 45^{a}$ (11)	292 ± 31^{b} (18)	235 ± 49^{c} (18)	134 ± 38^{d} (14)
or					
(j fry^{-1})	NSW	$^{b}403 \pm 41^{a}$ (10)	294 ± 25^{b} (18)	224 ± 28^{c} (18)	160 ± 36^{d} (15)
	HSW	$^{ab}366 \pm 31^{a}$ (10)	284 ± 27^{b} (16)	215 ± 37^{c} (16)	108 ± 34^{d} (10)

The ANOVAs indicated significant interactions between factors, and only the results of a posteriori tests are shown. Different letters on the left indicate significant differences among parental groups in non-fertilized eggs. Different letters on the right indicate significant differences among stages within families. NFW: fresh water, natural seasonal temperature variations; CFW: fresh water cooled to mimic seawater temperature variations; NSW: seawater, natural seasonal temperature variations; HSW: sea water, heated to mimic summer freshwater temperature variations.

species, an increase in the rearing temperature may decrease vitellin deposition and lipid metabolism in females, resulting in a decrease in active vitellogenesis (Cossins & Bowler 1987; Sargent, Henderson & Tocher 1989). Because the ovulation period was similar among our four experimental groups, a delay in ovulation could hardly explain the differences observed among experimental groups, in the energetic content of non-fertilized eggs. However, a temperature effect on active vitellogenesis cannot be dismissed.

NSW was also the best environment to ensure male reproductive success. Salinity was a key factor since males kept in salt water showed higher sperm counts and higher osmolality. However, cold temperature rearing was associated with higher glucose concentrations. Seminal glucose is an important energy source for sperm motility (Piironen & Hyvärinen 1983). The combination of cold and salt water in our experiment produced milt with improved physico-chemical characteristics. Results from other studies on the physico-chemical characteristics of sperm in salmonid fishes are contradictory. Seawater rearing of coho salmon, *Oncorhynchus kisutch* (Walbaum), had no effect on sperm (Clarke *et al.* 1977). In Atlantic salmon, raising males in sea water during summer resulted in a drop in fertilisation success even though the males were returned to fresh water 2–6 months before spawning (Haffray *et al.* 1995). In the present study, salinity was associated with an increase in seminal fluid osmolality. In Atlantic salmon, Haffray *et al.* (1995) were not able to demonstrate a relationship between seminal plasma osmolality and low fertility. On the other hand, elevated sperm motility and fertilisation rate were correlated to high seminal plasma Na^{+} and

Cl^- concentrations in rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Munkittrick & Moccia 1987), and Atlantic salmon (Aas, Refstie & Gjerde 1991).

An improvement in gamete quality is best expressed in terms of fry survival and, as may be expected from the earlier discussion on eggs and sperm characteristics, fry from broodstock raised in NSW showed the highest survival. However, fry survival dropped dramatically in families produced by NFW or HSW broodstock. Again, cooler temperatures found in salt water cannot be the only factor involved because cooling fresh water produced intermediate results. However, even in the NSW group, egg survival was low (50% on average), indicating that we still have to improve broodstock rearing conditions. In the Fraser strain, the percentage of egg survival has been found to be low (De March 1995). Further work is needed to determine if this is related to genetic factors or to broodstock particular requirements.

Egg mortality at the first stages of development may be related to egg retention in the abdominal cavity or to poor fertilization success (Billard & Breton 1977; Papst & Hopky 1984). Mortality related to gamete ripening occurs during the first steps of embryonic cleavage (Papst & Hopky 1984). Our mortality results at 24-h post-fertilization indicate higher mortality in eggs produced by broodstock reared in cold water, so it may be suggested that oocyte maturation was faster in cold water. Egg retention in the abdominal cavity would be one of the main factors determining the ultimate egg quality in salmonids (Papst & Hopky 1984; Springate, Bromage, Elliott & Hudson 1984; Gillet 1991; Bromage *et al*. 1992; Gillet & Breton 1992). Because ovulation progress was surveyed weekly for each female we do not think that egg retention was a major problem here. Our results showed less than 10% mortality in the first 24 h after fertilization and that families with the highest mortality at 24 h were the same ones that showed the best percentage survival both at the eyed stage and at hatch. These results support the idea that short-term mortality was not linked to poor quality overmature eggs. Egg survival at the eyed stage has been used to assess per cent fertilization or spermatozoids quality (Levanduski & Cloud 1988; Aas *et al*. 1991; Haffray *et al*. 1995). In the present work, best survival at the eyed stage was observed in eggs produced by NSW broodstock.

The rearing environment of male and female broodstock had consequences on embryonic development even though incubation and fry rearing conditions were identical. Lipid and protein use was delayed until the eyed stage in embryos produced by broodstock previously raised in NFW while eggs from NSW broodstock used these reserves starting from fertilization. Eggs produced by HSW and CFW broodstock mainly used proteins until they reached the eyed stage after which all energy sources were used until exogenous feeding. These patterns of energy source utilization could explain the differences in survival at the different developmental stages. Eggs from NSW breeders contained 40% more lipids and were the only ones to use a significant portion of them during the first stage of development. This strategy seems to be efficient as these eggs showed the best survival at this stage. However, eggs from NFW broodstock used less lipid and protein reserves during first development stages. The results obtained suggest that the use of lipids or proteins during embryonic development is linked to their initial concentrations at spawning. Moreover, there seems to be a link between the pattern of lipid or protein utilization and the duration of embryonic development. When proteins and lipids are used simultaneously at the early stage, as in NSW, embryonic development is shorter.

We conclude that gamete quality and reproductive success are influenced both by the temperature and the salinity of the broodstocks' summer rearing environment and that in geographical areas like ours, where summer fresh water could be inappropriate for Arctic charr reproduction, coastal water can provide adequate rearing conditions to ensure the production of high quality gametes. Our results also indicate that egg energy content is a better indicator than egg diameter of improved development, growth, and survival of eggs and fry.

Acknowledgments

We thank Ms Nathalie Morin and Ms Agnès Thomas-Collignon for helpful technical assistance. This study was financed by a CORPAQ-3525 grant.

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