Projekt "Smoltvindue hos ørred, *Sálmo trutta*"

(projekt nr. 1329 jf. Handlingsplanen for Fiskeplejen 1998)

af

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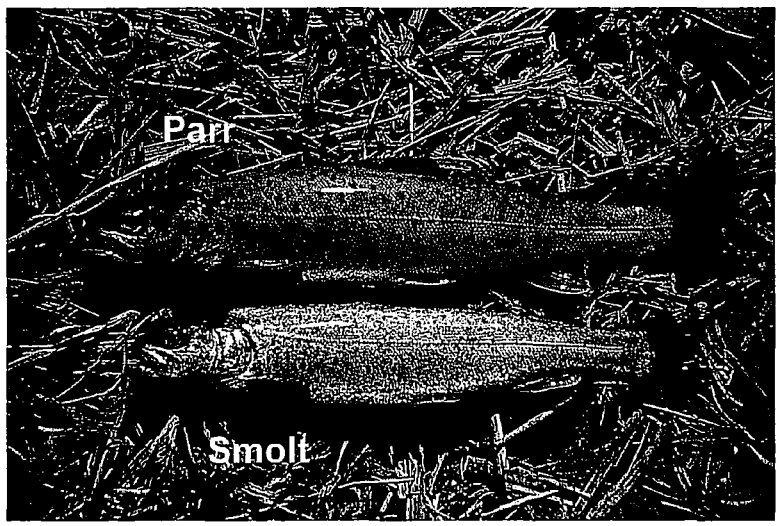

Artikel 1: Evaluation of Smoltification and Seawater Tolerance in Danish Hatchery-reared Brown Trout (*Salmo trutta*)

Artikel 2: Survival and Growth of Hatchery-reared Brown Trout (*Salmo trutta*) after Direct Transfer to Coastal Water: Relation to Seasonal Timing

af

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Forord

Udsætning af ørred (*Salmo trutta*) har igennem de sidste 20 år udgjort en markant post på det samlede budget for fiskeplejen i Danmark. Udsætninger af smolt fordeler sig med ca. 80% mundingsudsætninger, dvs. udsætninger i den nederste del af vandløbet, og 20% direkte kyst-udsætninger, hvorved forstås udsætninger direkte i saltvand. Der er i Danmark ikke nogen tradition for, at objektive undersøgelser af ørredsmoltens udvikling indgår i fastlægning af udsætnings-tidspunktet. Der er derfor en vis sandsynlighed for, at resultatet af smolt-udsætninger kan forbedres ved i højere grad at tilgodese ørredens biologiske udvikling.

Forud for havvandringen gennemgår 1-2 årige vilde laksefisk en gennemgribende forvandlings-proces, som betegnes *smoltifikationen*. Denne proces omfatter ud over nogle få visuelle ændringer (blankhed, kropsform) en lang række af metaboliske, biokemiske og fysiologiske ændringer, som er særdeles velbeskrevet for adskillige laksefisk. Som kulmination på forvandlingsprocessen og mens fisken begynder sin nedvandring i åen, udvikler den sig til en funktionel saltvandsfisk, dvs. en fisk, der har stor kapacitet til at leve og regulere sin vand-/saltbalance i det salte miljø. Denne kapacitet kan undersøges ved en såkaldt saltvandstest.

Flere undersøgelser har vist, at vildtlevende ørreder gennemgår en mere intens forvandling end dambrugsopdrættede fisk (bl.a. Sundell *et al.*, 1998; Nielsen *et al.*, 1999). Dette kan skyldes forskellige forhold, bl.a. genetiske, idet der på mange ørreddambrug selekteres for kraftig vækst og ikke direkte smolt-relaterede parametre. Dambrugsmiljøet, med store fisketætheder og høj fodrings-intensitet, kan ligeledes være en medvirkende årsag til forringet smoltudvikling hos opdrættede ørreder.

Smoltudviklingen hos danske, opdrættede ørredstammer og dermed egnetheden som udsætnings-materiale er aldrig blevet videnskabeligt undersøgt. På denne baggrund tog forfatterne af denne rapport i 1997 initiativ til at lave en forundersøgelse af smoltudviklingen hos udvalgte danske ørredstammer (Nielsen og Madsen, 1998). Initiativet blev finansieret af projektmidler fra Ministeriet for Fødevarer, Landbrug og Fiskeri i henhold til Handlingsplanen for 1997 (projekt nr. 1329). 1997-undersøgelsen omfattede smoltunder-søgelser hos 7 ørredstammer (og 5 laksestammer). Resultaterne dannede baggrund for nærværende undersøgelse, som i princippet er en gentagelse og forbedring af 1997-undersøgelsen.

I 1998-undersøgelsen, som denne rapport beskriver, indgår to dele. Dels en undersøgelse af smoltifikationsprocessen hos 9 udvalgte ørredstammer, som benyttes til smoltudsætninger (artikel 1); dels en undersøgelse af overlevelse og vækst af en enkelt ørredstamme efter udsætning i saltvand (kystudsætning) i relation til udsætningstidspunktet (artikel 2). Resultaterne er afrapporteret på engelsk for at give en bredere læserskare mulighed for at få udbytte heraf.

Det var forfatternes intention, at nærværende undersøgelser skulle udgøre første fase i forbindelse med en revurdering af praksis for udsætning af smolt. Anden fase, som netop er blevet startet i form af et nyt 3-årigt projekt, tilstræber at vurdere sammenhængen mellem smoltens udvikling og dens skæbne (nedvandring) efter udsætning. Når dette projekt når sin afslutning, er det vores overbevisning, at der er dannet et solidt grundlag for at bruge objektive kriterier som retningslinier for design af lokale udsætningsplaner.

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maj 1999

Dansk resumé

Nærværende rapport beskriver to projekter, der blev gennemført med støtte fra Ministeriet for Fødevarer, Landbrug og Fiskeri (Handlingsplan for Fiskeplejen 1998, projekt nr. 1329). Det ene projekt undersøger smoltifikationsforløbet i 9 opdrættede, danske ørredstammer med henblik på optimering af smoltudsætninger. Dette projekt var en direkte opfølgning på en forundersøgelse af smoltkvaliteten i 1997. Det andet projekt undersøger overlevelse og vækst af en udvalgt ørred-stamme efter direkte udsætning i saltvand i relation til tidspunktet for udsætningen. Følgende konklusioner kan drages fra de to undersøgelser af smoltbiologien hos danske havørreder:

- Forsølvning er en upålidelig enkelt-indikator på saltvands(SW)-tolerance hos dambrugsørred. Især under afsmoltifikationen, hvor SW-tolerancen ofte mistes før et markant tab af sølv-farvning indtræder.
- Muskelvandindhold er en pålidelig indikator på SW-tolerance i 24-timers SW-tests. Denne parameter er velegnet til løbende undersøgelser ude på de enkelte dambrug.
- Alle 9 undersøgte ørredstammer viste en sæsonbetinget smoltudvikling (dvs. forøget SW-tolerance og forhøjet gælle Na^+,K^+-ATPase enzymaktivitet).
- Der var relativt små variationer i smoltintensiteten og timingen af smoltudviklingen mellem de undersøgte stammer.
- År-til-år variationen (1997-1998) i smoltudviklingen var minimal i de fleste undersøgte stammer.
- Alle ørredstammer begyndte at miste smoltkarakteristika (Na^+,K^+-ATPase enzymaktivitet og SW-tolerance) allerede i begyndelsen/midten af april måned.
- På grundlag af de foreliggende resultater foreslås det, at mundingsudsætning af ørredsmolt foregår inden SW-tolerancen og høj gælle-Na^+,K^+-ATPase-aktivitet mistes, dvs. ikke senere end slutningen af marts/begyndelsen af april måned i de fleste undersøgte stammer.
- Direkte kystudsætninger bør kun gennemføres, hvis SW-temperaturen er højere end 4°C.
- Direkte saltvandsudsætning af præsmolt (februar) og smolt (april) gav bedre overlevelse og vækst end udsætning af afsmoltificerede ørreder (juni).

Part 1:

Evaluation of Smoltification and Seawater Tolerance in Danish Hatchery-reared Brown Trout *(Salmo trutta)*

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Part 1: Table of contents

1. Introduction

Every year massive releases of hatchery-reared brown trout are carried out in Denmark. Traditionally, the choice of strain and time of smolt-release (coastal and riverine) are based on local habits developed during the last couple of decades rather than specific knowledge about the biological status and development of the fish. Until 1998, no basic information was available about the development and intensity of smoltification and thus suitability for release of different strains of Danish brown trout. On this background, a comprehensive research project supported by the *"Handlingsplan for Fiskeplejen 1997"* was initiated during the spring of 1997, in order to investigate the development and characteristics of smoltification in 7 strains of Danish hatchery-reared brown trout. The strains were selected in order to account for approximately 90% of all smolt releases in Denmark (G. Rasmussen, DFU, personal communication 1996). The main idea of the project was to use measures of 24-h seawater tolerance and the enzymatic activity of gill Na^+,K^+-ATPase as parameters indicative of smoltification with special reference to predict the optimal time for river and coastal smolt releases.

The project provided the first original information about the smoltification profiles of Danish hatchery trout. With regard to Danish smolt release practises, the project provided new information, that was intended for use in optimisation and fine-tuning of present programmes. The following points summarise the overall conclusions of the project:

- *Silvering is not a reliable predictive indicator of SW-tolerance in hatchery-reared brown trout.*
- *All strains of brown trout showed significant variations in SW-tolerance and gill Na^+,K^+-ATPase activity between February and June.*
- *The time and duration of the period with maximum SW-tolerance were different among the different strains indicating different smolt potentials.*
- *All strains started to lose smolt characteristics (Na^+,K^+-ATPase activity and SW-tolerance) in April.*
- *Estuarine and coastal releases of brown trout should be performed before smolt characteristics are lost, i.e. no later than mid April in most of the investigated stocks. River releases should be carried out no before the loss of migration tendency, i.e. when gill Na^+,K^+-ATPase activity is maximal, and therefore no later than the beginning of April.*

Before applying the interesting and rather surprising data obtained in the 1997 study (available in Nielsen & Madsen, 1998) for revision of local release programmes, we found it important to repeat the investigation of smolt development in a second year. An improved project was therefore initiated in the beginning of 1998, which would also elucidate between-year variation in the timing and duration of hatchery trout smoltification. Six of the strains of brown trout from the 1997 study were included in the re-investigation in 1998. In addition,

strains reared at the Trevad and Skibelund Hatcheries (both 1+ and 2+) were brought in. The main goal of the present research project was thus to re-investigate the timing of smoltification (development of seawater tolerance and gill Na^+,K^+-ATPase activity) in different strains of trout and to obtain information whether the process showed significant signs of between-year variations within the particular stocks due to random differences in climatic conditions at the hatcheries. This report describes the results of the 1998 study and extracts the major conclusions from the two years of smolt studies. For a more comprehensive background for the present study the reader is referred to Nielsen & Madsen (1998).

2. Materials and methods

2.1. Fish populations and hatchery conditions

In order to repeat the 1997-study, smolt development was investigated in the following hatchery strains of brown trout: Hårkær Hatchery (HAR), Spjarup 1 Hatchery (SP1), Vork Hatchery (VOR) and 3 strains from Vilhelmsborg Hatchery (THY, LV and VIL). For information concerning the origin of these strains, the reader is referred to Nielsen & Madsen (1998) and Hansen *et al.* (1997). Vestjysk Fiskepark (VFI) was left out compared to the 1997-study because of a milk-pollution of the rearing creek, which killed almost all 1+ brown trout smolts scheduled for release in 1998. Instead, two new strains were included: Skibelund Hatchery (SKI) and Trevad Hatchery (TRE). The stocks SKI and TRE are each year supplemented by wild trout caught in the Gudenå and Karup River systems, respectively. Specific information concerning the origin and genetics of these strains is found in Hansen *et al.* (1997) (in Hansen *et al.* SKI and TRE are named LYS and DR 1, respectively). Fish from all of the above strains were 1+, except SKI, where both 1+ and 2+ were included. The reason being that the stocking of the Gudenå River system has recently come to include both 1+ and 2+ age groups of SKI trout (hatchery personnel; personal communication, 1998). All trout stocks were reared in outdoor freshwater ponds and fed commercial trout pellets *ad libitum* according to local hatchery practices.

2.2.1. SW-tests: SW-tolerance

The development of SW-tolerance in the above strains of brown trout was investigated by sampling at regular (1-3 week) intervals from February until June 1998. Sampling dates are shown in Tables I-IX. Three days before a SW-test was to be conducted, the hatchery personnel transferred 20-22 trout of each strain (or age group) to a 400-l outdoor plastic tank containing aerated freshwater (FW) from the ponds. Food was withheld during this period. The hatchery managers were asked to choose fish randomly, representing the average size and morphology of the stock to be released as smolts in 1998. Unfortunately, this did not always prove to be the case, as discussed later. The SW-tests were conducted as 24-h SW challenge tests in outdoor 400-l plastic tanks supplied with air pumps. SW-tolerance was evaluated by transferring 10-12 fish of each strain (or age) from the FW tank directly to SW (30 ± 0.9 ppt., Tables I-IX) and sampling 24 hours later. Artificial SW was used (Red Sea salt) and the salinity was adjusted on location using a freezing point osmometer. In order to avoid extremely low water temperatures as experienced during the winter in 1997 (Nielsen & Madsen, 1998), the outdoor tanks were wrapped with insulating Rockwool mats. Maximum

and minimum SW temperatures during the 24-h SW-tests were recorded (see Tables I-IX) with electronic thermometers (Origin scientific).

2.2.2. FW-fish: smolt status and control fish

Smolt development was examined in 10 fish from the FW tanks on each sampling date. These fish were also used as control fish for SW-challenge tests.

2.3. Sampling

The sampling procedure was exactly the same as described in Nielsen and Madsen (1998). Gill tissue (for Na^+,K^+-ATPase activity analyses) and plasma and muscle samples (for ion-osmotic analyses) were taken from FW fish. Additional plasma and muscle samples were taken from 24-h SW fish. Condition factor (CF) was calculated as (100 x weight)/length^3.

2.4. Analyses

Plasma sodium concentration ($[Na^+]$) was determined by flame photometry (Instrumentation Laboratory 243, Lexington, MA) using samples diluted 200 times in 15 mM LiCl. Muscle water content was determined as weight loss after drying at 105°C for 48 hr and expressed as percentage of wet weight. Gill Na^+,K^+-ATPase activity was analysed in homogenates at 27°C by the method of McCormick (1993) using plate reader (Spectramax Plus, Molecular Devices, Sunnyvale, CA). Protein content in the tissue homogenates was measured by the method of Lowry *et al.* (1951).

2.5. Statistics

Statistical differences among groups were analysed using SYSTAT 5.03 (Systat, 1991, Evanston, IL). When necessary, transformation of data was done to meet the assumption of homogeneity of variances (Bartlett test). Seasonal differences were analysed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test. Differences in SW-tolerance between males and females within the strains and comparisons of overall SW-tolerance between strains were analysed by two-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference Test. Correlation analyses were performed on plasma $[Na^+]$ versus muscle water content, mean values of plasma sodium versus mean values of gill Na^+,K^+-ATPase activity and plasma $[Na^+]$ versus fish size. Significant differences were accepted if $P<0.05$.

3. Results

The pond water temperature was continuously recorded at the Vilhelmsborg Hatchery and varied as shown in Fig. 1. There was a gradual increase from 3°C in February to 15°C in June.

The water temperature was generally higher and more fluctuating in 1998 than in 1997. The maximum and minimum SW temperatures, the salinity used in the various SW-tests and morphometric data of fish on the different sampling dates are shown in Tables I-IX. The salinity generally varied between 29.1 and 30.9 ppt.

3.1. Development of SW-tolerance in the individual strains of brown trout

In the following, the degree of change in the level of plasma Na^+ and muscle water content (MWC) after transfer to SW for 24 h are used to evaluate and characterise SW-tolerance in the different strains of trout. Following 24-h SW exposure, plasma [Na^+] generally increases and MWC decreases temporarily. **The degree of change is fully dependent on smolt status. In general terms, small changes in plasma [Na^+] and MWC after transfer equates good SW-tolerance.**

HAR strain

The FW-values of plasma [Na^+] varied between 141 ± 1.8 and 150 ± 1.2 mM during the study period.

(Fig. 2A). HAR trout showed good SW-tolerance already from the beginning of the study in early February with relatively small elevations in plasma [Na^+] and decreases in MWC following the 24-h SW-tests (Fig. 2A,B). In March, the SW-tests (conducted at SW-temperatures <3°C!) induced greater increases in plasma [Na^+] and decreases in MWC than in February. In early April, the SW-induced disturbances in plasma [Na^+] and MWC were at the same low level as in February. From mid April through the rest of the study period, SW-transfer induced increasing disturbances in hydro-mineral balance, indicating loss of SW-tolerance in this strain.

