Master Thesis

Changes in gill Na^+K^+ATPase α subunit isoform expression during smoltification and in maturing male Atlantic salmon

For the Fulfilment of the Degree

Master of Science in Marine Biology

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Front page: painting by Brian Bradfield; Mature male Atlantic salmon
http://www.flickr.com/photos/34293299@N04/5266227417/in/photostream/

Abstract

Distinct freshwater and seawater chloride cells have been identified in salmon gills and recent studies also suggest that there are specific freshwater (α1a) and seawater (α1b) isoforms of the α subunit of Na^+K^+-ATPase (NKA). Salmon smolts adjust to seawater prior to migration and an upregulation of NKAα1b transcription have been seen while the smolts were still in freshwater. Studies of mature Pacific salmon (*Oncorhynchus spp.*) suggest that mature salmon adapt to freshwater while still in seawater and may thus loose the ability to hypo-osmoregulate. Consequently, maturing salmon kept in net pens may suffer from terminal dehydration and this might be a major fish welfare problem in aquaculture as many salmon mature before slaughter weight is achieved.

Thus, the aim of the present study is to detect possible changes in gill NKAα1a and gill NKAα1b expression in maturing male salmon kept in fresh- and seawater using Real-Time RT-qPCR.

Pre-smolts were exposed to a smolt inducing photoperiod, before half were transferred to seawater, while the rest remained in freshwater. The post-smolts were then exposed to either continuous light or short day photoperiod to induce high end low incidence of mature males, respectively.

The present study is the first in which changes in salinity specific NKAα1a and NKAα1b isoforms is used to detect a possible preparatory adaptation to freshwater in maturing male Atlantic salmon.

Our date coincides with previously seen changes in NKAα1a and NKAα1b in relation to smoltification and desmoltification as the expression of NKAα1b increased in smolts prior to seawater transfer and NKAα1a expression decreased. Furthermore, an increase in NKAα1a was seen in desmolting salmon while NKAα1b expression declined. These findings support the hypothesis of NKAα1b as the seawater adaptive isoform and NKAα1a as the freshwater isoform. In addition, an increase in NKAα1a were seen in maturing male salmon kept in seawater and NKAα1a levels were significantly higher in mature males than in immature females. No significant differences in NKAα1b were seen between mature males and immature females in seawater, but NKA activity was significantly lower in mature males than in immature females in seawater. This suggest that mature salmon adapt to freshwater while still in seawater and may consequently die from elevated plasma osmolality if kept in seawater after the onset of maturation.

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Tusen takk.

Christine Ranang Elgen
22. June 2011

1. Introduction

1.1 The lifecycle of Atlantic salmon

The anadromous Atlantic salmon (*Salmo salar L.*) is one of the teleosts that show the greatest variety in lifecycle strategies (Hutchings and Jones, 1998). Critical events, such as smoltification and age at first maturity may vary with several years, between and within populations (Hutchings and Jones, 1998, Fleming, 1996).

The salmon migrate from their river of origin to the ocean as smolts during spring and they return in the summer/autumn to spawn, after one to four years at sea (McCormick et al., 1998, Hutchings and Jones, 1998). During these migrations, the salmon undergo many complex physiological, behavioral, and morphological adaptations to survive in these two diverse habitats (McCormick et al., 1998).

1.2 Smoltification

As the juvenile salmon goes through smoltification, it will change from stream-dwelling and aggressive parr, to a pelagic and schooling smolt, perfectly adapted to the marine environment (fig. 1) (McCormick et al., 1998, McCormick and Saunders, 1987, Stefansson et al., 2008, Wedemeyer et al., 1980). To complete the transition from freshwater (FW) to seawater (SW), the salmon must change from hyper-osmoregulation to hypo-osmoregulation prior to and during migration (McCormick et al., 1998).

In most of the distribution range, smoltification takes place in April to May, with light and temperature as the two main triggering factors (McCormick et al., 1998). The increased day length is registered by the pineal gland and retina, which send signals to the hypothalamus through neurons, resulting in the release of growth hormone (GH) and thyroid stimulating hormone (TSH) from the pituitary (Ebbesson et al., 2003). GH stimulates the hepatic and peripheral insulin-like growth factor (IGF-1) production, adrenocorticotropic hormone (ACTH) the release of cortisol from the head kidney, while TSH stimulates the production of the thyroid hormones (TH); thyroxin (T_4) and tri-iodothyronine (T_3) (McCormick et al., 1998, Stefansson et al., 2008). The interaction of these hormones induce structural modifications known to be associated with seawater adaptation as they increase the number of SW chloride cells and thus Na^+K^+ATPase (NKA) activity, which is essential for seawater survival

(McCormick, 2001, Prunet et al., 1994). The smolt will enter seawater when it is behaviorally and psychologically ready. This period is referred to as the "smolt window" and is characterized by a high NKA activity and an ability to survive an immediate transfer to full strength SW (Stefansson et al., 2008).

The production of smolt for aquaculture purposes was previously season dependent, but methods using artificial light have made it possible to produce "out of season" underyearling smolts (Berge et al., 1995). Salmon farmers are now able to transfer fish to ocean net pens year round.

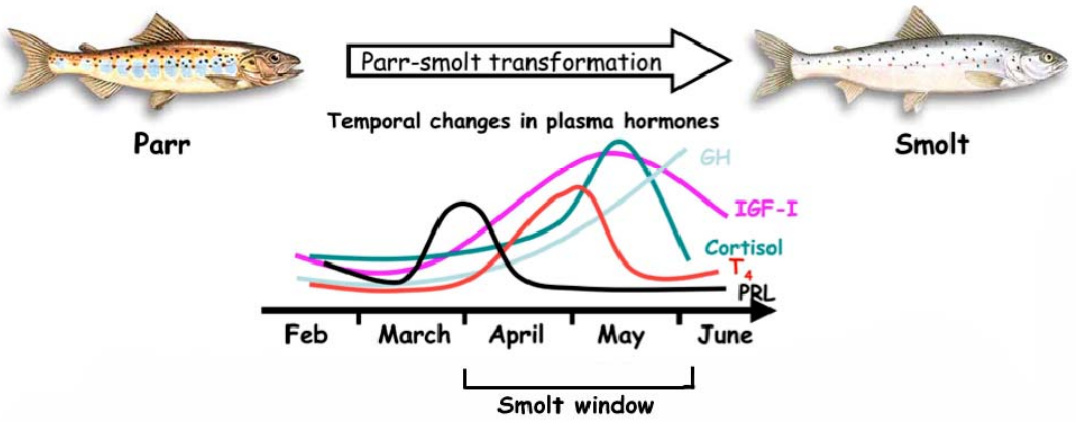

Figure 1: Parr-Smolt transformation and its related hormones. Notice the color difference in salmon parr and smolt. A normal developing fish is always perfectly adapted to their environment. The brown color base and aggressive behavior of the parr is in contrast to the silvery body and schooling behavior of the smolt. The graph shows the relative hormone levels of GH, IGF-1, Cortisol, T_4 and PRL in relation to a natural spring smolting and the smolt window (Nilsen, 2007, McCormick, 2001). Illustration modified from (Nilsen, 2007 Phd).

1.3 Puberty

Puberty is the developmental period during which an immature animal requires the capacity to produce offspring for the first time (Okuzawa, 2002, Taranger et al., 2010). Puberty in teleosts is associated with rapid gonad growth due to differentiation of germ cells, and culminates into the first spermiation or ovulation (Okuzawa, 2002). The onset of puberty in Atlantic salmon is triggered through several internal and external factors, such as light, temperature and energy stores (Taranger et al., 2010). Changes in the photoperiodic cycle stimulates the production and release of the neurohormone gonadotropin-releasing hormone (GnRH) from the hypothalamus, which then triggers the production of the pituitary gonadotropins: follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Schulz et al., 2006, Schulz et al., 2010, Okuzawa, 2002). LH and FSH will then activate the production of sex steroids and germ cells in ovaries and testis (Schulz et al., 2006, Schulz et al., 2010, Okuzawa,

2002). The proliferation and maturation of gametes requires energy, more so in females than in males (Fleming, 1996). Thus, most female Atlantic salmon are anadromous, so they may utilize the rich feeding grounds of the ocean (McCormick et al., 1998). Male salmon will either select to spawn as parr or as full-size males after one to four years at sea (Hutchings and Jones, 1998). Whether the salmon becomes mature after one year at sea or later depends on genetic factors, the energetic factors and the overall physiological status of the individual (Thorpe, 1986). When mature, the anadromous salmon will return to their river of origin and reproduce (Hutchings and Jones, 1998). Some will survive spawning and return to sea, while for others the energy cost is to great and death is inevitable (Fleming, 1996).

1.4 Early puberty and sexual maturation

Atlantic salmons may become mature as parr, prior to SW migration, after a few months in SW, at the "jack" stage and after 1.5 years in SW, at the "grilse" stage or after two or more years in SW as multi-sea-winter salmon (Jonsson and Jonsson, 2007, Taranger et al., 2010). Early puberty and maturation normally occur when food is available in sufficient amounts (Fleming, 1996, Hutchings and Jones, 1998) and the proportion of males that reach parr, jack and grilse maturation is normally higher than in females (Taranger et al., 2010). Because of this lifecycle variation, some farmed salmon may reach puberty at an early age, due to enhanced food availability and growth conditions in tanks or net pens (Taranger et al., 2010).

Early puberty in Atlantic salmon is a major economical and welfare problem in the aquaculture industry considering the negative impact puberty has on meat quality, growth and survival rate (Taranger et al., 2010). Although methods like photoperiodic control may delay puberty in salmon (Bromage et al., 2001), the commercial use of these methods may be compromised by unpredictable results (Taranger et al., 2010). Temperature and other uncontrollable factors may result in a significant number of mature fish in spite of the use of inhibitory photoperiod (Taranger et al., 2010). Consequently, a better understanding of the onset of puberty and fish welfare consequences is needed.

1.5 Photoperiodic control of sexual maturation

As mentioned, salmon require a substantial amount of energy for the production of gametes and to mature (Fleming, 1996). Thus, an internal biological threshold for

entering puberty is present to ensure that each salmon has stored enough energy to complete maturation (Fleming, 1996). The biological threshold is based on the physiological state of the animal (e.g. size, adiposity and gonad development) and is determined by both genetic and phenotypic factors (Taranger et al., 2010). Fast growing salmon are more likely to reach the threshold before the "critical window" controlled by circannual rhythms closes, than slow growing salmon (Taranger et al., 2010).

Advancing photoperiod, by increasing day length in winter or early spring, will reduce the proportion of salmon entering puberty as few individuals have reached the required threshold so early in the season (fig. 2) (Taranger et al., 1999). However, prolonged exposure to long days, or exposure to continuous light after summer solstice can have the opposite effect by increasing the number of fish who has reached the threshold (fig. 2) (Taranger et al., 2010). This model makes it possible to move forward or postpone puberty and sexual maturation (Taranger et al., 2010). Advancing puberty is first and foremost useful in an experimental context, making it possible to study maturation in smaller fish.

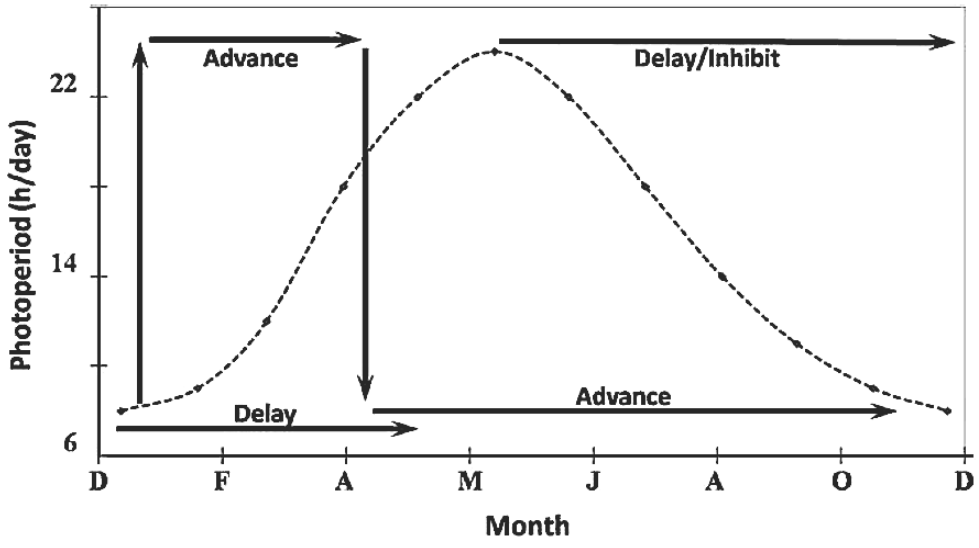

Figure 2: Photoperiodic control of puberty. The dotted line represents the natural photoperiod in the northern hemisphere. The arrows illustrates changes in photoperiod that may advance or delay circannual rhythms. Long days or continuous light early in the season will advance such rhythms and therefore delay puberty. Short days early in the season will delay the rhythm and advance puberty. Long days or continuous light after midsummer will postpone circannual rhythms and advance puberty, but short days from spring/early summer will advance such rhythms and delay puberty (Taranger et al., 2010).

1.6 Osmoregulation

Shifting between FW and SW is challenging as the two environments represent completely different osmotic challenges for the fish. In FW a stable plasma osmolarity is maintained through absorbing ions across the gill and excrete excess water, obtained through passive osmosis, by producing large volumes of dilute urine (Perry, 1997). In SW, the fish dinks water to avoid dehydration and ions are actively excreted trough the gill and (Evans et al., 2005).

Chloride cells (CC) play an important role in osmoregulation and adaptation to SW or FW in migrating fish species as they are the main site for ion secretion and ion uptake allowing maintenance of a stable plasma osmolality (McCormick, 2001, Perry, 1997). CC are located in the gill filament s, mainly in the afferent edge of filaments and in the interlamellar region (Evans et al., 2005). Distinct FW-type CC and SW-type CC are identified based on differences in function, morphology and specific antibodies (fig. 3) (Evans et al., 2005, Perry, 1997, McCormick et al., 2009). SW-type CC have an extensive tubular system, which is formed by invagination of the basolateral membrane (Evans et al., 2005). This tubular system, which almost fills the entire CC, have a high abundance of the enzyme Na^+K^+ATPase (NKA) (Evans et al., 2005). A study of Brown trout (*Salmo trutta)* revealed a correlation between increased NKA activity and increased number of SW-type CC after SW transfer (Seidelin et al., 2000) and the same is seen in Atlantic salmon (McCormick et al., 2009). In addition, multiple studies have shown that a high NKA activity is linked with seawater adaption and survival (Berge et al., 1995, Boeuf and Prunet, 1985, McCormick et al., 1995, Nilsen et al., 2007, Prunet et al., 1989, Stefansson et al., 1998).

It has been shown that GH and IGF-1 stimulate the differentiation of SWCC and more efficiently so in synergy with cortisol (McCormick, 1996, McCormick, 2001). Furthermore, TH has an indirect effect on SW adaption through the upregulation of corticoid receptors in the gill, which is further enhanced by GH (Shrimpton and McCormick, 1998, McCormick, 2001). Thus TH indirectly supports cortisol's ability to increase NKA activity (McCormick, 2001, Shrimpton and McCormick, 1998). In addition, prolactin (PRL) is considered the FW adaption hormone as it is antagonistic to GH and a reduction in PRL levels is seen in smolting salmon (fig.1) (Prunet et al., 1989), but there is still some uncertainty about the precise effect of PRL on CC and osmoregulation as inconsistent results of PRL exposure are reported (Manzon, 2002).

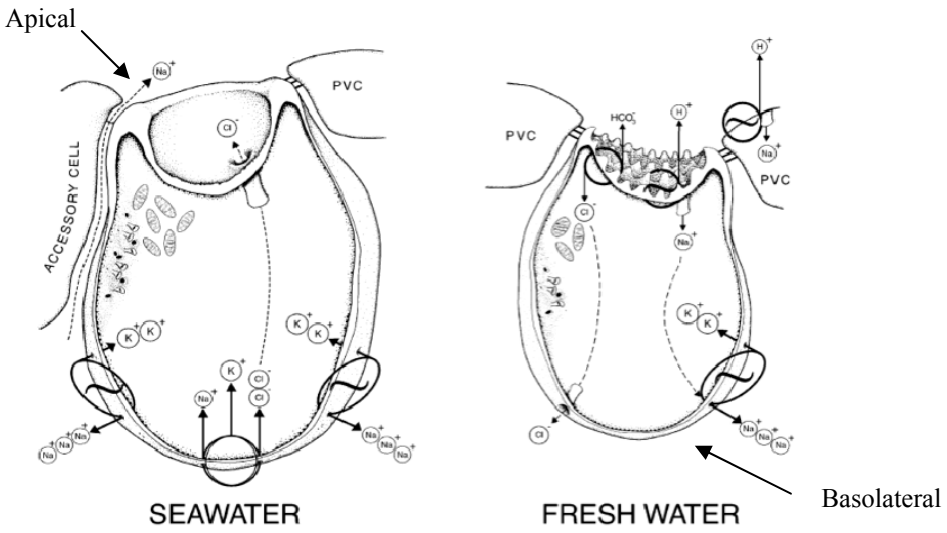

Figure 3: Seawater and freshwater chloride cells. The general morphology of SW CC and FW CC differ in several ways. The SW CC, unlike FW CC, form shallow tight junctions with accessory cells, are larger in size, have a higher NKA activity and a smooth apical pit (Evans et al., 2005). Illustrations modified from McCormick et al 2001.

NKA is, as mentioned, present in the basolateral membrane of both FWCC and SWCC, but in greater number in the latter (Evans et al., 2005). The functional enzyme has two essential subunits, α and β, and a FYXD protein (sometimes referred to as the γ subunit) (Skou and Esmann, 1992, Blanco and Mercer, 1998, Kaplan, 2002, Hirose et al., 2003, Geering, 1990, Geering, 2005, Tipsmark et al., 2008). The α subunit is the catalytic component and the binding site for Na^+, K^+, ouabain and ATP, while the β subunit is found to stabilize and support the correct folding of the α subunit and regulate the molecules affinity for Na^+ and K^+ (Blanco and Mercer, 1998, Richards et al., 2003, Mobasheri et al., 2000, Skou and Esmann, 1992, Kaplan, 2002). NKA hydrolyzes one molecule of ATP to ADP to exchange three intracellular Na^+ ions with two extracellular K^+ ions and the electrochemical gradient NKA maintain drives other Na^+ coupled transporters such as ion channels, co-transporters and counter transporters (fig. 3) (Blanco and Mercer, 1998, Mobasheri et al., 2000). Four different isoforms of the α (α1 - α4) and β (β1 - β4) subunit have been found in mammals (Blanco and Mercer, 1998) while five different isoforms of NKA (α1a, α1b, α1c, α2, α3) have been found in salmonids (Richards et al., 2003, Gharbi et al., 2005) and all except α2 have been found in Atlantic salmon gill tissue (Nilsen et al., 2007).

Richards et al (2003) discovered that the expression of NKA α1a and α1b isoforms change during adaption to SW and FW in rainbow trout. The level of α1a mRNA

decreased when the fish was transferred to SW, whereas the amount of α1b mRNA increased during SW transfer (Richards et al., 2003). The same has been shown for mRNA (Nilsen et al., 2007, Mackie et al., 2005, Madsen et al., 2008) and protein (McCormick et al., 2009) abundance in Atlantic salmon. Further, Gill NKAα1b mRNA levels increased in the early stages of smolting and remained high after SW transfer, while the levels of NKAα1a decreased during smoltification (Nilsen et al., 2007). The overall increase in NKA activity during smolting may therefore be the result of an increase in NKAα1b abundance (Nilsen et al., 2007). This change in isoforms indicates the presence of distinct FW and SW isoforms of NKA, which may play different roles in salinity acclimation in salmonids (Richards et al., 2003, McCormick et al., 2009). Although several studies have looked at NKA isoform change in relation to smoltification and SW transfer (Mackie et al., 2005, Madsen et al., 1995, Nilsen et al., 2007, Madsen et al., 2008, Richards et al., 2003), there is very little research done on isoform change during sexual maturation and FW adaption in salmonids (Shrimpton et al., 2005). What is known is that wild caught homing Pacific salmon (*Oncorhynchus spp.*) show a reduction in NKA activity even when kept in SW and fail to survive for more than a week in SW while they adapt perfectly to FW (Hirano et al., 1990, Uchida et al., 1997, Shrimpton et al., 2005). Additionally, decrease in NKAα1a were seen in salmon migrating upstream, which corresponded with lowered level of NKA activity (Shrimpton et al., 2005).

1.7 Motivation

Based on the findings of Shrimpton et al. (2005) Uchida et al. (Uchida et al., 1997) and Hirano et al. (Hirano et al., 1990)it would be interesting to see if NKA activity and NKAα1b and NKAα1a isoforms change in a similar manner in maturing Atlantic salmon. If mature Atlantic salmon show sings of preparatory acclimation to FW while still in SW it might represent a fish welfare problem as the salmon is in danger of obtaining a fatally high plasma osmolality caused by reduced hypo-osmoregulating ability (Hirano et al., 1990)

Consequently, it is necessary to get information regarding changes in hypo-osmoregulatory ability during the maturation cycle, the timing of these alterations and the underlying endocrine and molecular mechanisms.

Scientists at The Institute of Marine Research discovered that a high water temperature (16°C) and continuous light induce a high incidence of male post-smolt maturation in Atlantic salmon, a few months after SW transfer (P.G Fjelldal and T Hansen, in prep). Using this method will allow us to study an "out of season entry" into puberty under highly controlled settings and test a model that will complete the whole lifecycle of the Atlantic salmon within a year.

1.8 Aim of the study

Atlantic salmon is a major aquaculture species and most stages of the lifecycle have been thoroughly researched. The endocrine, physiological and molecular changes during the transition from freshwater to seawater during smoltification are well understood (Mackie et al., 2005, Nilsen et al., 2003, Sardet et al., 1979, Stefansson et al., 1998, Stefansson et al., 2009, Stefansson et al., 2007, Thrush et al., 1994, Tipsmark et al., 2008, McCormick, 2001) thus creating a good base for studying similar changes during puberty and maturation.

This experiment was part of a larger study funded by the Norwegian Research Council where the main objective is to obtain more information concerning molecular and endocrine changes prior to and during early puberty in Atlantic salmon males and shed light on possible fish welfare problems associated with such. Hence, the hypothesis of this thesis is:

- There are changes in the expression level of NKAα1b and NKAα1a during the sexual maturation in male salmon.
- There are differences in expression level of NKAα1b and NKAα1a in mature males and immature fish (males and females).
- Salinity will affect NKAα1b and NKAα1a expression.

To test these hypotheses we will utilize well-established methods of molecular biology, including Real-Time qPCR using specific primers for NKAα1b and NKAα1a.

2. Material and Methods

2.1 Experimental design

The samplings took place at Matre Research facility (61° N), which is owned and run by the Institute of Marine Research, Bergen. The experiment started the 29th of September 2010 with 1600 Atlantic salmon pre-smolt distributed in 16 experimental tanks of 500 L with 100 fish in each tank. The fish were kept under continuous light (LL) in freshwater until November 5th, when an out of season smoltification regime was initiated, in which all tanks received 6 weeks of short day photoperiod (LD 12:12 09.00-21.00), followed by 4 weeks of LL. Studies show that this light regime will induce out of season smoltification and is utilized in commercial fish farming (Berge et al., 1995, Arnesen et al., 2003).

On January 10th, after the completion of smoltification, 8 tanks were moved back to LD 12:12 and 8 tanks were kept on LL. The tanks were supplied with either 35 ‰ seawater or freshwater, creating four experimental groups with four replicate tanks (fig. 4).

It is expected that LL and LD 12:12 photoperiod will induce high and low incidences of male post-smolt maturation, respectively, while females will remain immature and thus function as immature controls within each group.

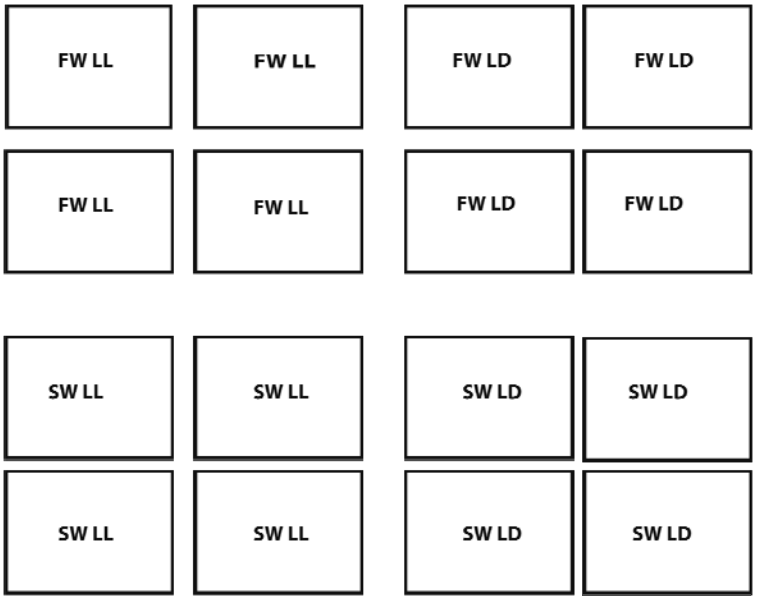

Figure 4: Experimental setup. Eight tanks were kept on LL and eight tanks were kept on LD 12:12 after completion of smoltification. These two groups will then be divided into seawater (SW) and freshwater (FW) groups, creating four groups with four replicates in each group.

2.2 Sampling

The experiment consisted of a total of eight samplings; four from the smolting phase and four from the maturing phase (table 1).

Table 1: Overview of sampling dates and number of fish sampled.

Sampling nr	Date	Fish sampled
1	29.09.10	20
2	03.11.10	20
3	08.12.10	20
4	05.01.11	20
5	26.01.11	80
6	16.02.11	80
7	09.03.11	80
8	29.03.11+ 30.03.11	160

For the first four samplings, 20 fish were collected each time. On January 10th, 2 ½ weeks prior to sampling 5, the fish were separated into the four treatment groups illustrated in figure 4. Twenty fish were sampled from each group, resulting in 80 fish in sampling 5, 6 and 7. The number of immature males was limited at the end of the experiment. Hence, it was decided to sample 160 fish for the 8th sampling to ensure a sufficient number of non-mature males.

The fish were collected using a dip net and transferred to 15 L buckets containing 1g of the anesthetic metomidate (Syndel, Victoria, BC, Canada). When the fish were sedated, the fork length and total body weight were recorded and 2.5 ml of blood was extracted from the caudal vein. The blood was centrifuged (3000 x g, 5 min, 4C°) and three plasma aliquots, A, B and C, were collected and put on dry ice. After blood sampling, the head was cut off, internal organs removed and gonad weight recorded.

Several tissue samples such as pituitary, kidney and intestine were taken as this experiment provided material for a number of studies, but only gill samples will be mentioned further.

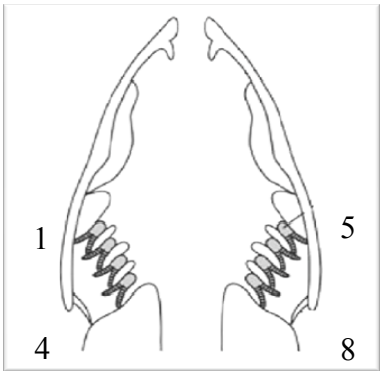

Figure 5: Gill arches. The image shows a salmon head from above, illustrating the numbering and placement of sampled gill arches. Gill arch 1-4 is on the left side, whilst gill arch 5-8 is on the right side. Illustration modified from: animaldiversity.ummz.umich.edu/site/resources/Grzimek_fish/structure_function/v04_id131_con_gillfun.jpg/view.html.

The gill arches were sampled using tweezers and a scissor, cutting the gill arches off one by one. The gill arches are numbered 1 to 8 (fig. 5), with 1 being the anterior left arch, 4 the posterior left arch, 5 the anterior right arch and 8 the posterior right arch. The cartilage was cut off from gill arches 1 and 7 and the soft tissue was put in tubes marked G1. Gill arch nr 4 and nr 8 were not used, since there was sufficient amount of tissue and the posterior gill arches were often damaged when the head was removed. Gill arch 2 was put in tube marked G2. Gill arch 6 was put in tube marked G3. Gill arch 3 and 5 was put in tubes marked G4. The gill size increased rapidly and in sampling 7 and 8 the content of tubes originally containing two gill aches was reduced to one gill arch. Only gill arch 1, 2, 5 and 6 were then used.

The tubes were marked with following labels, and with the correct number of the sampled fish:

- G1 was frozen on dry ice for Western blots
- G2 contained 4 % Paraformaldehyde (PF) in 0.1M Sørensen buffer for histology.
- G3 contained SEI buffer for NKA activity
- G4 contained RNA*later* (Ambion, Austin, TX, USA) for RNA isolation.

Plasma samples, G1 and G3 were kept on dry ice in a Styrofoam box during transportation back to UiB. These samples were stored on -80°C. Samples in RNAlater and PF were left in a fridge over night. Gill sample from G2 were fixated in 4% PF, embedded in Tissue-tek (Sakura Finetek, Alphen aan then Rijn, Nederland) and stored at -80°C.

Selected samples from tubes marked G4 were used for quantification of EF1a, NKAα1a and NKAα1b gene expression, while samples from G3 were analyzed for

NKA activity at Havbruksinstituttet AS, Bergen High Technology Center (http://www.hi.no/index.php).

2.3 Selecting samples

For gene expression twelve individuals, six immature females and six immature males were selected from sampling 1 to 4, giving 48 samples from the smolting phase. The individuals from sampling 5 to 8 were selected, post sampling, based on gender and gonado-somatix index (GSI) values (see below). Fish with a GIS above 0.05 were considered maturing, as elevated levels of 11-ketotestosterone have been observed in salmon reaching GSI of 0.05 and above in previous experiments (Andersson, Taranger, personal communication). The fish were then separated into three gender/maturity groups: maturing males, immature males and immature females. For each treatment group, six fish were selected from each gender/maturity group, if there were enough representatives. This gave 223 samples from sampling 5 to 8.

2.4 Condition factor and sexual maturation

The condition factor was calculated to using equation 1.

Equation 1: Condition factor

$$CF = \frac{\text{Total body weight (g) x 100}}{(\text{Fork length (cm)})^3}$$

To access the degree of maturation was the gonado-somatic index (GSI) was calculated using equation 2.

Equation 2: GSI

$$GSI = \frac{\text{Gonad weight (g) x100}}{\text{Total body weight (g)}}$$

2.5 RNA isolation in gill tissue

Gill tissue from sample tubes marked G4 was used to extract mRNA according to the following procedure (Chomczynski, 1993). A standardized piece of gill tissue of approximately 80 mg were put in pre marked Fast-Prep vials containing 1 ml of TRI-reagent (Sigma-Aldrich, St. Louis, MO, USA) 0.6 mg of ceramic beads and kept on ice for 5 min, before they were put in the Fast-Prep-120 (Thermo scientific, Waltham, MA, USA) for 20 sec on speed 4. The homogenized tissue was kept on room

temperature for 5 min before 200 µl of chloroform was added. The tubes were vortexed for 1 min and put in a pre-cooled (4°C) 5415R centrifuge (Eppendorf, Hamburg, Germany) for 15 min at 12000 x g.

The supernatant containing total RNA was transferred to new pre marked 1.5 ml tubes and 500 µl isopropanol were added. The tubes were inverted five times each and left at room temperature for 10 min, before centrifugation for 10 min at 12000 x g at 4°C The supernatant was removed and the remaining pellet was washed with 500 µl 80 % ethanol and centrifuged for 5 min at 7600 x g. The supernatant was decanted, the samples flash spun and the last drop of ethanol removed. The pellet was air dried for 5-10 min and reconstituted in 100 µl sterile nuclease free water. To ensure that the pellet was completely dissolved, the tubes were heated up to 55-60°C on a hot plate for 2-3 min and vortexed.

Total RNA quantity and purity was determined using NanoDrop-1000 spectrophotometer (Thermo Scientific, NC, USA). Overall, the purity of the total RNA samples was satisfactory, with 260/280 absorbance ratio being 1.8 or higher. However, the 260/230 ratio indicated small residual amounts of organic compounds in the sample (See section 5.2 for extended details). Total RNA in samples from sampling 1 – 4 were precipitated by adding 10 µl 3M Sodium Acetate, (NaAc, pH 5.2) and 250 µl ice cold 100% ethanol, as they were to be stored at -80°C for more than two weeks. Prior to cDNA synthesis, the precipitated samples were collected from -80°C and immediately centrifuged for 30 min, 12000 x g at 4°C. The supernatant was decanted and RNA dissolved and quantified as described above.

2.6 RNA integrity

The integrity of twelve isolated and precipitated RNA samples was treated with Agilent RNA6000 Nano reagents and measured with Agilent 2100 bioanalyzer (Agilent technologies, Santa Clara, CA, USA), using the Agilent RNA Nano protocol according to the manufacturer's instructions.

1 µl of Agilent RNA6000 Nano dye concentrate was added to an aliquot containing 65 µl of Agilent RNA6000 Nano filtered gel matrix (gel spun through a filtered tube) and the tube was vortexed and centrifuged at room temperature for 10 min at 13000 x g (Eppendorf, Hamburg, Germany). 9 µl of the Agilent RNA6000 Nano gel-dye

solution was added to one well, marked G on the LabChip (Caliper Life Sciences, Hopkinton, MA, USA).

The LabChip was then placed on the Agilent 2100 bioanalyzer chip priming station (Agilent technologies, Santa Clara, CA, USA) and a permanently connected syringe was placed above the LabChip well to disperse the liquid by applying pressure. 9 µl of gel-dye mixture was then added to two other marked wells. 5 µl of Agilent RNA 6000 Nano marker was applied to all wells without gel-dye. The Agilent RNA 6000 ladder consisting of six RNA transcripts with lengths of 0.2, 0.5, 1.0, 2.0, 4.0 and 6.0 kb, was collected from the freezer and heated to 70°C for 2 min along with the samples. 1 µl of ladder was added to the ladder well and 1 µl of each sample was added to their respective wells. The LabChip was then vortexed for 60 sec at 24000 rpm in an IKA vortex (IKA, Staufen, Germany) and placed in the Agilent 2100 Bioanalyzer. Results were obtained using the 2100 expert software (Agilent technologies, Santa Clara, CA, USA) and the RNA integrity number (RIN) noted. The RIN values showed excellent RNA integrity in all samples (Bustin and Nolan, 2004b).

2.7 DNase treatment

The samples were treated with RQ1 RNase free DNase (Promega Cat. No M6101) to remove traces of DNA. In a total reaction volume of 10 µl, 6.5 µl of total RNA (5 µg RNA), 2.5 µl RQ1 RNase-free DNase and 1 µl reaction buffer was used for the DNase treatment (table 2) per sample. The samples were then incubated at 37°C for 30 min in a PCR machine (Applied Biosystems, Foster city, CA, USA) before the DNase reaction were inactivated by adding 1 µl of DNase stop solution followed by incubation of samples at 65°C for 10 min. The RNA was then used for cDNA synthesis.

Table 2: Components of master mix used in DNase reaction (Promega Cat. No. M6101)

Component	Volume per sample
10x reaction buffer	1µl
RQ1 RNase free DNase 1	2.5 µl
RNA templat	X µl (5 µg RNA)
RNase free H_2O to total Volume 10 µl	
Sum	10 µl

2.8 cDNA synthesis

cDNA synthesis was performed using the Reverse Transcription Core kit (EUROGENTEC RT-RTCK-05, Liege, Belgium) following the manufactures instructions.

