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THE EFFECT OF A SHORT TIME EXPOSURE TO DIFFERENT TEMPERATURE AND SALINITY REGIMES ON SURVIVAL OF MATURING ATLANTIC SALMON AND EYED EGGS, AND CHANGES IN BLOOD AND SEMINAL PLASMA DURING THE SPAWNING PERIOD

by

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ABSTRACT

Maturing Atlantic salmon, transferred from brackish water two weeks prespawning, were held under four different temperature and salinity regimes through their spawning period.

Values of blood glucose and haematocrit were measured every week during this period. Inorganic components (K+ and Cl-) were analysed from coelomic fluid, blood and seminal plasma.

The dry matter of the eggs at spawning, were measured. The mortality of the broodfish and the eyed eggs were also recorded.

Cold seawater was the most unfavourable environment to the broodfish, as they suffered high mortalities and high levels of chloride, potassium and blood glucose. These results probably reflected problems with osmoregulation. Cold brackish water appeared to be the best environment to both eggs and broodfish, although the males had problems at low temperatures. The haematocrit values were highest among males, but decreased among both males and females during the investigation period. Neither haematocrit, nor the dry matter of the eggs seemed to be affected by this short time exposure to different water qualities.

Abstract

Des saumons de l'Atlantique matures ont ete transferes d'un milieu saumâtre deux semaines avant le fraie et fûrent disposes selon quatre regimes de temperature et de salinite differentes.

Les valeurs d'hematocyte et du glucose sanguin fûrent enregistrees à chaque semaine durant cette periode. Certains composants inorganiques (K+ et CL-) ont ete analyses à partir du liquide coelomique, du sang et du plasma seminal.

Le poids sec des oeufs à la ponte, obtenus des geniteurs provenant des differents environnements fût enregistre. Les mortalites des geniteurs ainsi que des oeufs au stade oeillee fûrent aussi
recueillies.

L'eau froide saline s'est averee de loin le milieu le moins favorable aux geniteurs, ces poissons ont d'ailleurs demontre les plus fortes mortalites ainsi que des taux en chloride, potassium et glucose sanguin les plus eleves. Des problemes relies au phenomene d'osmoregulation sont sans doutes à l'origine de ces faibles performances. L'eau froide saumâtre semblerait être le meilleur milieu tant pour les geniteurs que pour les oeufs, toutefois les mâles ont semble affecter par les basses temperatures. Les valeurs d'hematocyte etaient plus elevees parmis les mâles, notons que ces valeurs ont deline chez les deux sexes au cours de l'experimentation. Le changement rapide de milieu n'a pas semble affecter les taux d'hematocytes ainsi que le poids des oeufs.

INTRODUCTION

There are problems in the Norwegian Atlantic salmon (Salmo salar) farming industry, in production of egg of good quality.

Earlier experiments at Matre Aquaculture Station have shown that the environment under which the reared Atlantic salmon are kept, are of great importance to the survival of both the mature fish and the eggs until the eyed stage. Ulgenes et al. (1984) found that the mortality of the Atlantic salmon broodstock was lower in both brackish and fresh water than in seawater. The eggs stripped from brackish and fresh water had also a lower mortality until the eyed stage.

Gall (1980) reports that the salinity regime under which the maturing salmon are kept , are critical to the hydration and normal development of the eggs and sperm. Allee (1980) found a close relationship between high osmolarities in bloodserum and mortality of the broodfish (Oncorhunchus kisutch) kept in seawater during the maturing period. According to Finstad et al.(1985), temperature affects the sea water tolerance of rainbow trout (S. gairdneri).

The purpose of this experiment was to investigate the levels of organic and inorganic components in different body fluids of Atlantic salmon broodstock, kept at four different shorttime temperature and salinity regimes during the spawning period. It was further investigated how these environments influenced the survival of the mature salmons and the eggs.

