Master Thesis

Age Composition and Genetical Affiliation of a Sea Trout (*Salmo trutta f. trutta*) Smolt Population in a Stream in Schleswig-Holstein

Im Ein-Fach-Masterstudiengang, Fach Biologie der Mathematisch Naturwissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel

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1 Abstract

Juvenile sea trout (*Salmo trutta f. trutta*) spend their first years of life in freshwater habitats of little streams and rivers. Here they go through diverse developmental stages before they finally migrate to marine waters. The developmental stage during the first seaward migration is called "smolt". In this study, a smolt population was trapped during a spring migration in 2016. The smolt trap was erected in the Lipping Au, a little freshwater stream in northern Germany in Schleswig-Holstein discharging into Flensburg Fjord (Baltic Sea), in which natural sea trout reproduction occurs. Three kilometres before the estuary after the union of all tributary streams, the trap captured 2167 smolts during spring seaward migration in a period of two month. Among others scale samples for age determination and tissue samples (fin clip) for parent-offspring assignment tests were taken. In the Baltic Sea area of Schleswig-Holstein, there is a lack of information concerning age structures of smolt populations and the only study dates back decades. There is also little information about the success of stocking programs of early sea trout life stages that are part of the sea trout management in Schleswig-Holstein for more than 30 years. On the one hand, this study determined the age structure of a subsample (n=834) of the spring smolt migration via scale reading. On the other hand, a subsample of 951 smolts was genotyped in the laboratory. The DNA of smolts and adult fish used for stocking programs in 2013 and 2014 (n=255) was extracted from fin clips. A PCR amplified 12 established microsatellite primers before capillary electrophoresis and analysing the output with the programs GeneMarker v1.91 and Colony v2.0.6.1. to identify parent-offspring matches and according to this a stocking background. Scale reading showed that 85.85 % of the down migrating smolts were aged 1+. Remaining 118 smolts aged 2+ and no smolts older than this were found in the subsample. Main migration activity happened during three periods with rising water level. Inside these events older smolts migrated significantly earlier than younger smolts. Microsatellite analyses assigned 22 smolts with a stocking background (parent pair assignment to male and female of the parental stocking pool). That is a proportion of 2.31% of the subsample and a survival rate of 0.02 % of 120,000 stocked fry. An effective population size of 3040 individuals was estimated for the study system Lipping Au combining the two years. This study provides a valuable pilot study for estimating the age structure of a smolt population and the survival success from stocked fry to migrating smolts in a little stream in Schleswig-Holstein. However, a replication of this study is necessary to compare the results among different years with different environmental conditions.

Zusammenfassung

Junge Meerforellen (*Salmo trutta f. trutta*) verbringen die ersten Jahre ihres Lebens in den Süßwasserhabitaten kleiner Bäche und Flüsse. In dieser Zeit durchleben sie diverse Entwicklungsprozesse und Entwicklungsstadien und wandern schließlich in marine Gewässer ab. Das Stadium abwandernder Jungforellen wird als „Smolt“ bezeichnet. In dieser Studie wurde eine Smoltpopulation während der Abwanderung im Frühjahr 2016 gefangen. Eine Smoltfalle wurde an der Lipping Au, einem kleinen Bach mit natürlicher Meerforellenreproduktion in Schleswig-Holstein mit Mündung in die Flensburger Förde (Ostsee), installiert. Drei Kilometer vor der Mündung, nach Vereinigung aller Nebenflüsse, wurden innerhalb von zwei Monaten (April und Mai) 2167 Smolts gefangen. Unter anderem wurde den Fischen eine Schuppenprobe zur Altersbestimmung (Schuppenlesen) und eine Gewebeprobe (Flossenabschnitt), zur Untersuchung der genetischen Zugehörigkeit, abgenommen. Im Bereich der Schleswig-Holsteinischen Ostsee besteht ein großer Mangel an Informationen zu Altersstrukturen von Smoltpopulationen und die einzige Studie darüber liegt über 40 Jahre zurück. Auch Informationen über den Erfolg von Besatzmaßnahmen früher Lebensstadien der Meerforelle, welche seit über 30 Jahren Teil des Meerforellenmanagements in Schleswig-Holstein sind, gibt es wenige. Diese Studie definierte zum einen die Altersstruktur einer Frühjahrs-Smoltabwanderung anhand einer Stichprobe von 834 Individuen, zum anderen wurde eine Stichprobe von 951 Smolts in Laborarbeit genotypisiert. Hierfür wurde die DNA der gefangenen Smolts und die der Elterntiere (n=255) für die Besatzmaßnahmen 2013 und 2014 aus Flossenabschnitten extrahiert. Eine PCR integrierte dann 12 Mikrosatelliten (Primer) bevor die Proben sequenziert und schließlich mit den Programmen GeneMarker v1.91 und Colony v2.0.6.1 analysiert wurden um mögliche Verwandtschaftsverhältnisse und somit eine Herkunft aus Besatzmaßnahmen zu identifizieren. Mit Hilfe des Schuppenlesens wurden 85,85% der abwandernden Smolts als einjährig (1+) eingestuft, verbleibende 14,15% als zweijährig (2+). Keine älteren Smolts befanden sich in der Stichprobe. Die Hauptaktivität der Smoltabwanderung fand während drei Perioden mit steigenden Pegelständen statt. Innerhalb dieser drei Perioden wanderten ältere Smolts signifikant früher ab als jüngere Smolts. Die Mikrosatellitenanalyse wies 22 Smolts der Stichprobe eindeutig dem Elternpool der für die Besatzmaßnahme verwendeten Laichfische zu und identifizierte somit 2,31% der Stichprobe als Besatzfische. Von 120.000 besetzten Fry wurde somit eine Überlebensrate von 0,02% ermittelt. Des Weiteren wurde eine effektive Populationsgröße von

3040 Individuen für die Lipping Au ermittelt welche die Jahre 2013 und 2014 miteinschließt. Diese Arbeit stellt eine wertvolle Pilotstudie zur Bestimmung der Altersstrukturen von Smoltpopulationen und zur Ermittlung von Überlebensraten (von Fry zum Smolt) in einem kleinen Schleswig-Holsteinischen Bach dar. Dennoch sind Wiederholungen dieser Studie nötig um die gesammelten Ergebnisse über die Jahre, bei unterschiedlichen Umweltbedingungen vergleichen zu können.

2 Introduction

The sea trout (*Salmo trutta f. trutta*) is an anadromous fish species native to the whole Baltic Sea and its river systems. Beside an increasing importance for fisheries the sea trout is a popular target for sports anglers. The coastlines and rivers of Baltic States like Denmark, Sweden and Germany are favoured destinations for angling tourists which represent a considerable portion of the total tourism of these areas, especially in low seasons.

Due to its high requirements to water quality and habitat properties the sea trout is known to be an important indicator organism. Juvenile trout inhabit freshwater habitats like little streams and rivers which are highly sensitive eco systems. Anthropogenic transformation, pollution and destruction of Baltic- and North Sea running waters in the past made such habitats become rare. The habitat loss consequently initiated a decrease of natural reproduction and population size. Nowadays, Baltic states invest a lot to understand and protect sea trout populations with the aim to increase the population size again. To fulfil this, the first focus of attention should be the protection and improvement of habitat renaturalization of freshwater habitats. This is the place of reproduction and the nursery ground for juvenile trout, the most sensible point of the life cycle of sea trout. Later on, it should be also the abundance of the spawner population.

2.1 This study

The conclusions of this thesis, performed in the study system Lipping Au, should help closing some parts of the sea trout knowledge-gaps we have in Schleswig Holstein. One research objective deals with an important population-structure characteristic which is of high relevance for the ecology of sea trout: 1) What is the age composition (age structure) of juvenile sea trout (smolts) when migrating from streams into the Baltic Sea? The second objective targets the success evaluation of sea trout fry releases, one of the management options conducted since decades to enhance sea trout abundance in freshwater: 2) What is the contribution of individuals from stocking initiatives in comparison to wild reproduced individuals during a spring smolt-run season?

In spring of 2016 a “smolt trap” was set-up into the Lipping Au. The trap, collecting down migrating smolts in a capture box, covered the whole width of the stream and was placed three kilometres upstream of the estuary to the Baltic Sea. All small tributaries of the Lipping Au

converge upstream of the trap into the river. The fish caught were sampled every day over the 61-day trapping season and were again released downstream of the trap after sampling. Among others scale- and genetic (fin-clip tissue) samples were taken of more than 900 out-migrating sea trout.

To improve our current understanding of ecological processes related to sea trout in Baltic Sea running freshwater streams of Schleswig-Holstein, we determined the time the trout spend in fresh water by scale reading. Age structure is an important characterization for the population because it is closely related to vulnerability to environmental threats (droughts, floods, strong winter, etc.), anthropogenic influences (pollution, damming, erosion, siltation etc.) and to biotic factors (density dependence → food availability, predation etc.). The effects of these influences can be very different in heterogenous and homogenous smolt populations.

Genetic samples of the trapped fish were taken to perform parentage analysis using microsatellite marker. Like many European streams, the system Lipping Au has been part of continuous stocking programs with juvenile trout for the last 10 to 15 years at least. Since 2013 the parental generation of the stocked fry is known. The genetic information of the adults is the key to perform parent-offspring analysis with juvenile sea trout from this system. Beside information of the stocking success, a microsatellite analyses of young out-migrating sea trout will also give information about the genetic diversity of the population inside the Lipping Au.

2.2 Taxonomy and distribution of *Salmo trutta* species

In Germany and surrounding European countries many fresh- and saltwater habitats are inhabited by native trout belonging to the family of Salmonidae. Looking at various habitats, we find different forms of trout differing in body form, coloration and migratory or non-migratory habits (Wheeler 1969). Even if subdivided in different forms they belong to only one polymorphic species (*Salmo trutta*) (Elliott 1989), first described by Linnaeus (1758). The brown trout (*Salmo trutta f. fario*) for example is a residential non-migrating form of this species using streams and rivers as main habitat. There are also isolated forms in lakes and lake-river systems (*Salmo trutta f. lacustris*). The sea trout (*Salmo trutta f. trutta*) is the marine form with a migratory behaviour and is known to be the origin of *Salmo trutta* variations (Gehlhaar 1972). The separation of the different forms is very unstable or even undetectable. Especially in areas where different habitats collide and mating between forms is a common happening, no genetical difference can be found while isolated forms, spawning in geographically separated localities differ genetically from each

other and from anadromous forms (Hindar et al. 1991). Even in little streams discharging into Baltic Sea waters the separation of the forms is of doubtful value because, in some populations, the eggs of female sea trout are fertilized by sperm from smaller male resident trout that have never left the native stream (Elliott 1989).

Originating from sea trout, the *Salmo trutta* variations are originally native to Europe but have been successfully introduced in at least twenty-four countries outside Europe (Elliot 1989). Nowadays the distribution of the marine form *Salmo trutta f. trutta*, which is the main subject of this study, covers the European North Atlantic coast from North Spain to Iceland and the Norwegian coastline to Russia, the whole Baltic Sea region and the Black Sea (Muus et al. 2013) (Figure 1). During glacial periods in the past the population was pushed southward up to the Mediterranean Sea and North Africa. With the retreat of the glacial ice the sea trout resumed the northern habitat again and vanished in the now warmer regions of the Mediterranean Sea, leaving isolated residential freshwater forms still existing in the Mediterranean lakes and rivers with comparatively cold waters (Gehlhaar 1972).

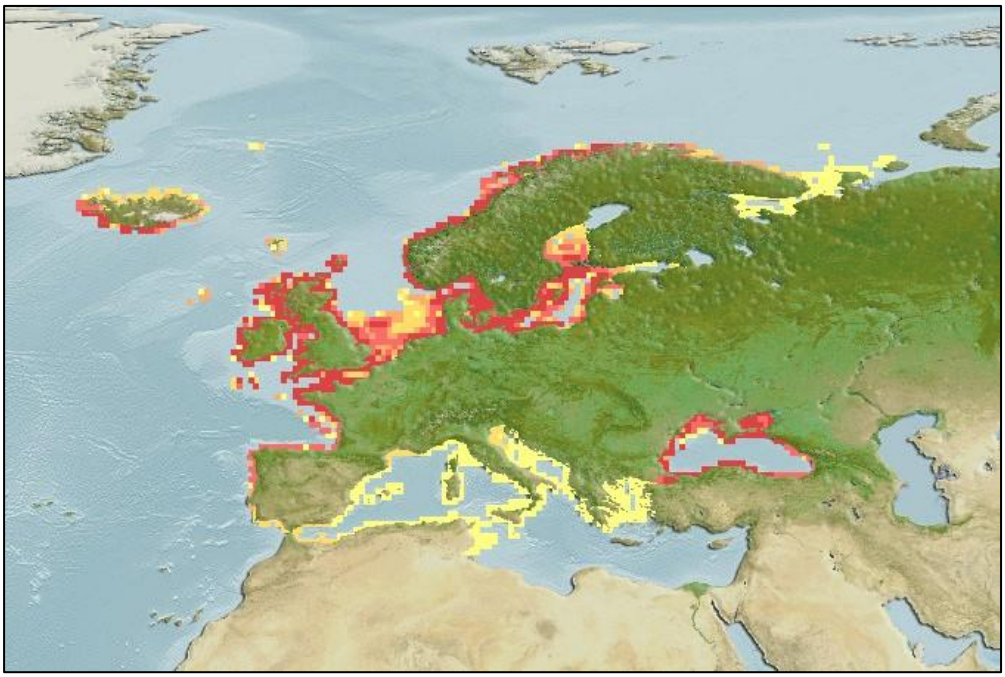


Figure 1: Distribution of *Salmo trutta* species in Europe (resource: (www.fishbase.org). Colours indicate the probabilities of occurrence (red=high, yellow=low).

2.3 Reproduction and lifecycle

The sea trout is an anadromous fish species. Adult fish spend most of their life in saltwater habitats and migrate to fresh water for spawning. Like all other species of the genus *Salmo*, sea trout spawn in late autumn and early winter in cold fresh-waters of little streams, providing clean water with high oxygen content and gravel substrate. The timing of the spawning run is a very individual process. While some fish start the fresh water run in mid-May, the majority migrates to fresh water during high water levels in the end of October and November which depends on climate conditions and latitude (Klemetsen et al. 2003). Not the entire population takes part on the yearly spawning activity. Some fish stay in the salt water for up to four years before returning to fresh water for spawning while other return after six months at marine waters. The main spawning activity happens in November/December and can last until the end of January and beginning of February in exception. After moving upstream until they find shallow water and gravely grounds males and females pair up. The female fish digs a depression in the gravel by lying on her side and flapping her tail, using the upward force generated combined with the flow of water to displace stones downstream (Thomson 2015). Simultaneously to the oviposition of the female the male trout fertilises the eggs by releasing sperm into the water. After completing the egg laying process, the female moves further upstream repeating the flapping movement to cover the eggs with gravel. The spawning process can be repeated several times. When the spawning terminates the often exhausted and injured fish moves downstream back to salt water regions where it starts feeding again and recovers quickly. *Salmo trutta* is a multiple spawner and some fish return every year to freshwater for spawning.

Embedded in the gravel the eggs are saved from drifting and direct UV-radiation while a constant waterflow ensures the oxygen supply. The eggs hatch after approximately 440 degree days (Elliott 1994). Further freshwater development is subdivided in various stages until the fish migrates to the sea. Hatched trout remain in the gravel receiving their nutrition from a yolk sac until it is consumed. This stage of development is called “alevin”. Once the yolk sac is depleted the fish emerge from the gravel and start active feeding. This is the “fry” stage. The development from fry to “parr” begins after a few weeks from emergence (Elliott 1994) but is a gradual transition which is hard to determine. Parr usually establish feeding grounds where they prefer to stay in and which they defend from other parr. As they grow, their energy demand increases and they increase the size of their territory (Thomson 2015; Klemetsen et al. 2003). Parr show a colourful phenotype

with reddish fins and typical dark marks on lateral sides (Figure 2). The last fresh water stage and simultaneously the first salt water stage is the “smolt”. Compared to the colourful parr stage, smolts (Figure 3) show a silvery phenotype when entering marine waters for growth and maturation (Petereit et al. 2013). The cycle is completed when these fish return to their place of birth, this time for spawning.

Figure 2: Shown is a juvenile trout of ca. 5 cm body length in the parr stage. The fins of parr show a reddish colouration and the body shows typical parr marks on lateral sides.

Figure 3: The smolt stage can differ in age, size and colouration. This picture shows three smolts during migration between 13 cm and 25 cm. The two upper smolts show typical colouration while the smallest fish is darker and more colourful.

The switch from freshwater to marine environment is a key event during their life cycle and involves complex physiological and morphological changes in a process termed “smoltification” (Thomson 2015; Klemetsen et al. 2003). The changeover from parr to smolt also happens in a gradual transition and not every migrating juvenile trout fits into the phenotype we expect of a smolt. One generally accepted trigger that onsets smoltification is the day length (Hoar 1976). The smolt migration varies with temperature and latitude and generally occurs during a few weeks between April (south) and July (north). Main migration events are highly correlated to the water level and water flow is important in explaining day-to-day variations in smolt runs (Jensen et al. 2012). Another environmental factor for the migration is the water temperature which regulates the rate and duration of migration (Hoar 1991). Beside the spring peak migration there may be a presmolt migration during the high water levels in autumn (Aarestrup et al. 2017). The main mean smolt length and age correlate with the mean annual water discharge of the stream and with the latitude (Jonsson et al. 2001). The higher the latitude towards the north, the longer the time before smoltification of parr which is probably as an effect of decreasing water temperature resulting in a slower growth rate (L'Abee-Lund et al. 1989). The age of migration can vary from 1-year smolts in warmer regions to up to 7-year smolts in northern regions with long winters while the range of variation in smolt ages seems to increase with the river size (Okland et al. 1993). The crucial factor for timing of migration in smolts seems to be the body size and not the age of the smolt (Bohlin et al. 1996).

2.4 Determining smolt age

As mentioned above the ages of migrating smolts can vary within a population and are highly variable in populations from different latitudes and river systems. These factors make it impossible to obtain reliable information about the individual smolt age by just examining length-frequency data. For age determination in marine fish usually scales or otoliths are used. These hard structures show growth zones that can be observed to determine the exact age. Jonsson (1976) showed that otoliths are more representative for determining ages in brown trout for individuals aged 3 years and more. However, Rifflart et al. (2006) confirmed that scale reading is a reliable estimation with low error rates for age determination in brown trout. In this study, the individual smolt ages were determined by scale reading. The major advantage of scale samples in comparison with otolith samples is the fact that sampling is not a lethal process for the smolt. After taking scale samples the smolts can be released without negative side effects. Missing scales will be replaced shortly.

The first full year in the juvenile trouts life is completed in springtime of the following year after hatching. Of course, the time of hatching varies every year depending on the egg development in differing temperatures. This makes the exact age of a fish undetectable. However, the literature usually agrees to the 1st of April as fixed date that indicates the end of a full year in every trouts life (Gehlhaar 1972). Every uncompleted year is defined as "Plus Growth". A smolt caught in the smolt trap (capturing after the 1st of April) has the minimum age of "1+". The parental generation of this fish spawned in winter of 2014. "1" indicates the completed year and "+" the growth of an uncompleted year (Elliott, Chambers 1996). A "2+" smolt spend 2 completed years in freshwater and was caught 2 years and a bit after hatching. The parental generation spawned in winter 2013 (Figure 4).

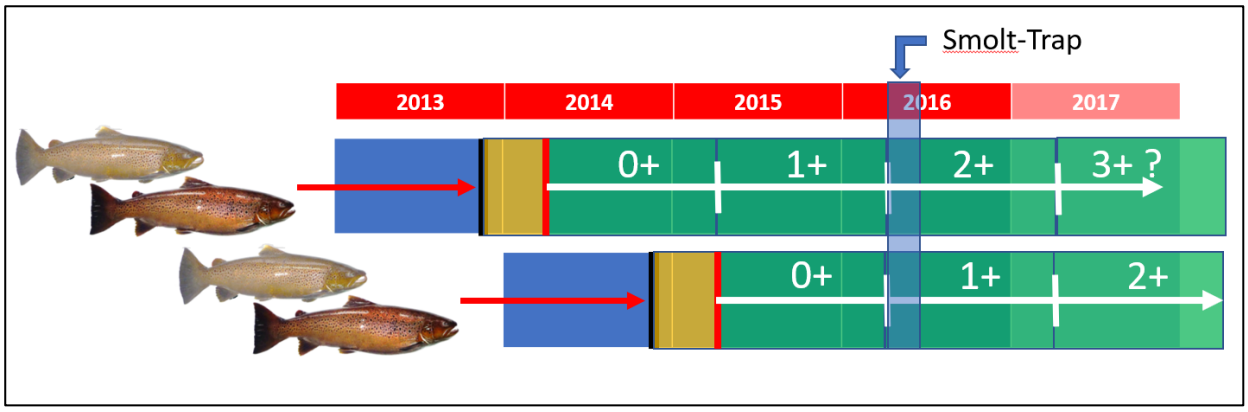


Figure 4: Shown is an overview explaining the smolt age composition of two smolt generations and their belonging to parental generations before, during and after smolt trapping. The black bar indicates the actual spawning followed by a yellow field explaining egg stage. The red bar indicates the hatching date. White arrows show the time juvenile trout spend in their birth stream and white bars indicate a completed year of live. The transparent blue field indicates smolt trap activity.

