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Faculty of Technology, Natural Sciences and Maritime Sciences
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# Scale and growth analysis of Sea trout (*Salmo trutta L.)* using object j caught in Etneelva in 2019.

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This thesis is worth 60 study points

# Summary

There is a decline in sea trout *(Salmo trutta L.)* population in central Norway since 1970s. While the reason behind this reduction until now unknown but marine mortality can be one of the reasons. Sea trout, known as anadromous brown trout, shares its life both in fresh water and sea water and shows a complex life history. Young sea trout migrates to sea and undergo smoltification commonly at age two, where they attain proper growth and return to freshwater during winter or late autumn for spawning or over-wintering. Sea trout, unlike Atlantic salmon is less studied and is very little is known.

In this study, age and growth of sea trout caught in Etneelva in 2019 is estimated by reading scales. Use of scales to estimate age and growth patterns has been very popular since 1980's as they are less expensive, non-lethal and less-time consuming as compared toother calcified structures. 1236 sampled sea trout scale images were collected from Etne lab and analysed using a free image processing software called image j and its macro objectj.4 out of 1232 were excluded from sample due to insufficient information and other 237 scale were replacement scales they were not used in back calculation as it underestimates the age estimation. Annuli in scales of sea trout are used to determine age and reconstruct growth histories. Back- calculation based on the assumption that fish length/growth is proportional to otolith or scale length is done. In salmonids, linear relationship is assumed between scale and body. Results showed that sea trout caught in etneelva was of age 1-15years. Majority of the young parr smolted at age three and four and a most at age two which

was a common age for smolts. Rapid variations in smolt growth rate was noticed for the first 3 years in sea after smoltification. This may be due to change in water temperature and food availability. Back-calculated first winter length for most sea trout was between 40-60 mm, but some were very high for first year length. Back- calculated winter length for sea trout showed a high variation in the growth between the age eight, nine, ten and eleven. likewise, back- calculated smolt size differs a lot at the age three and four.

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I would like to thank Fjeldheim Per Tommy from Etne lab who provided sea trout scales sample. Also guiding me how to read and interpret scales which was completely new to me. Also, I cannot thank enough Eivind who helped me with study protocol and guidance through my entire thesis.
Last, I want to thank my family and friends for supporting and inspiring me to complete my thesis.

Bø i Telemark, 15.11.2020

Sangita Bhattarai

# 1.Introduction

There is a decline in sea trout *(Salmo trutta L.)* population in central Norway since 1970s. While the reason behind this reduction until now unknown but marine mortality can be one of the reasons. Atlantic salmon being in the same species to sea trout had been studied more in comparison. Knowledge on population, migration pattern, age and growth are important for effective management and stock assessment.("<Current-Status-and-Review-of-Freshwater-Fish-Aging-Procedures-Used-by-State-and-Provincial-Fisheries-Agencies-with-Recommendations-for-Future-Directions.pdf>,")
As the fish grows, it gains age and age and growth are corelated. Age assessment from calcified structures (scales, otoliths, fin rays, bone of the jaw etc.) has been a major factor for effective fish stock management and population dynamics("<10_chapter 6.pdf>," ; Campana & Thorrold, 2001; Haglund & Mitro, 2017; van der Meulen, West, & Gray, 2013; Závorka, Slavík, & Horký, 2014). Scales shows life history similar as tree rings. Use of scales to estimate age has been popular since early 1980's as they are non-lethal, less time consuming, inexpensive and easy to sample multiple times (Elliott & Chambers, 1996; Stolarski & Hartman, 2008) unlike otoliths .Beside its advantages, scale reading age estimates has low accuracy and precision in comparison to otoliths especially in slow growing fish(van der Meulen et al., 2013). Likewise, in Salmonids, it is often difficult to locate the first-year annulus and also annuli are closely placed in slow growing fish which makes the scale reading difficult("<Scalesvsspines.pdf>,").

Scale- based age estimation and back calculation relies on the annuli counts of scale and provides information on previous growth histories to calculate individual annulus length(Heidarsson, Antonsson, & Snorrason, 2006;

Pierce, Rasmussen, & Leggett, 1996). Back calculation depends on the body -scale growth relationship (Heidarsson et al., 2006; Li et al., 2008) and its formula using scales was first developed by Dahl (1907) and Lea (1910). According to Francis (1990), there are two main techniques to back calculate length at age :1) direct method, where length of fish and length of calcified structure at capture is noted, and 2) by regression method.

## 1.1. Aims and Objectives

Atlantic salmon and other fish have been studied a lot and gained quite attention but much less is known about sea trout. Sea trout scales can provide age and growth estimates and growth histories can be constructed. However, very little study has been done. Information regarding populations, marine migrations or human marine activity effect on marine migrations are poorly studied. The overall purpose of this master thesis is to determine different life history characteristics of Sea trout (*Salmo trutta L.)* in Etneelva river, based on the information obtained from scales and sampling information (length at capture, weight, Date and the location). To be more specific study focus on:

- Age and Growth structure analysis.
- Migration patterns.
- Back-calculation winter length.

# 2.Methods

## 2.1. Study species

Figure 1: Sea trout("<Celtic_sea_trout_project_-_summary.pdf>,")

Sea running form of brown trout, sea trout (*Salmo trutta L*) shares both freshwater and sea water life and is known as anadromous and adopts many alternative life histories(Ramey, 2007). Anadromous Brown trout belongs to the family Salmonidae and is predominant to Europe, North Africa and western Asia. Sea trout are found in small streams to large rivers and in marine area.

Sea trout, specially smolts undergo seaward migration  from fresh water in spring  and return in late summer or autumn for spawning or overwintering(Olsen, Knutsen, Simonsen, Jonsson, & Knutsen, 2006). This seaward migration is seen as a life history strategy in order to gain growth, fecundity and fitness(Harvey, Glover, Wennevik, & Skaala, 2020). Sea trout live in fresh water for almost 1-8 years (commonly for 2-4 years) before migrating towards sea and can attain body size up to 10-25 cm and undergo smoltification. Smolting level in sea trout can depend on the body size and the origin e.g. juveniles with rapid growth can migrate to sea even without smolting("<Environmental+influences+on+life+history+strategies+in+partially+anadromous+brown+trout.pdf>,").

Sea trout usually feed during summer at sea but variations can be seen as they can also spend their winter at marine water. This results variation in spawning patterns, some may spawn right after first summer but some may after two or three summer. Sea trout may spawn two or more times throughout their life and hence are called iteroparous(Eldøy et al., 2015). The recorded sea life in sea trout is up to 13 years. ("<REPORT Sea lice sea trout review.pdf>,").

## 2.2. Location and data collection

The Etneelva river is located on the south western of Norway in Etne municipality, Hordaland county (UTM zone 32, 331719 east,6619892 north). From Stordalsvatnet to Etnefjorden, the river is about 7km long. The river includes a large tributary stretch of 13 km for anadromous salmonoids. Etneelva is also known as Nordelva ("<Fjørtoft master.pdf>,").

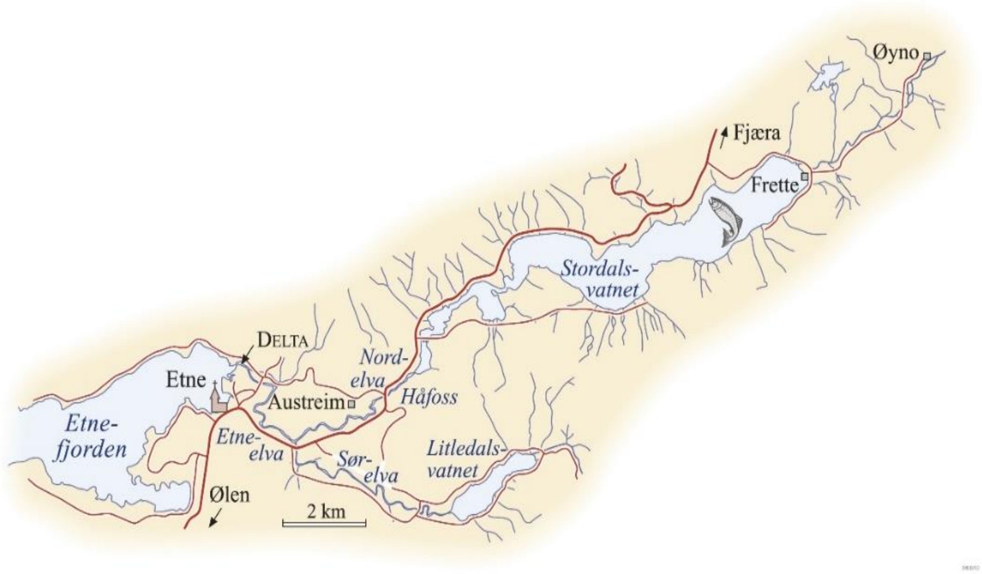


Figure 2. The Etneelva river and its tributaries. (source: google)

Age and growth of fish were determined by reading scales. Total 1236 high resolution scales image of sea trout were collected from Etneelva river located at the south western, Norway in Etne municipality as my secondary data. All scale images were obtained by the help of Per tommy at Etne from April to November 2019. Out of 1236 sample ,4 sample were excluded due to insufficient information. Total 1232 sea trout scales were read using a free image processing program called image j and its macro object j. 237 sea trout scales were regenerated, so they were not included for age determination and kept in separate group. Only 995out of total, were used for back calculation. All measurements were done along the longest axes of the scale, focus to the edge of the scale (figure 7).

