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DTU Aqua Report no. 376-2020

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Circulus formation rate in scales from sea trout (*Salmo trutta* L.)

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Colophon

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Indhold

Preface

This report is based on a comprehensive study of the sea trout stock in River Simested, River Rye and Liver Å, which was carried out in the years 1982-2002. The basic material of the study is scale samples taken by electrofishing in the autumn for spawner sea trout.

The ageing of these scales became more difficult and more uncertain than was needed in the later analysis. Therefore, it was decided to supplement the normal ageing by counting the number of circuli on the scales, and if possible find a pattern in these that could impart additional accuracy to the ageing.

For this purpose, a larger number of scales from sea trout with a known number of days in the sea were needed. This material was found at DTU Aqua, as in connection with a major release program of sea trout smolt in 1958-66, scales had been taken from the marked and recaptured trout.

However, this material contained only recaptures from sea trout above the legal size (40 cm), as fish under this size was discarded by the fishery. As sea trout only reach the legal size in the winter after release in the spring, the entire first summer and the first autumn were thus missing in the material.

The material was therefore supplemented with scales from a study of the sea trout's food intake, which was carried out in the years 1994-96 by Kaare Manniche Ebert in connection with his thesis at DTU Aqua. Selected scales from the above study of River Simested were also included.

The primary purpose of the study has thus been to provide a scientific basis for the use of circuli counts as a supplementary tool in the assessment of sea trout scales. The studies from 1958-66 were led by State Biologist Knud Larsen. The scale material from that time was made available by Department Head Ole Christensen DTU Aqua. The scale material from the survey in 1994-96 was provided by Kaare Manniche Ebert from the Danish Sports Fishermen's Association. The material from River Simested was obtained by electrofishing for mother fish. This was done in close collaboration with Aalestrup Sportfisher Club and Viborg Sportsfisher Club.

The project has received funding from many different sides. The 1958-66 surveys were funded by state funds. The study from 1994-96 was financed by internal funds from DTU Aqua. The study of the three North Jutland streams (1982-2002) was financed by internal funds from Aalborg University.

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Aalborg, November 2020

Jens-Ole Frier
Associate Professor Emeritus

English summary

Circulus formation rate in the scales of sea trout during different times of the year has been studied in populations from several Danish marine areas. Analysis of the structure in the scales can provide information on fish age, age at seaward migration, number of spawns, and growth rate during different life stages.

The circulus formation stops completely from the beginning of December to the middle of April. This period is represented on the scale by a winter check. A zone with small distance between circuli is seen centrally for this winter check. This zone is normally accounted for as a winter zone, but it is formed before December. During the rest of the year from April to December, the circulus formation rate is approximately one circulus per week. This is true for both the first and second year at sea. The statistical variance in circulus counts between individuals is constant throughout the growing season, and most of the differences between these circulus counts consequently can be related to differences in starting date after winter or after smolt run.

The constant circulus counts during the growing period make it possible to relate a circulus to a certain period of this season, but the winter stop in circulus formation rate make this impossible for the winter period. The winter stop most probably is related to coldwater temperatures. The duration of the period with cold water may vary considerably from north to south within the distribution area of the trout. Consequently, the onset and stop of circulus formation may also vary considerably with latitude. In the present study, however, analysis of several different populations from different marine areas show only minor differences between annual numbers of circuli in immature trout.

Due to an early stop in circulus formation related to the maturing processes, spawning changes the number of circuli formed during the spawning years considerably. This stop takes place earlier and earlier in the year as the sea age of the trout increases. The difference in circulus counts between small 0SW spawners and immature trout allows for an almost complete separation of these two groups without internal dissection of the trout.

Circulus counting also allows for separating between spawning marks, winter checks, summer checks, and other checks in the scale because these phenomena can be dated.

Growth rates calculated from scales are possible for short periods using circulus counts, but precaution has to be taken because of the possible lack of linear relationship between scale growth and fish growth during small periods of time.

Dansk resume

Vi har i denne undersøgelse studeret dannelsen af ringe (circuli) på havørredskæl gennem året i forskellige områder af de danske farvande. Analyse af skællenes struktur kan give information om havørredens aktuelle alder, dens alder ved udvandring til havet, antallet af gydninger samt væksthastighed i forskellige livsstadier.

Dannelsen af ringe på skællene stopper fuldstændig om vinteren fra begyndelsen af december til midten af april. På skællene er denne periode repræsenteret af et vinterstop. Skællet vokser ikke, og der dannes derfor heller ikke nogen nye ringe. Før dette stop ses en zone på skællet, hvor ringene ligger tæt. Denne zone repræsenterer efteråret, selvom den ofte kaldes vinterzonen. Resten af året vokser skællet og danner ca. en ring om ugen. Dette gælder både for det første og andet år i havet.

Tiden mellem dannelsen af ringe i vækstperioden varierer kun lidt mellem de enkelte individer, og størstedelen af de forskelle, der findes på antallet af ringe pr. år mellem fiskene, skyldes forskelle i, hvornår dannelsen af ringe starter om foråret, og hvornår den slutter om vinteren. Det er således teoretisk muligt at sætte dato på de forskellige ringe. Hvis dette skal gøres med stor nøjagtighed kan det være nødvendigt at kalibrere starten og slutningen på vækstsæsonen ved indsamling af fisk fra forårsmånederne og fra vinteren. I dette studie finder vi dog ikke store forskelle mellem de forskellige geografiske områder, og vi konkluderer derfor, at en ring med tilstrækkelig nøjagtighed kan henføres til et tidspunkt med en måneds nøjagtighed. Ved ørredudsætninger, hvor udsætningstidspunktet er kendt, kan man regne med at begrænse denne nøjagtighed til en uge.

Modningsprocessen og gydevandringen stopper ringdannelsen. Derfor dannes der betydeligt færre ringe i år med gydning. Det betyder, at man kan skelne mellem gydefisk og umodne fisk, der vandrer op i vandløbet om efteråret eller om vinteren, ved at tælle antallet af ringe, selvom man ikke kan se forskellen på fiskens ydre ved indsamlingen.

Ringtælling gør det også lettere at skelne mellem gydemærker, vintermærker og sommerstop i skællet, fordi man kan datere fænomenerne.

På grundlag af ringtællinger vil det også være muligt at beregne ørredens vækst i kortere tidsintervaller. Dette skal dog gøres med en vis varsomhed, da der ikke nødvendigvis er en lineær sammenhæng mellem ringdannelsesraten og vækstraten på alle årstider.

1. Introduction

Analysis of the structure in scales from anadromous brown trout or sea trout (*Salmo trutta* L.) provides information on fish age, age at seaward migration, number of spawns, and growth rate during different life stages (Nall, 1930). The analysis is primarily based on the circular circuli that fishes produce on the upper side of the scales (Meunier, 2002). When the growth of the brown trout is fast, these circuli are far apart, while slow growth result in a smaller circuli distance. Thus, the main scale pattern becomes an alternation between periods with: (i) fast growth and large distance between circuli, and (ii) periods with slow growth and small distance between the circuli. In addition, it is normally possible to distinguish the freshwater period with slow growth from the sea period with faster growth. During spawning the trout resorbs some calcium from the scales, resulting in an eroded margin. When growth and scale formation resumes, this part of the scale is reestablished, but the process leaves a distinct and irregular mark named a spawning mark. By counting these marks, the number of spawnings can be established. The calculation of the growth rate depends on the proportionality between scale radius and fish length (Dahl, 1910). This provides a possibility to calculate the fish size at the end of each year. The annual growth is calculated as the difference in size (i.e. length) between two succeeding years.

The scale reading method described above has been known for a century (Dahl, 1910, Nall, 1930). It has been used on a vast amount of different fish species (Carlander, 1987) and the method is standard in salmonid ecological research. With some moderation, other calcium structures in the fish can be used for the same purpose, and e.g. otoliths have been widely used for aging of commercial fishes. Although otoliths have been used for aging salmonids, scale reading has been preferred in many cases due to the possibility for nonlethal sampling. Many salmonid populations are so small in numbers, that even killing a few fish can have a considerable effect on population size.

From the early days of scale reading, it became clear that several different factors could influence the readings (Nall, 1930; Alvord, 1954). If a salmonid has a period with slow growth during summer this might incorrectly be interpreted as a winter period. Moreover, if a fish has a fast growth rate in freshwater this period might incorrectly be taken as a stay in saltwater, and if a fish experience bad conditions like cold water or food shortage during winter, the scale may erode and form a false spawning mark. In older sea trout, erosion at spawning might be so pronounced, that it removes an earlier spawning mark, thus making the trout one year younger. All these disturbing factors are present in sea trout scale reading, and often make it difficult to determine age, number of spawnings and growth rate with the necessary accuracy. This accuracy is especially challenged in older specimens with a complex life story (Carlander, 1987).

The appearance of new micro-methods for analysis of chemical components in e.g. scales makes it gradually possible to conduct these analyses on very small areas of intact scales (de Pontuall and Geffen, 2002). Relating these measurements to environmental variables such as temperature, salinity, or oxygen concentration might well be a major goal for future investigations in ecology and migration. This reinforces the need for age determination with finer time resolution than years. To be able to tell in which month a given spot on the scale is formed would undoubtedly be very useful.

To improve the accuracy and increase the time resolution of scale reading it will be necessary to supplement the standard readings with new measurements. An obvious candidate for this would be direct counting of circuli. The rate of formation of circuli is not directly proportional to the growth rate but rather the distance between circuli is, hence the difference between fast summer growth and slow winter growth can be discerned. On the other hand, we also know that circulus formation rate is not always constant in time (Friedland et al, 2005). Several authors have shown that different environmental factors have considerable influence on the circulus formation rate. Food availability influences the rate in four species of pacific salmon (Bilton and Robins, 1971a; Bilton and Robins, 1971b; Bilton, 1974), and other measurements have shown differences between different ages (Wells *et al.*, 2003). Temperature per se might also have influence in brown trout (Skurdal and Andersen, 1985) and in cod *Gadus morhua* (Dannevig, 1957). Although circulus formation rate obviously is influenced by a variety of different environmental factors, it might still be both possible and useful to find conditions, seasons, and larger geographic areas where the formation rate is approximately constant.