2.4.1 Why estimating the age of fish?

"Management of fish populations for restoration or conservation requires an assessment of the present status, and reliable knowledge of the spatial and temporal dynamics of the population" (Rifflart et al. 2006). This information includes, among others, knowledge about age composition. This knowledge can be used to measure the rates of various processes affecting these fish. For example, the information of age combined with information of size and weight enables the detection of growth rates for the entire population and for every single age class in particular. Comparing abundances of age classes between different years gives the opportunity to detect

mortality rates and the variability of annual spawning success. When calculating the reproductive rates of fish, it is important to know how long it takes the specie to mature. To receive this information, age determination is essential. Finally, determining smolt ages of one stream enables the estimation of the population age structure and gives information about the composition of age classes. Acquired knowledge of age structures of several years could detect the effects of different management strategies or the influence of global warming on sea trout populations for example.

2.5 *Salmo trutta f. trutta* in the Baltic region and Schleswig-Holstein

Anthropogenic activities like the construction of hydroelectric-power dams, watergates and other barriers in the past have impaired or completely restricted migratory routs of descending and ascending anadromous fish in Europe (Serrano et al. 2009). Other factors like logging and overfishing lead to a diminishing or even extinction of sea-running brown trout in European rivers (Jonssonn et al. 1999). Especially in little streams, the pollution and the destruction of natural habitats in the past massively decreased the number of suitable spawning grounds for *Salmo trutta* species. Even if adult fish spawn in polluted rivers and survive the spawning run, the eggs die by lack of oxygen or fungal infections as a consequence of sedimentation and pollution. Nowadays, many important steps have been done to prevent this species with its immense importance for fisheries and angling tourism from extinction. Renaturation, strict wastewater regulations and stocking programs have stabilized European sea trout populations. In the year of 2012 a number of 642 Baltic Sea running streams and rivers hosted sea trout populations (Skrupskelis et al. 2012). However, the importance of this species as an indicator organism for intact eco systems is still underestimated in Germany and Schleswig-Holstein.

Petereit et al. (2013) found that at least 43 streams of Schleswig-Holstein discharging into the Baltic Sea host historically or currently sea trout populations (Figure 5). However, the lack of actual general information (population size/ structure) about the sea trout in Schleswig-Holstein leads to a gap of knowledge compared to many European neighbouring countries. Filling this knowledge gap would help improving the management of sea trout population in the Baltic Sea region. In August 2015 the "SMARRT" („Schleswig-Holsteinische **Sm**olt- und P**arr** Produktion in **T**heorie und Praxis") project started, which is financed by the fond of the Schleswig-Holstein angling licence fee. This research project should increase and generate basic knowledge about sea

trout ecology and partly evaluate the success of employed management methods in Schleswig-Holstein.

2.5.1 Lipping Au- the study system

One of the major targets of "SMARRT" is to collect data about stocking success and smolt production of the Lipping Au in Schleswig-Holstein in northern Germany. The Lipping Au is the main stream of a river system with two smaller tributaries, the Bolthofter Au and the Bordeskuhler Au, and a larger branch- the Esgruser Mühlenstrom. In total, this partly restored river system covers an area of 49,7 km^2 and discharges into the Baltic Sea close to Gelting (Figure 5 and 6). The mean yearly discharge (MQ) is 0,43 m^3/s, minimum discharge (MNQ) 0,03 m^3/s and the maximum discharge (MHQ) is 5,65 m^3/s (www.umweltdaten.landsh.de). It can be characterised as a typical stream for this region of the Baltic Sea. Recording to the Water Framework Directive (WFD) the river habitat Lipping Au is categorised as "intermediate" to "good". The ecological status is categorised as "moderate" to "poor" and the water quality as "intermediate". Sample collection for this study took place at a smolt trap located at the main stream after the union of all tributary streams, thus theoretically representing the smolt production of the whole river system.

Figure 5: Map of the Lipping Au (red line) and the location of the smolt trap (white arrow). Inside picture shows the location of the smolt trap in the Baltic Sea region. (resource: Google Earth)

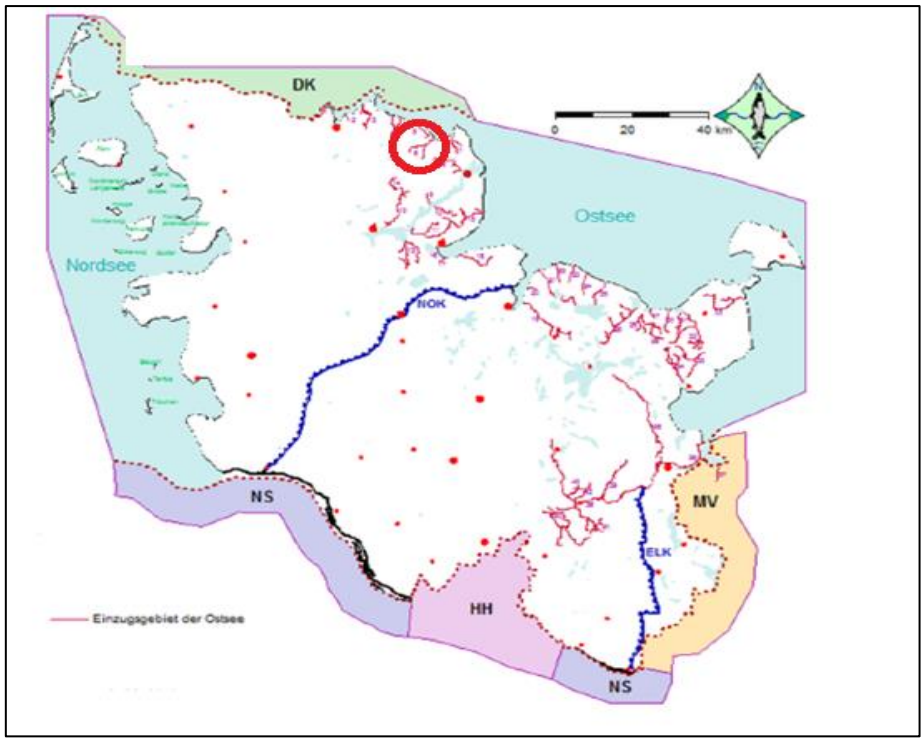


Figure 6: Map of Schleswig-Holstein showing Baltic Sea streams (red lines) with historic and recent trout occurrence. The red circle indicates the area of the Lipping Au, leading into the Flensburg Fjord close to Gelting (resource: Petereit et al. 2016)

2.6 Stocking

To prevent sea trout populations from extinction, supportive breeding and stocking programs became a common alternative in Baltic States to compensate the decline of natural reproduction. Supportive breeding increases the survival of eggs and larvae inside a protected captive environment (Ryman et al. 1991). Stocking is defined as the intentional release in the natural environment of individuals produced in hatcheries (Ruzzante et al. 2004). In German river systems, stocking of *Salmo trutta* forms is a known procedure for more than 100 years. The success and the effects of stocking programs are intensively discussed in fishery conservations. On the one hand stocking gives the opportunity to reintroduce sea trout populations to renatured streams where the natural population vanished before. Here the aim could be a continuous stocking of juvenile trout until the population is stable and reproduces naturally. On the other hand, supportive breeding can be used to provide native populations in their reproductive output. However, stocking events should always be considered as intervention in a natural process with unknown effects.

Hansen (2002) for example, showed that the range of stocking in terms of hatchery-wild introgression can vary from near zero introgression to almost complete displacement of native populations. The survival rate of hatchery fish, which is commonly assessed as worse than the natural, is an essential information to calculate stocking success and effects (Bernaś et al. 2014). An accepted point in stocking hatchery fish is that the success of cultured fish increases with the amount of time spent in the wild (Jonsson & Jonsson 2006). Due to the chances of survival most stocking programs nowadays release fry or parr stages. When stocking smolts, the proportion of fish migrating successfully to the coast is significantly higher of wild fish compared to fish of hatchery origin (Serrano et al. 2009). The Lipping Au is a stream with natural reproduction but has also been stocked with mostly fry for several years. In the years of 2014 and 2015, 120.000 (60.000 in 2014 and 60.000 in 2015) fry were stocked to the entire system Lipping Au from a parental pool captured in the Lipping Au during the spawning seasons of 2013 and 2014.

Spawners of 2013 and 2014 were collected in late November of each year by electro fishing in the lower section of the Lipping Au. These fish were measured (length,weight), sexed, fin clipped and finally transported to the hatchery FBA – Altmühlendorf. Here the fish were narcotised, strip spawned and the eggs were artificially fertilised with the half dry method. After stripping the adult fish recovered in storage containers at FBA before they were brought back and released to the Lipping Au. More details to the hatchery proceeding are presented by Albrecht (2016).

2.7 Using microsatellites for determination of stocking success

To assess the proportion between wild smolts and smolts originating from a stocking program supporting a natural reproducing population in the Lipping Au, different methods can be used. In this study, microsatellite markers of more than 900 captured smolts during migration were examined and compared with parental DNA of individuals that were used for the stocking programs in 2013 (n=98) and 2014 (n=149). This parent-offspring assignment test was realised using microsatellite DNA analysis. Microsatellites are often randomly cloned parts of the nuclear DNA consisting of repetitive units of two to five base pairs. Most of these units are located outside of genes which makes them neutral for selection (Estoup et al. 1998). A high mutation-rate and the neutrality of the loci against selection cause a high variability and a multitude of alleles in the course of time (Halliburton 2004). Comparing the allele-length of several (at least 10) microsatellites between smolt individuals and the potential parental pool, an indication of direct

ancestry can be obtained. This would indicate a smolt with stocking background. Undetected smolts result from natural reproduction with unknown parents. Microsatellites used in this study originate from the literature (Hansen et al. 2000; Koljonen et al. 2014; Nilsson et al. 2008) and were collected and approved in a previous study by Albrecht (2016).

2.8 Scientific Research Questions

1) What is the individual age composition of a spring smolt-run in a typical small river system discharging into the Baltic Sea in Northern Germany, characterised by both natural reproduction and supportive breeding by fry stocking?

2) What is the fraction of genetically assignable smolts derived by the anthropogenic supportive breeding program in relation to the total spring smolt-run?

3 Material and methods

3.1 Field work and smolt trap

Figure 7: Smolt trap of the Lipping Au. The bow net covers the whole width of the stream concentrating the down moving fish in the centre where a creel net leads the fish to a self-made capture box

To capture the migrating smolts in spring time, a smolt trap (Figure 7) was activated three and a half kilometres upstream of the estuary. The trap started operating on the 4th of April in 2016 during the 7th of June the same year and was controlled and maintained every day. The aim of the trap was to capture as many migrating sea trout smolts as possible. Using a bow net, covering the whole width and depth of the stream, the down moving fishes were concentrated in a self-made capture box anchored in the middle of the stream at the end of the net. To protect the bow net from holes, probably caused by rats, the creel part of the net, leading to the capture box, was exchanged with a plastic creel after a few weeks of capturing. The corpus of the box was made of wood while the bottom part was made of perforated metal plates, ensuring a constant water circulation inside the box. The length of the box was 150 cm, the width 60 cm, the heights 70 cm and the water level inside the box was strongly linked to the water level of the stream. To preserve the captured fish

of UV-radiation, predation and stress, the box was covered with a lid removable for collecting the fish and daily maintenance. For selective capturing, the box integrated a by-catch exit which enables mammals, birds and amphibians to leave the trap. To inhibit escaping of the smolts back into the net when opening the lid for collection or maintenance, an integrated closing mechanism blocked the entrance of the box. Once the entrance was blocked, fishes remaining outside the box in the entrance tube and the channels of the bow net were collected and transferred to the box. Every day, during control and maintenance the trap stayed inactive. The time of inactivity varied every day depending on capturing success and efforts for maintenance (holes in the net, much floating refuse in the net…). To collect the fish from the box, the entrance was blocked and the lid was opened. Then a small number of smolts was removed (using a spoon net) from the box into a transport bucket, filled with fresh river water and brought to the field station where it was placed in the shade. To keep the oxygen level at a constant rate, the bucket was prepared with an O_2-dispenser.

3.1.1 Narcotic bath

To facilitate the treatment, realise a reasonable number of measurements, prevent stress and injuries of the smolts, a narcotic was used. Therefor two to three smolts were transferred from the bucket to a narcotic bath (Ethyl 3-aminobenzoate methanesulfonate). Here 0,8g to 1,0g of the narcotic (the higher the water temperature the higher the concentration of the narcotic) was dissolved in 10 litres of river water. The smolts were removed from the narcotic bath after tilting to the side (time varied by water temperature and concentration of narcotic bath, usually 100 to 180 seconds). To observe the time the smolts spent in the narcotic bath a stopwatch was used. Then the narcotised smolts were washed and put into a measure box filled slightly with water to cover the fish and protect it from drying out.

3.1.2 Measurements

3.1.2.1 Size

The individual maximum size was recorded using an integrated scale at the measure box (Figure 8). The recorded total length (TL, mm) of the fish is defined by measuring the maximum body span from the nose to the tip of the tail fin in natural position. Smolts with a total body length of at least 120 mm were used for further genetic treatments and scale reading. Smolts with a total

body length below 120 mm were used only for length-frequency (LF) and length-weight (TL and WW) data collection.

3.1.2.2 Weight

Wet weight (WW, g) of the fish was recorded using a balance (Figure 9). It was placed in a windshield and tared regularly.

Figure 8: A smolt during size measurements on the integrated scale of the measure box.

Figure 9: After size, the weight of every fish was recorded using a field scale

3.1.3 Tissue samples

For genetic analysis the adipose fin, or a part of it, was removed with a scissor (Figure 10) and put into a labelled container filled with ethanol (98%). For later identification, each fish got a unique number (Genentical Number) written on each container used. The samples were later stored at -20°C until DNA extraction.

3.1.4 Scale samples

To determine the age of the smolt, scale samples were taken. After removing the scales from the epidermis, using a forceps for slightly scratching the skin and loosen the scales (Figure 11), these were stored dry in separate paper bags (otolith bag). All samples originate from the same body parts of the fish, underneath the back of the dorsal fin, before the adipose fin above the lateral line (Celtic Sea Trout Project) (Figure 12). Further treatments took place which justify the narcosis but were not of concern for this study.

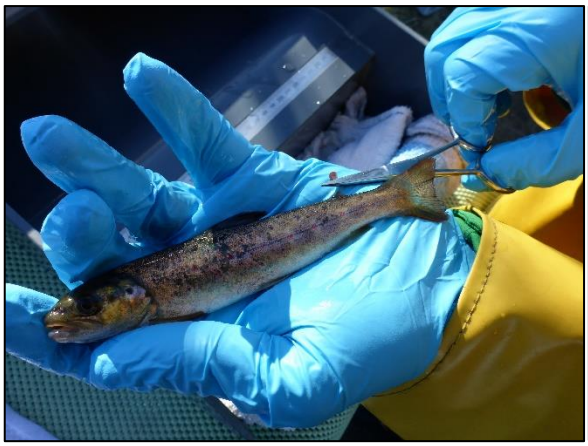


Figure 10: The adipose fin was removed with a scissor and later used for genetic analysis.

Figure 11: Scale samples for age determination were taken with a foreceps

Figure 12: Scale samples were taken from the identical side and identical area of each fish (indicated by red area)

3.1.5 Rehabilitation and releasing procedures

Following to the mentioned procedures, the smolts were transferred to a freshwater bucked, again prepared with an O_2-dispenser. Here the smolts were kept until the main effects of the narcosis passed and a normal behaviour of the fish was to be observed. For final rehabilitation, the buckets were transferred to a storage system integrated in the river (Figure 13). The buckets, placed at a slow flowing and shallow area of the stream, were prepared with little holes to guaranty a circulation of fresh water and to synchronise the water inside the bucket with the water of the stream. To reduce stress during rehabilitation the buckets were covered with a lid. When rehabilitation was provided, the smolts were released downstream of the smolt trap (Figure 14). Places for the release were chosen carefully, ensuring slow current, adequate depth and a high occurrence of hiding spots (rocks, roots, vegetation).

Figure 13: Bucket storage system integrated in the stream for final recovering of smolts after narcotic bath.

Figure 14: Releasing procedure of smolts at slow current parts of the stream with high frequency of hiding possibilities.

3.1.6 Length frequency (LF)

A maximum of 50 smolts with a total body length over 120 mm per day were treated like mentioned above. If the daily catch exceeded 50 smolts or if the smolts were smaller than 120 mm they were used only for LF-analysis. Therefore, the fish were slightly narcotised (max. 1 min. in narcotic bath) subsequently measured for size and weight.

3.2 Parental generation and fry stocking

During many spawning seasons spawners were collected via electro fishing in the lower section of the Lipping Au. That also happened in the spawning season of 2013 and 2014. All spawners were fin clipped (genetic tissue), sex-determined, weight- and length-measured. The fin-clip tissues were although stored in ethanol (98%) containers marked with a unique genetic number. After measurements, the adult trout were transported to the "Fischbrutanstalt-Altmühlendorf" (FBA). Here the spawners were tranquilised and strip spawned. For artificial fertilisation no beforehand selection of specific spawner phenotypes was performed.

For artificial fertilising the half dry method was performed. Therefore, the eggs of one female (preferred method, sometimes more females were used) were stripped into a bowl. After that a male spawner was stripped dispersing its sperm into the same bowl. Using the half dry method, ovary fluids of female and sperm fluids of male must be included in the bowl. After that the eggs and the sperm were immediately and gently poured by hand for around 60 seconds. Subsequently,

water was added to the fertilized eggs. The eggs absorb water for the first one or two hours before the egg shell hardens.

After strip spawning the adult fish rehabilitated in water tanks at the FBA for a couple of days and were then relocated to the lower Lipping Au.

Around 12.000 eggs were deposited into a drawer inside of an incubation cabinet to guaranty continuous flow of 5 °C cooled water. Here the eggs developed for about 60 – 70 days. During development dead eggs were removed regularly. The eggs started hatching after further 20 – 25 days. One to two weeks after hatching the yolk-sac fry were released to small tributaries of the Lipping Au system.

In springtime of 2014 60.000 yolk-sac fry originating from the 2013 parental pool were released at four different tributaries of the Lipping Au. The same amount of yolk-sac fry was released in 2015. In total 120.000 yolk-sac fry were released in 2014 and 2015.

3.3 Scale reading (SR) of sea trout smolt scales

3.3.1 Age determination of smolts by scale reading

For age determination of the smolts caught in the trap, dry stored scale samples, taken like shown above, were photographed using a compound microscope mounted with a digital camera and the Image Pro Insight software. The scale reading procedure mainly followed the instructions given by: “Manual on Sea Trout Ageing, Digital Scale Reading and Growth Methodology” by the Celtic Sea Trout Project, published 2010 and: “A Guide to the Interpretation of Sea Trout Scales” by the Institute of Freshwater Ecology, published 1996. The mentioned literature manly describes the scale reading procedure of adult fish. The following text is focused just on the scale reading procedures of smolt scales. These scales represent the time a young trout spent in the fresh water of its birth stream. Compared to reading of adult fish scales, for example of multiple spawners, reading of smolt scales is not that multifaceted and speculative due to a shorter lifetime the smolt has. While the life of adult trout can be very diversified by habitat changes between fresh and sea water, the spawning events or the skipping of it, the habitat of smolts is mostly limited to a freshwater habitat only. However, even in a smolt population there is a high diversity of individual growth and behaviour which makes the scale reading of smolts a versatile procedure. The following manual of reading smolt scales is based on just one smolt population originate from the Lipping Au. Comparing smolt populations of different streams, areas or countries is not considered in the following text. The order of reading scales was linked to the capture date starting with the 5th of April proceeding day by day until the beginning of June. During scale reading size and weight of the fish were not known to guarantee an unbiased reading process.

3.3.2 Preparation of scales

Not many scales are suitable for age determination by scale reading so the suitable scales were chosen carefully. Many scales are replacement scales, showing unproportionable growth and structures and are not representative for the whole lifespan of the fish. Other scales show mechanical damage and erosion or consequences of fungal attacks during storage and should also not be used for age determination by scale reading.