To synthesize cDNA, 23.8 µl of master mix and 1.2 µl DNase treated RNA (500 ng) were added to each PCR tube (table 3). The tubes were incubated (PCR 2700, Applied biosystems, Foster City, CA, USA) for 10 min at 25°C, then 30 min at 48°C, before 5 min at 95°C. The cDNA were then stored at -20°C.

Table 3: cDNA syntheses, master mix (Eurogentec Cat. No. RT-RTCK-05, Liege, Belgium)

Components	Volume per sample	Final concentration	Volume for 22 samples
10x Reaction buffer	3 µl	1x	66 µl
25 mM $MgCl_2$ solution	5 µl	5 mM	110 µl
2.5 mM dNTP solution	5 µl	500µM each dNTP	110 µl
Random nonamer	1.5 µl	2.5 µM	33 µl
RNase inhibitor	0.6 µl	0.4 U/µl	13.2 µl
H_2O	12.95 µl	384.9 µl	284.9 µl
Euroscript Reverse transcriptase	0.75 µl	1.25 U/µl	16.5 µl
RNA template 5 µg	1.2 µl		
Total volume	30 µl		

2.9 Real time quantitative PCR: quantification of gene expression

Real-time quantitative PCR (qPCR) was performed to determine the relative expression of NKAα1a and NKAα1b levels in the gill tissue, using the Chromo4 Continuous Fluorescence Detector (Bio-Rad, CA, USA) and MJ Opticon Monitor Analysis Software Platform (version 3.1, Bio-Rad).

2.0 µl of cDNA from the 48 samples obtained from sampling 1 to 4 was collected in one tube to get one sample of stock cDNA to be used for dilution series. 50 µl of stock cDNA was added to 200 µl of nuclease free water. Then, 40 µl of the 1:5 diluted cDNA was added to 360 µl of water, giving a dilution of 1:50. This was repeated with the 1:50 dilution and then with the 1:500 giving a ten-fold dilution series (fig. 7).

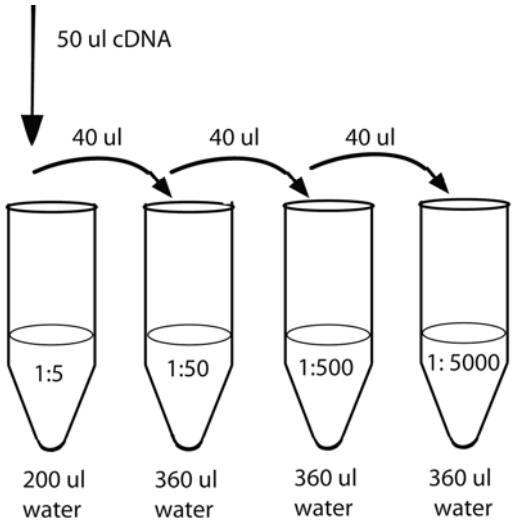

Figure 7: The figure illustrates how a ten-fold dilution series of cDNA was generated.

The qPCR reactions were performed in a total volume of 25 µl, with 12.5 µl SYBR green (Applied Biosystems, Foster city, CA, USA), 0.5 µl of 10µM specific forward and reverse primers (table 4), 6.5 µl of H_2O and 5 µl of cDNA template diluted 1:20.

Table 4: Primer sequences. The specific primers used for the RT-qPCR and their respective nucleic acids sequence.

Gene	Primer sequences	Gene Bank Acc. No
EF1α	5'- CACACGGCCCACAGGTACA-3' R 5'- CCCCTCCAGGACGTTTACAAA-3' F	AF321836
NKAα1b	5'-TGCAGCTGAGTGCACCAT-3'R 5'- GCTACATCTCAACCAACAACA-3' F	CK879688
NKAα1a	5'- CCAAAGGCAAATGAGTTTATATCAT-3' R 5'- CCAGGATCACTCAATGTCAT-3'F	CK878443

Each 96-welled plate (Bio-Rad, CA, USA) contained two Non Template Control (NTC) samples, followed by triplicates of the dilution series and duplicates of the actual samples. The thermal cycling protocol consisted of 10 min at 95 °C, followed by 45 cycles at 95 °C for 15 seconds and 60 °C for 1 min. All in all, 271 samples were analyzed for each gene, giving 6.5 plates per gene. A threshold of 0.012 was set manually for EF1α, and at 0.013 for NKAα1a and NKAα1b. The threshold was within the exponential phase and above the background noise for all assays (Bustin, 2000).

The obtained Ct values were imported to Microsoft Excel® and the mean values of the sample duplicates were used for quantification of gene expression. The amplification efficiency (E) was determined using the slope of the regression line generated by the log cDNA input (0.5 μg RNA template) versus Ct values from cDNA dilution series. The regression line and slope for each plate was calculated using Sigmaplot version 12 (Systat Software, San Jose, CA, USA). To reduce the efficiency variation in EF1α were the average slope of all plates used for calculation the efficiency. The regression lines were used for calculating efficiency in NKAα1a and NKAα1b using the following equation (Pfaffl, 2004):

$$\mathbf{E = 10^{(-1/slope)}}$$

To calculate relative expression in NKA1b and NKA1a were the following equation used (Pfaffl, 2004):

$$\text{ratio} = \frac{(E_{ref})^{CP_{sample}}}{(E_{target})^{CP_{sample}}} \div \frac{(E_{ref})^{CP_{calibrator}}}{(E_{target})^{CP_{calibrator}}}$$

Where:

E_{target} = Efficiency of the target gene (NKAα1a or NKAα1b)

E_{ref} = Mean efficiency of the reference gene (EF1α)

CP sample = Mean Ct values from target gene of a specific individual

CP sample = Mean Ct values from reference gene of a specific individual

CP calibrator = Mean Ct value for NKAα1a or NKAα1b from the first sampling

2.10 Statistical analysis

All statistical analyzes were performed in Statistica version 10 (StatSoft, Inc, Tulsa, OK, USA). As the experiment consisted of two stages, the results and statistical analysis were divided in the same manner. A one-way ANOVA was used to test for differences in fork length, body weight, GSI, condition factor and gill NKA activity between tanks in each sampling to eliminate differences between tanks. A Dunnett's post hoc test was performed if significant differences were picked up by the one-way ANOVA.

Smolting stage:

A factorial ANOVA was performed to test differences in fork length, body weight, condition factor, GSI, NKA activity, NKAα1a and NKAα1b mRNA expression between genders and sampling. Gender and samplings was used as predictor variables and all the variables mentioned above were used as response variables. In case of significant ANOVAs, A Newman-Keuls post-hoc test was applied to reveal where the significant differences were. Samples displaying values that were more than 2 times the standard deviation (2 S.D.) of the mean were considered outliers and excluded from the dataset (Zar, 1996). The data was tested for homogeneity of variances and normality of distributions using Levene's F-test and Normal probability plot of residuals with a Shapiro-Wilk test, respectively. When necessary, data were log transformed to better fit the assumptions of ANOVA.

Maturation stage:

A factorial ANOVA was performed to test differences in fork length, body weight, condition factor, NKA activity, NKAα1a and NKAα1b mRNA expression between mature males (MM) and immature females (NF) among each sampling, within each photoperiod/salinity group. The response variables were the same as in the smolting stage and maturation group and samplings was used as predictor variables. When finding differences in GSI between treatments groups were only data from MM used and treatment was used as a predictor variable instead of gender. Very few immature males (NM) were found after sampling 5, as almost all males became mature. For this reason, the NM group is not used for statistical analyzes. However the few NM are included in the graphical presentation of the results. Just as for the smolting data, samples displaying values that were more than 2 times the standard deviation (2 S.D.) of the mean was considered outliers and excluded from the dataset. The data was tested for homogeneity of variances and normality of distributions using Levene's F-test and Normal probability plot of residuals, respectively. When necessary, data were log transformed to better fit the assumptions of ANOVA.

3. Results

3.1 Smoltification stage

The results were divided into two categories; the smolting stage (sampling 1 to 4) and the maturation stage (sampling 5 to 8). The 16 tanks received the same treatment during the smoltification stage and no significant difference in fork length, body weight, condition factor and GSI was found between tanks in each sampling. Slightly elevated levels of NKA activity were seen in one tank in sampling 1 and one in sampling 2. A one-way ANOVA found the two tanks to be significantly different (ANOVA $p<0.05$) from the other tanks in the sampling (appendix III). Although a significant difference was found, this was only for one response variable and in only two tanks (appendix III). Consequently, all tanks were treated as one group for the smolting stage.

3.1.1 Fork length

A significant increase in fork length (cm) in males and females was observed during smoltification and a factorial ANOVA revealed significant (ANOVA $p<0,001$) differences between samplings (fig. 8, appendix III). The fork length had increased significantly in both males and females in relation to the first sampling, but the length in sampling 3 did not differ from sampling 4 in neither males nor females. There was no significant difference in length between genders (appendix III).

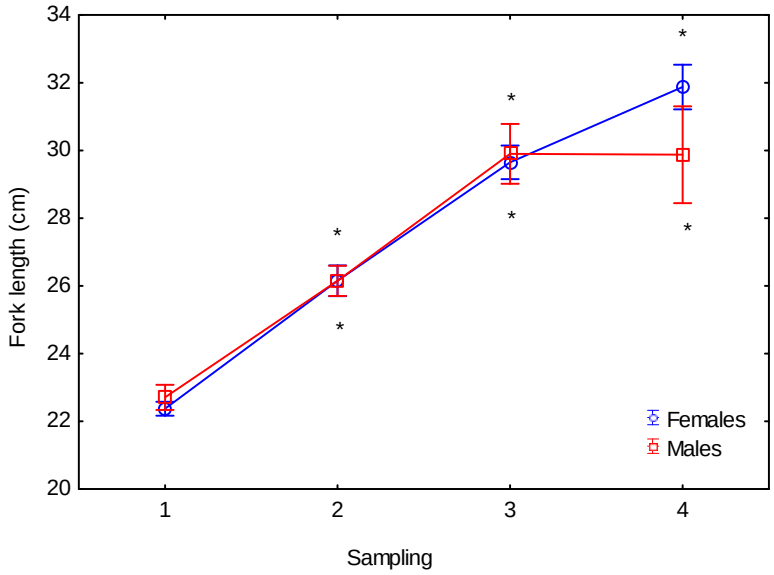

Figure 8: Fork length of Atlantic salmon males and females during smoltification stage. Asterisks (*) indicate significant differences in time within gender group in relation to the first sampling date. Data is presented as mean ± s.e.m. Females: n = 8 – 10 for each sampling, Males: n = 10 – 12 for each sampling.

3.1.2 Body weight

The body weight of both males and females increased significantly (ANOVA $p<0.001$) (fig.9, appendix III) throughout the smolting stage, as observed for fork length. The weight remained stable between sampling 3 and 4 and no significant difference was detected between the two samplings (appendix III).

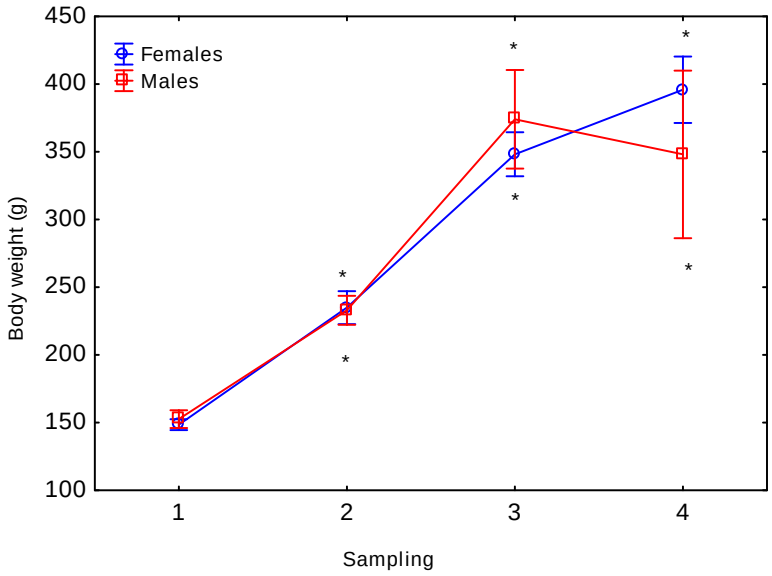

Figure 9: Body weight of male and female Atlantic salmon during smoltification. Asterisks (*) indicates difference in time within gender group in relation to first sampling. Data are presented as mean ± s.e.m. Females: n = 8 – 10 for each sampling, Males: n = 10 – 12 for each sampling.

3.1.3 Condition factor

The condition factor remained steady for the first three samplings but dropped significantly (ANOVA $p<0.001$) between sampling 3 and 4 (fig. 10, appendix III). However, both males and females follow the same pattern and no significant difference was observed between them (fig 10, appendix III).

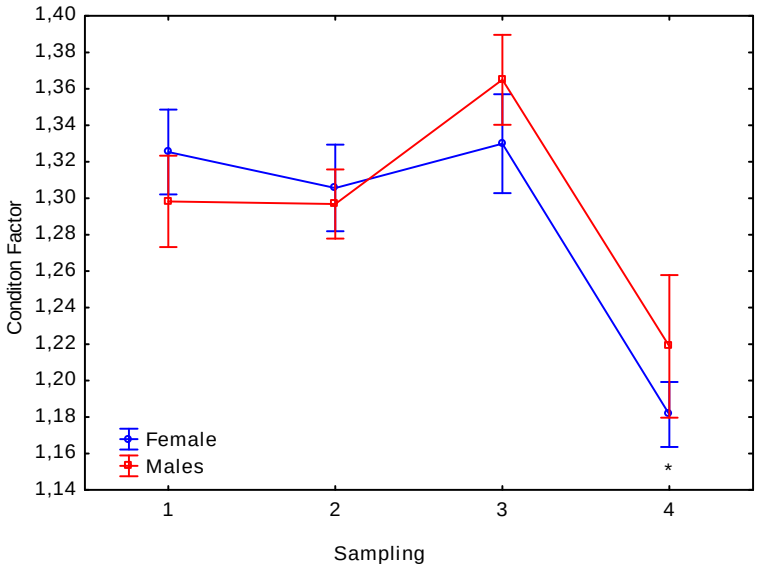

Figure 10: Condition factor of Atlantic salmon males and females during smoltification. No significant difference between males and females was found. Data is presented as mean ± s.e.m. Females: n = 8 – 10 for each sampling, Males: n = 10 – 12 for each sampling**.**

3.1.4 Gonadosomatic Index

The gonadosomatic index (GSI) remained stable in females and showed a slight increase in males throughout the smolting stage, and the GSI in males in sampling 3 and 4 was significantly higher compared to sampling 1 (fig. 11, AVOVA $p<0.05$) (appendix III). The factorial ANOVA revealed a significant difference (ANOVA $p<0.001$) between males and females, with females having a slightly higher GSI than males (appendix III).

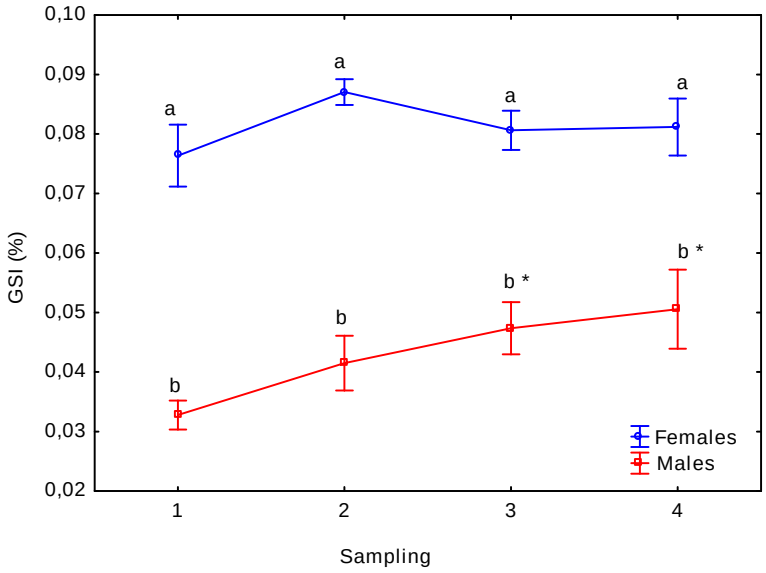

Figure 11: GSI (%) of Atlantic salmon males and females during smoltification. Asterisks (*) indicates difference in time within gender group in relation to the first sampling. Differences between gender groups are represented by letters. Data is presented as mean ± s.e.m. Females: n = 8 – 10 for each sampling, Males: n = 10 – 12 for each sampling.

3.1.5 Gill NKA activity

Gill NKA activity remained stable for the first two samplings and then dropped significantly (ANOVA $p<0.001$) in the third sampling (fig. 12, appendix III). The activity level increased significantly for sampling 4 in relation to sampling 3 (appendix III). No significant differences between males and females were found (appendix III).

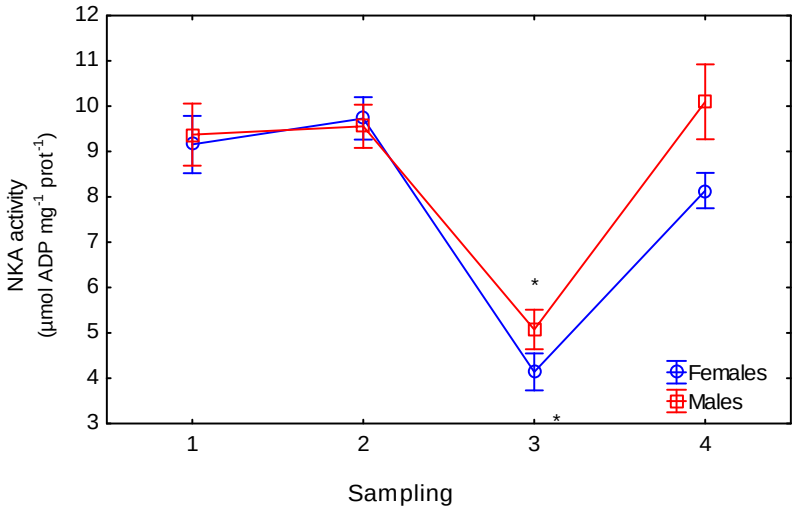

Figure 12: Gill NKA activity (µmol ADP mg^{-1} $prot^{-1}$) of Atlantic salmon males and females during smoltification. Asterisks (*) indicates difference in time within gender group in relation to the first sampling. Data is presented as mean ± s.e.m. Females: n = 8 – 10 for each sampling, Males: n = 10 – 12 for each sampling.

3.1.6 Gill NKAα1b gene expression

The relative NKAα1b gene expression level decreased in both males and females from sampling 1 to 3, but not significantly (fig. 13). A factorial ANOVA revealed a significant difference among samplings (ANOVA $p<0.05$, appendix III) and the post hoc test found that difference to be between females in the first sampling and between males in the 3rd sampling (appendix III). The NKAα1b gene expression in both genders seemed to reached the lowest point in sampling 3 and increased slightly for sampling 4 (fig. 13). No differences between genders were found (appendix III).

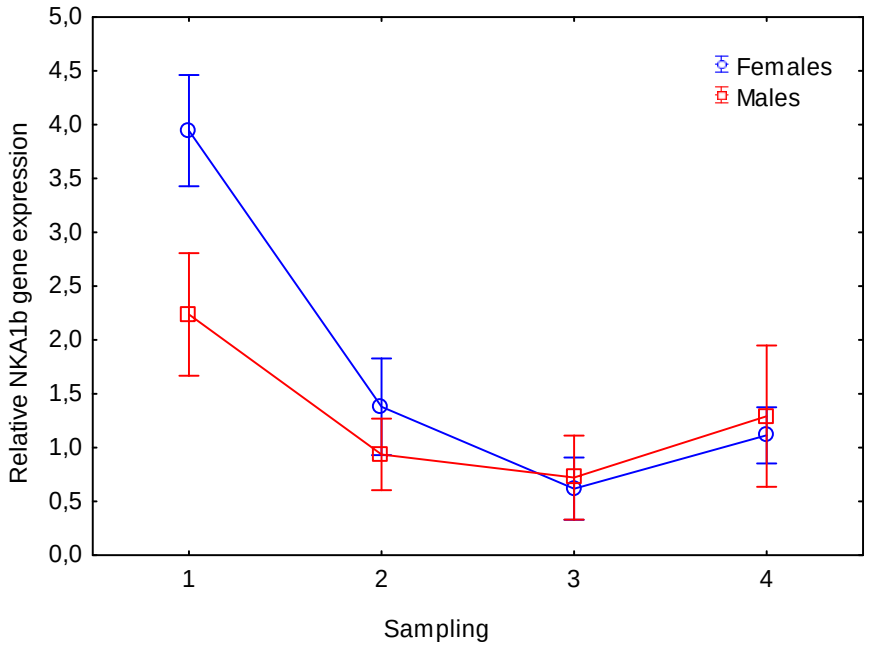

Figure 13: Gill NKAα1b gene expression of Atlantic salmon males and females during smoltification. Data is presented as mean ± s.e.m., Females: n = 6 for each sampling, Males: n = 6 for each sampling. No differences in relation to samplings 1 was found within each gender, but a difference was found between females in the 1st sampling and males in the 3rd sampling.

3.1.6 Gill NKAα1a gene expression

The expression of NKAα1a remained low throughout sampling 1 to 2 and increased significantly for males and females in sampling 3, before a decrease was observed at sampling 4 (fig. 14). A factorial ANOVA revealed significant differences between samplings (ANOVA $p<0.0001$) and the following post hoc test found the expression level of NKAα1a in males and females in sampling 3 to be significantly higher than in sampling 1 (fig 14, appendix III). No significant difference between genders was found (appendix III).

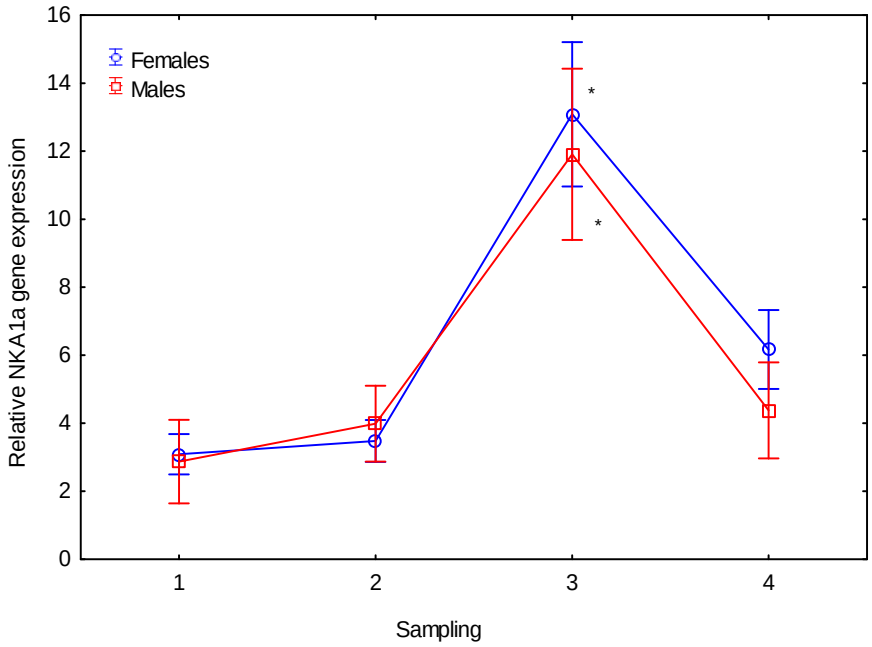

Figure 14: Gill NKAα1a gene expression of Atlantic salmon males and females during smoltification. Asterisks (*) indicates difference in time within gender group in relation to the first sampling. Data is presented as mean ± s.e.m. Females: n = 6 for each sampling, Males: n = 6 for each sampling.

3.2 Maturation stage

The results from the maturation stage are divided into the three maturation categories: immature females (NF), immature males (NM) and mature males (MM) and are presented for each salinity/photoperiod combination (treatment group). As mentioned in section 2.11, only NF and MM were used for statistical analyses, due to a low n for NM in several of the treatment groups.

3.2.1 Percentage of mature males

As the tanks contained both males and females and no selections was done during sampling, was the gender of the sampled fish was completely random and this sometimes gave a low number of one of the genders. Following is an overview of the number of males sampled in each treatment group in each sampling (table 5). In general, the LL groups have a higher percentage of mature males, but almost all groups show a maturation percentage above 40. In sampling 8 almost all sampled males were mature (table 5).

Table 5: Sampled males. The table shows the number of sampled males and the percentage of mature males within each group.

Sampling	Treatment	No. of males	No. of mature males	% Mature males
5	SWLL	5	5	100.0
5	SWLD	10	5	50.0
5	FWLL	10	9	90.0
5	FWLD	11	3	27.3
6	SWLL	10	9	90.0
6	SWLD	7	4	57.1
6	FWLL	9	4	44.4
6	FWLD	10	8	80.0
7	SWLL	11	11	100.0
7	SWLD	10	7	70.0
7	FWLL	6	3	50.0
7	FWLD	9	4	44.4
8	SWLL	17	15	88.2
8	SWLD	17	16	94.1
8	FWLL	18	17	94.4
8	FWLD	24	17	70.8

3.2.2 Fork length sampling 5-8

SWLL

The fork length increased significantly for both NF and MM in SWLL throughout the maturation stage (fig. 15). The length remained stable in both NM and MM and no major peaks or lows were observed (fig. 15). The factorial ANOVA found a significant difference between samplings (ANOVA $p<0.001$), but not between maturation groups (appendix III). Two NM were found in sampling 8 and they seemed to have the same length as NF and MM (fig. 15).

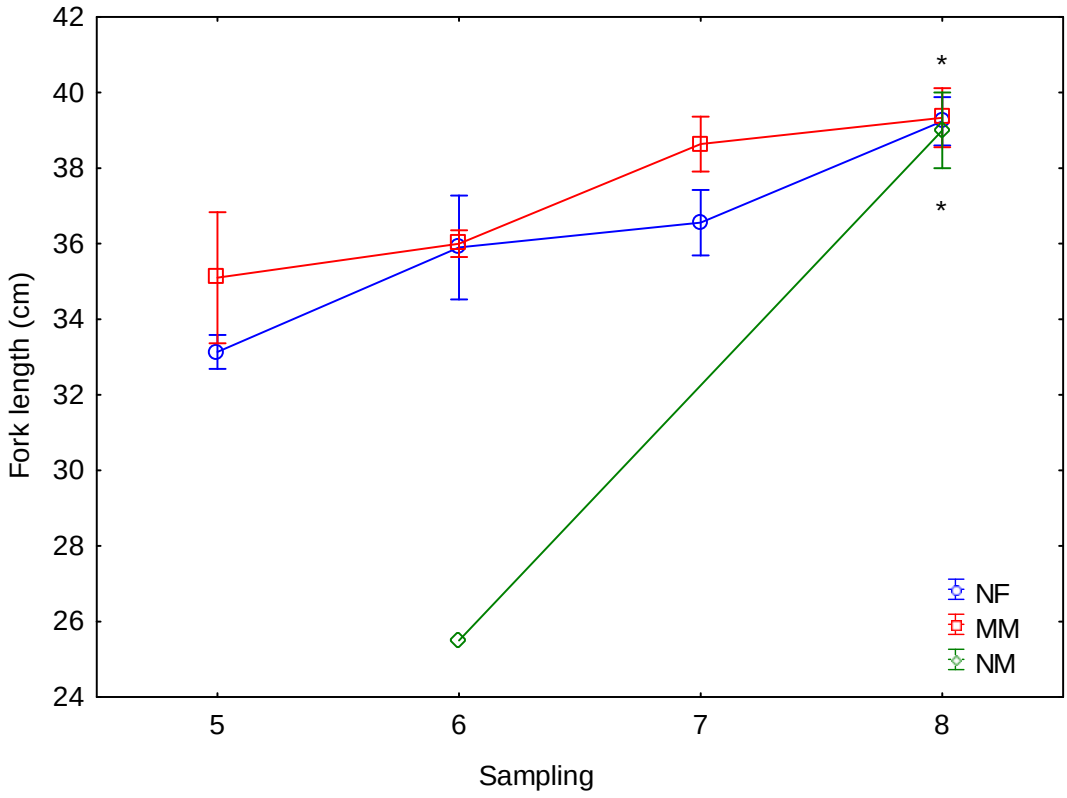

Figure 15: Fork length of Atlantic salmon SWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. No difference between NM and MM were found. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 23 in sampling 8. MM: n = 5 in sampling 5 and n = 9 in sampling 6, n = 11 in sampling 7 and 17 in sampling 8.

SWLD

The fork length of NF was significantly longer in sampling 7 and 8 compared to sampling 1 (ANOVA $p<0.0001$, fig. 16, appendix III). The length of MM increased significantly between sampling 5 and 6 and was significantly higher in sampling 6 compared to NF (ANOVA $p<0.0001$, appendix III, fig. 16). However, the length of MM did not differ from NF in sampling 7 and remained similar till sampling 8.

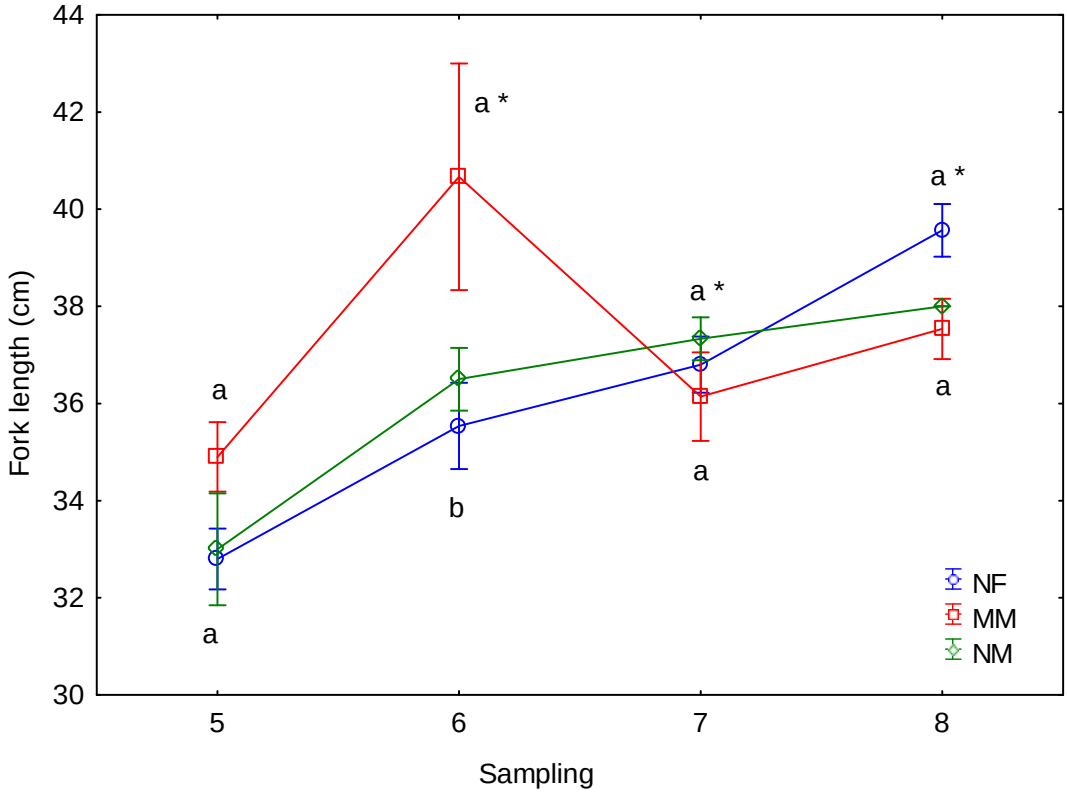

Figure 16: Fork length of Atlantic salmon SWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 23 in sampling 8. MM: n = 10 for sampling 5, n = 7 in sampling 6, n = 10 in sampling 7, n = 17 in sampling 8.

FWLL

The fork length increased significantly for MM and NF in FWLL throughout the experiment (fig. 17). NF and MM displayed similar growth curves, but MM was significantly longer in sampling 5 (fig. 17, appendix III). A factorial ANOVA revealed significant differences between samplings (ANOVA $p<0.0001$), NF and MM (ANOVA $p<0.05$) and in the interaction between samplings and MM and NF (ANOVA $p< 0.05$) (fig. 17, appendix III). The growth curves of NF and MM did not display any peaks or low points as seen in FWLD (fig. 16, 18).

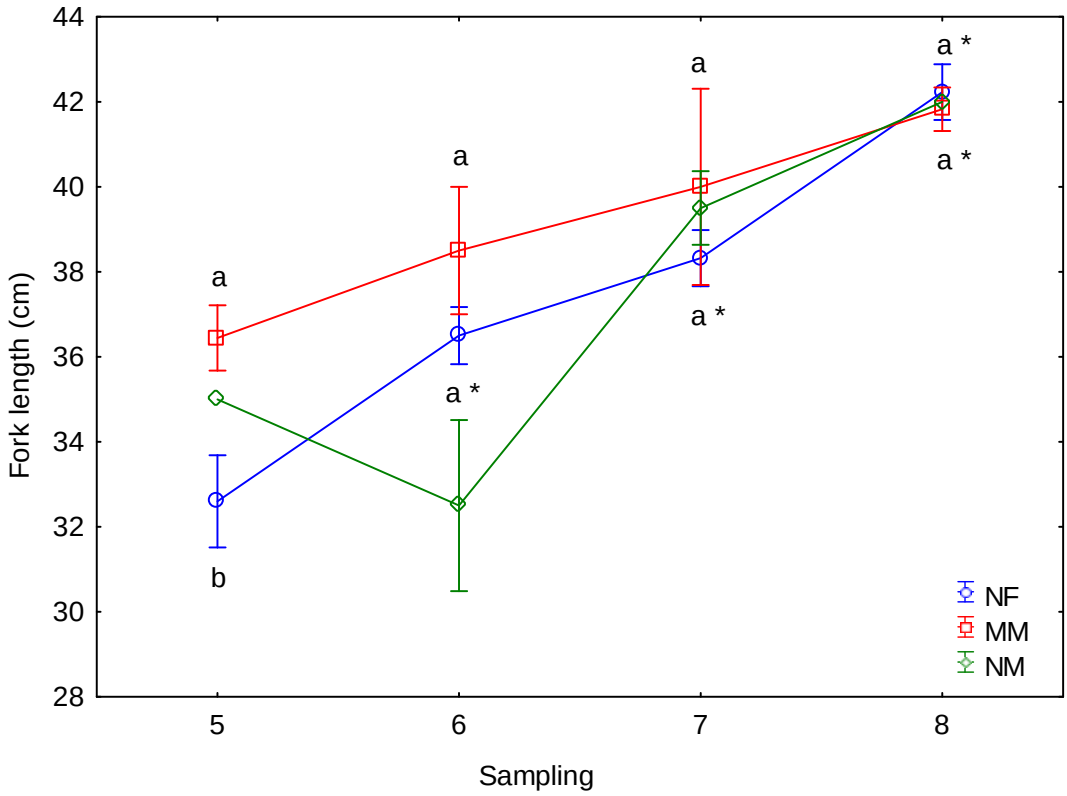

Figure 17: Fork length of Atlantic salmon in FWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 22 in sampling 8. MM: n = 10 for sampling 5, n = 9 in sampling 6, n = 6 in sampling 7, n = 18 in sampling 8.

FWLD

The fork length increased significantly for NF in FWLD throughout the study, but not for MM (fig. 18). The length increased significantly between sampling 5 and 6 for NF, but not for MM (fig. 18). The MM was significantly longer than NF in sampling 5 and since MM did not increase significantly between sampling 5 and 6, NF and MM had the same mean length inn sampling 6 (fig. 18).