Plenty of food and near maximal growth rate of sea trout can be found everywhere in the transitional zone between the brackish Baltic Sea and the North Sea (Frier, 1994). This area is shallow with water of increasing salinity from the Baltic Sea (app. 5-10‰) to the North Sea (33‰). The transitional area is also an area with large variation in surface temperature from 0ºC in winter to 25ºC in summer. The shallow bays and fjords have the largest variation while the main area shows more moderate oscillations. The food is plentiful all over the area. A variety of crustaceans (e.g. *Gammarus* sp. and different species of shrimps), worms (*Neriis* sp. and *Arenicola* sp.), fish fry, and small adult fishes like different species of gobies (*Gobius sp.*) dominate the shallow areas. Sprats (*Sprattus sprattus* L.), herring (*Clupea harengus* L.) and different species of sandeels (*Ammodytes sp.*) dominate the pelagic waters. All these animals are important food items for the sea trout (Rasmussen and Pedersen, 2018). Larger sea trout tend to be pelagic preying mainly on fishes, while the small specimens are more coastal preying on a variety of coastal food items (Rasmussen and Pedersen, 2018). It has not yet been possible to find major differences in the primary (before first spawning) growth rate of sea trout anywhere in these waters (Frier, 1994). The growth rate is at the same time high (app. 20 cm per year) (Frier, 1994). The area studied here looks in all respects to be well suited for an investigation of circulus formation rate during optimal growth conditions (i.e. temperature, oxygen level, and food availability) in a temperate climate.

The sea trout from the datasets in this study have all lived in areas with large variation of abiotic factors. Salinity declines from The North Sea to the Baltic, temperature varies with the seasons and occasional periods with oxygen depletion may occur. Any major dependence on circulus formation rate by any of these factors should therefore if present clearly were observed.

This paper investigates if it is possible to find a constant circulus formation rate during the growth period in sea trout living in an environment with plenty of food and varying abiotic factors.

2. Materials and methods

Three datasets was used in this analysis. Dataset 1 consists of fish above the legal limit (40 cm) and covers the time from November in the first year at sea and the following years. Dataset 2 covers the post-smolt period until the first winter. Dataset 3 covers differences between spawners and immature trout taking part in the spawning run. While dataset 1 consists only of domesticated and released trout caught in the sea, dataset 3 consists of wild trout caught in River Simested, and dataset 2 consists of a mixture of wild and domesticated fish caught in the sea.

Dataset 1 (Table 1) consists of data from a large release and recapture experiment conducted in the Danish marine areas from 1958 to 1966. Brown trout smolts were released during spring at 11 different localities along the Danish coasts. These fish were domesticated trout coming from the same hatchery. The fish were marked externally by removal of the left or right pectoral fin and internally by batch tagging. When recaptured and recognized by the external mark, the fish were sent to The Danish Institute for Fishery and Marine Research (now DTU Aqua) for batch identification by the internal mark. The fish were then measured (length, mass) and analyzed (stomach content and scales were taken). Only fish above the legal limit of 40 cm were taken, while fish smaller than 40 cm were released after catch.

From these releases below 10% were recaptured in total and scales were only sampled in 8 localities during the release years 1960-62. We do, however, have scales from 1,229 sea trout, and this scale collection have been analyzed for circulus formation (Table 1).

For the general analysis the data from different localities and different smolt ages has been grouped.

Table 1. Dataset 1. Released and recaptured domestic brown trout from the large-scale release, mark, and recapture experiment conducted from 1958 to 1966. Only fish from the years 1960-62 have been included in this analysis.

PLACE	RELEASE YEARS	SMOLT AGE	NUMBER	AVERAGE LENGTH cm	AVERAGE MASS g	ANALYZED NR.
Ejby Isefjord	1960-66	1	3,964	16.3	36	103
Ejby Isefjord	1958-66	2	10,435	24.8	144	302
Fjellebroen	1958-62	2	8,473	24.2	133	249
Hjarbæk Fjord	1958-66	2	9,482	24.7	143	136
Korsør	1958-62	2	8,500	24.0	131	306
Storstrømmen	1959-62	2	6,410	23.7	130	27
Ringkøbing Fjord	1960-66	1	3,969	16.6	41	41
Ringkøbing Fjord	1959-66	2	8,500	24.8	151	108
Sebbersund	1958-62	2	8,500	24.3	132	49
Vejle Fjord	1959-62	2	6,494	23.4	126	52
Total	1958-66	1	7,933	16.4	38	144
Total	1958-66	2	66,794	24.3	137	1,229

Dataset 2 (Table 2) consists of scale samples from a large number of small sea trout caught by commercial fishery in the Limfjord in the Northern part of Denmark during the years 1994-96. Contrary to dataset 1, the exact date for migration from freshwater to sea is not known from these trout. These samples, however, cover the first year after seaward migration, thus supplementing dataset 1 with fish below the legal limit (40 cm). It must be emphasized, that these fish are judged to be at their first year at sea by standard scale reading, but this will normally result in a very limited number of reading errors when dealing with sea trout of such small size.

Table 2. Dataset 2. Immature sea trout caught by commercial fishery during their first year at sea.

MONTH	NUMBER	AVERAGE LENGTH cm	AVERAGE MASS g
January	1	29.0	339
February	0		
March	1		
April	10	23.2	133
May	1	24.0	140
June	7	31.4	411
July	44	26.9	262
August	9	29.3	317
September	4	31.0	331
October	23	32.6	454
November	10	30.7	349
December	2	29.3	266
Total	112		

Dataset 3 (Table 3) consists of scales from a number of sea trout caught by electrofishing during the years 1982-2002 in River Simested, which flows into the Limfjord in the central part of the fjord. During the spawning run in November, they were caught as either 0SW (half year in sea), 1SW (one and a half year in sea) mature fish, or immature fish entering freshwater. These samples have primarily been included to explore possible differences in circulus formation between 0SW spawners and the immature trout, and secondarily to reveal possible differences in circulus formation rate between domesticated fish from dataset 1 and wild sea trout. The two groups of mature fish were restricted to males because spawning males were easily recognized from external characters. The mature males were differentiated from the immature trout using external characters, but the mature 0SW females could sometimes be taken for 0+ immature trout. To minimize this problem the measurements of 0+ immature trout were restricted to 1-year smolts. Only a very limited number of female smolts from this group mature after only one summer at sea. The exclusive use of 1-year smolts for immature fish is the reason for the relative small average length of these fish (Table 3).

Table 3. Dataset 3 from River Simested. All trout were caught by electrofishing during November.

DATASET	TYPE OF SEA TROUT	SMOLT AGE	SEX	NUMBER	AVERAGE LENGTH cm
3	Immature fish – 0+	1	immature	119	33.6
3	Mature fish – 0SW.	1 and 2	males	113	38.2
3	Mature fish – 1SW.	1 and 2	males	107	48.5

All scale samples were taken from the zone below the dorsal fin and above the sideline of the trout. All readings of scales have been conducted either on scales rinsed in tap water immediately after catch or on dry scales rinsed in KOH. The scales were photographed through a microscope using different adequate magnifications. The different measurements were made using imaging software (Axiovision ver. 4.7). The number of circuli has been counted along the middle axis of the scale using one scale from each trout. All counting of circuli in this report is from the saltwater period. The circuli from the freshwater period has not been dealt with, because some of the trout (dataset 1) has spent this period in a hatchery.

The analysis involves the use of standard statistical methods included with Microsoft Excel 2010. The detailed regression analysis presented in Table 4 has been conducted using Sigmaplot 13.0.

The analysis in this study follows a main path where circulus formation during the first winter at sea is analyzed using dataset 1. After that, circulus formation rate during the post-smolt summer is analyzed using dataset 2. Finally, dataset 3 is included to provide information on circulus formation rate in spawning sea trout and the difference between 0SW spawners and immature trout of the same age entering freshwater during their first winter at sea.

Throughout this report, standard nomenclature has been used to differentiate between different groups of spawning and non-spawning trout. Thus, 0SW trout enters freshwater for spawning during their first summer at sea, and 1SW trout enters freshwater for spawning during their second summer at sea. Immature trout might enter freshwater in winter without spawning, but might also stay in seawater.

3. Results

The scale collection sampled in connection with the saltwater release experiments in 1960-62 (dataset 1) has been analyzed for circulus formation. These fish were released all over Danish saltwater (Table 1) and the recaptures covers approximately the areas of release. The recaptured fish were divided in immature and mature specimens. The mature fish consist partly of fish from autumn and winter, where the maturity was judged by direct examination of gonads, and partly of fish from the following spring and summer that were judged as postspawners by the presence of spawning marks on the scales supported by external and internal examination.

3.1 Immature fish

The immature subsample consists of 94 trout from the first and second year at sea after stocking. The measured counts of circuli all come from the last year at sea. The fish were released in the beginning of May with one exception from the middle of April. Figure 1 shows the results.

