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PHYSIOLOGICAL CHANGES IN BLOOD AND SEMINAL PLASMA DURING THE SPAWNING PERIOD OF MATURING RAINBOW TROUT HELD UNDER DIFFERENT TEMPERATURE AND SALINITY REGIMES, AND THE EFFECT ON SURVIVAL OF THE BROODSTOCK AND THE EYED EGGS.

by

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ABSTRACT

Maturing rainbow trouts reared in sea cages were transferred to tanks and kept under nine different temperature and salinity regimes. Blood and seminal fluids were regularly sampled from the fish, and different organic and inorganic components were measured. The survivel of both the broodstock and the eyed eggs were recorded. Dry matter of the eggs were measured as well.

Sexual differences were noted according to the progressive changes of glucose, chloride, haematocrit and the survivel of the broodstock. Cold temperatures delayed the sexual maturation independent of salinity, and only few females spawned at these temperatures. The broodstock reared at cold temperatures in freshwater and sea water suffered from high mortality and high levels of blood chloride or blood glucose. Brackish water appeared to be the most favourable environment, especially for the broodstock reared at the warmer temperatures. The survivel of the broodstock and also of eggs from females stripped at this salinity was high. Analysis of the physiological components were more stable compared to fish reared at the other salinities.

INTRODUCTION

Sexual maturation in fish and development of gonads involve major physiological changes. Experiments have shown that the environment in which the broodstock is reared, is of great importance to the survival of both the mature fish and the eggs.

Craik and Harway (1986) reported a higher mortality of incubated eggs when the broodstock of Atlantic salmon was held in sea lochs until the time of stripping, compared to exposure to freshwater for one month before stripping. At Matre Aquaculture Station it was found that the mortality of the Atlantic salmon broodstock was lower in both brackish and fresh water compared to seawater (Ulgenes et al., 1984). The mortality of the eggs until the eyed stage was also lower when stripped from brackish water and fresh water.

According to Billard et al. (1981) salmonid gamete physiology is affected by temperature. He reported that the fertilization rate decreased slightly when artifical insemination was carried out near 0°C. Saunders et al. (1983) studied the effect of marine water temperature on the timing of sexual maturation in Atlantic salmon reared in sea cages. Measured in degree-days, salmons exposed to low winter temperatures (1-3°C) was adversly affected by a delayed sexual maturation.

The purpose of this experiment was to investigate the physiological changes of blood and seminal fluids when the broodfish was exposed to different temperature and salinity regimes. The effects on the survival of the mature rainbow trouts and the time of spawning were investigated. Also, the survival of the eggs until the eyed stage, and some egg quality criteria were investigated.

MATERIALS AND METHODS

Experimental design

Maturing, female rainbow trouts reared in netpens at seawater, were transported from Austevoll to Matre in the middle of December 1985. They were acclimated to brackish water (ca.15 ‰) in tanks of 3 m in diameter. In the first week of January mature rainbow trouts of both sexes reared in netpens at Matre, were transferred from brackish water to the same kind of tanks. At this time the broodstock was splitted into 9 groups through a combination of three different salinities and three different temperatures as followes:

Fresh water	(FW)	1. Cold -	2. Medium -	3. Warm temperature
Brackish water	(BW)	4. "	5. "	6. "
Sea water	(SW)	7. "	8. "	9. "

Warm FW was obtained from the cooling system at the BKK hydroelectric powerplant in Matredal, and cold FW was obtained from the Matre river. In tank 2 a combination of these two was used. Warm seawater was taken from the Matre Fjord and temperatures in tank 7 and 8 adjusted by use of a heat exchanger. The brackish water (tank 4-6) was obtained by a combination of FW and SW in a mixing battery and temperatures adjusted by the heat exchanger.

6 - 7 males and 13 - 14 females, weighing from 1 to 6 kg, were placed in each tank and individually labeled by Floy Anchors (Flo tags & Manufacturing, Seattle). The fish were adapted to their new water qualities within a weeek.

During the first three months the salinities in FW, BW and SW were 0, 13-17 and 28-30 ‰ respectivly. Snow melting later in the spring (April to June), resulted in dilution of the SW and led to a decrease in salinity in BW and SW which were now 10-13 and 23-26 ‰ respectivly. Fig.1 illustrates the changes in salinity in BW and SW during the experimental period. Because of small variances the data from each salinity is presented as means of the three temperature regimes.