SP1 strain

The FW-values of plasma [Na^+] varied between 144 ± 1.2 and 152 ± 2.5 mM during the study period (Fig. 3A). The SW-tolerance of the SP1 strain increased gradually from early February until mid March, by when the strain had obtained maximum SW-tolerance with minor SW-induced changes in plasma [Na^+] and MWC (Fig. 3A,B). The period with maximum SW-tolerance lasted until mid April, where after the SW-induced disturbances in hydro-mineral balance became gradually more severe.

VOR strain

The FW-values of plasma [Na^+] varied between 149 ± 1.2 and 155 ± 1.1 mM during the study period (Fig. 4A). SW-tolerance was relatively good in February where only small post-transfer changes in plasma [Na^+] and MWC were seen (Fig. 4A,B). In mid March, SW-exposure resulted in larger plasma ion- and MW-changes, where after the SW-tolerance gradually increased and reached a maximum around April. SW-tolerance then regressed through the rest of the study period.

THY strain

The FW-values of plasma [Na^+] varied between 139 ± 1.5 and 150 ± 2.0 mM during the study period (Fig. 5A). THY trout had good SW-tolerance from the beginning of the study in February until mid April, where after the trout experienced gradually poorer SW-tolerance (Fig. 5A,B).

LV strain

The FW-values of plasma [Na^+] varied between 140 ± 0.8 and 152 ± 1.9 mM during the study period (Fig. 6A). The SW-induced elevations of plasma [Na^+] and decreases of MWC were low and fluctuating through February and March, reaching the lowest levels in the period between late March – mid April (Fig. 6A,B). The LV trout then began to lose SW-tolerance and experience a severe post-transfer disturbance in hydro-mineral balance.

VIL strain

The FW-values of plasma [Na^+] varied between 142 ± 1.6 and 152 ± 1.2 mM during the study period (Fig. 7A). The VIL strain exhibited good SW-tolerance from the beginning of the study in February lasting until late April (Fig. 7A,B). From early May onwards VIL trout gradually lost SW-tolerance and experienced significant elevations of plasma [Na^+] and decreases in MWC following SW-transfer.

SKI strains (1+ and 2+)

The FW-values of plasma [Na^+] varied between 146 ± 1.6 and 154 ± 1.3 mM in 1+ trout and between 151 ± 1.2 and 156 ± 2.1 mM in 2+ trout during the study period (Fig. 8A). The 1+ SKI trout showed good SW-tolerance with relatively small post-transfer elevations in plasma [Na^+] and MWC from February until late April. From early May onwards, SW-exposure resulted in greater changes in plasma [Na^+] and MWC (Fig. 8A,B). By comparison, post-transfer elevations in plasma [Na^+] were generally higher and showed less seasonal variation than in 1+ trout of the same strain.

TRE strain

The FW-values of plasma [Na^+] fluctuated between 140 ± 1.0 and 146 ± 1.1 mM during the study period (Fig. 9A). TRE trout had good SW-tolerance from the beginning of the study in February and the trout showed only small post-transfer changes in plasma [Na^+] and MWC until early May, where a significant decrease in SW-tolerance was observed. Through the rest of the study period, the SW-induced disturbances in hydro-mineral balance became gradually more severe (Fig. 9A,B).

The following lowest levels of plasma [Na^+] following SW-exposure were measured in the different strains (in mM, test salinity and sampling date in brackets): **HAR**: 171.9 ± 3.5 (29.8

ppt., 1 April), **SP1**: 153.1 ± 2.0 (30.2 ppt., 16 March), **VOR**: 165.6 ± 1.7 (30.0 ppt., 1 April), **THY**: 167.2 ± 2.6 (30.2 ppt., 26 February), **LV**: 160.4 ± 1.8 (30.1 ppt., 2 April), **VIL**: 164.6 ± 2.2 (30.1 ppt., 2 April), **SKI 1+**: 164.2 ± 3.0 (29.9 ppt., 26 February), **SKI 2+**: 170.4 ± 2.0 (29.9 ppt., 26 February), and **TRE**: 163.4 ± 1.8 (30.3 ppt., 1 April).

3.1.1. Correlation between MWC and plasma [Na^+]

When combining data from all fish in all strains exposed to 24-h SW tests, a highly significant linear correlation between muscle water content and plasma [Na^+] was found ((P<0.0001, Fig. 10).

3.1.2. Comparison of the SW-tolerance in males and females

There was no significant difference in the SW-tolerance between males and females in any of the strains during the study period (P>0.05, two-way ANOVA).

3.1.3. FW fish: Gill Na^+,K^+-ATPase activity (smolt development)

Gill Na^+,K^+-ATPase activity showed significant changes during the spring of 1998 in all of the investigated strains of brown trout.

HAR: Gill Na^+,K^+-ATPase activity increased significantly during February and reached a plateau at the end of the month (Fig. 2C). Enzyme activity decreased from early April through May.

SP1: Gill Na^+,K^+-ATPase activity increased significantly from the first sampling in early February until mid March, where peak levels were observed (Fig. 3C). Enzyme activity decreased from mid April through May.

VOR: Gill Na^+,K^+-ATPase activity showed a small increase from early February and peaked in late March (Fig. 4C). After that, the activity gradually decreased until the last sampling in June. Variations in enzyme activity in VOR trout were relatively minor to those observed in other strains.

THY: Gill Na^+,K^+-ATPase activity increased significantly during February and reached a high level plateau at the end of the month (Fig. 5C). At the end of April the activity decreased significantly and remained low through May and June.

VIL and LV: In both VIL and LV trout, gill Na^+,K^+-ATPase activity increased gradually between February and early May, where peak levels were observed (Fig. 6C & 7C). This was followed by an abrupt and significant decrease through May and June.

SKI 1+: Gill Na^+,K^+-ATPase activity was relatively high and unchanged from the first sampling date in February until late March, after which the activity started to decline (Fig. 8C). By late April, a significantly lower enzyme level was observed, and the decline continued until the last sampling point in June.

SKI 2+: With a few exceptions, the pattern of variation in enzymatic activity in this age group was more or less similar to that observed in the 1+ age group (Fig. 8C). Enzyme activity was, however, generally lower in this group. This difference was significant from February until mid April.

TRE: Gill Na^+,K^+-ATPase activity increased significantly in February and reached a relative high and stable level at the end of the month (Fig. 9C). In mid April a significant decrease was observed and from late April through May and June the activity declined further.

The following peak smolt gill Na^+,K^+-ATPase activities were measured in the different strains (dates in brackets): **HAR**: 6.60 ± 0.58 (25 February), **SP1**: 4.40 ± 0.29 (25 March), **VOR**: 3.66 ± 0.52 (1 April), **THY**: 5.23 ± 0.52 (2 April), **LV**: 4.64 ± 0.33 (15 April), **VIL**: 4.05 ± 0.41 (28 April), **SKI 1+**: 4.64 ± 0.25 (26 March), **SKI 2+**: 2.58 ± 0.26 (26 March), **TRE**: 7.10 ± 0.52 (1. April).

3.1.4. Correlation between gill Na^+,K^+-ATPase activity and plasma $[Na^+]$ (SW-tolerance)

By comparing mean values of gill Na^+,K^+-ATPase activity in FW fish with mean plasma $[Na^+]$ from 24-h SW fish on the same date, a significant negative correlation was found for each strain except for the THY strain (Fig. 11). Exponential and linear curves were fitted to the data, the best fit being shown in Fig. 11.

4. Discussion

4.1. SW-tolerance

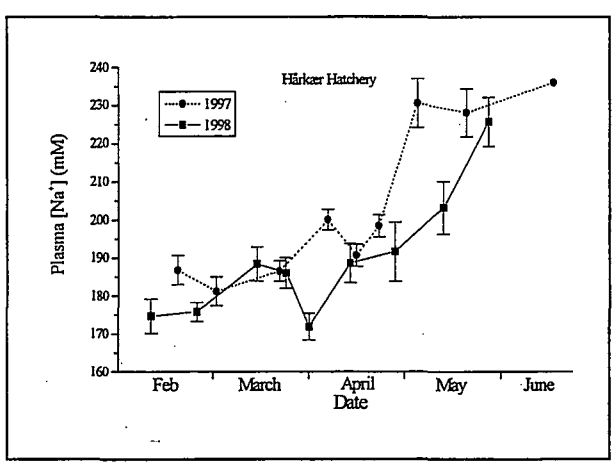
The overall aim of the present study was to characterise the development of smoltification in selected hatchery strains of brown trout most commonly used for riverine and coastal stocking programmes in Denmark. The study was basically designed to repeat a similar investigation carried out in 1997 (see Nielsen and Madsen, 1998) and thus made it possible to further examine between-year variation in the timing and duration of smoltification in the particular strains of brown trout. In the 1997-study we developed and reported reliable methods to test the progress of smolt development at the hatchery. Twenty-four-hour SW challenge tests as developed by Hogstrand & Haux (1985) (measured as changes in muscle water content and plasma $[Na^+]$) proved useful to reveal the development of SW-tolerance in hatchery trout, and the development of gill Na^+,K^+-ATPase activity, in addition to matching SW-tolerance, was also found to be a useful predictive measure of smolt development. Trout were generally exposed to test salinities between 29.1 and 30.9 ppt (Tables I-IX), which is a most effective salinity to be used for discrimination of the peak smolt stage from pre- and post-smolt stages of brown trout. Near-freezing SW-temperatures may seriously bias the test results in a SW-test and thus the conclusion about SW-tolerance. In order to avoid extremely low SW temperatures as experienced in the 1997-study (see Nielsen & Madsen, 1998), the outdoor SW-tanks were therefore wrapped with insulating Rockwoll mats. Near-freezing SW-temperatures were thus avoided (Tables I-IX).

In accordance with the results obtained in 1997, all strains of hatchery-trout showed significant seasonal changes in SW-tolerance during the study period from February until June (Fig. 2-9). Even though the specific date and duration of maximum SW-tolerance differed among the investigated strains, there was a clear overall trend for optimal SW-tolerance to develop in early spring (late March-early April) followed by a period of regressing SW-tolerance throughout the rest of the study (late April-June). In some strains (HAR, THY, VIL, TRE) the occurrence of de-smoltification (i.e. loss of SW-tolerance) was more pronounced than the occurrence of smoltification (i.e. improved SW-tolerance), indicating that smolt development might already have begun by the time of first sampling in February. In other strains (SP1, VOR, LV) SW-tolerance showed an increase during February-March and the occurrence of improved SW-tolerance and loss of SW-tolerance occurred at more or less similar rates. Gill Na^+,K^+-ATPase activity profiles were more similar between strains with respect to qualitative changes. In all strains except SKI 1+ and SKI 2+, there was a clear surge in gill Na^+,K^+-ATPase activity during the study period, indicating smolt development. In most strains (HAR, SP1, VOR, THY, SKI 1+, TRE), enzyme activity increased steadily from the first sampling until peak values were reached in late March. After that, enzyme activity rapidly decreased within 1-2 weeks, indicating the onset of de-smoltification. In two strains (LV and VIL), however, peak activities of Na^+,K^+-ATPase occurred markedly later – i.e. in late April, and again were followed by a sharp decline within a few weeks. Under the present rearing conditions, LV and VIL may thus represent strains with a later smolt development than all other strains investigated. There was also a marked variation in the intensity of maximal smolt development between the strains. Lowest plasma sodium levels, indicative of peak SW-tolerance, varied from 153 mM (SP1) to 172 mM (HAR) suggesting different smolt potentials in these two strains of trout held under the present rearing conditions.

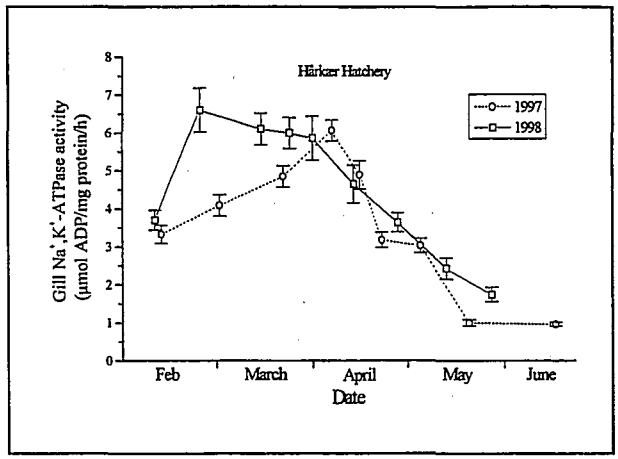
4.2. Comparison of smolt development in 1997 and 1998

In order to better compare the differences in smolt development between years, the results (plasma $[Na^+]$ and gill Na^+,K^+-ATPase activity) from 1997 and 1998 are presented below in the same figures for each hatchery. In light of the close relationship between plasma $[Na^+]$ and muscle water content (see section 4.1.1.), only the former parameter is shown as an index of SW-tolerance.

Hårkær Hatchery (HAR)



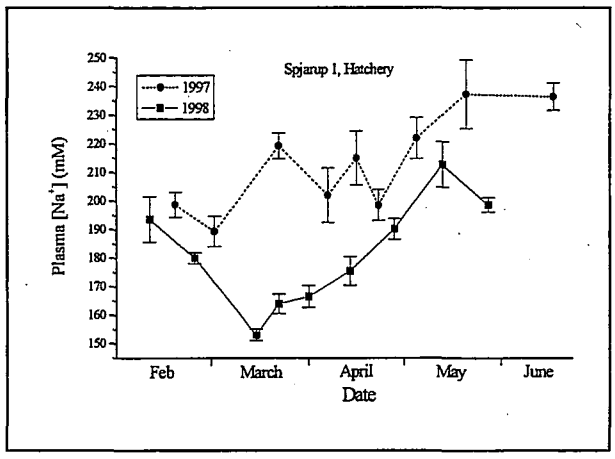
SW-tolerance profiles were similar in the two years. HAR trout had relatively good SW-tolerance from early February with the smallest elevations in plasma $[Na^+]$ following 24-h SW-exposure. The period of highest SW-tolerance lasted until mid April, and was followed by an abrupt decline in the SW-tolerance. Gill Na^+,K^+-ATPase activity profiles followed slightly different patterns early on in the two years. High levels were, however, seen in late March in both years,



followed by a rapid decline through April and May. In 1998, the plateau of high activity was reached earlier than in 1997.

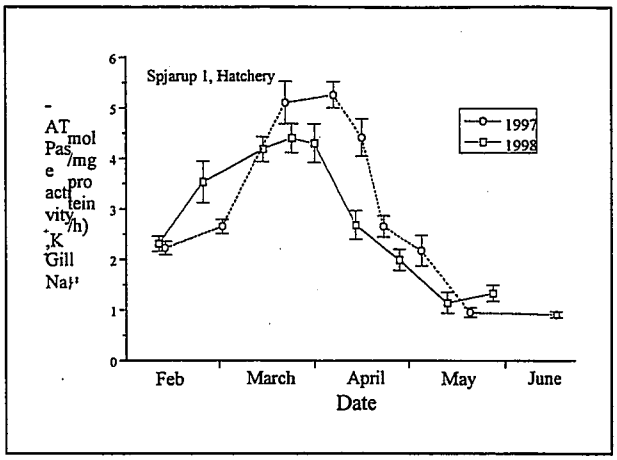
The strain at the Hårkær Hatchery has been domesticated for more than 20 years and breeding material has been selected for high growth rate and silvery coloration (Hatchery personal 1997; pers. comm.). The growth of the strain is therefore likely to be uniform from year to year and during the two springs of investigation the trout followed a similar growth pattern. The water temperature was only measured continuously during the study course at the Vilhelmsborg Hatchery, where the water temperature was higher in 1998 than in 1997. The water temperature at Hårkær Hatchery is likely to have been correspondingly higher in 1998, which may have contributed to the slightly earlier development of maximum gill Na^+,K^+-ATPase activity and SW-tolerance in 1998. In contrast, de-smoltification followed a very parallel pattern in the two years, despite evidence that this is greatly affected by elevated water temperatures (e.g. McCormick *et al.*, 1994, Duston *et al.*, 1991). *The results obtained in 1998 support the findings in 1997 of an unexpected early de-smoltification and suggest that releases of HAR smolts should be carried out no later than late March - early April, in order to obtain optimal migration and survival.*

Spjarup 1 Hatchery (SP1)



In 1997, the SW-tolerance of SP1trout was seriously biased by a period of extremely low SW-temperatures in March and April (see Nielsen & Madsen, 1998). SW-temperatures less than 2°C, impair the apparent SW-tolerance of salmonids (see Nielsen & Madsen, 1998). Before and after this period, however, the SW-tolerance profiles were more or less parallel in the two years, with loss of SW-tolerance starting in early-mid April. In 1998, all SW-tests were conducted at SW-temperatures >4°C (Table II) and these data are thus considered to be most representative of the SW-tolerance in this strain. In 1998, the trout began to lose SW-tolerance in early April.