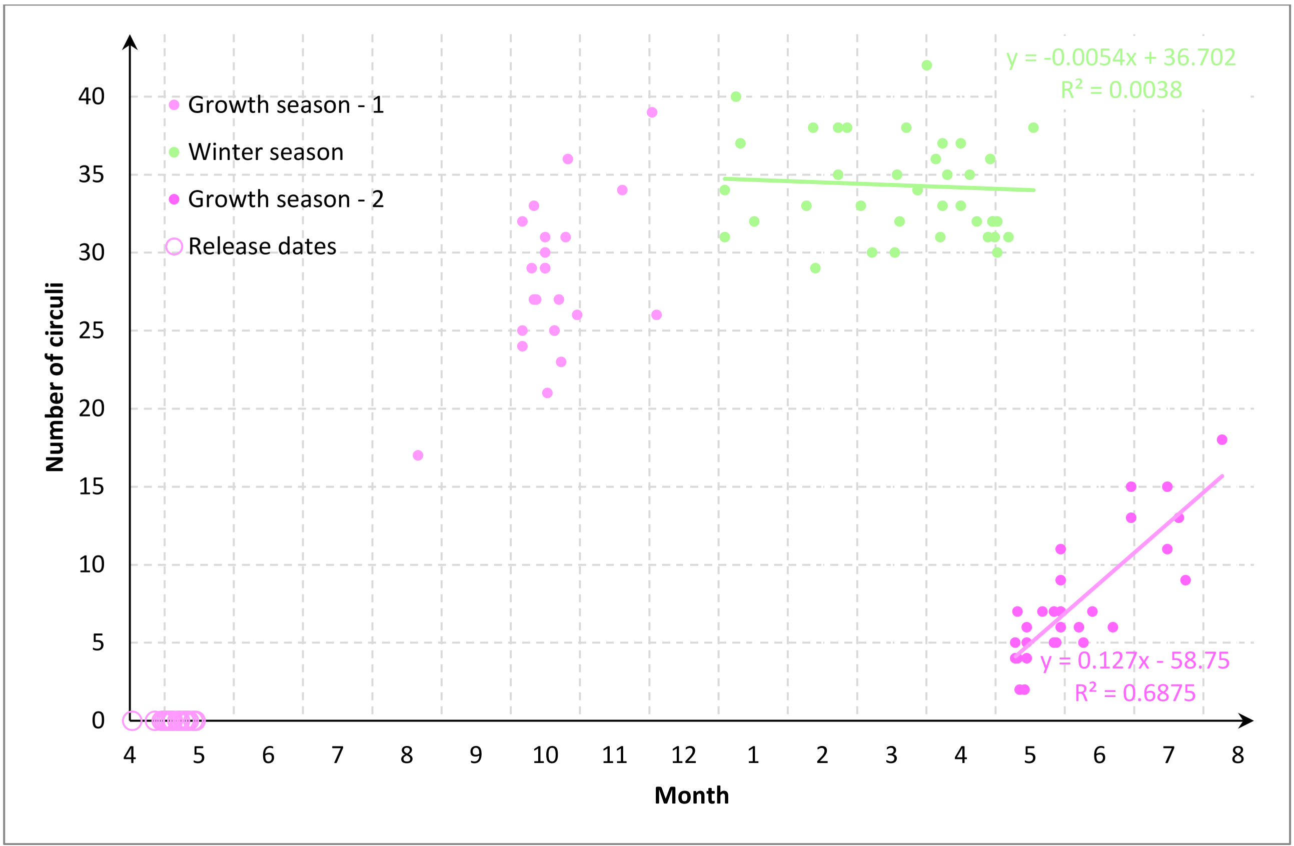

Figure 1. The number of circuli formed in immature sea trout during the whole season. Results are from the dataset 1. The releases were conducted from 1961-62. The fish was sampled by various methods (angling and commercial fishery). Fish caught during their first or second year at sea are included, but circuli counts are only from the year where they were captured. The equations for the regression lines use day-number in the calendar year as units although the x-axis shows month to illustrate the annual seasons.

Circulus counts from growth season 1 (Figure 1) cannot be used for calculation of circulus formation rate, because the dataset only includes fish of more than 40cm. Although the released smolts were quite large, this size (i.e. 40 cm) would not be reached for all specimens until the end of the first growing season in October and early November.

There is no sign of circulus formation during the winter period as shown by the very low correlation coefficient for the regression line. The production of circuli per year deduced from the average number found during the winter pause is 34.3±3.4 (S.D.), (n=39).

The circulus formation rate for growth season 2 in Figure 1 amounts to approximately 1 per week (1 per 7.87 days). The calculated average onset of circulus formation is at day number 463 (7th of April in the second year at sea).

From the trout collected by the commercial fishery in the Limfjord during the years 1995 and 1996 (dataset 2) it is possible to extract (from scale reading) a population of 106 individuals that were caught the same year as they migrated to saltwater. From these fish, a relation was established between days at sea and the number of circuli formed. Only fish caught during the growing season has been included because the fish do not make circuli during the winter season (Figure 1).

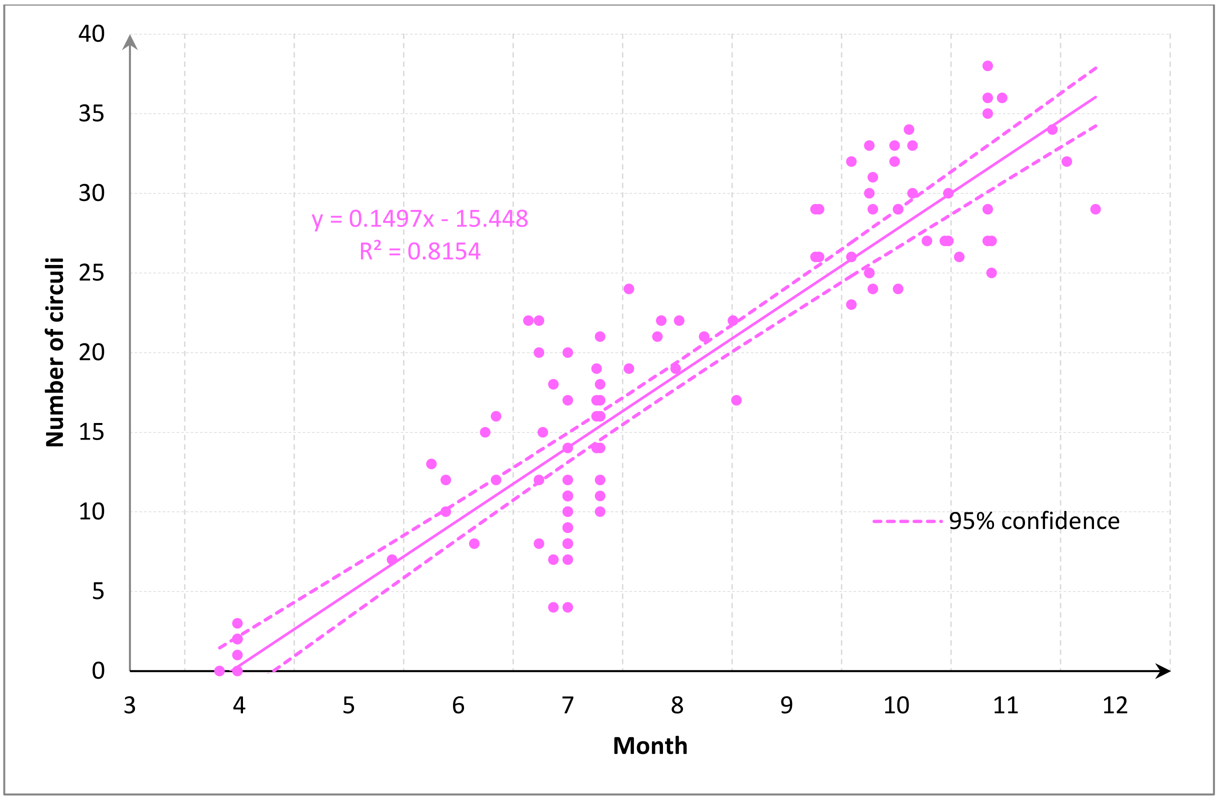

Figure 2. The number of circuli formed during the growing season. Results from sea trout sampled from commercial fishery during the seasons 1995 and 1996 (dataset 2). Only fish from the first year at sea are included. The equations for the regression lines use day-number in the calendar year as units although the x-axis shows month to illustrate the annual seasons.

The increase in circuli over time in Figure 2 amounts to approximately 1 per week (1 per 6.68 days), and the calculated average onset of circulus formation is at day number 103 (12th of April). This is slightly faster than the rate found for the second year at sea in Figure 1 (1 per 7.87 days). The slope is tested different from 0 and has a standard error of 0.00698 (Table 4) or approximately 5%. The regression line gives a value of approximately 35 circuli in the beginning of December. This fits well with the average winter value of 34.3 calculated from dataset 1 (Figure 1)

The regression analysis of the data from dataset 2 (Figure 2) is shown in detail (Table 4, Figure 3) because constancy of circulus formation with time is the most important underlying assumptions for using circulus counts in scale analysis.

Table 4. Results from the regression analysis of dataset 2.

N = 106	R = 0.903	R^2 = 0.815	Adj. R^2 = 0.814	Standard Error of Estimate = 4.231	
Regression line	Coefficient	Std. Error	t	P	
Constant	-15.448	1.637	-9.434	<0.001	
Slope	0.150	0.00698	21.435	<0.001	
Analysis of Variance:	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	8226.416	8226.416	459.478	<0.001
Residual	104	1861.999	17.904		
Total	105	10088.415	96.080		
Durbin-Watson Statistic = 1,510	**Passed**		**No autocorrelation**		
Normality Test (Shapiro-Wilk)	**Passed**		(P = 0.253)		
Constant Variance Test:	**Passed**		(P = 0.766)		

Table 4 shows no significant deviation from the presumptions concerning a linear regression. No significant autocorrelation is found. No deviation from a normal distribution of the data is found and the variance shows no significant deviation from being constant during the studied growing period.