The scales were carefully scratched of the otolith bags using a forceps or a taxidermy needle. Then the scales were placed on a slide with a drop of distilled water. Using the microscope, the not suitable scales and air bubbles sticking to the scales were removed from the slide using the

taxidermy needle. After that the drop of water containing the suitable scales was covered by a slip and observed in detail. If a scale was used for age determination it was adjusted to the correct direction with the caudal part showing upwards (Figure 15). The final scale used for age determination was photographed and saved as tif. picture named as the identical genetical number on the otolith bag. If necessary a second picture was taken using a higher resolution focusing on difficult scale structures. After scale reading of each fish, the slide was rinsed with water and wiped carefully with a paper towel. Slide, hands and tools must be free of scales to avoid cross-contamination. Scales that have been used once were disposed and not restored.

3.3.3 Scale area for age determination:

The interpretation of scale structures is limited to a specific area of the scales surface. Looking at the middle of the scale, directed with the caudal part pointing upward, an angle of around 60° (between 330° NW and 30°NE) should be observed (Figure 15). Remaining parts of the scales surface do not show representative growth and arrangement of concentric lines. The interpretation starts from the middle of the scale continuing to the outer edge.

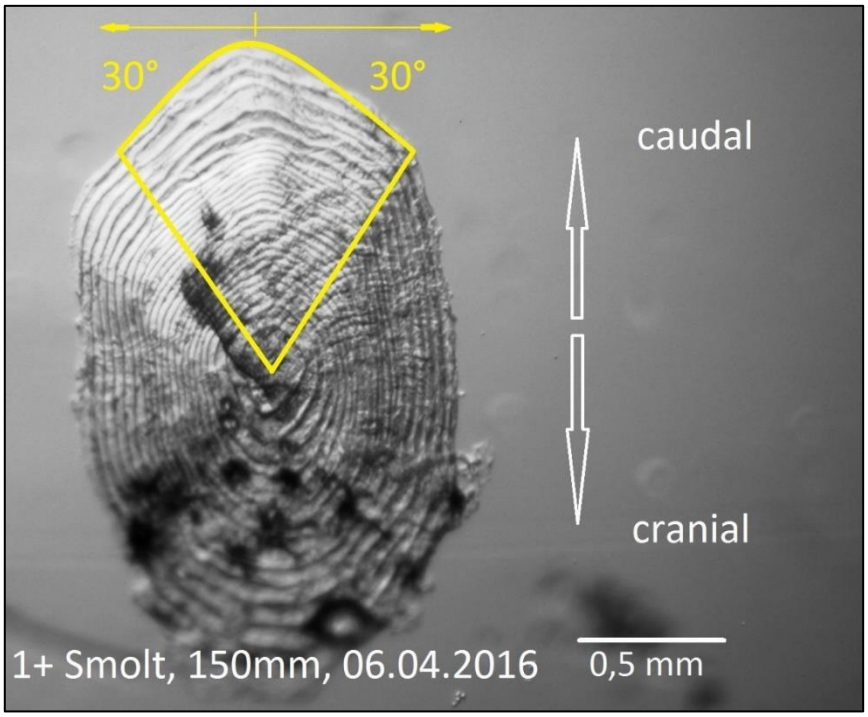


Figure 15: Example for scale reading on a typical smolt scale (Age: 1+, Size: 150 mm, Date of capture: 06.04.2016). The scale should be placed with the caudal part pointing upward. The area of reading structures is limited to an angle of ~ 30° to both sides from the nucleus upward.

3.3.4 Structures of smolt scales

3.3.4.1 Nucleus

The so-called “Focus” or “Nucleus” is the basis of each fish scale and is usually located towards the proximal anterior, exposed portion of the scale in the centre of the concentric lines (Figure 16). This spot represents the beginning of body growth in fish.

3.3.4.2 Circuli

The focus is surrounded by dark concentric lines, the so called “Circuli” (Figure 16). The circuli represent growing events. In times of fast growth, usually during spring and summer, the circuli show wide gaps between each other. On the contrary to the wide gaps during fast growth, there are compacted gaps between the circuli during slow growing periods in winter time.

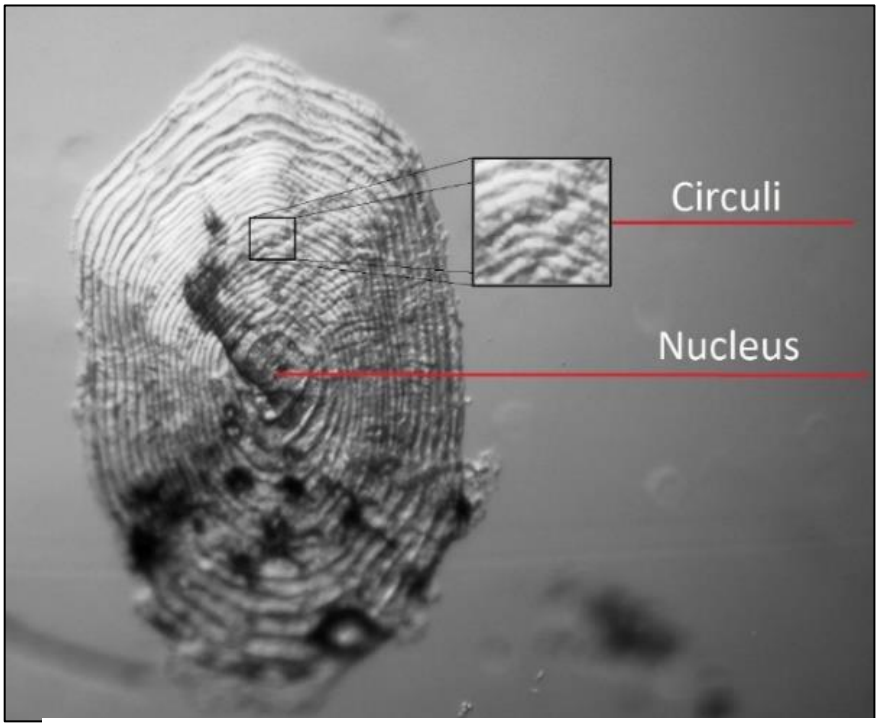


Figure 16: The centre of the scale is indicated by the Nucleus which is surrounded by concentric lines, the circuli.

3.3.4.3 Light bands (LB) and dark bands (DB)

Depending on the exposure of the photograph, the slow-growing period looks darker ("Winter Dark Bands") than the fast-growing period ("Summer Light Bands"). This is due to the distance between the circuli. Wide gaps look lighter because of a higher light reflection of the wide areas between circuli. The closer together the circuli the lower is the reflection of light and the darker looks the area. The DB mostly includes less circuli than the LB. A LB (summer) followed by a DB (winter) covers a complete year of the fish´s life cycle. These structures can be found in smolt scales but are mostly hard to determine and play a more significant role for determining age of adult trouts.

3.3.4.4 Annual Zone (AZ) and Annulus:

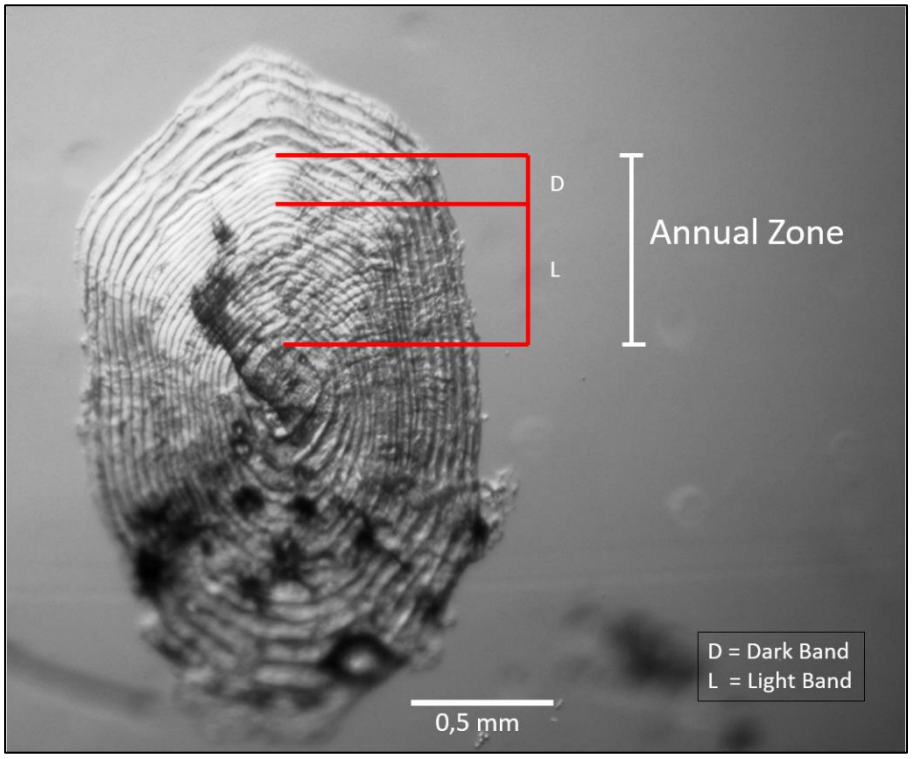


Figure 17: Different sequences of spaces between circuli represent the growing periods during winter- and summer- time. The picture shows the identification of an Annulus with DB and LB. Compacted circuli at the end of a DB represent the end of a year in the smolts life.

The "Annual Zone" is determined as the area including a LB and a DB (Figure 17). The so-called "Annulus" is the end of the slow growing period including the last circuli of the winter dark band. The contrast of the fast-growing period (spring/summer) following an annulus (winter) generates a striking indication for finding completed years on the smolts scale. The quantity of annulus´s on a scale is identical to the number of winters the fish experienced and therefore representative for

the age of the fish. The annulus is the most significant structure for determining smolt age. Figure 17 shows a typical smolt scale of a fish aged 1+. At the end of the annual zone the fish starts its second year of life. Figure 18 shows two annual zones of a 2+ smolt. The first annual zone is smaller than the second when the fish starts growing faster in the second summer.

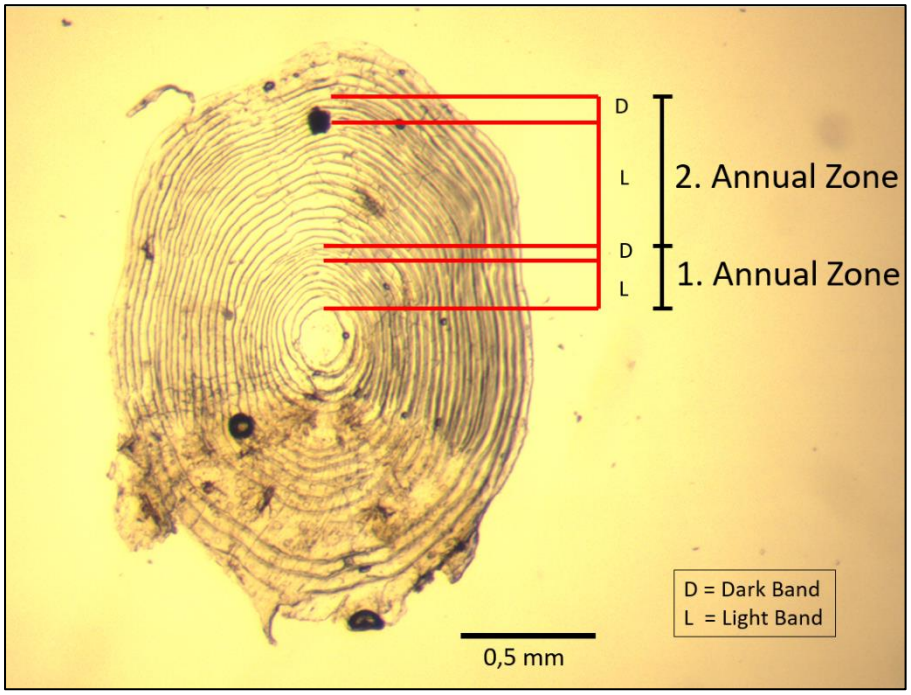


Figure 18:

The picture shows the identification of two annuli on a scale of a two-year-old smolt (2+). Dark bands (D) and light bands (L) are indicated with the red marks. Compacted circuli at the end of a dark band representing the end of a year in the smolts life.

3.3.4.5 Plus growth (PG)

An uncompleted AZ at the outer edge of the scale indicates an incomplete year of growing and is called “Plus Growth”. The area of PG gets wider the later in year the scale samples were taken. Scale samples taken in spring time, simultaneously with the hatching time, show no or just little PG. Compared to the gaps between the circuli of existing AZ, circuli of PG usually show wider gaps due to typical fast growth at the beginning of the second (or third) year. Figure 19 and 20 show two smolt scales with different portion of PG. The scale on Figure 19, sampled in early April shows little plus growth compared to the scale on Figure 20 which was sampled in early May.

Figure 19: Scale of a smolt with capture date on 5th of April. Scale shows little PG portion indicated by red arrow.

Figure 20: Scale of a smolt with capture date on 1st of May. Scale shows higher PG portion indicated by red arrow.

3.3.4.6 Replacement Scales:

These scales are characterised by the extraordinary size and form of the nucleus. Reasons for the loss of scales can be variegated but often relate to predation and other physical impacts. Once a fish lost a scale, the gap in the skin is filled with a new scale as fast as possible. The new scale growth until the gap is closed and then starts to grow simultaneously to the original scales. During replacement, no concentric lines were generated. The earlier in life the scale was replaced, the smaller is the clear tissue of the nucleus. A just recently replaced scale shows a large area of clear tissue (Figure 21). Beside an unproportionable nucleus, replacement scales vary and differ in sizes compared to original scales. Replacement scales should not be used for age determination since clear tissue of the nucleus could cover crucial structures for interpretation. Figure 22 shows two scales of the same fish. Left-hand side scale is a replacement scale with unproportionable size of clear tissue of the nucleus compared to an original scale on the right-hand side. Figure 23 shows the differences in determined ages on both scales. The original

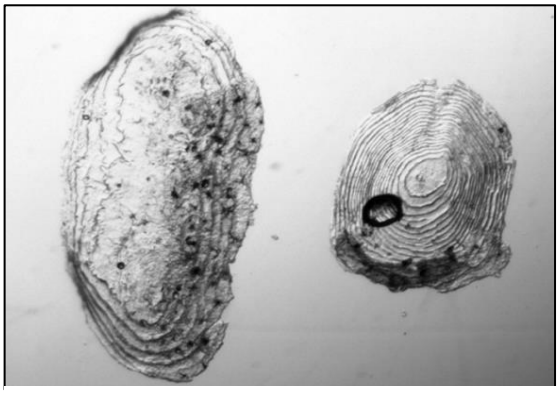


Figure 21: Two scales of the same smolt. Left scale is a replaced scale showing unproportioned form and size of Nucleus compared to the original scale (right scale)

scale shows a compacted area of circuli which can be identified as an annulus. Comparing with the replaced scale this annulus is covered by clear tissue. Determining the fish´s age on both scales ends in different results. While the original scale shows an age of 2+ years, the replaced scale just shows 1+ years because the first year is covered by the nucleus.

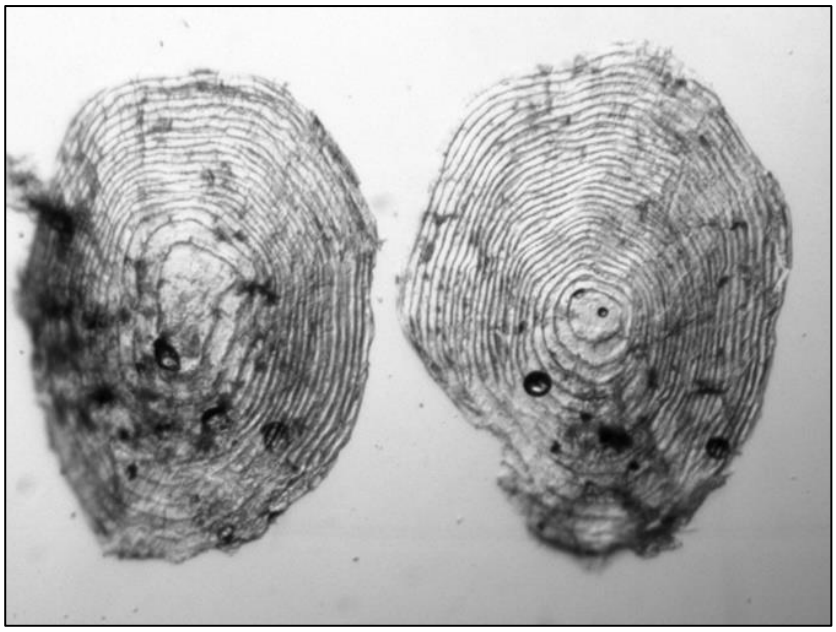


Figure 22: The picture shows two scales of the same fish. The left scale is a replaced scale identified by unproportionable nucleus. The scale on the right is an original scale.

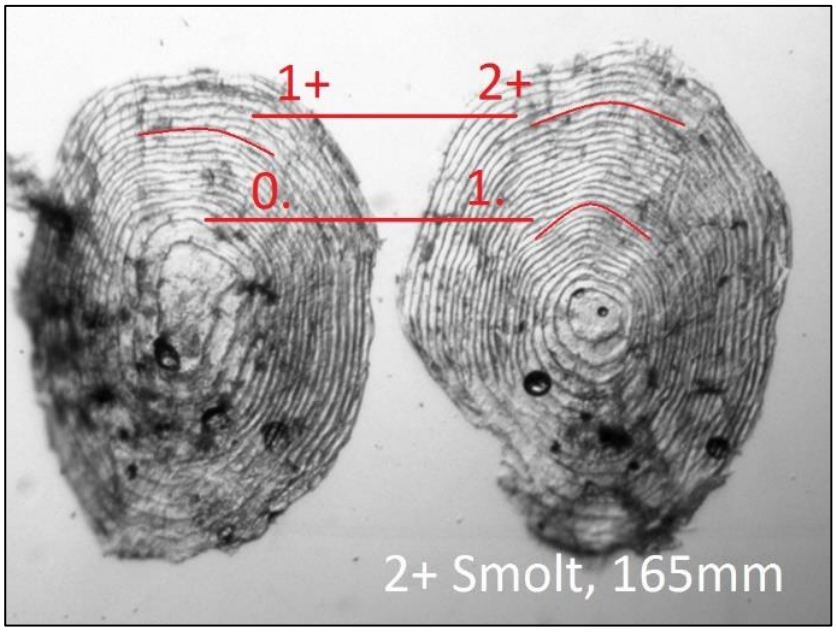


Figure 23: Example for wrong age determination on replacement scales. The clear tissue of the replaced scales nucleus (left) hides essential information and leads to wrong age (1+) by showing one annulus while the original scale (right) shows two annuli (2+).

3.3.5 Difficulties in scale reading:

When reading scales, there are typical patterns expected in the structures of scales. First, we expect the fish to grow faster during summertime and slower during wintertime. Second, we expect a clear mark between these two growing periods looking at the scale. In reality, these patterns are often hidden and sometimes really hard to observe in the structures of the scale. Also, there are fish that grow slower in summertime then they do in wintertime and there are fish that grow at a constant rate independent of the time of year. These scales are complicated to interpret and the correctness of the indicated age of the fish cannot be guaranteed. Another reason for mistakes in the interpretation is the biased attitude that expects large scales to be from old smolts. Examples in hatchery reared smolt show that total body length between the individuals of the same bred reared under the same conditions for one year can vary from ~7 cm to almost 30 cm.

3.3.5.1 Slow summer growth:

If the area around the nucleus starts with a composition of compacted circuli, this could be due to a slow summer growth. A slow growing period during summer is often followed by a faster growing period in winter which is against the expectation that fish grow faster during warm water conditions. This sequence observed on the scale is hard to differentiate from an annulus and often leads to wrong results of age determination. An indication for slow summer growth could be the quantity of compacted circuli which is usually lower in slow summer growth than in a total year of growing.

3.3.5.2 Constant growth:

Some smolt scales show the same growth rate all over the year. The scales of these smolts show no winter or summer bands. The reason for this steady growth and the equal arrangement of the circuli is highly speculative. For determining the age of a fish with a steady growth, the focus of observation should be the annulus. With the beginning of a new year usually the growing behaviour changes and the gaps between the circuli get wider. Figure 24 shows the scale of a 194 mm smolt. This fish was determined as 1+ even if the size of the smolt fits to a 2+ or even 3+ smolt. The constant arrangement of the gaps between the circuli hardly show any slow growing periods which could indicate a winter.