The fork length decreased in all maturation groups in the seventh sampling (fig. 18). The curve of MM showed the same trend as NF and NM, but did not display as distinct peaks. A factorial ANOVA revealed differences between samplings (ANOVA $p<0.0001$), and in the interaction between samplings and NF and MM (ANOVA $p<0.0001$), but not between NF and MM alone (fig. 18, appendix III). NF is significantly longer than MM in sampling 8 (appendix III).

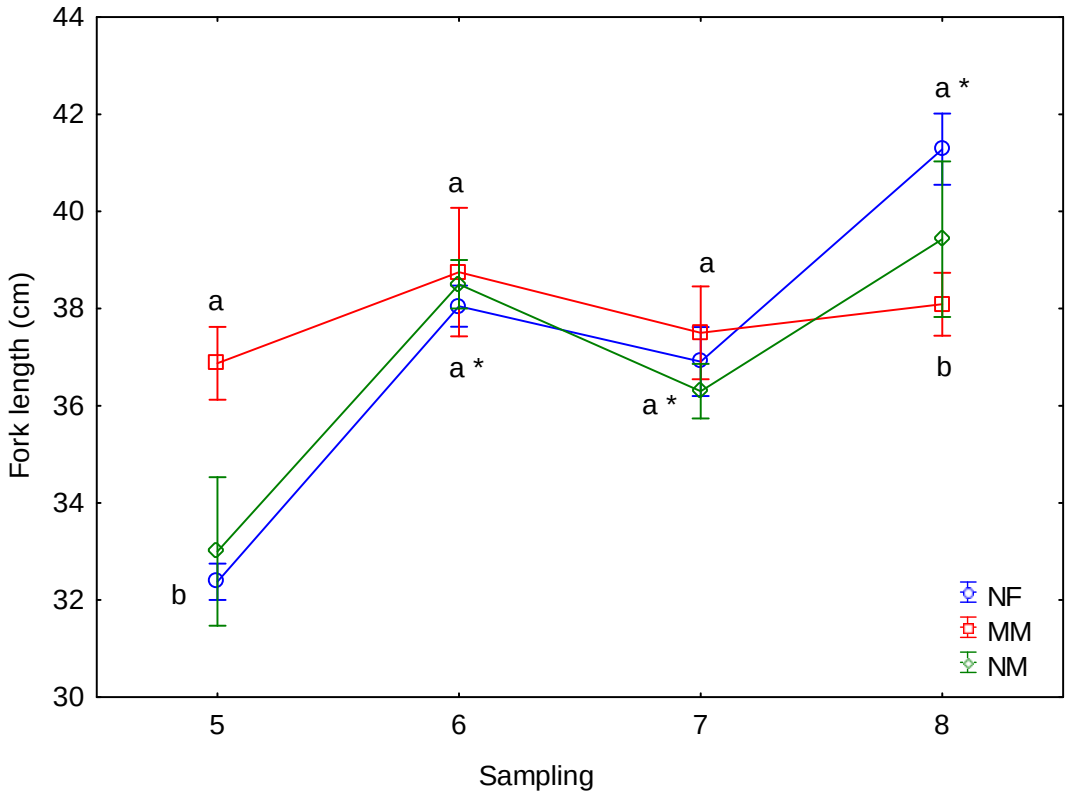

Figure 18: Fork length of Atlantic salmon in FWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-in sampling 8. MM: n = 11 for sampling 5, n = 10 in sampling 6, n = 9 in sampling 7, n = 24 in sampling 8.

3.2.3 Body weight sampling 5-8

SWLL

The weight increased significantly in both MM and NF through the maturation period (fig. 19, appendix III). Just as in fork length of MM in SWLL, there was an even increase in body weight, without any major peaks or lows.

The weight of NF increased steady throughout the maturation stage and NF was significantly heavier in sampling 8 than in sampling 5 (fig. 19, appendix III). A factorial ANOVA revealed a significant difference between samplings (ANOVA $p<0.001$) and between NF and MM (ANOVA $p<0.05$), but the following post hoc test showed no significant differences between MM and NF within specific samplings (fig. 19, appendix III).

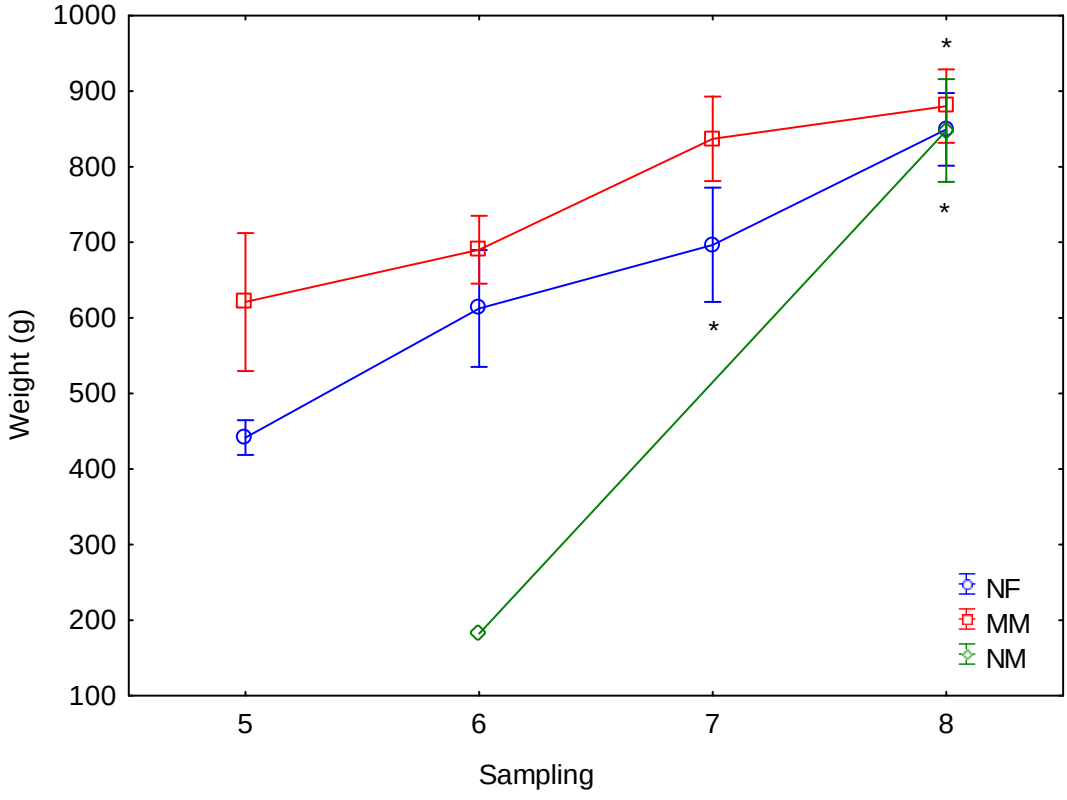

Figure 19: Body weight of Atlantic salmon in SWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8.MM: n = 5 in sampling 5 and n = 9 in sampling 6, n = 11 in sampling 7 and 17 in sampling 8.

SWLD

The weight curve of SWLD is similar to the fork length curve of SWLD, in which MM peaks at sampling 6 and was significantly heavier than NF (fig. 16 and 20). NF grows steady, and was significantly larger in sampling 8 than they were in sampling 5 (fig. 20, appendix III). The same can not be said for MM, which all in all did not increase significantly in weight. The factorial ANOVA found significant differences between samplings (ANOVA $p<0.0001$) and in the interaction between maturation groups and samplings as males and females differed in sampling 6 and 8 (ANOVA $p<0.0001$) (appendix III).

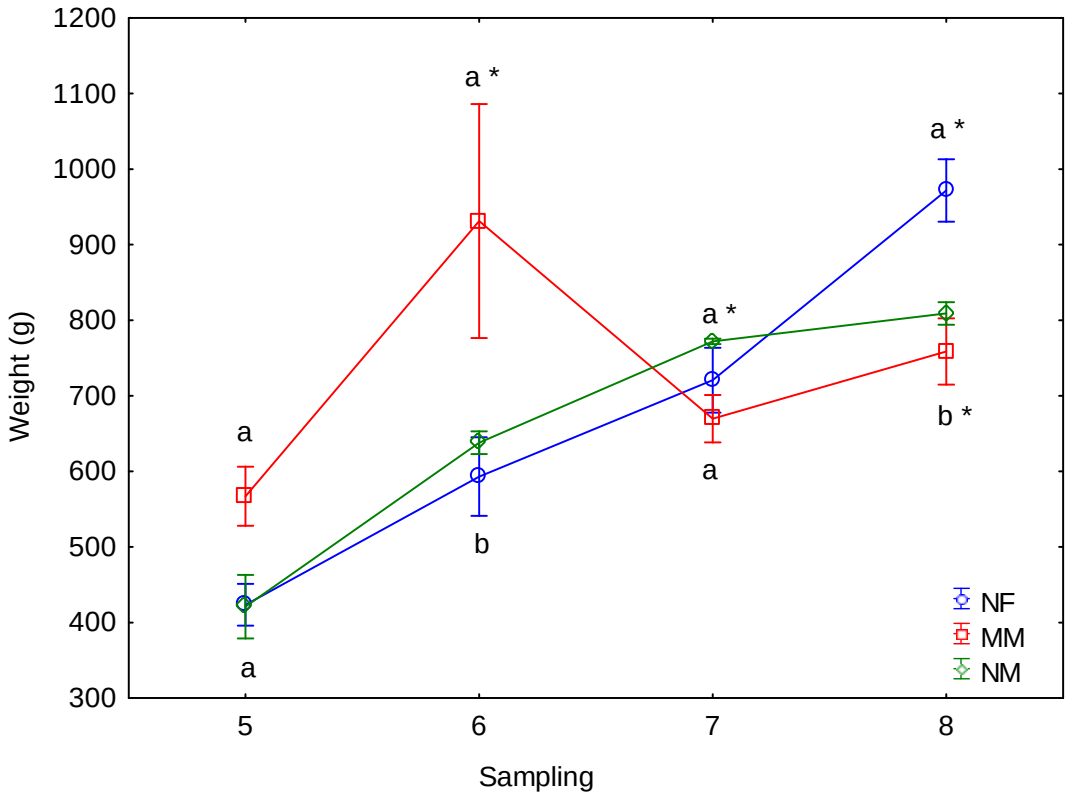

Figure 20: Body weight of Atlantic salmon in SWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 10 for sampling 5, n = 7 in sampling 6, n = 10 in sampling 7, n = 17 in sampling 8.

FWLL

Both NF and MM showed a significant increase in weight throughout the maturation stage and the factorial ANOVA revealed a significant difference in body weight between NF and MM in FWLL (ANOVA $p<0.001$, fig. 21, appendix III). MM was slightly higher than NF until sampling 8, where NF was equally heavy as MM. As with fork length in FWLL, there was no specific peaks or lows in the weight curve (fig. 21).

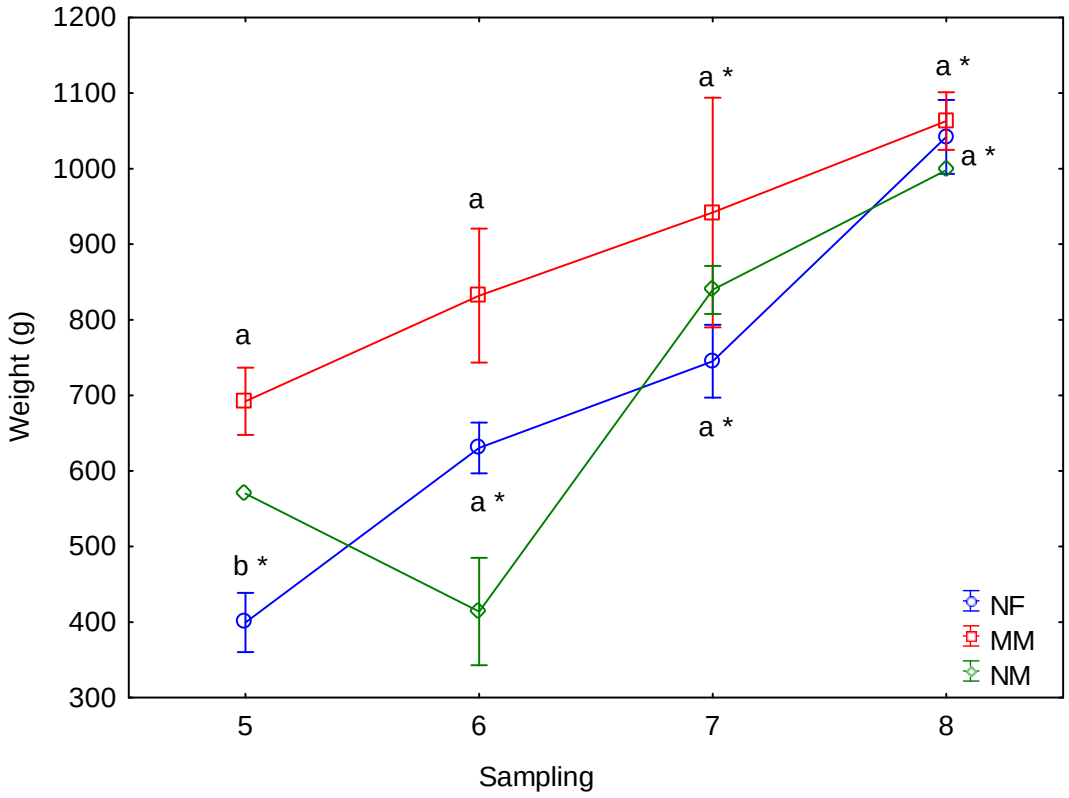

Figure 21: Body weight of Atlantic salmon in FWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 10 for sampling 5, n = 9 in sampling 6, n = 6 in sampling 7, n = 18 in sampling 8.

FWLD

Just as in SWLL and SWLD, the weight of MM was higher than the weight of NF in sampling 5 (fig 22). The weight of MM peaked slightly in sampling 6, but flattened out and ended up significantly lower than NF in sampling 8 (fig. 22, appendix III). A factional ANOVA revealed a significant difference between samplings (ANOVA $p < 0.0001$), but not between NF and MM, although the post hoc test found differences between NF and MM in sampling 8 (appendix III).

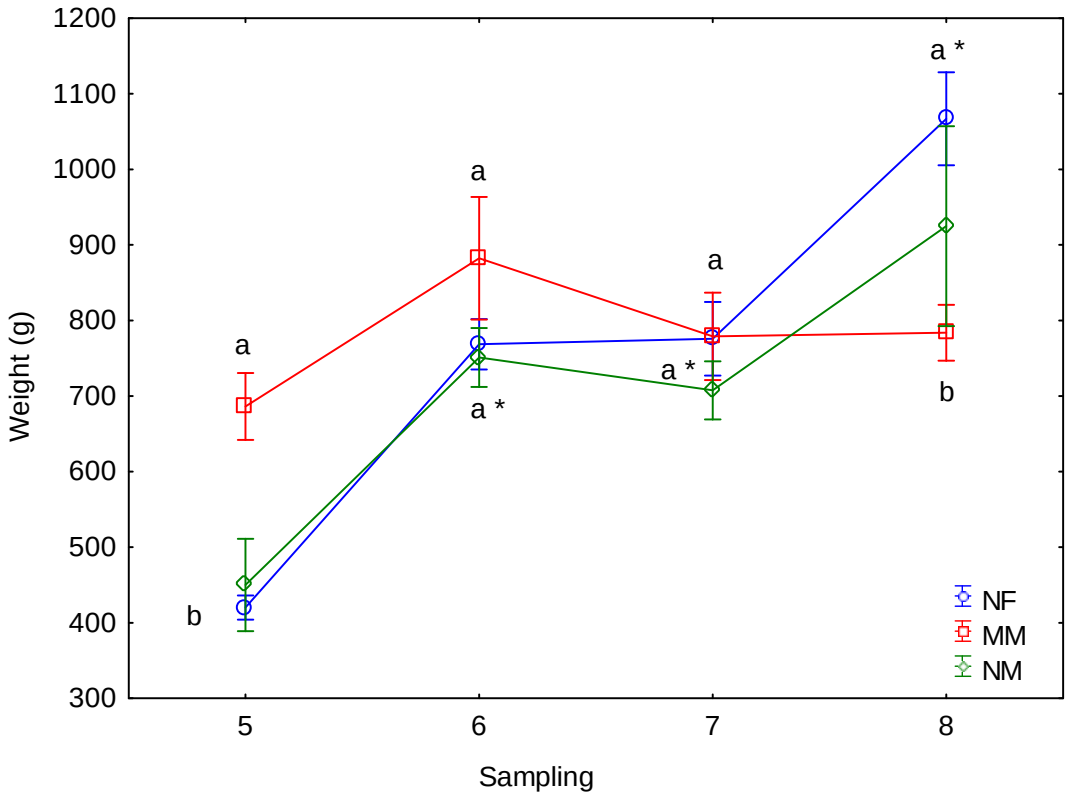

Figure 22: Body weight of Atlantic salmon in FWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 11 for sampling 5, n = 10 in sampling 6, n = 9 in sampling 7, n = 24 in sampling 8.

3.2.4 Gonadosomatic index sampling 5-8

As the mature and immature individuals are separated by a GSI of 0.5, all immature fish will have a GSI below 0.5 and the difference between NF and MM will always be significant. The GSI is therefore presented for MM, with all treatment groups in one graph (fig 23).

The GSI of SWLL and SWLD was significantly higher than FWLL in sampling 5, but the GSI of FWLD did not differ from any of the other treatment groups. In the following samplings it was the treatments with the same light regime that had the most similar development (fig. 23). The GSI peaked at sampling 6 for SWLD and FWLD, whereas the peak for SWLL and FWLL was observed sampling 7 (fig. 23). The GSI of SWLL and FWLL remained elevated after the peak, but the GSI of FWLD and SWLD decreased a great deal after peaking at sampling 6 (fig. 23). A factorial ANOVA showed significant differences between treatment groups (ANOVA $p<0.05$) and samplings (ANOVA $p<0.0001$) and in the interaction between them (ANOVA $p<0.0001$) (appendix III).

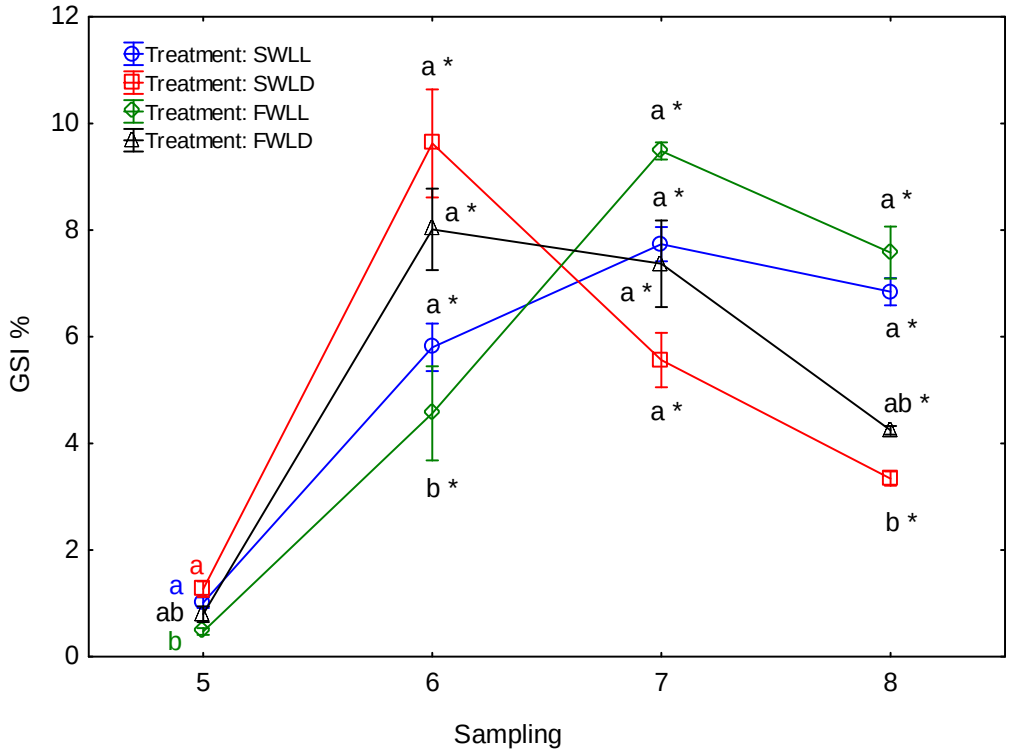

Figure 23: GSI of Atlantic salmon MM in all treatment groups during maturation. Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between treatments in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: for n see table 5.

3.2.5 Condition factor sampling 5-8

SWLL

The condition factor of MM was quite high in sampling 5 and remained stable throughout the experiment, with no significant changes (fig. 24) (appendix III). The condition factor of NF started out significantly lower, but increased until sampling 7, remaining stable (fig. 24). A factorial ANOVA showed significant differences between NF and MM (ANOVA $p<0.001$) and between samplings (ANOVA $p<0.01$) (fig. 24, appendix III).

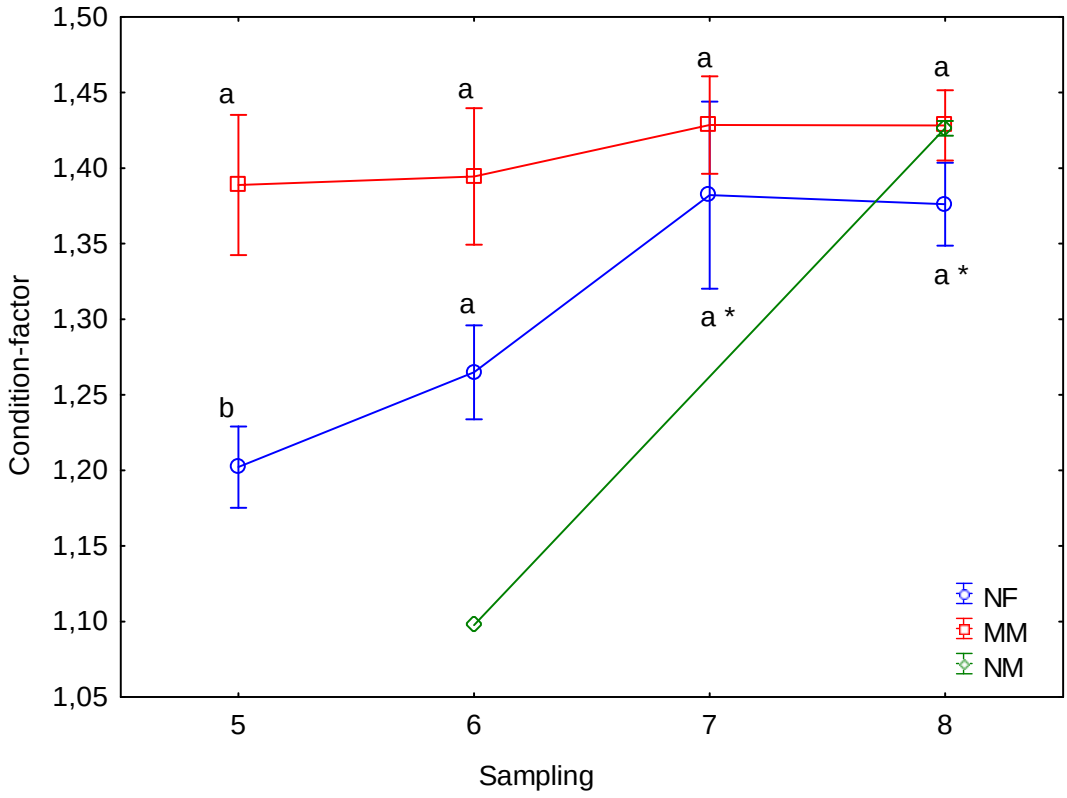

Figure 24: Condition factor of Atlantic salmon in SWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 5 in sampling 5 and n = 9 in sampling 6, n = 11 in sampling 7 and 17 in sampling 8.

SWLD

The condition factor was quite high for MM in this treatment group and remained relative stable throughout the experiment (fig. 25). However, there was a slight decline in sampling 8, causing MM to be significantly lower than NF in sampling 8 (fig. 25). The condition factor of NF was lower than MM until sampling 7, where NF went above MM. A factorial ANOVA revealed significant differences between samplings (ANOVA $p < 0.0001$), but not between MM and NF (fig. 25, appendix III).

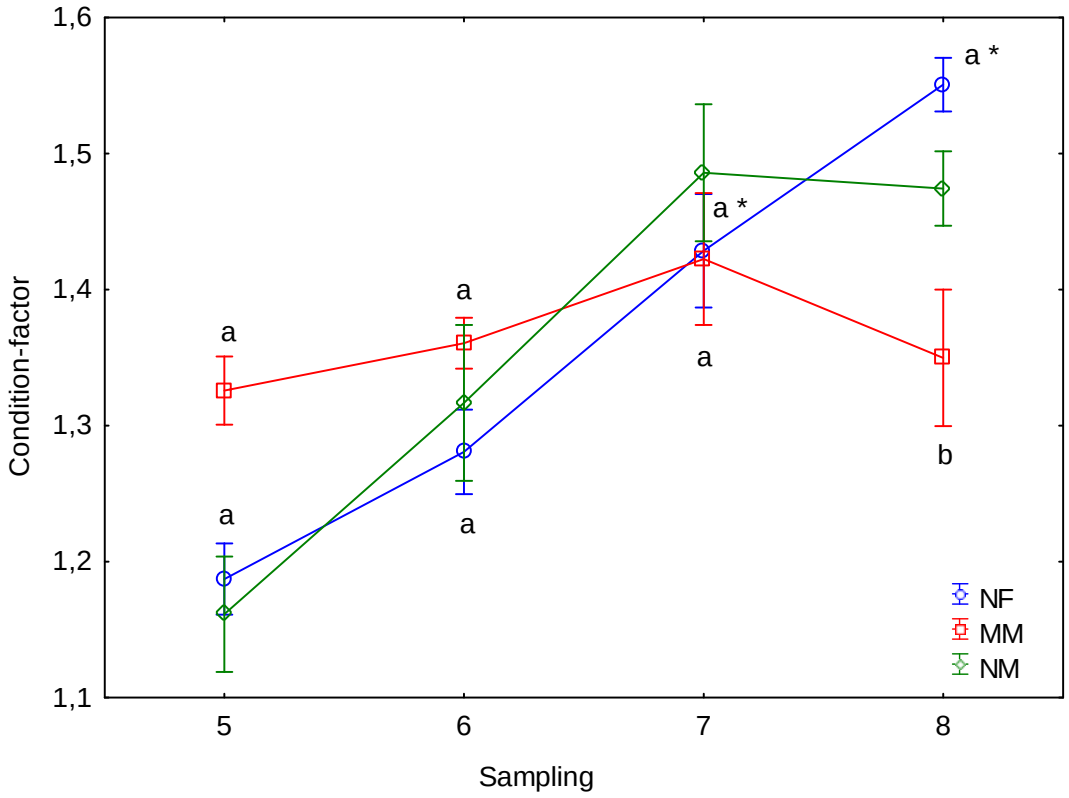

Figure 25: Condition factor of Atlantic salmon in SWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 10 for sampling 5, n = 7 in sampling 6, n = 10 in sampling 7, n = 17 in sampling 8.

FWLL

The distance between the condition factor in MM and NF in sampling 5 was greater here than in the other treatments and the gap remained large until sampling 8 (fig. 26). Unlike in treatment SWLD and FWLD, did the NF line never cross and go above the MM line (fig. 26). In addition, the pattern of the lines resembles the pattern seen in SWLL. A factorial ANOVA revealed significant differences between MM and NF (ANOVA $p<0.01$) and between samplings (AVOVA $p<0.001$) (appendix III)

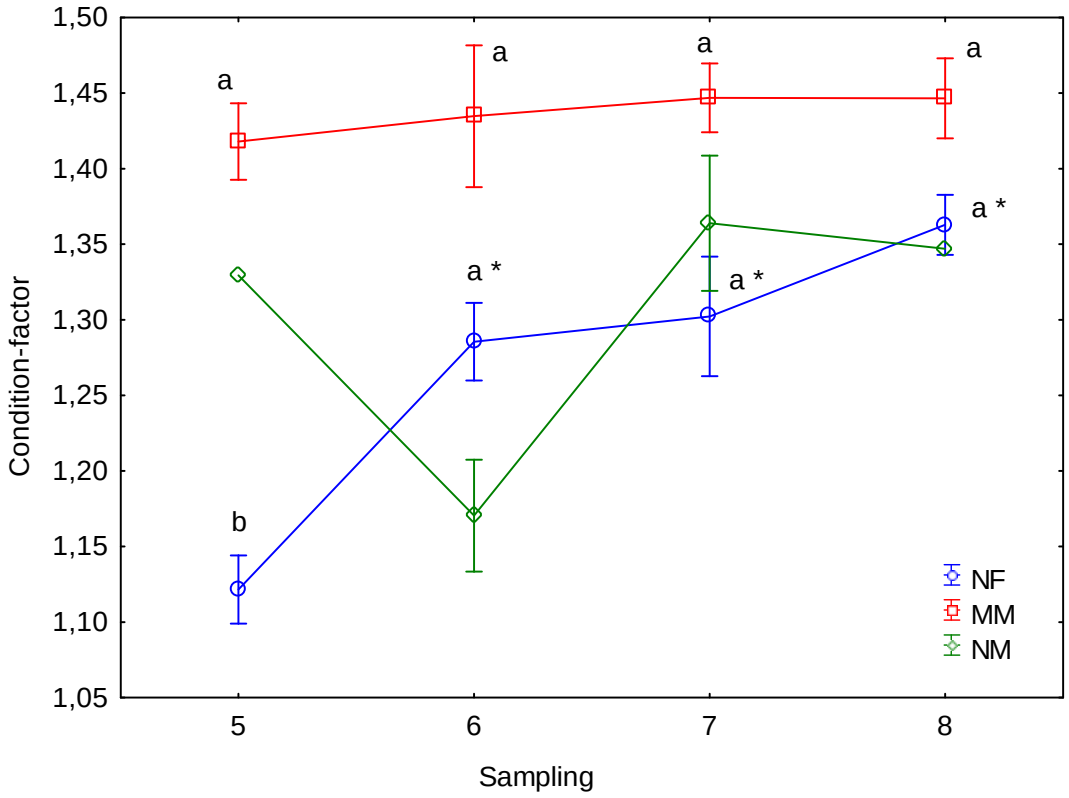

Figure 26: Condition factor of Atlantic salmon in FWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 10 for sampling 5, n = 9 in sampling 6, n = 6 in sampling 7, n = 18 in sampling 8.

FWLD

Once more, the condition factor of MM is above NF in sampling 5, but as seen in SWLL and SWLD, MM remained quite stable as NF increases (fig. 24, 25, 27). Nevertheless, there was a significant increase in condition factor for MM between sampling 5 and 6, not seen in the other treatments (fig, 27). A factorial ANOVA revealed no significant difference between MM and NF, but a difference between samplings (ANOVA $p<0.00001$, appendix III).

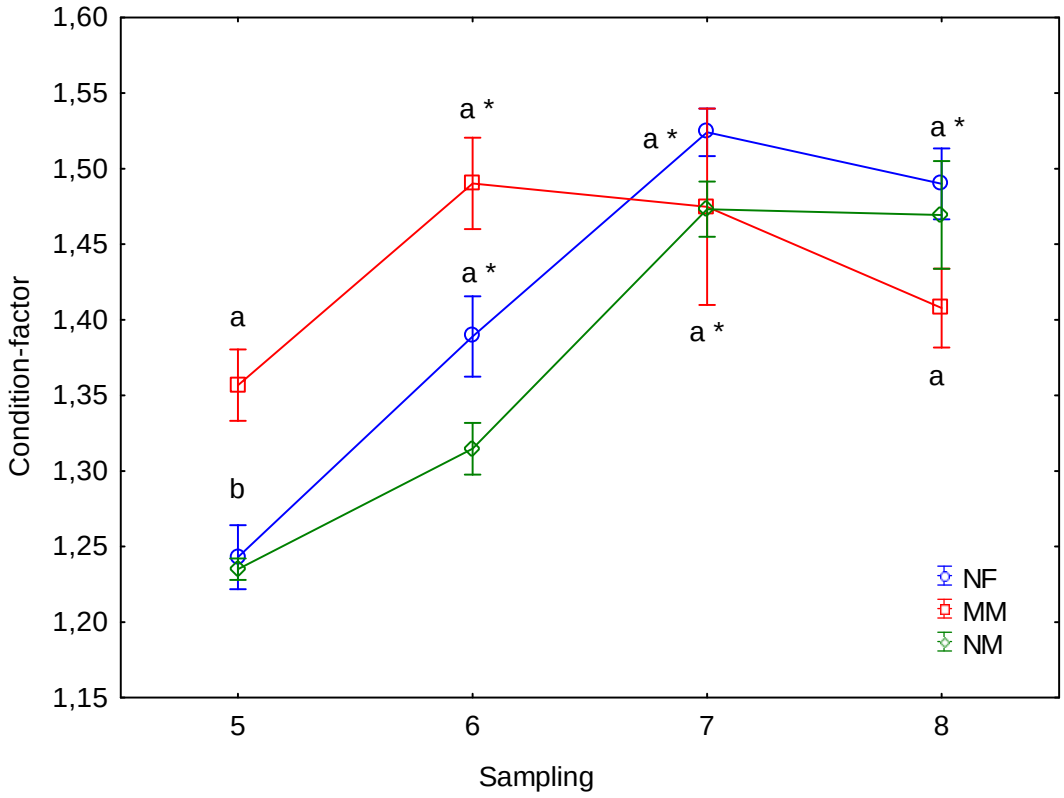

Figure 27: Condition factor of Atlantic salmon in FWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 11 for sampling 5, n = 10 in sampling 6, n = 9 in sampling 7, n = 24 in sampling 8.

3.2.5 Gill NKA activity sampling 5-8

SWLL

The gill NKA activity was similar for MM and NF in sampling 5, but was significantly different between groups in sampling 6, since the NKA activity increased in NF, while it dropped in MM (fig. 28) (appendix III). Even though enzyme activity in NF increased in sampling 6, it followed MM and dropped at sampling 7. The NKA activity for both NF and MM reached their lowest level in sampling 7, as the activity level for both increased at sampling 8 (fig. 28). The factorial ANOVA found significant difference between samplings (ANOVA $p<0.0001$ and maturation groups (ANOVA $p<0.001$) (appendix III).