MATERIALS AND METHODS

Experimental design

Mature Atlantic salmon (Salmo Salar), reared in netpens, were transferred from brackish water to landbased, round tanks of 3 meter in diameter, 1 nov. 1985. The salinity of the brackish water was measured the last 10 days before transference. It varied between 9.2 ‰ at 0 m. to 25.8 ‰ at 5 m. The broodstock was splitted into four groups, and reared in the following water qualities:

1) Warm seawater (WS)
2) Cold seawater (CS)
3) Warm brackish water (WB)
4) Cold brackish water (CB)

5 females and 5-6 males , weighing from 1 to 5 kg, were placed in each tank, and adapted to the tanks within 3-4 days before we started to adjust the new water qualities. The fish were individually marked by floy tags.

In case 1 we used warm seawater from the Matre fjord, whereas the seawater in case 2 was cooled by exchanging warm seawater against cold river water in a heat exchanger. In case 3 we took advantage of cooling water from the turbines at the BKK hydroelectric powerplant in Matredal, and in case 4 we used water from the Matre river. During the first three weeks, the salinities were (15-17 ‰) in the seawater tanks, and 5-7 ‰ in the other tanks. This was adjusted to 26 ‰ and 8-10 ‰, respectively, in the 48. week, and finally to around 30 ‰ and 14-16 ‰ in the 49. week, using sea water from the Matre fjord. In the last week slight decreases in salinities were observed in all tanks, except that for cold seawater. The temperatures were about 1-4°C in the cold water tanks and 7-8 °C in the warm water tanks.

Sampling and chemical analysis

Once a week from week 46, all the fishes were anaesthetized with benzocaine (Wedemeyer 1971), and 2-5 ml blood, dependent on the size of the fish, was sampled from the duct of Cuvier (Lied et al. 1975). Blood was transferred to heparinized tubes, and haematocrits were measured within half an hour after bleeding the fish. Haematocrit values were recorded, using the method of Wintrobe (1974).

At the same time 4-5 ml. of the semen was collected by gently pressing the abdomen of males. Coelomic fluid from females was collected according to the same method, just before and after spawning. Care was taken to prevent contamination with urin, any sample with yellowish colour was discarded.

Eggs were collected by hand stripping. The broodfish from different environments were crossbred.

The blood plasma was separated after sentrifugation at 4,000 rpm. for 10 min at 8 °C. In order to remove the spermatozoa, the milt was sentrifuged at the same speed for 10 min. The supernatant from milt, and coelomic fluid from females were centrifuged for at 10,000 rpm. for 10 min. Plasma from blood and semen , and coelomic fluid were immediately frozen at -20 °C.

Determination of labile substances, like glucose in the blood, was conducted on samples which had been thawed only once.

Potassium in blood and semen was analyzed by atomic absorption spectrophotometer (Perkin Elmer, Model 300). Chloride concentrations in blood, semen and coelomic fluid were determined using Radiometer CMT 10 coulometric chloride titrator.

Seminal fluid was analyzed for K+ using acid extraction for ion elution (Lutz,1971). Glucose was determined enzymatically with Sigma test kit (Glucose UV-test, hexokinase method). Dry matter of the eggs at spawning and mortality at the eyed stage, were used as egg quality criteria. Dry matter of the eggs was measured after freeze drying.The mortality of the broodfish during the investigation period was registered.

RESULTS

Haematocrit

There were generally sinking haematocrit values of both sexes at all the water qualities, during the whole experimental period (Tab. 1a,b). The values of the males were generally higher than those of the females.

Table 1a: Mean % RBC (± SD) of females, reared in four different water qualities. The values are sorted, according to the week of spawning. 0 denotes the spawning week.

	GROUPS			
	WB	WS	CB	CS
WEEK -2		51		
WEEK -1	42.5 (2.1)	35.3 (16.6)	35.0	
WEEK 0	29.0	26.3 (10.3)	36.0	43.0
WEEK +1	32.3 (1.1)	30.9 (15.8)	36.8	43.0
WEEK +2	32.0 (0.7)	27.9 (11.2)	30.3 (2.5)	41.0
WEEK +3	27.0 (1.4)	30.0 (9.5)	21.8 (7.4)	32.0
WEEK +4	23.0 (4.9)	15.8 (15.1)	16.8 (4.6)	29.0
WEEK +5			25.0	18.5

Table 1b: Mean %RBC (± SD) of males, held under four different water qualities. The values are displayed chronologically.