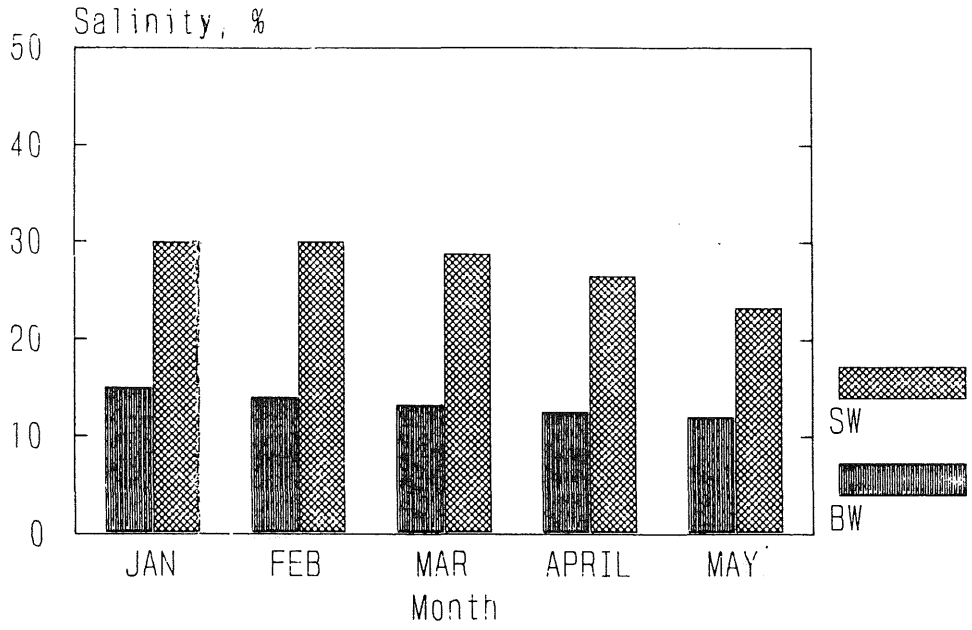

Fig.1 Salinity at monthly intervals in the spawning period.

The climate this spring was extremly cold. Due to clogging of the heat exchanger by mud and algae the efficiency of the heat exchanger periodically decreased which resulted in an unintended increase in the cold water temperature. Generally, the temperatures were higher in BW and SW compared to FW. The changes of temperature are illustrated in FW, BW and SW in each individually tank (Fig. 2-4).

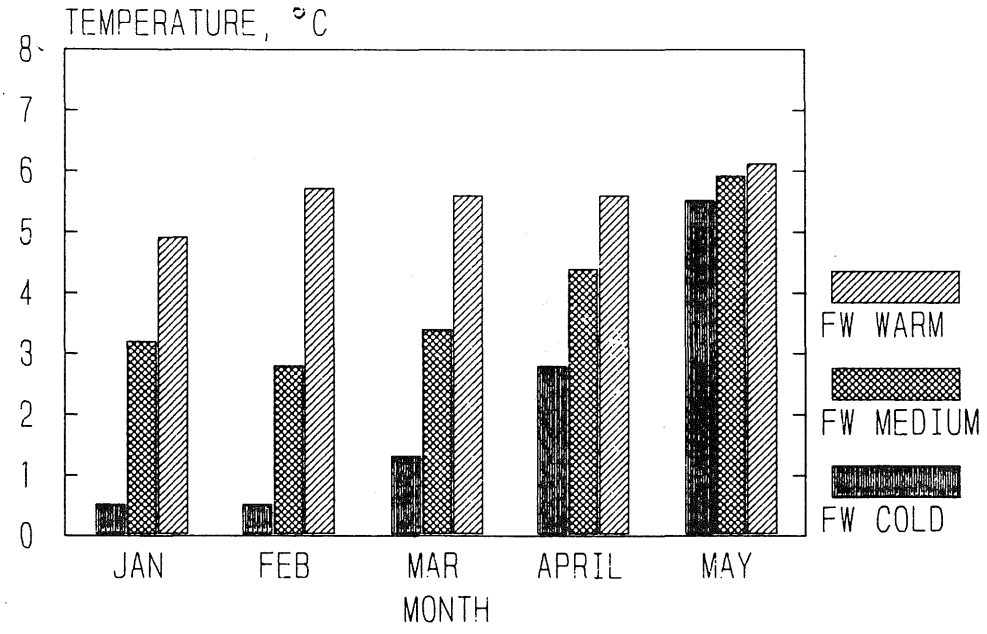

Fig.2 Temperature changes in FW at each of the three temperature regimes in the investigation period.

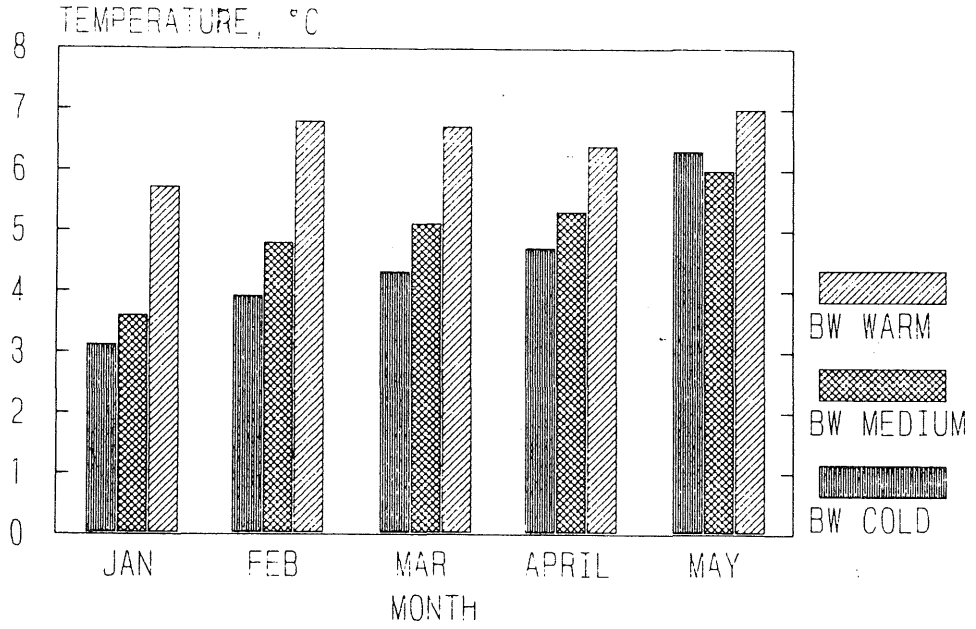

Fig.3. Temperature changes in BW at each of the three temperature regimes in the investigation period.