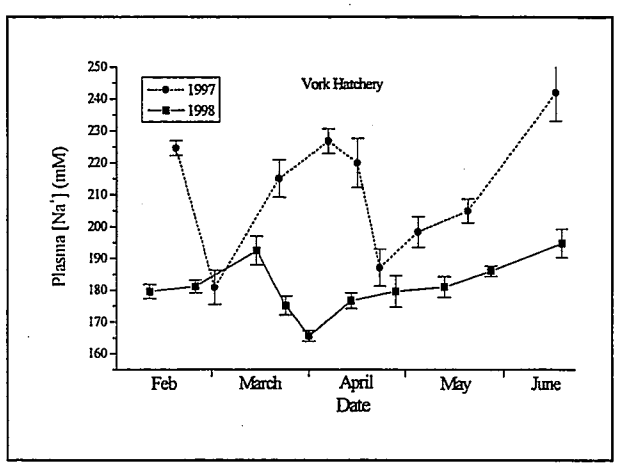
Gill Na^+, K^+-ATPase activity profiles followed more or less the same pattern in the two years with more or less similar high levels in March–early April and decreasing levels through mid April. It is noticeable, however, that the decrease in activity began approximately 14 days earlier in 1998 than in 1997. As explained above, higher water temperatures may be the explanation for this earlier onset of de-smoltification in 1998. The strain at the Spjarup Hatchery has been domesticated for more than 20 years without any supplementation with trout from outside this hatchery (hatchery personnel, 1997; pers. comm.). This fact is likely to be the explanation for the relative similarity of the smoltification profiles observed in the two

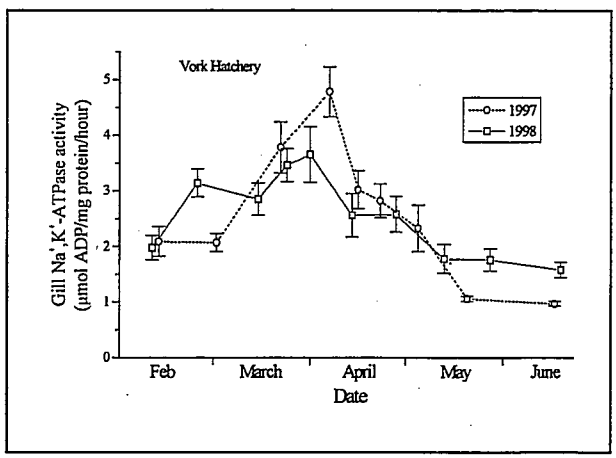


years. The present results also show, however, that despite genetic stability in the population, the timing of smoltification and de-smoltification does not follow a completely fixed pattern but may display some year-to-year variation. *Taken together, the results from 1998 and 1997 demonstrate an early de-smoltification of the SP1trout and suggest that the release of SP1 smolts should be carried out no later than late March.*

Vork Hatchery (VOR)

As for SP1, the SW-tolerance data for VOR were seriously biased by the extremely low SW-temperatures in 1997 (see Nielsen & Madsen, 1998). The samplings in late March until mid April, were negatively affected by SW-tempera-tures of less than 2-3°C (see Nielsen & Madsen, 1998). Extremely low temperatures were avoided in 1998. The apparent SW-tolerance of the VOR strain changed less dramatically than in 1997, and remained at approximately the same intermediate level throughout the spring. This unusual "prolonged" smoltification profile may have been biased by an unfortunate subjective size selection of the trout by the hatchery personnel. The sampled fish are thus not representing the average size in the population. Morphometric data (Table III) indicate that the hatchery personal may have selected oversized individuals at the beginning of the study and undersized individuals later on. This has lead to an almost identical average size of the trout provided for the experiments in the period March until May 1998. There is evidence that the fastest-growing individuals in a population will smoltify and migrate earlier than the slower growing individuals (e.g. Økland *et al.*, 1993), and a subjective size-selection is therefore unfortunate and very critical in assessing the average smolt status of the whole population.





The surge in gill Na^+, K^+-ATPase activity of VOR trout was also more pronounced in 1997 than in 1998. This could be explained by our suspicion of a subjective size selection in 1998 as mentioned above. During the spring of both years, however, highest enzyme activity was observed in

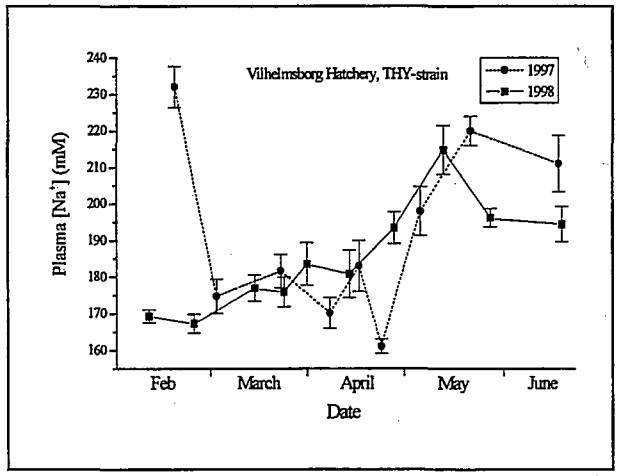
March-early April. The period of peak activity was shorter in 1997. *Based on the results obtained from the present two years study, it is suggested that releases of VOR smolts should be carried out no later than late March - early April.*

Vilhelmsborg Hatchery (THY, LV, VIL)

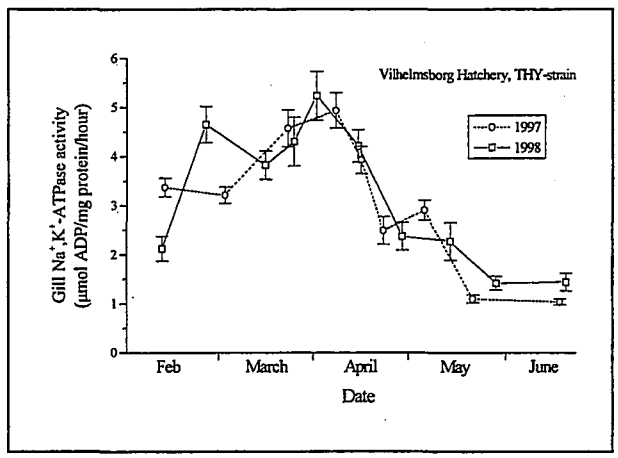
At the Vilhelmsborg Hatchery, two F1-stocks (THY and LV) and 1 domesticated stock (VIL) are reared and used for smolt releases.

THY strain

The SW-tolerance profile of the THY trout was very similar in 1997 and 1998. SW-tolerance was high through March and the first half of April, and was then lost rather abruptly in late April and early May. SW-tolerance began to regress approximately 14 days earlier in 1998 than in 1997, which could either arise from the slightly higher water temperatures in 1998 or reflect genetic differences in these F1 fish. As fish were chosen subjectively by the hatchery personnel, our data do not allow any comparison of the growth pattern between the 2 years of investigation.

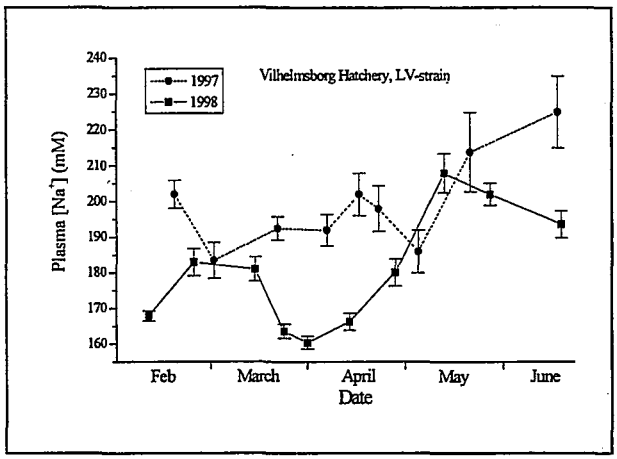


Gill Na^+, K^+-ATPase activity profiles were very similar in the two years of investigation. In 1998, though, a high level plateau was reached earlier than in 1997, whereas enzyme activity regressed rapidly in mid April in both years. Despite the above-mentioned possibility of genetic variation between these F1 offspring of wild caught trout, the smoltification profiles of the THY trout were surprisingly similar in the two subsequent years. The degree of genetic variation among different age classes of F1-trout is likely to be higher, if the parental fish belong to large water systems with several tributaries. The fact that the parental fish of the THY strain are caught in a small water system (Storå River) is likely to diminish genetic variation among different age classes of trout. *Our data from the two years suggest that optimal smolt development occurs in late March-early April and that releases of THY smolts should take place no later than early April.*

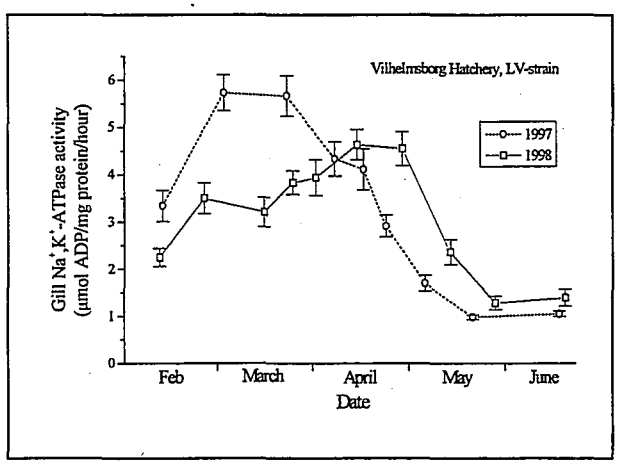


LV strain

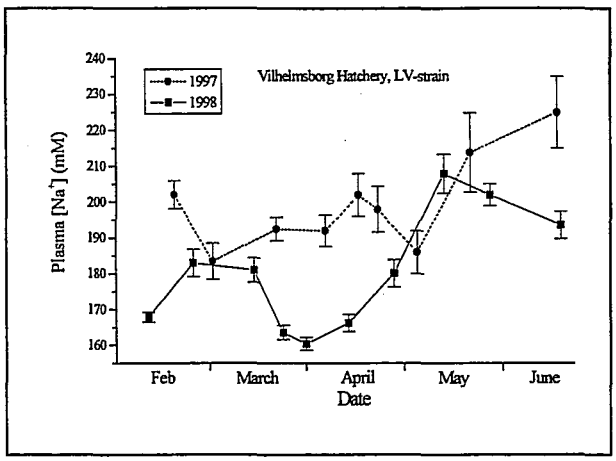
The SW-tolerance profiles of the LV strain did not follow the same pattern during spring of 1997 and 1998. Again, we suspect the extremely low SW-temperatures to be the major factor responsible for the abnormal profile in 1997. In 1998 the SW-temperatures were not critically low at any time (Table VI), and the SW-tests revealed a well-defined period of improved SW-tolerance from mid March until mid April. After that, SW-tolerance regressed within a few weeks.



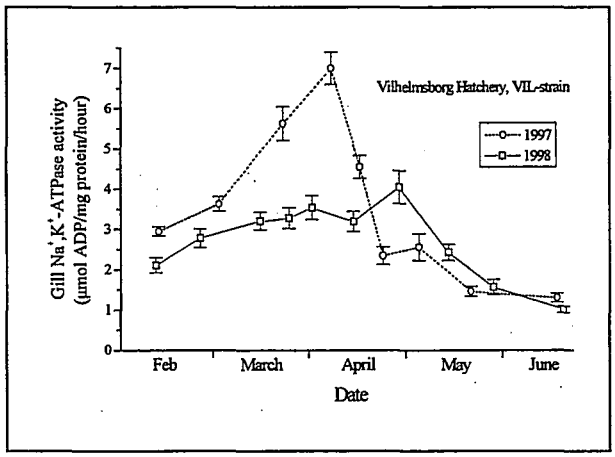
Gill Na^+, K^+-ATPase activity showed a spring surge in both 1997 and 1998. There was, however, marked differences in the timing of the surge in the two years. In 1997, the activity peaked in March, while peak activities were reached in late April in 1998. In both years apparent de-smoltification occurred abruptly after the peak enzyme activities were reached in early April and mid May, respectively. The major difference in smolt development (i.e. gill Na^+,K^+-ATPase activity) between the two years is unlikely to be due to the rather small climatic differences between years. On the other hand genetic differences between F1 stocks may have contributed to the observed difference. The most obvious explanation, however, is the rather subjective size selection of the sampled fish done by the hatchery personnel. Thus the sampled fish did not represent the same modal growth group. Sampling of smaller fish late in the season (where the larger individuals had been released!) may have prolonged the apparent duration of the smoltification period in the LV trout. A direct comparison between the two years is further complicated by the fact, that the trout provided for sampling by the hatchery personnel during the first months in 1997, were approximately 4 cm larger than the trout selected at the same time in 1998. *In taking these uncertainties into consideration, it is still suggested that release of LV smolts takes place no later than early-mid April for optimal results of the release.*



VIL strain



In 1997, SW-temperatures were critically low during March and early April, leading to an apparent poor SW-tolerance in spite of high gill Na^+,K^+-ATPase activity in this period (see below). This problem was avoided in 1998, and the SW-tolerance profile was changed accordingly. In 1998, the VIL trout showed good SW-tolerance from late February until mid April. In both years, SW-tolerance began to regress in late April-early May. The morphometric data (Table IV) suggest, however, that the relatively wide period of good SW-tolerance in 1998 may have been affected by non-random size selection of fish from the main stock.



The temporal increase in gill Na^+,K^+-ATPase activity in VIL trout was more pronounced and well-defined in 1997 than in 1998, where the plateau of highest activity lasted for several months. Stemming from a domesticated stock, the observed differences are the likely result of the size selection, which could have biased and prolonged the duration of the "average" smoltification period in the VIL trout. Decreasing levels of gill Na^+,K^+-ATPase activity was observed in mid April in 1997, while an activity decrease was measured approximately one month later in 1998. *In taking the unfortunate selection of fish into account, it is suggested that VIL smolts should be released no later than early-mid April.*

Skibelund Hatchery (SKI 1+, 2+)

The breeding stock of trout at the Skibelund Hatchery is each year supplemented by wild spawners caught by electrofishing in the Gudenå River system. Only 1+ trout are normally used for smolt releases in the Gudenå River but since this strategy has failed to give satisfactory returns of mature spawners (hatchery personnel; pers. comm. 1998), attempts were made to increase the returns by supplementing the smolt releases with 2+ trout in 1998. Smolt development was therefore investi-gated in both 1+ and 2+ trout in 1998.

When reared under the same conditions, the 1+ trout (size: 12-16 cm) showed an overall better SW-tolerance and higher gill Na^+,K^+-ATPase activity through the whole season than the 2+ trout (size: 18-25 cm) of the same genetic strain (Fig. 5A and C). At the same time, 1+ trout showed a more distinct seasonal variation in these parameters (i.e. smolt development) than the 2+ trout. The SW-tolerance of the 2+ trout was surprisingly poor and unchanged through the season, and gill Na^+,K^+-ATPase activities were the lowest recorded in any of the investigated strains (1.5–2.6 µmol ADP/mg/h). Our data thus suggest, that smoltification was

less intense and the level of SW-tolerance was poorer in 2+ than in 1+ trout of this strain. This was an unexpected finding, as SW-tolerance of immature 2+ smolts is better than 1+ smolts in Baltic salmon, *S. salar* (Lundqvist *et al.*, 1986). In addition, size was expected to have a general positive effect on general SW-tolerance due to a more favourable surface-to-volume ratio (Hoar, 1988). Only few studies have investigated parallel smolt development in 1+ and 2+ age groups of the same strain reared under identical conditions. Langdon & Thorpe (1985) reported different seasonal patterns of change in SW-tolerance, gill Na^+,K^+-ATPase activity and size and number of chloride cells in immature 1+ and 2+ Atlantic salmon (*S. salar*) smolts. In their study, the 2+ smolts developed good SW-tolerance without simultaneous increase in gill Na^+,K^+-ATPase activity. In contrast to this, a 2-fold elevation in gill Na^+,K^+-ATPase activity was observed in 1+ smolts before any of these survived the SW-tests. Enzyme activity and SW-tolerance reached the same levels, however, in 1+ and 2+ smolts but differed in the timing. This suggests that elevated gill Na^+,K^+-ATPase activity is not as important for achieving high SW-tolerance in 2+ Atlantic salmon smolts as in 1+ smolts. This is probably explained by the more favourable surface-to-volume ratio in larger fish. In contrast to this, Muona & Soivio (1992) observed a similar development of gill Na^+,K^+-ATPase activity in 1+ and 2+ smolts of Atlantic salmon.