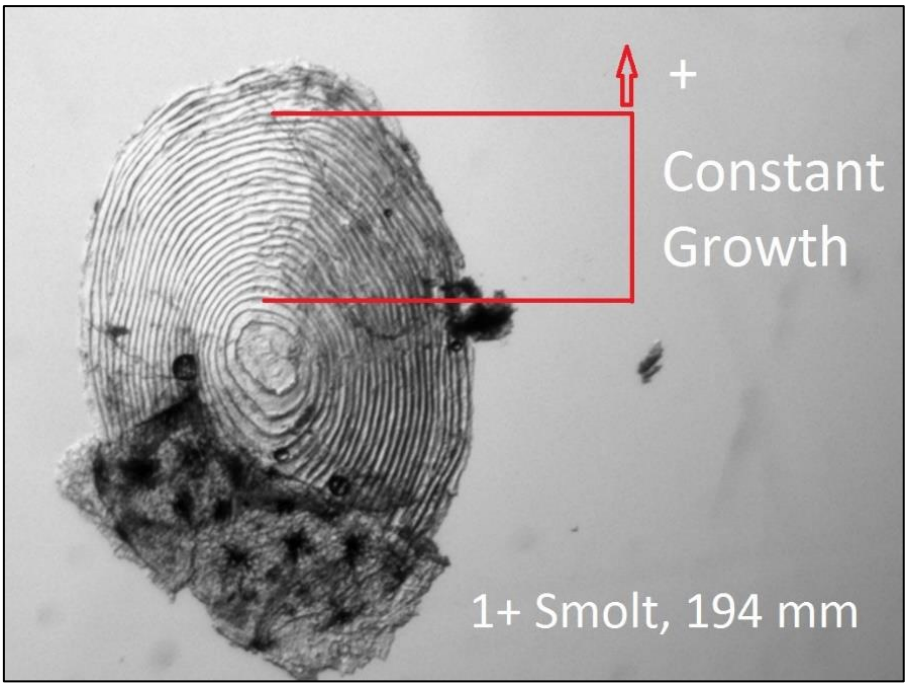


Figure 24: The AZ of the scale of a 194 mm smolt does not show any LB or DB inside the red marker bars. The only sign for the beginning of a new AZ is the + Growth (red arrow).

3.4 Laboratory work

For microsatellite DNA analysis, more than 900 smolts were compared with 279 potential parental individuals that were used for hatchery reproduction in 2013 and 2014.

3.4.1 DNA extraction

For DNA extraction, the "All-round kit: MN-Genomic DNA from tissue" (Machery-Nagel GmbH & Co. KG, Düren, Germany) was used, following the instructions of the user manual "NucleoSpin® 96 Tissue".

3.4.1.1 Preparation of samples

The adipose fin (finclip of adults) was removed from the storage container filled with 98% ethanol and cut in a half. One half of the sample was replaced in the container and kept as backup, the other half was placed in a tube of the round-well block. If the sample was too small, the whole tissue was used and no backup was kept. After every sample the tools (forceps, scissor, and scalpel) were cleaned using a paper towel and ethanol, to avoid cross contamination. A total of 96 samples was stored in one round-well block. For exact attribution, the adjustment of the samples was recorded carefully.

3.4.1.2 Lyse samples

To set free the nucleic acids, enzymes and buffers were used, to dissolve the connective tissues and cell membranes of the sample. In this step, each tube of the round-well block was filled with 200 µL enzyme-buffer solution prepared of 25 µL Proteinase K and 180 µL Lysis Buffer T1 for each tube. After filling, the tubes were sealed using cap strips and the block was placed in the centrifuge. A brief spin (15 s; 1,500 ×*g*) collected the samples at the bottom of the tubes, completely covered by the solution. Finally, the block was placed in an incubator and left over night, slowly shaking at 56°C.

3.4.1.3 Isolation of DNA

After lysis, each tube of the round-well block was added with 400 µL Buffer/ 98% ethanol mix, sealed with cap strips and again briefly centrifuged (10 s; 1,500 ×*g*). The lysates then were carefully (without moistening the rims to avoid cross contamination) transferred from the round-well block to a tissue binding plate. The binding plate was sealed with a self-adhering foil and

centrifuged for 10 minutes at 5,600-6,000 ×*g*. High chaotropic salt conditions of the buffer remove (reversible) the hydrate shell of the DNA and make it bind to the silica membrane. Once the DNA is bound to the membrane, two following washing steps remove contaminants and unwanted components like PCR-inhibiting molecules (Figure 25). During washing steps, the salt concentrations stay high to keep the binding of DNA and membrane steady. After centrifuging twice (first washing step 2 min., second washing step 4 min.) the DNA remains on the membrane while the contaminants get washed off. To make sure there is no ethanol left on the membrane, the tissue binding plate was incubated for 10 minutes at 70°C. For elution of DNA, 100 µL of a preheated Buffer (70°C) was dispensed on each membrane and incubated for one minute at room temperature. The low salt concentration of the elute buffer effects a regeneration of the DNA´s hydrate shell and consequently interrupts the binding of DNA and silica membrane. After centrifuging for 2 minutes at 5,600-6,000 ×*g*, the remaining DNA gets washed out and concentrated in a PCR plate.

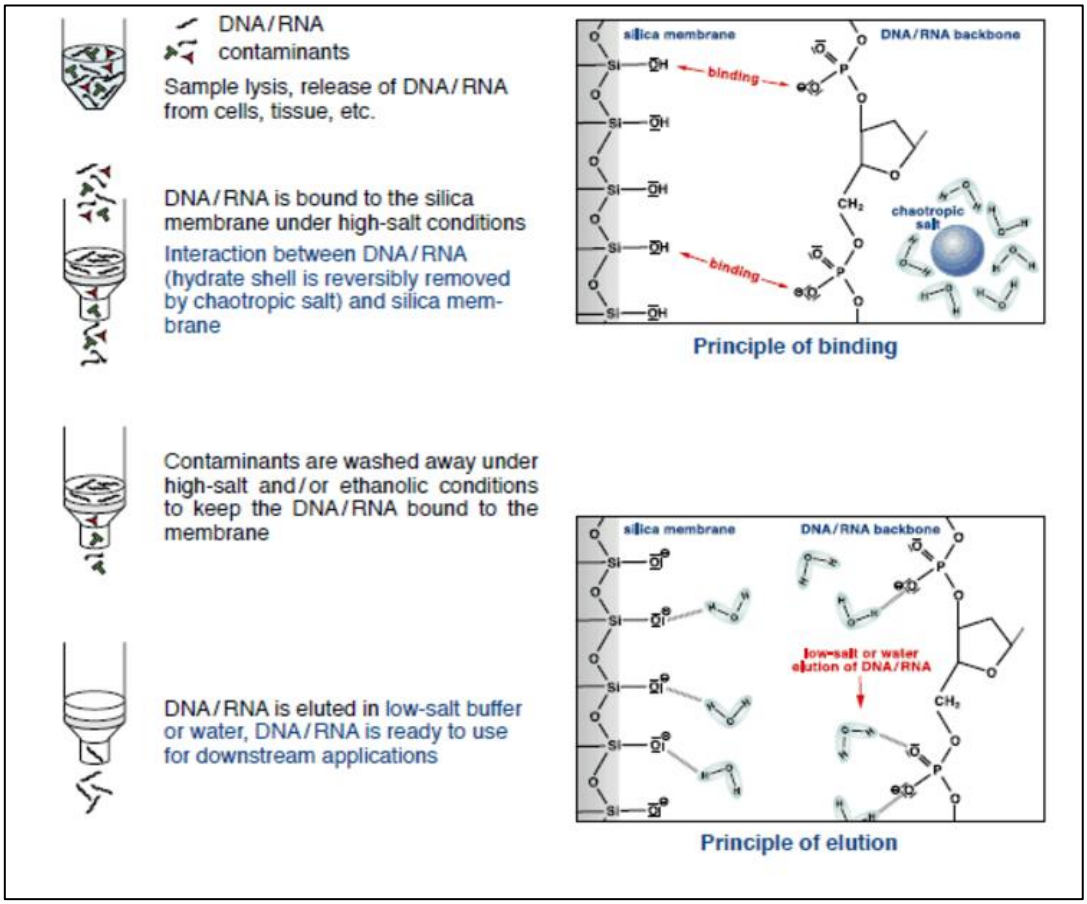


Figure 25: Washing steps during DNA isolation. The DNA is bound to a silica membrane while unwanted cell containments get washed away in different steps. Finally, a low-salt buffer solution washes DNA of the membrane into a container (resource: Eurofins Genomics)

3.4.2 Microsatellites

Microsatellites used in this study originate from a similar previous study by Albrecht (2016) and were collected from the literature. 14 Microsatellites (by Eurofins Genomics) were split up into two pools with seven microsatellites per pool (Table 1).

Pool 1	**Dye**	**Size Range [nm]**	**c [µM]**	**µl in PCR [µl]**	**100µl Pool [µl]**	**from Stock [µl]**	**1:10 predilution [µl]**	**final addition [µl]**
SSsp2201	6FAM	202-354	0.03	1.3	1.3	0.13	1.3	2.6
Ssa197	NED	126-154	0.02	0.2	0.2	0.2	2	4
Ssa407	NED	206-300	0.15	1.5	1.5	1.5	15	30
Ssosl417	PET	174-194	0.04	1.4	1.4	2.8	2.8	2.8
OneU9	VIC	198-208	0.03	1.3	1.3	1.3	13	26
Ssa85	VIC	100-124	0.02	0.2	0.2	0.2	2	4
Str73INRA	VIC	140-148	0.04	1.4	1.4	1.4	14	28
							Total [µl]:	**97.4**
							H_2O [µl]:	**2.6**
Pool 2	**Dye**	**Size Range [nm]**	**c [µM]**	**µl in PCR [µl]**	**100µl Pool [µl]**	**from Stock [µl]**	**1:10 predilution [µl]**	**final addition [µl]**
Str15INRA	6FAM	222-228	0.05	1.1	1.1	0.11	1.1	2.2
Strutt58	6FAM	100-186	0.3	3	3	3	-	6
Ssosl311	NED	131-161	0.07	0.7	0.7	0.7	7	14
SSsp1605	NED	310-590	0.04	0.4	0.4	0.04	0.4	0.8
Str60INRA	PET	98-102	0.05	0.5	0.5	0.5	5	10
BS131	VIC	143-171	0.03	0.3	0.3	3	-	6
Ssosl438	VIC	100-108	0.07	0.7	0.7	3	-	6
							Total [µl]:	**45**
							H_2O [µl]:	**55**

Table 1: Listed are the two primer pools containing seven primers per pool. The table also shows name, dye, the size range (nm), the predilution and the final addition (µl) of each primer.

3.4.3 Polymerase chain reaction and amplification

The amplification of the primers was realised with a polymerase chain reaction using the Qiagen Multiplex PCR Kit (from Qiagen, Germany).

1 µl of the extracted sample DNA was added to 5 µl multiplex master mix and 4 µl of RNAse free water including 0.02 - 0.3 µM of each primer making a total of 10 µl multiplex PCR reaction mix.

An ABI thermal cycler was used to carry out the amplifications. The initial heat-activation was 95°C for 15 min. For denaturation 30 cycles for 30 s at 94°C followed. The annealing and the extension were carried out at 60°C for 90 s and 72°C for 60 s. The PCR was terminated after 30 min of final extension at 60°C.

3.4.4 Sequencing

1 µl of the PCR product was put together with 8,75 µl HiDi Formamide and 0.25 µl of a dye size standard (GeneScan™ 500 LIZ™). Before sequencing the plates were denatured for 2 min at 95°C. The sequencing was carried out using an Applied Biosystems 3130xl Genetic Analyser in a w/EDTA 10x buffer with 25 ml of running buffer and POP7 Polymer.

3.4.5 Genotyping and genetic population analysis

For genotyping the program GeneMarker v1.91 was used. After importing the sequencing data, it visualizes the alleles for the 14 different loci of the primer.

For each primer pool (Multiplex 1 and Multiplex 2) a unique panel prepared in a previous work by Albrecht (2016) was used. The program calls every section of each marker showing the exact loci of the allele (Figure 26). Based on possible interfering signals or false interpretation of the program, every sample was observed in detail and corrected manually when necessary. This process was done for 976 smolts and 255 (187 females, 68 males) potential parents that were used for the stocking. This procedure showed inappropriate scoring with too much missing data on two markers (Str15INRA and SSsp1605). After dismissing these two markers a primer pool of 12 marker remained.

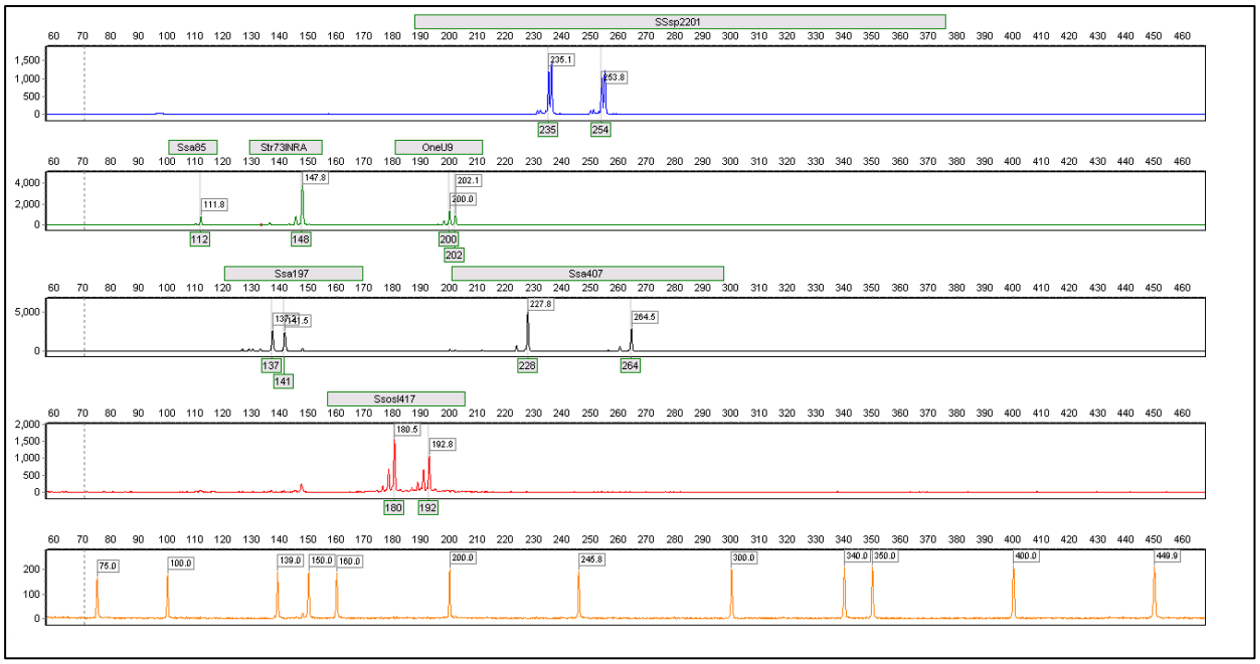


Figure 26: Screenshot from GeneMarker v1.91 showing microsatellite markers of primer pool 1. 4 dyes showing up with several peaks for each microsatellite marker. Names of the marker are shown in the grey bars above the peaks. Orange peaks shows the size standard (GeneScan™ 500 LIZ™).

After calling the alleles of every single individual the data of GeneMarker was exported to the program Colony v2.0.6.1. for final parent-offspring assignment. “Colony is a computer program implementing full-pedigree likelihood methods to simultaneously infer sib ship and parentage among individuals using multilocus genotype data” (Jones, Wang 2010). The program uses a maximum likelihood approach to receive sib ship and parentage relationships.

The exported input files were separated in three different files. One file was the offspring file including the data of 976 smolts. The other two were including the data of the parental generation split in female and male.

When setting up Colony for the parent-offspring assignment (POA) a marker error rate file must be entered. Representative error rates used for this study were detected by Albrecht (2016) (Table 7 and 8, Appendix Page 65). Therefor 20 samples were sequenced and genotyped two times, independently from each other. Comparing the results of both runs the error rate of each primer was estimated. These were in between 0% and 5% between the different primer.

3.5 Statistical methods for data analysis

To determine or refuse differences of the age, size and weight composition in different migration events or between the different smolt ages the following statistical tests were used:

3.5.1 D'Agostino & Pearson normality test

The D'Agostino & Pearson normality test was used to determine the data as normally distributed or not. The output of this test decided about further options for statistical treatment of the data. The test was performed using the program GraphPad Prism 7.

3.5.2 Mann-Whitney U Test

The U-Test from Mann and Whitney is a nonparametric test. It investigates the data of two samples for significant differences by comparing their medians. This test was carried out using the program GraphPad Prism 7.

3.5.3 Chi^2-Test

This test is used to compare frequencies of normally distributed data. It determines if observed frequencies differ from expected results or not.

4 Results

4.1 Total capture of smolt trap

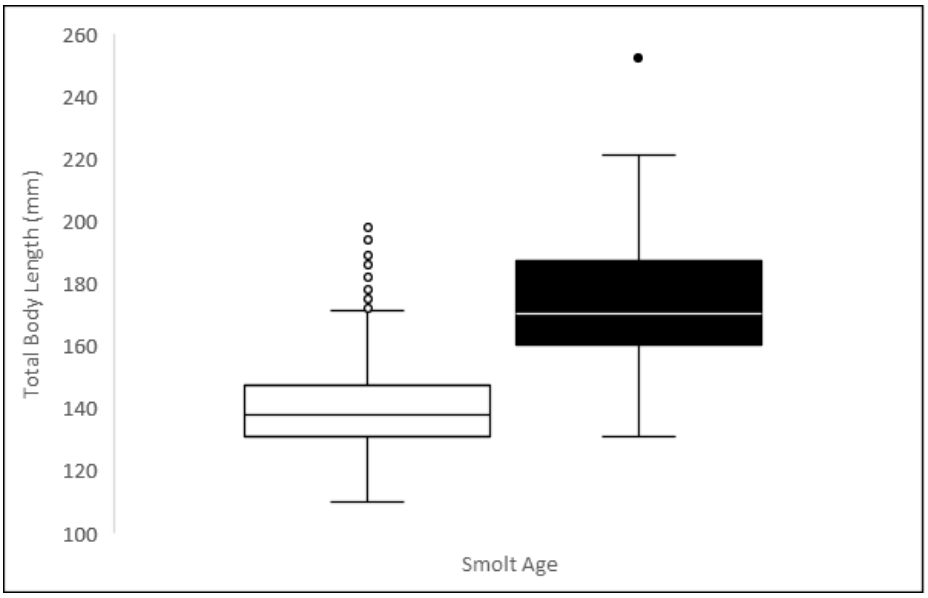
During the whole smolt capture period from the beginning of April 2016 to the beginning of June 2016, 2167 migrating sea trout were trapped. 1191 of these fish were mostly used for LF data collection only. The remaining 976 fish were also used for LF analyses but among others for age determination by scale reading and genetical analyses. The mean total body length of the entire smolt catch was 138,35 mm and the mean weight was 25,13 g. The mean smolt used for scale reading and genetical analysis was 148,08 mm in size and 29,60 g in weight.

4.2 Age distribution in total

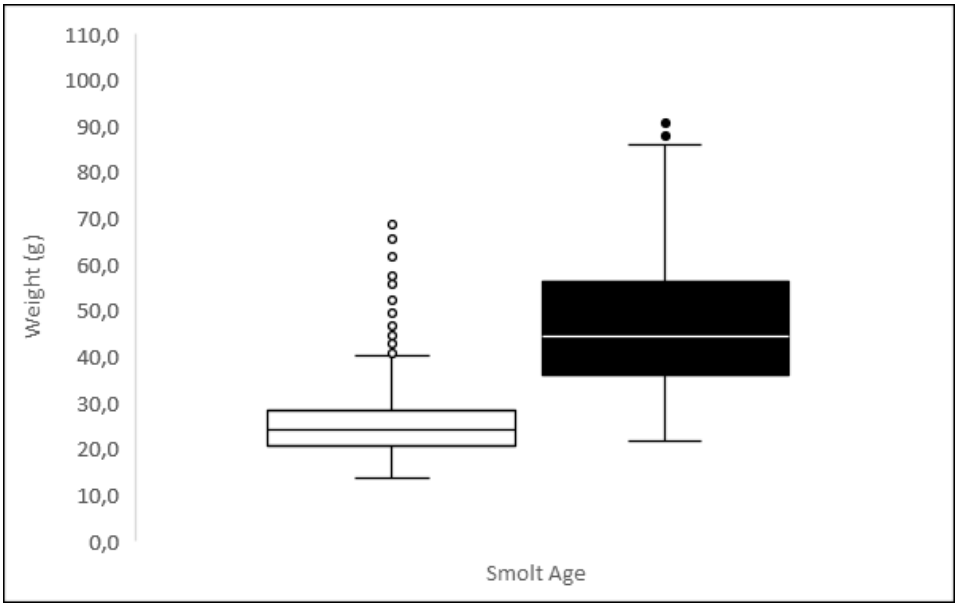
A total of 834 scale samples were analysed. 142 scale samples were not suitable for age determination (missing scales, fungal contamination, replacement scales) and a final evaluation of the fish age is missing. Of 834 suitable scales, 716 fish (85,85%) were determined as an age of one year (+) and 118 (14,15%) as two years (+). No migrating smolts older than 2+ years were found in the samples.