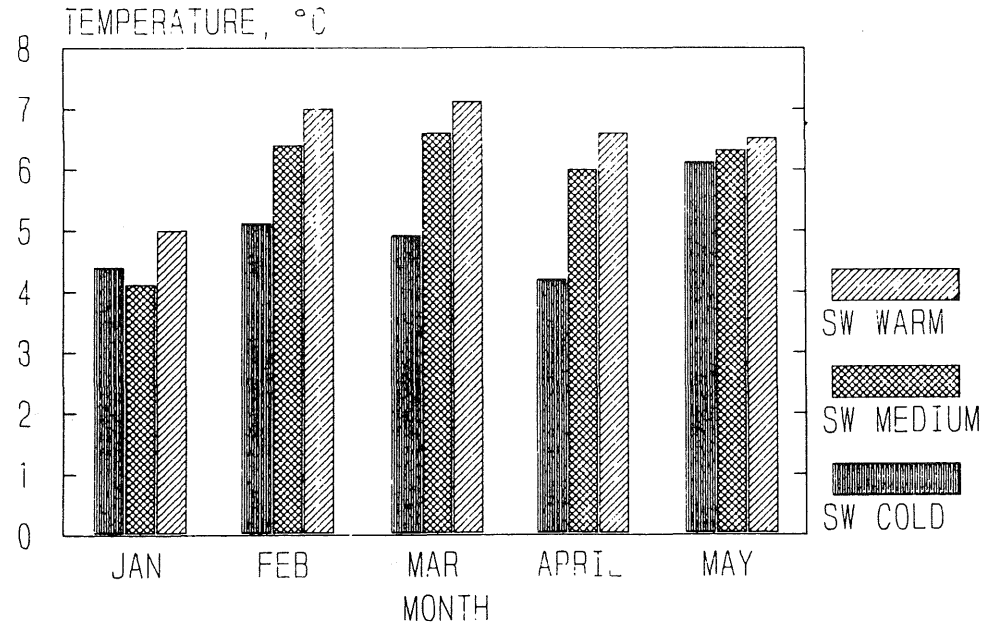

Fig.4. Temperature changes in SW at each of the three temperature regimes in the investigation period.

Sampling and chemical analysis

Every second week from the middle of January, 2 - 5 ml blood was withdrawn from all the fishes from the duct of Cuvier (Lied et al., 1975). The sampled fish were anaesthetized with benzocaine prior til handling (Wedemeyer, 1971). Semen (4-5 ml) and coelomic fluid was collected, and blood plasma and the seminal fluids treated as described by Albrektsen et al. (1986). The survival of fish, number of spawners and time of spawning were recorded for every water quality.

Eggs were collected by hand stripping, and the broodfish from different environments were crossbred. The eggs were incubated in small hatching trays of 10 * 10 cm^2 with a salinity of 7 - 8 ‰ , and a water input of 2 L/min. The fertilized eggs were measured by shocking at the eyed stage, and dead eggs were removed. Dry matter in the egg were measured at the time of spawning.

Haematocrits were measured according to the method of Wintrobe (1974). Glucose was determined enzymatically in blood plasma with Sigma test kit (Glucose UV-test, hexokinase method). Chloride consentrations in blood and coelomic fluid were analysed by use of Radiometer CMT 10 coulometric chloride titrator.

Statistics

ANOVA oneway-analysis of variance and Student's t-test were carried out using a BMDP statistical computer program.

RESULTS

Broodstock survival

Mortality was highest (100 %) among males reared in SW, and the mortality was independent of temperature (Fig. 5). The survival of females reared at the same salinity was temperature dependent with the highest mortality (80 %) at the coldest temperature (Fig.6). In FW the total mortality of the broodstock was high (80-90 %) and independent of sex at the coldest and the warmest temperature (Fig. 7-8). For both sexes death occurred earlier at the coldest temperature. Only 43 % of the females died in FW of medium temperature. The unexpected death in May of 8 females reared in FW of warm temperature might be explained by a sudden change in the environment. At the two coldest temperatures in BW only 15 % of the fish died. No mortality occurred among fish held in warm brackish water.

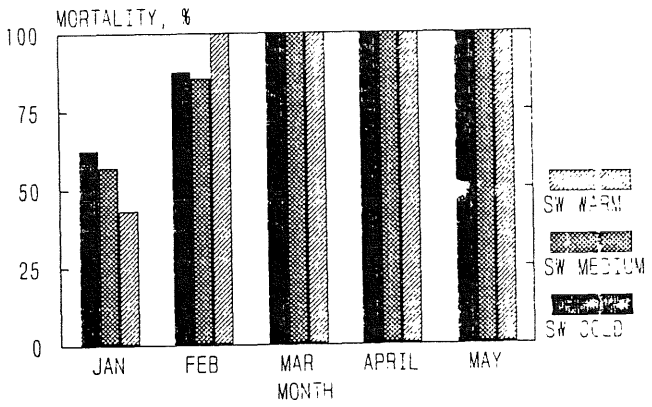

Fig.5 Mortality (%) of males reared in SW.