	GROUPS			
	WB	WS	CB	CS
WEEK 46	55.7 (5.6)	53.2 (8.1)	53.4 (2.9)	51.0 (6.6)
WEEK 47	41.8 (7.2)	38.2 (10.1)	36.0 (14.6)	46.0 (16.8)
WEEK 48	41.3 (4.8)	41.8 (6.5)	39.2 (5.4)	42.6 (7.3)
WEEK 49	36.6 (6.0)	32.5 (15.6)	32.6 (7.7)	35.7 (11.3)
WEEK 50	33.1 (5.6)	26.9 (8.0)	27.7 (10.5)	32.0 (2.8)
WEEK 51	25.5 (8.2)	21.3 (6.1)	22.5 (11.4)	28.3 (1.5)

Potassium in seminal fluid

The potassium values were generally increasing (Table 2), although quite variably, until week 50, whereafter it declined in several of the males.

Table 2: Mean (± SD) potassium concentrations (mmol/l) in seminal fluid of males, reared in four different water qualities.

	GROUPS			
	WB	WS	CB	CS
WEEK 46	14.6 (3.9)	20.0 (5.1)	16.0 (7.2)	19.1 (2.8)
WEEK 47	19.0 (2.6)	22.8 (3.0)	17.6 (7.3)	18.1 (5.8)
WEEK 48	21.7 (5.8)	21.9 (7.1)	17.9 (8.4)	17.4 (8.3)
WEEK 49	19.6 (1.5)	24.3 (5.1)	17.4 (2.6)	25.2 (8.1)
WEEK 50	22.6 (3.1)	29.6 (3.9)	22.3 (1.7)	22.1 (8.6)
WEEK 51	25.6 (4.4)	22.6 (4.2)	20.8 (5.4)	20.0 (9.3)

Chloride

The chloride concentrations in blood plasma from the females, reared in warm seawater and cold brackish water, were quite stable during the investigation period (Fig. 1a), whereas those from cold seawater were increasing, and reached a maximum concentration the last week of 202 mmol/l. The chloride concentrations in blood plasma from the males (Fig. 1b) were increasing in all the groups, but particularly in the seawater reared. Those reared in cold seawater showed the highest maximum of 167 mmol/l.

The chloride concentrations in coelomic and seminal fluid increased in all the groups during the experimental period. The highest value of 192 mmol/l were observed in seminal fluid from males reared in cold seawater, and of 157 mmol/l in coelomic fluid from females in warm seawater.

Blood glucose

The main trend among the females was a short rise in blood glucose levels in the week of spawning, followed by decreasing levels thereafter (Fig. 3a). An exception to this trend was among those reared in cold seawater, where the glucose levels continued to rise to 371 mg/dl, five weeks past spawning. The males also showed a temporary increase in glucose levels (Fig. 3b), which in brackish water thereafter declined to values below the first observations. In seawater, however, the glucose levels continued to rise. Broodfish reared in cold seawater showed the highest values, with a maximum in week 49 of 272 mg/dl.

Dry matter of eggs

The largest value of 42.5 % dry matter was measured on eggs stripped from warm brackish water (Table 3), but this value was not significantly different from the others.

Table 3: Mean (± SD) dry matter of eggs at spawning, in % of wet weight.

	GROUPS			
	WB	WS	CB	CS
%	42.5 (1.5)	40.3 (2.2)	40.8	40.6

Egg survival

The highest survival was seen among the egg groups where one or both the parents were reared in cold brackish water (Table 4). The highest mortality (36.6 %) until the eyed stage was observed in the groups were both parents were held in warm brackish water.

Table 4: Percentage survival of the eggs until the eyed stage, after crossing parents from similar or different environments.