In accordance with our data, Zaugg & McLain (1972) found elevated gill Na^+,K^+-ATPase levels in hatchery reared 1+ steelhead trout (*O. mykiss*) during the spring and no elevations in 2+ trout. This suggest an impaired smolt development and may imply an impaired migration tendency of the 2+ trout compared with the 1+ trout. In contrast, Muona & Soivio (1992) measured a 2-fold increase in gill Na^+,K^+-ATPase activity in 2+ brown trout smolts, so the present lack of increasing gill Na^+,K^+-ATPase activity is not a general feature of smolting in 2+ brown trout. The above species difference in the development of gill Na^+,K^+-ATPase activity in 1+ and 2+ fish, is likely to reflect the less anadromous life history pattern of the brown trout compared to the strongly anadromous Atlantic salmon. Further, there is evidence that the decision to migrate (i.e. smoltify) or reside in the river (and become sexually mature) is influenced by food availability, and thus fat deposition and growth rate of the fish (Rowe and Thorpe, 1990). Favourable growth conditions for an extended period of time (2 years) may therefore explain the impaired smoltification of 2+ fish of the SKI strain, and these fish may not be suitable for smolt releases. *Our data suggest, that 1+ trout of the SKI strain should be released as smolts very early in the season and no later than mid March.*

Trevad Hatchery (TRE)

The strain of trout at Trevad Hatchery is first generation offspring from wild spawners caught in the River Karup Aa (world famous for its return of very large mature sea trout). Good SW-tolerance was observed from the beginning of the study in February until early April, after which it gradually regressed (Fig. 4A,B). Gill Na^+,K^+-ATPase activity increased to the highest levels of all strains investigated (7.10 ± 0.52) between late February and early April and then declined abruptly within two weeks in April. Such high enzyme levels may reflect a very anadromous life history of this strain, being in line with the fact that TRE fish are F1 siblings of anadromous wild fish. Unfortunately, this rather "normal" smolt profile may have been biased by a serious misunderstanding by the hatchery personnel. All the larger 1+ trout (avg. size 13-14 cm) were released as smolts in early April (when smolt development was maximal!) and only significantly smaller individuals (less than 10 cm) were left in the hatchery for further sampling, e.g. 10.2 ± 0.2 cm on 14 April (Table III). These fish represent the slow growing lower modal group and are smaller than the minimum size normally

required for smolting (Tanguy *et al.*, 1994). The apparent drop in gill Na^+,K^+-ATPase activity may thus be influenced by this bias in fish size. It is noticeable though, that there was not a dramatic loss of SW-tolerance but only a slight impairment by the succeeding sampling. The relatively good SW-tolerance and elevated gill Na^+,K^+-ATPase activity in the lower modal growth group compared with the levels seen at the end of the study, suggest that these small individuals (<10 cm) of the TRE strain did indeed undergo smoltification. This is in line with observations of a relatively small average size of migrating smolts in the Karup Aa River (app. 13 cm in 1997; Stig Pedersen, pers. comm. 1999), and smoltification at a rather small size may be an adaptive feature of this genetic strain of brown trout. *Based on the present smolt profiles and the above considerations, it is recommended that TRE trout are released as smolts no later than late April.*

4.3. Correlation between muscle water content (MWC) and plasma $[Na^+]$

Using data from every fish exposed to SW, significant correlation's (P<0,0001) between muscle water content and plasma $[Na^+]$ were found for all the investigated strains of brown trout (Fig. 10). These results are in accordance with the finding in 1997 (Nielsen & Madsen, 1998) and *indicate that MWC is a very useful and reliable tool to evaluate the development of SW-tolerance in brown trout.*

4.4. Gill Na^+,K^+-ATPase activity as a predictive measure of smolt development

In all strains except THY, there was a significant statistical correlation between gill Na^+,K^+-ATPase activity and the ability of brown trout to osmoregulate in 24-h SW tests (Fig. 11). Compared with the 1997-study, the correlations are better in 1998. The possible reasons for this is better homogeneity between FW- and SW groups with regard to size, standardised feeding procedure before SW-tests, and the general avoidance of critically low SW-temperatures. *In both years, however, it can be concluded that gill Na^+,K^+-ATPase activity is generally a reliable a predictive measure of SW-tolerance in brown trout.*

4.5. Comparison of the SW-tolerance in males and females

There was no significant difference in the SW-tolerance of males and females in any of the stocks during the study period (two-way ANOVA).

4.6. Effect of low SW-temperature on the SW-tolerance of brown trout smolt

The experience from both years of investigation is that SW temperatures below 2-3°C lead to a severe impairment of the SW-tolerance of brown trout smolts, regardless of the Na^+,K^+-ATPase activity in the gills. *It is therefore recommended, that direct coastal releases of hatchery trout is carried out only when the SW temperature is higher than 4°C.*

4.7. Factors affecting between-year variation in smolt development

In the present two-year study, there were a few examples of slight differences in the timing of peak smolt development. Only a few studies have examined such variation in smolt development in hatchery-held salmonids. Zaugg & Mahnken (1991) observed between-year variation in gill Na^+,K^+-ATPase activity profiles in chinook salmon (*Oncorhynchus tshawytscha*) during a 5 year study. Dickhoff *et al.* (1995) reported significant variation in smolt quality in chinook salmon between years. From laboratory studies, several physical and biological parameters are known to influence the rate, intensity and duration of smoltification, and variations in any of these parameters at the hatcheries are likely to affect smolt development. Growth rate is an important initiating factor of smoltification (*S. trutta*: Fahy, 1990; *S. salar*: Whitesel, 1993), and Berge *et al.* (1995) suggested that growth rate may be important for the development of gill Na^+,K^+-ATPase activity in under-yearling Atlantic salmon. Dickhoff *et al.* (1995) ascribed differences in growth rate to account for the observed between-year differences in smolt development in chinook salmon. Although growth rate is genetically determined to some extent, environmental variation is important in determining the development and behaviour of the fish (Mangel, 1994). The primary single physical factor affecting growth rate and thus smoltification in hatchery-held salmonids is water temperature. Increasing water temperature accelerates both the initiation of smoltification (e.g. Solbakken *et al.*, 1994) and the succeeding de-smoltification (e.g. McCormick *et al.*, 1994; Stefansson *et al.*, 1998; Hoar, 1988). Rearing density at the hatchery may also affect smoltification via effects on the hierarchical structure, competitive interactions, and general stress level within the fish population. High rearing densities appear to retard smolt development (plasma thyroxine levels, gill Na^+,K^+-ATPase activity and SW-tolerance (Schreck *et al.*, 1985; Patino *et al.*, 1986; Soivio *et al.*, 1988). Even though unlikely to be a major source of variation, differences in rearing densities at the hatcheries could thus contribute to variation in smolt development. Phenotypic expression of key smolt features such as migratory tendency is under both environmental and genetic influence (Jonsson, 1985). Hansen *et al.* (1997) observed no significant differences in haplotype frequencies among age classes of the same domesticated strains of brown trout, but significant differences were found between F1 stocks. F1 stocks of trout, that are regularly supplemented by genes from wild spawners (as THY, LV and TRE), are likely to display greater between-year variation in smolt development compared with domesticated stocks. THY showed some variability in smolt development that may be ascribed genetic variability in 1997 and 1998, whereas LV did not show such variability. *Smolt intensity varied between the investigated strains, but the present data do not suggest that smolt quality is unequivocally improved in strains using F1 siblings compared to purely domesticated strains.*

5. Conclusions

The following conclusions can be drawn from two years of investigation of smolt development in Danish hatchery-reared brown trout:

- Silvering is an unreliable single indicator of SW-tolerance in hatchery trout. Especially during de-smoltification, where SW-tolerance is lost before any significant loss of silvering.
- Muscle water content is a reliable measure of SW-tolerance in 24-h SW tests. This parameter can easily be measured by the hatchery personnel.

- All 9 investigated strains of brown trout showed significant seasonal smolt development (i.e. increasing SW-tolerance and gill Na^+,K^+-ATPase activity).
- Small between-strain variations were found in smolt intensity and timing of smolt development.
- Between-year variation in smolt development was negligible in most strains.
- All strains began to lose smolt characteristics in early-mid April.
- SW test temperatures of less than 2-3°C negatively affect SW-tolerance irrespective of current smolt status.
- Based on the present results, it is suggested, that all releases of brown trout smolt take place before loss of SW-tolerance and gill Na^+,K^+-ATPase activity; i.e. no later than late March - early April in most of the presently investigated stocks.
- Direct coastal releases should only be carried out when SW temperatures are higher than 4°C, irrespective of smolt status.

As a final remark to the reader, it is important to emphasise that laboratory assessments of smolt development only give measures of acute SW-tolerance. At present, only preliminary information is available about concurrent levels of SW-tolerance, gill Na^+,K^+-ATPase activity and migratory behaviour. In other words, the present data do not provide any evidence about migratory patterns as a function of smolt status. Presently, it can only be assumed that the two events correlate in time, or at least that migratory activity is ceasing when SW-tolerance is regressing (i.e. de-smoltification). In order to provide data for this question, studies have recently been initiated (in collaboration with Kim Aarestrup, DIFRES, Silkeborg) to investigate the link between smolt status and post-release behaviour of hatchery reared trout.

6. Acknowledgements

We would like to thank the following hatchery personnel for their help and co-operation during the project: P. and T. Ebbesen (Haarkær), O. Jøker (Spjarup 1), F. Skov (Vork), K. Kristensen (Vilhelmsborg), T. and F. Andersen (Skibelund) and T. Johansen (Trevad). Ms. H.A. Petersen and J.S. Hansen and H. Blenstrup (Odense University) are acknowledged for excellent technical assistance. This project was supported by *the Ministry of Food, Agriculture and Fisheries* according to the *Danish Programme of Rehabilitation and Stocking (Handlingsplan for Fiskeplejen) 1998* (project no. 1329).

7. Figures and Tables

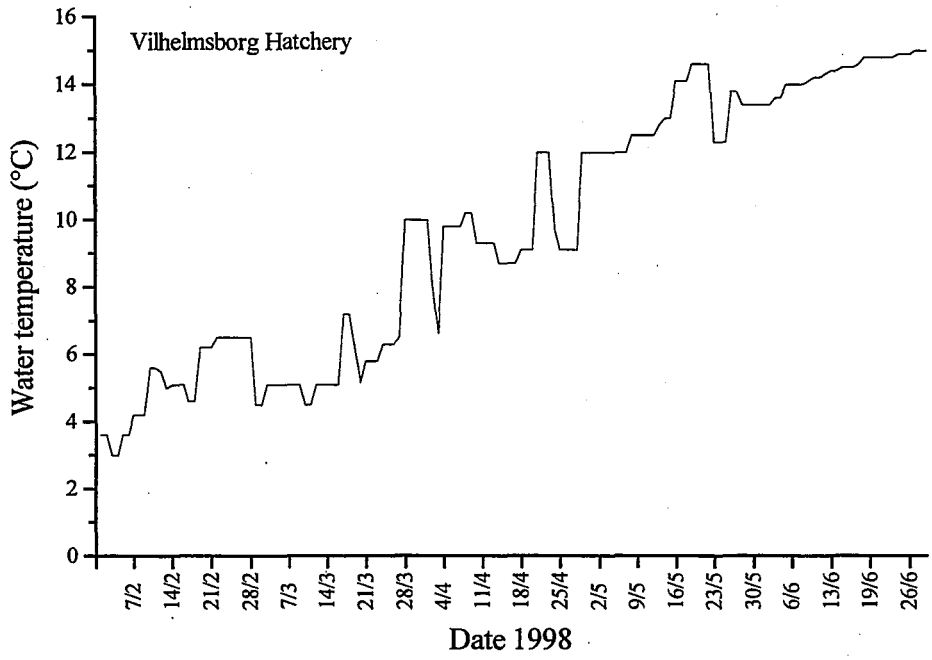
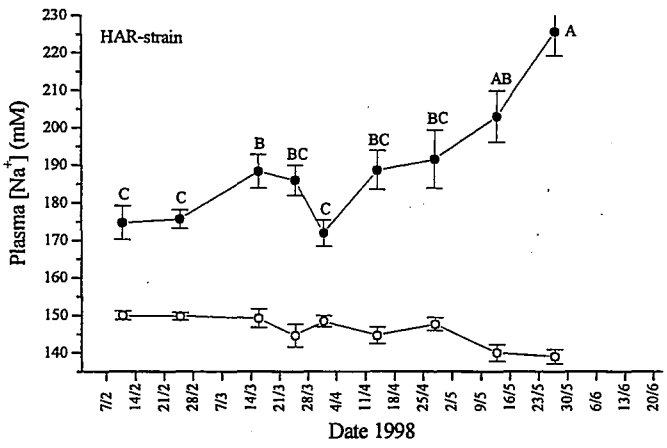


Fig. 1: Water temperature (°C) in the FW ponds at the Vilhelmsborg Hatchery.

A



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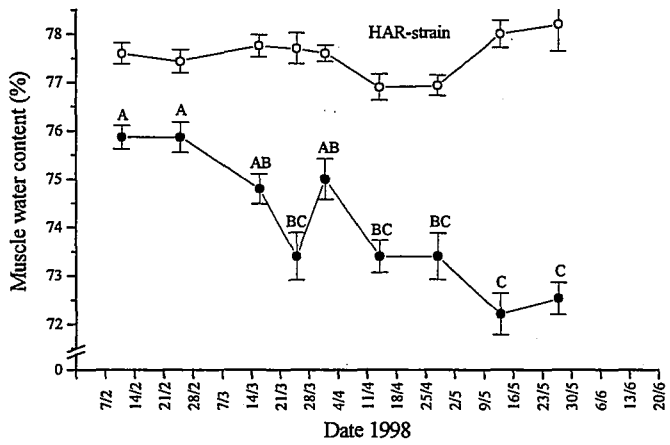

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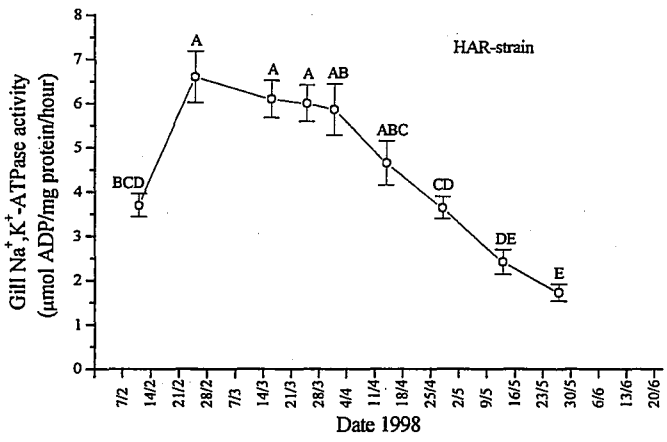
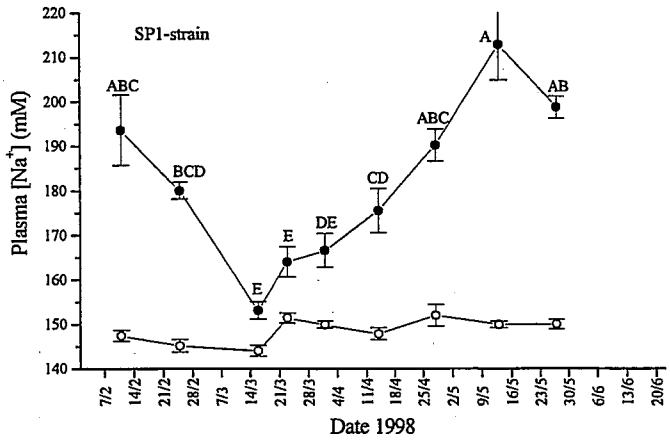


Fig. 2: Smoltification in HAR trout, 1998. Seasonal changes in (**A**) plasma [Na^+] (mM) and (**B**) muscle water content (%) in FW-fish (o) and 24 h after transfer to 30 ‰ SW (•); **C**: gill Na^+,K^+-ATPase activity (µmol ADP mg^{-1} h^{-1}). Values are mean ± SE of 8-10 fish. Values with shared letters are not significantly different (P>0.05).