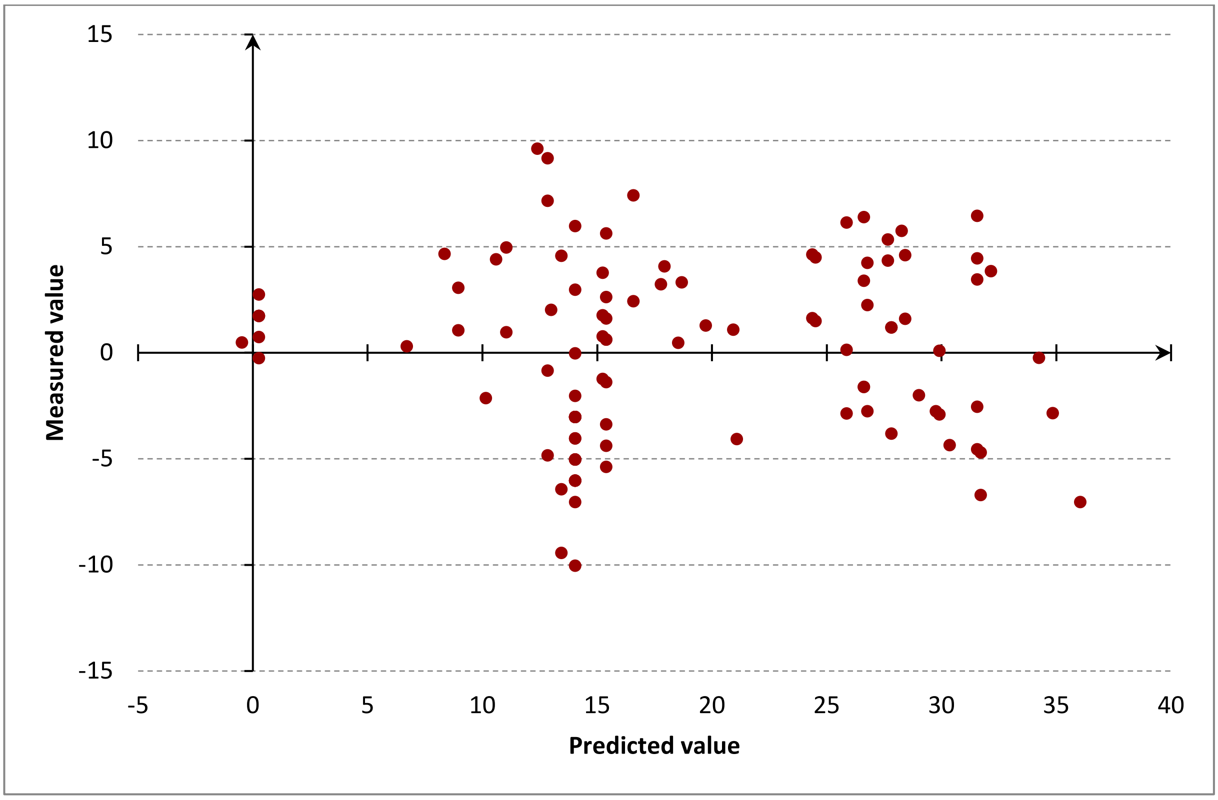

Figure 3. Residual variances for dataset 2 from Figure 2.

The linearity of the regression in Figure 2 means, that the formation rate is not obviously dependent of any seasonal factor like growth or temperature during the growing period. This can be judged by visual inspection from Figure 3. A systematic deviation from the straight regression line would imply a non-even distribution around the x-axis. In this case, the points in the middle would be skewed either under or over the axis while the points at both ends would be skewed the opposite way. The figure shows no sign of such skewness.

If external factors such as temperature and food availability plaid a significant role for circulus formation rate during the growth season the relation between circulus count and time should be nonlinear.

A combined diagram has been constructed for the circulus counts of immature trout from both datasets (Figure 4).

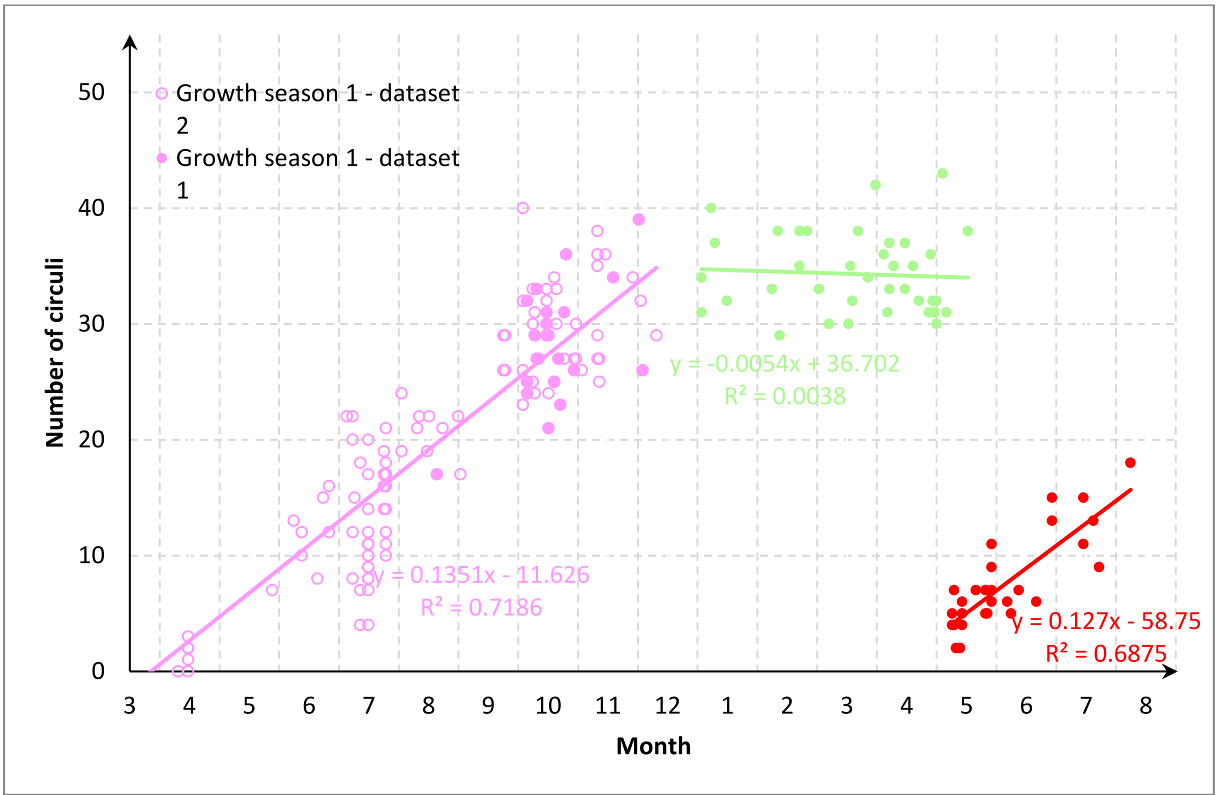

Figure 4. The number of circuli formed in immature sea trout. Both populations from dataset 2 (open dots) and dataset 1 (filled dots) are included. All circulus counts are from the last year at sea. In growth season one, trout the circuli are counted from the check at the end of the freshwater period, while in growth season two, trout the circuli are counted from the winter check after the first winter at sea. The equations for the regression lines use day-number in the calendar year as units although the x-axis shows month to illustrate the annual seasons.

The average increase in circuli during the first year at sea calculated from the combined datasets is 7.40 days per circulus. The average onset date for circulus formation in spring is day number 86 (26th of March).

3.2 Mature 0SW fish

The mature fish from dataset 1 were caught during their second year at sea and the maturity was judged by internal inspection. They were divided in two groups according to their spawning pattern. This was done by means of scale reading of spawning marks. The fish without any spawning marks were 1SW trout and the fish with a spawning mark from the year before were 0SW trout.

The circulus counts from the mature 0SW trout were treated the same way as the immature trout. The results are shown for two separate periods. The growing period lasted from April to August and the spawning period from September to March (Figure 5).

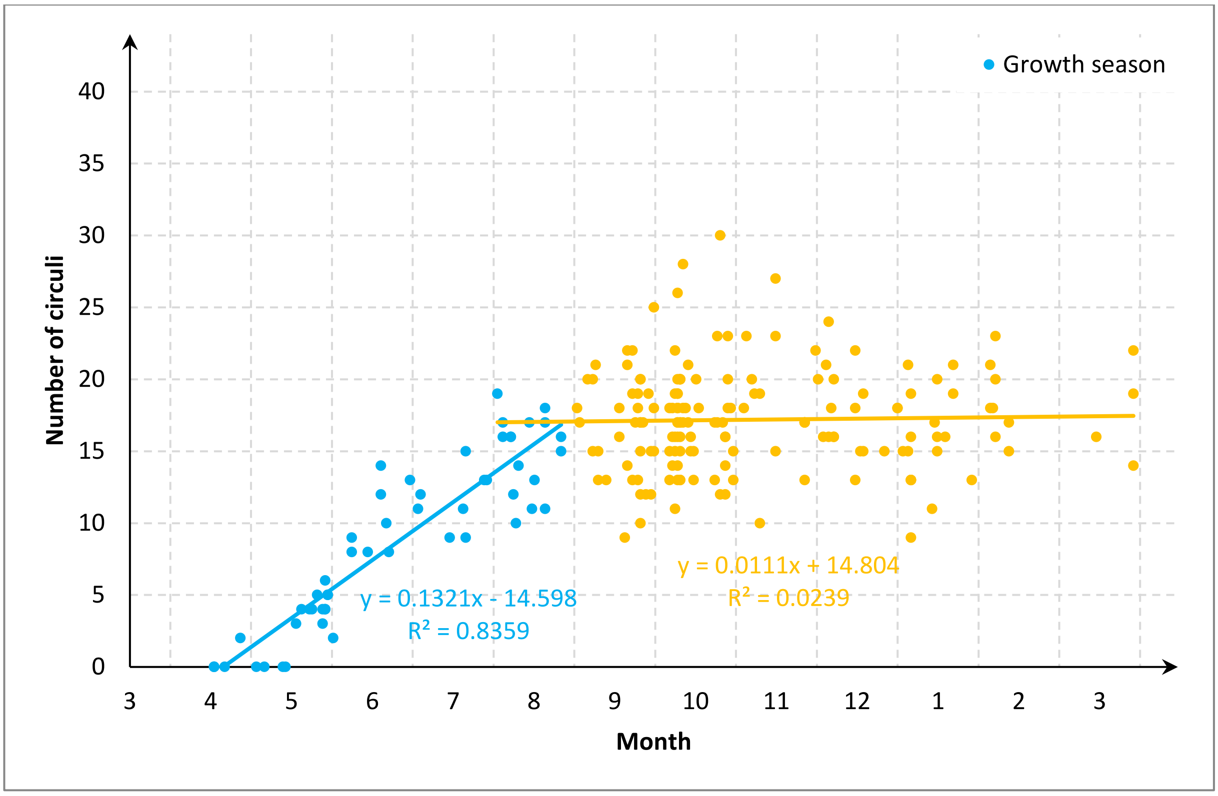

Figure 5. Dataset 1. The number of circuli formed in 0SW sea trout during the year after their first spawning. The spawning depicted is their second spawning. The equations for the regression lines use day-number in the calendar year as units although the x-axis shows month to illustrate the annual seasons.