### 2.3. Scale analysis

Scale images were analysed using a free image processing program, Image J and using its plugin Object J. Image J can read, process and analyse images in the form TIFF, PING, JPEG etc., as stack which makes analyse easy. (https://imagej.nih.gov/ij/docs/intro.html).object .

Object J is the macro in image J which was designed to count tree rings, measure eucalyptus leaves and cell (https://imagej.nih.gov/ij/docs/intro.html). This plugin is developed to measure the annuli count in otolith and determine past growth histories.("<Tutorial_Otoliths_ObjectJ.pdf>,")

#### 2.3.1. Installation

- Image J was installed in computer(https://imagej.nih.gov/ij/download.html).
- Then object J was downloaded and installed(http://sils.fnwi.uva.nl/bcb/objectj/download/current/objectj_.jar

#### 2.3.2. setting a project file

##### 2.3.2.1. loading images in object J

A copy of project file otoliths_1.10.ojj was moved to the folder that contains scales to be analyzed. Object j becomes visible by dragging project file to image j window. All the images are linked from the folder. Choose object J>show project window>linked images choose one by one images or a folder to link images.

#### 2.3.3. Analyse

As the images are linked, sequence was started by double clicking image. Press f1 or go to (image j >object j>start sequence). Cursor becomes green itself which mark the core, red cursor measures the vector and the blue marks each increment in scale (in any order). Backspace key can be used to delete the last point just in case if a point is missed. Sequence was finished by pressing f2(or go to image j>object j>year and quality). Positive number can be used for start year and negative for the end. I used negative value (-2019) which is fishing year. After year, scale quality is labelled from 1 to 3 which explains very good, good and poor respectively.

Since my images were of high resolution, year annotation became too small so following steps were changed. Go to ImageJ>ObjectJ>" Show Embedded Macros" and adjust line 6 "font Size". click "Install in ObjectJ menu". To apply the changes in the image, select the Object J-finger-tool, press the blue increments, and press F2 to set the year again. Overlay was saved and measuring scales continued.

### 2.3.4. Output results

Once all scales are analysed, project is saved and results were obtained pressing f3 or go to object j> create output table. All the data's obtained are exported to excel for further calculations. An organized result table is obtained which explains Sample name, Quality, Age birth year of fish, diameter of focus, order of increment of each annulus and Increment of each annulus.

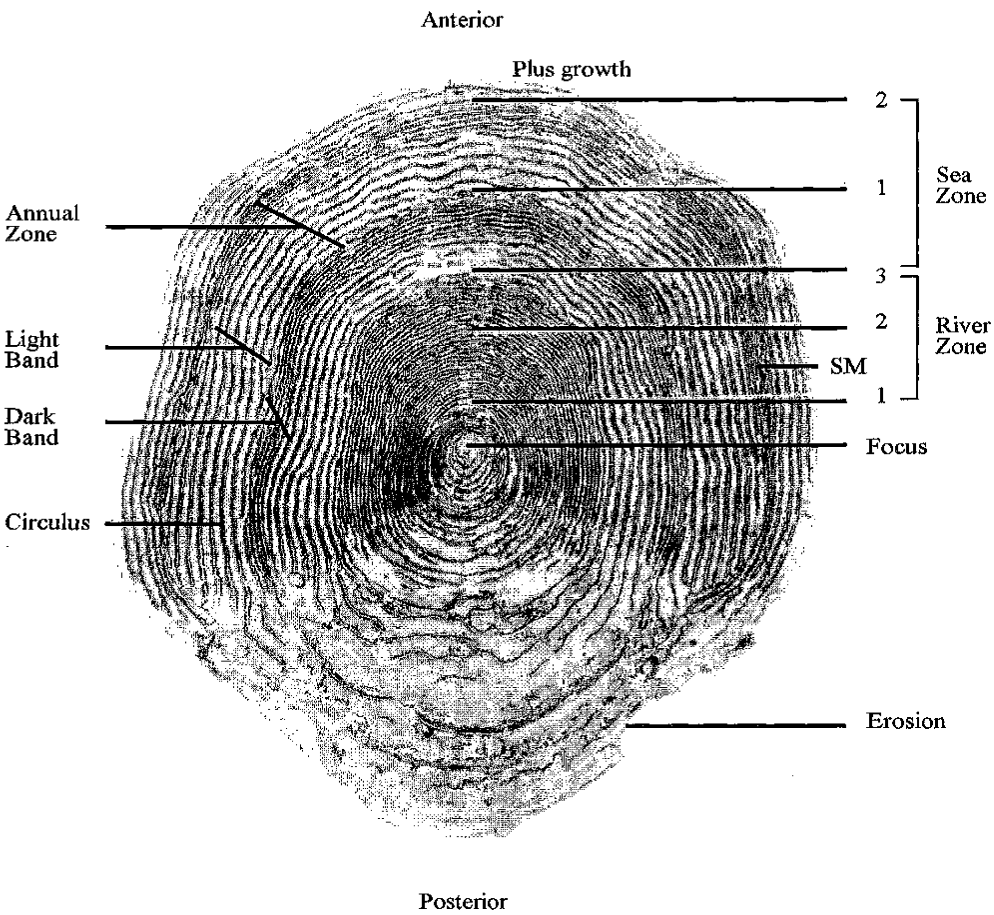


Fig 3: An overview of sea trout scale with different features. (Elliott & Chambers, 1996)

Sea trout gets their first scale at the length 30-40 mm. Generally, a newly born fish lacks scale, focus is established first and represents the size of the scale. As the fish grows, an addition ring is formed around focus called circulus. Rapid growth is seen during summer and the circuli are widely spaced due to high temperature and nutrients present. Whereas, slow growth is observed during winter and circuli are closely packed. This uneven seasonal growth results darker distinct band, a transition between two annual zone called annuli. Each annulus explains a complete year life and thus counting these annuli, age can be estimated(Elliott & Chambers, 1996).

## 2.4. Scale reading and interpretation

Age determination is done by scale reading and scale is read by counting number of annuli. Annual growth on scale is distinguished by the formation of dark and light bands representing winter and summer growth. The main important steps to follow while reading scales are: 1) choosing best scale, 2) identify the end of the river zone ,3) count number of annuli in river zone and any run-out is noted and 4) count annuli number and spawning marks in sea zone.

It is important to choose a best quality scale for reading and age determination. A best scale should have a well distinguished focus and annuli both in fresh water and marine zones with restricted erosion and spawning marks at the posterior position of the scale.(Elliott & Chambers, 1996)

The fresh water zone is distinguished by the short distances between the circuli and forking of the circuli. Whereas, sea water zone is identified by increased spacing of circuli.