A



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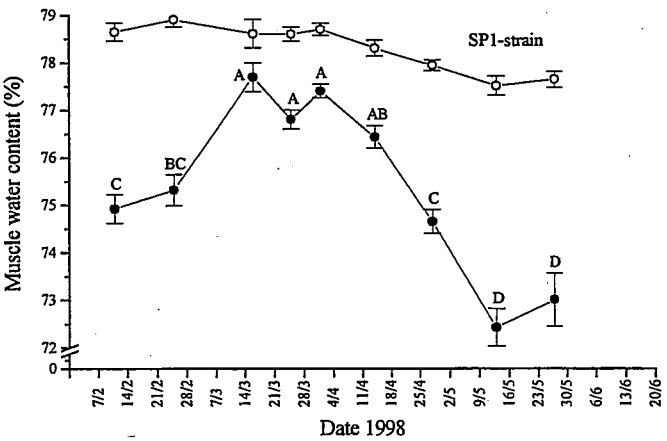

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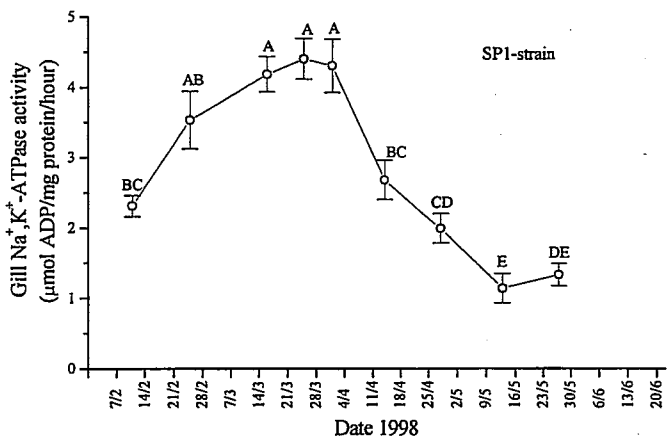
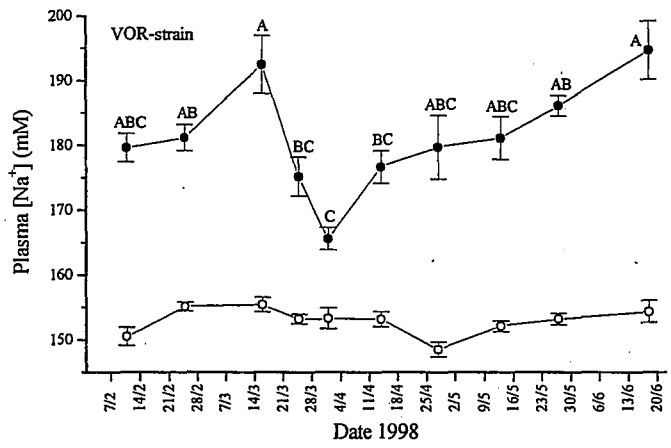


Fig. 3: Smoltification in SP1 trout, 1998. Seasonal changes in (**A**) plasma [Na^+] (mM) and (**B**) muscle water content (%) in FW-fish (o) and 24 h after transfer to 30 ‰ SW (•); **C**: gill Na^+,K^+-ATPase activity (µmol ADP mg^{-1} h^{-1}). Values are mean ± SE of 8-10 fish. Values with shared letters are not significantly different (P>0.05).

A



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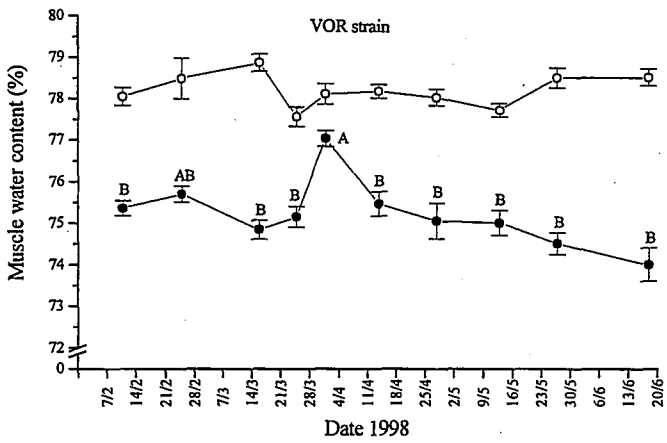

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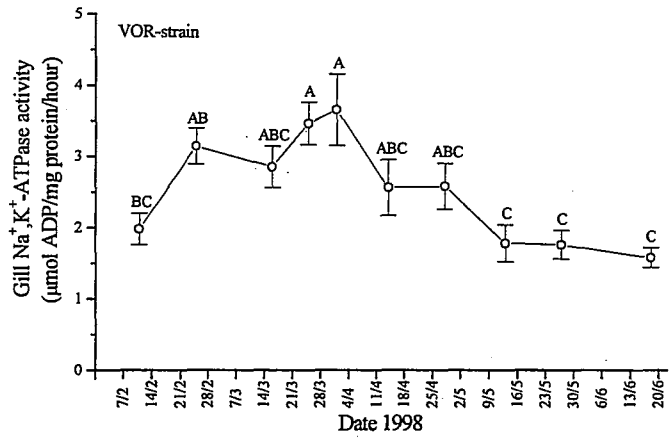
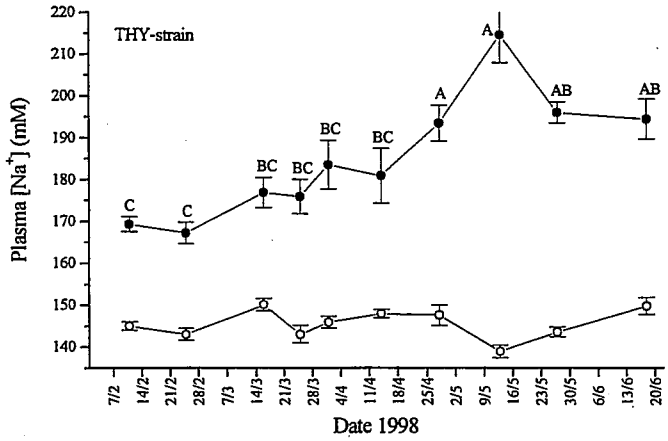


Fig. 4: Smoltification in VOR trout, 1998. Seasonal changes in (**A**) plasma [Na^+] (mM) and (**B**) muscle water content (%) in FW-fish (o) and 24 h after transfer to 30 ‰ SW (•); **C**: gill Na^+,K^+-ATPase activity (µmol ADP mg^{-1} h^{-1}). Values are mean ± SE of 8-10 fish. Values with shared letters are not significantly different (P>0.05).

VILHELMSBORG HATCHERY: THY-STRAIN

A



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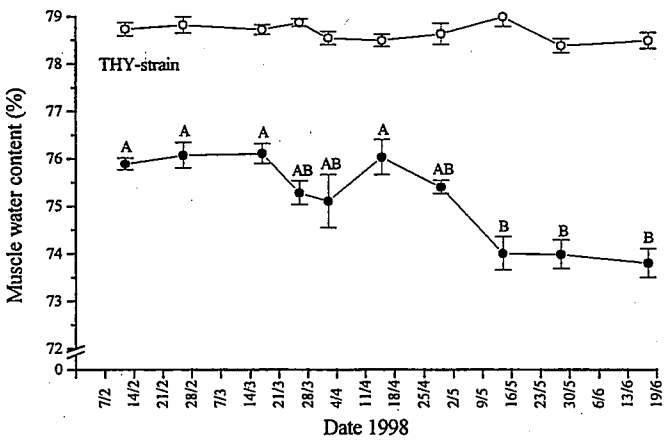

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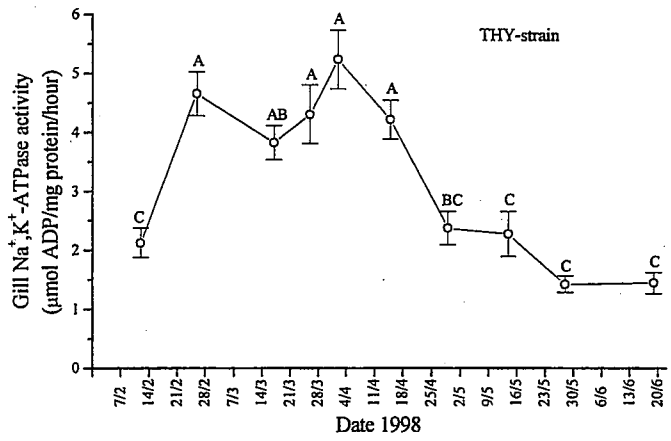
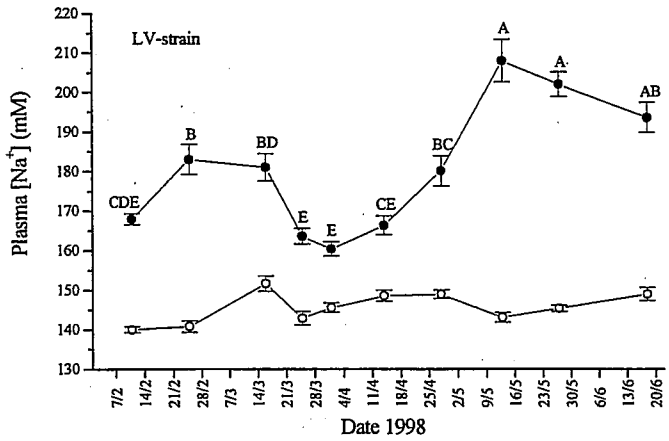


Fig. 5: Smoltification in THY trout, 1998. Seasonal changes in (**A**) plasma [Na^+] (mM) and (**B**) muscle water content (%) in FW-fish (o) and 24 h after transfer to 30 ‰ SW (•); **C**: gill Na^+,K^+-ATPase activity (µmol ADP mg^{-1} h^{-1}). Values are mean ± SE of 8-10 fish. Values with shared letters are not significantly different (P>0.05).

VILHELMSBORG HATCHERY: LV-STRAIN

A



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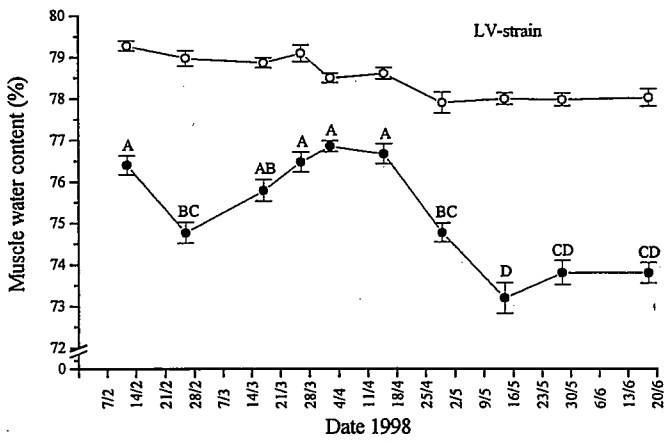

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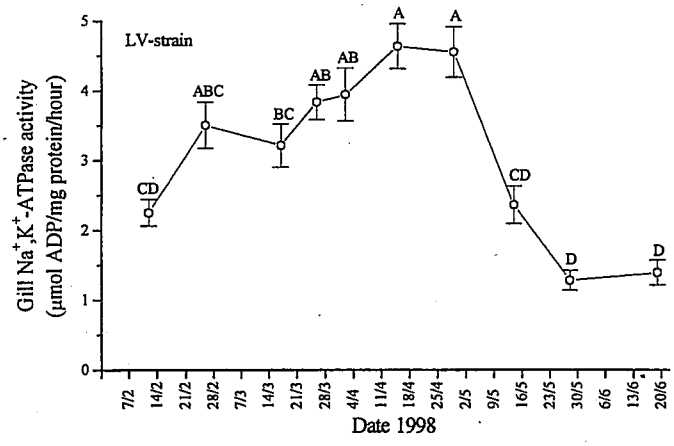
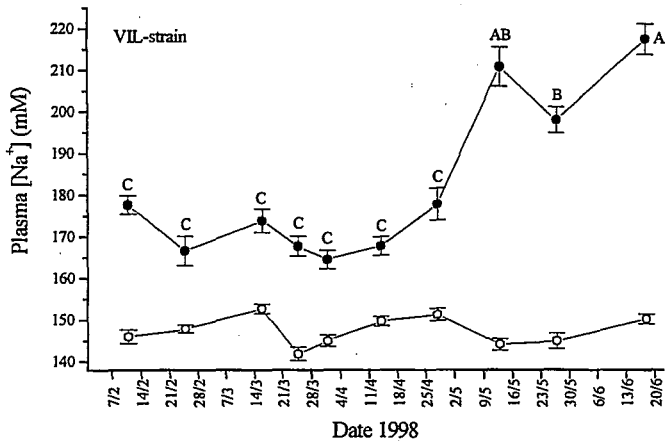


Fig. 6: Smoltification in LV trout, 1998. Seasonal changes in (**A**) plasma [Na^+] (mM) and (**B**) muscle water content (%) in FW-fish (o) and 24 h after transfer to 30 ‰ SW (•); **C**: gill Na^+,K^+-ATPase activity (µmol ADP mg^{-1} h^{-1}). Values are mean ± SE of 8-10 fish. Values with shared letters are not significantly different (P>0.05).

A



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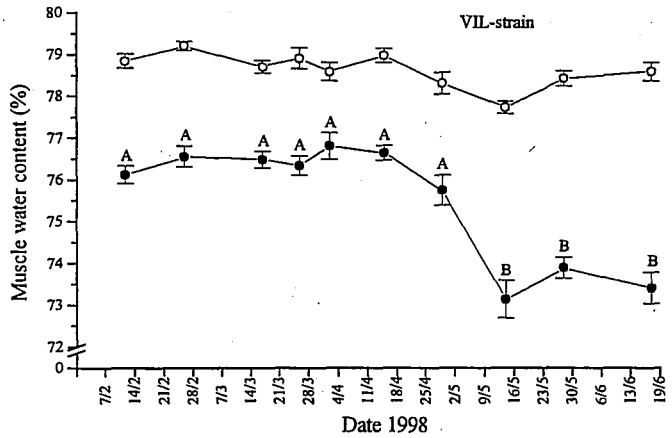


C

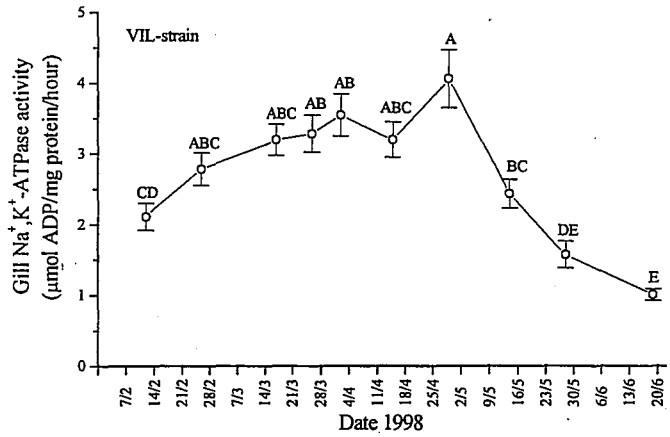
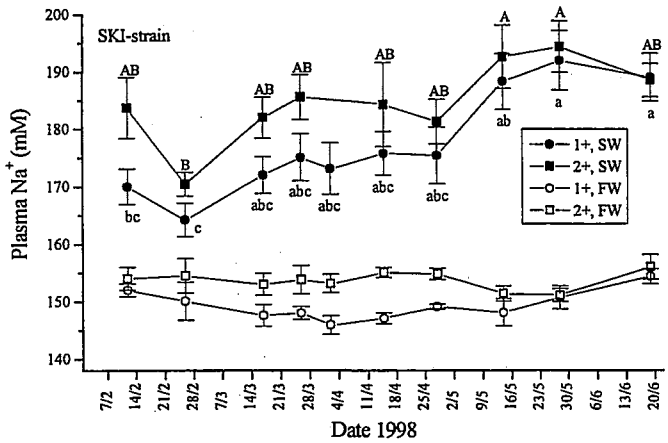


Fig. 7: Smoltification in VIL trout, 1998. Seasonal changes in (**A**) plasma [Na^+] (mM) and (**B**) muscle water content (%) in FW-fish (o) and 24 h after transfer to 30 ‰ SW (•); **C**: gill Na^+,K^+-ATPase activity (µmol ADP mg^{-1} h^{-1}). Values are mean ± SE of 8-10 fish. Values with shared letters are not significantly different (P>0.05).

SKIBELUND HATCHERY, Gudenå, 1+ & 2+ trout

A



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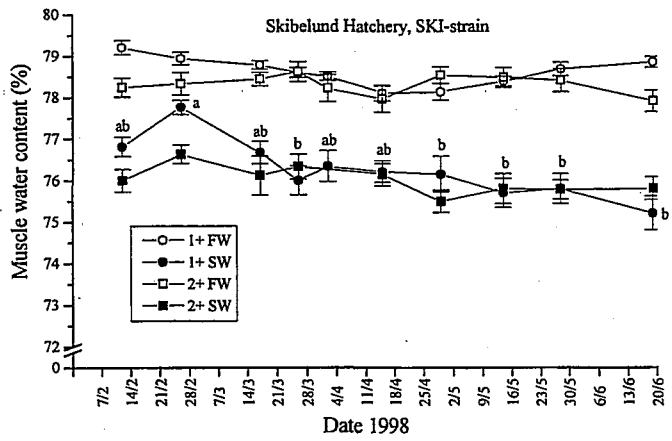

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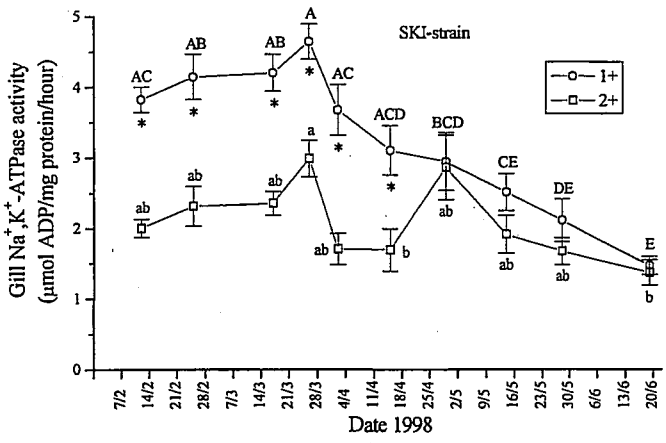
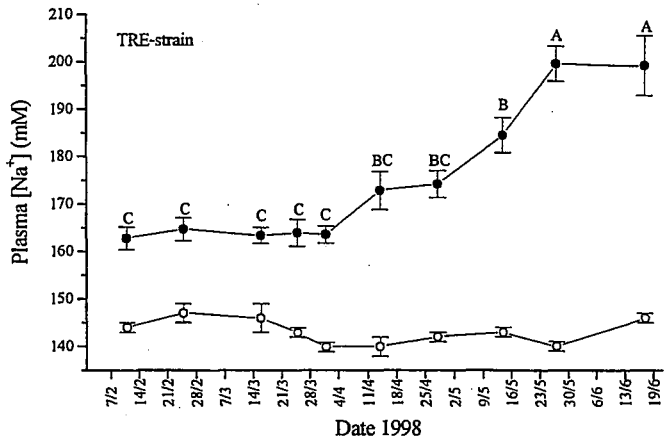


Fig. 8: Smoltification in SKI trout (1+ & 2+), 1998. Seasonal changes in (**A**) plasma [Na^+] (mM) and (**B**) muscle water content (%) in FW-fish (1+, o and 2+, □) and 24 h after transfer to 30 ‰ SW (1+, • and 2+, ■); **C**: gill Na^+,K^+-ATPase activity (µmol ADP mg^{-1} h^{-1}). Values are mean ± SE of 8-10 fish. Values with shared letters are not significantly different (P>0.05). Asterisks indicate significant difference between activity in 1+ and 2+ trout.