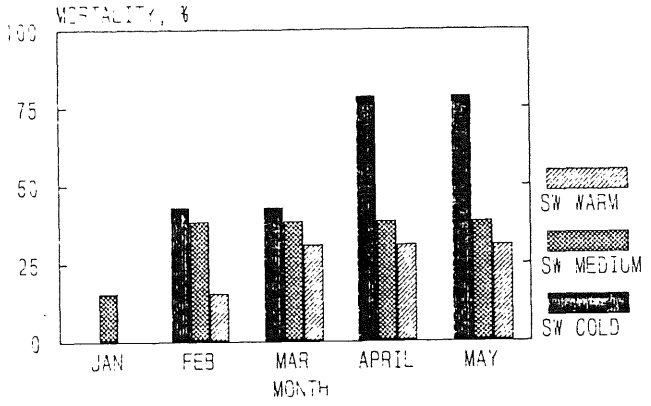

Fig.6 Mortality (%) of females reared in SW.

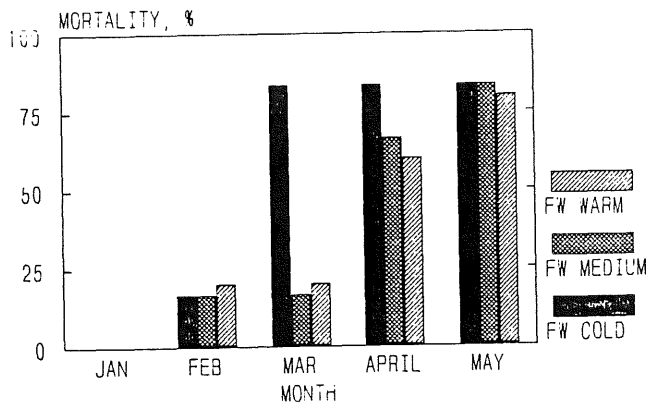

Fig.7 Mortality (%) of males reared in FW.

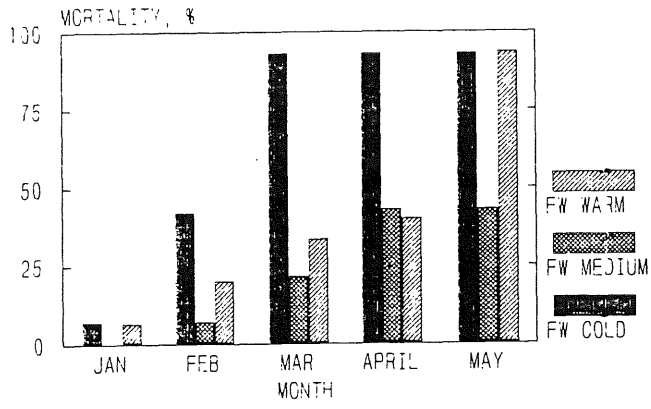

Fig.8 Mortality (%) of females reared in FW.

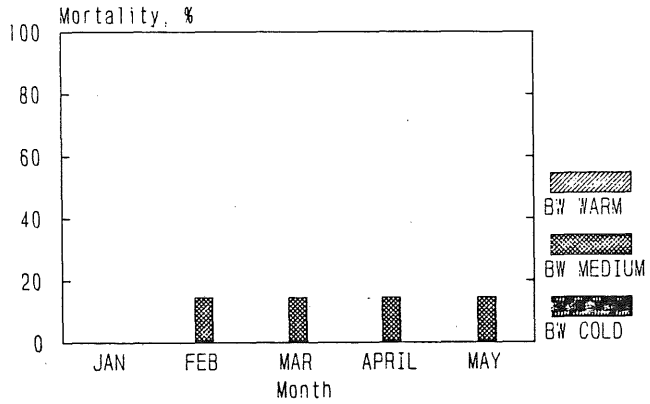

Fig.9 Mortality (%) of males reared in BW.

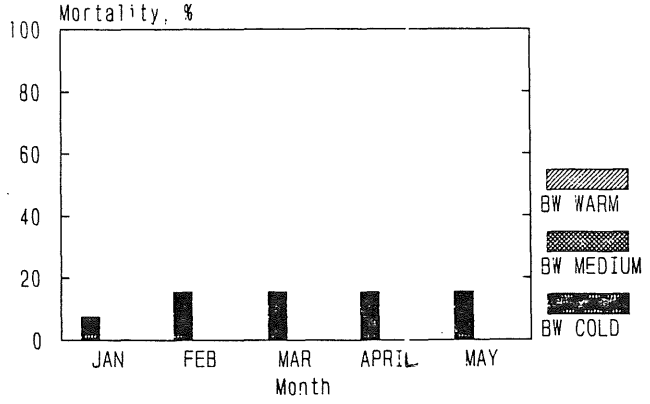

Fig.10 Mortality (%) of females reared in BW.

Haematocrit

The haematocrit values of males were not significantly different between temperatures at the same salinity ($p > 0.05$). Haematocrit values are presented at each salinity as means (% RBC) of the three temperatures (Table 1). SEM and n are given at each salinity. Haematocrits of males in January was significantly higher in FW compared to the other two salinities ($p < 0.05$), but no significantly differences were found in the following samplings. In the two last samplings statistical testing was not performed with only two males left in FW. Anaemia seem to be more marked in FW than BW with a total loss in haematocrit of 80 and 53 % respectivly.

Table 1. Haematocrit (% RBC) of males in the spawning period.

	FW			BW			SW		
Month	RBC	SEM	n	RBC	SEM	n	RBC	SEM	n
Jan	59.3	1.7	13	49.9	3.8	12	54.4	1.1	19
Feb	47.1	2.4	15	43.4	2.1	18	38.7	2.0	4
Mar	27.2	2.7	7	34.8	2.7	16	29.6		1
April	16.0	0.1	2	26.5	3.2	14			
May	12.0	1.5	2	23.3	4.0	11			

Anaemia in females reared at the different salinities, were not as serious as observed for males (Table 2), except in SW where a loss in haematocrit of 50 % was recorded during the spawning period. Haematocrit values of spawners are presented in BW and SW as means (% RBC) of the three temperatures with SEM and n given.