The increase in circuli over time for the second growth period after first spawning shown in Figure 5 amounts approximately to 1 per week (1 per 7.57 days). The rate is slightly faster than for immature trout during the corresponding second year at sea (1 per 7.87 days) (Figure 1). The calculated average onset of circulus formation is at day number 475 (19th of April in the second year after release).

The fish do obviously not produce new circuli during the second spawning period ($R^2 = 0.0239$). The number of circuli produced during the second year after release deduced from the average number found during the spawning pause is 17.2 ±3.6 (S. dev.), (N =160).

Using circulus counts from the first spawning on the same 0SW fish as above (caught during their second spawning) it is possible to compare the annual circulus counts during the first and second spawning period. These results can also be compared to the relative few data from fish caught during their first and third spawning. These data are all summarized in Table 5. The table includes both fish where circulus counts are from the recapture year (red numbers) and circulus counts from the year before catch (blue numbers).

Table 5. Dataset 1. Annual circulus counts from 0SW trout caught at different times after release. They have spawned 1-3 times before being caught. The counts are all taken from the last spawning apart from the 160 fish spawning for the second time. From these fish, circulus counts from the first spawning are also included (blue numbers). Tested differences between the groups are shown by small letters. Groups with identical letters cannot be tested different (5% significance level), while groups with different letters can.

Year of catch after release	First	Second	Second	Third
Spawning number	1	1	2	3
N	8	160	160	15
Average circulus count	22.88	21.34	17.19	13.80
S. dev.	2.80	3.88	3.59	3.66
t-test	a	a	b	C

The most noticeable result from this comparison is the decrease in annual circulus number produced during successive spawnings (or sea age). The number decreases from around 23 to around 17 and further to 14 from the first to the third spawning. This can be compared to the 35 circuli per year formed in immature fish (Figure 1). The average number found for the first spawning using two different methods are not significantly different (5% level) (a-a), while the differences between the first, second, and third year are all significantly different (5% level) (a-b-c). All the counts are significantly different from the counts found in immature fish.

3.3 Mature 1SW fish

The circulus counts from the mature 1SW trout from dataset 1 were treated the same way as the immature trout and the mature 0SW trout. The results are shown for two separate periods. The growing period lasted from April to August and the spawning period from September to March, Figure 6.

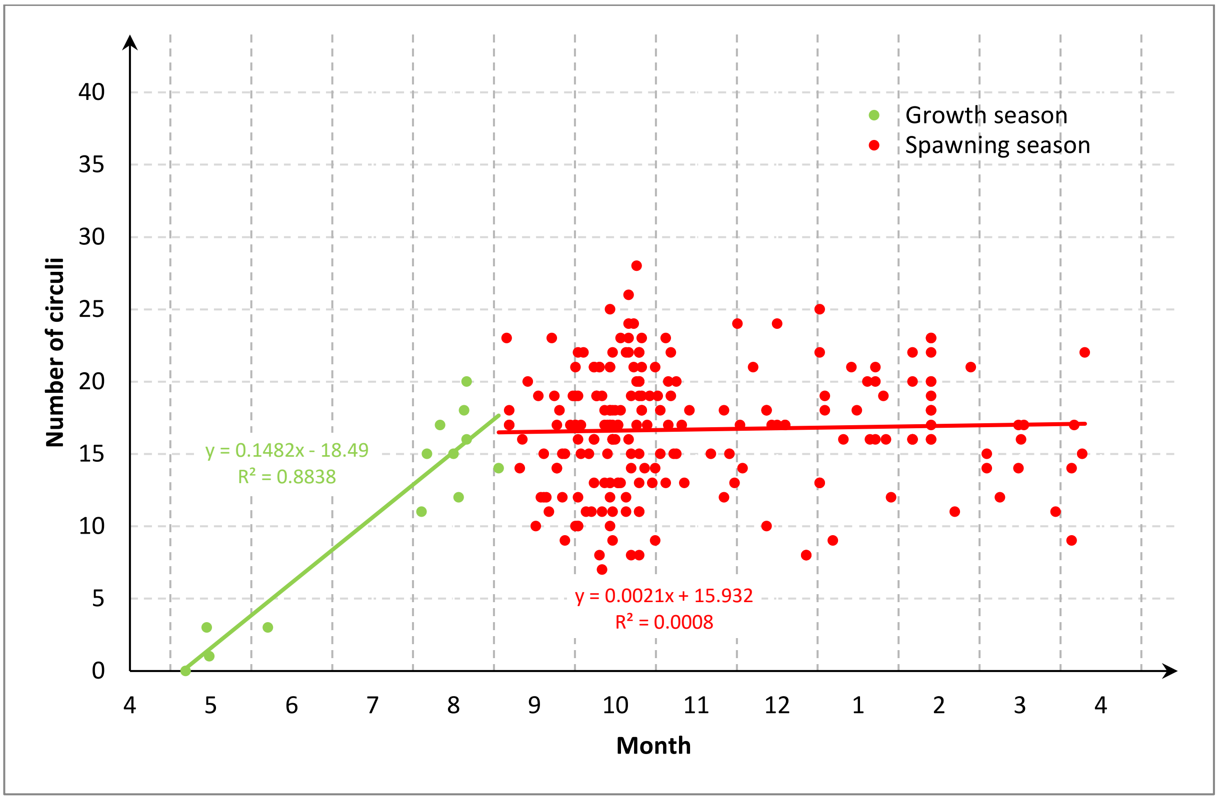

Figure 6. Dataset 1. The number of circuli formed in 1SW sea trout during their second year at sea. Only results from fish caught in the second year at sea are included. The equations for the regression lines use day-number in the calendar year as units although the x-axis shows month to illustrate the annual seasons.

The increase in circuli over time for the second growth period shown in Figure 6 is based of few measurements but amounts approximately to 1 per week (1 per 6.75 days). The calculated rate is faster than in Figure 1 (1 per 7.87 days) and Figure 5 (1 per 7.57 days). The calculated average onset of circulus formation is at day number 489 (3th of May in the second year after release).

The production of circuli in the second year after release calculated from the average number found during the spawning pause is 16.7 ±4.1 (S.D.), (n=214).

Using circulus counts from the first year at sea on the same 1SW fish as above it is possible to compare the annual circulus counts during the first and second year at sea (immature and first spawning respectively). These results can also be compared to the relative few data from fish caught at their third year at sea (second spawning). These data are all summarized in Table 6. The table includes both fish where circulus counts are from the recapture year (red numbers) and circulus counts from the year before catch (blue numbers).

Table 6. Dataset 1. Annual circulus counts from 1SW trout caught at different times after release. They all spawned in the second year after they were released. They have spawned 1-2 times before being caught. The counts are from the last year at sea apart from the immature period, where the counts have been made on the first year at sea after release. Tested differences between the groups are shown by small letters. Groups with identical letters cannot be tested different (5% significance level), while groups with different letters can.

Year of catch after release	Second	Second	Third
Spawning number	Immature	1	2
N	213	214	7
Average circulus count	33.58	16.70	13.57
S. dev.	4.87	4.12	2.38
t- test	C	a	b

The most noticeable result from this comparison is the significant decrease in annual circulus counts in 1SW trout between the second (1. spawning) and third year (2. spawning) at sea. The number decreases from around 17 to around 14. There is also a significant decrease in circuli counts between the first year at sea (immature period) and both of the following years. The number decreases from around 34 to around 17 or 14 respectively (Table 6). This value for immature fish can be compared to the value of around 34 measured on dataset 1 (Figure 1). These values are not significantly different.

3.4 Comparison with wild trout from River Simested

The general character of the results above between different Danish populations of sea trout has been investigated by supplementing dataset 1 with a third subpopulation of trout from River Simested (Dataset 3). Circuli from this population were counted from immature trout, 0SW mature trout, and 1SW mature trout. Results from the two datasets are summarized in Table 7.

Table 7. Annual circulus counts from the dataset 1 and from River Simested (dataset 3). The results are grouped according to the number of years they have spent at sea. Four subgroups have only spent the summer at sea (0+), three subgroups have spent 1.5 year at sea (1+), and two subgroups have spent 2.5 years at sea (2+). Immature trout, 0SW trout, and 1SW trout are included. Tested differences between the groups are shown with small letters. Subpopulations with identical letters cannot be tested different (5% significance level), while subpopulations with different letters can.

Population	Dat. 1	Dat. 3	Dat. 1	Dat. 3	Dat. 1	Dat. 1	Dat. 3	Dat. 1	Dat. 1
Counting year	Last	Last	Last	Last	Last	Last	Last	Last	Last
Years at sea	0+	0+	0+	0+	1+	1+	1+	2+	2+
Spawning type	Imm.	Imm.	0SW	0SW	0SW	1SW	1SW	0SW	1SW
Spawning number	0	0	1	1	2	1	1	3	2
N	39	119	8	117	160	214	108	15	7
Average circulus count	34.31	32.50	22.88	19.15	17.19	16.70	15.13	13.80	13.57
S. dev.	3.41	4.08	2.80	3.29	3.59	4.12	4.14	3.66	2.38
Subpopulation no.	1	2	3	4	5	6	7	8	9
t-test	a	a	b	b	c	c	c	d	d

The fish do split into two significantly different groups during the first year at sea. The spawning trout (0SW group – subpopulation 3 and 4) makes an average of around 20 circuli, while the immature fish subpopulation 1 and 2) makes on average 33-35. During the second year at sea, all trout in the subsamples are spawners. They make in average 15-17 circuli with no significant difference between the 0SW fish spawning for the second time (subpopulation 5), the 1SW fish spawning for the first time (subpopulation 6), and The River Simested trout (subpopulation 7) spawning for the first time. During the third year at sea, the released trout (dataset 1) makes on average 14 circuli, again with no significant difference between the 0SW fish spawning for their third time (subpopulation 8) and the 1SW fish spawning for their second time (subpopulation 9).