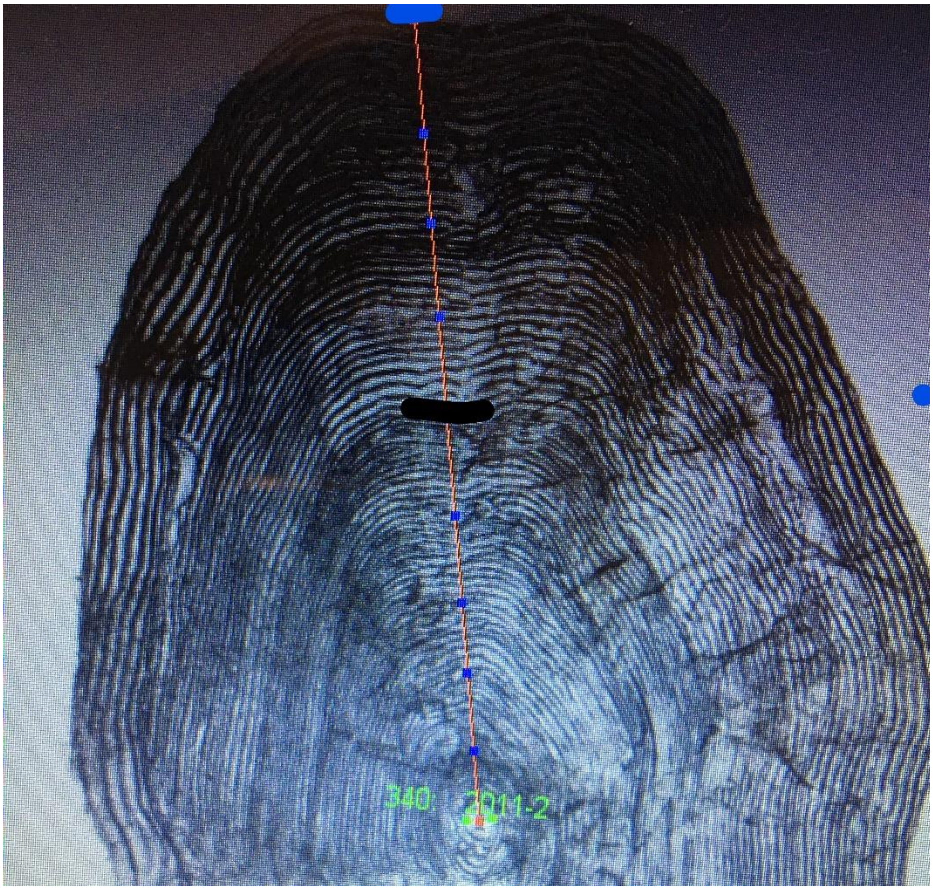


Figure 4. Scale of sea trout(A0172) caught from Etneelva in 2019. The longest axes of the scale from the focus (bottom red dot) to the edge of scale (upper red dot), fresh water zone (black line) and sea zone (top blue line). Each blue dot represents annuli.

A number of sampled fish included previous spawners and were identified by the erosions at the edge of the scale during the sexual maturation phase. More erosion is seen in older fish and also when the fish is captured at the time near to the reproduction. This result formation of spawning marks that erodes the sea winter band completely and it makes the age estimation difficult in previous spawners. Spawning marks are more present in sea trout as compared to lake and river trout("<Guide of Scale reading in Brown trout.pdf>,").

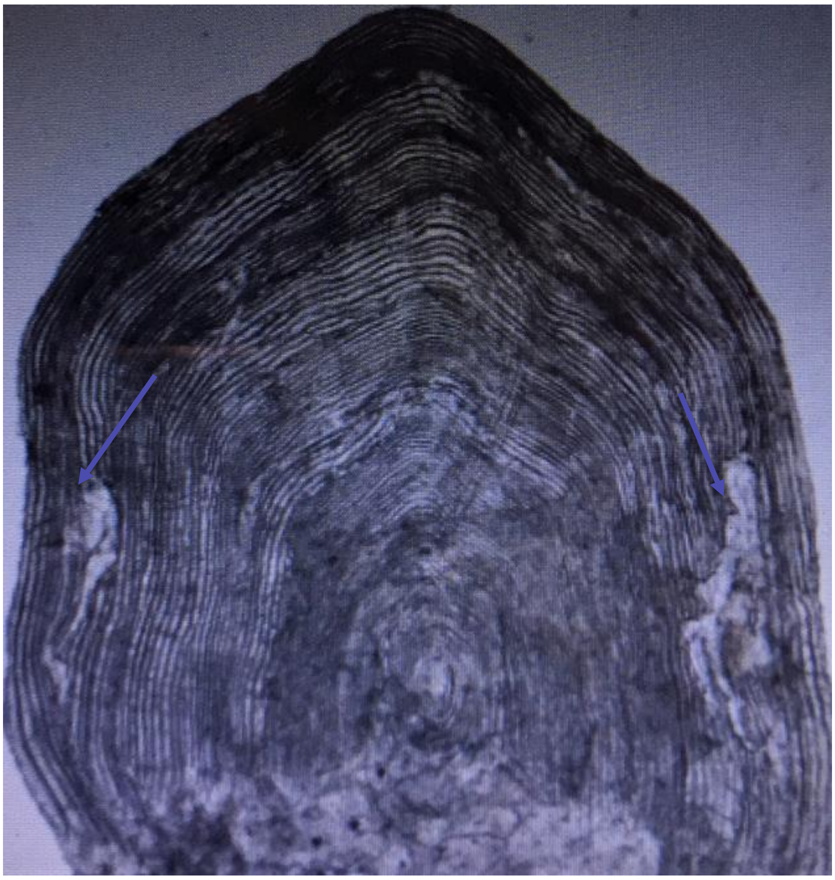

Figure 5. Scale from sea trout caught in Etneelva river showing spawning marks at the side edge (blue arrows).

When a fish loses its scale due to some environmental factor or some injuries, a new scale is regenerated. On a replaced scale, a disorganised centre having a scar is noticed i.e. an open centre with an absent focus. These scales are useless for the interpretation and age determination as they falsely underestimate the age value.(Elliott & Chambers, 1996).

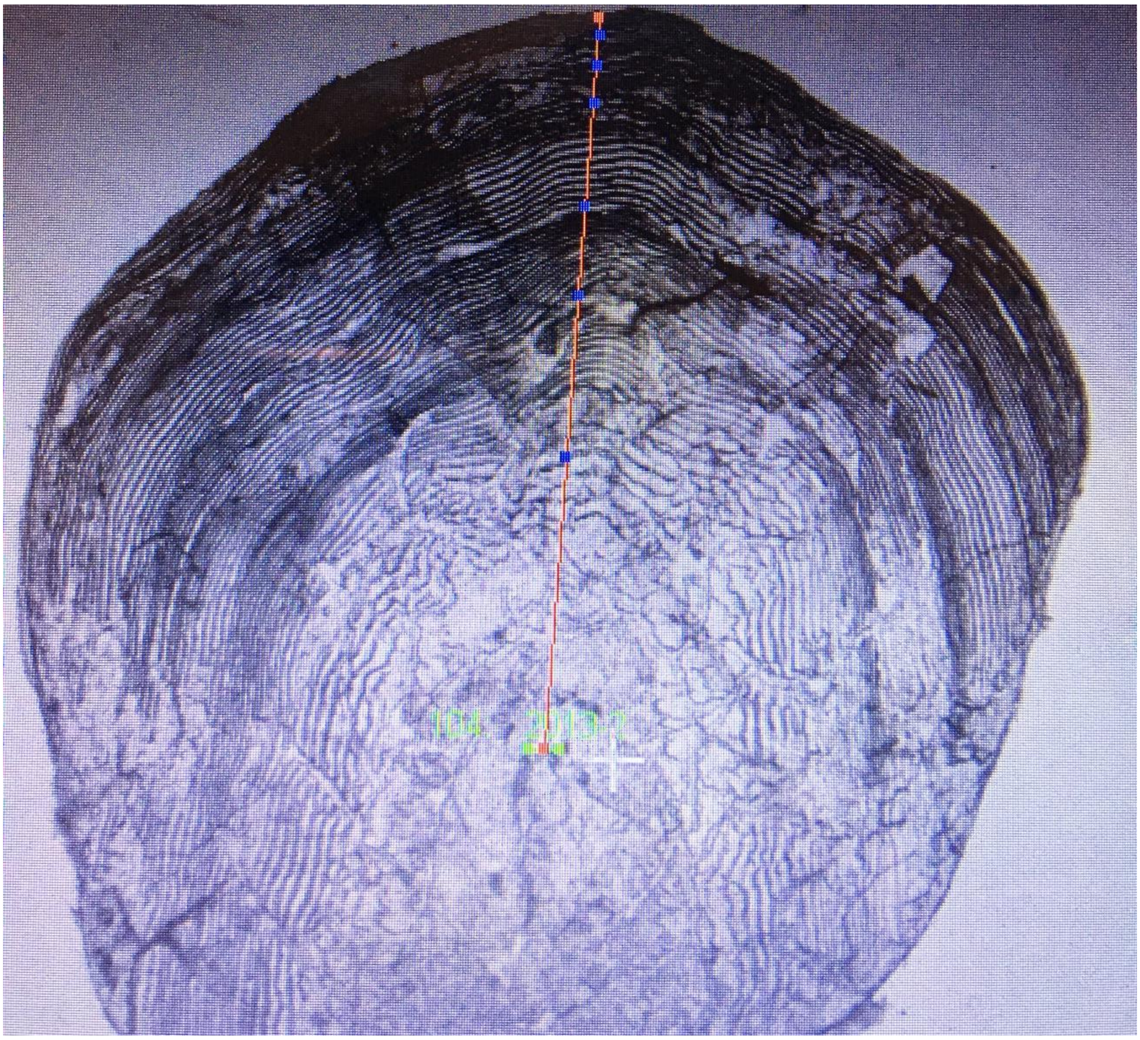


Figure 6. Replacement scale (A104) from sea trout caught at Etneelva with disorganised and open centre.