A



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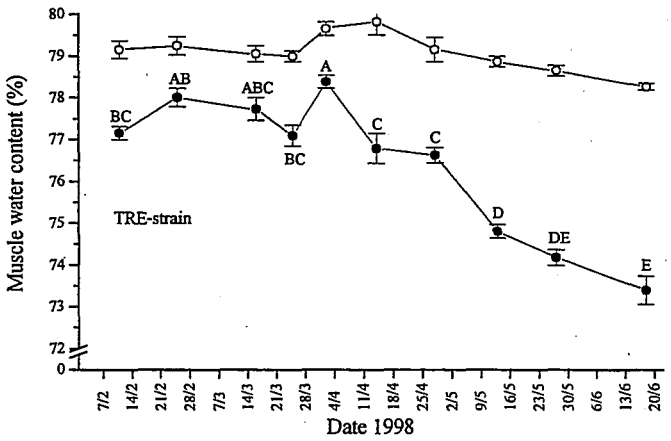

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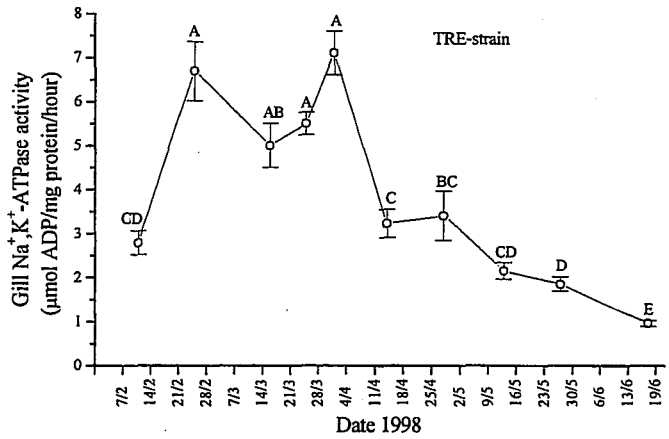

Fig. 9: Smoltification in TRE trout, 1998. Seasonal changes in (A) plasma [Na^+] (mM) and (B) muscle water content (%) in FW-fish (o) and 24 h after transfer to 30 ‰ SW (•); C: gill Na^+,K^+-ATPase activity (µmol ADP mg^{-1} h^{-1}). Values are mean ± SE of 8-10 fish. Values with shared letters are not significantly different (P>0.05).

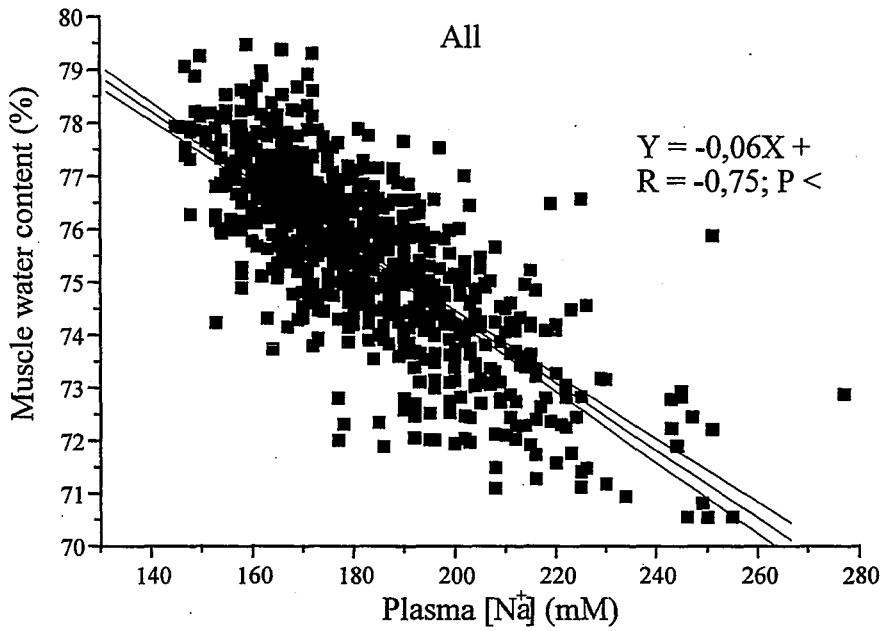

Fig. 10: Linear regression with 95 % confidence bands between muscle water content (%) and plasma [Na^+] (mM) in the all strains of *S. trutta* 24 h after transfer to SW.

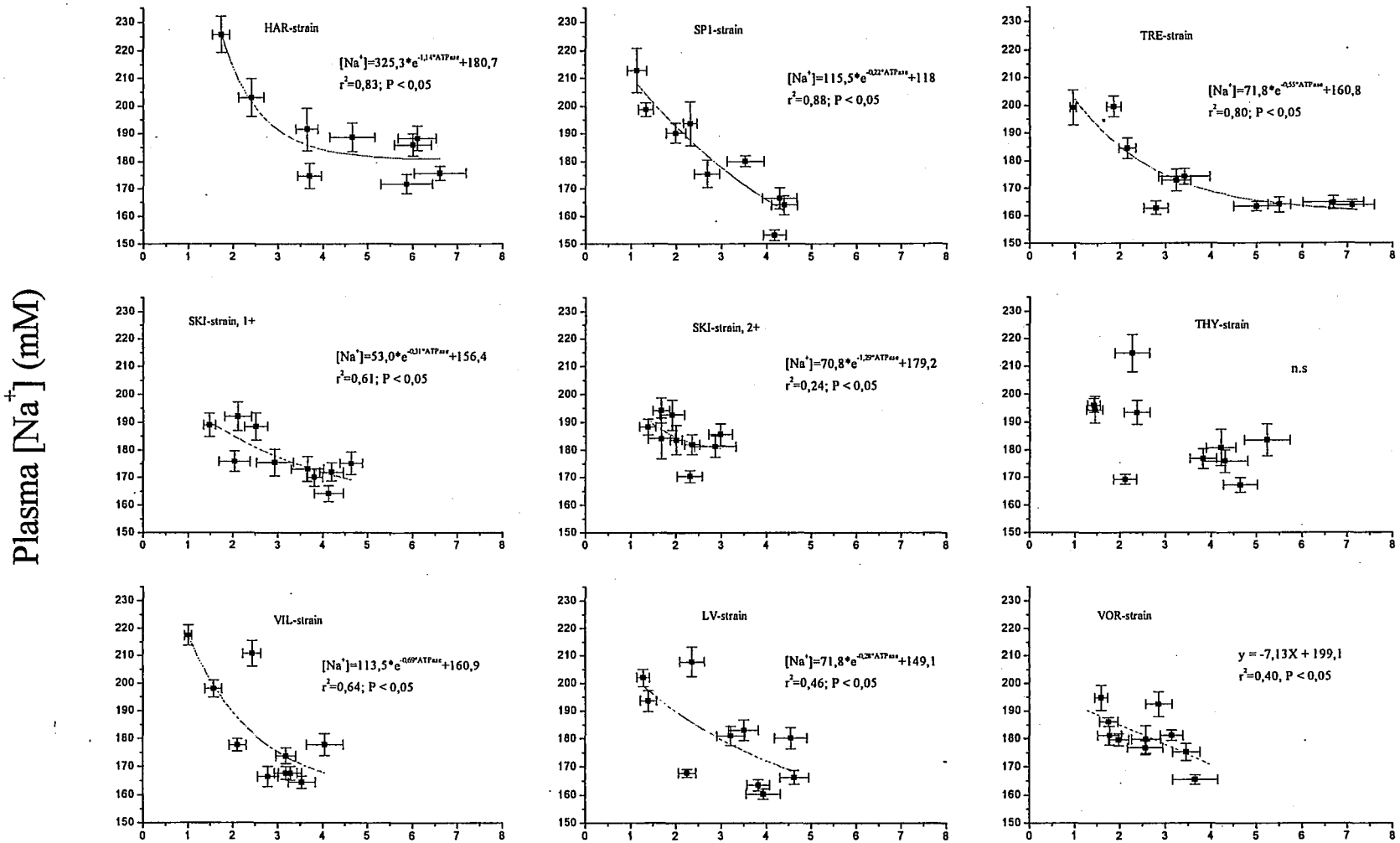

Fig. 11. Correlations between mean values of plasma [Na^+] after 24 h SW exposure and mean values of Na^+,K^+-ATPase activity in gills from the FW-group at the same sampling date at the different hatcheries.

Table I. Hårkær Hatchery. Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	11/2	25/2	16/3	25/3	1/4	14/4	28/4	13/5	27/5	
Max SW-temp. (°C)	3,6	5,2	2,8	2,6	6,8	5,0	7,8	12,2	11,7	
Min SW-temp. (°C)	3,5	5,1	2,3	2,4	6,5	4,8	7,6	11	10,2	
Salinity (‰)	29,8	29,7	29,8	29,1	29,8	30,0	30,0	30,4	30,9	
FW-group, Length (cm)	17,9 ± 0,26	19,1 ± 0,14	18,8 ± 0,38	19,3 ± 0,19	19,5 ± 0,26	20,3 ± 0,33	20,5 ± 0,2	20,3 ± 0,46	20,4 ± 0,25	
SW-group, Length (cm)	18,0 ± 0,23	18,9 ± 0,17	18,3 ± 0,32	18,9 ± 0,18	19,0 ± 0,19	19,6 ± 0,25	19,9 ± 0,4	20,2 ± 0,36	20,1 ± 0,33	
CF	1,16 ± 0,02	1,17 ± 0,01	1,18 ± 0,02	1,2 ± 0,01	1,17 ± 0,02	1,18 ± 0,02	1,17 ± 0,01	1,15 ± 0,02	1,07 ± 0,02	

Table II. Spjarup 1 Hatchery. Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	11/2	25/2	16/3	25/3	1/4	14/4	28/4	13/5	27/5	
Max SW-temp. (°C)	6,5	7,5	6,4	4,7	10,1	6,8	10,3	13,3	12,5	
Min SW-temp. (°C)	6,3	6,9	4,7	4,2	9,4	6,3	8,7	12,7	11,9	
Salinity (‰)	30,1	30,0	30,2	30,1	30,2	30,3	30,4	30,6	30,9	
FW-group, Length (cm)	14,1 ± 0,14	14,3 ± 0,2	14,5 ± 0,1	15,0 ± 0,18	16,0 ± 0,2	16,4 ± 0,2	17,8 ± 0,14	17,1 ± 0,17	17,4 ± 0,22	
SW-group, Length (cm)	14,3 ± 0,2	14,1 ± 0,18	14,3 ± 0,13	14,8 ± 0,2	16,2 ± 0,2	17,1 ± 0,3	17,2 ± 0,3	16,5 ± 0,3	16,7 ± 0,24	
CF	1,08 ± 0,03	1,08 ± 0,02	1,06 ± 0,02	1,08 ± 0,01	1,12 ± 0,02	1,1 ± 0,02	1,18 ± 0,02	1,15 ± 0,01	1,18 ± 0,02	

Table III. Vork Hatchery. Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	11/2	25/2	16/3	25/3	1/4	14/4	28/4	13/5	27/5	18/6
Max SW-temp. (°C)	7,1	6,6	4,9	3,3	7,2	5,9	9,0	11,8	10,8	11,2
Min SW-temp. (°C)	6,5	6,0	3,9	3,0	6,7	5,4	7,8	11,3	10,2	-
Salinity (‰)	29,9	29,4	29,3	30,0	30,0	29,2	29,4	29,9	30,9	30,5
FW-group, Length (cm)	15,3 ± 0,33	16,2 ± 0,42	17,0 ± 0,19	17,0 ± 0,32	17,2 ± 0,52	17,0 ± 0,37	16,8 ± 0,25	17,8 ± 0,36	19,1 ± 0,49	17,0 ± 0,59
SW-group, Length (cm)	15,5 ± 0,24	15,6 ± 0,46	17,6 ± 0,29	16,4 ± 0,35	17,1 ± 0,31	17,4 ± 0,35	16,6 ± 0,43	16,8 ± 0,51	18,2 ± 0,56	18,4 ± 0,74
CF	1,13 ± 0,01	1,15 ± 0,01	1,19 ± 0,02	1,12 ± 0,01	1,1 ± 0,02	1,1 ± 0,02	1,12 ± 0,03	1,11 ± 0,02	1,23 ± 0,03	1,14 ± 0,03

Table IV. Vilhelmsborg Hatchery. **THY strain.** Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	12/2	26/2	17/3	26/3	2/4	15/4	29/4	14/5	28/5	19/6
Max SW-temp. (° C)	5,7	7,2	5,7	5,4	8,4	6,4	8,9	10,9	11,2	11,5
Min SW-temp. (° C)	5,4	6,6	4,1	4,8	8,0	6,2	8,7	10,5	10,4	11,3
Salinity (‰)	30,0	30,3	30,6	29,4	30,1	30,1	30,0	30,3	30,6	30,8
FW-group, Length (cm)	12,9 ± 0,16	13,4 ± 0,4	13,6 ± 0,1	13,7 ± 0,27	14,5 ± 0,25	14,5 ± 0,28	15,0 ± 0,28	14,9 ± 0,34	14,3 ± 0,2	16,2 ± 0,18
SW-group, Length (cm)	13,4 ± 0,2	13,6 ± 0,4	14,0 ± 0,3	14,0 ± 0,29	14,5 ± 0,24	14,6 ± 0,2	14,9 ± 0,28	14,5 ± 0,28	15,3 ± 0,31	16,6 ± 0,27
CF	1,01 ± 0,01	1,07 ± 0,01	1,02 ± 0,01	1,04 ± 0,01	1,1 ± 0,04	1,07 ± 0,02	1,03 ± 0,01	1,09 ± 0,03	1,06 ± 0,02	1,17 ± 0,02

Table V. Vilhelmsborg Hatchery. **LV strain.** Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	12/2	26/2	17/3	26/3	2/4	15/4	29/4	14/5	28/5	19/6
FW-group, Length (cm)	12,3 ± 0,1	12,7 ± 0,22	12,8 ± 0,17	12,6 ± 0,14	13,4 ± 0,16	13,9 ± 0,17	15,5 ± 0,17	15,8 ± 0,19	15,7 ± 0,14	17,9 ± 0,2
SW-group, Length (cm)	12,3 ± 0,2	12,5 ± 0,12	13,1 ± 0,18	12,8 ± 0,18	13,5 ± 0,14	14,1 ± 0,2	15,1 ± 0,29	15,3 ± 0,19	15,2 ± 0,27	17,9 ± 0,29
CF	1,08 ± 0,01	1,04 ± 0,04	1,11 ± 0,01	1,07 ± 0,02	1,1 ± 0,01	1,08 ± 0,02	1,07 ± 0,02	1,06 ± 0,02	1,11 ± 0,01	1,18 ± 0,02

Table VI. Vilhelmsborg Hatchery. **VIL strain.** Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	12/2	26/2	17/3	26/3	2/4	15/4	29/4	14/5	28/5	19/6
FW-group, Length (cm)	12,7 ± 0,24	13,1 ± 0,18	13,6 ± 0,16	13,7 ± 0,18	14,2 ± 0,2	14,6 ± 0,16	15,3 ± 0,19	15,8 ± 0,22	15,4 ± 0,19	16,6 ± 0,16
SW-group, Length (cm)	12,5 ± 0,24	13,0 ± 0,23	13,4 ± 0,17	13,4 ± 0,42	14,2 ± 0,15	14,4 ± 0,11	15,4 ± 0,17	15,2 ± 0,22	15,7 ± 0,13	16,3 ± 0,23
CF	1,08 ± 0,01	1,09 ± 0,02	1,12 ± 0,02	1,12 ± 0,01	1,09 ± 0,01	1,14 ± 0,02	1,08 ± 0,01	1,08 ± 0,02	1,06 ± 0,02	1,1 ± 0,01