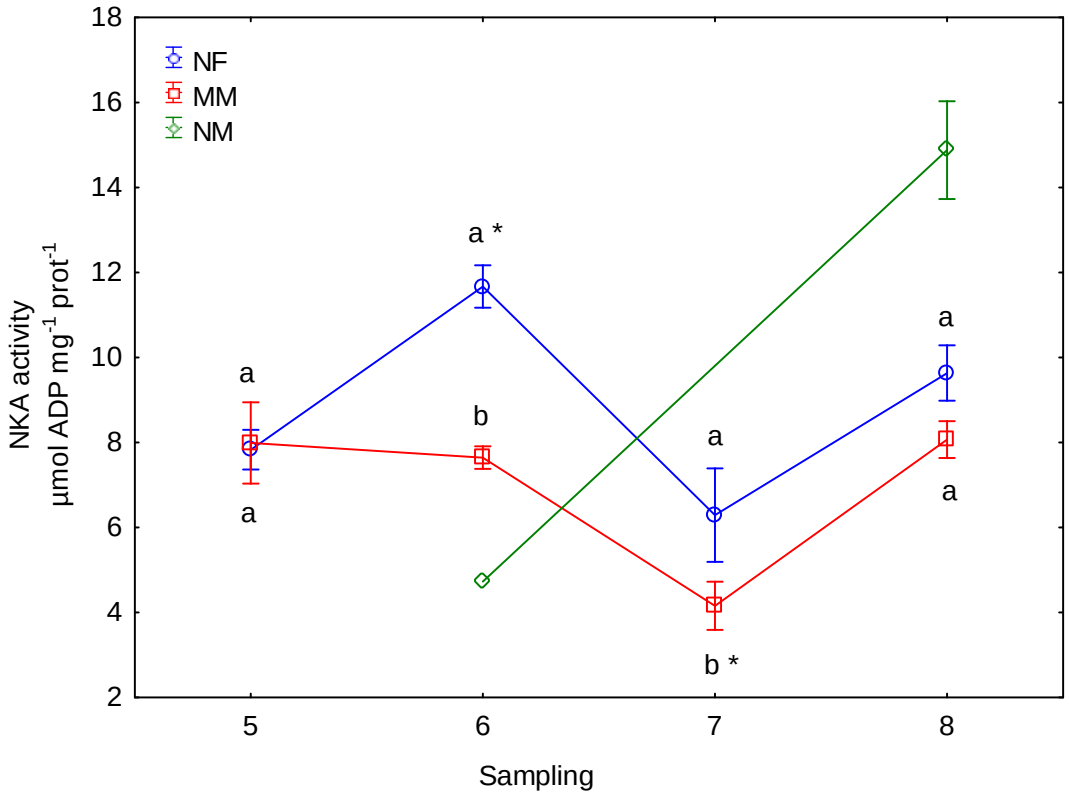

Figure 28: Gill NKA activity (µmol ADP mg^{-1} $prot^{-1}$) of Atlantic salmon in SWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 5 in sampling 5 and n = 9 in sampling 6, n = 11 in sampling 7 and 17 in sampling 8.

SWLD

The Gill NKA activity level peaked in both NF and MM at sampling 6 followed by a decrease in sampling 7, and increased again for sampling 8 (fig. 29). The enzyme activity level in NM seemed to follow the same trend as NF, but there was a greater variation in activity within each sampling, probably due to low n. A factorial ANOVA found significant differences between samplings (ANOVA $p<0.0001$) and maturation groups (ANOVA $p< 0.01$) (appendix III).

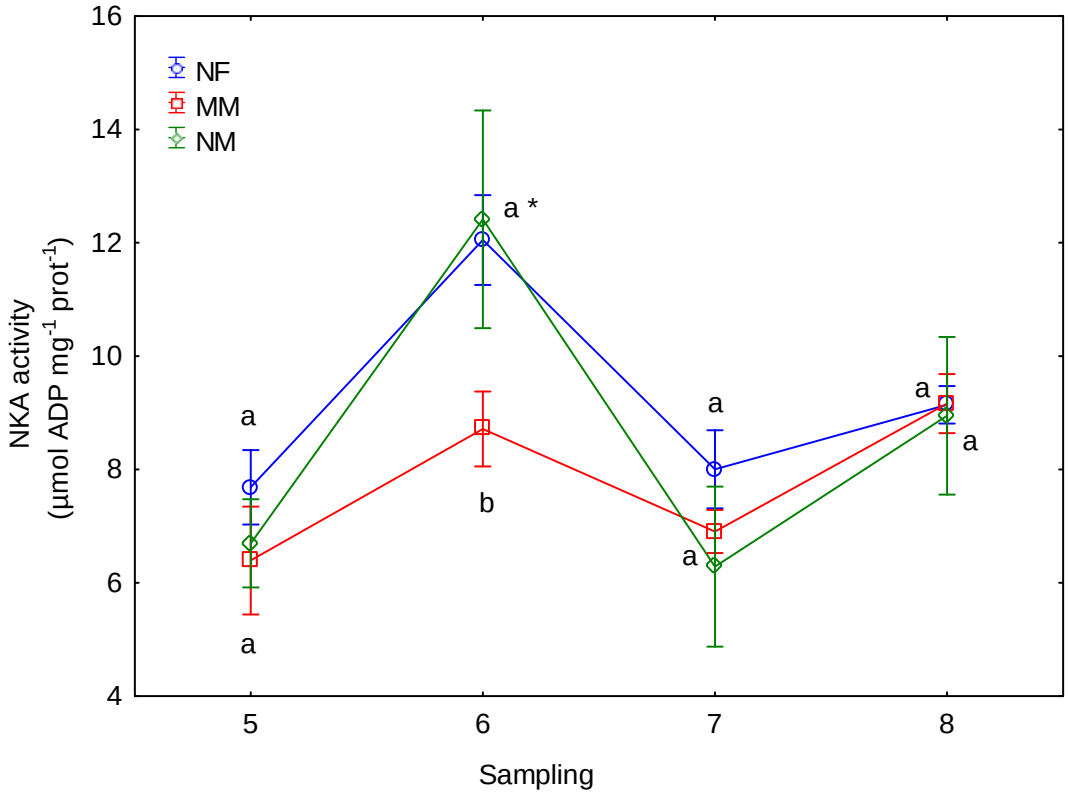

Figure 29: Gill NKA activity (µmol ADP mg^{-1} $prot^{-1}$) of Atlantic salmon in SWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 10 for sampling 5, n = 7 in sampling 6, n = 10 in sampling 7, n = 17 in sampling 8.

FWLL

The activity level in FWLL resembled the activity level seen in FWLD. As in the other treatment groups, the activity level of NF was significantly higher than in MM (fig. 30) (appendix III). The peaks and lows were not as prominent here as in the other groups, but the activity level in both NF and MM in sampling 7 were significantly lower compared to the first sampling (fig. 30, appendix III). NM is the group that stands out, with very high activity in sampling 6 and very low activity in sampling 7 (fig. 30). The factorial ANOVA revealed significant differences between samplings (ANOVA $p<0.0001$) and maturation groups (ANOVA $p<0.0001$) (appendix III).

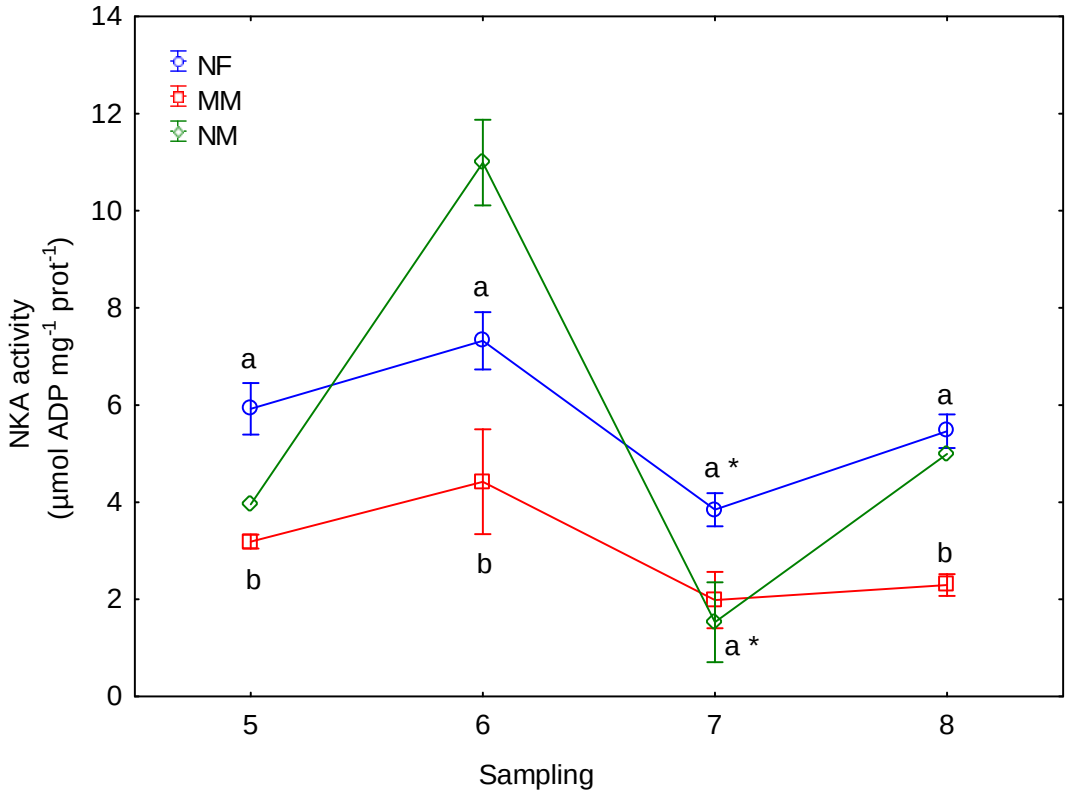

Figure 30: Gill NKA activity (µmol ADP mg^{-1} $prot^{-1}$) of Atlantic salmon in FWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 10 for sampling 5, n = 9 in sampling 6, n = 6 in sampling 7, n = 18 in sampling 8.

FWLD

There was a significant difference between the activity level of NF and MM in sampling 5 and the difference remained until sampling 8, except for sampling 7 where no significant difference was found (fig. 31) (appendix III). Compared to SWLL and SWLD, one can see a similar trend in activity level, but the levels were much lower in FWLD. Another thing that also differs from the SW groups is that only NF has an increased NKA activity level in sampling 8, while MM remained low. The NKA activity in NM was almost completely level with no apparent trend. The factorial ANOVA revealed significant differences between samplings (ANOVA $p<0.0001$) and maturation groups (ANOVA $p<0.0001$) (appendix III).

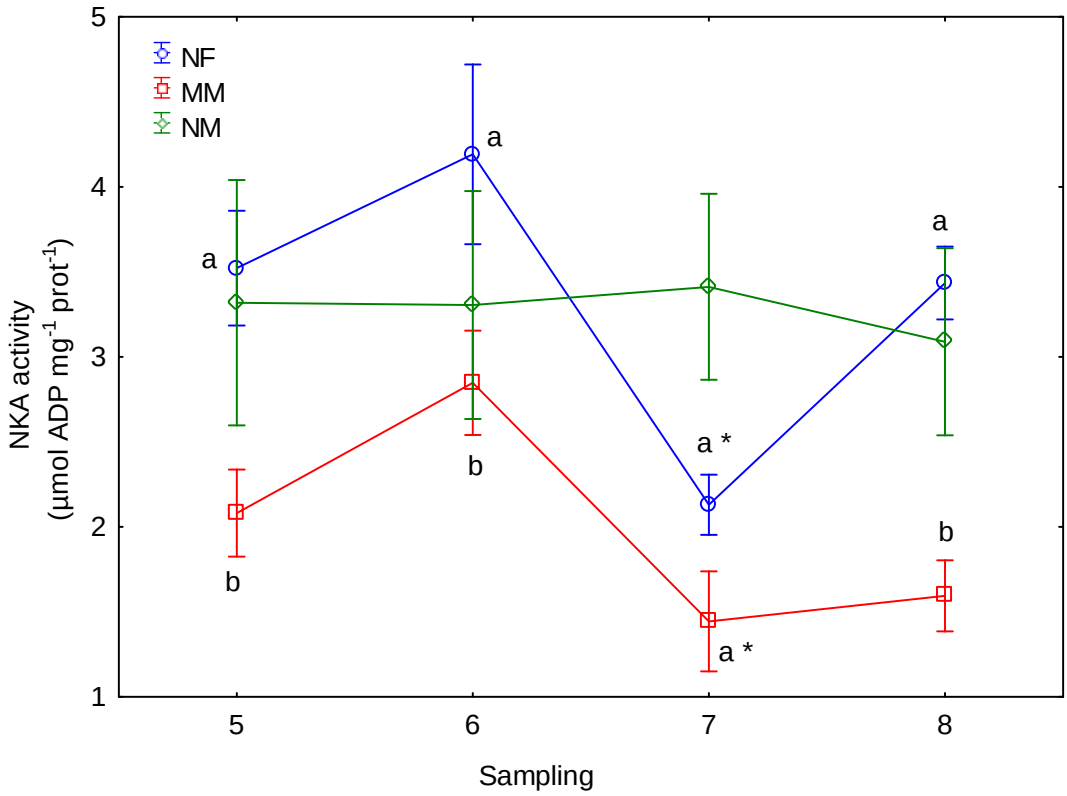

Figure 31: Gill NKA activity (µmol ADP mg^{-1} $prot^{-1}$) of Atlantic salmon in FWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling. Differences between NF and MM in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 8-15 in samplings 5-7 and 16-23 in sampling 8. MM: n = 11 for sampling 5, n = 10 in sampling 6, n = 9 in sampling 7, n = 24 in sampling 8.

3.2.6 Gill NKAα1b gene expression sampling 5-8

SWLL

The NKAα1b expression level in NF was stable trough the whole maturation period, (fig. 32). If one compares the expression level in sampling 5 to the α1b expression level in sampling 4 (fig. 13), it is clear that the expression level has dropped between these two samplings. No significant differences were found between maturation groups or between samplings (appendix III) although the expression level for MM dropped considerably in sampling 8 (fig. 32).

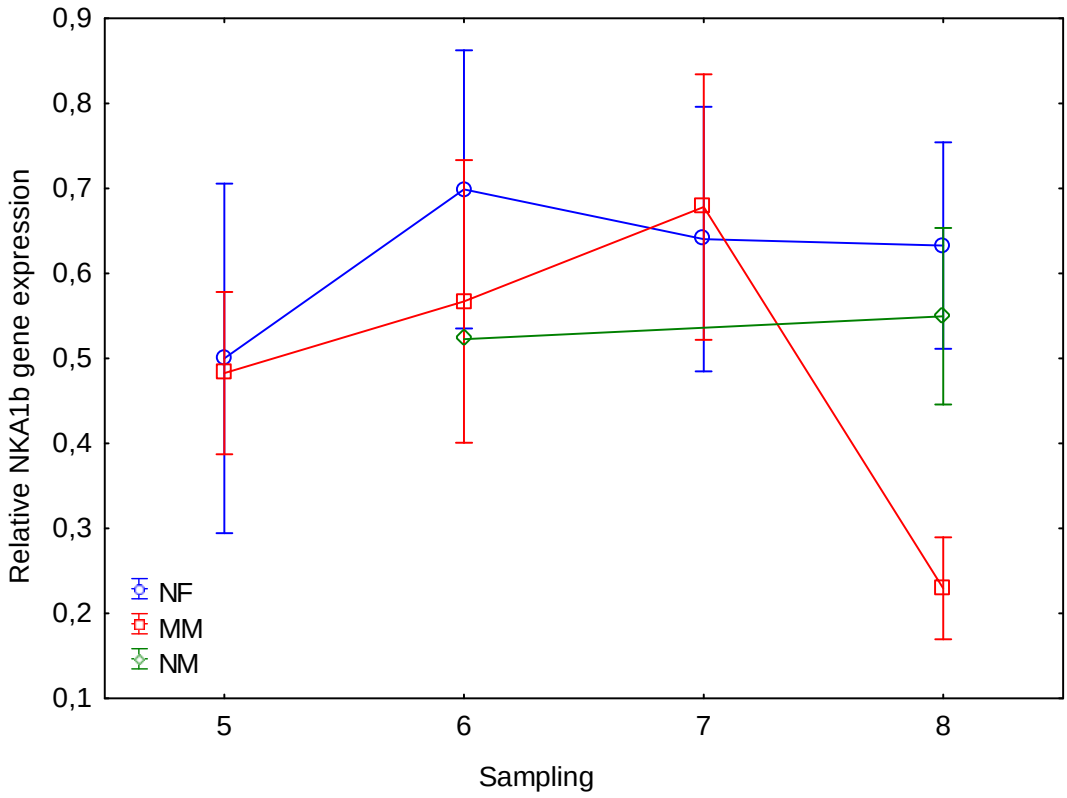

Figure 32: Gill NKAα1b gene expression of Atlantic salmon in SWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Data is presented as mean ± s.e.m. NF: n = 5-6 in all samplings. MM: n = 5-7 in all samplings

SWLD

The NKAα1b expression level of NF increased until sampling 7 where it peaked, before dropping at sampling 8 (fig. 33). The α1b level of MM remained fairly stable, but this also decreased slightly at sampling 8 (fig. 33). The expression level of NM seems different from the two other groups, with a low expression level in sampling, followed by a peak in α1b expression at sampling 7 (fig. 33). The factorial ANOVA revealed no significant differences between samplings nor between maturation groups (appendix III).

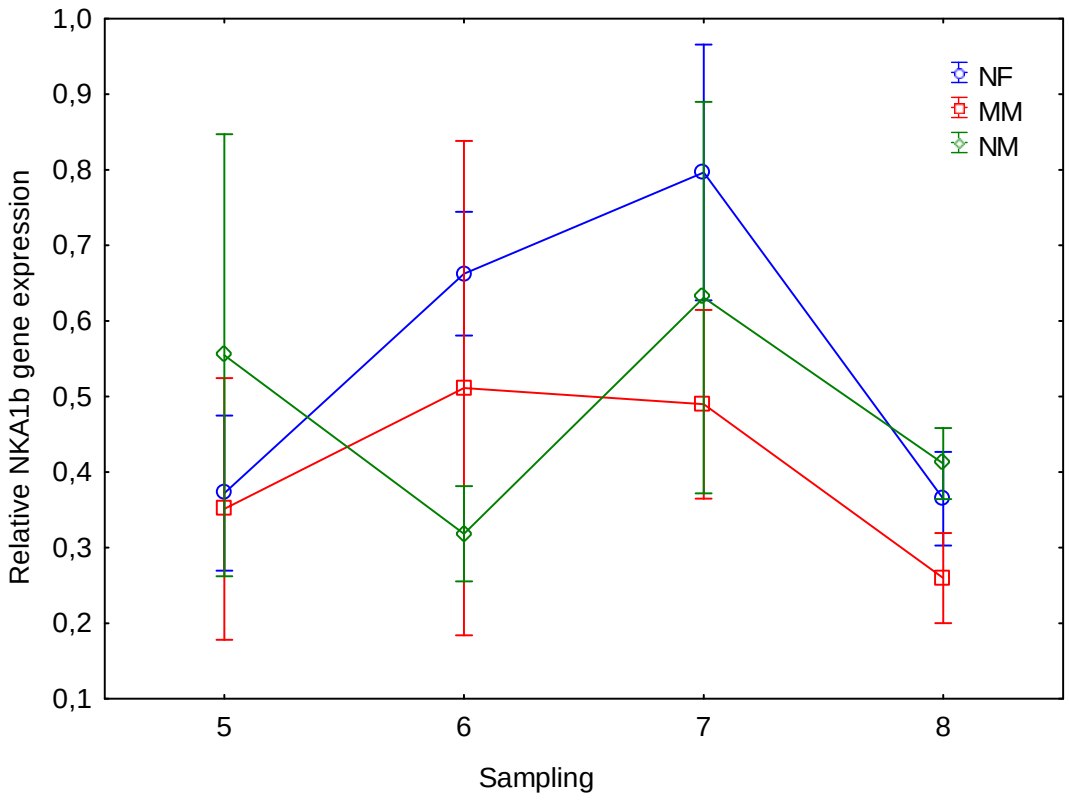

Figure 33: Gill NKAα1b gene expression of Atlantic salmon in SWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Data is presented as mean ± s.e.m. NF: n = 5-6 in all samplings. MM: n = 5-7 in all samplings except for sampling 6, where n = 3.

FWLL

The NKAα1b expression levels in MM in FWLL remained low during the entire experiment and thus seem to differ from all the other treatments (fig. 34). In contrast, the α1b expression level of NF followed a similar trend observed in SWLD and FWLD, with a significant peak in sampling 6 and then a steady decline till sampling 8. The factorial ANOVA found the difference between NF and MM to be significant (ANOVA $p<0.0001$), and the following post hoc found the expression level for NF in sampling 6 to be significantly different from sampling 1 (appendix III).

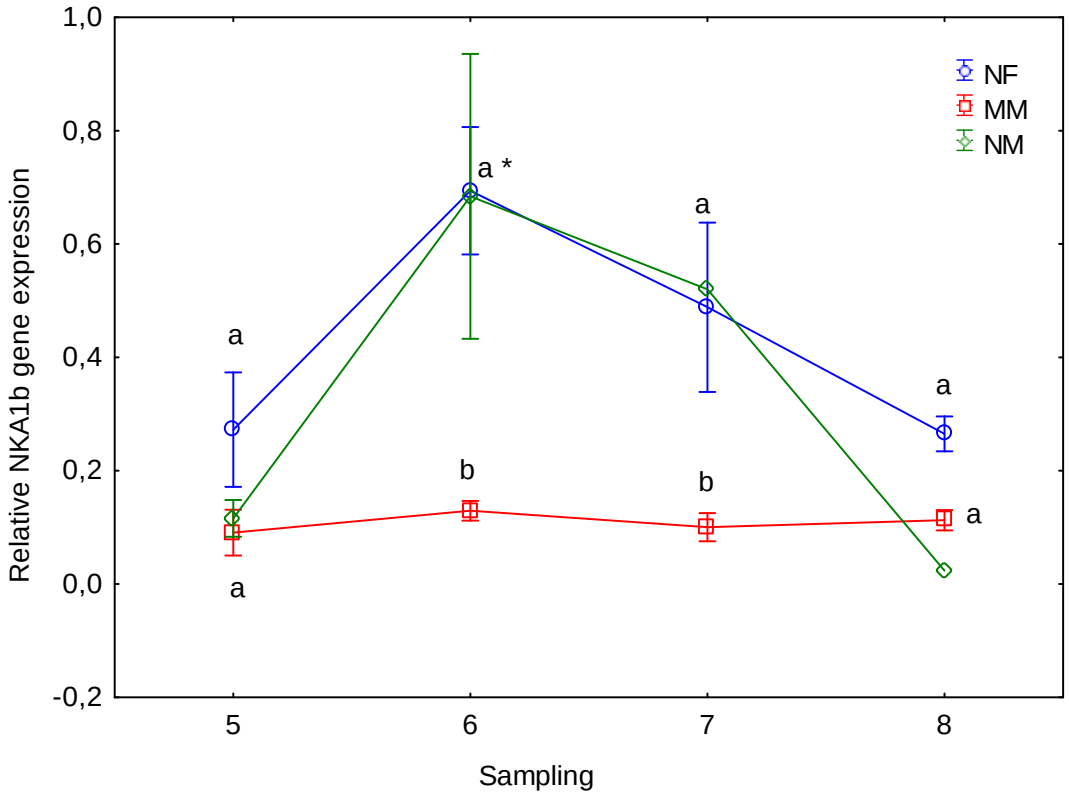

Figure 34: Gill NKAα1b gene expression of Atlantic salmon in FWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling (sampling 6 for MM). Differences between maturations groups in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 5-6 in all samplings. MM: n = 2 in sampling 5 and n = 3 in sampling 6, n = 5-6 in sampling 7 and 8.

FWLD

The NKAα1b expression levels in FWLD resembled the expression level in SWLD, with NKAα1b being expressed higher overall in NF than in MM. There were no specific peaks for NF and MM, but there was a slight increase in both groups at sampling 6, before they decline steadily until sampling 8 (fig. 35). It seemed that the NKAα1b levels of NM were at its highest in sampling 6, before it plunged down at a possible low in sampling 7 and then seemed to remained low throughout of the experiment (fig. 35). No significant differences were found between samplings or between maturation groups (appendix III).

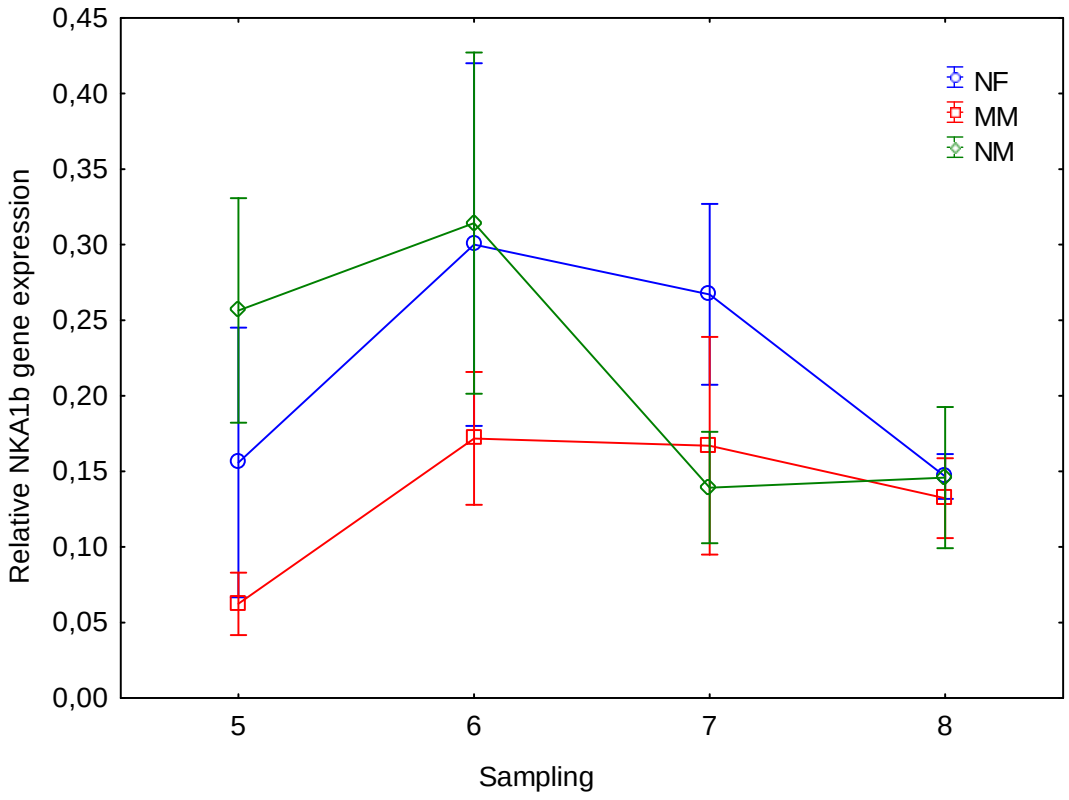

Figure 35: Gill NKAα1b gene expression of Atlantic salmon in FWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Data is presented as mean ± s.e.m. NF: n = 5-6 in all samplings. MM: n = 4 in sampling 5 and n = 3 in sampling 7, n = 5-6 in sampling 6 and 8.

3.2.7 NKAα1a gene expression sampling 5-8

SWLL

The NKAα1a gene expression results for SWLL are affected by the great variation for NF in sampling 6 and for MM in sampling 8. The level of NF peaks in sampling 6 but is low in the other samplings (fig. 36). The expression level in MM stays low until sampling 8, where the mean level and variation increased. The factorial ANOVA found a significant difference between samplings (ANOVA $p<0.05$), but the following post hoc test could not detect which samplings that differed (appendix III). No significant differences were found between maturation groups (appendix III).

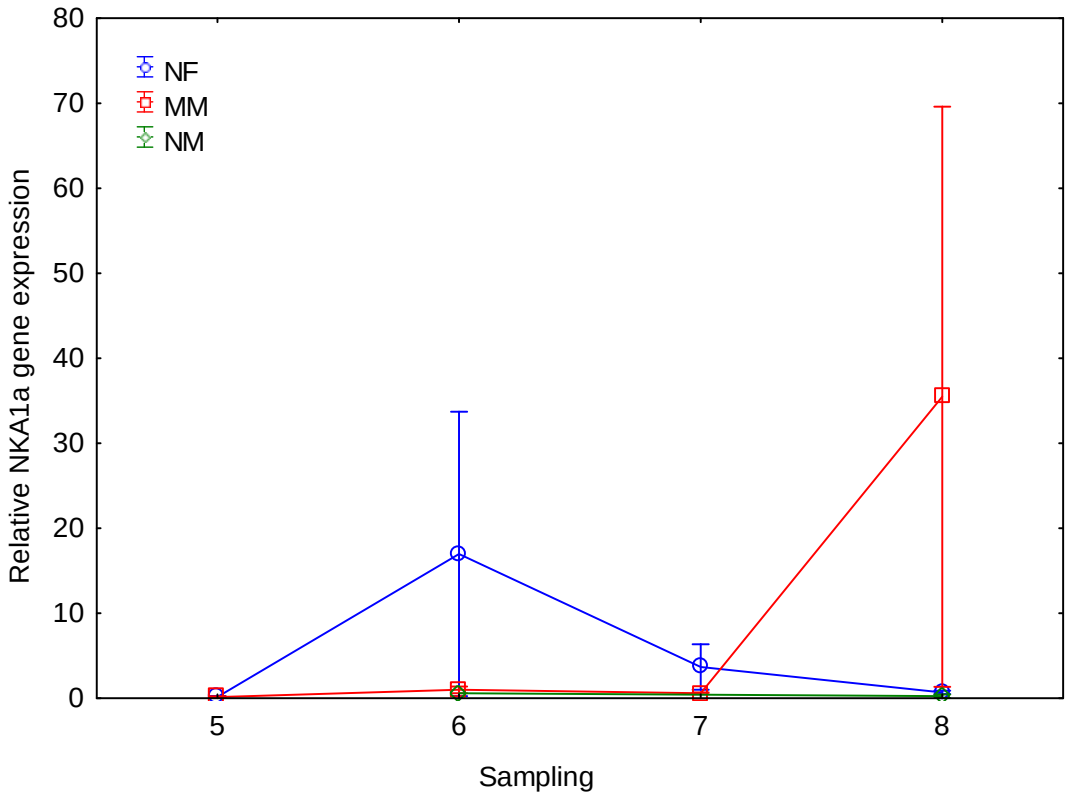

Figure 36: Gill NKAα1a gene expression of Atlantic salmon in SWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Data is presented as mean ± s.e.m. NF: n = 5-6 in all samplings. MM: n = 5-7 in all samplings

SWLD

The expression level of MM increased significantly from sampling 6 through 7 and 8 and was significantly higher than NF in sampling 7. Although the expression level in NF does not seem to increase all that much, did the post hoc test revealed a significant increase in sampling 7 and 8 compared to sampling 5 (fig. 37, appendix III). The factorial ANOVA revealed significant differences between samplings (ANOVA $p<0.0001$) and between maturation groups (ANOVA $p<0.001$) (appendix III).

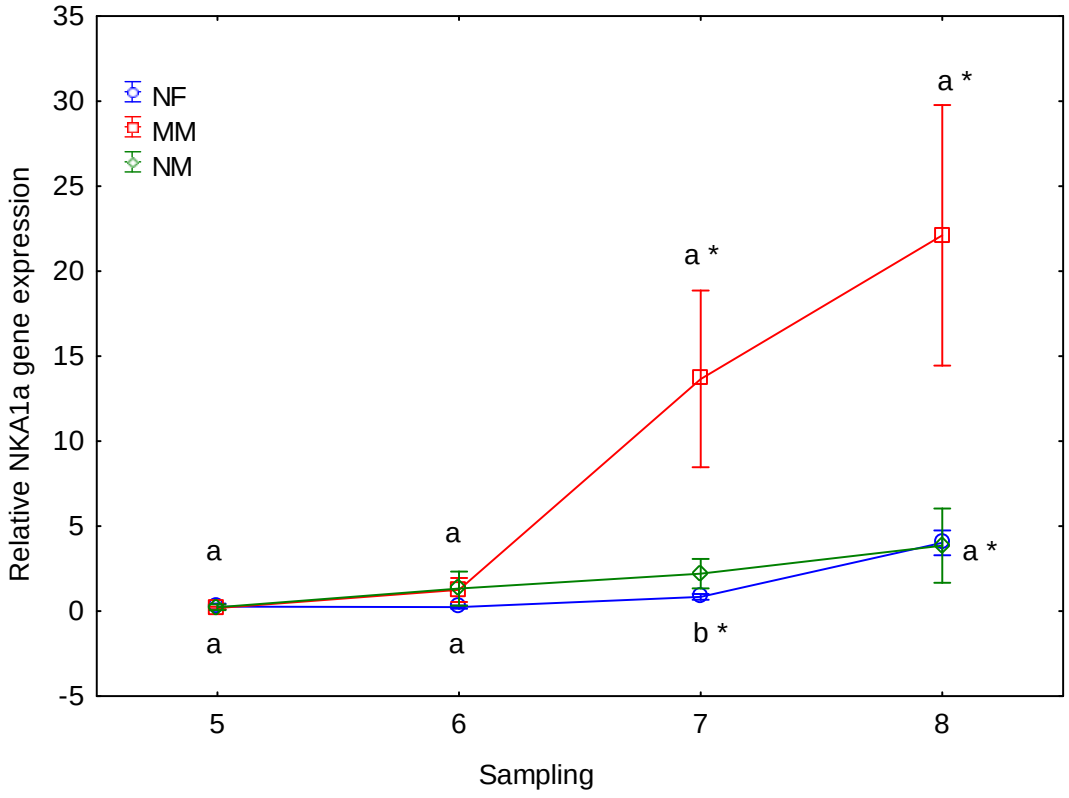

Figure 37: Gill NKAα1a gene expression of Atlantic salmon in SWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling (sampling 6 for MM). Differences between maturations groups in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 5-6 in all samplings. MM: n = 5-7 in all samplings except for sampling 6, where n = 3.

FWLL

The expression level of NF remains quite high throughout the experiment with a slightly lower expression in sampling 7 (fig. 38). The level of expression in NF is also significantly higher compared to MM in sampling 5 (fig. 38). The expression level in MM shows a steady increase from sampling 5 to sampling 8. The factorial ANOVA revealed significant differences between samplings (ANOVA $p<0.01$), maturation groups (ANOVA $p<0.01$), and in the interaction between them (ANOVA $p<0.05$) (appendix III).

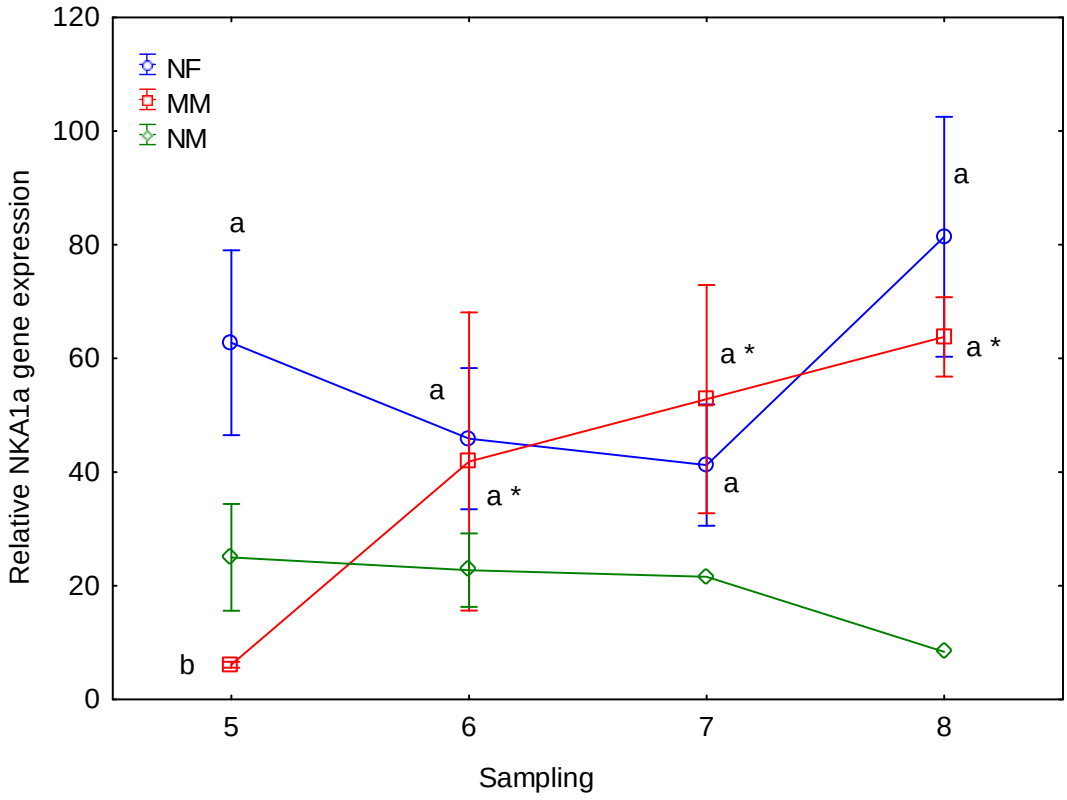

Figure 38: Gill NKAα1a gene expression of Atlantic salmon in FWLL during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling (sampling 6 for MM). Differences between maturations groups in a sampling are marked with letters. Data is presented as mean ± s.e.m. NF: n = 5-6 in all samplings. MM: n = 2 in sampling 5 and n = 3 in sampling 6, n = 5-6 in sampling 7 and 8.