## 2.5. Age determination and back calculation

Age determination is done by scale reading and scale is read by counting number of annuli. Annual growth on scale is distinguished by the formation of dark and light bands representing winter and summer growth. We can easily back calculate length at each annulus if the growth rate of bony structure and fish body is same. There are many model proposed to back calculate but the first model was developed by Dahl and lea(Heidarsson et al., 2006). Back calculation is based on the assumption that fish length/growth is proportional to otolith or scale length. In salmonids, linear relationship is assumed between scale and body ("<Atlantic Salmon Scale Reading Guidelines.pdf>," ; Ryan, Shephard, Gargan, & Roche, 2019). Length at age can be estimated by simple equation:

$$Ln = L*(Sn/S)$$

where Ln is length for age to be estimated, L is the length of fish at capture, Sn is the length of scale at nth annulus and S is the total scale length from focus to edge.

(a)

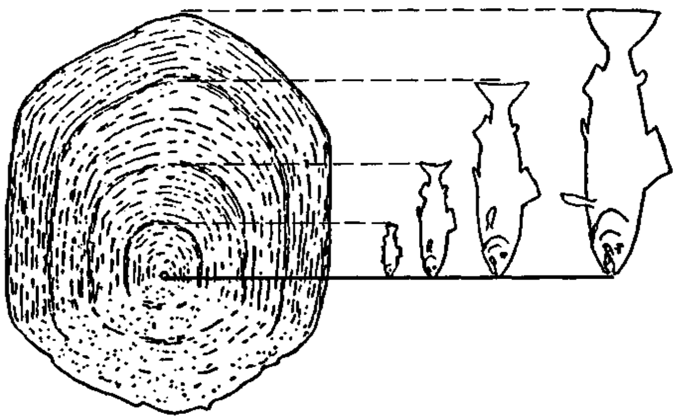

Fig.7: Back calculation of length from scale.

a. Direct proportionality between fish length and scale length.

(Elliott & Chambers, 1996)

## 2.6. Statistical analysis

Using excel further calculations were done and plots were made. With the obtained data, I looked for the following metrics and relationships:

1. length distributions for each sampled trout.
2. Back -calculated first winter length distributions.
3. Back- calculated total length as a function of fish age.
4. Mean back -calculated length as a function of fish age.
5. Smolt -age distribution.
6. Smolt length distribution.

Since my sample size was large, it was hard to make lineplot in one for all the individuals in Excel. So, I illustrated my data in 4 different line plot. Sample size and sample id all noted down of each plot.

# 3. Results

## 3.1. Individual growth trajectories

All sampled sea trout were of relatively similar sizes before their first year (figure 8,9,10,11). But for sample A060, A066, A426, A825, A826, A850, A861, A864 A938, A1085 and A1103, length for the first year was greater than 70 or around 100mm which was highest for this species. Rapid increase in body size of most sea trout was seen after second winter as shown in all 4 figures. More rarely the growth spurt occurred after third and fourth year. Significant decrease in the growth rate can be observed after its fifth or sixth year. Though the size for sampled sea trout at its first year were of similar sizes, variation in size increased during the following several years. For all the sea trout of same age group the size was relatively similar but the variations in back- calculated length was considerably noticeable. For example. Sea trout sample A01 and A055 both of age 8 and similar body length at capture had variations in back- calculated length.

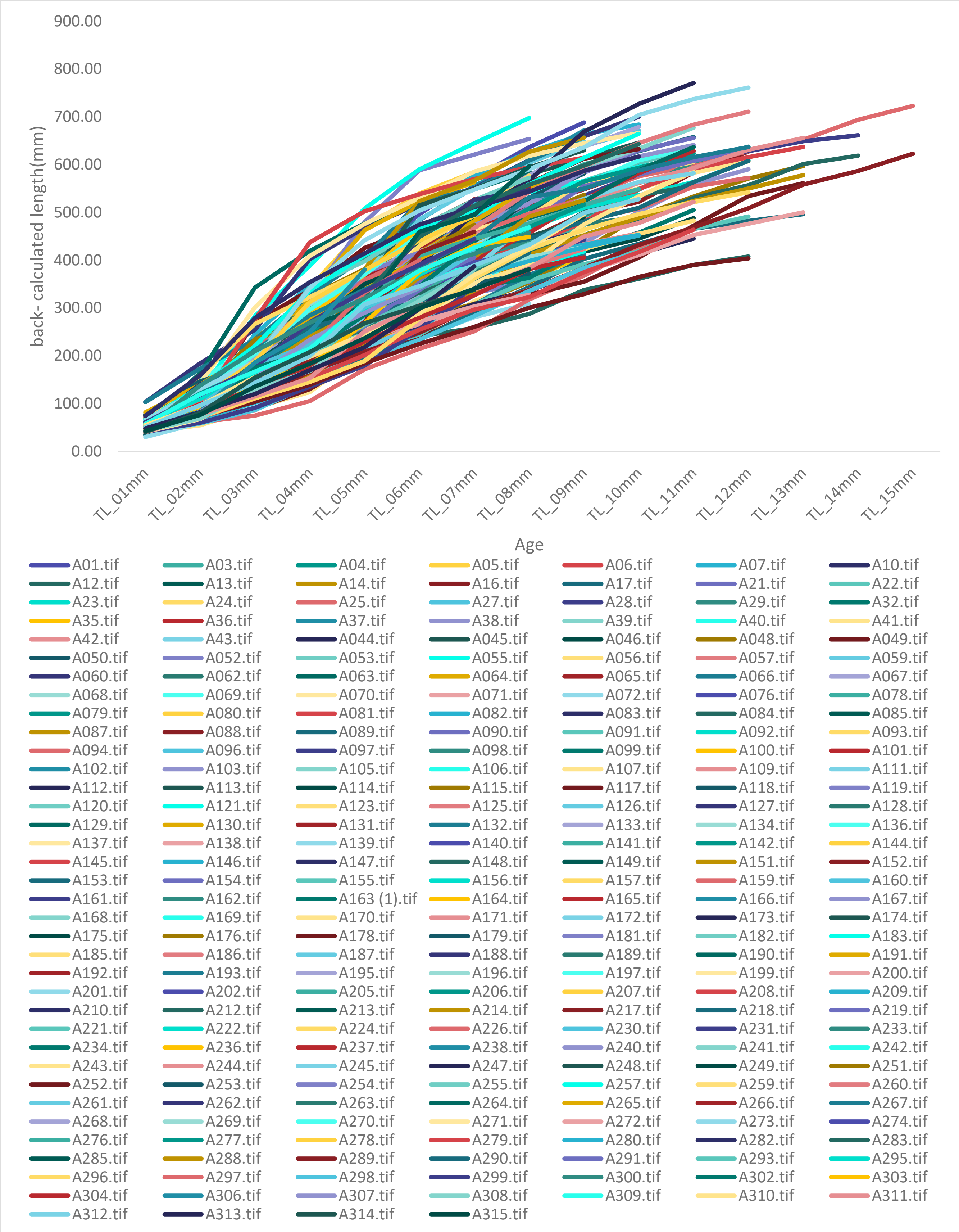


Figure 8. Back- calculated length in milli-meters as a function of age in years for sea trout caught in Etne elva in 2019. Each of 249 sampled sea trout

has individual line color. Total length at every year was back calculated using distance between each annuli and total fish length obtained from scale reading.