The results from Table 7 were tested pairwise against each other using Students t-test (Table 8). The results are shown in three groups. First group involves testing the release experiment against the Simested measurements. The second group tests a possible decline of circulus counts with number of spawnings for fish at the same age. The third group tests decline in circulus counts with sea age.

Table 8. Pairwise testing of circulus counts from different populations, maturity stages, and sea ages using students T-test. The numbers showing the test pairs are the subpopulation numbers from Table 7.

Population	Sea age	Maturity	Test pair	P	Circuli difference
Release – Simested	0+	Immature - Immature	1-2	0.0081	1.80
Release – Simested	0+	1. spawning - 1. spawning	3-4	0.0094	3.72
Release – Simested	1+	1. spawning - 1. spawning	5-7	0.0016	2.06
Release - Release	0+	Immature - 1. spawning	1-3	0.0000	11.43
Simested - Simested	0+	Immature - 1. spawning	2-4	0.0000	13.35
Release - Release	1+	1. spawning - 2. spawning	5-6	0.2153	0.50
Release - Release	2+	2. spawning - 3. spawning	8-9	0.8703	0.23
Release - Release	0+ - 1+	all spawning - all spawnings	3-5,6	0.0000	5.97
Simested - Simested	0+ - 1+	all spawning - all spawnings	4-7	0.0000	4.02
Release - Release	1+ - 2+	all spawning - all spawnings	5,6-8,9	0.0003	3.18

The first group of results (test pair 1-2, 3-4, 5-7) reveals the difference between the two populations (dataset 1 and dataset 3). They are significantly different in all three paired tests. The numerical differences between the averages are small although.

The second group of results (test pair 1-3, 2-4, 5-6, 8-9) tests the differences resulting from spawning. During the first year at sea, a major difference develops between the spawners and the immature fish. The numerical differences between these two groups are large for both dataset 1 and dataset 3 (Simested). During the following year at sea, the circulus counts for first time spawners (1SW-6) and second time spawners (0SW-5) of the same sea age are not significantly different. This is also the case for fish measured during their third year at sea comparing second (1SW-9) and third time spawners (0SW-8).

The third group of results (test pair 3, 4, 5 against 6; 4 against 7; and 5, 6 against 8, 9) shows the diminishing circulus counts with the sea age of spawning trout. For both populations and for both sea ages there is a significant drop in circulus counts with sea age.

The use of circuli counts as a tool for scale analysis is illustrated in Figure 7 where the frequency distribution for both mature and immature trout are depicted using the samples of more than 100 individuals from River Simested.

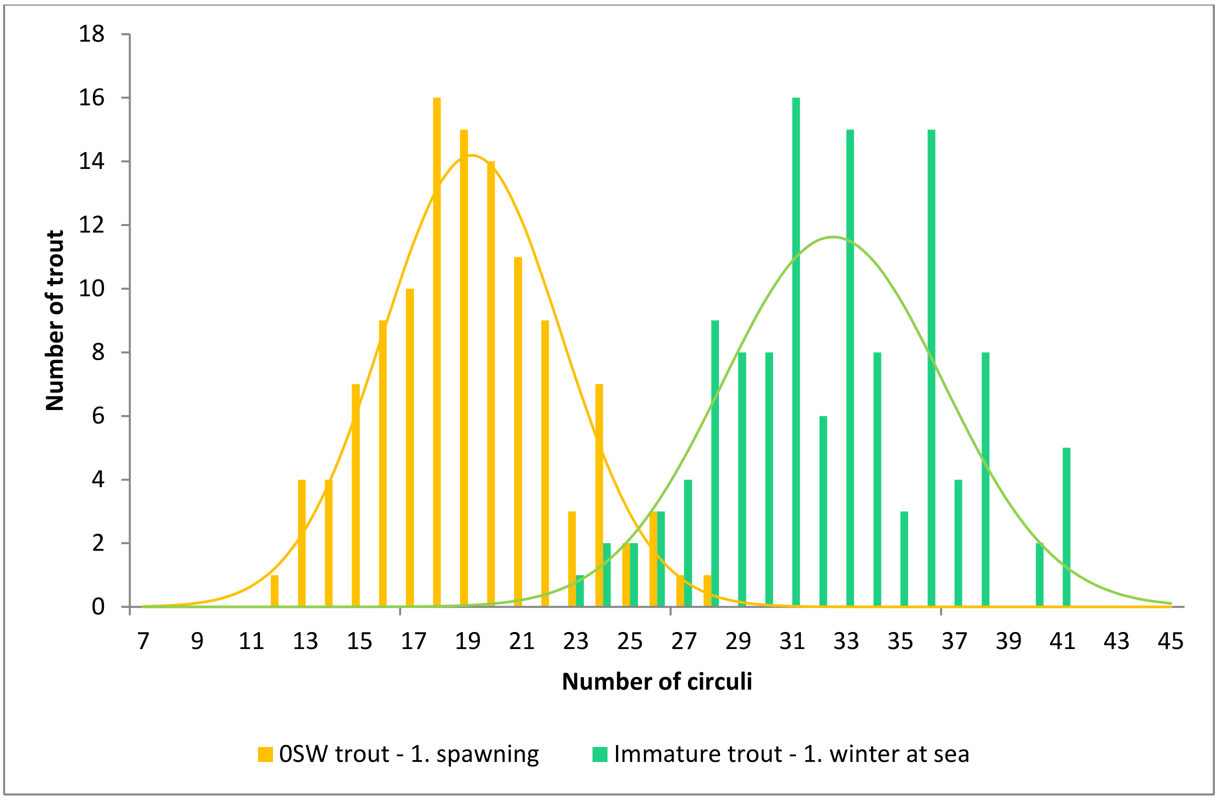

Figure 7. Dataset 3. Frequency distribution of circulus counts from 0+ sea trout.

Figure 7 shows a very small overlap of circulus counts from immature and mature trout of the same age.

4. Discussion

The primary purpose of this study was to provide a supplementary tool for scale reading by using circulus counting. The secondary purpose was to provide the basis for increased time resolution in scale reading. Both of these purposes ideally call for a constant circulus formation rate independent of environmental factors, possible hatchery treatment, and geographical or genetic differences.

The most obvious deviation from a constant circulus formation rate takes place during the winter period where no circuli were formed (Figure 1). This means that the winter period is represented by a single circulus (check) on the scale and that the compression of the distance between circuli seen during the last part of the season takes place during the autumn (Figure 8).

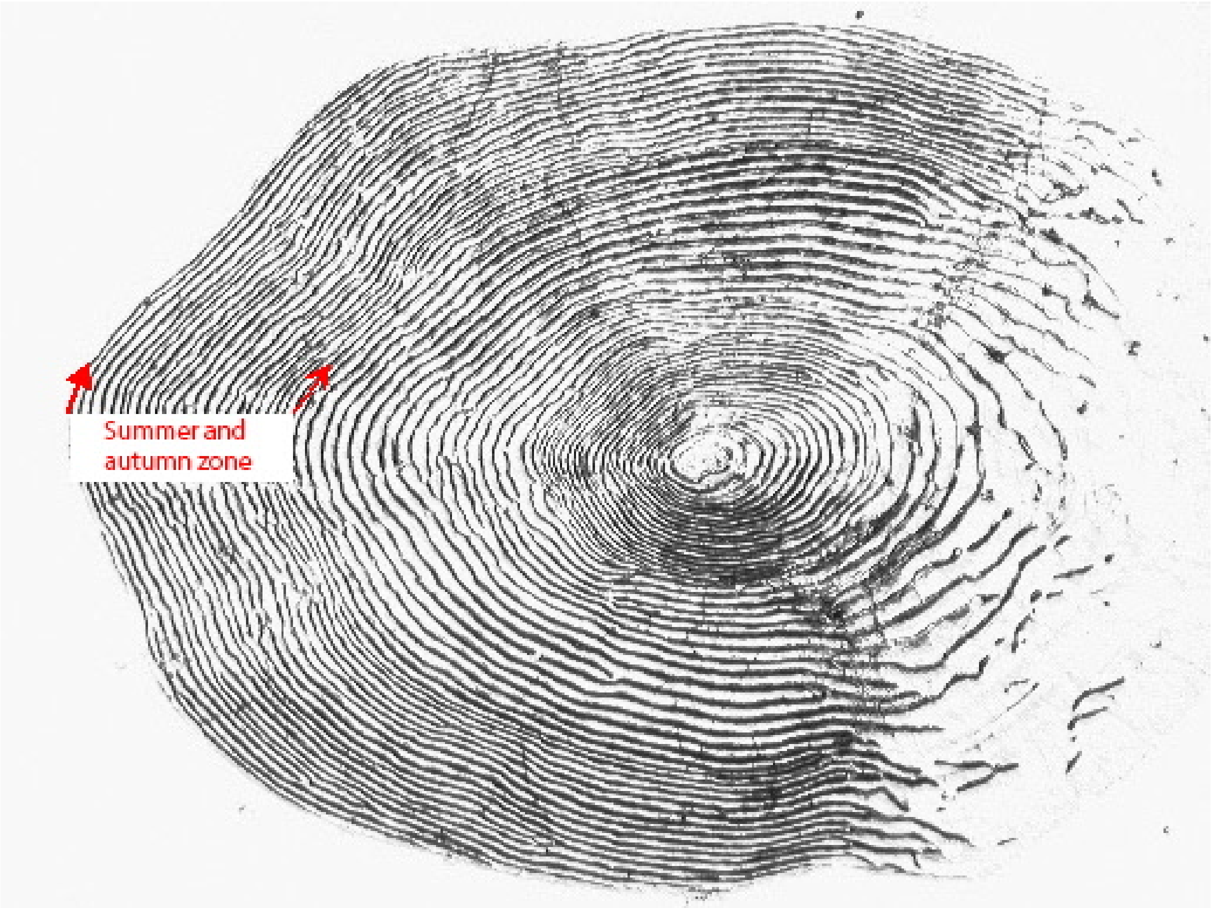

Figure 8. Sea trout from River Simested (dataset 3). 35 cm long immature fish caught by electrofishing on Nov. 11 2001. One year in freshwater and 7 to 8 month in the sea. The zone between the arrows corresponds approximately to the period from June 1 to Nov. 11. Some erosion can be seen along the scale margin although the fish is immature.