Fertilizations (Female x male)	%
WS x WS	73.1
WS x WB	94.1
WS x CB	100.0
CS x CS	83.3
WB x WB	63.4
CB x CB	100.0
CB x WS	96.6

Broodstock survival

Mortality following 18 nov. was highest among the cold seawater reared (54 %), and was sex independent. No mortalities occured among fish held in cold brackish water. The mortalities were highest before stripping, where 8 females (immature) and 6 males died, whereas 3 males died after stripping.

Table 5: Survival of the brood fish during the investigation period.

DATE	GROUPS			
	WS	CS	WB	CB
1 NOV	13	13	12	12
13 NOV	13	13	12	11
14 NOV	11	13	12	10
21 NOV	11	12	9	10
27 NOV	10	10	9	10
4 DEC	10	7	9	10
5 DEC	9	7	8	10
7 DEC	9	6	8	10

DISCUSSION

One should keep in mind that very few spawners were observed in each tank (1-4), whereas the number of males were higher (7). It is likely to assume that high variation within results , was caused by small groups of broodfish and stress from frequent blood sampling.

The observed decline in %RBC (Table 1a,b) is in good agreement with the observations of Triplett and Calaprice (1971). They observed a gradual decline in %RBC during migration of pacific salmons. However, they observed a remarkable increase some time after spawning which was not observed in our investigation, probably because our fish only was sampled four weeks after spawning.

We did not see any particular difference between fish in brackish or seawater, conserning the levels of % RBC. The levels of % RBC observed, were consequently due to the maturation, and independent of these water qualities.

The levels of % RBC in male rainbow trout, has been reported to be higher than for females during the spawning period (Love, 1980). This is in agreement with our findings.

Inorganic components

The levels of K+ in seminal fluid greatly exceeded values of blood K+ (results not treated statistically) . This observation correspond with that found in mature pacific salmon during freshwater adaptation (Hirano et al. 1979). Our results of K+ in semen and blood are, however, generally lower.

Sampling is reported to cause an increase of K+ in blood plasma (Railo et al. 1984), because of an efflux of K+ from cell to plasma. K+ is further reported to increase with increase in temperature at salinities of 0 and 15 ppt. (Byrne et al. 1971). At 30 ppt. K+ values were about the same between 1 and 10 °C, but increased at 14 °C.

In the present investigation, the difference in K+ among fish reared at different water qualities, was very small. The WS-levels tended to be the highest, whereas brackish water values, starting at a lower level, also tended to increase. In cold seawater, a decline in values was followed by a sudden increase at the time full strenght seawater (30 ppt.) was obtained. Whether this is a response to stress or due to problems with osmoregulation, is however uncertain.

A marked increase of chloride was measured in seminal fluid and blood of males in seawater, especially in those reared in the cold seawater. The levels of chloride in seminal fluid of broodfish reared in seawater, was lower than that in blood plasma at the start of the present investigation, but in the four last weeks of the experimental period, these levels exceeded the blood plasma values. This was probably due to the slow adjustment of the seawater to around 30 ppt.

Hirano et al. (1978) and Morisawa et al. (1979) found higher values of chloride in seminal plasma and coelomic fluid, compared to values in blood plasma in mature chum salmon (O. keta), during adaptation to freshwater. This does not concur with our findings as only slight increases of chloride concentrations were observed in blood and seminal plasma in brackish water, and values for seminal fluid never exceeded those for blood plasma.

Values of chloride in blood plasma of females and males showed the same trend, but slightly higher values were observed among the females. An exception to this trend was seen among fish in warm seawater, where chloride values declined during the experimental period. The reason for this is however uncertain.

It was suggested by Gall (1980) that the salinity regimes at maturity is critical for hydration and normal development of the eggs and sperm.

Atlantic salmon, exercised at 30 ppt., showed large increases of both Na+ and Cl- (and hence osmolality),probably due to intake of seawater (Byrne et al. 1972). According to these authors, plasma osmolalities and ionic concentrations observed for exercised salmons at 15 ppt. were similar to those of unexercised fish. The test salinity of 15 ppt. was close to isoosmotic medium, and the osmoregulatory cost was small.