Table VII. Skibelund Hatchery (1+ trout). Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	12/2	26/2	17/3	26/3	2/4	15/4	29/4	14/5	28/5	19/6
Max SW-temp. (° C)	6,4	7,1	4,9	4,8	5,7	4,8	8,3	12,8	10,9	11,4
Min SW-temp. (° C)	5,8	6,2	4,5	3,7	2,9	4,1	8,1	11,2	10,1	10,4
Salinity (‰)	29,8	29,9	29,5	30,0	30,2	29,2	29,8	29,6	29,6	30,1
FW-group, Length (cm)	12,0 ± 0,29	13,3 ± 0,18	12,6 ± 0,23	13,0 ± 0,36	13,2 ± 0,6	13,2 ± 0,38	14,7 ± 0,27	14,9 ± 0,33	15,4 ± 0,28	16,3 ± 0,32
SW-group, Length (cm)	11,7 ± 0,26	12,8 ± 0,3	12,6 ± 0,27	13,3 ± 0,26	12,5 ± 0,51	12,8 ± 0,37	14,5 ± 0,5	13,25 ± 0,33	14,4 ± 0,58	16,1 ± 0,54
CF	1,1 ± 0,02	1,1 ± 0,02	1,13 ± 0,02	1,07 ± 0,01	1,06 ± 0,02	1,21 ± 0,07	1,12 ± 0,02	1,11 ± 0,02	1,12 ± 0,02	1,06 ± 0,03

Table VIII. Skibelund Hatchery (2+ trout). Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	12/2	26/2	17/3	26/3	2/4	15/4	29/4	14/5	28/5	19/6
FW-group, Length (cm)	20,1 ± 0,72	20,5 ± 0,66	21,5 ± 0,59	21,0 ± 1,11	20,3 ± 1,1	21,7 ± 0,55	20,0 ± 0,76	23,7 ± 0,89	22,8 ± 0,56	24,6 ± 0,85
SW-group, Length (cm)	18,0 ± 0,56	23,0 ± 0,62	23,2 ± 0,67	20,1 ± 0,75	21,6 ± 0,47	22,9 ± 0,49	22,0 ± 0,4	21,5 ± 0,76	23,1 ± 0,82	25,2 ± 0,8
CF	1,07 ± 0,02	1,18 ± 0,02	1,2 ± 0,03	1,23 ± 0,02	1,06 ± 0,02	1,14 ± 0,03	1,09 ± 0,40	1,12 ± 0,02	1,12 ± 0,03	1,17 ± 0,03

Table IX. Trevad Hatchery. Max & min water temperature and salinity in SW tank, fork-length (FW- and SW-groups) and condition factor (CF, based on FW-groups) on the different sampling dates in 1998.

Date	11/2	25/2	16/3	25/3	1/4	14/4	28/4	13/5	27/5	18/6
Max SW-temp. (° C)	6,1	7,0	5,8	5,0	8,9	6,1	10,3	13,2	12,4	13,6
Min SW-temp. (° C)	5,9	6,6	5,0	4,6	-	5,8	10,1	12,9	12,1	12,3
Salinity (‰)	30,0	30,0	30,1	30,2	30,3	30,4	30,5	30,1	30,7	30,8
FW-group, Length (cm)	13,1 ± 0,6	14,1 ± 0,7	13,2 ± 0,44	13,2 ± 0,3	12,8 ± 0,23	10,0 ± 0,3	11,6 ± 0,2	12,0 ± 0,19	12,4 ± 0,13	13,4 ± 0,13
SW-group, Length (cm)	14,3 ± 0,4	12,9 ± 0,2	15,6 ± 0,65	13,0 ± 0,42	12,6 ± 0,2	10,4 ± 0,3	11,9 ± 0,3	12,1 ± 0,16	12,7 ± 0,37	12,7 ± 0,18
CF	0,99 ± 0,01	0,95 ± 0,03	1,01 ± 0,03	0,99 ± 0,01	1,01 ± 0,01	1,01 ± 0,02	1,08 ± 0,02	1,04 ± 0,04	1,11 ± 0,02	1,12 ± 0,01

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Part 2:

Survival and Growth of Hatchery-reared Brown Trout (*Salmo trutta*) after Direct Transfer to Coastal Water: Relation to Seasonal Timing

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1. Introduction

Each year, direct releases of hatchery-reared brown trout into coastal water are carried out in Denmark; in 1997, approximately 300.000 trout were released (N. Thomassen, pers. comm.). Around the Danish island Funen, most direct coastal releases take place in early June (hatchery personnel, pers. comm.). The fate of the trout following release into seawater is unknown. When released directly into seawater (SW), hatchery-reared trout have to deal with several factors that may reduce their survival: water- and ion-regulation, foraging in a new environment and the presence of predators. Each of these factors represents a potential hazard but their combined action may have a synergistic impact on the mortality of the released fish. The ability to avoid a predator can be expected to be dramatically altered by osmotic or nutritive imbalances. These factors must also be suspected to act as selective forces on wild brown trout during their SW migration; however, in wild fish the timing of the SW encounter is controlled by the endogenous rhythm of smoltification and not by man-made decisions. The salinity of the coastal waters around Funen fluctuates because of changes in run-off and wind direction but seldom exceeds approximately 25 ppt. By using 24-hour 30 ppt SW-tests, a previous study (Nielsen & Madsen, 1998) revealed seasonal changes in the SW-tolerance of the strain of brown trout (VOR, Vork Hatchery, Egtved) most frequently used for direct coastal releases around Funen. The osmoregulatory disorder in trout following SW-transfer is, however, salinity-dependent (Hoar, 1988), and the relatively low salinity around Funen may not cause similar severe osmoregulatory problems for the trout as observed in the high-salinity tests mentioned above. Coho salmon (*Oncorhynchus kisutch*), however, have an impaired appetite and reduced growth when exposed to approximately 25 ppt SW, outside the period of maximal SW-tolerance (Young *et al.*, 1989).

The main question addressed in the present study was whether the timing of direct release into natural coastal saltwater under controlled laboratory conditions has any major effect on survival and long term growth performance of hatchery-reared brown trout.

2. Materials & Methods

In order to imitate coastal releases around Funen as closely as possible, the experiments were carried out, under controlled conditions, at the Centre for Aquatic Biology (CAB), Odense University (Kerteminde, Denmark). The marine station is situated at the dockside of the harbour of Kerteminde. Indoor experimental tanks were used and continuously supplied with running, natural seawater of the salinity and temperature in the sea near Kerteminde. Batches of hatchery brown trout were transplanted to the CAB at different dates of the spring of 1998 and transferred to seawater. After a recovery period of a few days, feeding was initiated. They were fed *ad libitum* every day and weighed and measured at regular intervals. Growth and survival were recorded.

2.1. Experimental fish and design

Approximately 300 hatchery-reared brown trout (VOR-strain) were transported on a fish trailer (300 l freshwater oxygenated with pure oxygen) from the Vork Hatchery (Jutland) to the Centre of Aquatic Biology in Kerteminde and directly transferred to seawater in the

experimental tanks (2000 l) on different dates. Photo period, water temperature and salinity followed the ambient during the study period (Fig. 1). Water temperature and salinity of the seawater were measured by a stationary data logger placed just outside the CAB near the water intake. Based on the results from the SW-challenge tests at the Vork Hatchery (Nielsen & Madsen, 1998), SW-transfers were made before, during and after the period of maximal SW-tolerance. The transfer dates were February 20th, (twice in early April), April 29th and June 19th, 1998. Following transfer to the experimental tanks, trout were fed commercial trout pellets daily *ad libitum* using request pellet feeding systems similar to those used at the Vork Hatchery. The different groups of trout were kept in separate tanks during the study for variable periods of time. Mortality was recorded, and fork length and weight of the trout were measured to the nearest mm and g, respectively, at monthly intervals after the transfers. Condition factor (CF) was calculated as (100 x weight)/length^3.

3. Results

The idea of the study was to have SW-transfer groups representing hatchery trout at the pre-smolt, smolt and de-smolted stage. Thus the original plan was to transfer groups in late February (pre-smolts), early April (smolts) and June (de-smolts). Unfortunately, however, technical problems with the seawater pump system in the newly build marine station caused the death of all the fish transferred in early and mid-April. Another group of trout was therefore transferred on April 29th (late smolts), but these fish also died because of another failure in the seawater supply system. Thus successful transfers were made in late February and mid-June, while data from the April-group are rather limited because of the above problems.

3.1. Seawater transfer dates

On February 19th, 325 trout were transferred to 27 ppt seawater at 5 °C (Fig. 1). On April 3rd, 300 trout were transferred to 15 ppt seawater at 5 °C. All these fish died a few days later because of a failure in the seawater supply system. On April 17th, 300 trout were transferred to 12 ppt seawater at 6 °C. All these fish died a few days later due to a failure in the seawater supply system. On April 29th, 390 trout were transferred to 12 ppt seawater at 9,3 °C (Fig. 1). On June 19th, 260 trout were transferred to 18 ppt seawater at 13 °C (Fig. 1).

3.2. Mortality

February transfer (pre-smolts)

There was no immediate mortality in the February group following seawater transfer. These trout had a high survival rate during the study period (Fig. 2) and in mid-July 84% of the fish had survived.

April-transfer (late smolts)

The April-group suffered a mortality of 1% during the first 5 days of seawater exposure (Fig. 2). From early May until June, the mortality increased to 23% of the transferred trout. The mortality occurred evenly throughout this period and on June 12th, 74% of the fish had survived. Unfortunately, another failure in the seawater supply system killed the remaining fish shortly afterwards.

June-transfer (de-smolts)

The June-group suffered a mortality of 8% during the first 5 days following seawater transfer (Fig. 2). In the following period, the fish continued to die off at a steady rate, reaching a total mortality of 34% by July 15th. Hereafter, the mortality ceased, and a further 1% of the fish died from July until early September. Taken together, 57% of the trout survived from June until September in this group.

3.3. Growth and performance of seawater-transferred trout

February transfer

The relative length growth rate of the trout was relatively low (3.87 - 3.47%/month) between February and early May (Fig. 2; Table I). In the next period between May 5th and June 12th, length growth rate increased to 8.76%/month (Table I) and further increased to 12.96%/month between June 12th and July 15th. The relative weight growth rate increased steeply from 7.56%/month (Table I) between February 23rd and March 31st , reaching the highest rate of 51.98%/month in the period between June 12th and July 15th . The condition factor (CF) was constant at 1.10 ± 0.01 between February 23rd and March 31st , whereafter it increased to 1.30 ± 0.01 from early May and the rest of the study period (Fig. 5). Changing CF reflects differences in the relative growth of length and weight.

April transfer

The surviving trout had a good growth and appetite, resulting in relative length and weight growth rates of 9.4 and 42.3 %/month, respectively, between May 5th and June 12th (Table I, Fig. 3 and 4). CF increased from 1.12 ± 0.01 to 1.22 ± 0.01 (Fig. 5) in this period.

June transfer

Between June 24th and July 15th the surviving trout had length and weight growth rates of 1.64 and 3.95%/month, respectively (Table I; Fig. 3 and 4). Between July 15th and September 2nd the relative length and weight growth rate increased steeply to 8.41 and 33.18%/month, respectively. CF was 1.14 ± 0.01; 1.11 ± 0.01 and 1.15 ± 0.01 on the 3 sampling dates, respectively (Fig. 5).

4. Discussion

The present study aimed at investigating the fate of hatchery-reared brown trout after direct release into low salinity seawater at ambient conditions. The main purpose was to analyse growth and survival in relation to the time of release during smolt development. Therefore, a series of SW-transfers were carefully scheduled to take place before (February), during (April) and after (June) maximal smolt development, as assessed at the hatchery by 24-h high salinity SW-transfer tests. Unfortunately, this original plan was hampered by several accidents all of which were technical in nature as described in *Results*. Due to water supply problems, pump problems etc. all fish died in the April transfers, at the time of maximal smolt development, and solid data were only obtained from release experiments at suboptimal smolt development. The results presented in this report are therefore only fragmentary, and any conclusion may at best be based on the most obvious trends in the data-set.

The growth estimates are based on the mean size of several hundred fish in a particular transfer group. This estimate was not corrected by the length and weight of trout that died during the study course (dead fish were often partly rotten when discovered), and it is unknown whether the trout experienced a size-dependent mortality following transfer to seawater. Size-dependent mortality would give a biased picture of the growth of the different groups of trout, However, Nielsen & Madsen (1998) found no effect of size on the SW-tolerance of hatchery brown trout before, during and after the period of maximal SW-tolerance.

There were large seasonal differences in the survival of brown trout following direct seawater transfer.

4.1. February-transfer

Trout transferred in February displayed the best survival of all transfer groups (Fig. 2: 84 % of the fish survived from February until July) even though this group by far was exposed to the highest salinity at the time of transfer (Fig 1). Based on the SW-test at the hatchery, these trout had not developed maximal SW-tolerance at the time of seawater transfer and should be considered pre-smolts. The development of hypo-osmoregulatory mechanisms, however, proved sufficient to tolerate transfer to 27 ppt, 5°C seawater. During the first month in seawater these trout had a relatively low weight and length growth (Fig. 3 and 4). The initial low growth is probably caused by a combination of high salinity and low water temperature during that period. Following transfer to high salinity water, salmonids are reported to experience an acute though temporary suppression of appetite (Boeuf, 1993). The feeding activity of the Feb-trout was low during the first period of SW-acclimation (personal observation). Arnesen *et al.* (1993b) found increasing percentage of empty stomachs with increasing salinity (20 to 35 ppt) during the initial period after seawater transfer in Arctic charr (*Salvelinus alpinus*, L.). A similar appetite and growth suppression was observed for 30 days following transfer of Atlantic salmon (*Salmo salar*, L.) to high salinity water (30 - 33 ppt) Usher *et al.*, 1991). During the initial period of acclimation to high salinity water, the trout are faced with a high metabolic cost of osmoregulation (Morgan & Iwama, 1991), leaving less of the ingested food (energy) for growth. The temperature in this period varied between 3.5 and 5°C, which is in the lower end of the temperature range (4-19°C) in which feeding and growth has been shown to occur in brown trout (Elliott, 1994).

After the initial period with low growth rate, the growth increased in parallel with the increasing water temperatures through the spring and early summer. The trout had the highest growth rate in the period where water temperature fluctuated around 15°C. This agrees with Elliott (1975), who showed that optimal feeding and growth in brown trout occurs around 15°C. The salinity of the seawater was below 19 ppt throughout this period. The condition factor increased after the initial period with low growth rate and was high during the rest of the study (Fig. 5). An increase in CF is generally assumed to reflect an improvement in nutritional state (Koskela *et al.*, 1997) and the increase probably reflects fat deposition in fast growing fish. Maladapted SW-transferred salmonids (socalled stunts) are normally pin-head shaped and have a very low condition factor (Bolton *et al.*, 1987), which reflects malnutrition. Conversely, a high condition factor reflects good adaptation to seawater and good nutritional state.

4.2. April transfer

Because of the death of 2 groups of trout, the April-transfer did not take place until late April. At this time, SW-tolerance of the trout tested at the Vork hatchery had started declining. These tests were, however, done on fish 16.8 ± 0.3 cm in length, while the mean size of the fish transferred to seawater at Kerteminde was 14.7 ± 0.1 cm and thus significantly smaller. Almost 100 of these fish were smaller than 13.5 cm when measured in early May, and it is likely that these fish were too small for smolting during this spring. The seawater was 12 ppt at the time of transfer and there was a mortality of 1% during the first 5 days of seawater exposure (Fig. 2). From early May until June, however, 23% of the trout died in this group. The salinity did not exceed 16 ppt during this period and it is unlikely that osmoregulatory failure was the direct cause of mortality in this group. In late May, samples showed that the surviving trout had high muscle water content (data not shown) indeed confirming that osmoregulatory homeostatis was maintained. The cause of death is unknown but may well be related to the timing of SW-transfer. The surviving trout grew at about the same rate (length and weight, respectively) from May until mid-June (Table I) as the fully acclimated Feb-trout. The April-trout were smaller than the Feb-trout, however, resulting in a higher relative growth rate (Table I) in this period of favourable water temperature. Condition factor increased in this period (Fig. 5), reflecting good nutritional state and acclimation to seawater.

4.3. June transfer

At the time of seawater transfer in June, the trout at the Vork hatchery had completely lost the SW-tolerance, and the fish were thus considered de-smolts. The June-transfer took place in 18 ppt, 13°C seawater and resulted in the death of 8% during the first 5 days (Fig. 2). In this period the salinity increased to 19.5 ppt, and osmoregulatory stress is likely to have contributed to the observed mortality. The mortality progressed at a steady rate until July 15th, where 34% of the fish had died. During this period, the surviving trout had poor growth and appetite, compared with the Feb-trout and considering the favorable temperature (Fig. 1, 3 and 4; Table I). Together with the decreasing condition factor (Fig. 5), this suggests maladaptation to the moderate salinity water (15-21 ppt). The elevated water temperatures during that period (14-17°C) may have contributed to the osmoregulatory stress of the trout since earlier studies have shown an interactive effect of high temperature and salinity on seawater acclimation. Johnsson & Clarke (1988) found a negative effect of high temperature on seawater survival

and Hogstrand & Haux (1985) obtained 100% mortality in fully smolted sea trout transferred to 25 ppt SW at 20°C, but no mortality at 15°C.