This phenomenon was also reported for *Oncorhyncus kisutch* (Fisher and Pearcy, 2005). Other authors report a total stop for circulus formation in low temperatures (Jensen and Johnsen, 1982). In this study, the period with no circulus formation in immature fish lasts from the middle of November to the middle of April (Figure 1 and Figure 4). It is obvious that the length of this period will differ on a larger geographical scale due to the different duration of the winter period. This calls for some kind of local calibration before using circulus counts in other geographical regions. Collection and circulus count of post-smolts during their first year at sea is the most obvious tool for this.

The total lack of circulus formation during the winter period combined with different duration of this period opens for alternative fitting of many published data calculated on a yearly basis. This includes papers that report a varying and decreasing rate of circulus formation rate (Wells *et al.*, 2003; Todd *et al.*, 2013).

Spawning also stops circulus formation. In this material stop of circulus formation takes place in August before the spawning during the following winter. The number of circuli in the year of spawning becomes smaller as the sea age of the fish increases (Figure 5, Figure 6, Table 5, Table 6, Table 7, and Table 8). As the scale formation rate is approximately the same during the growing period for all subsamples (Figure 2, Figure 4, Figure 5, Figure 6) the stop in circulus formation must take place at different times during the season. Altogether, this means that the sea trout stops making circuli earlier and earlier during the succeeding spawning years as their sea age increases. In this material, this tendency is not correlated with number of spawnings but only with sea age (Table 8). The result indicates that the trout may mature earlier in the year as their sea age increase.

The circulus formation rate during the growing season is remarkably uniform and all values found are close to 1 circulus per week. The standard error on the circulus formation rate is approximately 5% (Figure 2, Table 4), but the residual variance around the regression line (Figure 3) is quite large. However, an analysis of variance reveals no tendency for this variance to become larger during the growing period (Table 4). This is interesting as two explanations for the variance could be postulated: (i) a difference in circulus formation rate between individuals, or (ii) a difference between individuals of the date where circulus formation starts. The first hypothesis would lead to an increase in variance through the season, while the other one would not because all the variation would be present from the start. The data from this investigation therefore points to the second hypothesis as an explanation for the major part of the variation, while the circulus formation rate itself shows smaller variation. The value of the circulus formation rate is consistent with most findings from the growing season (Bilton and Robins, 1971b; Hubley *et al.*, 2008; Jensen *et al.*, 2012).

Given a fairly constant circulus formation rate, it is possible to relate every circulus on the scale to a point in time. In this way, a scale calendar can be constructed. This calendar has a resolution of approximately one week, when the onset of circulus formation is known (release experiments), while a month will be a more fair value when it is necessary to include the variance in the onset of circulus formation in spring. This is a large improvement from the resolution of one year that is the present status, where only the age in years is known.

This finding can be used for several purposes. Firstly, it can improve the accuracy in scale reading, secondly it can make scale based growth calculations possible within the growth season, and thirdly it can make it possible to relate micro chemical samples taken in very small areas of the scale to time of the year (Figure 9).

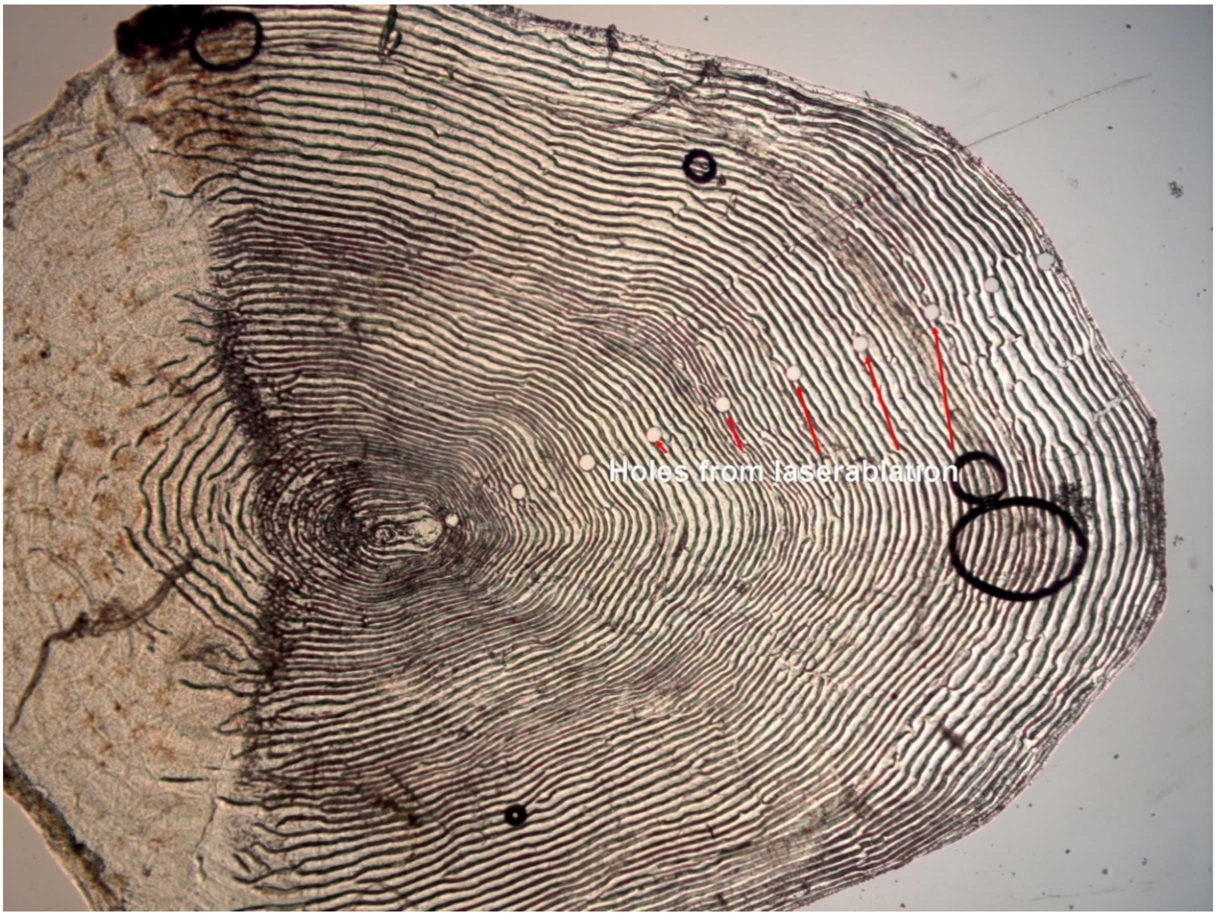

Figure 9. Sea trout from River Simested. 50 cm female. Caught on electrofishing Nov. 6. 2010. The figure shows holes from laser ablation made to analyze for relative proportion of selected chemical elements. Circulus counting helps in relating the different holes to different seasons.

Salmonids make resorption of scales in many different situations (Dannevig and Dannevig, 1937; Bilton, 1974; Kato, 1977; Ottaway and Simkiss, 1977). These resorptions are seen as so-called checks or repaired irregularities of the scale rim when scale formation is resumed. The most pronounced of these are the spawning marks associated with spawning, but also a less pronounced check associated with the winter period is normally found (Figure 8, 10, and 11). Besides that, early summer checks and prewinter checks are found in this material (Figure 10 and 11). The basic role of circulus counting in scale reading is to place these check at different times of the year, thereby supporting their discrimination from spawning marks. In many situations, this becomes a necessity for judging the correct age and spawning story of sea trout.

Within all the scales read during this investigation, a large part gave rise to reading problems during the first year at sea.

Counting from the seaward migration approximately 1/3 of the fish showed a zone with dense circuli from circulus number 5-10 to circulus 15-20. Using standard scale reading very conservatively this might well be judged as the first winter, while circulus counting tells us that it might be in the period from late May throughout July (Figure 10). Because of this, it is clear that this zone must represent a period with reduced scale growth. A possible explanation could be that some of the sea trout experience sub-lethal high temperatures in the inner part of bays and fjords.