4.3 Size and weight distribution on different ages

Excluding the LF smolts, the average 1+ smolt was 140,27 mm in total body length and 25,63 g in weight. The average 2+ smolt was 173,40 mm in total body length and 48,53 g in weight (Graph 1 and 2). The Mann-Whitney U-Test showed a significant difference between 1+ and 2+ smolts in both, length ($p<0,0001$) and weight ($p<0,0001$) (Table 2).



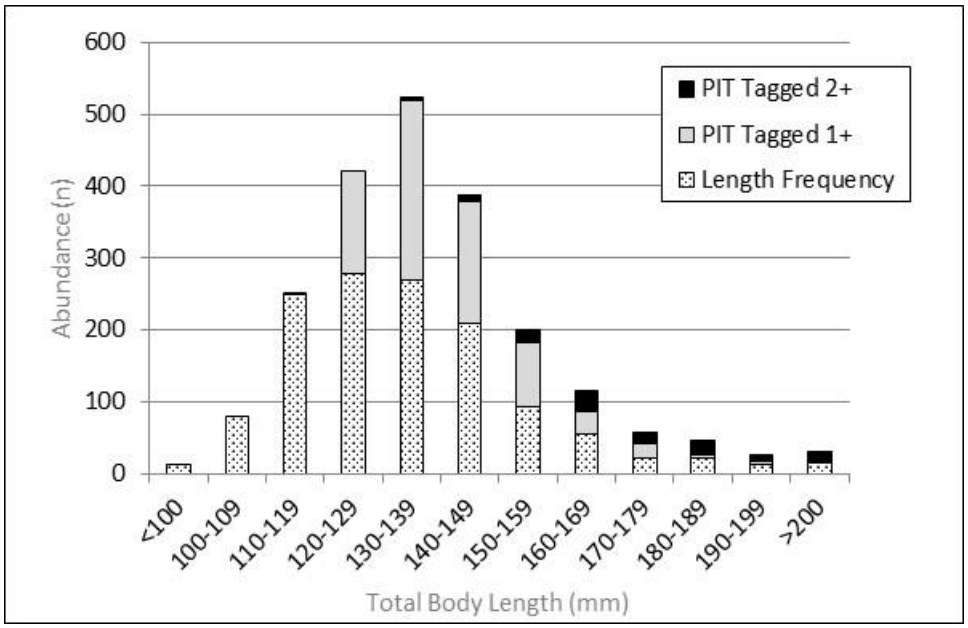
Graph 1: Total body length (mm) distribution of total smolt catch with two different ages (1+ white, 2+ black). Boundaries of the boxes indicate the 25th percentile and the 75th percentile. The line within the box shows the median value. Whiskers indicate the 90th and 10th percentiles. Circles show outliers.



Graph 2: Weight (g) distribution of a total smolt catch with two different ages (1+ white, 2+ black). Boundaries of the boxes indicate the 25th percentile and the 75th percentile. The line within the box shows the median value. Whiskers indicate the 90th and 10th percentiles. Circles show outliers.

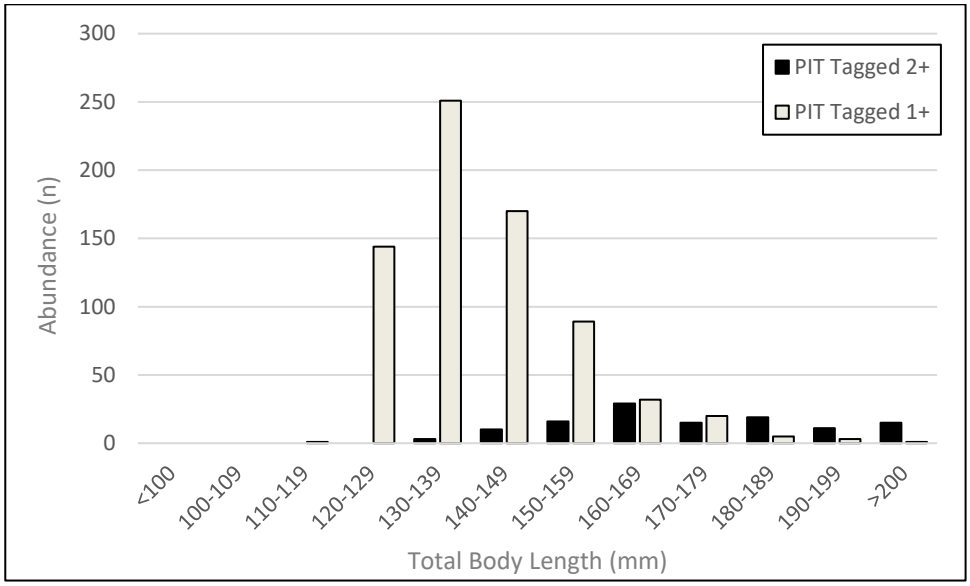
4.4 Age distribution on total body length cohorts

Graph 3 shows the entirety of all migrating sea trout smolts captured with the smolt-trap and their distribution on the body sizes summarized in 10 mm size cohorts. There is no information about age and genetical affiliation of the LF bars (dotted).



Graph 3: LF of 2167 sea trout smolts. Dotted bars indicate the smolts that were examined only for LF analyses while the grey bar represents the abundance of 1+ smolts and the black bars 2+ smolts. The x-axis shows the total body length from <100 mm to >200 mm in 10 mm size cohorts

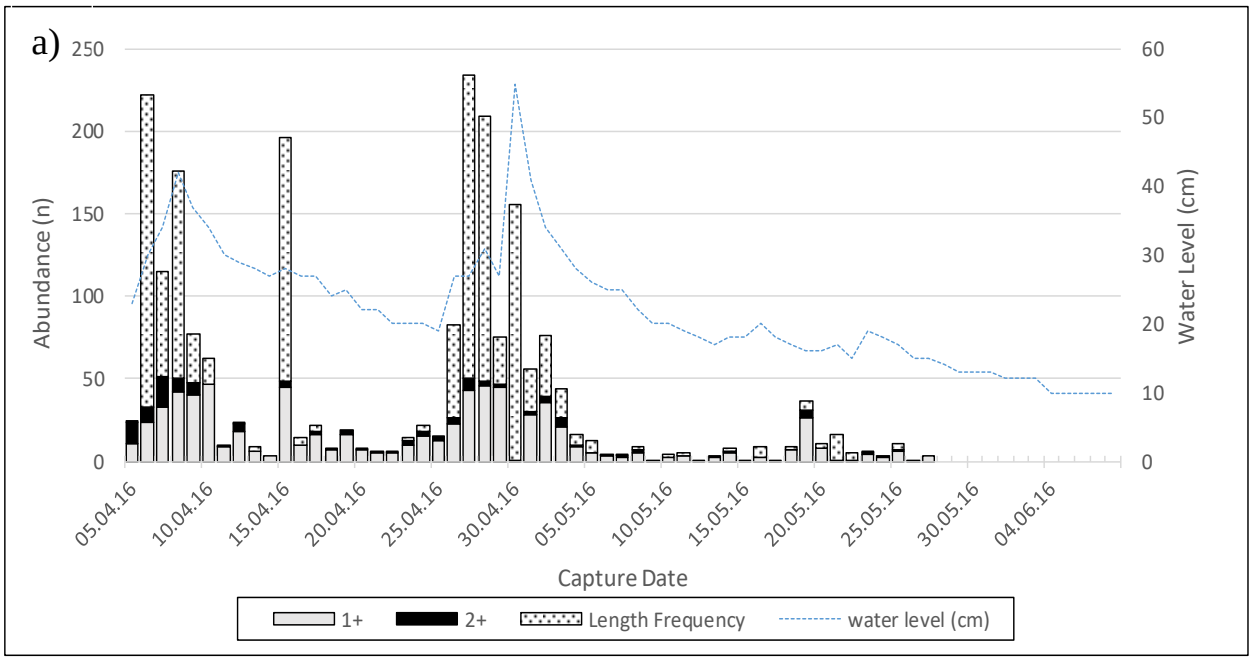
Graph 4 shows the distribution of the fish ages on the total body length only. The quantity of 1+ smolts is decreasing the bigger the size of the smolts while the number of 2+ smolts is increasing with the size. At the three size ranges of 120 mm to 159 mm there are distinctly more 1+ smolts than there are 2+ smolts. These three cohorts represent 66,13 % of all age determined smolts. However, looking at the body size range from 120 mm to 159 mm there are 2,25 % smolts older than 1+ years. From a total body length of 160 mm to 179 mm there is a similar proportion of both ages. From a total body length of 180 mm upward, the proportion of 1+ smolts decreases and the two ages clearly show a domination by 2+ smolts.

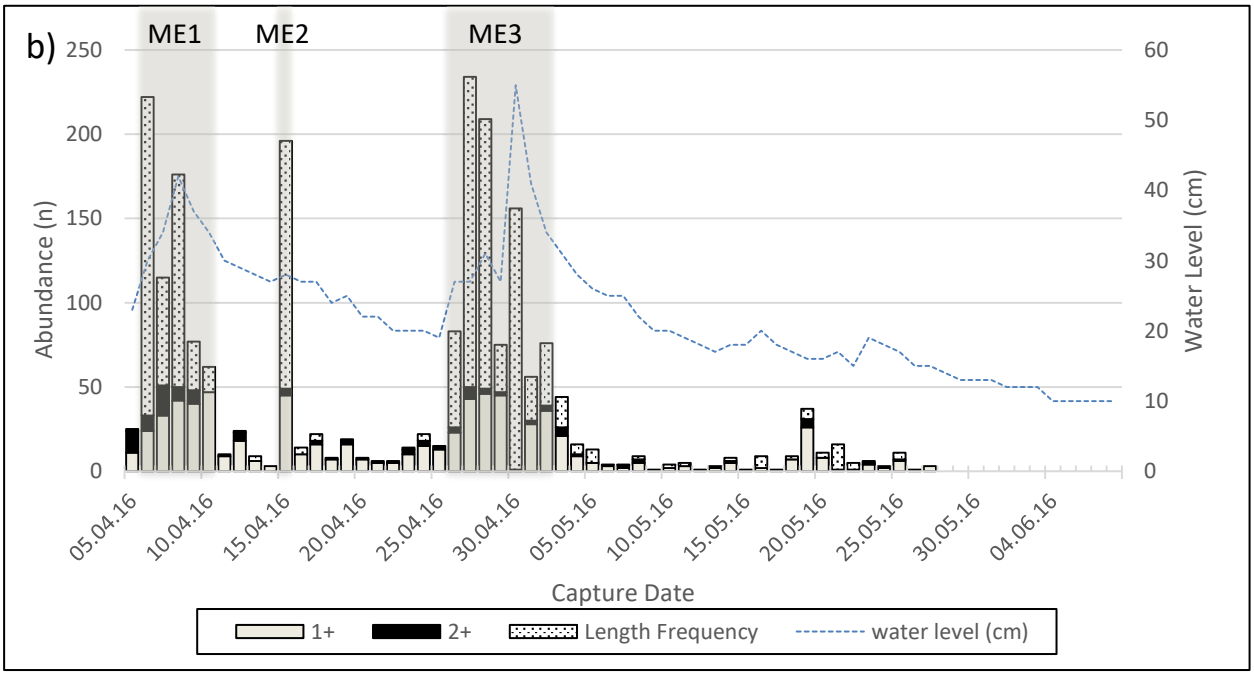


Graph 4: The graph shows the abundance of the two smolt ages found with scale reading and their distribution on the total body length. 1+ smolts are characterised by the grey bar and 2+ smolts by the black bar. The x-axis shows the total body length

4.5 Age distribution during migration events (ME)

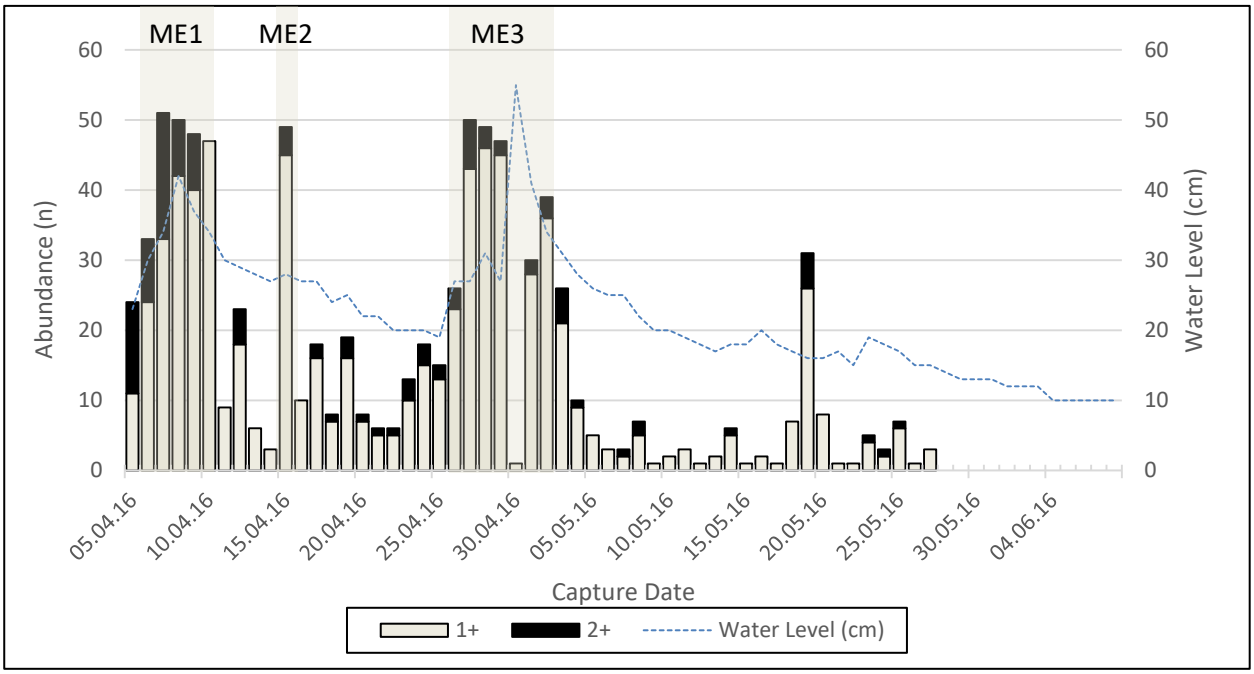
Looking at the total migration through the whole capture period of the trap, there are different migration activities to be observed. Graph 5 a) shows the total catch (*n*) of every day subdivided in three classes (LF, 1+, 2+). The main migration can be characterised by three migration events (ME1, ME2, ME3) including every day with a total catch of at least 50 individuals (Graph 5 b)). These events include 65,53 % of all individuals captured during trap activity. ME1 is the period including the 06th of April to the 10th of April (*n=652*). ME2 is limited to the 15th of April (*n=196*) and ME3 includes the 26th of April to the 2nd of May (*n=572*).





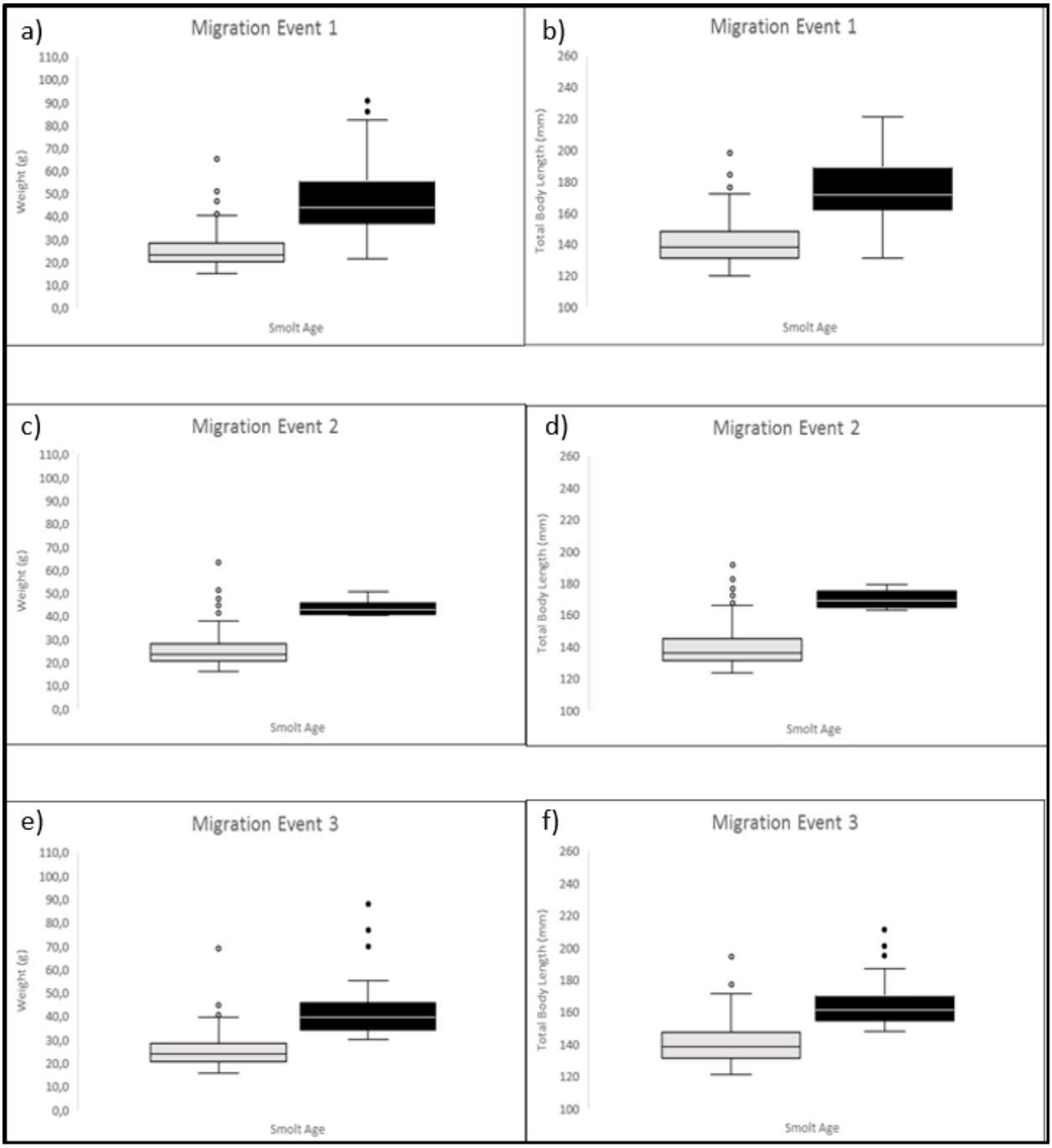
Graph 5 a) and b): The graphs show the abundance (left y-axis) of the entirety of smolts for every day (x-axis) of smolt trapping. The dotted bars indicate the abundance of LF, black bars indicate 2+ smolts, grey bars 1+ smolts. The blue dotted line represents the water level during smolt trapping (right y-axis). Graph b) additionally contains grey areas indicating the migration events ME1, ME2 and ME3

Comparing only the age composition of 1+ and 2+ smolts of the three ME´s, transferred from above, there is a difference in the composition of 2+ smolts in ME1 and ME3 ($Chi^2(1, 5\%, N=470)$, $p= 9,62$). Here significantly more 2+ smolts migrate at ME1 than at ME3. There is no difference of the 2+ compositions comparing ME1 with ME2 ($Chi^2(1, 5\%, N=278)$, $p= 2,69$) and ME2 with ME3 ($Chi^2(1, 5\%, N=290)$, $p= 0,00$).



Graph 6: The graph shows the abundance (left y-axis) of only the age determined smolts for every day (x-axis) of smolt trapping. The black bars indicate 2+ smolts, grey bars 1+ smolts. The blue dotted line represents the water level during smolt trapping (right y-axis). The grey areas indicate the Migration Events ME1, ME2 and ME3

Comparing weight and total body length between age class 1+ and 2+ inside the ME´s (Graph 7 a) - f)), there is a significant difference in both, weight and size between the ages in every ME (Table 2, Mann-Whitney U-test).