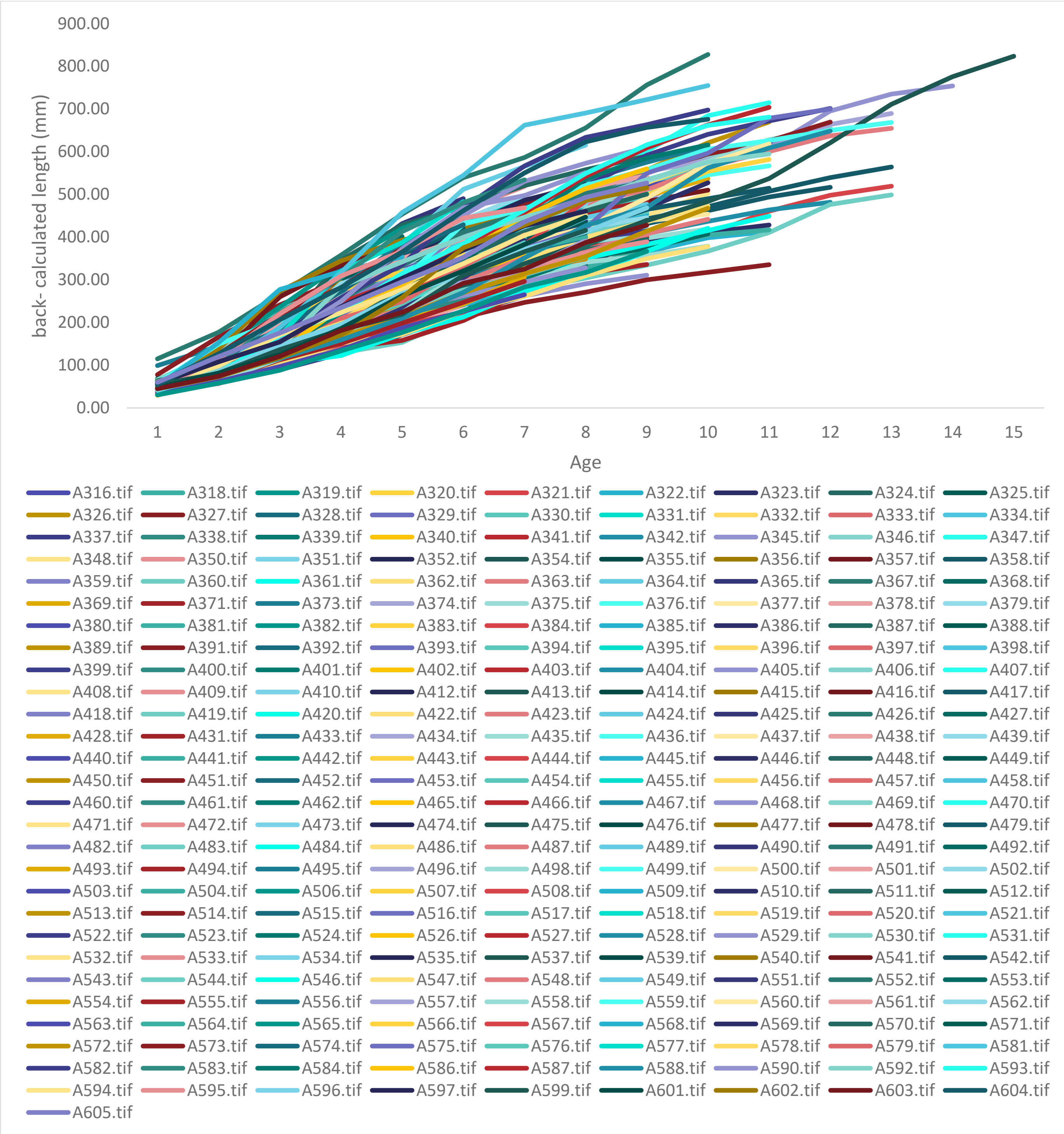

Figure 9. Back- calculated length in milli-meters as a function of age in years for sea trout caught in Etne elva in 2019. Each of 253 sampled sea trout has individual line color.

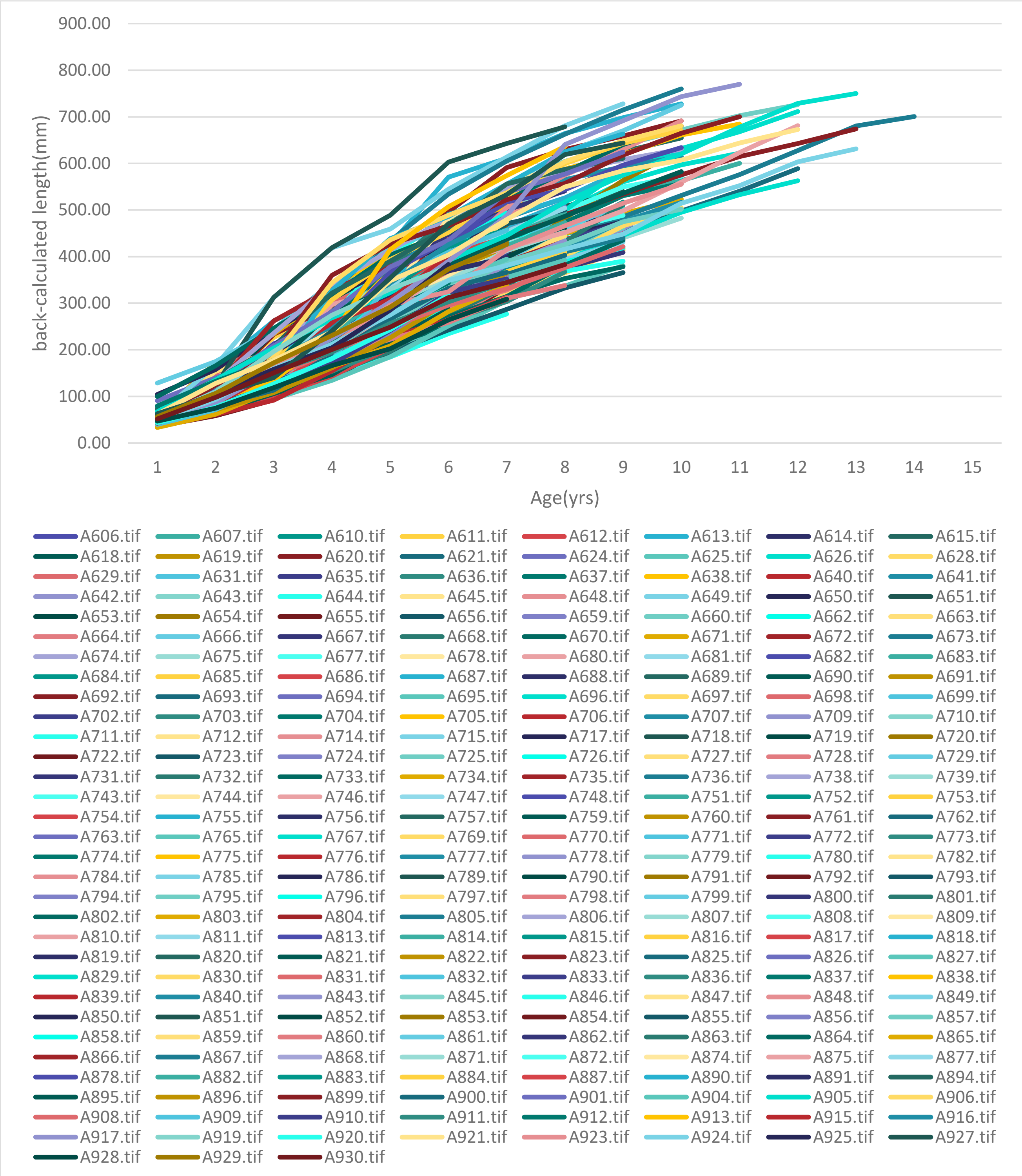


Figure 10. Back- calculated length in milli-meters as a function of age in years for sea trout caught in Etne elva in 2019. Each of 251 sampled sea trout has individual

line color. Total length at every year was back calculated using distance between each annuli and total fish length obtained from scale readings.