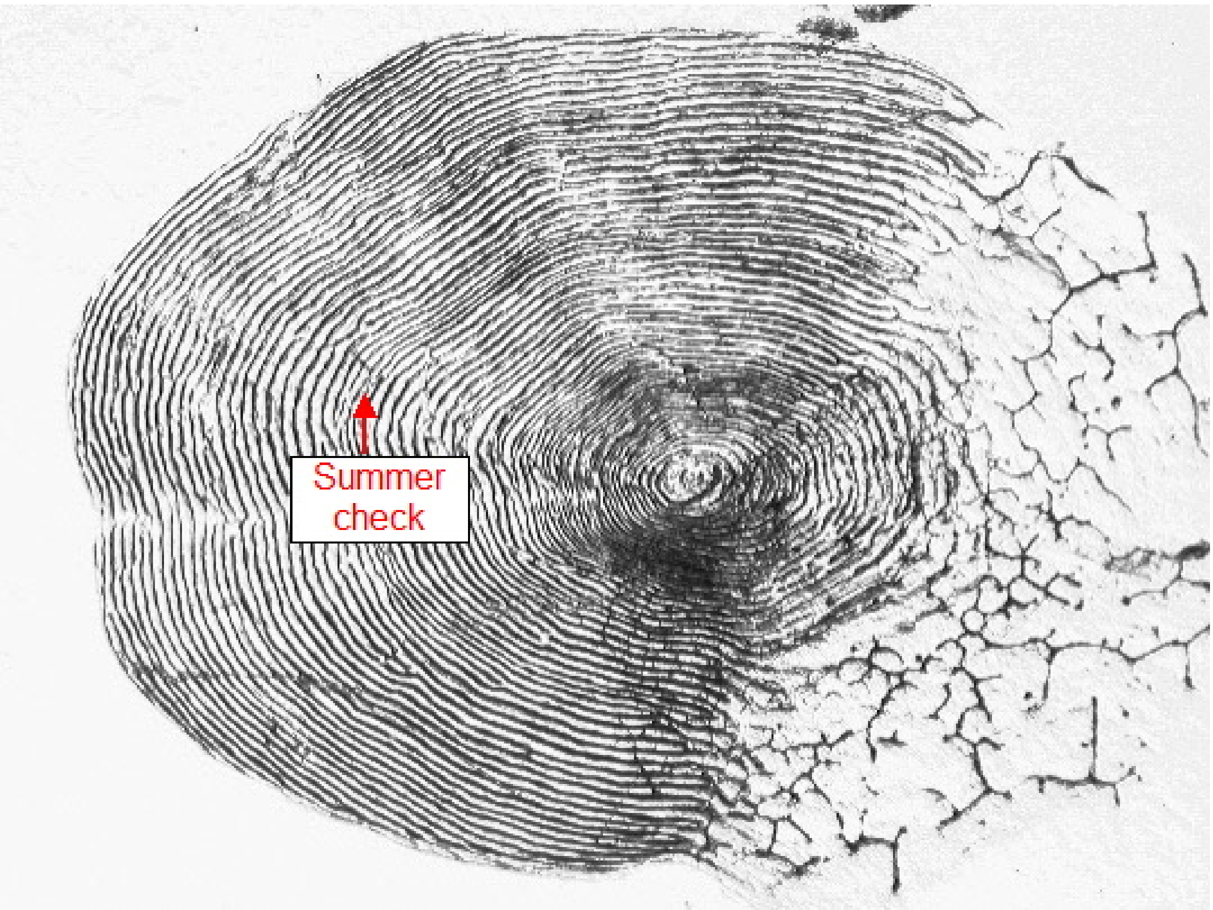

Figure 10. Immature sea trout from River Skals (10 km south of River Simested). 39 cm long caught by electrofishing Nov. 14 1998. The zone "check" can be dated to the month of May by circuli counting. Notice also the slight erosion of the scale margin.

In many Danish streams, scale samples from spawning sea trout are taken during November or December, when electrofishing of spawners for hatchery based breeding takes place. At this time of the year, it is often impossible to distinguish small 0SW spawners from immature trout without dissection. Scale reading could be of some help here, but nearly all of the investigated trout shows a distinct winter stop in scale formation, often accompanied by a slight erosion of the scale rim. This could easily be taken as a spawning mark because spawning marks in small trout generally are less distinct than in larger fish. However, circuli counting reveals a large difference in circulus number between immature trout and 0SW trout, with only a small overlap between the two populations (Figure 7). In this case, the combination of external inspection of the fish, evaluation of the erosion, and circulus counts in the scales will provide a sufficiently accurate distinction between these subpopulations. Circulus counting becomes increasingly important when dealing with scales from larger and older sea trout, because the early markings on the scales from these fish tend to be somewhat eroded. Many sea trout in this study show two winter checks with slight erosions in the rim (Figure 11). Standard scale reading might relate the last of these to spawning in the following year although the erosions are not nearly as large as in most spawning marks. In the material from the release experiment (dataset 1) where the age of the fish are known, these two winter checks can be seen already in December. Conclusively these checks must be formed within the first year. The last of them corresponds to the winter stop. Two winter checks are also seen in trout of unknown age, and only circulus counting reveals that the first one is formed during autumn.

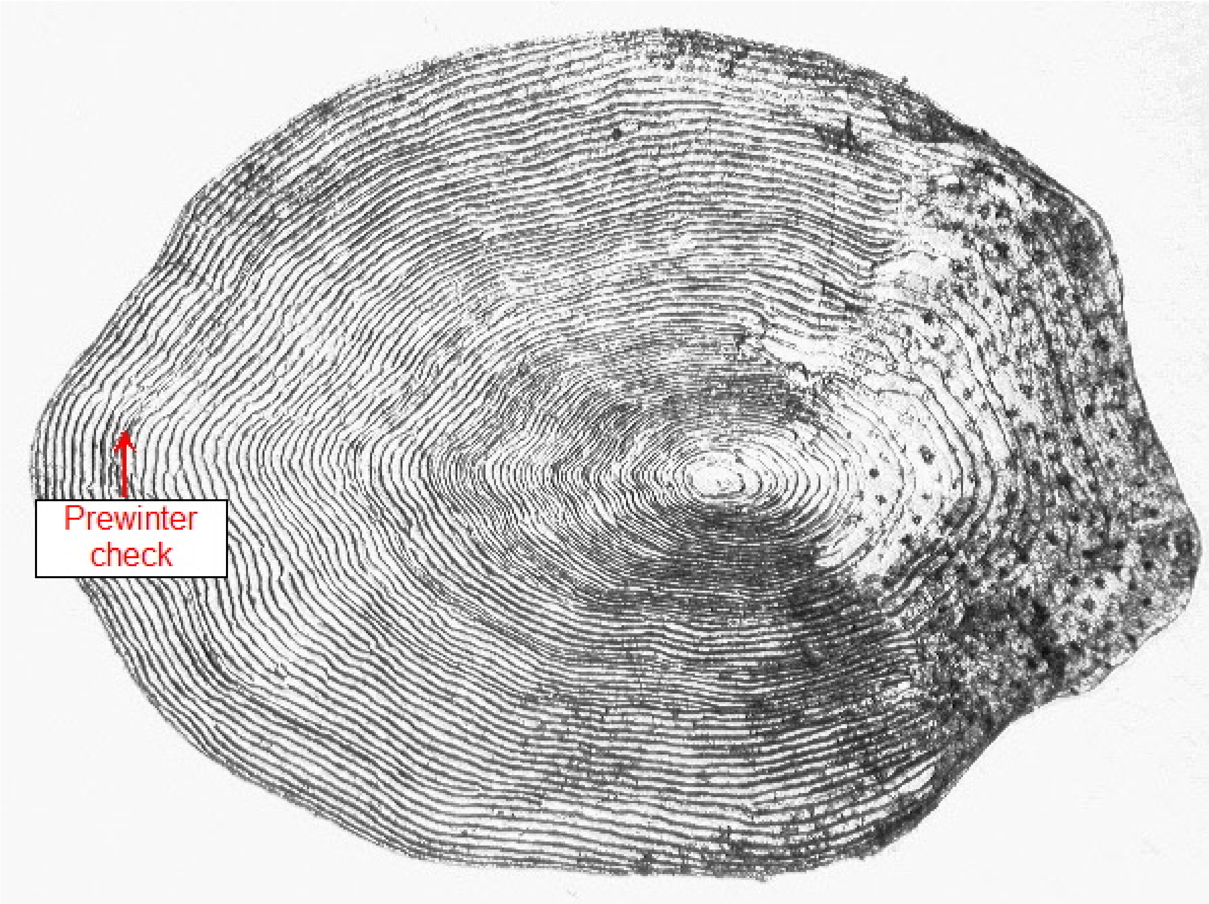

Figure 11. Immature sea trout from the release experiment (dataset 1). The fish were released in May and recaptured March 29 the following year after almost one year at sea. The scale shows a prewinter check from late November and a proper winter check with scale erosion later. The scale also shows a zone of denser circuli during summer.

As depicted above scales from sea trout in the investigated geographical areas, show a high diversity of different patterns, and the depicted scale samples only show the most common ones. For some investigations, a high accuracy of scale reading is a prerequisite for meaningful analyzes (i.e. calculation of annual mortality of repeating spawners or calculating differences in the abundance of different cohorts among the adult fish. Circuli counting might be a helpful supplementary tool in these connections.

Other scale reading problems might be present in other populations of sea trout in other areas. This can be caused by climatic constrains or the presence of other limiting abiotic or biotic factors, that influences scale growth and circulus formation. Circulus counting might not always cope with these, but on the other hand, it might be a helpful tool when a proper calibration has been performed.

The present investigation involves datasets collected over a period of 50 years. It cannot be ignored, that climate change during this period could change the calibrated values. Especially the onset and stop for circulus formation in spring and winter might change considerably with only slight changes in water temperature in shallow water areas. Collecting postsmolt in the spring and immature trout in winter could consequently be necessary when dealing with the investigated populations nowadays.

Growth calculation from scales is based on proportionality between scale radius and fish length. Because it is often useful to relate growth calculations to certain periods in the life story of the trout, a correct determination of both age and life history by scale reading is most often necessary. As concluded above, circulus counting improves scale reading, thus also improves the accuracy of growth calculations from scales. The use of circulus counting in connection with the scale based growth calculations goes beyond this although, because it also gives improved time resolution. This makes it possible to make growth estimates for smaller time periods, and for instance compare early growth in spring with late growth in autumn. Calculating weekly or monthly growth from scale measurements and circulus counts calls for some precaution. For short periods, the growth of scales is not necessarily isometric with the growth of fish (Kato, 1977). Using the distance between circuli as a proxy for estimating relative growth rates during the season has been conducted by several authors in different salmonids: (Friedland and Haas, 1996; Wells *et al.*, 2003; Hubley *et al.*, 2008) - *Salmo salar*; (Fisher and Pearcy, 2005) – *Oncorhyncus kisutch*; (Fukuwaka and Kaeriyama, 1997) – *Oncorhyncus nerka*. These applications do not necessarily call for a complete isometry between growth rate and circulus formation rate, because they focus on comparing relative growth from different periods or from different subpopulations.

Methods for measuring the concentration of substances in very small areas on the scale are either optical or based on laser ablation of a small area (Coillie and Rousseau, 1975; de Pontuall and Geffen, 2002). Both methods are at present improving in accuracy. In connection with salmonid ecology, they have been concentrated on giving the relative composition of specific chemical elements and their isotopes, but future applications might also include other chemical compounds. In many cases, the use of these methods need knowledge of the life history of the fish investigated in order to explain the findings. The whole idea is of course to be able to relate the analyzed spot on the scale to a certain life situation of the fish. In this case, an improved time resolution of the scale reading will be most valuable.

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