Graph 7 a)-f): Graph a), c) and e) show the weight (g) distribution of the two different ages (1+ white, 2+ black) inside the ME´s. Graph b), d) and f) show the total body length (mm) distribution. Boundaries of the boxes indicate the 25th percentile and the 75th percentile. The line within the box shows the median value. Whiskers indicate the 90th and 10th percentiles. Circles show outliers

	N 1+	N 2+	Mean 1+ length (mm)	Mean 2+ length (mm)	Mean 1+ weight (g)	Mean 2+ weight (g)	P value length	P value Weight
Total	*716*	*118*	*140,27*	*173,40*	*25,63*	*48,53*	*0,0001*	*0,0001*
ME1	*186*	*43*	*138,00*	*171,00*	*23,20*	*43,90*	*0,0001*	*0,0001*
ME2	*45*	*4*	*136,00*	*169,00*	*23,40*	*42,55*	*0,0054*	*0,0056*
ME3	*219*	*19*	*138,00*	*161,00*	*23,70*	*39,40*	*0,0001*	*0,0001*

Table 2: Shown are number of individuals (N), mean length (mm) and mean weight (g) of the two age groups (1+, 2+) in the total catch and in three different migration events (ME1, ME2, ME3). The p-values on the right indicate statistical differences in length and weight between the age groups in the total catch as well as in the three migration events.

4.6 Detection of hatchery bred individuals

Of 976 analysed smolt samples 951 (=97,43%) remained for final genetic analysis. 25 samples were excluded because of missing data after scoring or failure in extraction of DNA or missing samples. Of 255 potential parents from the years 2013 and 2014, 68 male samples and 187 female samples were included to the analysis. No parental samples were excluded.

96 (=10.09%) of the total individuals (n=951) could be related to the known parental pool (Appendix Page 74, Table 9). Out of these 96 individuals 22 (=2.31%) could be assigned to both, father and mother, 39 individuals to a single mother and 35 to a single father. Following results will concentrate on the 22 individuals with parent-pair detection (Appendix Page 76, Table 10).

These 22 individuals make up a portion of 0.02 % of the total fry stocking (n=120,000).

The mean length of these 22 individuals was 158,68 mm which was 10,60 mm larger than the mean smolt (148,08mm). With a total body weight of 38,55 g it was also 8,95 g heavier than the mean smolt (29,60g).

Every single individual was assigned to two parents used for the parental pool in 2014. 10 males were detected as fathers and 9 females as mothers responsible for the 22 offspring. That makes 12.10% of the total parental pool (n=157) for hatchery reared fish in 2014 effectively responsible for the recaptured offspring.

Parental pool	Parent pair	Single male	Single female	total
Pool 2013	*0*	*1*	*7*	*8*
Pool 2014	*22*	*34*	*32*	*88*
total	*22*	*35*	*39*	***96***

Table 3: Shown are number of individuals detected as parents (at 97% precision) for 96 captured smolts with possible stocking background. The table is divided in parent pair (both parents detected), single male (only paternal detection) and single female (only maternal detection) on two different spawning pools (2013 and 2014).

4.7 Sib ship of genotyped individual

Sib ship relations were examined for all analysed smolts (n=951). Over the total data set 10 individuals could be assigned to one to three full-sibs and two individuals to a half-sib. The probability for the detection was >97% (Appendix Page 77, Graph 12 and 13 (for all detections without >97% probability)).

4.8 Effective population size (EPS)

Beside POA, Colony determined the EPS (N_e) using the data of offspring and parental generations. The EPS was determined three times:

1. For the total subsamples of 951 smolts representing the whole smolt population (including the parental pools 2013 and 2014 as Colony input data). The colony output showed an EPS of 3040 when assuming random mating and 2576 when assuming non-random mating (Figure 27).

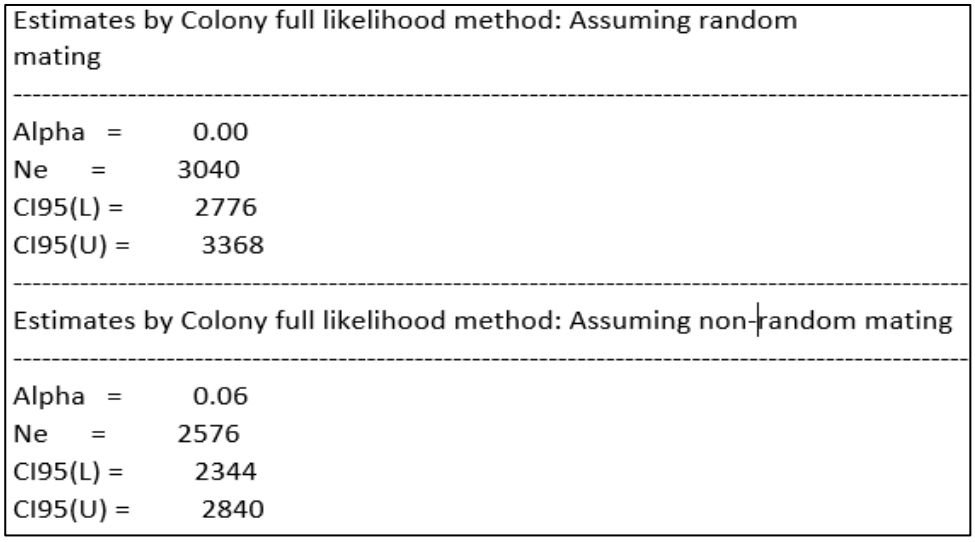


Figure 27:

N_e estimated by Colony and pairwise sib ship assignment for the minimum of spawners within the Lipping Au based on the analysis of n= 951 caught smolt

2. For the 1+ smolt generation estimated by scale reading including 702 offspring samples (parental generation from 2014 as Colony input data). An EPS of 2192 when assuming random mating and 1858 when assuming non-random mating was detected (Figure 28).

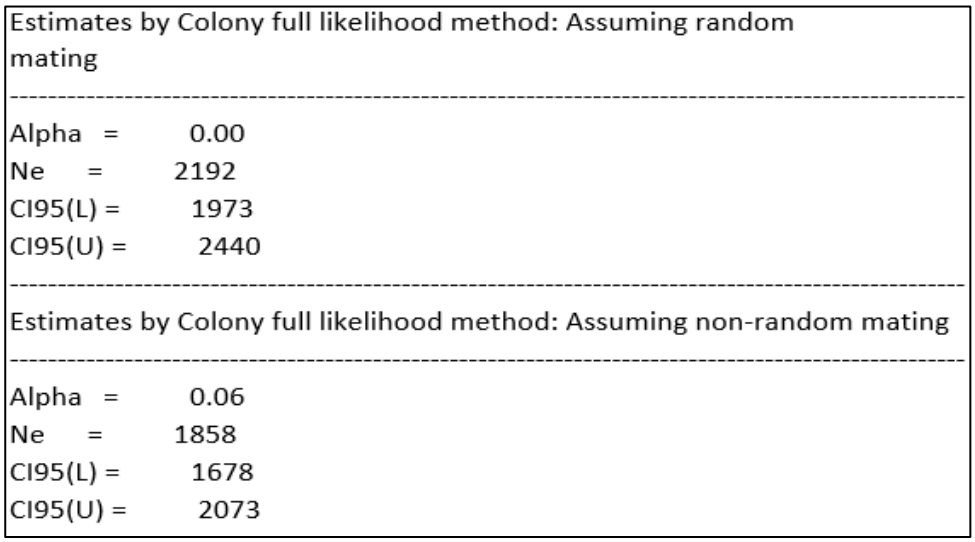


Figure 28:

N_e estimated by Colony and pairwise sib ship assignment for the minimum of spawners within the Lipping Au based on the analysis of n= 702 caught 1+ smolt and n= 157 spawners

3. For the 2+ smolt generation with 118 offspring samples (parental generation from 2013 as Colony input data). An EPS of 359 when assuming random mating and 312 when assuming non-random mating was detected (Figure 29).

```
Estimates by Colony full likelihood method: Assuming random
mating
-----------------------------------------------------------------------------------------------------
Alpha  =      0.00
Ne     =     359
CI95(L) =     282
CI95(U) =     476
-----------------------------------------------------------------------------------------------------
Estimates by Colony full likelihood method: Assuming non-random mating
-----------------------------------------------------------------------------------------------------
Alpha  =      0.05
Ne     =     312
CI95(L) =     242
CI95(U) =     415
```

Figure 29:

N_e estimated by Colony and pairwise sib ship assignment for the minimum of spawners within the Lipping Au based on the analysis of n= 118 caught 2+ smolt and n= 98 spawners

4.9 Estimated smolt ages by POA

Because of the parental information (pool 2013 or pool 2014) of smolts with identified stocking background age estimation was possible by POA. 86 (89,58%) of 96 smolts could be detected as offspring of a father and/or mother of the 2014 parental pool (age 1+) and 10 (10,42%) as offspring of the 2013 parental pool (age 2+). 22 individuals detected with a parent pair can be assigned to the 1+ age group because they originate from the parental generation of 2014. None of these individuals with both detected parents showed a mixed parental pool.

4.10 Comparing age determination by SR and POA

The POA used for age estimation showed partly different results compared to age estimation by SR. For all 96 smolts both methods estimated the same age for one individual in 75%. For 22 offspring (Appendix Page 76, Table 10) with parent pair, three mismatches were detected but both methods agreed in 86% (n=19).

Looking at mean body length and body weight of the different age groups within the detected 96 offspring, there was a considerable difference in the results of POA and SR. Table 4 (1+

generation) and table 5 (2+ generation) show the results of the two different methods comparing mean size and weight of the age classes. Here the 1+ generation by SR is smaller than the one estimated by POA. In the 2+ generation the proportion changed. 2+ smolts, age determined by SR, are clearly larger and of greater weight than 2+ smolts determined by POA. When comparing both age classes, in SR the 1+ generation is smaller than the mean total and smaller than the 2+ generation. POA age estimation shows a larger 1+ smolt than the mean total and also a larger and heavier mean 1+ smolt than mean 2+ smolt.

Weight/size	Mean total (n=951)	Mean 1+ SR (n=716)	Mean 1+ POA (n=86)
Weight (g)	*32,10*	*32,40*	*33,10*
Total-body-length (mm)	*150,04*	*146,20*	*151,45*

Table 4: Shown are mean weight (g) and total-body length (mm) of the total smolts (n=951) and the 1+ age group estimated by SR (n=716) and POA (n=86).

Weight/size	Mean total (n=951)	Mean 2+ SR (n=118)	Mean 2+ POA (n=10)
Weight (g)	*32,10*	*39,50*	*23,50*
Total-body-length (mm)	*150,04*	*161,24*	*135,86*

Table 5: Shown are mean weight (g) and total-body length (mm) of the total smolts (n=951) and the 2+ age group estimated by SR (n=716) and POA (n=86).

5 Discussion

This study succeeded as a pilot study to estimate the age of a sea trout smolt population during springtime migration to marine waters in Schleswig-Holstein. It shows that scale reading is an appropriate method for estimating smolt ages. It also succeeded as the first investigation for estimating stocking success in the pilot system Lipping Au. The results of scale reading and microsatellite analysis together gave latest information for a better understanding of the fresh water stages of sea trout and will help to plan stocking procedures in the future.

5.1 Total catch

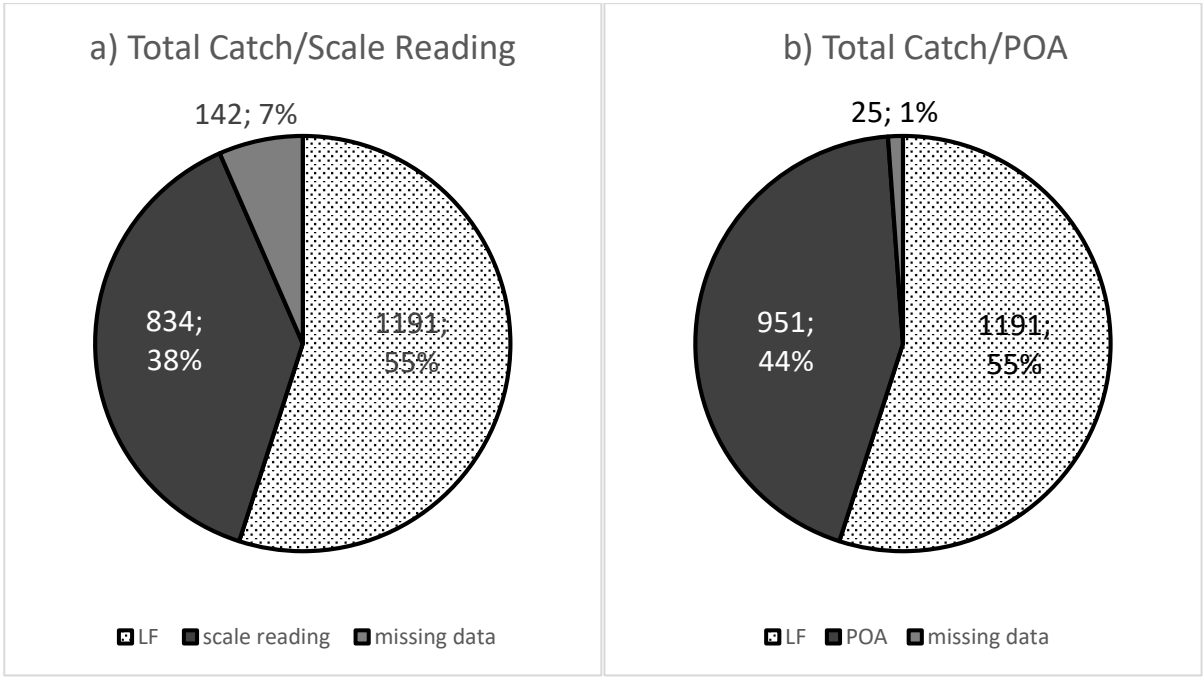
The total catch of 2167 sea migrating smolts seems to be a representative number explaining the smolt output of the Lipping Au during a springtime migration. In 2017 the smolt trap project was repeated and a similar total number of smolts was detected. However, the detailed results of this study do not consider the total smolt population of the Lipping Au but just analysed age and genetics of sampled smolts with a total body length of >120mm. Also, not every smolt that showed the appropriated size was sampled when the daily catch exceeded 50 smolts. That excluded 1191 (55%) migrating smolts from most of this study (Graph 8 a) and b)). So, it should be considered that mean smolt length and weight are supposed to be lower than described because the majority of the excluded smolts were smaller than 120 mm.

The trap was erected on 06th of April 2016. In 2017 the trap was activated three weeks earlier and migration activity was clearly visible already in March. According to that it should be considered that a portion of early spring time migration stayed unvalued in 2016. Like mentioned in the introduction, the feasibility of an autumn presmolt migration is a current topic frequently discussed in sea trout research. Aarestrup et al. (2017) trapped smolts during a presmolt migration in October. In one month of trapping they caught more than 2000 smolts representing 20% of a common springtime migration of the same stream. Due to high water levels and consequently inactivity of the trap they suggested a likely greater number of presmolts. These are further indications that this study just concentrates on a portion but maybe on the majority of the total smolt population of the Lipping Au. However, the maintenance of a smolt trap requires a lot of time and manpower which in most cases just allows short-time operations. Added to this, a fishtrap integrated into a stream or a river is highly susceptible to high water levels and currents. The highest water levels can be

expected in autumn. A smolt trap operating in autumn should be a lot more robust to resist the current and the high emerge of detritus which raises the costs and the manpower of trapping.

The capture success of the trap was assumed to be high but not every migrating smolt could be trapped during activity of the trap. Holes in the net, uneven sealing on gravely bottom and escapes during maintenance and captivity represent opportunities for fish to migrate downstream without being counted. Especially high-water levels with strong current enabled fish to avoid the trap. Here the water level overruns the reach of the collecting bow-net so fish could pass over or next to it. However, in 2016 only one-day was counted on which the trap was not operating in reliable condition. The water level of 55cm allowed an overflowing of the net. Therefore, the catch was reduced significantly (30.04.2016).

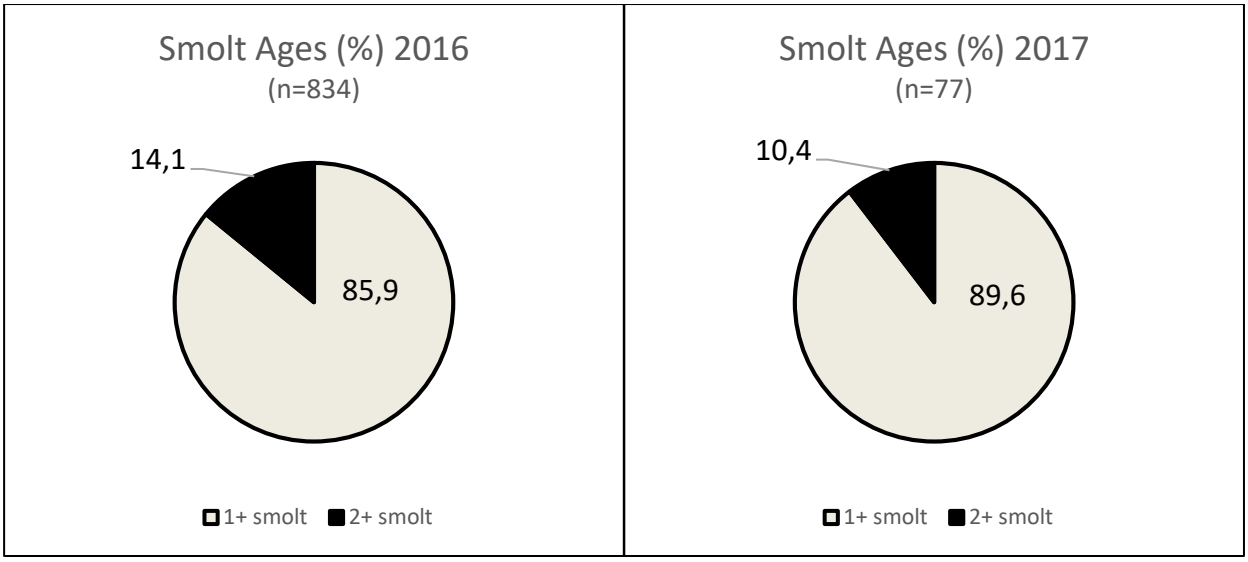
Finally, autumn presmolt migration, early springtime migration and unknown migration events during high-water periods all over the year, so as undetected migration during trapping should be considered as missing data concerning the whole smolt population of the system Lipping Au.



Graph 8 a) and b): Shown are the proportion of migrating smolts captured with the smolt trap in 2016 that were used for LF data collection only (dotted), for a) SR (dark grey) and b) POA (dark grey) and with missing data (light grey).

5.2 Total distribution of smolt ages (by SR)

The scale reading method for estimating smolt age is expected to be a highly reliable model for characterising the age structure of a smolt population. Unbiased results can be expected due to the unknown individual information (size, weight) during reading procedure. Comparing the scale reading results of this study with a following investigation by Kramer (2017), there is a high agreement in the age structure of migrating sea trout smolts of two different springtime migrations in the same river system. After repeating smolt trapping in springtime 2017, the age of 10 randomly chosen smolt scales of every week of trapping (total of 80 scales) were determined. Within these 80 control samples there were 10,60 % detected as an age of 2+. The major age was 1+ in both studies and there were also no smolts older than 2+ in the control-samples of 2017 (Graph 9 and Graph 10). Like mentioned above, the actual age distribution of the whole smolt population is supposed to shift towards the 1+ age group when small not-sampled smolts are expected to be young smolts (1+).



Graph 9: Age distribution of 834 down migrating smolts trapped in 2016 in %. Grey field indicates 1+ smolts, black field indicates 2+ smolts.

Graph 10: Age distribution of 77 down migrating smolts trapped in 2017 in %. Grey field indicates 1+ smolts, black field indicates 2+ smolts. (Taken with permission and modified from Kramer (2017)).

When reading fish scales for age determination mistakes in the interpretation of very few individuals could be expected. Most of the scales showed a striking annulus and the interpretation of the age was considered as highly accurate. As mentioned in the materials, however, some scales

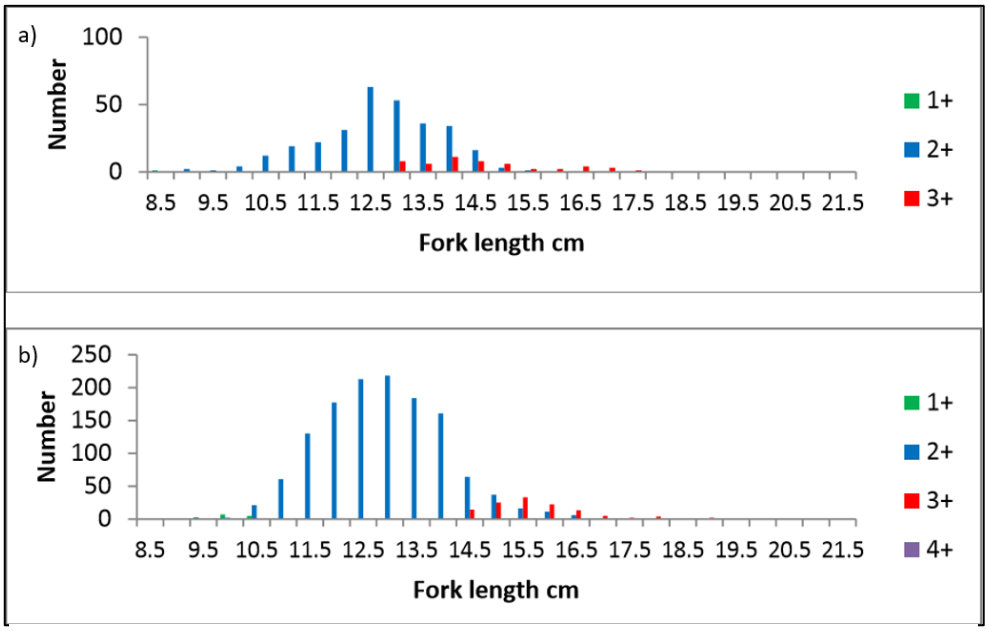
were difficult for interpretation. The annulus, especially of large scales mostly of large smolts, could be hard to determine when the frequency of circuli was high and the growth rate was constant. These scales could be misinterpreted.