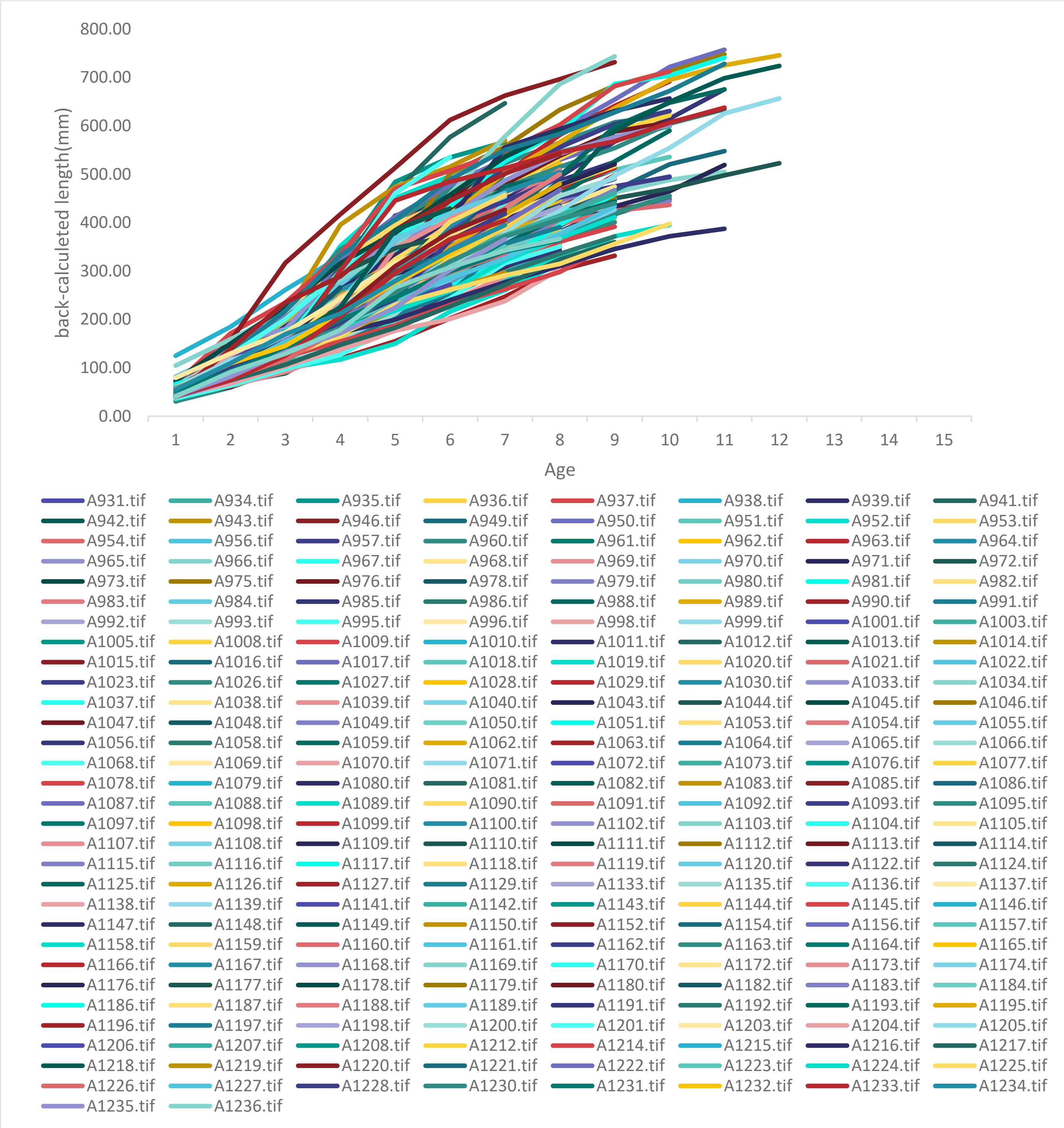


Figure 11. Back- calculated length in milli-meters as a function of age in years for sea trout caught in Etne elva in 2019. Each of 242 sampled sea trout has individual line

color. Total length at every year was back calculated using distance between each annuli and total fish length obtained from scale reading.

The frequency of back- Calculated first winter length of sea trout from Etne 2019 showed considerable differences (figure 12). Majority of sea trout back calculated lengths were 40 -60.mm at the first winter year, which is common for this species. Out of 995 back calculated sea trout number, 35 were more than approximately 70 mm and 40 below 40 mm at the end of their first winter.

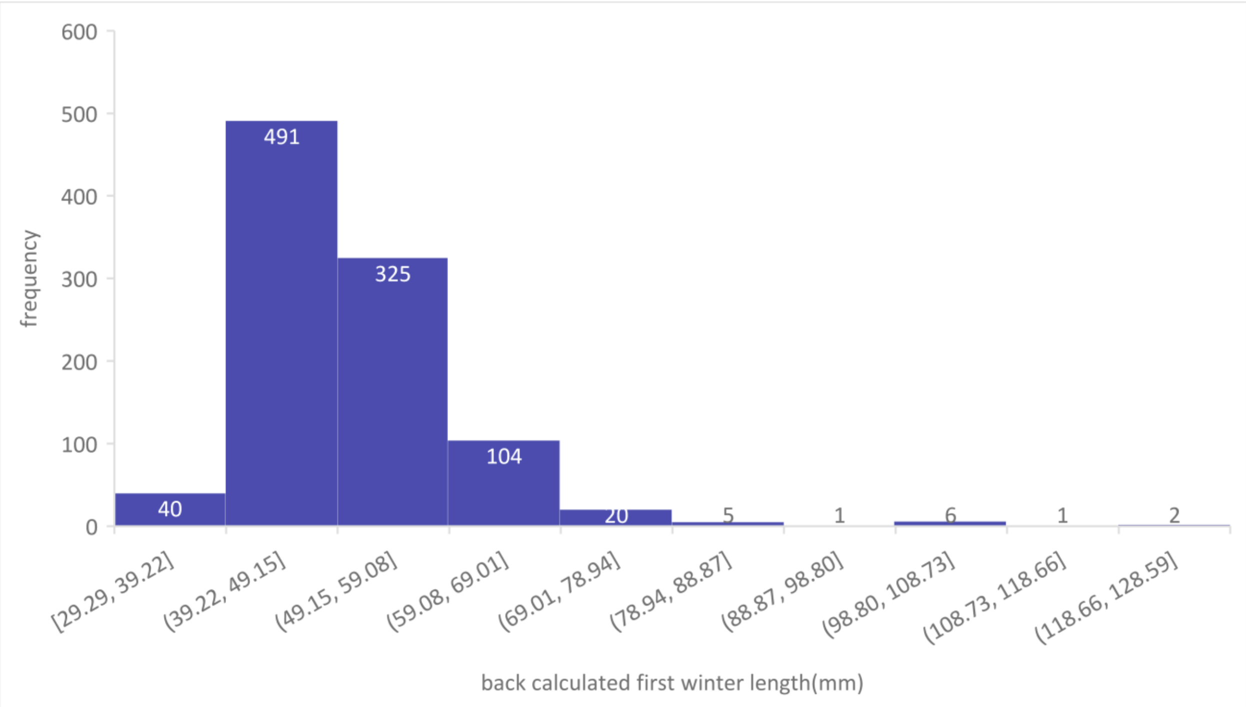


Figure 12. Back-calculated first winter length distributions in millimeters caught in Etneelva in 2019.

Sea trout growth rate in Etneelva varies through the years. Growth rate is stable for the first six to seven years and is decreased by the following years. With the increasing age, growth rate is decreased (figure 13). The variation in size is lowest in the first winter and is increased by the following years, highest during ten, nine, eleven and eight years. Also, considerable variation in length can be seen during twelve, seven and six years. Boxes includes number of observations, horizontal line represent the medians, whiskers show the variation in sizes and dots explains the outliers. During first winter, the variations in back-calculated first winter is relatively small but increased following the ten years and decreasing again to the age of fifteen.