It is not possible to directly compare blood characteristics of exercising fish with those of mature broodfish, but it is quite obvious that the broodfish were stressed, because of both the physiological processes going on , and caused by repeated handling, anaesthetizing and blood sampling. Moreover, the mature salmon do not eat, and are thus not supplemented with new energy. It is reasonable that the broodfish in brackish water had the smallest problems with osmoregulation, and hence showed a better survival.

Sampling stress is believed to cause notable changes in the blood status of rainbow trout (Railo et al. 1984). According to them, sampling at short duration (normally a few minutes), caused significant changes in the haematocrit values, gas tension, pH and ionic balance of rainbow trout. They found that plasma K+ increased notably, whereas glucose and chloride decreased. This was not observed for chloride in our samples, and it is reasonable to conclude that the environmental conditions made greater influence on the broodfish than did the weekly

sampling, although this factor should not be neglected.

The Cl- concentrations are consequently caused by the water qualities, and does not seem to be influenced by the maturation. It is also evident that seminal and coelomic fluid, compared to blood plasma, are stronger influenced by the salinity and temperature regimes.

Organic components

Mature males showed a different pattern of glucose concentration in blood plasma, with generally higher values than the females. Cold temperatures seems to affect the liberation of glucose in blood, with increasing levels of glucose during the experimental period, especially marked at cold seawater. At the two warmer water qualities, a different tendency was observed with a decrease of glucose level in the second week of sampling, and then an increase to higher levels, before a gradually decline.

Carbohydrates are stored in the liver as glycogen, a polysaccaride built of glucose units. When required, the glycogen is broken down and transported to the muscles as glucose. 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.

The reaction to stress in higher vertebrates has been shown to involve release of adrenaline and corticosteroids. The control of blood glucose levels involves both these hormones (and insulin). There is evidence that these hormone substances also occur in teleost fish. The rise in blood glucose, resulting from stress, has been documented among a large number of species. These have been investigated under a wide range of conditions like capture from the wild (Pleuronectes platessa, Wardle 1972) and handling (O. kisutch, S. gairdneri, Wedemeyer 1972). While

the response of blood glucose to adrenaline is rapid, the hormones of the pituitary adrenocortical axis (like cortisol) cause a rise in glucose which develops more slowly and is of longer duration (Love, 1980).

Most of the energy used by salmon during spawning migration originates from lipid reserves, but a change in carbohydrates have also been reported. Blood glucose of mature chum salmon decreased by approximately 40% during freshwater adaptation (Hirano et al. 1978). This corresponds well with our findings at WS and CB, whereas the gain at WB was a bit lower (30 %). The very high level of glucose at CS was probably due to stress, caused by handling and failure of osmoregulation. For males, it seems that cold temperatures are more stressing to the fish than the warmer. The high levels of glucose at CS correspond well to those found for females.

The dry matter of the eggs were not significantly different between the groups. The mean dry weight value (40.9 $\pm$ 1.8 %) of all the groups is, however, within the range of normal values of eggs at Matre Aquaculture Station. It is therefore concluded that this shorttime exposure of the broodfish to different environments, did not influence the dry weight development of their respective eggs.

The high survival of the eggs where one or two of the parents were reared at cold brackish water, were in agreement with the survival of the broodfish at that water quality. Ulgenes et al. (op.cit.) also found higher survival of eggs stripped from brackish water. The lowest egg survival was seen at WB and WS, while the survival at CS was higher (83 %). This is not in agreement with the survival of the mature salmon, but could be due to a coordination effect between salinity and temperature, which should be further investigated, on larger groups of broodfish.

Both osmoregulation and maturation are processes with high energetic cost to the broodfish. An unfavourable environment can consequently affect the maturation or make the osmoregulation temporarily ineffective.

Some mortality (8 %) was observed before we started to adjust the water qualities. This was probably due to the stress of handling and acclimation to the new environment.

It is concluded that cold brackish water is the most favourable environment to the Atlantic salmon broodfish, compared to full seawater in the temperature intervals investigated. These findings confirm the observations of Ulgenes et al.(1984).