Jonsson & L´Abee-Lund (1993), showed that age of European smolts during migration is correlated to the latitude. They found that there are mostly 2+ smolts at a latitude of 54°N whereas there are mainly 4+ to 5+ smolt at 70°N. Looking at the results of the Lipping Au (latitude: 54,5°N) in this study we mostly found 1+ smolts. This result fits to the expectation that Lipping Au smolts migrate in an early age but it does not exactly fit to the results of Jonsson & L´Abee-Lund (1993) which expected a mean age of 2+ for this latitude. However, Jonsson (2001) also showed that smolt age and variation in smolt age inside a population increased with mean water discharge. The Lipping Au is considered as a small stream with comparatively low water discharge. Including this information, a lower mean smolt age than 2+ and a low variation in age could be expected in the Lipping Au. Gehlhaar (1972) found a mean smolt age of 1.6 years in another small stream in Schleswig-Holstein. The Farver Au is a comparative stream with the latitude of 54,3°N. The results of the Gehlhaar study strengthen the results of this study to be assumable. Gehlhaar explained the difference in smolt age between the Farver Au (1.6 years) and the Ranzau (2.1 years), another stream in Schleswig-Holstein, with different mean in water discharge and water temperature. The discharge of the Ranzau is 10 times higher than the one of the Farver Au. This again would strengthen the assumption that smolt ages of little streams are low. In southern and eastern Baltic States like Poland and Lithuania a mean smolt age of 1+ is likely in comparatively little streams with low water discharge and high water temperatures. Skrupskelis et al. (2012) for example, studied smolt populations in three different river systems (55°N to 55,3°N) in Lithuania and he also found a higher 1+ smolt density than 2+ smolt density in at least one of three rivers.

5.3 Size and weight distribution on different smolt ages and total body length cohorts

Okland et al. (1993) and Skrupskelis et al. (2012) confirm that mean length of the smolts increases with age which strengthens the results of this study (mean 1+: 140,27mm; mean 2+: 173,40mm). Graph 11 a) and b) show the age distribution of migrating sea trout smolts on different size cohorts (fork length (cm)) in two different years sampled in a Danish stream with a latitude of 56,4°N (Rasmussen 2016). Here the migration was dominated by 2+ smolts that might be due to the higher

latitude or elevated discharge. Comparing these results with age to size distribution of migrating smolts of the Lipping Au in 2016 (Graph 4), there is a high agreement of the structure of the graphs (Fork length: the distance from the tip of the snout to the fork of the tail. Total length: distance between the tip of the snout to the tip of the longer lobe of the caudal fintip. Graphs can be compared when expecting the distribution of graph 11 a) and b) to shift to the right because smolt are bigger when measured in total length). Both studies confirm that the size difference between the smolt ages is significant. They also confirm that a smolt population is dominated by one age group which is supposed to be the younger generation (Lipping Au: 1+, Rasmussen 2016: 2+).



Graph 11 a) and b): Shown are the age structures of springtime smolt migrations of two different years (a and b) in a Danish stream. Blue bars indicate 2+ smolts and red bars 3+ smolts (directly taken from: Rasmussen (2016)).

5.4 Age distribution during ME´s

Three main migration events could be detected during the trapping period of 2016. Graph 5 a) and b) show, beside abundance (n) and capture date, the water level (cm) during the whole capture period. Every ME seems to correlate with the increase in water level. A higher capture rate could be expected with rising water levels which seem to be responsible for the ME´s. According to Jonsson (1991) the water level/ waterflow is one main trigger for the seaward migration of smolts.

The migration during high water levels is significantly higher compared to low water levels. Waterflow seems to be responsible for the timing and the intensity of migration.

Bohlin et al. (1993) found that smaller individuals of a springtime migration migrated later than those individuals initially bigger and body length at migration decreased with migration time. Looking at the age structures during three migration events (Graph 6), significantly more 2+ smolts were detected in ME1 than in the ME3. The SR results of this study confirm these results of Bohlin et al. (1993).

5.5 Detection of hatchery bred individuals

5.5.1 Parent pairs and natural reproduction

Like shown in the result section 22 individuals out of 951 sampled smolts could be specifically related to a father and a mother of the known parental pool. All 22 individuals were assigned to the parental generation of 2014 and no individual showed a mixed parental generation of 2013 and 2014 which indicates a high reliability of this result. Remaining 929 individuals originate from natural reproduction. 22 detected smolts with stocking background make 2.31% of the sampled 951 individuals. If a stable proportion of survivors is expected for the whole catch (n=2167) a total of 50 individuals could originate from the stocking program. In a previous study Albrecht (2016) detected 3.39% parr with stocking background in a subsample of >500 individuals of a 800 meter section where 10.000 hatchery bred fry were released into parts of the Habernisser Au, a small stream with comparable discharge and stream length located about 10 km north of the Lipping Au. Baer and Rösch (2008) who marked trout fry otoliths with alizarin recaptured 4.80% parr in 2002 and 8.90% in 2003. The recapture rate seems to be unstable but the results of the mentioned studies strengthen the results of this one. However, this result is only a subsample of a smolt population that is known to be bigger than just 951 smolts. Here again 55 % (LF data) of the captured spring migration, the early spring migration and the pre-smolt migration in autumn were excluded from sampling.

The high portion of smolts without hatchery background (n=951) indicates a high and successful rate of natural reproduction. That means that just a very small portion of the spawner population was used for the stocking programs and a high portion spawned and reproduced naturally in the Lipping Au system.

5.5.2 Single parent detection

Now it needs to be explained how 74 smolts could be assigned to a single parent. Since genetic analysis of every single parent of both parental pools succeeded, the single assignment cannot be explained by missing data of the parental generation. The most plausible explanation for 74 smolts with a single parent could be a repeated natural spawning of some fish of the parental pool. *Salmo trutta* is known to be a repetitive spawner that tend to be faithful to their home river (Thomson 2015). Most fish return to their natal stream even if a small portion of 1-3% is known to stray between different rivers (Harris & Milner 2006). Gehlhaar (1972) tagged 123 adult trout during spawning season in a stream of northern Germany (Ranzau). In the following spawning season 25.25 % could be recaptured via electro fishing. This and many other studies indicate a high portion of repeated spawning that should be considered discussing the results of this study. The parental pool used for stocking programs was released into the Lipping Au a few days after stripping. If a high survival rate is considered many of these fish will return for spawning to the Lipping Au the next and following years. The following example should explain the false detection of wild fish as hatchery bred individuals:

A male sea trout was captured during spawning season 2013 via electro fishing. It was measured, fin clipped and transported to the FBA where it was stripped and its sperm was used to fertilize eggs to produce hatchery bred fry. After a few days of rehabilitation this male spawner was re-transported to the Lipping Au where it migrated back to Baltic waters and recovered. In spawning season 2014 the same male spawner re-entered the Lipping Au as a repetitive spawner. This time it reached the natural spawning grounds without being captured before and successfully reproduced naturally with a female of unknown genetical information. Now there are two possible generations of offspring that could be genetical assigned to that male sea trout. The hatchery bred offspring of this male show an age of 2+ years when recaptured in the smolt trap in spring 2016. The natural produced offspring of the same male show an age of 1+ years when trapped. Now there are parent-offspring assignments detected for this male spawner, some with a parent pair and

some with a single assignment. The chances of a hatchery bred background is highly likely for individuals with a parent pair. Both parents were captured and the chance for a repetitive natural pairing of these two individuals is highly unlikely (but cannot be excluded). The background of offspring with single assignment is dubious because the whole parental pool is known and a detection should always show male and female. The first possibility is a mistake by the program with a false assignment which is supposed to be unlikely. Second possibility is the correct assignment of the program leading to a wrong interpretation. The smolts do really originate from this male but developed naturally in the redds of the Lipping Au and not in the FBA and do not originate from any stocking programs. Here the genetical information of the female is missing and the program just detected the male. This possibility is considered as bias mistake for detection of hatchery bred individuals in this study and is the reason why 74 individuals with single parent assignment should be considered as offspring of natural reproduction.

5.5.3 Stocking success

When looking at the stocking success, 0.02% of 120,000 stocked fry were recaptured as smolts inside the subsample. If the same portion of smolts with hatchery background is expected for the whole catch (n=2167) a total of 0.04 % survivors can be expected. Albrecht (2016) detected a stocking success of 0.38% from fry to parr. On first sight that seems to be a very low rate but it should be considered that mortality rates in fry stages are really high and just a little portion growth up to smolt stage. For example is the natural egg to smolt survival in Atlantic salmon around 1.7% in rivers without anthropogenic influences (Chadwick 1982). Bohlin et al. (2002) mentioned that population growth generally is believed to be negatively density-dependent. Around 97.7% of the trapped smolts originate from natural reproduction. This could indicate a high natural density of juvenile trout inside the Lipping Au. If now hatchery bred individuals were introduced in a habitat where natural reproduction is successful and density high, the niches of this habitat might already be occupied by wild juvenile trout. The introduced hatchery bred fry would be the first to starve or die on predators which would explain the low stocking success.

5.6 Sib ship detection

In the previous study Albrecht (2016) sampled parr inside an 800m section of the Habernisser Au. These 800-meter included spawning redds with natural reproduction. Just a little portion of the

effective population size spawned in this area. The genetic diversity is low when compared with the complete system. The result should be a high portion of siblings in this area.

In this study, the subsample represents the entire smolt production of the Lipping Au including all spawning redds in all tributaries and including the entire effective population size. The genetic diversity is higher in the entire system than it is in an 800-meter section. The expectation should be a high portion of siblings inside the 800-meter section of a tributary and a low portion of siblings in the entire system Lipping Au.

Albrecht (2016) assigned 49 individuals to one or two full sibs and 45 individuals to one, two or three half sibs inside a subsample of 472 parr. In this study, the number of full sibs was 10 and the number of half sibs was 2. This result absolutely fits the expectation explained above.

5.7 Effective population size (EPS)

The estimated EPS of 2576 for 951 sampled offspring expecting non-random mating is high but not illegitimated. When splitting the population in age classes there is a total estimated EPS of 2170 when assuming non-random mating, put together by the 1+ generation (EPS of 1858) and the 2+ generation (EPS of 312). The high number of spawners needed to reach a EPS of this size might be hard to believe for a little stream like the Lipping Au. In 2013 a video counting project started at the Hellbach in Mecklenburg-Vorpommern with the aim of defining the number of individuals entering the stream for spawning. The Hellbach has a similar mean water discharge to the Lipping Au. The project counted 2300 spawners in one spawning season and demonstrates that small streams can inhabit a large sea trout population (WGBAST (ICES) 2016). Albrecht (2016) found an EPS of 245 in the subsamples of the Habernisser Au. Combining all tributaries of the stream a EPS of 2576 seems to be a comprehensible result.

5.8 Comparing methods for age determination of smolts

Main focus for age determination was on the SR method. The idea of a comparing SR results with these of the POA based on the expectation of a higher detection of smolts with a stocking background. Finally, there are just 22 individuals with a parent pair assignment that really can be compared with the scale reading results. The uncertain origin of 76 individuals with a single parent detection might lead to the low agreement of 75% of both methods. Especially in that case the results of age determination by SR are more reliable than the determination by POA. If comparing

mean weight and mean length of 1+ and 2+ smolts (of the total 96 individuals) detected with the two different methods, there are great differences between the two groups. The detection by SR led to a mean smolt in both age groups that highly fits to the expectation of size and weight (1+=32,40g/146,20mm; 2+= 39,50g/161,20mm). The results show that 2+ smolts are clearly bigger than 1+ smolts. The detection by POA did not fit the expectation and clearly detected a smaller 2+ smolt than the mean 1+ smolt (1+=33,10g/151,45mm; 2+=23,50g/135,86mm).

If only the age of 22 individuals with parent pair were compared, the agreement of both methods rises to 86%. Just three individuals differ in estimated age. Even if the SR method is supposed to be more reliable than the POA method, in this case it seems to be the opposite. POA detected all 22 individuals as 1+ generation because every single individual was assigned to the parental pool of 2014. SR agreed in 19 individuals but detected three individuals as 2+ generation. Table 6 shows the data of the three individuals that show the mismatches.

offspring	probability of origin	age POA	age SR	agreement of age (yes/no)	weight (g)	length (mm)
4994	1,00	1	2+	no	43,50	164,00
5223	1,00	1	2+	no	62,30	194,00
5250	1,00	1	2+	no	33,80	157,00

Table 6: Shown are the information of three individuals that show mismatches in the age classes detected by SR method and POA method.

The table shows that all three individuals are distinctly bigger than the mean total smolt (138,35mm) and the mean 1+ smolt by SR (140,27mm). A scale of a large smolt is usually a big scale. Looking at a big scale could mislead the reader to think he is looking at the scale of a large and consequently old smolt. This could affect the bias of the reader and thereby the result. Even if the smolt just shows an age of one year the scale shows a high frequency of circuli that could be misinterpreted. A large smolt of 1+ experienced great growing. If this growing was not a constant occasion but split in various events the scale will be difficult for interpretation. Different frequencies in the circuli might accidently lead the reader to detect more winter dark bands than existing. That again would lead to a wrong age detected by SR. The age detection by POA is a total unbiased method done by the program Colony not using any other information than the

genetics. Finally, in this case the POA results should be considered correct and all 22 parent pair offspring were detected 1+.

5.9 Conclusions and outlook

The individual age composition of a spring smolt-run in a typical small river system discharging into the Baltic Sea in Northern Germany, characterised by both natural reproduction and supportive breeding by fry stocking is dominated by smolts aged 1+ (85.85%) and first migration to marine waters happens early compared to northern Baltic States. The fraction of genetically assignable smolts derived by the anthropogenic supportive breeding program in relation to the total spring smolt-run is low (2.31%) compared to natural reproduction (97.69%). However, the results represent a subsample and missed parts of the natural smolt production and do not include fish smaller than 12cm.

Using microsatellites is a capable method to distinguish between hatchery used spawners and pure natural spawners. Including 74 smolts detected with a single parent assignment would have increased the proportion of stocking background smolts inside the subsample to 10.09% and the survival success to 0.08%.

The habitat Lipping Au seems to have improved over the last 15 years due to intensive renaturation and removal of barriers and dams to allow free and undisturbed up-migration for spawning. This seems to allow a reproduction without anthropogenic assistance in this recent years. However, previous years cannot be evaluated and current issues with water quality and increasing siltation puts pressure to keep going with restoration measures and aims of the EU water frame work directive. Further habitat improvement and spawning area building would help to even enhance the production capacity. Examples were anthropogenic stocking has been reduced (Denmark) or even stopped (Estonia) show that habitat improvement is a major step towards a maximum reproduction without stocking.

Like mentioned in the discussion the smolt trap was erected for the second year already in mid-March 2017. Again, many smolts were captured during seaward migration and with this a lot of samples and data were collected that need evaluation in the future. The scale reading protocol of Kramer (2017) was a first step to compare the age structures of a smolt migration between two

years. However, this was just a subsample of the actual sample size and a final analysis should include a similar number of scales like in this study.

The replication of the parent-offspring assignment experiment is recommended. The previous study of Albrecht (2016) helped to compare stocking success between developmental freshwater stages (fry, parr and smolt). To compare the stocking success between smolt migrations of different years the genetic analysis should be repeated more often with a similar number of samples. This would give the opportunity to evaluate the influence of anthropogenic activity, portion of natural reproduction, biotic and abiotic factors on the stocking success.

Finally, a better understanding of the success and the effects of stocking events could help to optimise this procedure for Baltic streams in Schleswig-Holstein.

6 Appendix

Table 7 and 8: shown are the marker error rates for each marker (Colony input data). Table 7) = marker pool 1, Table 8) = marker pool 2

7)

Marker (P1)	SSsp2201	Ssa85	Str73INRA	OneU9	Ssa197	Ssa407	Ssosl417
Error rate	0.0300	0.0300	0.0300	0.0300	0.0533	0.0364	0.0545

8)

Marker (P2)	Strutta58	Ssosl438	BS131	Ssosl311	Str60INRA
Error rate	0.0245	0.0500	0.0352	0.0280	0.0497

Figure 27 to 42: GraphPad Prism 7 output for statistical analysis:

	1	2
Number of values	716	118
Minimum	110	131
25% Percentile	131	159,8
Median	138	170
75% Percentile	147	187
Maximum	200	252
Mean	140,3	173,4
Std. Deviation	13,38	20,83
Std. Error of Mean	0,5	1,917
Lower 95% CI of mean	139,3	169,6
Upper 95% CI of mean	141,3	177,2
Sum	100435	20461
D'Agostino & Pearson normality test		
K2	145,4	10,82
P value	<0,0001	0,0045
Passed normality test (alpha=0.05)?	No	No
P value summary	****	**

D'Agostino & Pearson normality test for length (mm) of two smolt age classes (1+,2+) estimated by SR

Table Analyzed	1 and 2 length
Column B	2
vs.	vs,
Column A	1
Mann Whitney test	
P value	<0,0001
Exact or approximate P value?	Approximate
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	263247 , 84949
Mann-Whitney U	6561
Difference between medians	
Median of column A	138, n=716
Median of column B	170, n=118
Difference: Actual	32
Difference: Hodges-Lehmann	32

Mann-Whitney U test for differences in length (mm) of two smolt age classes (1+,2+) estimated by SR

	1	2
Number of values	716	118
Minimum	13,7	21,5
25% Percentile	20,4	35,73
Median	24	44,15
75% Percentile	28,4	56,55
Maximum	68,7	130,3
Mean	25,64	48,53
Std. Deviation	7,784	17,43
Std. Error of Mean	0,2909	1,604
Lower 95% CI of mean	25,07	45,35
Upper 95% CI of mean	26,21	51,71
Sum	18358	5727
D'Agostino & Pearson normality test		
K2	283,7	38,85
P value	<0,0001	<0,0001
Passed normality test (alpha=0.05)?	No	No
P value summary	****	****

D'Agostino & Pearson normality test for weight (g) of two smolt age classes (1+,2+) estimated by SR

Table Analyzed	1 and 2 weight
Column B	2
vs.	vs,
Column A	1
Mann Whitney test	
P value	<0,0001
Exact or approximate P value?	Approximate
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	262799 , 85396
Mann-Whitney U	6113
Difference between medians	
Median of column A	24, n=716
Median of column B	44,15, n=118
Difference: Actual	20,15
Difference: Hodges-Lehmann	20,3

Mann-Whitney U test for differences in weight (g) of two smolt age classes (1+,2+) estimated by SR

	1	2
Number of values	186	43
Minimum	120	131
25% Percentile	131	161
Median	138	171
75% Percentile	148	190
Maximum	198	221
Mean	140,6	174,5
Std. Deviation	13,95	22,03
Std. Error of Mean	1,023	3,36
Lower 95% CI of mean	138,6	167,8
Upper 95% CI of mean	142,6	181,3
Sum	26153	7505
D'Agostino & Pearson normality test		
K2	36,3	0,4821
P value	<0,0001	0,7858
Passed normality test (alpha=0.05)?	No	Yes
P value summary	****	ns

D'Agostino & Pearson normality test for length (mm) of two smolt age classes (1+,2+) estimated by SR including individuals of ME1.