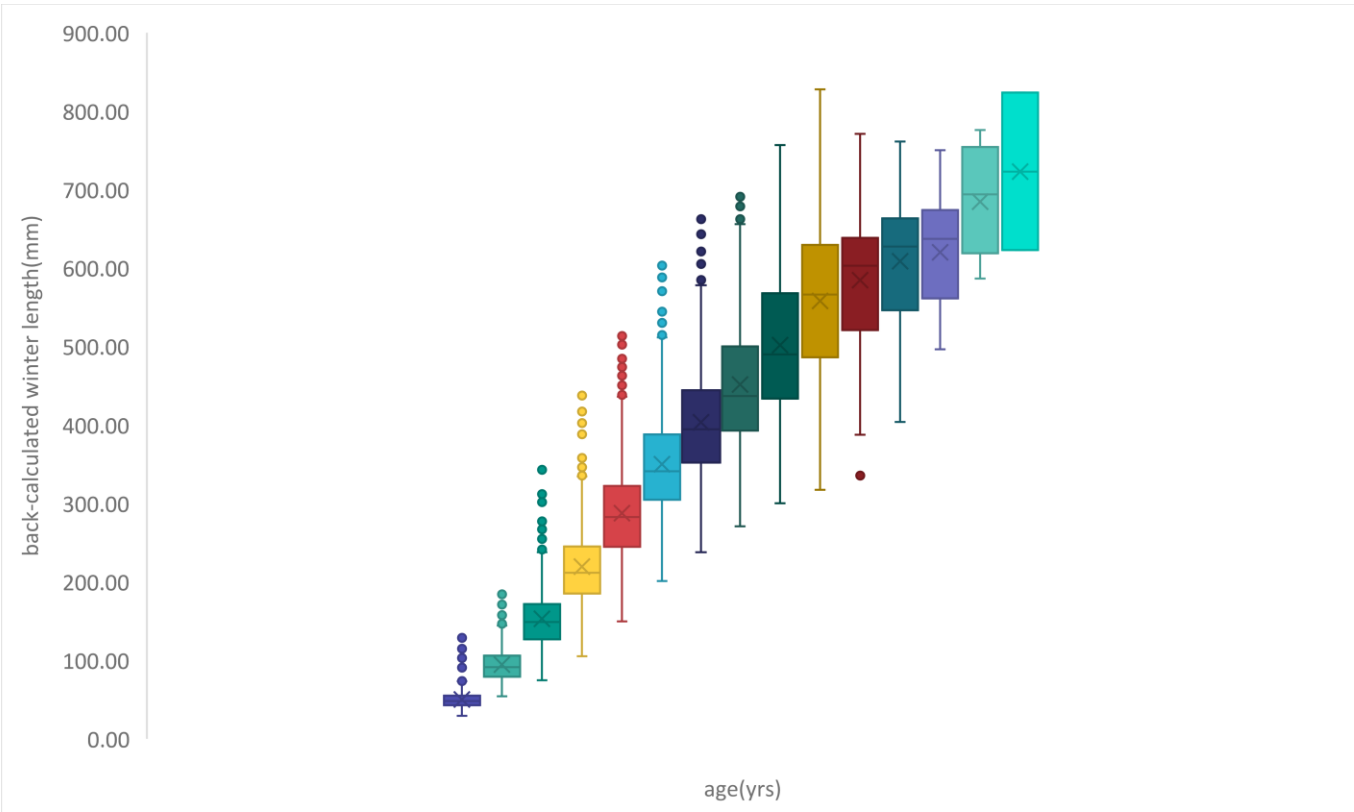

Figure 13. Correlation between back -calculated length(mm) for sea trout caught in Etneelva in y- axis and winter age(years) in X-axis. Boxes entail the majority of observations, horizontal line shows the median, whiskers show variations in length and the dot explains the outliers.

The average mean back -calculated length for sea trout caught in Etneelva in 2019 was 50mm. Rapid increase in mean length was observed from their second year upto six year(figure 14). With a stable increase in mean size during the age seven to the age ten, a decline in the mean length in the following three years was noticed. Mean back calculated length again increased at the age fourteen and fifteen.

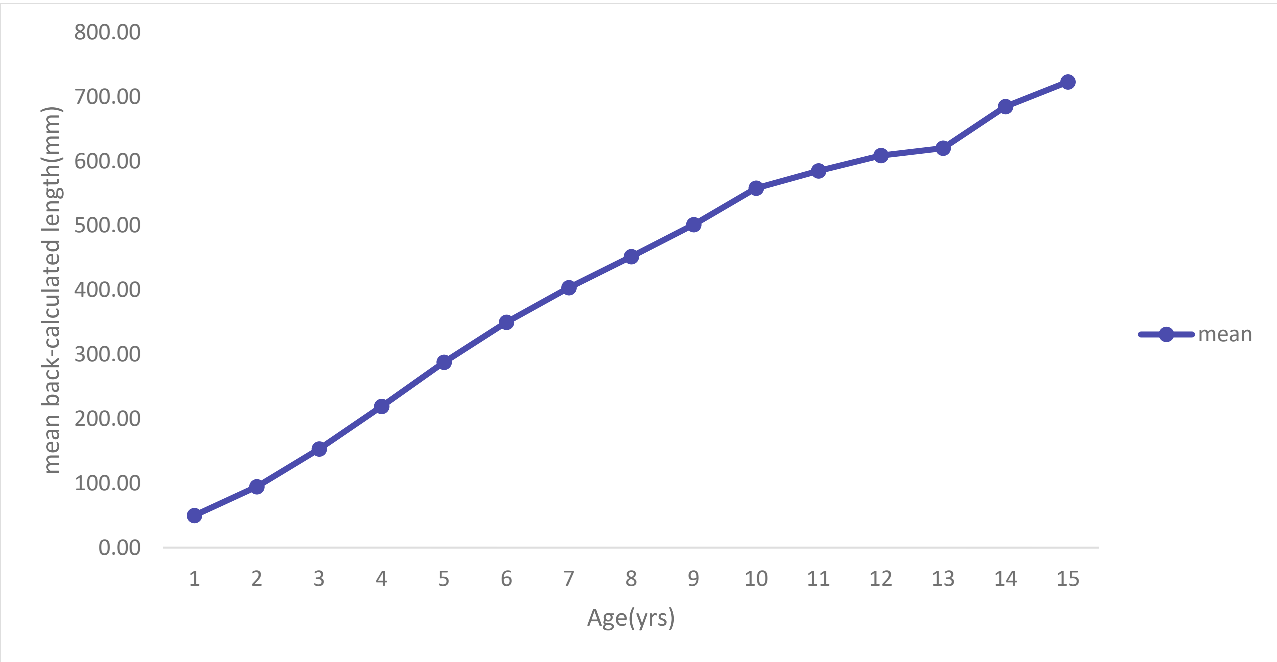


Figure 14. line-plot showing mean back -calculated length (mm) in y-axis as a function of winter age (yrs) in X-axis of sea trout caught in Etneelva 2019.

## 3.2. Age and size at smolt

Smolt age for sea trout caught in Etneelva 2019 range from one to six year. Majority of the sea trout smolted at the age of three or at four which is common age for this Species (figure 15)("<REPORT Sea lice sea trout review.pdf>,"). Most of the sea trout also smolted at age two. As juvenile sea trout can spend 1-8 years in Fresh water and up to 10-25 cm before seaward migration(Harvey et al., 2020) .However, some sea trout remain in fresh water up to five and six years. Also, three sampled sea trout smolted earlier at the age of one. Considerable differences are seen in back calculated first winter length among sea trout that smolted at the age three and four.

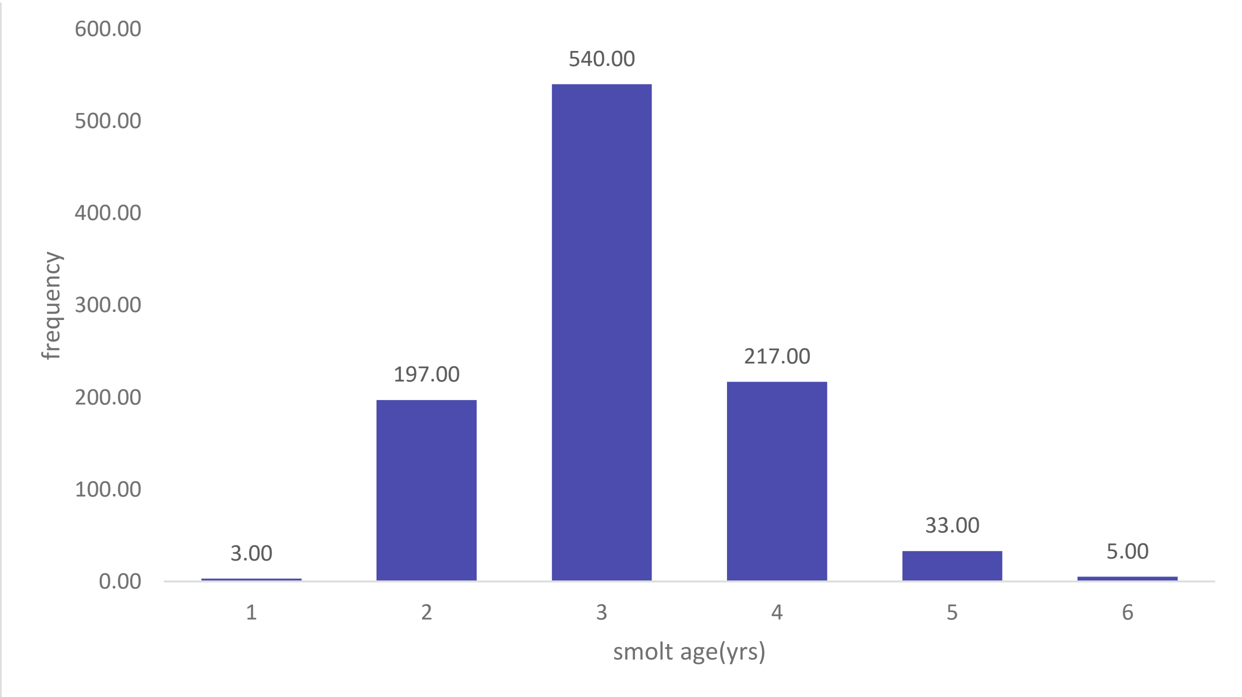


Figure 15. Smolt age distribution for sea trout caught in Etneelva in 2019.