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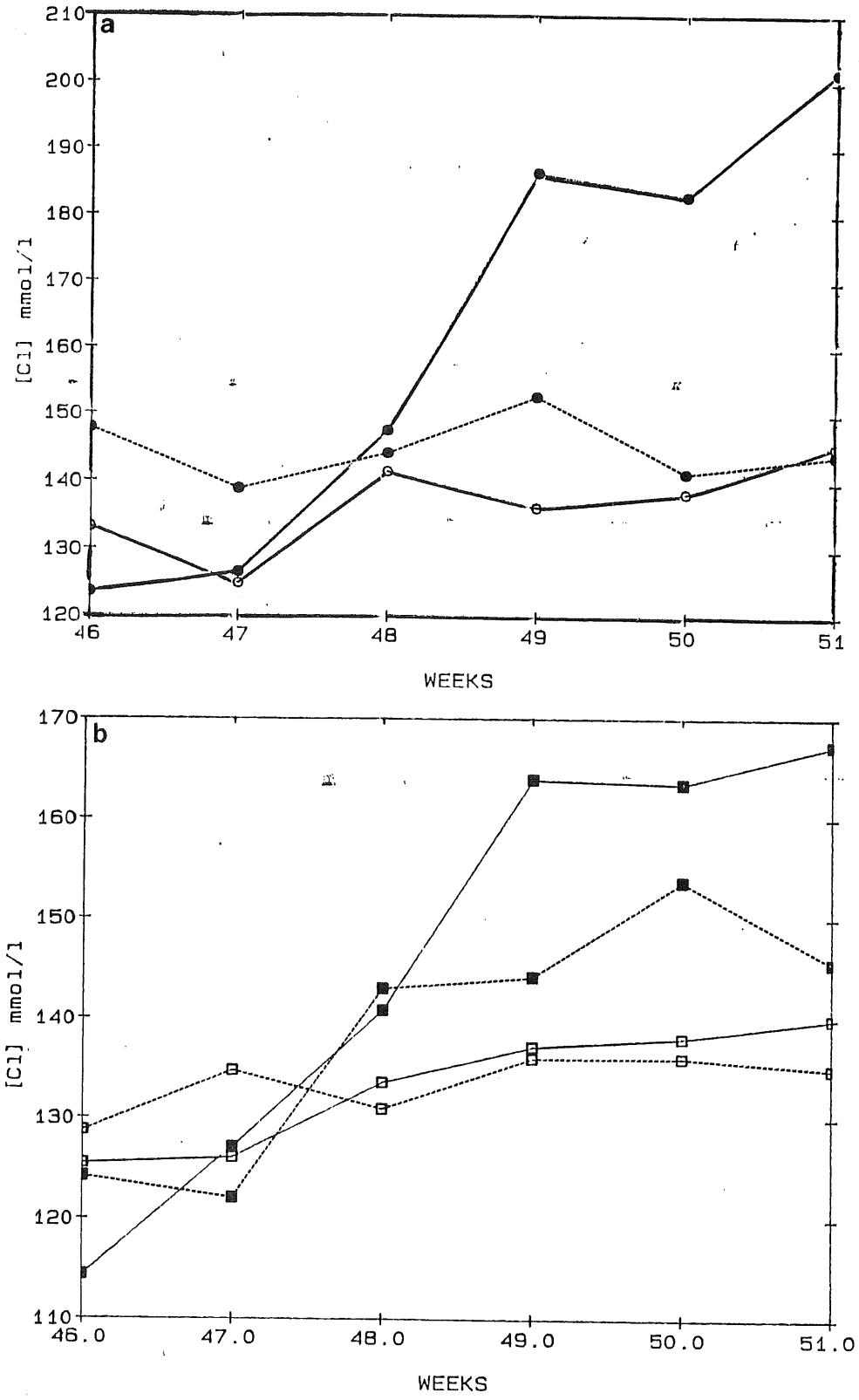
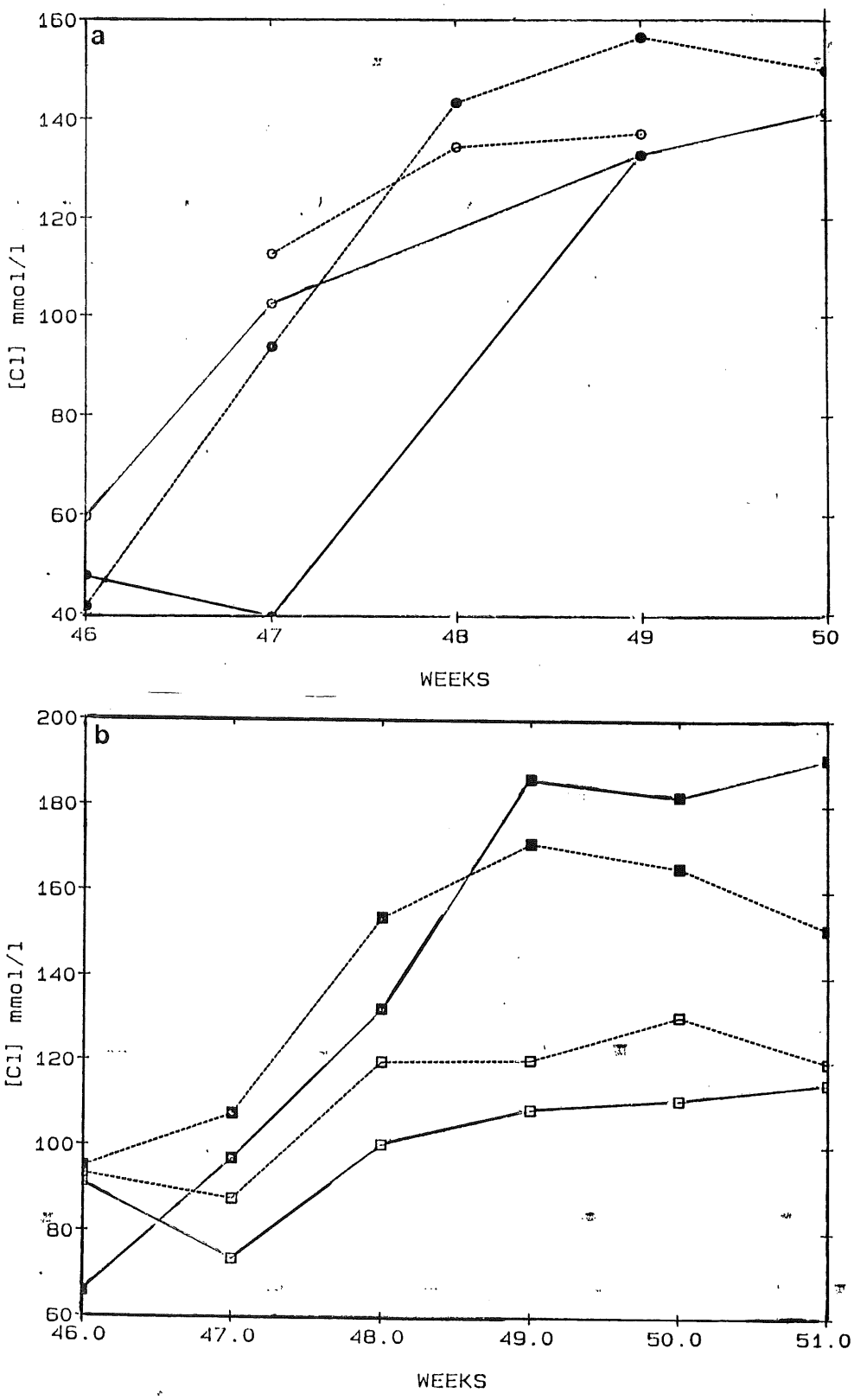