FWLD

Both NF and MM follow the same trend in this treatment group, with an expression that increases slightly for sampling 6, goes slightly down for sampling 7 before it peaks at sampling 8 (fig. 39). The factorial ANOVA found significant differences between samplings (ANOVA $p<0.001$), but revealed no significant difference between maturation groups (appendix III).

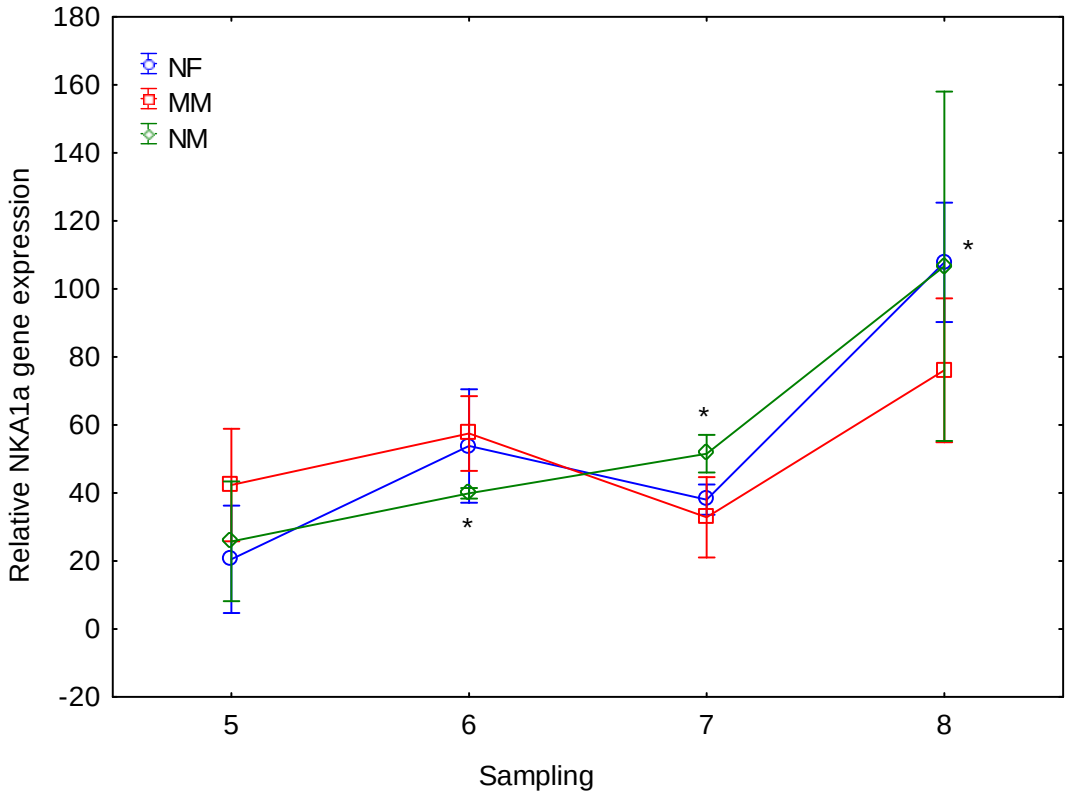

Figure 39: Gill NKAα1a gene expression of Atlantic salmon in FWLD during maturation. The fish are divided into the three maturation groups; immature females (NF), immature males (NM) and mature males (MM). Asterisks (*) indicates difference in time within maturation group in relation to the first sampling (sampling 6 for MM). Data is presented as mean ± s.e.m. NF: n = 5-6 in all samplings. MM: n = 4 in sampling 5 and n = 3 in sampling 7, n = 5-6 in sampling 6 and 8.

4. Discussion

Atlantic salmon go through several changes during smoltification, many of which occur when the salmon are still in FW. Some time after SW migration, the salmon will reach sexual maturation and migrate back to FW to spawn. Data from the current study give an insight to the osmoregulatory changes that take place during smoltification, SW transfer, desmoltification and maturation which will increase the understanding of osmoregulation and the expression of the NKAα1a and NKAα1b subunit isoforms in Atlantic salmon during these changes. The results from the present study will be discussed in the mentioned order.

4.1 Smoltification

Production of out of season smolts for intensive fish farming is widespread and the method has been perfected over many years. The parr are normally kept on LL for a period of time, before a LD photoperiod regime is initiated to induce smoltification (Sigholt et al., 1995), as changes in photoperiod are one of the main triggers for initiation of smoltification (McCormick et al., 1998). It is suggested that lack of photoperiod signals inhibit the development of the light-brain-pituitary axis and prevent smoltification through lack of endocrine control (Ebbesson et al., 2007). Several tests confirm this dependency on a period of short day length, as parr kept only on LL do not display increased NKA activity and fail to adapt to SW (Stefansson et al., 2007, Bjørnsson et al., 1989). Nevertheless, salmon from the present study showed several signs of a successful smoltification, which will be discussed below.

As expected, males and females increased in both length and weight during the smolt period, but there was no significant growth between sampling 3 and 4. Sampling 3 was conducted at the end of the artificial winter period (LD 12:12) and sampling 4 was performed after reintroduction to LL. Salmon exposed to continuous light grow faster due to higher plasma GH levels compared to salmon held in LD or simulated natural photoperiods (SNP) (Bjørnsson et al., 2000, Krakenes et al., 1991, McCormick et al., 1995), but a LD period is necessary to induce smolting and to maintain rapid growth after SW transfer (Berge et al., 1995, Endal et al., 2000, Sigholt et al., 1995, Stefansson et al., 2007).

The slow growth during LD is reflected by a decrease in condition factor (CF) in the same period. The reduction in CF is expected during smoltification (Bjørnsson et al., 1989, Sigholt et al., 1995) and is explained as a result of increased catabolism and growth in length unmatched by weight (McCormick and Saunders, 1987). The latter is explained as an ocean adaptive morphological change, giving the fish a more streamlined profile (Thorpe et al., 1982).

The gonadosomatic index (GSI) was the only variable showing differences between males and females during smoltification. Immature females have a higher GSI than immature males, since they have larger gonads (Mattson, 1991). Contrary to what was seen in females, GSI in males increased significantly during smolting. A reduction in day length may induce precocious maturation in male parr (Skilbrei and Heino, 2011, King et al., 2003, Berrill et al., 2003) and the significant increase in GSI in sampling 3 in males suggests that the males respond to the LD treatment and prepare for maturation. This could mean that the photoperiod regime used for production of 0+ smolt may induce puberty in large males (Skilbrei and Heino, 2011). Nevertheless, males never reached the GSI level of females during smoltification and no mature male parr was found.

In the two first samplings, when fish were still on LL, NKA activity was fairly high, before it dropped significantly under LD 12:12 in sampling 3. Previous studies on underyearling smolts show a similar reduction in NKA activity after the commencement of LD (Berge et al., 1995). Gill NKA activity increased after reintroduction to LL, suggesting that smoltification was initiated as elevated NKA activity levels is strongly associated with SW adaptation (Boeuf and Prunet, 1985, McCormick et al., 1998, McCormick and Saunders, 1987). Gill NKA activity was equally high prior to LD as after, which might be related to the fact that the parr was larger than anticipated, preceding the LD treatment. Large parr adapt better to abrupt SW transfer than small parr signifying body mass as an important factor in SW acclimation (Bjerknes et al., 1992).

The lowest point for gill NKAα1b expression was in sampling 3, the same as for gill NKA activity. This is in coherence with the previously found correlation between elevated levels of NKA activity and high NKAα1b protein (McCormick et al., 2009) and gene expression (Nilsen et al., 2007). Moreover, as NKA activity increased in sampling 4, enhanced gill NKAα1b expression was also expected since the NKAα1b

is related to NKA activity (Nilsen et al., 2007, Stefansson et al., 2007, Richards et al., 2003). Although a slight increase is seen in NKAα1b expression, it is not significant nor is it as profound as the increase seen in NKA activity. This could suggest that NKAα1b mRNA already has been translated into protein, giving a higher NKA enzyme activity than NKAα1b expression. During smoltification there will be a high turnover of NKAα1b to increase NKA activity and prepare the salmon for SW transfer. After SW acclimation NKAα1b will return to a "steady state" of transcription, maintaining NKA activity at a stable level (Meyer et al., 2004). Nilsen et al. (2007) detected high NKA enzyme activity in smolts one month after SW transfer while NKAα1b transcription declined indicating that NKAα1b had reached the steady state of transcription necessary to maintain a high NKA activity.

The expression of gill NKAα1a showed a significant peak in sampling 3, at a time when both gill NKAα1b expression and NKA activity were at their lowest. During smoltification, gill NKAα1a expression levels decline whilst gill NKAα1b expression and NKA activity levels rise (Nilsen et al., 2007). Previous studies of NKAα1a found changes in NKA isoforms as a response to salinity change in both rainbow trout (*O. mykiss*) (Richards et al., 2003) and Atlantic salmon (Bystriansky et al., 2006, Nilsen et al., 2007), but no data exist on NKA isoform alteration in relation to immediate changes in photoperiod. The hormonal alteration created by the change in light may contribute to NKA isoform change since there are often higher values of the FW adaptive hormone PRL and low amount of SW adaptive hormones like GH present during winter (McCormick, 2001, Nilsen et al., 2008, Prunet et al., 1989). Furthermore, the antagonistic relationship between NKAα1a and NKA activity supports the general understanding that a high NKA activity is more closely linked to the α1b isoform than the α1a isoform of NKA (Madsen et al., 2008, McCormick et al., 2009, Nilsen et al., 2007, Richards et al., 2003, Stefansson et al., 2007). Recently discovered structural and thermodynamic differences between NKAα1b and NKAα1a support hypothesis of NKAα1a as the main isoform for ion uptake while NKAa1b is the main isoform for ion excretion (Jorgensen, 2008)

4.2 Post smolts

After completion of smoltification the tanks were divided into FW and SW with either LL or LD light treatment. In the wild or in commercial farming, the smolts would at

this point migrate or be transferred to a marine environment. Consequently, immature females (NF) exposed to SW in this study follow a common SW adaptation process and are expected to show the characteristics of a post-smolt in SW.

The significant increase in general body size through sampling 5-8 was seen in both LL and LD and they showed approximately the same development. Both LL and SW transfer is found to increase GH levels (Arnesen et al., 2003, Bjørnsson et al., 2000, Sakamoto et al., 1993), which consequently may affect the overall growth of the salmon. Although one thus might anticipate a more profound length and weight gain in the LL group compared to the LD group, prolonged exposure to LL treatment is also known to eventually reduce growth rate (Berge et al., 1995, Saunders et al., 1985). The two known effects of LL might counterbalance each other, giving a similar growth for LL and LD treated salmon.

Even though no differences were seen between the photoperiod treatments in relation to weight and length there seems to be a difference between light regimes in CF.

The CF for NF in SWLD showed a steady increase throughout the SW acclimation period and showed no sign of flatting out, while CF for NF in SWLL stabilized between sampling 7 and 8 and never reached as high CF as NF in SWLD. It is possible that the mentioned negative effects of LL is displayed in the CF although no differences in seen in body size between the light treatments (Berge et al., 1995, Saunders et al., 1985).

Two weeks after salinity change, neither NF from the SWLL treatment group nor from the SWLD group showed elevated levels of NKA activity compared to the 4th sampling. Madsen et al (2008) found elevated levels of NKA activity first after seven days in SW while Berge et al. (1995) found increased activity 96 h after introduction to SW. It seems there is great variation between different photoperiod treatments as to when the first signs of elevated NKA activity occur (Berge et al., 1995). Moreover, salmon that are transferred to SW after the initial NKA activity peak show a greater increase in NKA activity after SW transfer (Arnesen et al., 2003). The modest increase in NKA activity in this study may indicate that SW transfer was timed perfectly in relation to smolt status. After some time in SW, when the salmon is completely acclimatized the NKA activity level will stabilize (Singer et al., 2002) and the reduction in NKA activity in NF in SW groups after sampling 6 may indicate that the smolts are fully adapted to SW some time between sampling 6 and 7.

In coherence with pervious findings, there was an upregulation in gill NKAα1b expression in both SW groups after SW transfer (Madsen et al., 2008, McCormick et al., 2009, Nilsen et al., 2007, Bystriansky et al., 2006). Because of the variation within each point, few statistically significant changes were detected but a general trend can be seen. In both smolting salmon and in parr, amplification in relative NKAα1b gene expression was seen after SW transfer (McCormick et al., 2009, Nilsen et al., 2007). The effect of SW was more profound in these experiments than in the present results but some similarities can be seen. A drop in NKAα1b expression was seen in SWLD and may indicate that the smolts are adjusting to SW and high transcription of NKAα1b is no longer necessary to maintain a stable plasma osmolality. NKAα1b transcription has thus reached a steady state. A down-regulation of NKAα1b in SW acclimatized smolts was also noticed in Nilsen et al. (2007) and after one month in SW, NKAα1b expression level was equally high as in smolts kept in FW. The NKAα1b transcription level in SWLL remained high and did not decrease towards the end of the experiment. It can be hypothesized that elevated levels of GH in the LL treatment group may have stimulated NKAα1b transcription to remain high as GH is known to be involved in the proliferation and differentiation of SW-type CC (McCormick, 2001) and high levels of GH correlate with elevated NKAα1b transcription (Stefansson et al., 2007)

Gill NKAα1a transcription in NF was on a steady decline in both SW groups after smoltification was initiated. NKAα1a is associated with FW adaptation and its transcription is expected to decline during SW adaptation (Madsen et al., 2008, McCormick et al., 2009, Nilsen et al., 2007, Stefansson et al., 2007). However, an increase in NKAα1a expression is registered for NF in SWLL in sampling 6 and there is also a significant increase in NKAα1a expression in NF for SWLD from sampling 7 and onwards. Unlike previous findings the NKAα1a expression peak in SWLL seems to coincide with high expression of NKAα1b in the same group. When taking in to consideration the variation within NKAα1a expression in this particular sampling and the fact that all previous experiments of similar character contradicts this finding, one should be careful to interpret too much into the elevated levels of NKAα1a (Bystriansky et al., 2006, Madsen et al., 2008, McCormick et al., 2009, Nilsen et al., 2007, Stefansson et al., 2007). The small but significant increase in

NKAα1a in SWLD stands in relation to the decrease in NKAα1b and NKA activity and supports the previous description of the antagonistic relationship between these isoforms (Madsen et al., 2008, McCormick et al., 2009, Nilsen et al., 2007, Richards et al., 2003).

To summarize, based on increased NKAα1b and NKA enzyme activity it seems like the smolt transfer to SW was timed perfectly in relation to smolt status and that the smolts had reached the steady state of NKAα1b transcription after sampling 6 and thus were acclimatized to SW. In addition, NKAα1a level is low in SW, but increases towards the end of the experiment.

4.3 Desmoltification

When smolts are prevented from reaching SW they go through a desmoltification process where they are partially readapted to FW through the loss of hypo-osmoregulatory ability (Stefansson et al., 1998). Subsequently, in this study, NF smolts kept in FW are expected to show signs of desmoltification after several weeks. High temperatures (>14 °C) are known to accelerate desmoltification in salmon (Stefansson et al., 1998, Handeland et al., 2004), thus the high temperature (16°C) maintained during this trial is thought to speed up the desmoltification process. Just as for NF kept in SW, both FW groups grew steadily throughout the long-term FW phase although NF in FWLD did not increase in size between sampling 6 and 7.

The condition factor also increased in the FW groups and CF did not seem to be affected by the slow growth between sampling 6 and 7 in the LD group. Handeland et al. (2004) reported of decreasing condition factor in desmolting salmon and while the condition factor in the present study increased, it seemed to stabilize and even decrease slightly for NF in FW after sampling 7.

The gill NKA activity level was lower in the FW groups than the SW groups throughout sampling 5-8. NF in FWLL had a higher NKA activity in sampling 5 than FWLD. In addition the drop in NKA activity between sampling 6 and 7 was greater in FWLL than FWLD. Lowered NKA activity is a strong indication of desmoltification (Handeland et al., 2004, Stefansson et al., 1998) and thus is seems that desmoltification in FWLD already had begun in sampling 5, while desmoltification did not fully commence in FWLL before sampling 6. As both treatment groups were

kept at 16 °C the difference can only be explained by the different photoperiod treatments.

As discussed above, lowered NKA activity is often in coherence with lowered transcription of gill NKAα1b (Nilsen et al., 2007, Madsen et al., 2008, McCormick et al., 2009, Stefansson et al., 2007). Consistent with these findings, NKAα1b expression seemed to be lower in FWLD than in FWLL throughout sampling 5 to 8 and a drop in NKAα1b expression is seen in NF from sampling 6 and onwards in both FW groups. The higher NKA activity and NKAα1b expression level in LL can only be related to photoperiod treatment. It is possible that the elevated levels of GH in LL stimulate NKAα1b production and thus delay desmoltification (McCormick, 2001, Tipsmark and Madsen, 2009). In addition, NKAα1b, considered to be the SW specific isoform (Richards et al., 2003), is expressed lower in FW then in SW, giving support to this hypothesis.

The high levels of NKAα1a were found in FW groups throughout the study and support the suggestion of NKAα1a as the FW adaptive isoform (Nilsen et al., 2007, McCormick et al., 2009, Shrimpton et al., 2005, Richards et al., 2003). While there was a significant increase in NKAα1a transcription in the LD photoperiod group, there were no significant changes in the LL group. The high expression of NKAα1a in sampling 8 for both groups indicates full FW adaptation and is consistent with the low NKAα1b transcription in the same sampling and with previous findings of the antagonistic relationship between NKAα1b and NKAα1a (Madsen et al., 2008, McCormick et al., 2009, Nilsen et al., 2007, Richards et al., 2003, Shrimpton et al., 2005).

Conclusively, based on gill NKA activity, gill NKAα1b and NKAα1a expression data, desmoltification seems to begin earlier in the FWLD group compared to the FWLL group, which may be caused by a elevated GH levels in LL (McCormick, 2001, Bjørnsson et al., 2000).

4.4 Osmoregulation in maturing male Atlantic salmon

As presented in table 5, there were a high percentage of mature males in all treatment groups although there were slightly fewer in the LD groups than in the LL. A high percentage in the LD group was unexpected and is probably mainly connected to

large size of the smolts (see section 5.1). Consequently, few immature males were available and were thus not included in statistical analysis.

In previous studies on osmoregulatory changes in maturing salmon Pacific salmon have been used and sampled individuals were always wild caught (Makino et al., 2007, Shrimpton et al., 2005, Uchida et al., 1997). When comparing Atlantic and Pacific salmon one should always bear in mind that their salinity tolerance varies between genus in the salmon family and differences in lifecycle occur (Bystriansky et al., 2006, Gross, 1985). In addition, all wild caught individuals have been exposed to various environmental stimuli and one does not know the background for each fish. With that said, all are salmonids and similar changes in NKA subunits have been observed in Pacific and Atlantic salmon (Madsen et al., 2008, Nilsen et al., 2007, Richards et al., 2003, Shrimpton et al., 2005).

Puberty and sexual maturation in salmon are mainly controlled trough photoperiod cues and the activation of the light-brain-pituitary-gonad axis (Schulz et al., 2010, Schulz et al., 2006, Taranger et al., 1999). Moreover, puberty in males will eventually lead to elevated levels of androgens and it is hypothesized that endocrine changes during maturation may have an direct effect on osmoregulation (Makino et al., 2007). Hormone data are not presented in this thesis, but blood plasma samples from samplings are available and will be analyzed at a later date. Elevated levels of 11-KT have been seen in male salmon with a GSI above 0.5 (Andersson E, Taranger G.L., Personal communication, (Campbell et al., 2003) thus MM in this study are considered to be under the influenced of androgens.

The fork length of mature males increased significantly in the LL treatment groups but not in the LD groups, although a significant increase in length was seen in SWLD in sampling 6. These findings are consistent with previous observations where fish kept in LL were longer than fish kept on LD (Krakenes et al., 1991). Furthermore, in the 5th sampling, MM seems to be overall longer than NF, but only significantly so in the FW groups. The smolts that remained in FW were not exposed to the stress of a salinity change and it seemed like MM in the FW groups grew more between sampling 4 and 5 than NF in FW. This may have caused the significant difference in length between NF and MM in the FW groups in sampling 5. In addition, reduced growth has been observed in mature salmon (Saunders et al., 1982) and this is one of the reasons why mature fish are unwanted in commercial aquaculture (Taranger et al., 1999). Thus, the difference in length may have been related to maturation. When

examining GSI data one clearly seen that the peak in GSI for LD treatment was in sampling 6 while it was in sampling 7 for LL. Maturation seemed to commence earlier in LD possibly resulting in low appetite and poor growth. In addition, negative effect of maturation on growth may be less prominent in salmon kept on LL due to the fact that growth is stimulated by LL (Bjørnsson et al., 2000).

The pattern for growth in body weight reflects the changes in length growth with no significant weight gain for MM in the LD groups and significant growth in the LL groups. This is also in accordance with previous studies on photoperiod and growth (Saunders et al., 1989, Sigholt et al., 1995) although Duncan et al. (1999) reported of a higher weight gain in simulated natural photoperiod groups in comparison to LL.

The large body size upheld by MM in the LL groups was reflected in the CF, as CF in MM stayed above NF throughout the sampling. However, this is not the case in LD were the CF for MM remained fairly stable, but is passed by NF in sampling 7 and end up being significantly lower in sampling 8 in SWLD. There seems to be a connection between light and gender/maturation in relation to CF. Perhaps MM in LD consumed less feed as CF and feed uptake are proven to be correlated (Austreng et al., 1987, Kindschi, 1988). In addition, maturing grilse tend to cease feeding during the summer months, after growing rapidly in the spring (Kadri et al., 1997, Kadri et al., 1996). This is unlike immature fish, which grow slow in spring, but tend to increase appetite in relation to elevated temperatures (Kadri et al., 1997, Saunders et al., 1994). If this were true for this study, we should have seen a decline in CF in MM, but it remained stable. This could suggest that the MM consumes enough feed to maintain a stable CF, but not enough feed to increase CF.

There seems to be a correlation between photoperiodic treatment and when the GSI peak occurs. MM in both SWLD and FWLD reach their maximum GSI in sampling 6 while MM in SWLL and FWLL peak in sampling 7. Photoperiod treatment has variable effects depending on when the treatment is initiated, duration and strength of photoperiod and the physiological state of the animal (Oppedal et al., 1999, Schulz et al., 2006, Taranger et al., 1999). In this particular study smolts exposed to LD became mature earlier than smolts in LL. Some male smolts may have during smoltification reached the internal threshold for commencing maturation. Since salmon spawn in autumn, males that already were ready to mature and put on LD may have accelerated maturation as the reduced day length made them believe autumn was approaching. When planning this study, a low degree of male maturation was expected in the LD

group, but instead they matured earlier. This illustrates how unpredictable the outcome of photoperiodic treatment can be and why more knowledge is needed when it comes to the role of preventing precocious maturation in male salmon (Taranger et al., 2010).

NKA activity for MM seemed to follow the general pattern of NKA activity in NF but was lower in MM than NF in all treatment groups although not statistically lower in all samplings. In addition, NKA activity was lower in the FW groups, which is a common observation for NKA activity in smolts kept in FW (Bystriansky et al., 2006, McCormick et al., 2009, Nilsen et al., 2007, Stefansson et al., 1998). It may seem like MM prepare for FW with reduced NKA activity in a similar way that smolting parr prepare for SW by increasing NKA activity.

Because of the variation within each point, few statistically significant changes were detected, however, a general trend can be seen where NKAα1b expression in MM in general was lower in the FW groups than in the SW groups and it also seemed to be lower in MM than in NF. In addition, NKAα1b expression for MM in SWLL was equally high as for NF throughout sampling 5-7 before a drop in expression was seen in sampling 8. Even though NKAα1b expression for MM in SWLD was not significantly different from NKAα1b expression in NF, the trend suggests a lower expression in MM. The same can be said for NKAα1b transcription in MM in FWLD. Moreover, NKAα1b transcription in MM in FWLL was different than in the other treatment groups as there was no up or down regulation of NKAα1b throughout the experiment and the expression level in MM was significantly different from that of NF.

The lowered levels of NKAα1b in MM in relation to NF may indicate that elevated levels of maturation specific androgens like testosterone (T) and 11-ketotestosteron (11-KT) affect gill NKAα1b gene expression. An experiment done by Onuma et al. (2005) showed that sex steroids have a direct effect on the gene expression of PRL in masu salmon (*O. masou)* and PRL has been shown to reduce NKAα1b gene expression in Atlantic salmon (Tipsmark and Madsen, 2009).

Both immunohistochemistry using specific antibodies of α1a and α1b (McCormick et al., 2009) and In Situ hybridization (Madsen et al., 2008) have been used to characterize the size, abundance and localization of NKAα1a and NKAα1b in gill tissue. Utilizing the latter method, PRL was found to have a negative effect on SW-

type CC and especially NKAα1b (Tipsmark and Madsen, 2009). Consequently, the low NKAα1b expression seen in MM in relation to NF in this study may have been an indirect effect of elevated levels of androgens as NKAα1b expression in MM seemed to be lower at the end of maturation when the androgen level is high (Campbell et al., 2003).

NKAα1a expression for MM in SWLL was low until sampling 8 where two individuals had high NKAα1a transcription, creating great variation within the sampling. Because of this variation it was difficult to draw any conclusions about the NKAα1a expression in this treatment group. Nevertheless, the high expression in some MM may suggest the start of increased NKAα1a transcription in MM. To answer this, one more sampling would have been required. In SWLD NKAα1a transcription in MM was low in sampling 5 and 6, before NKAα1a expression increased significantly in sampling 7 and 8. The NKAα1a levels in MM did not follow the same trend as NF and the two were significantly different in sampling 7. NKAα1a expression in both NF and MM followed the same trend in FWLD and expression was high and stable with a slight increase in sampling 8. In contrast, NKAα1a transcription for MM in FWLL was completely different from NF. While the expression of NKAα1a started low and increased significantly for MM no significant changes were seen between MM and NF. In general, when NM were present they seemed to follow the same trend as NF, indicating NF as a trustworthy control group.

When Shrimpton et al. (2005) studied homing sockeye salmon (*O. nerka*) they found that gill NKAα1a expression increased while the salmon were still in SW and continued to rise while migrating up the river. The new discovery was not that NKAα1a transcription increases in FW, but that NKAα1a expression increased prior to FW transfer (Shrimpton et al., 2005). Furthermore, mature Chum salmon (*O. keta*) captured in SW just before river entry, failed to survive in SW for more than 5 days due to elevated plasma osmolality (Hirano et al., 1990, Uchida et al., 1997). Elevated plasma osmolality has been correlated with low NKAα1b expression and low NKA enzyme activity (Mackie et al., 2005, Bystriansky et al., 2006, Richards et al., 2003) suggesting that low NKAα1b transcription followed by low NKA activity in MM in SW may cause dehydration and death. Our results are consistent with these findings

as NKAα1a levels increase in MM kept in SWLD, suggesting a preparatory adaption for FW in mature salmon kept in SW.

4.5 Conclusion and further perspectives

The results from the present study support previous studies claiming NKAα1a to be the main isoform for FW adaptation (Madsen et al., 2008, McCormick et al., 2009, Nilsen et al., 2007, Richards et al., 2003, Stefansson et al., 2007) as NKAα1a expression decreased during preparatory SW adaptation and in smolts transferred to SW. In addition, NKAα1a increased in desmolting salmon and also in MM kept in FW and SW. The latter suggests that maturing salmon adapt to FW, which might lead to elevated plasma osmolality and death if they are kept in SW for a longer period of time after initiation of maturation (Hirano et al., 1990, Uchida et al., 1997).

Furthermore, the hypothesis of NKAα1b being the SW adaptive isoform (McCormick et al., 2009, Nilsen et al., 2007, Richards et al., 2003) and the isoform with the strongest correlation to gill NKA enzyme activity (Nilsen et al., 2007, Richards et al., 2003, Stefansson et al., 2007) has gained support. The NKAα1b expression increased during preparatory SW adaptation and continued to increase after SW transformation before SW acclimation was complete and NKAα1b transcription reached the level where it was able to maintain sufficient NKA activity to keep plasma osmolality stable. The lowered level of NKAα1b transcription in MM also suggest a preadaptation to FW in mature individuals, which may be fatal during prolonged exposure to SW.

Further studies should be conducted to uncover all physiological changes during maturation in Atlantic salmon including influence of androgens on osmoregulation and the welfare consequences of keeping mature salmon in SW. Some of these questions might be answered when hormone and plasma samples taken during this experiment are analyzed and compared to the present results.

Nevertheless, this study has given us new insight to salmon maturation and we can say that there are changes in the expression level of NKAα1b and NKAα1a during the sexual maturation in male salmon.

5. Methodological considerations

5.1 Experimental design

The experiment started 29th of September with 16 tanks containing parr kept on LL. After smoltification were the fish divided into four treatment groups, where each treatment group consisted of four replica tanks. The use of replicates in an experiment is necessary to reduce variance and improve the significance of the result. Based on previous characterization of the post-smolt maturation model (Fjelldal, Hansen, in prep) it was unexpected to discover a considerable proportion of mature males in the LD groups during maturation. This may be explained by the extra body weight the fish gained during the three extra weeks, as larger individual are more likely to enter maturation (Taranger et al., 2010). Consequently, few immature males were available for analysis and statistically comparing immature males with the other gender/maturation groups could give untrustworthy results. The females remained immature and were thus representing a normal post-smolt. This made them suited to be compared to the mature males.

5.2 RNA isolation and quality

Good quality RNA is vital for successful and reliable gene expression analysis as the real-time RT-PCR need DNA-free, un-degraded RNA (Bustin and Nolan, 2004a, Pfaffl, 2004). RNA is vulnerable to degradation by RNase enzymes, which may result in shorter fragments of RNA occurring in samples. This may again lead to loss of or errors in gene expression results. To secure an adequate RNA quality, gill tissue was submerged in RNA*later* (Ambion, Austin, TX, USA) immediately after dissection as RNA*later* rapidly permeates most tissues and stabilize and protect RNA (Tröβe et al., 2010). The gills grew large during the course of the experiment. The size of the sampled gills was subsequently reduced to fit in the sample tubes in sampling 7 and 8. Applied Biosystems (Applied Biosystems, Carlsbad, CA, USA) recommend the use of 1 ml RNAlater per 50 mg of tissue so ensure that RNA enters the tissue completely. Gills are "designed" to have maximum contact with the surrounding environment, thus less RNA*later* is required per mg to obtain full effect. Alternative tissue preservation for RNA isolation is freezing the tissue directly. This would be the preferred method if the same tissue were to be used for protein analysis, as RNA*later*

may complicate such work. For RNA isolation, RNA*later* considered to be the best preservation medium.

When performing RNA isolation the tissue was cut from cartilage in a standardized manner and transferred to Trizol-reagent (Sigma-Aldrich, St. Louis, MO, USA) as quickly as possible. Following homogenization, Trizol dissolves the cell component and preserves the RNA integrity (Chomczynski and Sacchi, 1987). Even though precautions were taken to ensure good RNA quality, we should not disregard factors that may have affected RNA quality and the integrity of the RNA should always be examined (see below)

The concentration and absorbance of isolated RNA were measured using a NanoDrop-1000 (Thermo Scientific, NC, USA). According to Bustin and Nolan (2004) is an A260/280 ratio greater than 1.8 is a sign of good RNA purity as it indicate a low protein contamination. However, a low A260/230 ratio suggested a residual contamination of organic compounds, such as phenol and alcohol (Bustin and Nolan, 2004a) from the RNA isolation protocol, which could affect downstream applications such as cDNA synthesis and quantitative PCR (Pfaffl, 2004) Preferably both ratios should be above 1.8, but this may be difficult to achieve and a ratio around 1.7 still indicates sufficient RNA purity for the protocols used in the present study (Nilsen, personal communication).

Although, the A260/280 and A260/230 reflects purity, it does not give information about the RNA integrity. To access the integrity the Agilent 2100 bioanalyzer (Agilent technologies, Santa Clara, CA, USA) were utilized, as it produces a RNA integrity number (RIN) based on the 18s and 28s ribosomal RNA bands (Bustin et al., 2005). The software generates a RIN number ranging from 1 to 10, with 1 being the lowest integrity and 10 being the highest. A RIN value above 5 is recommended for a successful and reliable real-time RT-PCR quantification (Bustin and Nolan, 2004b). For this study, twelve random samples were checked for RIN values and the majority of samples had a high RIN value (table 6), indicating very good RNA quality. The one sample with low RIN value was monitored closely throughout the experiment, particularly for the reference gene, but no notable negative effects were observed during subsequent analysis of the sample in question.

Table 6: RNA Integrity Numbers (RIN). Values from randomly selected samples analyzed with the Agilent 2100 Bioanalyzer. Absorbance ratios from the NanoDrop are also included.

Sample nr	Sampling	A260/280	A260/230	RIN value
47	3	2.03	2.29	10
6	1	2.05	2.30	10
33	2	2.04	2.30	8.8
31	2	2.04	2.33	9.9
3	1	2.03	2.37	10
71	4	2.01	2.32	4.4
67	4	2.04	2.36	9.9
77	4	2.04	2.31	8.3
11	1	2.04	2.37	10
22	2	2.06	2.26	10
9	1	2.06	2.28	10
75	4	2.02	2.31	8.3

5.3 DNase treatment and cDNA synthesis

As most RNA preparations may contain residual amounts of genomic DNA, gDNA, (Pfaffl, 2004), the isolated RNA was treated with DNase to remove all gDNA residues. Even the smallest DNA contamination may interfere with the desired "specific amplification" as the kinetic PCR have a tremendous amplification power (Pfaffl, 2004). This study used SYBR green as the staining dye, which bind to all double stranded DNA and emits a fluorescence signal. Thus it is important to remove all unwanted genomic DNA, as any contamination would give imprecise results (Bustin, 2005). With that said, treating RNA with DNase may result in RNA degradation and the DNase should always be removed and/or inactivated before any Reverse Transcriptase (RT) step (Pfaffl, 2004). The protocol used in this study includes an inactivation of the DNase enzyme. Moreover, the protocols for DNase treatment and cDNA synthesis are adjusted in a way they "dilute" the carryover contamination of residual proteins (DNase) into the cDNA synthesis step.

As RNA cannot serve as a template for PCR, the formation of a DNA template is necessary and RNA is used as the template for the formation of single stranded (ss) complementary DNA (cDNA) through reverse transcriptase (Pfaffl, 2004). The RT step is the source of the most variability in a kinetic RT-PCR experiment (Pfaffl, 2004) and it is crucial that all samples are treated in a standardized manner throughout the experiment. To minimize variation in the RT step, all reaction components were

added into one master mix, as described in the Material and Methods section, before aliquots of the master mix were added to the samples. This together with not changing manufacturer or batch number for reagents, primers and laboratory supply most likely minimized variation throughout the experiment.