Majority sea trout smolted at the age of three or four when they were around 150 mm (figure16). A small portion of sampled sea trout migrate towards sea when they were close to the size 250 and 300mm at the age five and six. Only three individual smolted at the age one showing no significant differences in back-calculated smolt length. Variations in body size was higher for the sea trout that smolted at the age three and four and lowest during the

age of one.

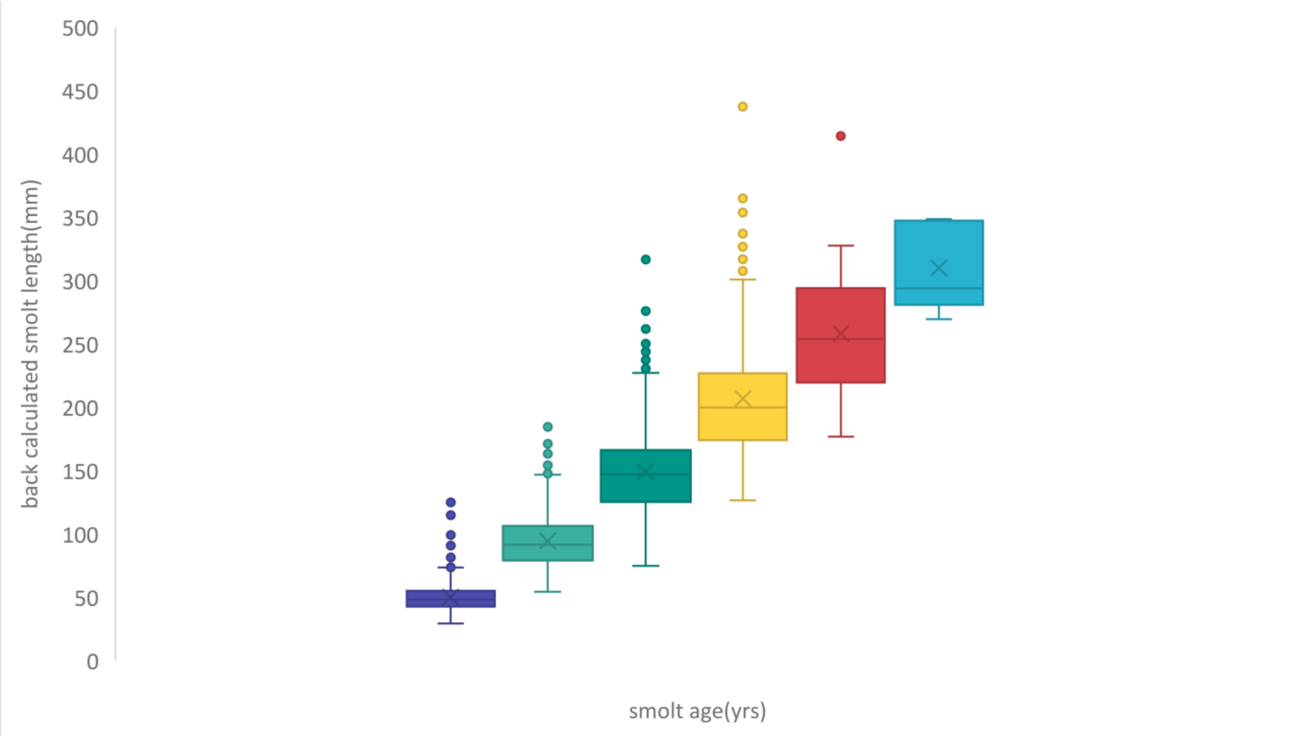


Figure 16. Back calculated smolt length in millimeters as function of smolt age for sea trout from etneelva in 2019. Boxes represents majority of observations; horizontal line show the medians, whiskers show variations in length and dots represents outliers.

# 4. Discussion

All the biological data obtained shows that sea trout age, size, smolt length, smoltification age, size at first year are normally similar to the parameters of the same studied species(Eldøy et al., 2015; Jonsson, 1985).

## Individual growth trajectories

Growth patterns for majority of the sampled sea trout is similar in comparison. We can see most of the sea trout were of relatively similar size before first winter (figure 8,9,10,11). First year length for high number of sea trout ranges from 40-60 mm which is common for most of the fish. However, for some first-year growth rate is comparatively high ranging 70mm to more than 100 mm. A rapid growth rate in sea trout aged two was seen as it was time of smoltification. A growth spurt at the age of three and four was noticed. A significant year to year variation in growth rate was noticed.

All the sea trout caught in Etneelva in 2019 ranges from 1-15 years. Sea trout has life expectancy of 20 years. We can say that all the sea trout are matured enough. However, for anadromous brown trout study have found longevity is closely 8 years in northern Norway which is supposed to increase with latitude. Life expectancy decreases with increasing water temperature. Growth rate in most of the sea trout (figure 13) in my study start slowing down from 8 years in the sea. As, the growth rate is size dependent and decreases with fish size.

In my study, the back -calculated smolt length varied a lot between 39mm and 327 mm (figure 16). Data obtained in this study shows a more stable growth rate among all sea trout. Higher variability can be seen in the smolt age, this can be due to difference in spawning timing and habitats, food supply and temperature. As explained in the following two study:

According to the study conducted between 102 European population, mean smolt age varies from 2.1 to 4.5 years respective in north and south. However, for Norwegian waters (58 and 70N) average smolt age differs from 1.5 and 5.6 years. The mean length for smolt in the same study conducted was 10.7 cm to 25.2 cm however in Norway size varied between 6 and 32 cm, this variation might be due to increase in latitude as a result of changing water temperature.("<Native_Trout_Workgroup_Atlantic_Salmon_Brown_Trout_and_Arctic_Charr_A_Review_of_Aspects_of_Their_Life_Historie_Klemetsen_et_al_2003.pdf>,").

According to Reidar Borgstrøm and Jan Hegennes, Considerable changes in sea trout size and age prior to smoltification might be due to variation in water temperature, food availability, predation change and low water flow.

Another Comparison can be done with the study done in small stream Årungselva, in southern Norway. In this study, growth rate in younger trout was high and smoltification occur earlier("<EarlySmoltification.pdf>,"). The mean size of 0+ sea trout in this study was 7.4cm whereas the average 0+ length is found 5.0 cm (figure 13 &14). Large variations between estimated growth patterns can be seen in two studies, former one showing higher growth rate.

Majority of the sea trout in my study smolted at the age three, four and most at two. However, some smolted at the age five and six. This is because smolt age is related to its size. So,fish that smolted late at age is relatively smaller in size and slow growing fish as compared to those smolting at early age("<Native_Trout_Workgroup_Atlantic_Salmon_Brown_Trout_and_Arctic_Charr_A_Review_of_Aspects_of_Their_Life_Historie_Klemetsen_et_al_2003.pdf>,").

# 5. Conclusion and recommendations

In this study, all the biological data obtained are quite similar to the studied parameters of the same species, despite some differences. Smolt age distribution, smolt length, back calculated first winter length all were considerably similar to the studied species. Variation in marine growth in most of the sea trout caught in Etneelva was seen. This may be due to salmon parasite associated with farmed Atlantic salmon which reduce marine mortality and sea life stages.

Studies on marine migration and survival of Atlantic salmon and other salmonoids species has been done but very less is known about sea trout. Detailed study on age growth pattern, seaward migration of sea trout is very important. Age determination by scale reading in slow growing salmonoids may result inaccuracy in age estimation. Reading same sea trout scales caught in Etneelva in 2019 to determine age can be a good option to reduce inaccuracy and biasness. Also, new sea trout scale samples from 2020 can be studied to see how the growth patterns has changed throughout year.

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