Table Analyzed	M1 1 and 2 length
Column B	2
vs.	vs,
Column A	1
Mann Whitney test	
P value	<0,0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	18151 , 8184
Mann-Whitney U	760
Difference between medians	
Median of column A	138, n=186
Median of column B	171, n=43
Difference: Actual	33
Difference: Hodges-Lehmann	34

Mann-Whitney U test for differences in length (mm) of two smolt age classes (1+,2+) estimated by SR including individuals of ME1

	1	2
Number of values	186	43
Minimum	15	21,5
25% Percentile	19,8	36,2
Median	23,2	43,9
75% Percentile	28,2	57
Maximum	65,3	90,7
Mean	25,35	48,64
Std. Deviation	7,891	17,88
Std. Error of Mean	0,5786	2,726
Lower 95% CI of mean	24,21	43,14
Upper 95% CI of mean	26,49	54,14
Sum	4715	2091
D'Agostino & Pearson normality test		
K2	76,29	3,339
P value	<0,0001	0,1884
Passed normality test (alpha=0.05)?	No	Yes
P value summary	****	ns

D'Agostino & Pearson normality test for weight (g) of two smolt age classes (1+,2+) estimated by SR including individuals of ME1.

Table Analyzed	M1 1 and 2 weight
Column B	2
vs.	vs,
Column A	1
Mann Whitney test	
P value	<0,0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	18134 , 8201
Mann-Whitney U	743
Difference between medians	
Median of column A	23,2, n=186
Median of column B	43,9, n=43
Difference: Actual	20,7
Difference: Hodges-Lehmann	21,2

Mann-Whitney U test for differences in weight (g) of two smolt age classes (1+,2+) estimated by SR including individuals of ME1.

	1	2
Number of values	45	4
Minimum	123	163
25% Percentile	130,5	163,5
Median	136	169
75% Percentile	145	177,5
Maximum	191	179
Mean	141,6	170
Std. Deviation	16,39	7,394
Std. Error of Mean	2,443	3,697
Lower 95% CI of mean	136,7	158,2
Upper 95% CI of mean	146,5	181,8
Sum	6373	680
D'Agostino & Pearson normality test		
K2	15,28	N too small
P value	0,0005	
Passed normality test (alpha=0.05)?	No	
P value summary	***	

D'Agostino & Pearson normality test for length (mm) of two smolt age classes (1+,2+) estimated by SR including individuals of ME2.

Table Analyzed	M2 1 and 2 length
Column B	2
vs.	vs,
Column A	1
Mann Whitney test	
P value	0,0054
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	1054 , 171
Mann-Whitney U	19
Difference between medians	
Median of column A	136, n=45
Median of column B	169, n=4
Difference: Actual	33
Difference: Hodges-Lehmann	33

Mann-Whitney U test for differences in length (mm) of two smolt age classes (1+,2+) estimated by SR including individuals of ME2.

	1	2
Number of values	45	4
Minimum	16	40,3
25% Percentile	20,35	40,48
Median	23,4	42,55
75% Percentile	28,3	49,05
Maximum	63,2	50,7
Mean	26,79	44,03
Std. Deviation	10,34	4,746
Std. Error of Mean	1,542	2,373
Lower 95% CI of mean	23,69	36,47
Upper 95% CI of mean	29,9	51,58
Sum	1206	176,1
D'Agostino & Pearson normality test		
K2	23,17	N too small
P value	<0,0001	
Passed normality test (alpha=0.05)?	No	
P value summary	****	

D'Agostino & Pearson normality test for weight (g) of two smolt age classes (1+,2+) estimated by SR including individuals of ME2.

Table Analyzed	M2 1 and 2 weight
Column B	2
vs.	vs,
Column A	1
Mann Whitney test	
P value	0,0056
Exact or approximate P value?	Exact
P value summary	**
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	1054 , 171
Mann-Whitney U	19
Difference between medians	
Median of column A	23,4, n=45
Median of column B	42,55, n=4
Difference: Actual	19,15
Difference: Hodges-Lehmann	19,85

Mann-Whitney U test for differences in weight (g) of two smolt age classes (1+,2+) estimated by SR including individuals of ME2.

	1	2
Number of values	219	19
Minimum	121	148
25% Percentile	131	153
Median	138	161
75% Percentile	147	171
Maximum	194	211
Mean	139,7	167,2
Std. Deviation	11,56	18,32
Std. Error of Mean	0,7811	4,204
Lower 95% CI of mean	138,1	158,4
Upper 95% CI of mean	141,2	176
Sum	30584	3177
D'Agostino & Pearson normality test		
K2	39,7	6,003
P value	<0,0001	0,0497
Passed normality test (alpha=0.05)?	No	No
P value summary	****	*

D'Agostino & Pearson normality test for length (mm) of two smolt age classes (1+,2+) estimated by SR including individuals of ME3.

Table Analyzed	M3 1 and 2 length
Column B	2
vs.	vs,
Column A	1
Mann Whitney test	
P value	<0,0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	24369 , 4073
Mann-Whitney U	278,5
Difference between medians	
Median of column A	138, n=219
Median of column B	161, n=19
Difference: Actual	23
Difference: Hodges-Lehmann	25

Mann-Whitney U test for differences in length (mm) of two smolt age classes (1+,2+) estimated by SR including individuals of ME3.

	1	2
Number of values	219	19
Minimum	15,5	29,9
25% Percentile	20,6	33
Median	23,7	39,4
75% Percentile	28,4	47,1
Maximum	68,7	87,6
Mean	25,18	44,22
Std. Deviation	6,71	16,57
Std. Error of Mean	0,4534	3,801
Lower 95% CI of mean	24,29	36,23
Upper 95% CI of mean	26,08	52,2
Sum	5515	840,1
D'Agostino & Pearson normality test		
K2	115,4	10,62
P value	<0,0001	0,0049
Passed normality test (alpha=0.05)?	No	No
P value summary	****	**

D'Agostino & Pearson normality test for weight (g) of two smolt age classes (1+,2+) estimated by SR including individuals of ME3.

Table Analyzed	M3 1 and 2 weight
Column B	2
vs.	vs,
Column A	1
Mann Whitney test	
P value	<0,0001
Exact or approximate P value?	Exact
P value summary	****
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of ranks in column A,B	24333 , 4108
Mann-Whitney U	243
Difference between medians	
Median of column A	23,7, n=219
Median of column B	39,4, n=19
Difference: Actual	15,7
Difference: Hodges-Lehmann	14,5

Mann-Whitney U test for differences in weight (g) of two smolt age classes (1+,2+) estimated by SR including individuals of ME3.

Table 9: List of 96 (=10,09%) of the total individuals (n=951) that could be genetical related to the known parental

offspring	father	mother	probability of origin	age POA	age by scale reading	agreement of age (yes/no)	capture date	weight (g)	length (mm)
4798	3400V14	#	1,00	1	1+	yes	42465	26,00	150,00
4814	3114V14	#	1,00	1	1+	yes	42465	30,60	150,00
4815	*	1100M13	1,00	2	1+	no	42465	23,90	140,00
4822	*	3066M14	1,00	1	1+	yes	42465	39,00	165,00
4824	3403V14	#	1,00	1	1+	yes	42465	17,00	124,00
4844	3101V14	3126M14	1,00	1	1+	yes	42466	27,50	146,00
4845	*	3186M14	1,00	1	1+	yes	42466	25,80	138,00
4851	3078V14	3126M14	1,00	1	1+	yes	42466	31,70	145,00
4865	*	3153M14	1,00	1	missing	missing	42466	27,30	150,00
4882	3111V14	#	1,00	1	missing	missing	42466	90,00	221,00
4884	3113V14	#	1,00	1	missing	missing	42466	41,70	181,00
4891	3065V14	3180M14	1,00	1	1+	yes	42467	40,10	164,00
4911	3401V14	3180M14	1,00	1	1+	yes	42467	33,10	157,00
4917	*	3077M14	1,00	1	1+	yes	42467	22,30	137,00
4923	*	3077M14	1,00	1	2+	no	42467	42,70	171,00
4932	3113V14	3153M14	1,00	1	1+	yes	42467	27,90	149,00
4934	*	3115M14	1,00	1	2+	no	42467	24,70	143,00
4946	3401V14	#	1,00	1	1+	yes	42468	38,80	166,00
4957	3101V14	3126M14	1,00	1	1+	yes	42468	19,10	127,00
4960	*	3124M14	1,00	1	1+	yes	42468	24,50	142,00
4981	3113V14	3392M14	1,00	1	1+	yes	42468	25,80	140,00
4994	3101V14	3126M14	1,00	1	2+	no	42469	43,50	164,00
5001	3394V14	#	1,00	1	1+	yes	42469	21,60	138,00
5010	3401V14	#	1,00	1	1+	yes	42469	25,40	146,00
5013	3061V14	#	1,00	1	1+	yes	42469	23,10	137,00
5019	*	3186M14	1,00	1	1+	yes	42469	22,60	141,00
5026	3101V14	#	1,00	1	1+	yes	42469	29,00	147,00
5027	3113V14	3159M14	1,00	1	1+	yes	42469	28,50	150,00
5045	3189V14	#	1,00	1	1+	yes	42470	16,80	121,00
5059	*	3129M14	1,00	1	1+	yes	42470	23,10	136,00
5066	*	3159M14	1,00	1	1+	yes	42470	19,40	132,00
5076	*	3129M14	1,00	1	1+	yes	42470	18,90	131,00
5111	3401V14	3180M14	1,00	1	1+	yes	42472	57,50	182,00
5114	*	3063M14	1,00	1	1+	yes	42472	72,10	190,00
5120	3111V14	#	1,00	1	1+	yes	42472	27,70	143,00
5134	*	3124M14	1,00	1	2+	no	42475	26,00	139,00
5137	1011V13	#	0,99	1	1+	yes	42475	22,50	135,00
5141	3400V14	#	1,00	1	1+	yes	42475	41,00	165,00
5160	*	3135M14	1,00	1	1+	yes	42475	28,50	144,00
5181	3399V14	#	1,00	1	1+	yes	42475	37,70	158,00
5185	*	3129M14	1,00	1	1+	yes	42476	26,50	145,00
5199	*	3060M14	1,00	1	2+	no	42477	38,30	166,00
5204	3101V14	3392M14	1,00	1	1+	yes	42477	60,60	178,00
5210	3401V14	#	1,00	1	1+	yes	42477	25,30	141,00
5214	3394V14	3153M14	1,00	1	1+	yes	42478	19,80	131,00
5220	3057V14	#	1,00	1	2+	no	42479	78,00	206,00
5221	*	1041M13	1,00	2	2+	yes	42479	20,50	133,00
5223	3401V14	3180M14	1,00	1	2+	no	42479	62,30	194,00
5232	3400V14	#	1,00	1	2+	no	42479	29,00	147,00
5241	3134V14	#	1,00	1	1+	yes	42481	38,00	160,00
5247	3395V14	#	1,00	1	2+	no	42482	30,70	146,00

pool. Also shown are parents, probability of origin, estimated ages by POA and SR, capture date and details to weight (g) and length (mm) of every individual.

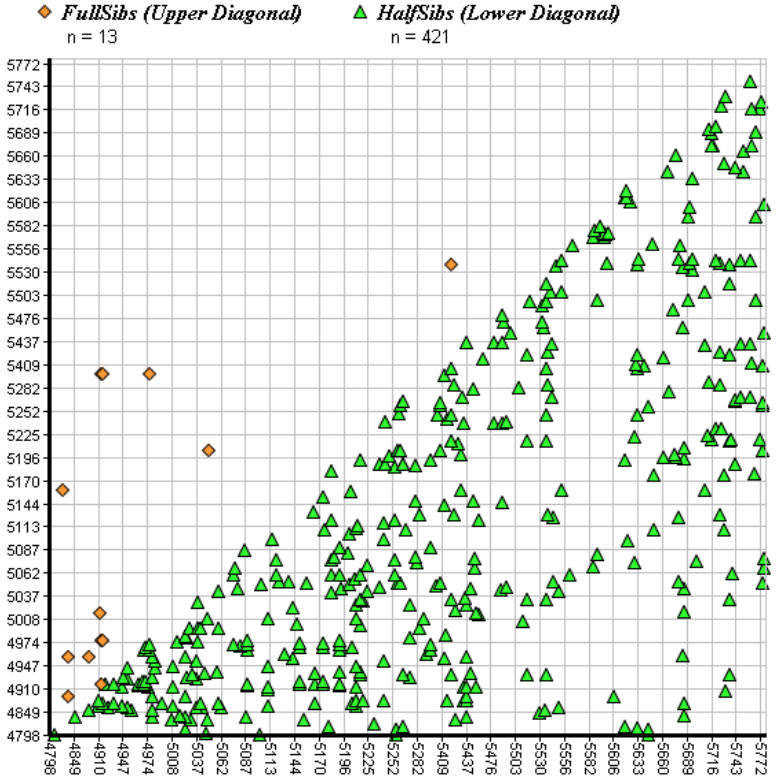
5250	3101V14	3153M14	1,00	1	2+	no	42482	33,80	157,00
5256	3114V14	3180M14	1,00	1	1+	yes	42483	52,90	181,00
5257	*	3129M14	1,00	1	1+	yes	42483	20,20	135,00
5265	3402V14	#	1,00	1	1+	yes	42483	20,90	132,00
5267	*	3046M14	1,00	1	2+	no	42484	49,90	173,00
5270	3114V14	3160M14	1,00	1	1+	yes	42484	56,40	180,00
5274	3113V14	#	1,00	1	2+	no	42484	36,20	162,00
5298	*	3180M14	1,00	1	1+	yes	42485	52,90	184,00
5301	3399V14	#	1,00	1	missing	missing	missing	missin	missing
5404	3395V14	#	1,00	1	2+	no	42486	45,30	165,00
5406	3400V14	3066M14	1,00	1	1+	yes	42486	40,20	160,00
5416	3065V14	3160M14	1,00	1	1+	yes	42486	24,40	145,00
5419	3395V14	3153M14	1,00	1	1+	yes	42486	45,10	170,00
5433	*	3180M14	1,00	1	1+	yes	42487	46,60	177,00
5435	3134V14	3180M14	1,00	1	1+	yes	42487	25,70	140,00
5478	*	3046M14	1,00	1	1+	yes	42488	40,60	164,00
5479	3403V14	#	1,00	1	1+	yes	42488	17,90	127,00
5491	3134V14	3081M14	1,00	1	1+	yes	42488	23,60	137,00
5496	3407V14	#	1,00	1	1+	yes	42488	22,40	135,00
5517	3396V14	#	1,00	1	1+	yes	42488	32,00	153,00
5537	3407V14	#	1,00	1	1+	yes	42489	41,70	165,00
5538	3395V14	#	1,00	1	1+	yes	42489	31,50	155,00
5559	*	1043M13	1,00	2	1+	no	42489	29,20	150,00
5606	*	3127M14	1,00	1	1+	yes	42492	16,20	122,00
5630	*	1041M13	1,00	2	1+	no	42492	27,40	136,00
5632	*	3046M14	1,00	1	1+	yes	42492	21,40	135,00
5645	3400V14	#	1,00	1	1+	yes	42493	25,20	135,00
5652	*	3063M14	1,00	1	1+	yes	42493	20,60	133,00
5654	*	3133M14	1,00	1	1+	yes	42493	20,00	131,00
5668	*	3115M14	1,00	1	1+	yes	42493	20,20	131,00
5670	*	1043M13	1,00	2	1+	no	42494	19,90	129,00
5671	*	1100M13	1,00	2	1+	no	42494	23,40	135,00
5676	*	3060M14	1,00	1	2+	no	42494	29,10	148,00
5682	*	1043M13	1,00	2	1+	no	42495	18,90	127,00
5686	*	3186M14	1,00	1	1+	yes	42496	19,50	129,00
5691	3401V14	#	1,00	1	1+	yes	42498	50,70	171,00
5710	*	3135M14	1,00	1	1+	yes	42504	23,30	136,00
5711	*	3097M14	1,00	1	1+	yes	42506	23,10	140,00
5723	1031V13	#	0,98	2	1+	no	42475	26,00	139,00
5728	*	3080M14	1,00	1	1+	yes	42509	22,00	131,00
5747	3403V14	#	1,00	1	1+	yes	42486	24,40	145,00
5748	3189V14	#	1,00	1	1+	yes	42486	45,10	170,00
5752	*	3115M14	1,00	1	1+	yes	42510	18,50	120,00
5761	3401V14	#	1,00	1	2+	no	42513	59,20	187,00
5773	1031V13	#	1,00	2	2+	yes	42506	23,10	140,00

Table 10: Shown are 22 (=2,31%) out of the total individuals (=951) that could be assigned to both, father and mother. Also shown are parents, probability of origin, estimated ages by POA and SR, capture date and details to weight (g) and length (mm) of every individual.

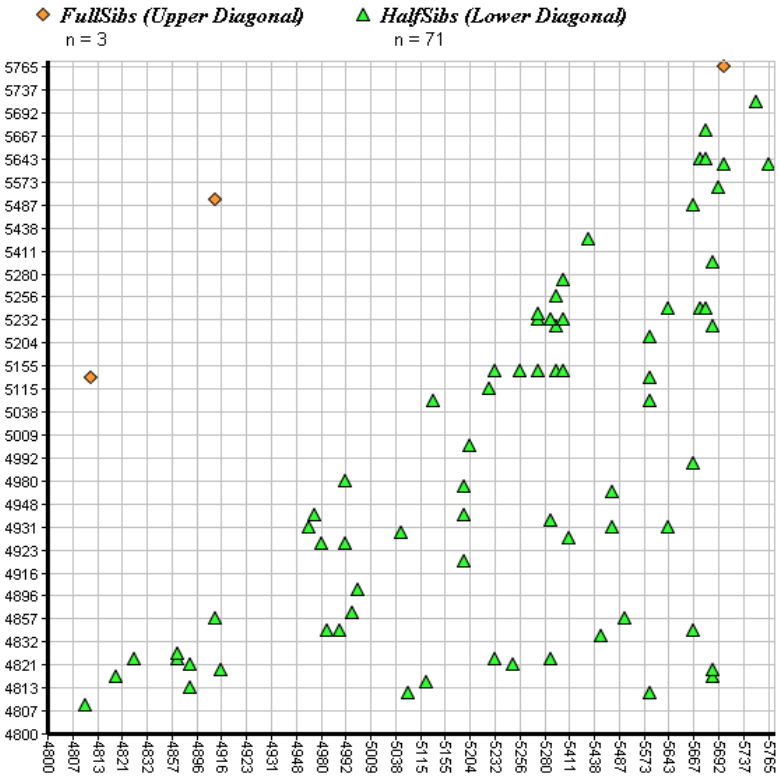
offspring	father	mother	probability of origin	age POA	age SR	agreement of age (yes/no)	capture date	weight (g)	length (mm)
4994	3101V14	3126M14	1,00	1	2+	no	42469	43,50	164,00
5223	3401V14	3180M14	1,00	1	2+	no	42479	62,30	194,00
5250	3101V14	3153M14	1,00	1	2+	no	42482	33,80	157,00
4844	3101V14	3126M14	1,00	1	1+	yes	42466	27,50	146,00
4851	3078V14	3126M14	1,00	1	1+	yes	42466	31,70	145,00
4891	3065V14	3180M14	1,00	1	1+	yes	42467	40,10	164,00
4911	3401V14	3180M14	1,00	1	1+	yes	42467	33,10	157,00
4932	3113V14	3153M14	1,00	1	1+	yes	42467	27,90	149,00
4957	3101V14	3126M14	1,00	1	1+	yes	42468	19,10	127,00
4981	3113V14	3392M14	1,00	1	1+	yes	42468	25,80	140,00
5027	3113V14	3159M14	1,00	1	1+	yes	42469	28,50	150,00
5111	3401V14	3180M14	1,00	1	1+	yes	42472	57,50	182,00
5204	3101V14	3392M14	1,00	1	1+	yes	42477	60,60	178,00
5214	3394V14	3153M14	1,00	1	1+	yes	42478	19,80	131,00
5256	3114V14	3180M14	1,00	1	1+	yes	42483	52,90	181,00
5270	3114V14	3160M14	1,00	1	1+	yes	42484	56,40	180,00
5406	3400V14	3066M14	1,00	1	1+	yes	42486	40,20	160,00
5416	3065V14	3160M14	1,00	1	1+	yes	42486	24,40	145,00
5419	3395V14	3153M14	1,00	1	1+	yes	42486	45,10	170,00
5435	3134V14	3180M14	1,00	1	1+	yes	42487	25,70	140,00
5491	3134V14	3081M14	1,00	1	1+	yes	42488	23,60	137,00

Graph 12 and 13: Colony output for sib ship detection showing every detection by the program without 97% probability. Graph 12 shows the sib ship relations inside the 1+ group, graph 13 inside the 2+ group. Orange dots indicate full sibs, green triangles indicate half sibs.

12)



13)



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(taken on: 04.08.2017)

8 Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne fremde Hilfe angefertigt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Die eingereichte schriftliche Fassung der Arbeit entspricht der auf dem elektronischen Speichermedium.

Weiterhin versichere ich, dass diese Arbeit noch nicht als Abschluss an anderer Stelle vorgelegen hat.

Kiel, den 19.10.2017 _____ (Jan-Philip Rathjen)