After the period of significant mortality and poor growth, the salinity decreased (Fig. 1) resulting in low mortality and a considerably increased growth rate of surviving trout (Table I). A comparison of the growth pattern with the other transfer groups during this period is not possible because of the accidental deaths of these. Taken together, only 57% of the trout transferred in June survived from June until September.

It was noted, that a few large and aggressive trout from the June-group were dominating in the tank and created intraspecific interactions for food (J.C. Rankin, pers. comm.). Food was distributed from a single point source, which is reported to intensify aggression (Davis & Olla, 1987). These aggressive individuals may have suppressed the feeding activity of the subordinates, which would lead to a skewness of the size distribution of the fish (Cutts *et al.*, 1998). Length frequency histograms (not shown) indicated a less pronounced unimodal population growth in this group compared with the others. Beside the effect of suppressed feeding activity, aggression in salmonids increases physiological stress in the subordinates (Pickering, 1992), and may have biased the growth potential negatively in this group.

5. Conclusions

The present study showed seasonal differences in the survival and growth potential of brown trout after direct transfer to seawater of naturally fluctuating salinity and temperature. Transfer of brown trout into high salinity water (27 ppt) at 5 °C in February (before development of maximum SW-tolerance) did not cause any immediate mortality. The early transfer group had the highest survival of all groups (84% of the fish survived from February until July) despite this group's by far being exposed to the highest salinity at the time of transfer. The Feb-trout showed a high and increasing unimodal population growth though the spring.

Transfer of trout in late April (at the time where hatchery trout had begun to lose SW-tolerance) into low-salinity water (12 ppt) did not cause osmoregulatory problems for trout released. Nonetheless, there was a 24% mortality until mid-June. The survivors achieved good growth rates at the same level as the trout transferred in February.

Abrupt transfer of trout in June (at the time where hatchery trout had completely lost SW-tolerance) into moderate-salinity water (18 ppt) at 13 °C, caused severe (osmoregulatory) problems for the trout and resulted in the loss of 34% until mid-July. During this period the surviving trout displayed poor growth and appetite. In the succeeding period the salinity decreased and the trout experienced a considerable increase in growth rate. From June until September, only 57% of the trout transferred in June survived.

Despite the variation in environmental parameters (salinity and temperature), the present data indicate that the timing of direct transfer of brown trout into seawater may be critical for the survival and growth performance of the released trout. In terms of survival and growth rate, release of pre-smolts gives far better results than the release of de-smoltified trout. As most coastal releases in Denmark are carried out at the time where hatchery-reared trout have more or less de-smoltified, i.e. in late May and early June (hatchery personnel pers. comm.), the

present data suggest that the release programme may be optimised by an earlier timing of coastal releases.

6. Acknowledgements

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7. Tables and Figures

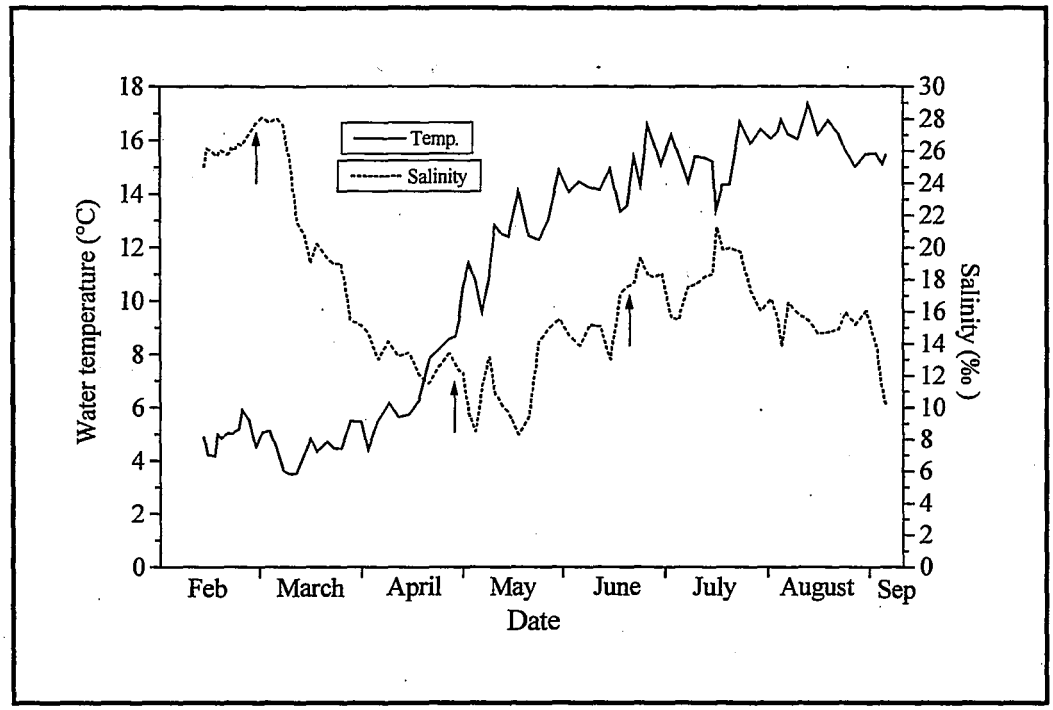

Figure 1. Daily mean water temperature (°C) and salinity (‰) of the sea water brown trout were transferred to and kept in. Arrows indicates the different transfer dates.

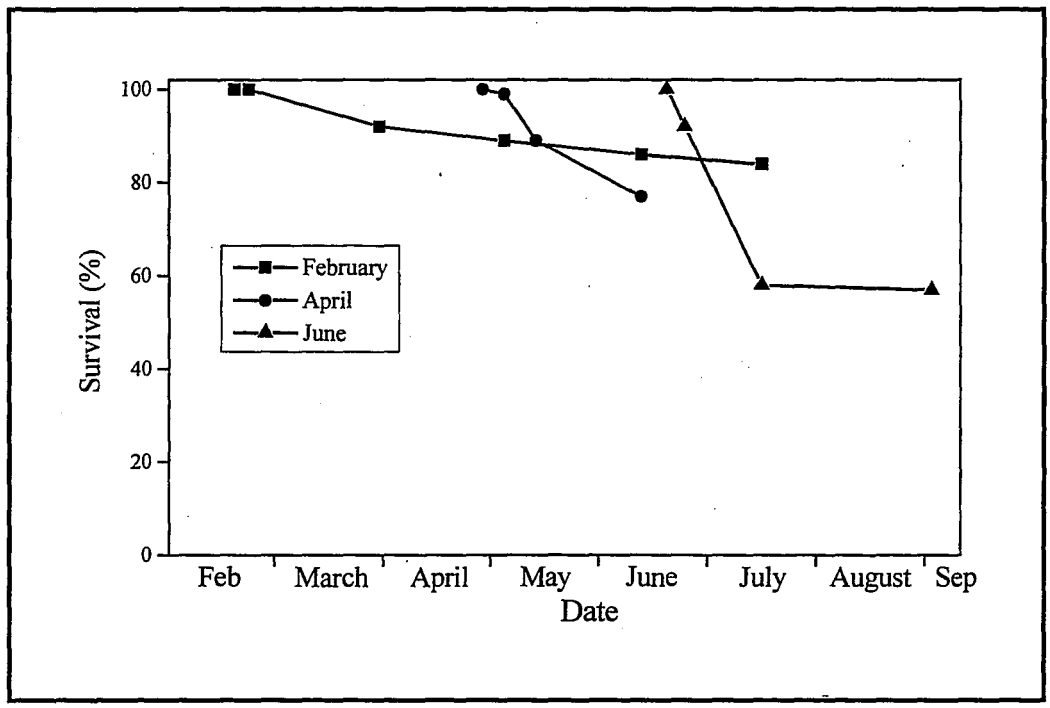

Figure 2. The percentage survival of the different groups of sea water transferred trout (*S. trutta* L.) as a function of time.

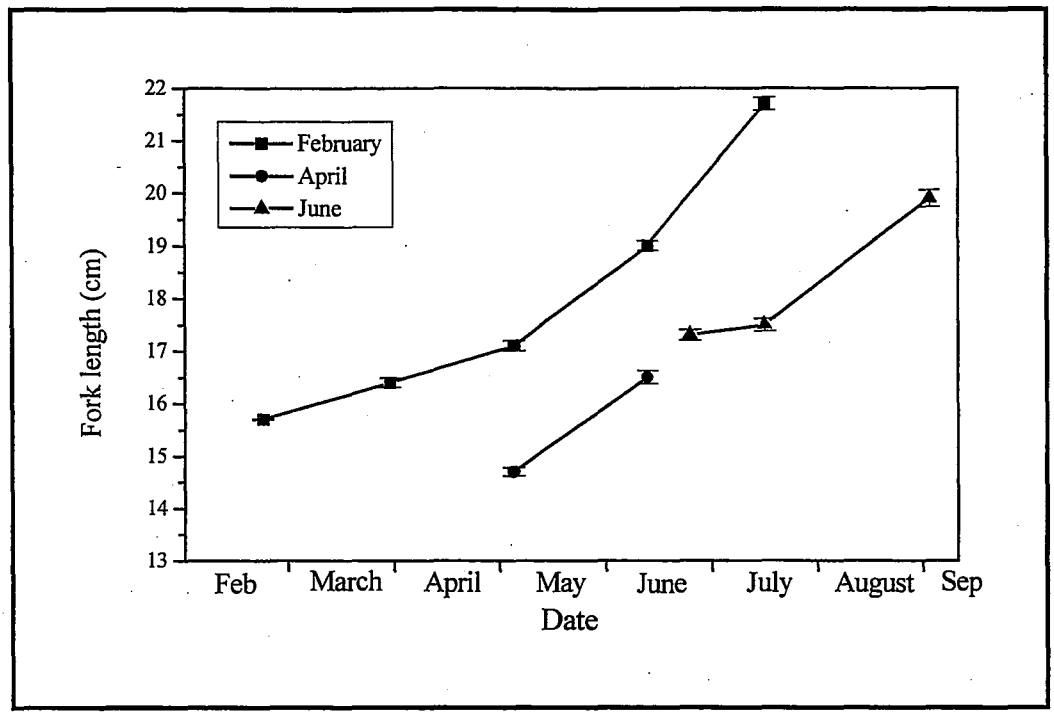

Figure 3. Mean fork length (cm) of the different groups of sea water transferred trout (*S. trutta* L.) as a function of time. Each point represents the mean value ± SEM of 156-393 fish.

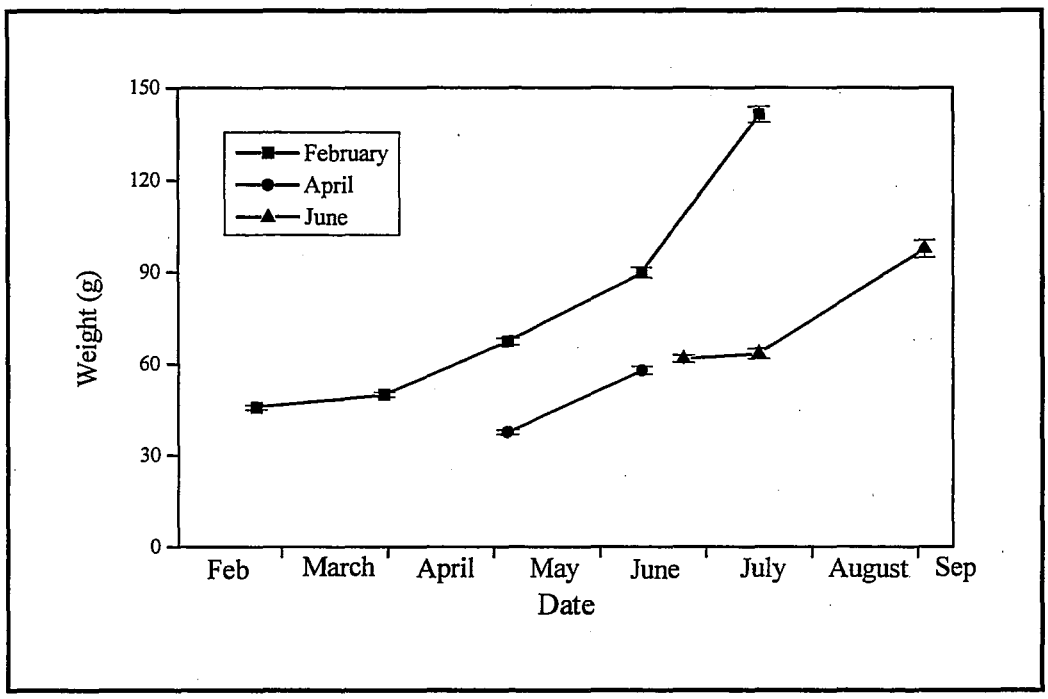

Figure 4. Mean weight (g) of the different groups of sea water transferred trout (*S. trutta* L.) as a function of time. Each point represents the mean value ± SEM of 156-393 fish.

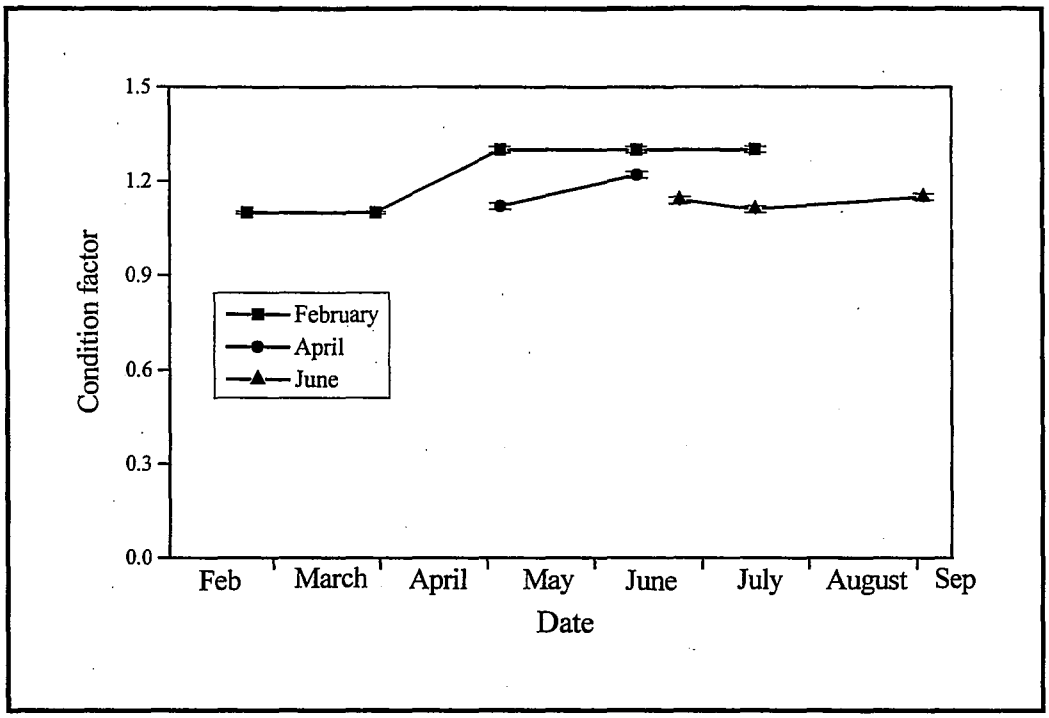

Figure 5. Mean condition factor of the different groups of sea water transferred trout (*S. trutta* L.) as a function of time. Each point represents the mean value ± SEM of 156-393 fish.

Table I. Mean length- (cm/month) and weight-increases (g/month), relative length- and weight-increases (%/month) of the different groups between the various sampling dates. e.o.e. means end of experiment.

	Length increase (cm / month)			Weight increase (g / month)			Relative length increase (% / month)			Relative weight increase (% / month)		
Date	**Feb. trans.**	**April trans.**	**June trans.**	**Feb. trans.**	**April trans.**	**June trans.**	**Feb. trans.**	**April trans.**	**June trans.**	**Feb. trans.**	**April trans.**	**June trans.**
23 Feb.												
	0.6			3.5			3.9			7.6		
31 Mar.												
	0.6			14.9			3.5			30.0		
5 May												
	1.5	1.4		17.8	15.9		8.8	9.4		26.5	42.3	
12 June		e.o.e.			e.o.e.			e.o.e.			e.o.e.	
24 June	2.5			46.7			13.0			52.0		
			0.3			2.4			1.6			4.0
15 July	e.o.e.			e.o.e.			e.o.e.			e.o.e.		
			1.5			21.0			8.4			33.2
2 Sep.			e.o.e.			e.o.e.			e.o.e.			e.o.e.

8. References

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