Haematocrit values of the only spawner reared in warm FW (tank 3) are given separately because these values are significantly higher compared to the two spawners reared in tank 2. In FW of medium temperature (tank 2) the levels of haematocrit were declining until the time of spawning (April) with a loss of 45 % followed by an increase in haematocrit. In BW the haematocrit levels declined slowly during the spawning period with a total loss of 20 %.

Table 2. Haematocrit (% RBC) of females in the spawning period.

	FW		BW			SW		
Month	RBC*	RBC**	RBC	SEM	n	RBC	SEM	n
JAN	54.8	55.2	45.4	2.8	8	42.9	2.0	10
FEB	37.4	41.1	41.5	3.3	11	33.5	1.6	10
MAR	33.4	47.6	38.8	2.4	11	29.4	3.9	8
APRIL	29.9	42.3	35.1	2.2	10	23.7	3.3	6
MAY	38.2		35.7	1.7	5	21.1	4.4	2

* : n = 2 (FW, medium temperature)

** : n = 1 (FW, warm temperature)

Glucose

High glucose levels in blood plasma of males were found in FW, especially at the coldest and the warmest temperatures (Table 3). In FW of medium temperature, the glucose consentration was significantly higher than in BW ($p < 0.05$), but lower and more stable compared to the other two temperatures in FW.

Table 3. Glucose (mg/dL Glc) in blood plasma of males reared at the three temperature regimes in FW.

	FW cold			FW medium			FW warm		
	Glc	SEM	n	Glc	SEM	n	Glc	SEM	n
Jan	267.7	40.2	4	237.9	31.6	5	185.7	20.8	4
Feb	759.5	100.2	5	209.3	12.7	3	387.0	77.8	4
Mar	701.4		1	235.5	51.9	2	512.9	51.9	2
April	420.6		1	209.8	13.7	4	423.9		1
May	219.6		1	217.4		1	195.6		1

Glucose data in BW and SW are presented as means of values at the three temperatures with SEM and n given (Table 4). The glucose levels in fish reared in BW were not changing with temperature ($p > 0.05$), except from sampling in March. High mortality in SW

makes the data less comparable to the other salinities. A fall in the glucose consentration occurred closly before death in SW.

Table 4. Glucose (mg/dL Glc) in blood plasma of males reared in BW and SW.

	BW			SW		
	Glc	SEM	n	Glc	SEM	n
Jan	142.0	14.3	4	228.5	23.2	13
Feb	143.4	9.5	14	102.7	8.5	4
Mar	160.6	15.0	14			
April	147.7	9.9	13			
May	119.0	10.5	12			

The glucose levels in blood plasma of female spawners were generally decreasing until the time of spawning (Table 5). Glucose values at each salinity are arranged around the time of spawning (0 = Week of spawning). As observed for males, high glucose levels were recorded in blood plasma of the only spawner reared in warm FW. The decline in glucose consentration among females reared in FW of medium temperature, in BW and in SW were 60.6, 35.7 and 27.6 % respectivly until the time of spawning. Glucose values varied significantly between the different salinities ($p < 0.05$) with the highest concentrations in FW and the lowest in SW. Only minor differences in glucose levels were observed between trouts reared at different temperature regimes. An increase in glucose was seen at all salinities at the time of spawning or shortly after.

Table 5. Glucose (mg/dL Glc) in blood plasma of females in the spawning period. (*:n=2 tank 2; **:n=1 tank 3)

	FW		BW			SW		
Week	Glc *	Glc **	Glc	SEM	n	Glc	SEM	n
-10			211.9	26.2	4	90.0	26.1	3
- 8	360.5	144.2	174.6	27.3	9	104.7	22.8	6
- 6	218.0	408.6	130.4	17.6	10	101.7	9.1	7
- 4	170.5	478.5	130.3	10.5	11	74.4	26.3	8
- 2	142.1	493.8	112.3	13.1	10	75.8	6.1	9
0	139.9	524.0	135.5	11.8	7	88.3	9.1	9
2	199.9	390.0	156.9	16.1	11	89.4	17.8	6
4	172.1	309.2	113.6	9.8	11	65.6	5.6	6
6			115.2	11.4	9	81.3	5.7	6

Chloride

The chloride levels in blood plasma of males were significantly different between the three salt regimes ($p < 0.05$), except at sampling in April. The highest chloride consentration were observed in SW and the lowest in FW. At all salinities the chloride levels were increasing through the investigation period, with the greatest variations in FW (Table 6). Chloride values are presented at each salinity as means (mmol/L chloride) of the three temperatures with SEM and n given.

Table 6. Chloride (mmol/L Cl) in blood plasma of males.

	FW			BW			SW		
	Cl	SEM	n	Cl	SEM	n	Cl	SEM	n
Jan	122.0	1.2	13	130.5	1.3	11	143.4	2.0	7
Feb	109.1	5.6	14	134.2	1.9	12	161.5	1.0	3
Mar	126.7	7.0	5	137.0	1.5	14			
April	134.8	4.5	5	139.9	1.2	12			
May	142.8	3.9	3	135.1	1.3	11			

Table 7. Chloride (mmol/L Cl) in seminal plasma of males.