A random nonamer primer was used in this experiment. The primer binds to RNA and acts as a starting point for DNA synthesis and the random primers will synthesize cDNA from all RNA present in the sample, such as ribosomal RNA (rRNA), mRNA and transcription RNA (tRNA) (Bustin et al., 2005). If the amount of mRNA is low, will this give a low concentration of primed mRNA in proportion to primed rRNA and its subsequent amplification may not be quantitative (Bustin et al., 2005). Other primers such as Oligo-dT and target-specific primers will only bind and synthesize cDNA from mRNA, but the transcription may not reach the PCR target sequence if secondary structures are present (Bustin and Nolan, 2004b). The use of specific primers is thus not recommended if one suspects RNA degradation or fragmentation as they are dependent on the exact nucleotide sequences they are designed for (Bustin and Nolan, 2004b). Random primers are used in about 30% of all RT-PCR assays and may produce good results if the cDNA synthesis is carried out in a careful, competent manner (Bustin et al., 2005, Bustin and Nolan, 2004b). When assays used in the present study were made, they were optimized for use together with MGB Taq-Man probes. As previous experiences have shown that cDNA synthesis using Oligo-dt primers may lead to inconsistencies in qPCR reactions with Taq-man assays (Nilsen, personal communication). While we used SYBRgreen for this experiment, our cDNA aliquots will be transported to other collaborating laboratories that use Taq-man. Thus, cDNA was synthesized using random primers.

5.4.1 Real-Time quantitative PCR, Quantification of gene expression

Real Time qPCR was in this study used to measure the expression of NKAα1a and NKAα1b in Atlantic salmon gill tissue. Real time RT-PCR is the most sensitive, specific and reproducible method for quantification of mRNA and is therefore frequently used (Bustin, 2000, Bustin et al., 2005). However, the quality of the RT-qPCR results is also dependent on the quality and consideration placed in the steps performed prior to qPCR. There are many potential pitfalls leading to variations and errors in qPCR. Such variations could stem from assay design, equipment, PCR

reagents, unspecific products, primer-dimers, amplification efficiency, presence of inhibitors and human error, all problems inherent to the amplification of genes and may therefore contribute to insecure results (Pfaffl 2004; Bustin et al. 2005).

Good lab routines and precision pipetting is also essential for preventing cumulative error and non-template control (NTC; sample well with water instead of cDNA template) samples should always be present on each qPCR plate (Bustin and Nolan, 2004b). The presence of NTC samples are important to detect possible contamination and should ideally be negative (Wong and Medrano, 2005). In the present study, four NTC samples displayed amplification like curves, but Ct values were high (around 35) and more than 10 Ct values from the actual samples, indicating low contamination levels. The amplification curves from the positive NTC differed from those of the actual samples. Hence, it was concluded that the four NTC wells that gave an amplification signal did not represent a potential problem for interpretation of our results.

The PCR reaction has four main phases: The ground phase, were fluorescence has not reached above background noise, the early exponential phase where fluorescence is rising above background noise, the exponential phase where the amount of DNA doubles in each step and the plateau phase were reaction components becomes limited and the fluorescence intensity is no longer useful for data calculation (Wong and Medrano, 2005). In real-time qPCR the amplification of DNA is monitored during the course of the reaction by observing the fluorescence of the added marker (Bustin, 2005). The registered quantity of fluoresces is proportional to the formed amount of double stranded DNA and the number of amplifications cycles required to achieve a specific amount of DNA is registered (Bustin, 2005). The more copies of the target there are at the beginning of the assay, the fewer amplification cycles are required for the fluorescence to reach the threshold level of detection (Bustin 2000; Bustin et al. 2005; Wong and Medrano 2005). The number of cycles needed to reach the threshold is referred to as threshold cycle (C_t) and is defined as the cycle when sample fluorescence exceeds a chosen threshold above calculated background fluorescence (Wong and Medrano, 2005). The threshold is set manually and must always be within the exponential phase and above background noise (Bustin, 2005). In this study, NKAα1a and NKAα1b where set at 0.013 and at 0.012 for EF1a, which was within the exponential phase for all plates.

Several probes and dyes may be used to detect DNA amplification in real time, some bind to specific sections of the DNA, whereas others binds to DNA in general (Bustin, 2000). For this study SYBRgreen (Applied Biosystems, Carlsbad, CA, USA) was chosen as the dye. SYBRgreen binds to the double stranded DNA and the bound dye emits a detectible fluoresce (Bustin, 2000). The amount of fluorescence will increase proportionally with the quantity of amplified DNA and is recorded in real-time (Bustin, 2000). As SYBRgreen binds to all dsDNA present, specific primers and low DNA contamination are required to ensure a reliable PCR result (Bustin, 2000). The isolated RNA was, as mentioned, treated with DNase and specific primers for NKAα1a, NKAα1b and EF1α were used in this study. High-quality results have been achieved using these assays and protocols in previous studies (Nilsen et al., 2007, Olsvik et al., 2005).

The PCR efficiency may have considerable impact on the final qPCR results (Bustin, 2005). It is therefore imperative that the efficiency is monitored during analysis in order to ensure accurate quantification results. In the present study, triplicate ten-fold dilution series of cDNA were used to generate regression slopes for each plate (see Materials and Methods) (Wong and Medrano, 2005), which gave a wide linear range in C_t values for all three genes. All of the experimental samples were within the linear range of the dilution series, allowing accurate quantification (Bustin, 2000).

All slopes for NKAα1a (fig 41) and EF1α (fig. 41) were found to be within a similar range. However one regression line for NKAα1b differed from the others. The efficiency was calculated using individual plates for NKAα1b and NKAα1a while the mean slope for all plates were used for EF1α (fig. 40). The NKAα1b plate that differed from the rest was closely monitored and no effect of plates was notable when analyzing the results.

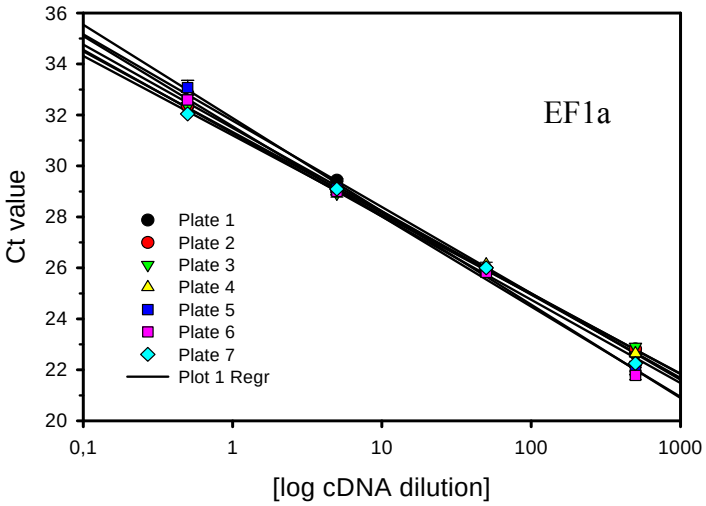

Figure 40: Efficiency curve for EF1α. The graph shows the efficiency curves for EF1α created by plotting Ct values from the dilution series against the log of input cDNA. For regression line equations see appendix II.

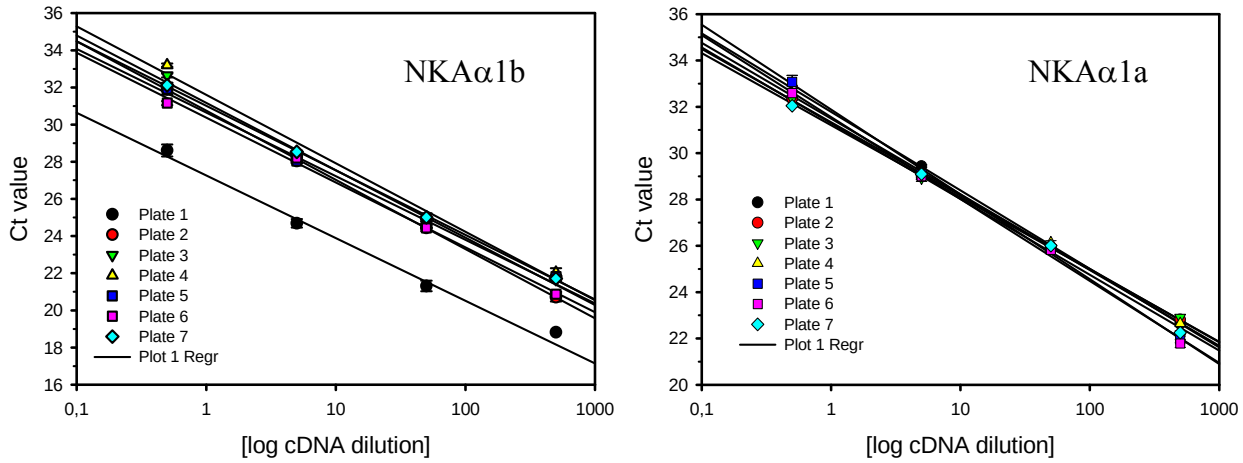

Figure 41: Efficiency curve for NKAα1a and NKAα1b. The graphs show the efficiency curves for NKAα1a and NKAα1b created by plotting Ct values from the dilution series against the log of input cDNA. For regression line equations see appendix II.

Some variation was observed between the duplicates of the experimental samples and mean Ct value with a standard deviation raised to the second power above 0.05 were monitored (Pfaffl, 2004). The real-time RT-PCR is less reliable when Ct values are in the upper limit of dependability of qPCR (≥35 Ct values) (Bustin 2002), but differences in duplicates were found in both low and high Ct values. All irregular samples were thoroughly monitored.

5.4.2 Normalization

As pointed out, variation in sample-to-sample and run-to-run may easily arise due to differences in amount of starting material between samples, variation in RNA integrity, difference in amplification efficiencies and variation of cDNA present in

each sample (Bustin, 2000, Pfaffl, 2004). These types of variations easily arise when samples are from different individuals and taken over a period of time, as was the case for this study (Bustin, 2000). To minimize such errors, must the gene expression level of the target gene must be normalized and efficiency corrected using a reference gene, also called housekeeping gene (Bustin, 2005, Bustin, 2000, Pfaffl, 2004, Wong and Medrano, 2005).

For this reason, this study uses relative gene expression as the quantification strategy, since this method is based on the expression level of a target gene compared to a reference gene (Pfaffl, 2004). The choice of reference gene depends on the tissue of interest (Olsvik et al., 2005), as the reference gene should be expressed at a stable level among different tissues of an organism and trough all stages of development and remain unaffected by the experimental treatments (Olsvik et al., 2005, Wong and Medrano, 2005, Bustin, 2000, Pfaffl et al., 2004, Pfaffl, 2004). Different endogenous control genes will behave differently in various tissues and experimental designs and one should thus test several potential reference genes for every examined tissue, under different treatments (Olsvik et al., 2005). The reference gene used for this study was Elongation factor 1a, a reference gene proven to be stable when tested on gill tissue and other organs in Atlantic salmon in the utilized laboratory (Nilsen et al., 2007, Olsvik et al., 2005). Unfortunately, variations in EF1a gene expression were detected in this experiment. When facing such a problem, it is possible to use the mean of all EF1a Ct values as the reference value instead of a sample-to-sample efficiency correction (Pfaffl, 2004) in combination with the mean slope from all EF1a plates.

Several mathematical methods are available for calculation of a target gene in relation to an adequate reference gene and two models are used for relative quantification (Pfaffl, 2004). The two models in question are the ΔΔCt and the efficiency-correlated Ct model (Pfaffl, 2001). As the ΔΔCt method should only be used for quick assessment of the data (Pfaffl, 2001), the efficiency correlated Ct model was used in this study. For this model, the relative expression of a target gene is calculated, based on its real-time PCR efficiencies and the Ct value difference of an unknown sample versus the reference gene (Pfaffl, 2004). The amplification efficiency is required for calculating the target quantity and therefore, the amplification efficiency for each plate was determined using the regression line slope from the dilution curves. As mentioned, some irregularities were found in the reference gene in this experiment. To reduce the influence of these variations the mean value of efficiency correlated Ct

values from NKAα1a and NKAα1b from sampling one were used as a calibrator for the other samplings. The efficiency-correlated data from sampling 1 were chosen as this sampling represents the biological initiation point for this experiment. The following equation was then used to calculate relative gene expression for NKAα1a and NKAα1b (Pfaffl, 2004):

$$\text{ratio} = \frac{(E_{ref})^{CP_{sample}}}{(E_{target})^{CP_{sample}}} \div \frac{(E_{ref})^{CP_{calibrator}}}{(E_{target})^{CP_{calibrator}}}$$

This method gave very good results and removed the chances for a false gene regulation expression due to variations in the housekeeping gene.

5.4 Statistical analysis

A two-way analysis of variance (ANOVA), followed by a Newman-Keuls test was chosen for analyzing the data of the present study. Since the ANOVA is a parametric test, it requires normally distributed data with an equal variance and this was tested using Normal probability plots with Shapiro-Wilks test and Levene's test for homogeneity of variance. Normal probability plots provide a quick way to visually inspect the pattern of data and see if they follow a normal distribution. The selected variable is thus plotted in a scatterplot against the values "expected from the normal distribution." If the observed values (plotted on the x-axis) are normally distributed, then all values should fall onto a straight line in the plot. If the values are not normally distributed, they will deviate from the line (Statistica, StatSoft, Inc., Tulsa, USA). To more easily assess the data a Shapiro-Wilks test was added to the normality plot, as this test produces a p-value where a value below 0.05 was considered significant and a normal distribution is rejected. The Levene's test was used, as it is a powerful way to check for homogeneity of variances. If the Levene's test is statistically significant, then the hypothesis of homogeneous variances should be rejected. All data used was normally distributed, but some data gave a significant result when tested for homogeneity of variance. To reduce variance, the body weight and relative NKAα1b gene expression data from the smolting period and relative NKAα1a and NKAα1b gene expression and GSI values from the maturation period were log transformed (Zar, 1996). Also, outliers in the NKAα1b and NKAα1a data were removed using the method of 2

standard deviation as the limit for outliers. After log transformation, some test groups still gave a significant result in the Levene's test (appendix III) and although nonparametric tests do not make assumptions about the nature of the distribution of the samples, parametric tests are more powerful than nonparametric ones as the latter will have a greater probability in committing a Type II error (Zar, 1996). Furthermore, the homogeneity of variances assumption is usually not as crucial as other assumptions for ANOVA (Zar, 1996). Thus, parametric tests were used for all groups, as commonly employed test as ANOVA are robust enough to allow us to disregard all but severe deviations from the theoretical assumptions (Zar, 1996). For GSI during the maturation stage, only MM were analyzed and differences between photoperiod/salinity groups were compared instead of gender/maturation differences.

Newman-Keuls test was used following a two-way ANOVA to detect differences between NF and MM in each sampling and to detect differences in samplings in reference to the first sampling. The Newman-Keuls test was chosen over Tukey test since it tends to detect more significant differences than the Tukey procedure. The Newman-Keuls test is therefore considered more powerful, although statisticians dispute over the merit of these tests.

Prior to the main analysis, a one-way ANOVA was performed on the mentioned response variables in materials and methods (section 2.11) within the sampled tanks from each sampling during the smolting stage. All tanks in the smolting stage were treated equally and the one-way ANOVA was utilized to detect potential differences between tanks in each sampling. Only one tank in sampling 1 and one tank in sampling 2 gave significant results in NKA activity and thus differed from the three other sampled tanks (appendix III). Two significantly different tanks in one of five response variables were not considered enough of a difference to discard all tanks from being one group during the smolting stage.

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Appendix I

Buffers

The Sørensen buffer consists of:

24 g of $Na_2H_2PO_4$ (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 1000 ml H_2O (solution 1) and 27.2 g Na_2HP_2 (Sigma-Aldrich, St. Louis, MO, USA) in 1000 ml H_2O (solution 2).

28 ml of solution 1 is then mixed with 72 ml of solution 2, resulting in a 0.2 M solution. Mix 500 ml of 0.2 M with 500 ml to get 1 L of 0.1 M Sørensen buffer.

Sørensen buffer consist of:

250 mM sucrose, 10 mM Na2–EDTA, 50 mM Mimidazole at pH 7.3

Appendix II

Efficiency data from the dilution series

Table A.II.1: Efficiency data of the dilution series from each plate for EF1α**.**

Plate no.	Slope	R2	Mean efficiency
Plate 1	-3.627	0.998	1.964
Plate 2	-3.505	0.996	1.964
Plate 3	-3.299	0.992	1.964
Plate 4	-3.303	0.982	1.964
Plate 5	-3.235	0.998	1.964
Plate 6	-3.379	0.998	1.964
Plate 7	-3.430	0.998	1.964
Plate 8	-3.544	0.997	1.964

Table A.II.2: Efficiency data of the dilution series from each plate for NKAα1b

Plate no.	Slope	R2	Efficiency
Plate 1	-3.371	0.979	1.980
Plate 2	-3.724	0.996	1.856
Plate 3	-3.628	0.991	1.886
Plate 4	-3.685	0.985	1.868
Plate 5	-3.426	0.992	1.958
Plate 6	-3.490	0.996	1.934
Plate 7	-3.474	0.997	1.940

Table A.II.3: Efficiency data of the dilution series from each plate for NKAα1a

Plate no.	Slope	R2	Efficiency
Plate 1	-3.270	0.994	2.022
Plate 2	-3.166	0.999	2.069
Plate 3	-3.155	0.998	2.075
Plate 4	-3.389	0.994	1.973
Plate 5	-3.661	0.995	1.876
Plate 6	-3.537	0.996	1.917
Plate 7	-3.269	0.996	2.023

Appendix III

Statistics

Test for homogeneity of variance sampling 1-4

Table A.III.1: Levene's Test for Homogeneity of Variances for **Fork length** (cm) for males and females in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

Gender	MS effect	Ms error	F	p
Males	8,675198	2,297765	3,775494	**0,018737**
Females	3,762528	0,772774	4,868861	**0,006064**

Table A.III.2: Levene's Test for Homogeneity of Variances for **Fork length** (cm) for each sampling. Significant values (p<0.05) are highlighted in bold.

Sampling	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
1	1,652836	1	1,652836	5,475984	18	0,304221	5,433004	**0,031586**
2	0,050000	1	0,050000	15,28200	18	0,849000	0,058893	0,810998
3	3,784500	1	3,784500	38,39600	18	2,133111	1,774169	0,199488
4	5,852083	1	5,852083	51,38542	18	2,854745	2,049949	0,169347

Table A.III.3: Levene's Test for Homogeneity of Variances for **Log Body weight** for males and females in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

Gender	MS effect	Ms error	F	p
Males	0,018335	0,005184	3,536928	**0,024152**
Females	0,004768	0,001964	2,428316	0,081236

Table A.III.4: Levene's Test for Homogeneity of Variances for **Log Body weight** for each sampling. Significant values (p<0.05) are highlighted in bold.

Sampling	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
1	0,004489	1	0,004489	0,014748	18	0,000819	5,478459	**0,030967**
2	0,000161	1	0,000161	0,032774	18	0,001821	0,088389	0,769639
3	0,007012	1	0,007012	0,081874	18	0,004549	1,541544	0,230324

4	0,023263	1	0,023263	0,127908	18	0,007106	3,273692	0,087131

Table A.III.5: Levene's Test for Homogeneity of Variances for **GSI** for males and females in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

Gender	MS effect	Ms error	F	p
Males	0,000140	0,000060	2,333036	0,090364
Females	0,000137	0,000042	3,279801	**0,031847**

Table A.III.6: Levene's Test for Homogeneity of Variances for **GSI** for males and females in each sampling. Significant values (p<0.05) are highlighted in bold.

Sampling	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
1	0,000199	1	0,000199	0,000515	18	0,000029	6,942465	**0,016818**
2	0,000222	1	0,000222	0,000699	18	0,000039	5,715081	**0,027959**
3	0,000001	1	0,000001	0,000861	18	0,000048	0,024640	0,877015
4	0,000031	1	0,000031	0,001590	18	0,000088	0,355134	0,558641

Table A.III.7: Levene's Test for Homogeneity of Variances for **Condition factor** for males and females in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

Gender	MS effect	Ms error	F	p
Males	0,002307	0,002670	0,864030	0,468608
Females	0,000280	0,003316	0,084588	0,968018

Table A.III.8: Levene's Test for Homogeneity of Variances for **Condition factor** for males and females in each sampling. Significant values (p<0.05) are highlighted in bold.

Sampling	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
1	0,000189	1	0,000189	0,046486	18	0,002583	0,073074	0,789987
2	0,000746	1	0,000746	0,024299	18	0,001350	0,552460	0,466906
3	0,000323	1	0,000323	0,043052	18	0,002392	0,135099	0,717489
4	0,007310	1	0,007310	0,037354	17	0,002197	3,327068	0,085777

Table A.III.9: Levene's Test for Homogeneity of Variances for **NKA activity** for males and females in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

Gender	MS effect	Ms error	F	p
Males	1,544946	1,431214	1,079466	0,370070
Females	0,112376	28,46134	0,138193	0,936509

Table A.III.10: Levene's Test for Homogeneity of Variances for **NKA activity** for males and females in each sampling. Significant values (p<0.05) are highlighted in bold.

Sampling	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
1	2,026868	1	2,026868	31,18766	18	1,732648	1,169809	0,293718
2	0,007015	1	0,007015	16,79023	18	0,932790	0,007520	0,931853
3	0,215421	1	0,215421	10,03808	18	0,557671	0,386287	0,542050
4	1,241090	1	1,241090	21,96908	17	1,292299	0,960374	0,340834

Table A.III.11: Levene's Test for Homogeneity of Variances for **Log NKA1b** for males and females in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

Gender	MS effect	Ms error	F	p
Males	92,77396	4,882840	0,824139	0,496711
Females	10,28135	3,551550	2,894890	0,062073

Table A.III.12: Levene's Test for Homogeneity of Variances for **Log NKA1b** for males and females in each sampling. Significant values (p<0.05) are highlighted in bold.

Sampling	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
1	4,811988	1	4,811988	17,83419	10	1,783419	2,698181	0,131493
2	3,641917	1	3,641917	8,806350	9	0,978483	3,722002	0,085776
3	0,258794	1	0,258794	93,98040	9	10,44227	0,024783	0,878384
4	1,515986	1	1,515986	39,63247	10	3,963247	0,382511	0,550089

Table A.III.13: Levene's Test for Homogeneity of Variances for **NKA1a** for males and females in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

	MS	MS	F	p
Gender	Effect	Error		
Males	0,602387	0,279776	2,153106	0,125437
Females	0,080182	0,035896	2,233729	0,117398

Table A.III.14: Levene's Test for Homogeneity of Variances for **NKA1a** for males and females in each sampling. Significant values (p<0.05) are highlighted in bold.

Sampling	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
1	0,022516	1	0,022516	0,266994	10	0,026699	0,843327	0,380064
2	0,026528	1	0,026528	0,341812	9	0,037979	0,698479	0,424927
3	0,544923	1	0,544923	4,001200	10	0,400120	1,361900	0,270283
4	0,897861	1	0,897861	1,667537	10	0,166754	5,384356	**0,042742**

Test for homogeneity of variance sampling 5-8

Table A.III.15: Levene's Test for Homogeneity of Variances for **Fork length** (cm) in sampling 5-8. Significant values (p<0.05) are highlighted in bold.

Sampling	SS	df	MS	SS	df	MS	F	p
5	2,904771	2	1,452385	125,5128	76	1,651484	0,879443	0,419194
6	15,79527	2	7,897633	263,4321	76	3,466212	2,278463	0,109397
7	4,331948	2	2,165974	172,1379	77	2,235557	0,968874	0,384088
8	0,782911	2	0,391455	415,8075	156	2,665432	0,146864	0,863531

Table A.III.16: Levene's Test for Homogeneity of Variances for **Fork length** (cm) for immature females (NF), immature males (NM) and mature males (MM) divided into the four treatment groups (SWLL, SWLD, FWLL and FWLD). Significant values (p<0.05) are highlighted in bold.

	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
SWLL-NF	31,91821	3	10,63940	156,3051	53	2,949152	3,607614	**0,019090**
SWLL-MM	15,59443	3	5,198143	69,84767	32	2,182740	2,381476	0,087847

SWLD-NF	15,36644	3	5,122146	79,33959	52	1,525761	3,357109	**0,025643**
SWLD-MM	5,002303	3	1,667434	30,65694	25	1,226278	1,359753	0,277881
FWLL-NF	5,566308	3	1,855436	144,4670	53	2,725792	0,680696	0,567765
FWLL-MM	4,136557	3	1,378852	56,48129	29	1,947631	0,707964	0,555085
FWLD-NF	23,56311	3	7,854369	70,29944	41	1,714620	4,580821	**0,007413**
FWLD-MM	10,39025	3	3,463417	60,62380	34	1,783053	1,942409	0,141321

Table A.III.17: Levene's Test for Homogeneity of Variances for **Body weight** (g) in sampling 5-8. Significant values ($p<0.05$) are highlighted in bold.

	SS	df	MS	SS	df	MS	F	p
Sampling 5	17465,12	2	8732,560	315068,1	76	4145,632	2,106448	0,128717
Sampling 6	18099,79	2	9049,897	871426,1	77	11317,22	0,799657	0,453180
Sampling 7	33545,95	2	16772,98	840077,8	77	10910,10	1,537381	0,221468
Sampling 8	47885,80	2	23942,90	2129804	157	13565,63	1,764967	0,174572

Table A.III.18: Levene's Test for Homogeneity of Variances for **Body weight** (g) for immature females (NF), immature males (NM) and mature males (MM) divided into the four treatment groups (SWLL, SWLD, FWLL and FWLD). Significant values ($p<0.05$) are highlighted in bold.

	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
SWLL-NF	173165,4	3	57721,82	657875,7	53	12412,75	4,650204	**0,005859**
SWLL-MM	51073,60	3	17024,53	289273,0	33	8765,848	1,942143	0,141979
SWLD-NF	82644,60	3	27548,20	428804,0	52	8246,232	3,340702	**0,026134**
SWLD-MM	59847,33	3	19949,11	134127,9	26	5158,766	3,867031	**0,020642**
FWLL-NF	97441,66	3	32480,55	531581,3	53	10029,84	3,238393	**0,029253**
FWLL-MM	14461,39	3	4820,464	230324,1	29	7942,210	0,606942	0,615846
FWLD-NF	208161,7	3	69387,23	399218,2	41	9737,030	7,126118	**0,000583**
FWLD-MM	34123,18	3	11374,39	284523,5	34	8368,338	1,359217	0,271740

Table A.III.19: Levene's Test for Homogeneity of Variances for **Log Gonadosomatic index** in sampling 5-8. Significant values ($p<0.05$) are highlighted in bold.

	SS	df	MS	SS	df	MS	F	p
Sampling 5	2,076043	2	1,038022	2,320874	77	0,030141	34,43861	**0,000000**
Sampling 6	0,072110	2	0,036055	0,856861	77	0,011128	3,240013	**0,044562**
Sampling 7	0,000348	2	0,000174	0,607541	77	0,007890	0,022077	0,978171
Sampling 8	0,176608	2	0,088304	1,599240	157	0,010186	8,668933	**0,000268**

Table A.III.20: Levene's Test for Homogeneity of Variances for **Log Gonadosomatic index** for immature females (NF), immature males (NM) and mature males (MM) divided into the four treatment groups (SWLL, SWLD, FWLL and FWLD). Significant values ($p<0.05$) are highlighted in bold.

	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
SWLL-NF	0,046260	3	0,015420	0,479601	53	0,009049	1,704034	0,177321
SWLL-MM	0,011621	3	0,003874	0,074574	33	0,002260	1,714150	0,183139
SWLD-NF	0,014035	3	0,004678	0,166630	52	0,003204	1,460004	0,236182
SWLD-MM	0,008260	3	0,002753	0,089010	26	0,003423	0,804233	0,502901
FWLL-NF	0,011878	3	0,003959	0,305536	53	0,005765	0,686832	0,564082
FWLL-MM	0,084517	3	0,028172	0,116298	29	0,004010	7,025069	**0,001083**
FWLD-NF	0,033923	3	0,011308	0,103021	42	0,002453	4,609974	**0,007073**
FWLD-MM	0,144940	3	0,048313	0,246418	34	0,007248	6,666138	**0,001162**

Table A.III.21: Levene's Test for Homogeneity of Variances for **Conditon factor** in sampling 5-8. Significant values (p<0.05) are highlighted in bold.

	SS	df	MS	SS	df	MS	F	p
Sampling 5	0,007874	2	0,003937	0,242705	77	0,003152	1,248973	0,292544
Sampling 6	0,003785	2	0,001892	0,248806	76	0,003274	0,578015	0,563457
Sampling 7	0,057144	2	0,028572	0,450247	77	0,005847	4,886283	**0,010050**
Sampling 8	0,023620	2	0,011810	1,146473	156	0,007349	1,607010	0,203787

Table A.III.22: Levene's Test for Homogeneity of Variances for **Condition Factor** for immature females (NF), immature males (NM) and mature males (MM) divided into the four treatment groups (SWLL, SWLD, FWLL and FWLD). Significant values (p<0.05) are highlighted in bold.

	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
SWLL-NF	0,050600	3	0,016867	0,289025	53	0,005453	3,092905	**0,034648**
SWLL-MM	0,003797	3	0,001266	0,114168	33	0,003460	0,365812	0,778114
SWLD-NF	0,014801	3	0,004934	0,207587	52	0,003992	1,235868	0,306106
SWLD-MM	0,051000	3	0,017000	0,290290	26	0,011165	1,522618	0,232121
FWLL-NF	0,020664	3	0,006888	0,230389	53	0,004347	1,584566	0,203992
FWLL-MM	0,015231	3	0,005077	0,063814	29	0,002200	2,307184	0,097430
FWLD-NF	0,014346	3	0,004782	0,056442	42	0,001344	3,558508	**0,022066**
FWLD-MM	0,006326	3	0,002109	0,127019	34	0,003736	0,564404	0,642214

Table A.III.23: Levene's Test for Homogeneity of Variances for **NKA activity** in sampling 5-8. Significant values (p<0.05) are highlighted in bold.

	SS	df	MS	SS	df	MS	F	p
Sampling 5	1,306487	1	1,306487	133,2556	67	1,988889	0,656893	0,420529
Sampling 6	11,73313	1	11,73313	177,5444	66	2,690067	4,361652	**0,040618**
Sampling 7	1,565574	1	1,565574	122,2275	67	1,824291	0,858182	0,357573
Sampling 8	19,58626	1	19,58626	378,1196	143	2,644193	7,407274	**0,007304**

Table A.III.24: Levene's Test for Homogeneity of Variances for **NKA activity** for immature females (NF) and mature males (MM) divided into the four treatment groups (SWLL, SWLD, FWLL and FWLD). Significant values (p<0.05) are highlighted in bold.

	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
SWLL-NF	18,78566	3	6,261887	129,4137	53	2,441767	2,564490	0,064333
SWLL-MM	5,183934	3	1,727978	25,43454	36	0,706515	2,445777	0,079668
SWLD-NF	7,933492	3	2,644497	85,29737	52	1,640334	1,612170	0,197751
SWLD-MM	4,325041	3	1,441680	28,29737	26	1,088360	1,324635	0,287730
FWLL-NF	0,928330	3	0,309443	48,19096	53	0,909263	0,340323	0,796241
FWLL-MM	4,494040	3	1,498013	8,601093	28	0,307182	4,876633	**0,007488**
FWLD-NF	4,791144	3	1,597048	12,76461	41	0,311332	5,129728	**0,004183**
FWLD-MM	0,450407	3	0,150136	9,858801	33	0,298752	0,502544	0,683152

Table A.III.25: Levene's Test for Homogeneity of Variances for **Log NKA1b** in sampling 5-8. Significant values (p<0.05) are highlighted in bold.

	SS	df	MS	SS	df	MS	F	p
Sampling 5	0,081521	1	0,081521	1,788712	36	0,049686	1,640700	0,208420
Sampling 6	0,230852	1	0,230852	2,374802	41	0,057922	3,985572	0,052561
Sampling 7	0,164442	1	0,164442	1,926703	41	0,046993	3,499314	0,068544

Sampling 8	0,000852	1	0,000852	1,037856	43	0,024136	0,035284	0,851885

Table A.III.26: Levene's Test for Homogeneity of Variances for **Log NKA1b** for immature females (NF) and mature males (MM) divided into the four treatment groups (SWLL, SWLD, FWLL and FWLD). Significant values ($p<0.05$) are highlighted in bold.

	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
SWLL-NF	0,079064	3	0,026355	0,448055	18	0,024892	1,058759	0,391085
SWLL-MM	0,687588	3	0,229196	0,892454	19	0,046971	4,879500	**0,011097**
SWLD-NF	0,075163	3	0,025054	0,363592	20	0,018180	1,378163	0,278264
SWLD-MM	0,072022	3	0,024007	0,780368	17	0,045904	0,522990	0,672268
FWLL-NF	0,156656	3	0,052219	0,243796	16	0,015237	3,427049	**0,042590**
FWLL-MM	0,024978	3	0,008326	0,204448	12	0,017037	0,488690	0,696568
FWLD-NF	0,259738	3	0,086579	0,324620	16	0,020289	4,267360	**0,021512**
FWLD-MM	0,214715	3	0,071572	0,336338	19	0,017702	4,043144	**0,022169**

Table A.III.27: Levene's Test for Homogeneity of Variances for **Log NKA1a** in sampling 5-8. Significant values ($p<0.05$) are highlighted in bold.

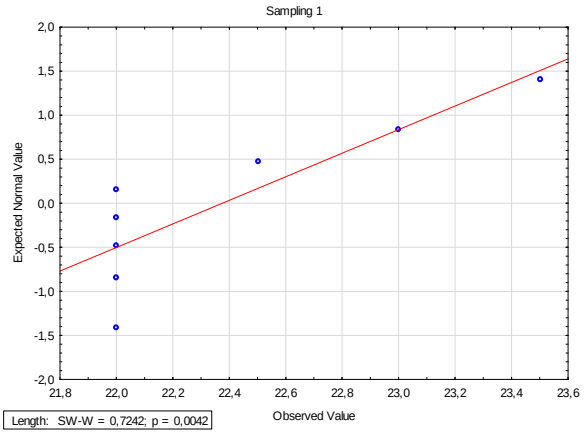
	SS	df	MS	SS	df	MS	F	p
Sampling 5	0,102895	1	0,102895	16,25055	36	0,451404	0,227945	0,635938
Sampling 6	1,174613	1	1,174613	9,229181	41	0,225102	5,218138	**0,027600**
Sampling 7	0,202780	1	0,202780	5,612415	42	0,133629	1,517488	0,224853
Sampling 8	0,163618	1	0,163618	9,219186	44	0,209527	0,780893	0,381671

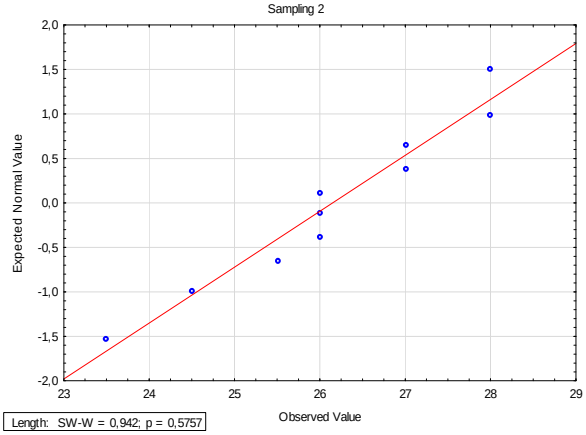
Table A.III.28: Levene's Test for Homogeneity of Variances for **Log NKA1a** for immature females (NF) and mature males (MM) divided into the four treatment groups (SWLL, SWLD, FWLL and FWLD). Significant values ($p<0.05$) are highlighted in bold.