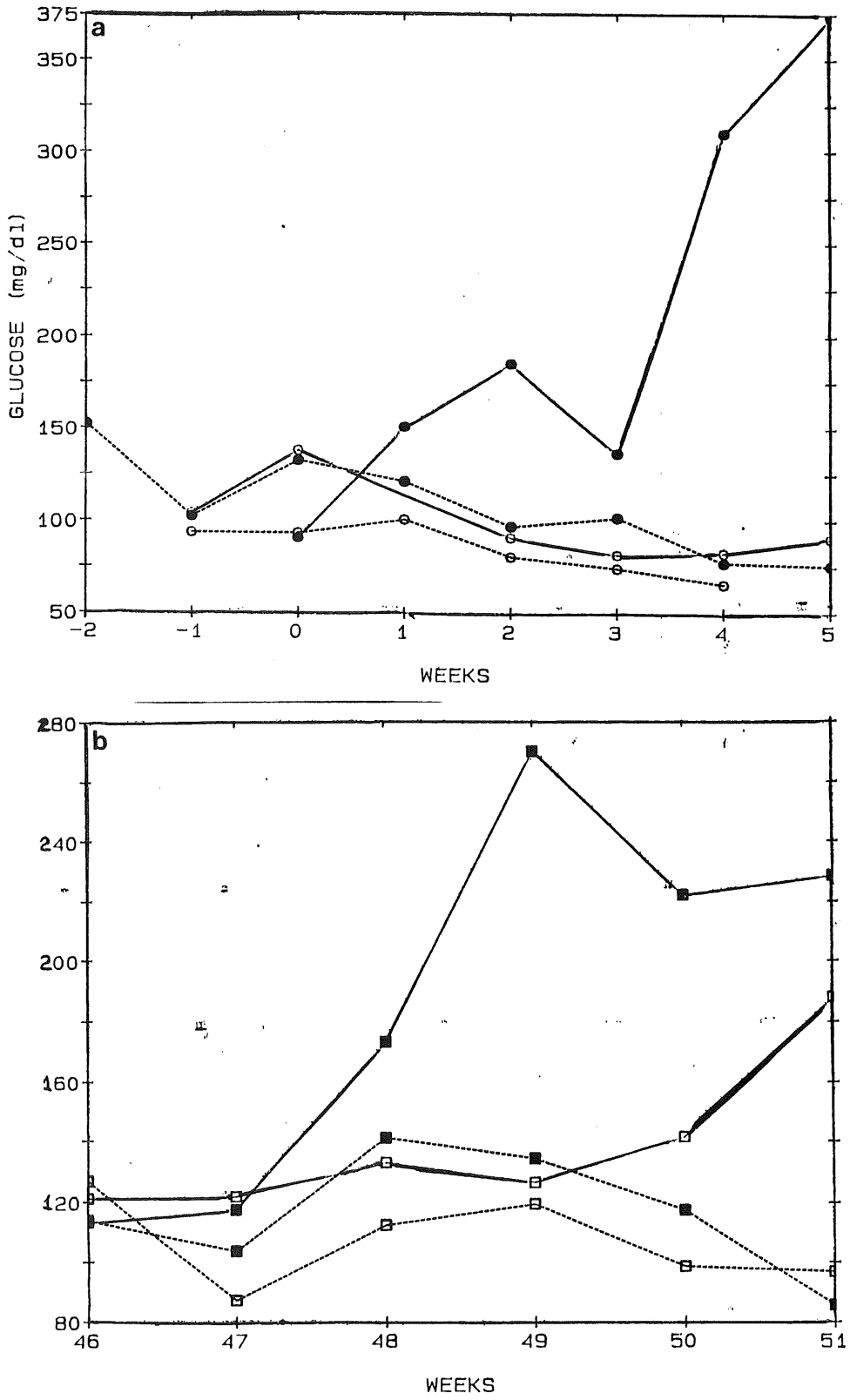
Figures 1a,b. Chloride concentrations in blood plasma of a) females (circles)

b) males (squares)
●, ■ = sea water. ○, □ = brackish water.
----- = warm water _____ = cold water



Figures 2a,b. Chloride concentrations in
a) Coelomic fluid (circles)

b) Milt (squares)
See also Fig.1.



Figures 3a,b. Glucose concentrations in blood plasma of

a) Females (circles)
b) Males (squares)
See also Fig.1.
Note different scales of axis.
The broodfish was stripped in week 0.