	FW			BW			SW		
	Cl	SEM	n	Cl	SEM	n	Cl	SEM	n
Jan	83.4	7.5	12	101.3	6.7	16	129.0	4.6	11
Feb	56.0	10.1	6	101.2	10.4	12	149.9	2.3	12
Mar	82.6	14.2	5	102.8	7.9	16			
April	123.0	8.2	6	111.6	6.4	16			
May	109.8	2.3	3	118.2	5.2	13			

The chloride levels in seminal plasma of males (Table 7) were significantly lower than in blood at all stages, but followed the same increasing trend as observed for blood. The individual variations of chloride in seminal plasma were greater than in blood, especially for trouts reared in FW. According to temperature, there were no significant differences of chloride in BW with the exception of February. Significantly differences of chloride were found between the different salt regimes ($p < 0.05$).

Table 8. Chloride (mmol/l Cl) in blood plasma of females arranged around the time of spawning, week 0.

	FW		BW			SW		
Week	Cl*	Cl**	Cl	SEM	n	Cl	SEM	n
-10	132.8		154.1	9.8	5	153.5	5.3	3
- 8	139.5	129.0	154.3	7.2	6	149.3	2.8	6
- 6	141.5	-	146.6	5.3	10	149.2	2.4	7
- 4	141.5	109.5	144.3	2.5	11	153.6	2.3	8
- 2	140.0	113.0	142.8	2.6	10	152.3	2.9	9
0	135.8	-	139.1	2.5	4	153.8	3.1	8
2	140.5	123.0	141.7	3.2	11	143.9	6.1	7
4	136.5	120.5	138.7	2.6	11	144.3	1.3	6
6	139.0	-	137.6	2.6	11	145.0	2.3	7
Chloride (mmol/L Cl) in coelomic fluids of females.								
0	128.0	2	123.4	5.8	9	121.6	12.1	6
2	119.0	2	120.5	6.9	12	113.0	7.9	6
4	111.5	2	131.4	4.1	8	132.0	9.5	3
6			139.7	2.6	8	128.0		1

* n = 2 (FW, medium temperature)

** n = 1 (FW, warm temperature).

As observed for males, the chloride concentrations in blood plasma of females were lowest in FW and generally highest in SW (Table 8). Values for females are arranged around the time of spawning, and can not be directly compared to males. However, sex differences are seen in FW and BW where the chloride levels are slightly elevated in females until the time of spawning, compared to males. In BW the chloride levels decreased slowly in the spawning period. A similar decrease in chloride was observed among females reared in FW of warm temperature. This was followed by an increase in chloride postspawning. In FW of medium temperature the chloride level remained higher and more stable. In SW, the chloride levels were high and quite stable until spawning, where a sudden fall occured.

At the time of spawning (week 0) the levels of chloride in coelomic fluids of females (Table 8) were lower and inversly correlated to the consentrations in blood plasma. Six weeks later the levels of chloride were isosmotic to blood in BW, but still lower at the two other salinities and actually falling in FW.

Analysis of egg

Values for dry matter and survivel of the eggs until the eyed stage are arranged according to the water quality where the female was reared (Table 9). The number of spawners are given at each salinity. Statistical testing between temperatures were only performed when at least three data from each temperature was present. All data given in percent was transformed by the formula asin (SQRT) before statistical testing was performed.

The largest value of dry matter in eggs (42.9 %) was measured from fish reared in FW, but this value was not significantly higher than dry matter of eggs from females reared at the other salinities.
A value of 45 % dry matter was measured in eggs stripped at the coldest temperature regimes in BW and SW (n = 2-3).

According to the survival of egg, no temperature differences was found in BW, but in SW of the coldest temperature, the survival of eggs was significantly higher compared to the other temperature regimes at the same salinity. Eggs from females reared in FW of warm temperature was not fertilized, but besides this no mortality occurred in FW.

Table 9. Number of spawners, and the dry matter (%) and mortality (%) in eggs stripped from the different environments.

	FW cold	FW medium	FW warm	FW mean
Number of spawners	0	2	1	
Dry matter,egg (%)	-	42.5(0.7)	43.9	42.9(0.6)
Egg survivel (%)	-	100.0(0.0)	-	100.0(0.0)

	BW cold	BW medium	BW warm	BW mean
Number of spawners	2	5	5	
Dry matter,egg (%)	45.0(4.1)	41.2(0.6)	41.9(0.7)	42.1(0.7)
Egg survivel (%)	91.7(8.4)	93.2(5.3)	89.3(5.1)	91.3(3.1)

	Sw cold	SW medium	SW warm	SW mean
Number of spawners	3	2	5	
Dry matter,egg (%)	44.9 *	41.8(1.2)	41.2(0.6)	41.8(0.6)
Egg survivel (%)	99.0(1.0)	81.7(1.7)	81.3(6.6)	86.2(4.3)

* n=1

DISCUSSION

Survival of the broodstock

The high mortality seen among fish reared in SW, especially of males (100 %), are higher but in agreement with earlier reports. A mortality of more than 30 % after stripping in SW is common in most fish farms in Norway, and represents a major problem in the salmon farming industry (Ulgenes and Nævdal, 1984). The high mortality experienced among fish reared in FW has not been reported by other workers, but can possibly be explained partly by the experimental situation (handling, stress) and partly by the very low temperature conditions this spring (Fig.2). Different from fish reared in SW, problems with heavily wounds and infections were not experienced in FW, but it was obvious that the fish had problem to mature naturally. Independent of salinity, the spawning occurred later in the spring (late February/March) at the coldest temperature regimes. A delayed sexual maturation was also found in Atlantic salmons exposed to low winter temperatures in sea cages (Saunders et al. 1983). In FW there were few spawners even at the warmer temperatures, and the females did not spawn until March. Two of the three spawners reared in FW died 2-4 weeks postspawning. No mortality were observed among spawners reared in BW. Among females reared at the two coldest temperatures in SW, three of the five spawners died within a week after stripping. The fish suffered from great injuries and infections. No death occurred among the five spawners reared in SW of the warmest temperature.

Broodstock reared under verifiable conditions and regularly handled with anaesthetising and blood sampling, will experience an unnatural stress situation. Repeated handling (stripping, sorting etc.) can easily cause damages to the skin with loss of the protecting mucous membrane. Secondarily, this might make the fish more susceptible to infections and cause some trouble with the osmoregulation in SW (Ulgenes and Nævdal, 1985). Interestingly, only the broodstock reared in BW seem to manage well under these conditions. According to Finstad et al. (1985) temperature affects the sea water tolerance of rainbow trouts. Low temperatures supressed the efficiency of the regulations mecanisms both in gills and kidneys and caused disruption of the osmoregulating capability. Analysis of chloride in blood plasma of fish reared in FW, did show that the

compensation mecanisms were out of control, especially at the colder temperatures. Independent of salinity, the mortality was higher among fish reared at the coldest temperatures.

Haematology

The trend towards anaemic conditions are in agreement with the observations of Lane (1979) who investigated the progressive changes in haematology of sexually mature rainbow trout. Likewise, sexual differences were noted with males having higher initial levels of haematocrit than females, and also were experiencing a more serious anaemia. The decline in haematocrit of males were independent of temperature, and only small differences were found according to salinity. Consequently, the variations across time were due to the maturation process. Lane (1979) postulated that the reduction in erythropoietic activity reflected the changes in metabolism and hormonal levels during the maturing period with cold temperatures and lower metabolic needs.

Triplett and Calaprice (1974) who investigated the changes in plasma constituents during natural spawning migration of Pacific salmons, observed a remarkable increase in haematocrit some time postspawning. Among females reared in FW of medium temperature, the decline in haematocrit until the time of spawning, was actually followed by an increase 2-6 weeks postspawning (Table 2). Unfortunatly only few females matured in FW, but still some difference were found conserning the changes in haematocrit among females reared at the different salinity regimes. The decline in haematocrit was lower among females reared in BW (20 %) compared to the other salinities (40 - 50 %). Due to large variation in time of spawning in BW, the individual variation in haematocrit around this time can not be seen. However, the decline in haematocrit across time in BW was more pronounced than the individual variation in haematocrit around the time of spawning. Females reared in SW experienced almost the same kind of anaemia as did males at this salinity. The levels of haematocrit were steadily declining in the spawning period.

Glucose

According to salinity, differences were found concerning the level of glucose in blood plasma of the broodfish. For both sexes glucose values were generally highest in FW and lowest in SW. Mature males showed a different change in blood glucose consentration during the experimental period, and with generally higher values than for females (Table 3 - 4). A decrease in blood glucose was found among females at all salinities during the spawning period. The gains in FW, BW and SW measured two weeks before spawning, were 60, 35 and 27 % respectively. Seasonal changes of blood glucose in connection with sexual maturity is found in different species of fish. Blood glucose of the mature salmon, *Oncorhynchus keta*, decreased by approximately 40 % when the fish was adapted to fresh water for 7 days (Morisawa et al., 1979). In the flounder, *Platichtys flesus L.*, an increase in liver glycogenolysis, but a decrease in blood glucose was found in response to estradiol (Sand et al., 1980). Glycogen is not very important as an energy reserve in fish, but it is probably deposited in the ovaries during development, and hence withdrawn from other parts of the body in females (Love, 1980).

No temperature differences in blood glucose were found among males reared in BW and SW, but temperature seem to have an adverse effect in FW with the highest levels of glucose at the coldest temperature. Notably, the levels of glucose in FW of medium temperature more closely resembled observations in BW than the levels of glucose in FW of the warmest temperature regime. In FW of both cold and warm temperature, the values of glucose for both sexes were high and increasing, and were not declining until late in the investigation period. An increase in blood glucose (Umminger, 1971,a; Leach and Taylor, 1977) and also an increase in liver glycogen (Jankowsky et al., 1984) is reported in different species of fish in response to low temperatures. The pituitary gland of fish is stimulated to secrete ACTH at low temperatures. Cortisol and adrenaline which is secreted in response to ACTH, stimulates to glycogenolysis and at low temperatures, gluconeogenesis, both resulting in an increased blood glucose (Seibert, 1985). According to Umminger (1971,a) the high serum glucose in freshwater-adapted killifish (*Fundulus heteroclitus*) probably reflected a protecting mecanism to ice formation by increasing the osmolality of the blood. The high glucose levels among fish reared in cold FW (0 - 1°C) supports

this theory, but can hardly explain the high glucose values also analysed in blood plasma of fish reared in warm FW. The complex osmoregulatory regulation can only be completely understood when both serum osmolality and serum electrolytes are measured in the same fish at the same time.

Exercise induced hyperglycemia is likely to be associated with increased adrenal activity. Both medullary (ACTH) and cortical hormones (cortisol, adrenaline) appear to be involved in the metabolic changes following handling or exercise. The rise in blood glucose resulting from stress has been documented in a large number of species under a wide range of conditions (Love, 1980). In our experiment the high glucose levels in some fish might be explained by the experimental conditions and also by individual variations.

Chloride

The chloride levels in blood and seminal plasma of males were significantly different between the three salinity regimes (Table 6 - 7). During the first month there was an increase in chloride in blood and seminal plasma of males reared in SW, and a decrease in chloride among males reared in FW. In BW where the salinity was nearly isosmotic to the blood, the chloride levels of males remained almost stable throughout the experimental period.

The salt balance within a fish is maintained almost entirely by the gills and the kidneys. The gill Na/K-ATPase is affected by salinity, temperature and an interaction of these two factors (Jürss et al., 1984). The activity of this enzyme in rainbow trouts at 8 and 16°C increased as the salinity of the environment increased, but no such increase was observed in fish adapted to 1 °C.
In most freshwater fish studied, serum inorganic ions decrease in the cold (Umminger, 1971,a; 1971,b). In the carp, *Cyprinus carpio*, organic substances like glucose is added to the serum in quantities sufficient to fully compensate for the reduction in electrolytes. In our investigation the chloride level in blood plasma of males reared in FW was inversly correlated to the glucose consentration and varied with temperature. The decrease in chloride in February among males reared in FW (Table 6) reflected a fall in chloride in blood plasma of males reared at the coldest temperature. At the same time the glucose level in blood plasma of these

fish increased approximately three times to 760 mg/dL (Table 3). Whether this reflects a compensation to increase the osmolality of the blood is uncertain without the data of osmolality. High glucose values was also observed in FW of the warmest temperature. Interestingly, the chloride level at this temperature remained "low" at approximately 120 mmol/L until April. The increase in blood chloride among males reared in FW of medium temperature was followed by a decrease in blood glucose. In SW the high and increasing chloride values where also accompanied by a fall in the glucose consentration. In BW only small variations were observed.

Sampling stress even at short duration is found to cause notable changes in the blood status of rainbow trout (Railo et al., 1984). Significant changes in the haematocrit values, gas tension, pH and the ionic balance has been reported. Plasma K^+ increased, while the glucose and chloride levels decreased. The osmoregulatory mechanisms consume energy, and there is evidence that a stressed fish may use so much energy in other ways that the osmoregulation is made temporarily ineffective (Love, 1980). In our investigation there is reason to believe that the environmental influences on the broodstock are greater than the regularly sampling, but this factor should not be neglected.

The chloride consentrations in blood and seminal plasma of males are counsequently caused by the water quality (salt, temperature) and of the experimental situation (handling, stress), and are probably little affected by the maturation prosess. This is in agreement with that found in Atlantic salmon (Albrektsen et al., 1986).

Different from males, the chloride values in blood plasma of females were generally falling in the spawning period independent of salinity (Table 8). The chloride level in blood plasma are higher than observed in the mature chum salmon (*O. keta*) during adaptation to freshwater (Hirano et al., 1978; Morisawa et al., 1979). Different from that investigation values for seminal fluid never exceeded those for blood plasma. This is in agreement with that found in the Atlantic salmon (Albrektsen et al.,1986).
As observed for males, a close relationship between glucose and chloride in blood plasma of females reared in FW was found. The high glucose level of the only spawner reared in warm FW was

accompanied by a very low chloride level (Table 5 and 8). As distinct from males, the chloride levels in coelomic fluid of females was not closely regulated to the variations in blood (Table 8). Only females reared in BW manage postspawning to rise the chloride consentrations to a level isosmotic to the blood. In SW 6 weeks postspawning this regulation was not completed, while the chloride levels in FW was still falling. These results might reflect the better survival of broodstock reared in BW compared to the other salinities. The nearly isosmotic condition in BW probably make the high energetic cost in connection with osmoregulation and the maturation process lower compared to the other salinities.

Egg analysis

The dry matter of eggs were not significantly different between the different water qualities (Table 9). The dry weights were slightly higher compared to the dry weights values of eggs from Atlantic salmon, 40.9 +/- 1.8 % (Albrektsen et al., 1986), but still within the range of normal values of eggs reared at Matre Aquaculture Station. According to these results it must be concluded that exposure of the broodstock to different environment in the spawning period did not significantly influence the development of dry weight in eggs. Due to few spawners, high values of dry matter (45 %) observed in eggs stripped at the coldest temperature regimes in BW and SW could not be statistically treated.

In this investigation it was found that the mortality of egg stripped from fish maturing in FW or BW was low (0 - 10 %) independent of temperature. In SW however, the mortality of eggs was influenced by temperature. The mortality of eggs until the eyed stage was approximately 20 % at the two warmest temperature regimes, while almost no mortality was seen among eggs stripped from the coldest temperature regime. This low temperature effect was also observed on the survivel of eggs from Atlantic salmon reared in BW (Albrektsen et al., 1986). The survivel of eggs differ from the survivel of mature rainbow trout where the highest mortality was observed at the lowest temperatures. The higher survivel of eggs stripped from BW and FW compared to SW have also been reported by other authors (Ulgenes et al., 1984; Allee, 1980).

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Both salinity and temperature and the complex interaction between these two parameters, do influence the survivel of both the broodstock and the eggs. Analysis of elecrolytes and other blood and seminal components should be further investigated in order to understand the complex osmoregulating mechanisms and the effects on broodstock during the maturing period. In our investigation it can be concluded that BW was the most favourable environment for the broodstock. Independent of temperature a high survivel of mature rainbow trout and of the eggs until the eyed stage was recorded.

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