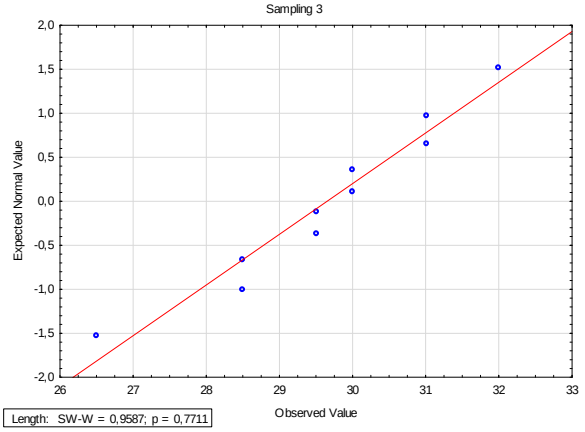
	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
SWLL-NF	1,677023	3	0,559008	5,274615	18	0,293034	1,907653	0,164546
SWLL-MM	1,703041	3	0,567680	3,194033	20	0,159702	3,554631	**0,032824**
SWLD-NF	1,033000	3	0,344333	1,684961	20	0,084248	4,087138	**0,020461**
SWLD-MM	0,314422	3	0,104807	2,552919	17	0,150172	0,697917	0,566071
FWLL-NF	0,008807	3	0,002936	0,275224	17	0,016190	0,181339	0,907591
FWLL-MM	0,326583	3	0,108861	0,245066	12	0,020422	5,330530	**0,014466**
FWLD-NF	0,385390	3	0,128463	0,709037	17	0,041708	3,080060	0,055454
FWLD-MM	0,242144	3	0,080715	0,919493	18	0,051083	1,580068	0,228947

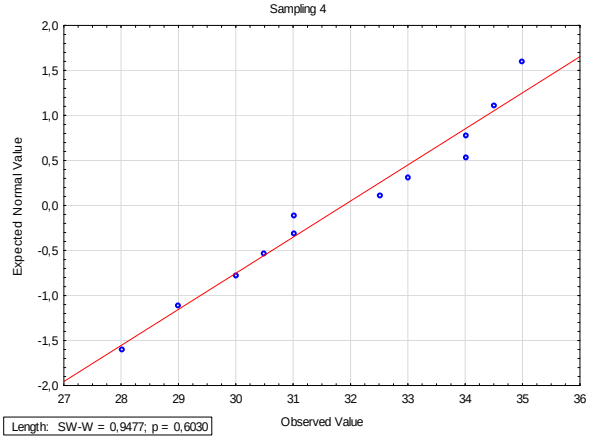
Test for normality, sampling 1- 4

Normal probability plot of residual for Fork length of females in sampling 1, 2, 3 and 4:

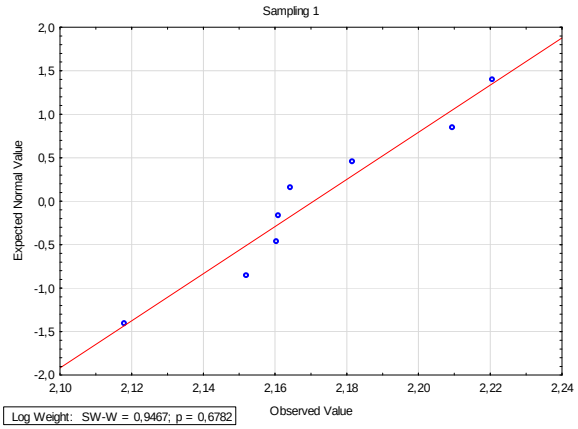


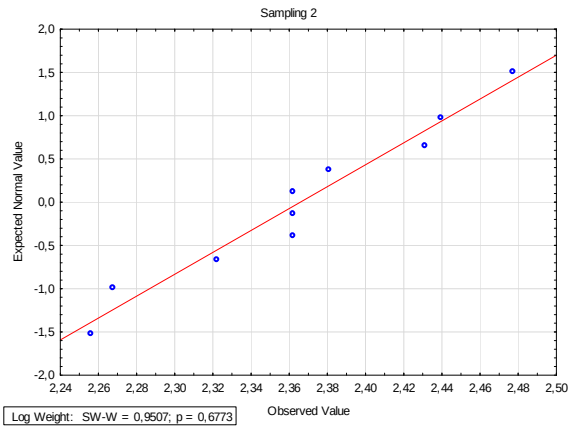


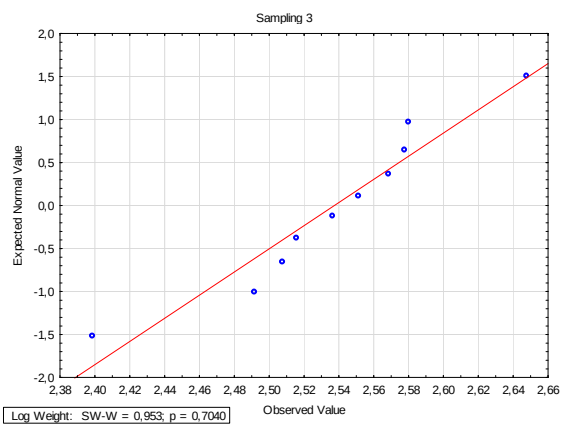


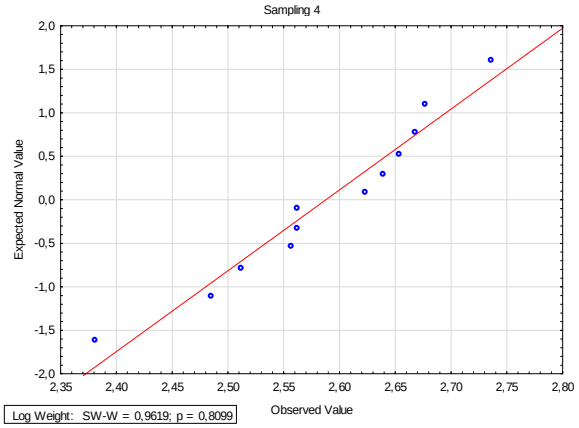


Normal probability plot of residual for Log body weight of females in sampling 1, 2, 3 and 4:

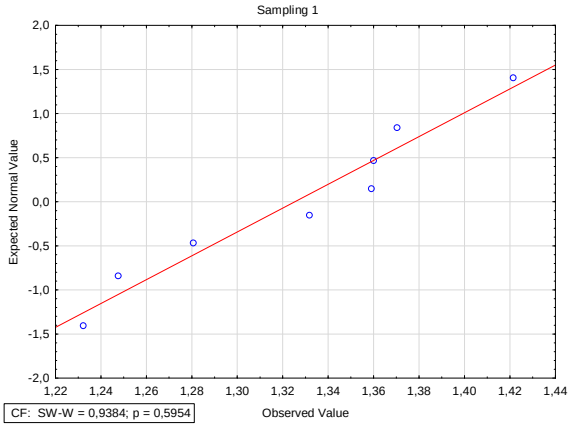


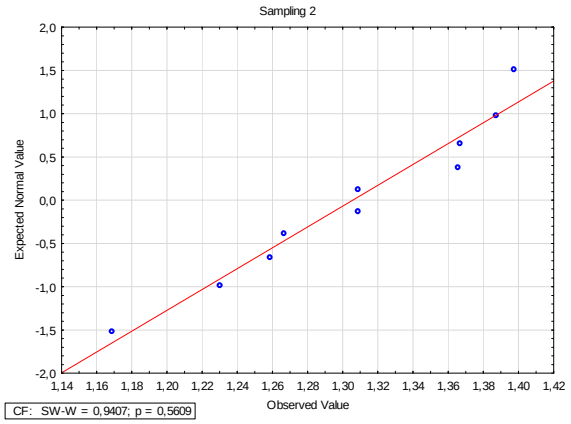


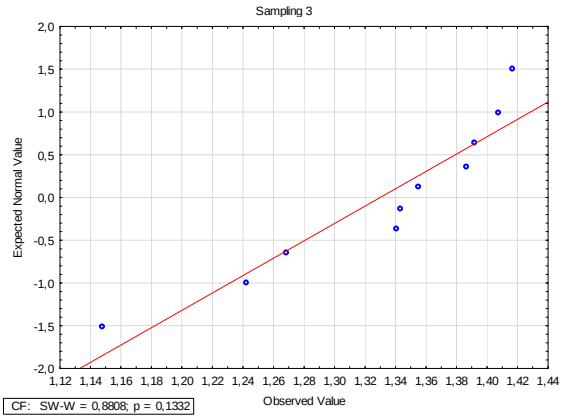


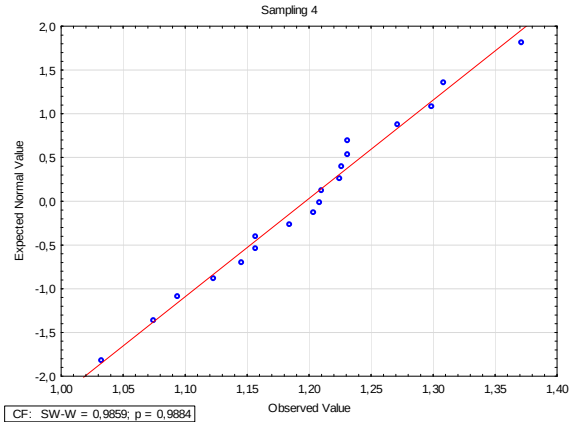


Normal probability plot of residual for Condition Factor of females in sampling 1, 2, 3 and 4:

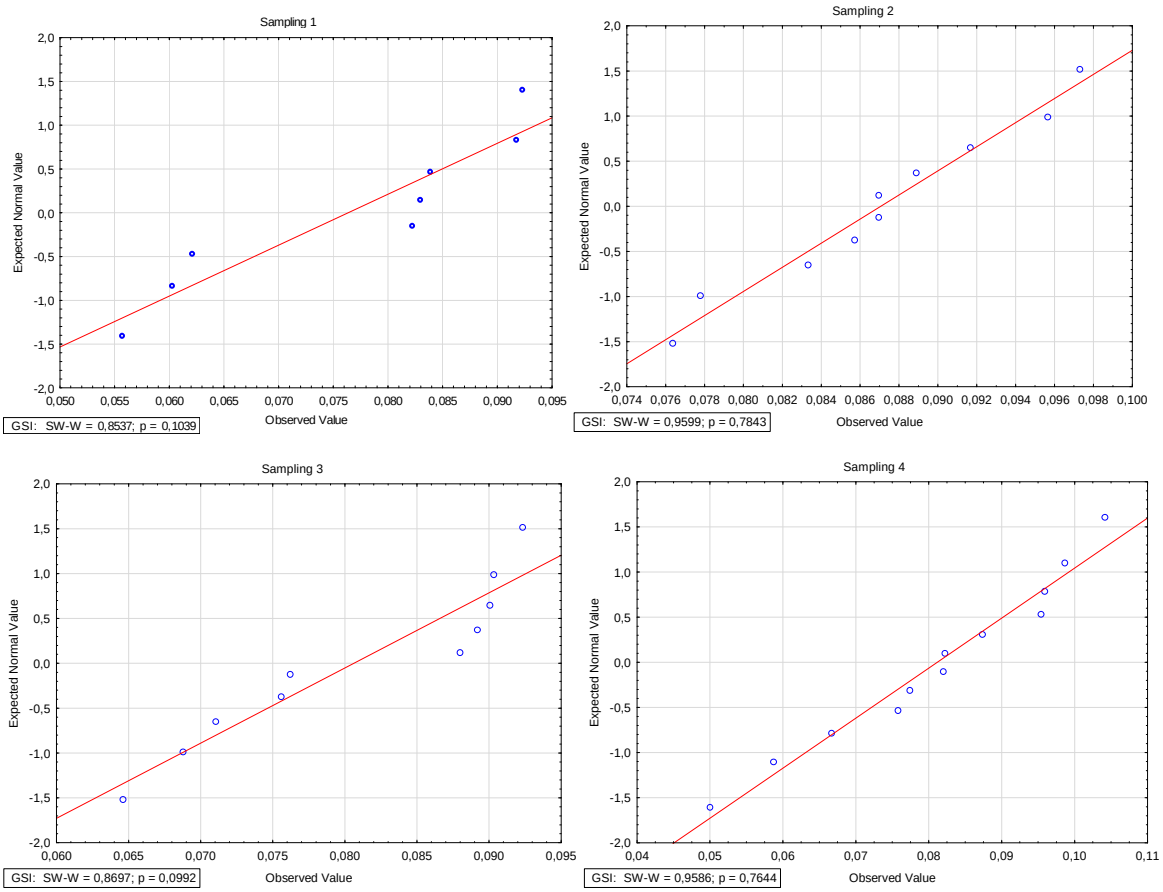




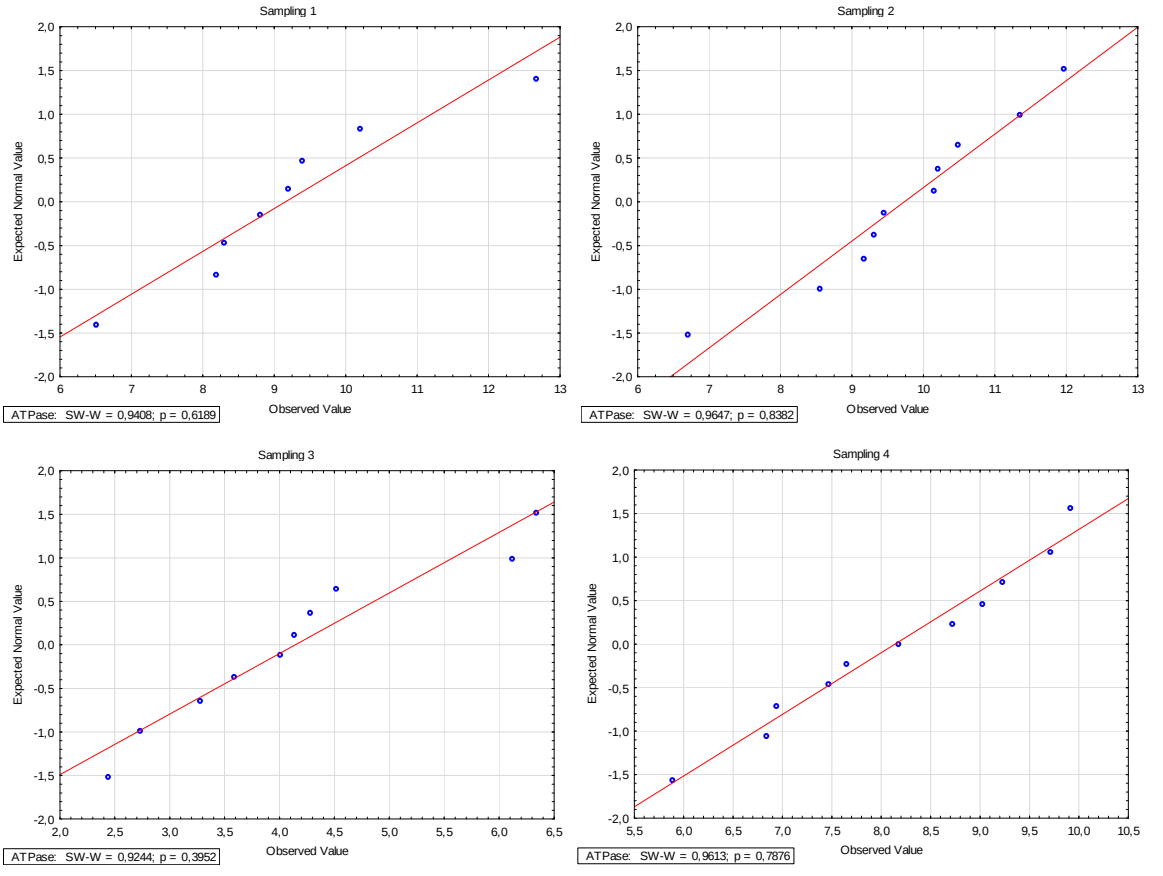




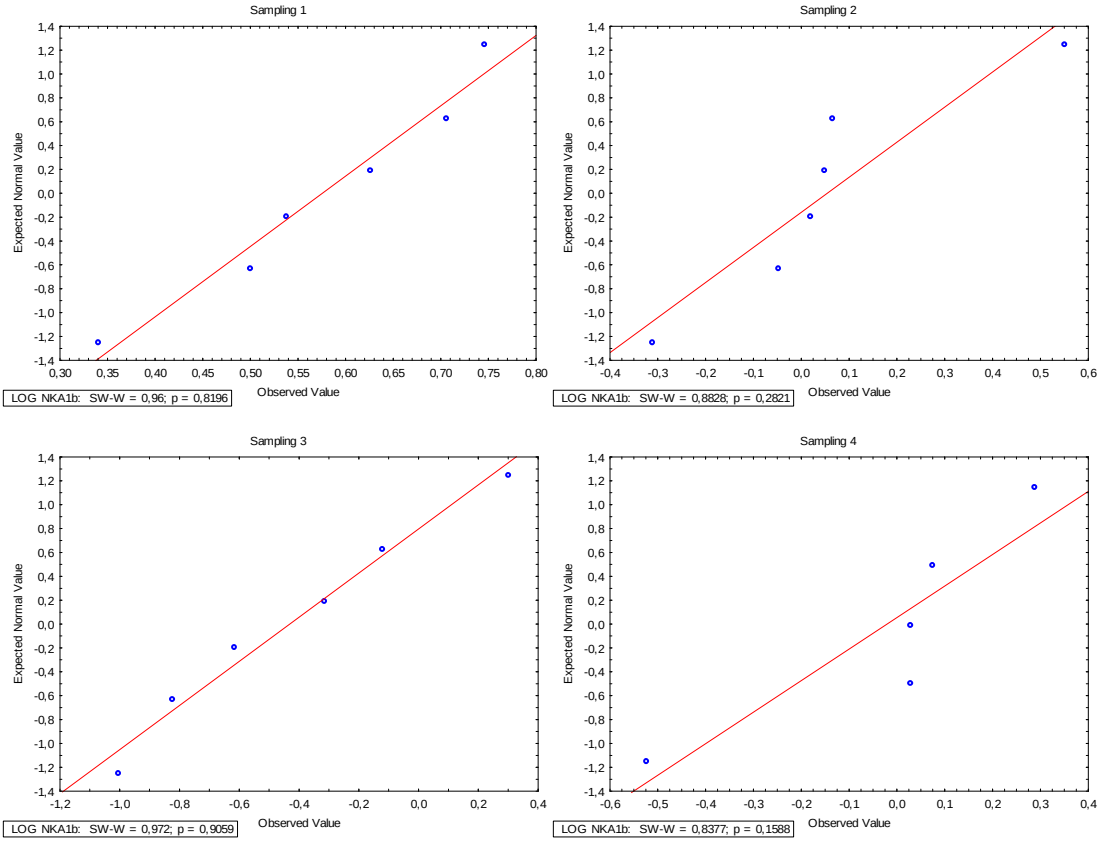
Normal probability plot of residual for GSI of females in sampling 1, 2, 3 and 4:



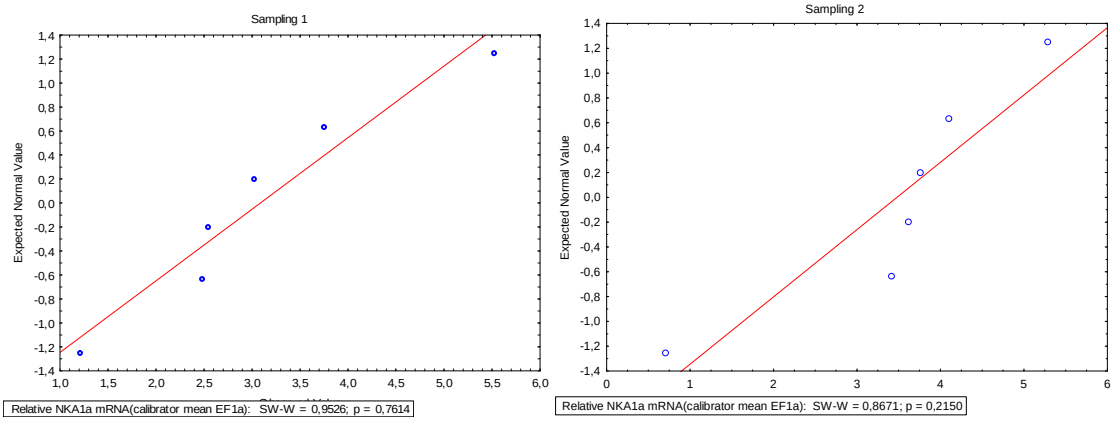
Normal probability plot of residual for NKA activity of females in sampling 1, 2, 3 and 4:

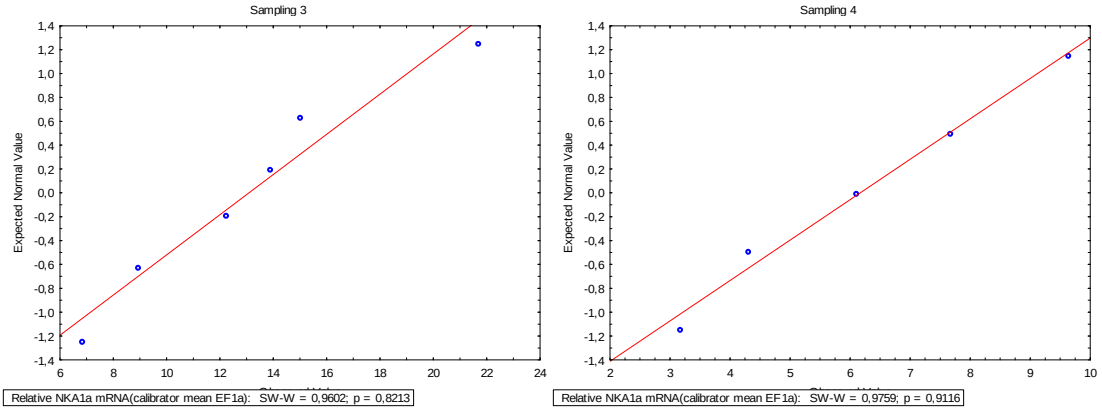


Normal probability plot of residual for Log NKA1b of females in sampling 1, 2, 3 and 4:

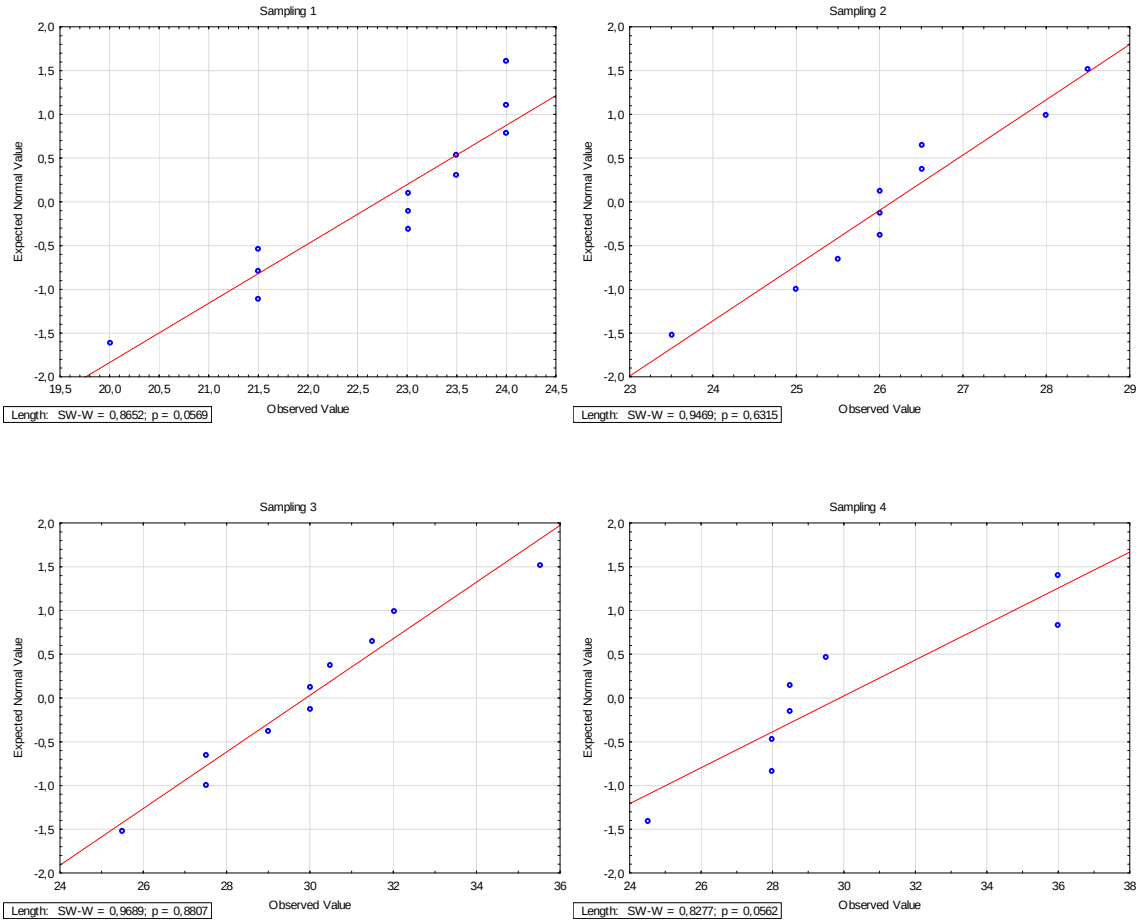


Normal probability plot of residual for NKA1a of females in sampling 1, 2, 3 and 4:

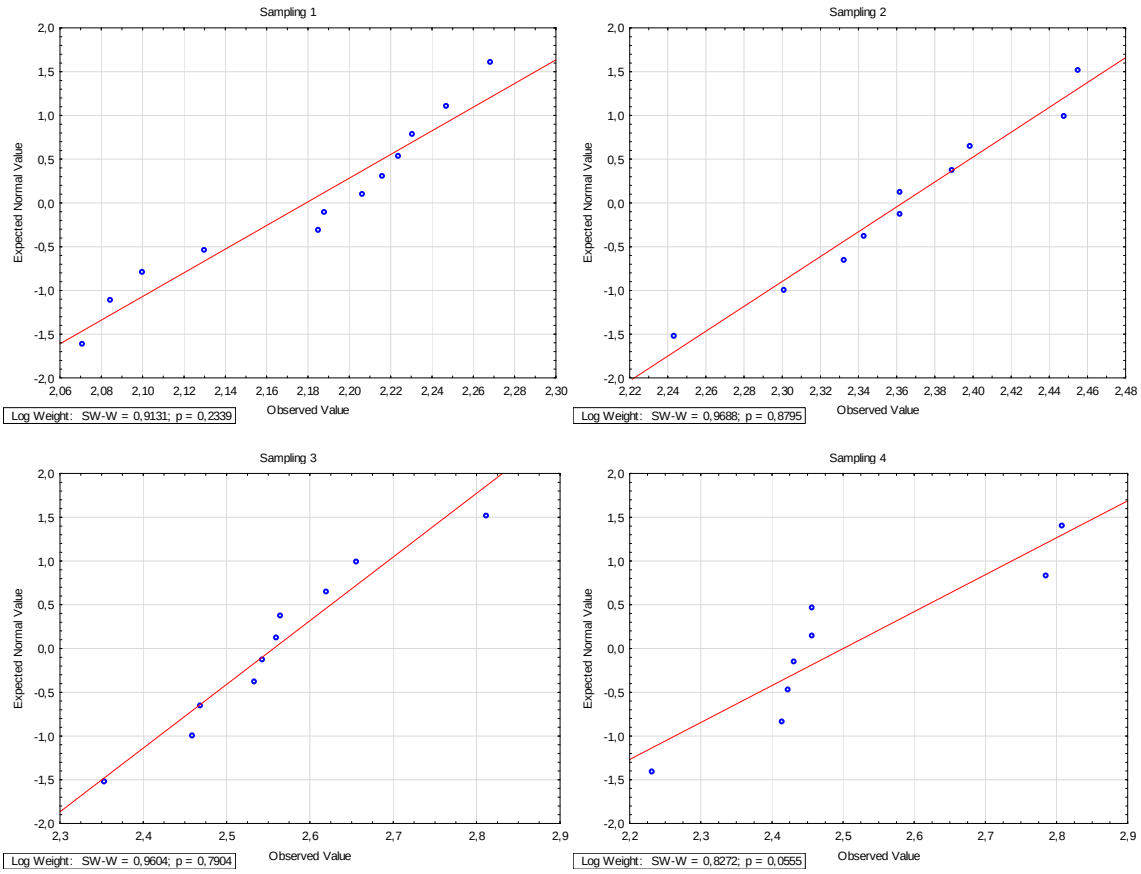




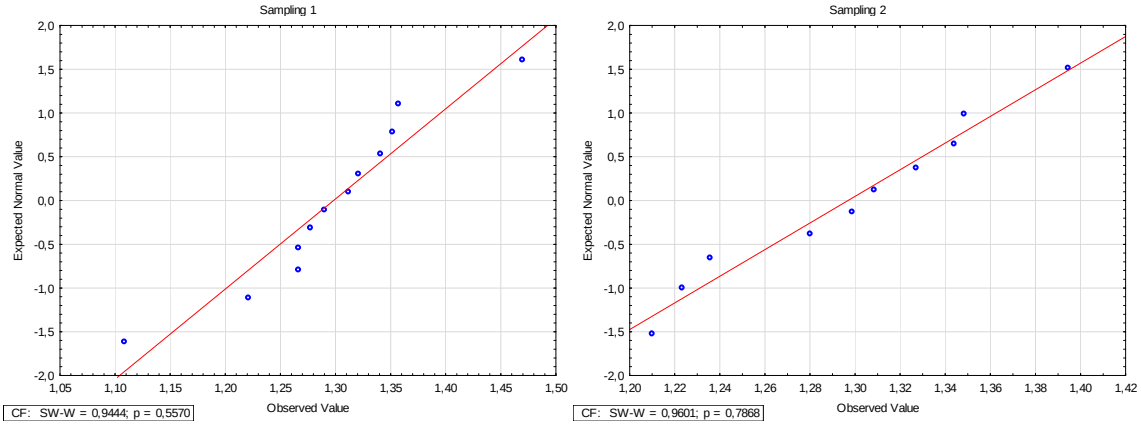
Normal probability plot of residual for Fork length of males in sampling 1, 2, 3 and 4:

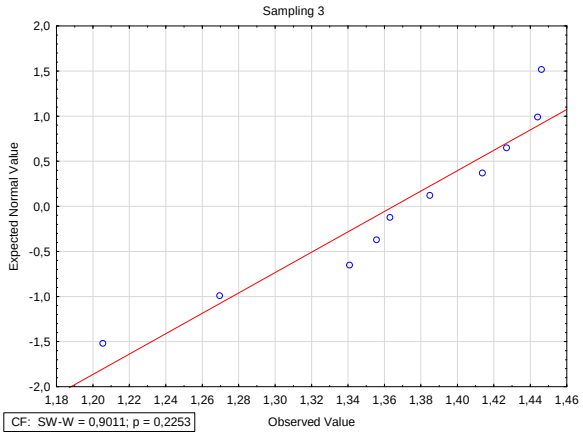


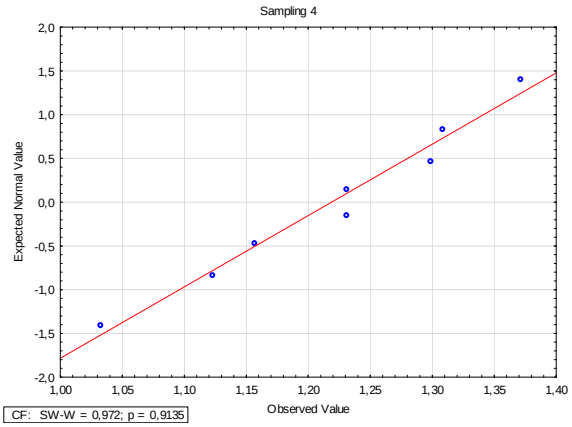
Normal probability plot of residual for Log body weight of males in sampling 1, 2, 3 and 4:



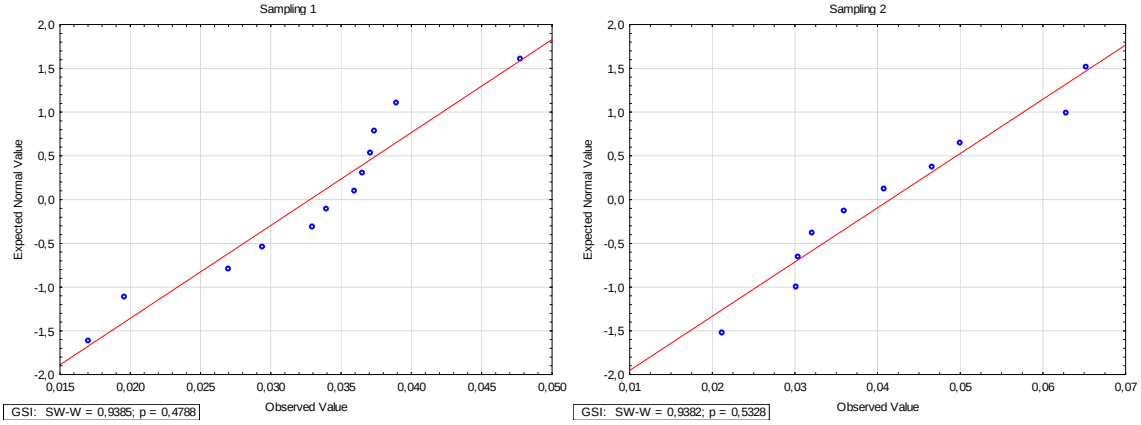
Normal probability plot of residual for Condition Factor of males in sampling 1, 2, 3 and 4:

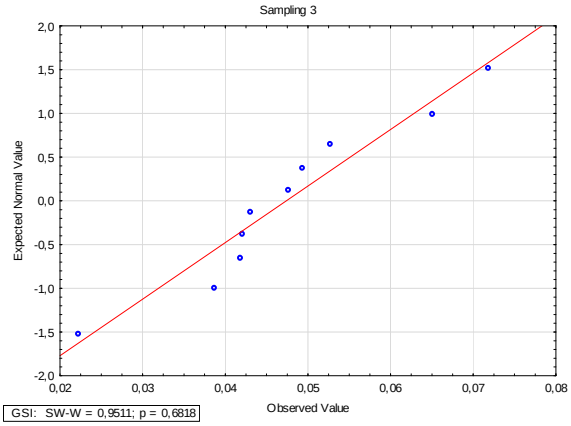


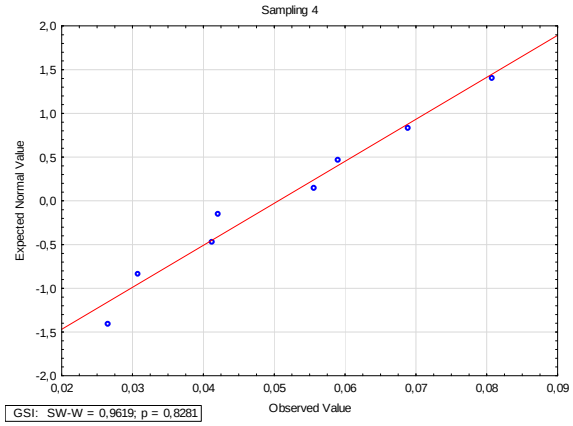




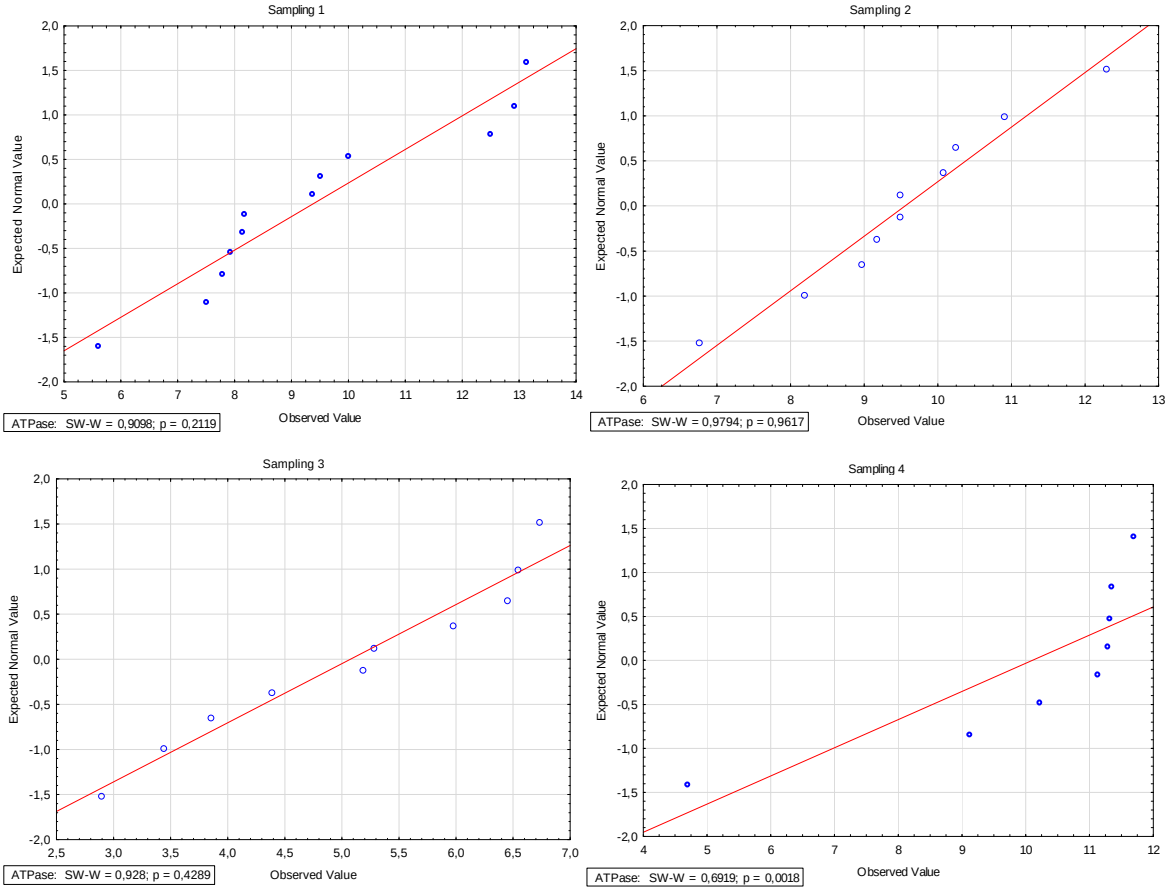
Normal probability plot of residual for GSI of males in sampling 1, 2, 3 and 4:



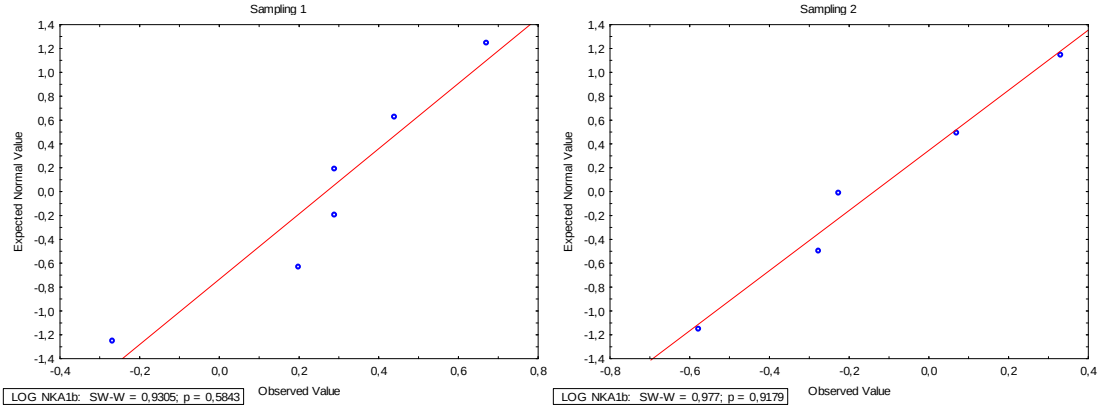


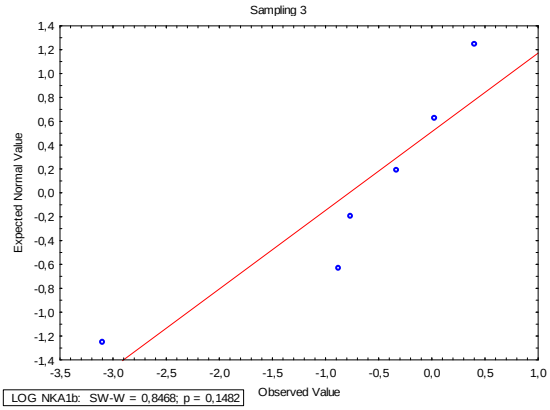


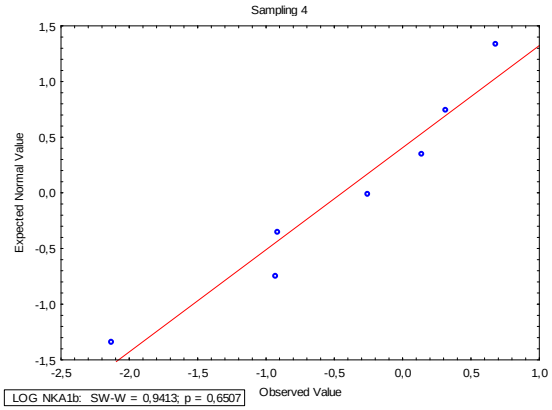
Normal probability plot of residual for NKA activity of males in sampling 1, 2, 3 and 4:



Normal probability plot of residual for log NKA1b of males in sampling 1, 2, 3 and 4:



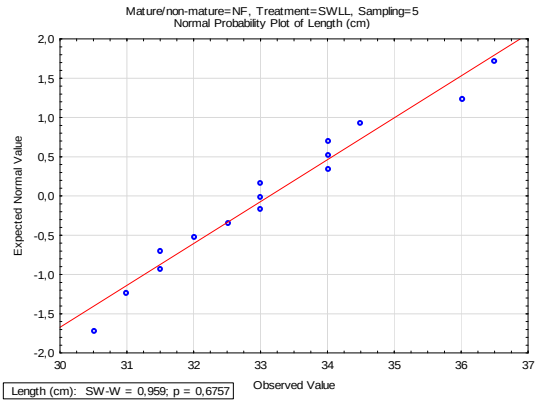


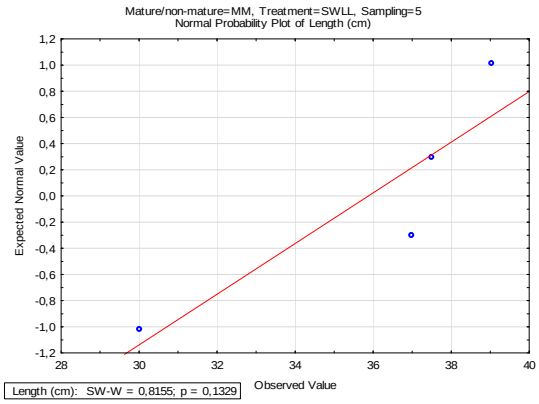


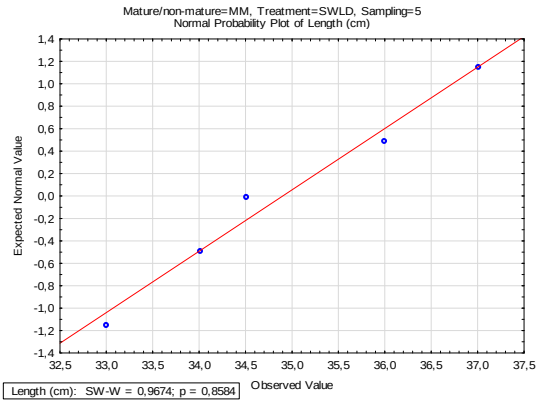
Normal probability plot of residual for NKA1a of males in sampling 1, 2, 3 and 4:

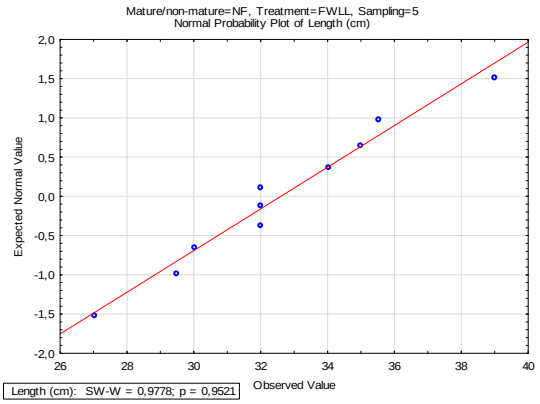
Test for normality sampling 5 - 8

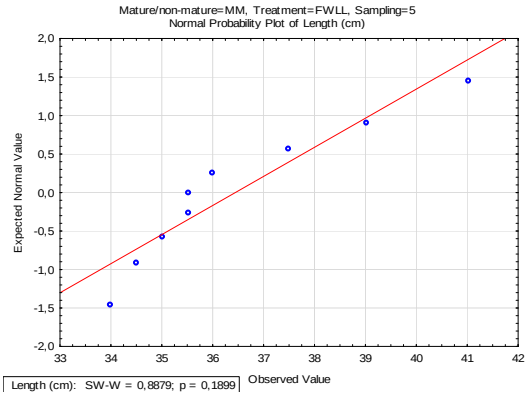
Normal probability plot of residual for fork length divided into samplings 5-8, treatment groups (SWLL, SWLD, FWLL and FWLD) and maturation category (NF and MM):

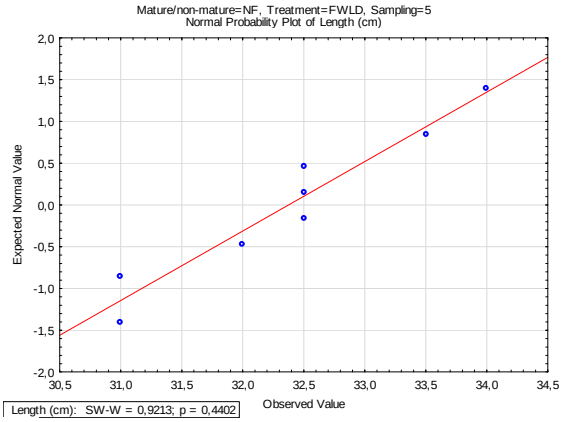


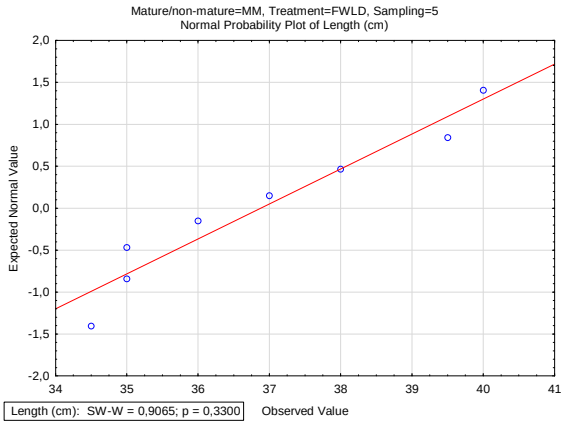


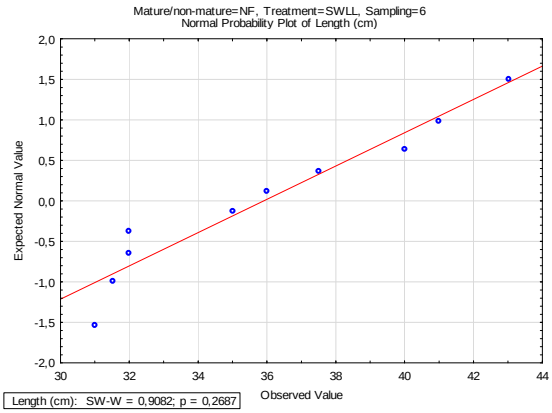


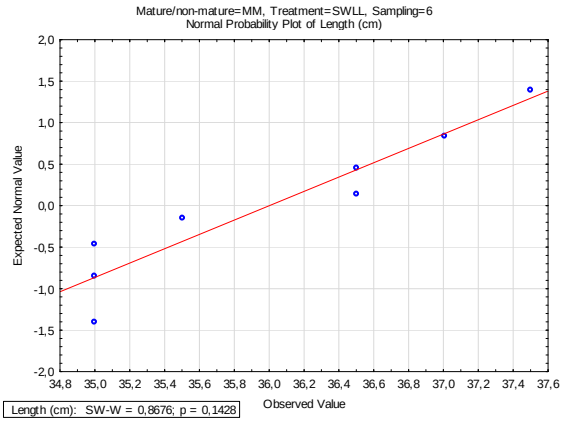


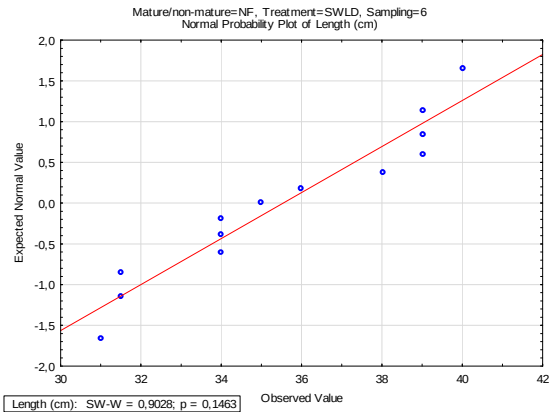

Mature/non-mature=MM, Treatment=FWLL, Sampling=5
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,8879; p = 0,1899

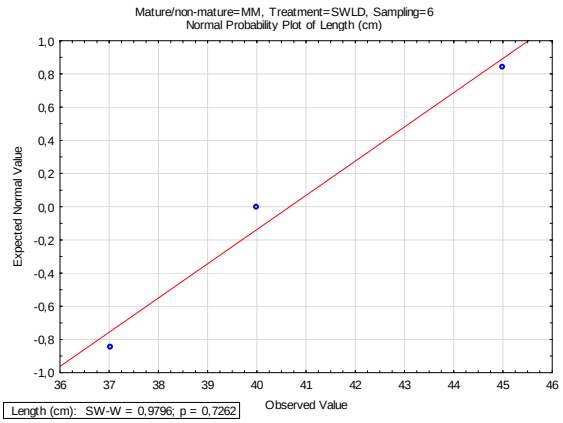

Mature/non-mature=NF, Treatment=FWLD, Sampling=5
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9213; p = 0,4402

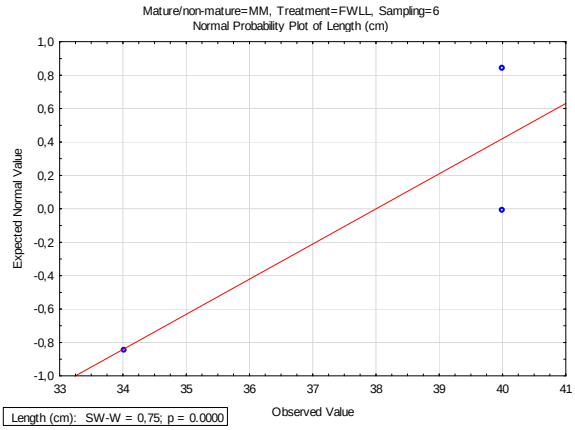

Mature/non-mature=MM, Treatment=FWLD, Sampling=5
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9065; p = 0,3300

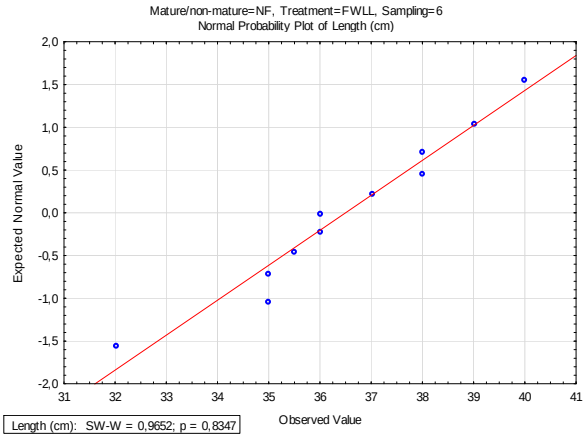

Mature/non-mature=NF, Treatment=SWLL, Sampling=6
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9082; p = 0,2687

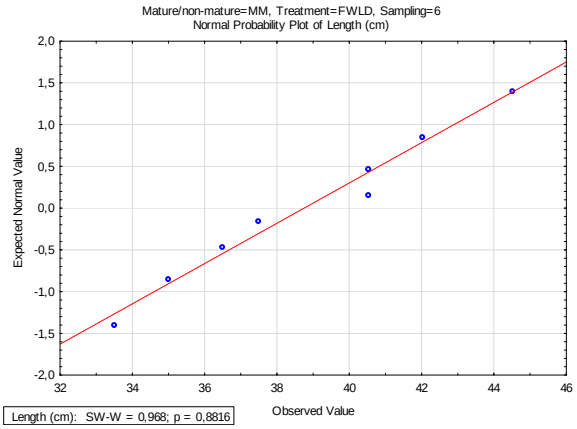

Mature/non-mature=MM, Treatment=SWLL, Sampling=6
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,8676; p = 0,1428

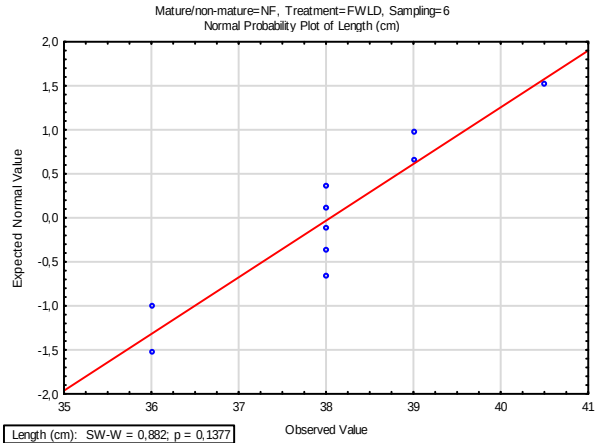

Mature/non-mature=NF, Treatment=SWLD, Sampling=6
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9028; p = 0,1463

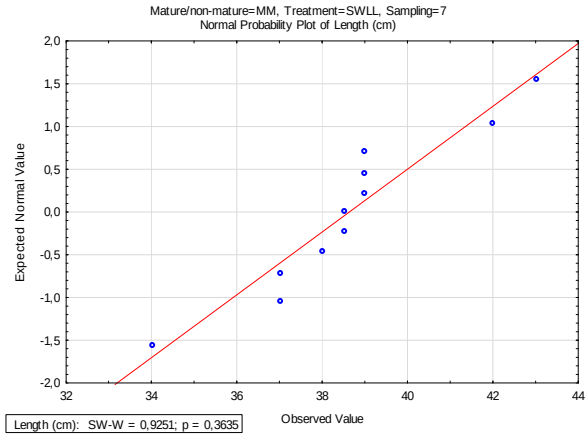

Mature/non-mature=MM, Treatment=SWLL, Sampling=6
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9796; p = 0,7262

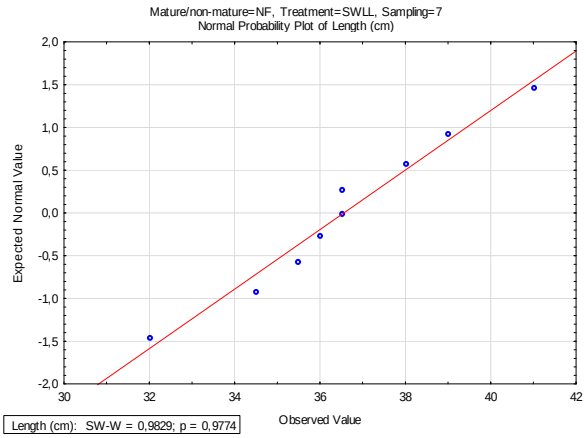

Mature/non-mature=MM, Treatment=FWLL, Sampling=6
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,75; p = 0.0000

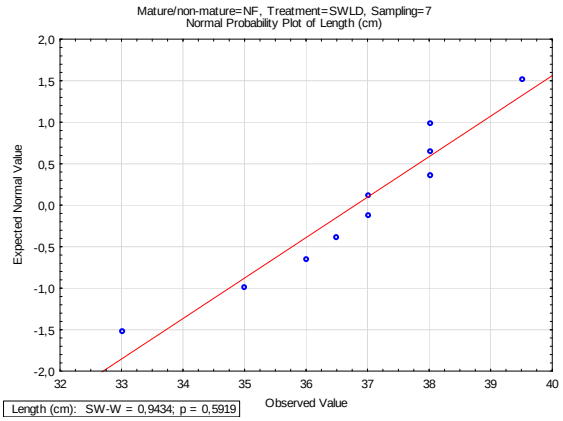

Mature/non-mature=NF, Treatment=FWLL, Sampling=6
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9652; p = 0,8347

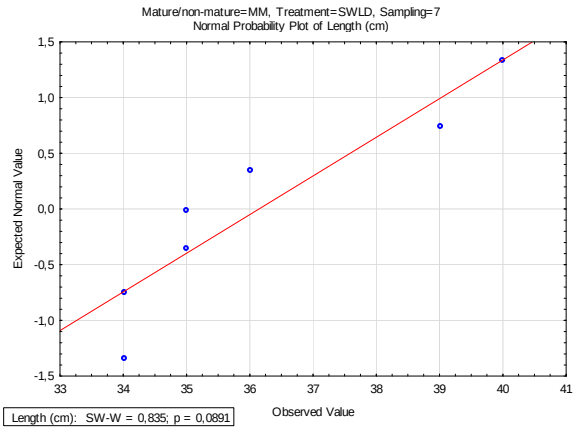

Mature/non-mature=MM, Treatment=FWLD, Sampling=6
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,968; p = 0,8816

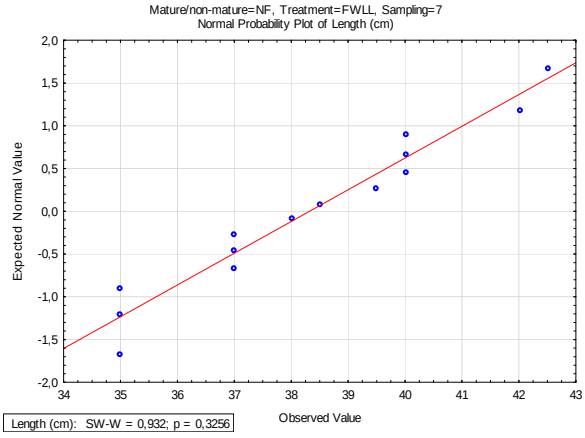

Mature/non-mature=NF, Treatment=FWLD, Sampling=6
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,882; p = 0,1377

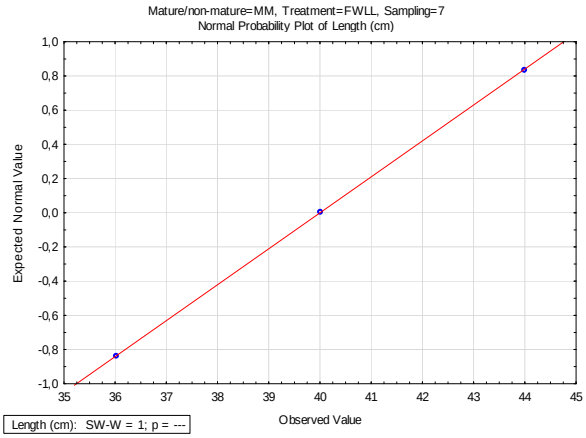

Mature/non-mature=MM, Treatment=SWLL, Sampling=7
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9251; p = 0,3635

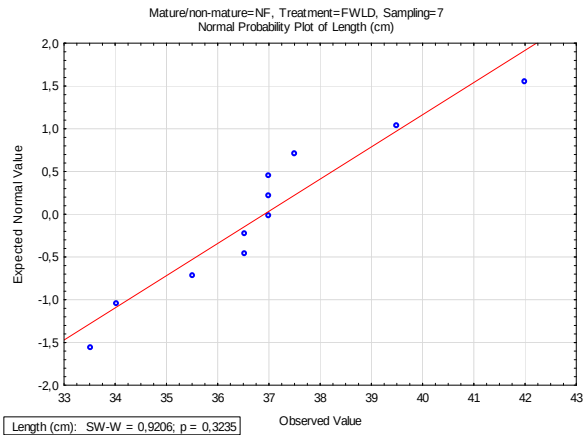

Mature/non-mature=NF, Treatment=SWLL, Sampling=7
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9829; p = 0,9774

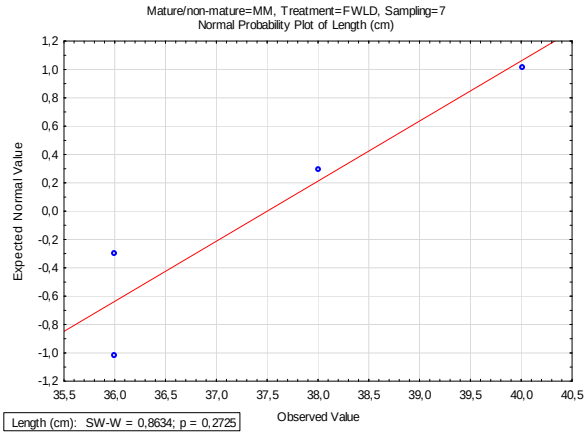

Mature/non-mature=NF, Treatment=SWLD, Sampling=7
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9434; p = 0,5919

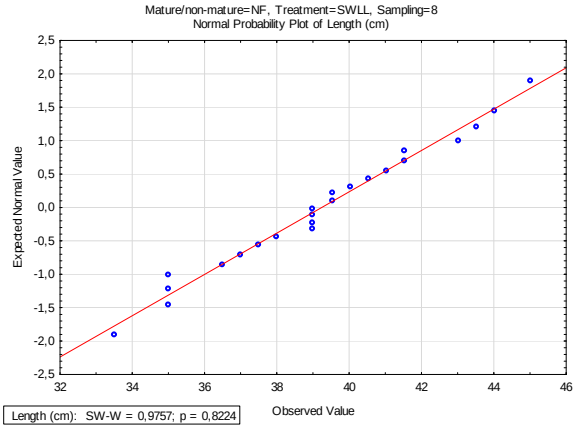

Mature/non-mature=MM, Treatment=SWLD, Sampling=7
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,835; p = 0,0891

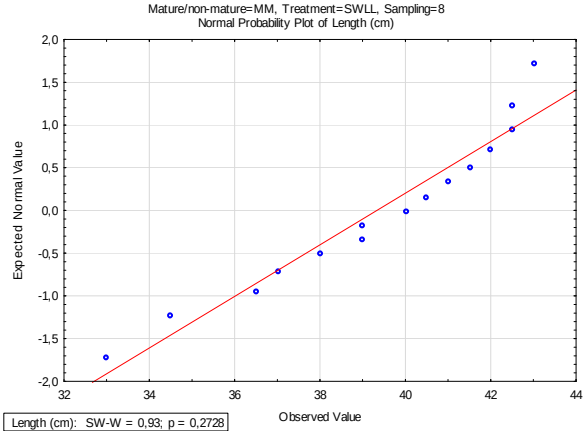

Mature/non-mature=NF, Treatment=FWLL, Sampling=7
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,932; p = 0,3256

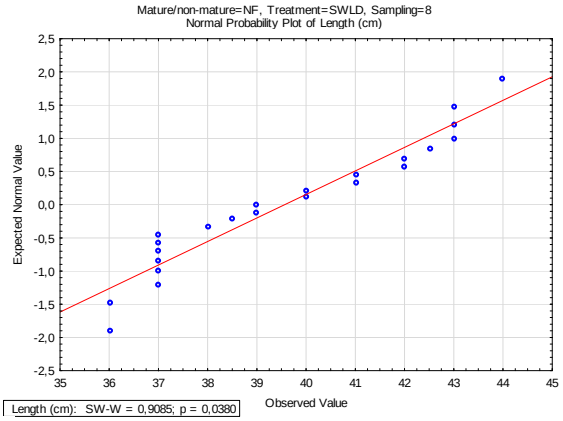

Mature/non-mature=MM, Treatment=FWLL, Sampling=7
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 1; p = ---

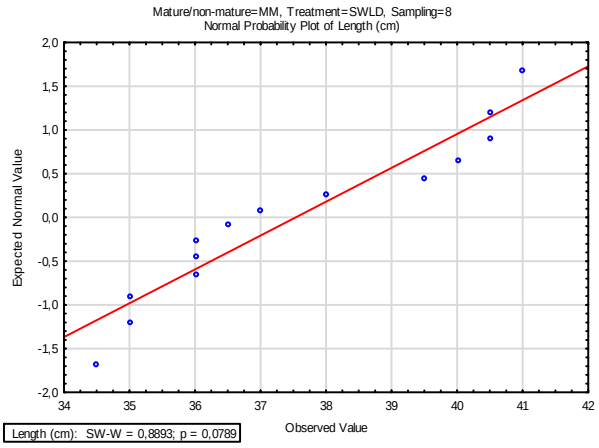

Mature/non-mature=NF, Treatment=FWLD, Sampling=7
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9206; p = 0,3235

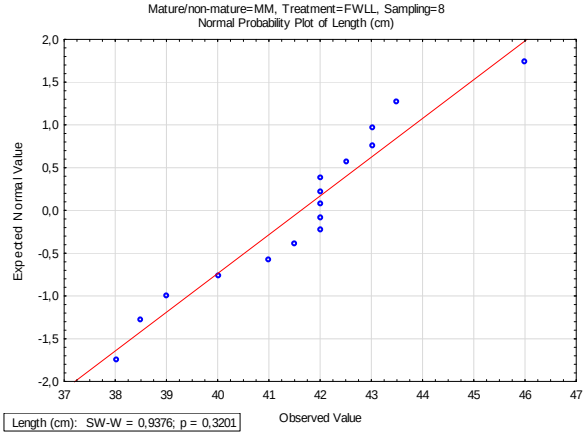

Mature/non-mature=MM, Treatment=FWLD, Sampling=7
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,8634; p = 0,2725

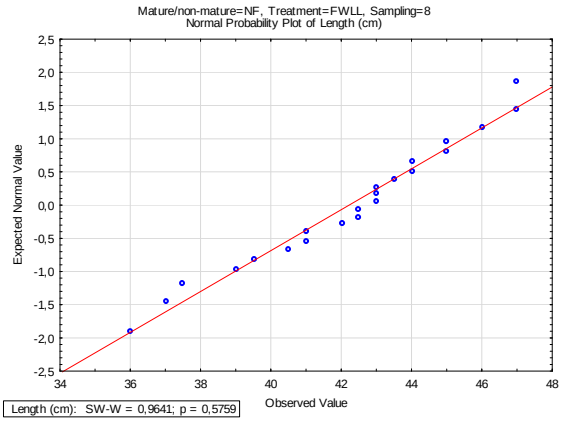

Mature/non-mature=NF, Treatment=SWLL, Sampling=8
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9757; p = 0,8224

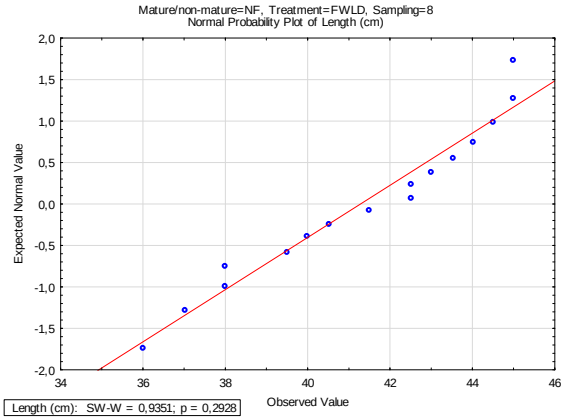

Mature/non-mature=MM, Treatment=SWLL, Sampling=8
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,93; p = 0,2728

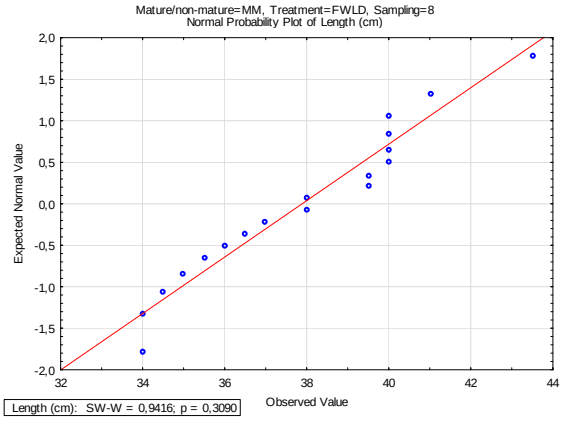

Mature/non-mature=NF, Treatment=SWLD, Sampling=8
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9085; p = 0,0380


Mature/non-mature=MM, Treatment=SWLD, Sampling=8
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,8893; p = 0,0789

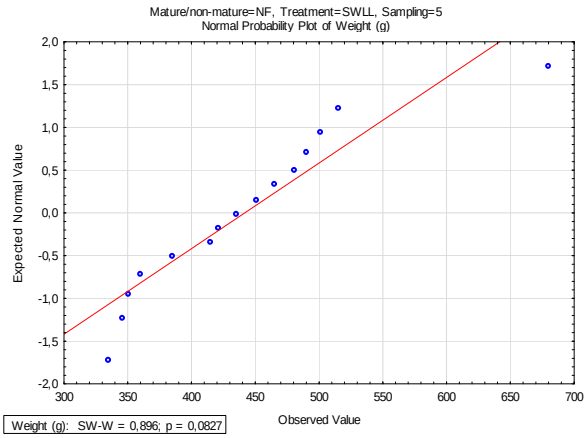

Mature/non-mature=MM, Treatment=FWLL, Sampling=8
Normal Probability Plot of Length (cm)
Expected Normal Value
Observed Value
Length (cm): SW-W = 0,9376; p = 0,3201

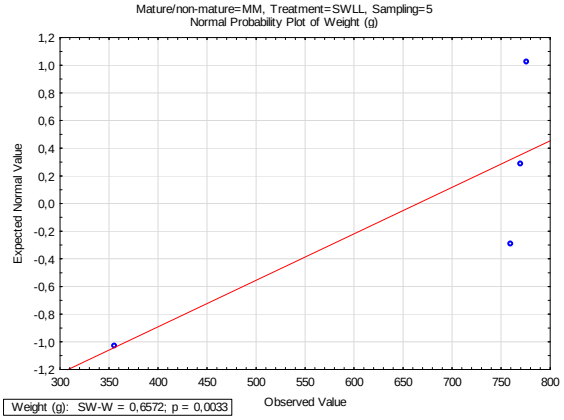


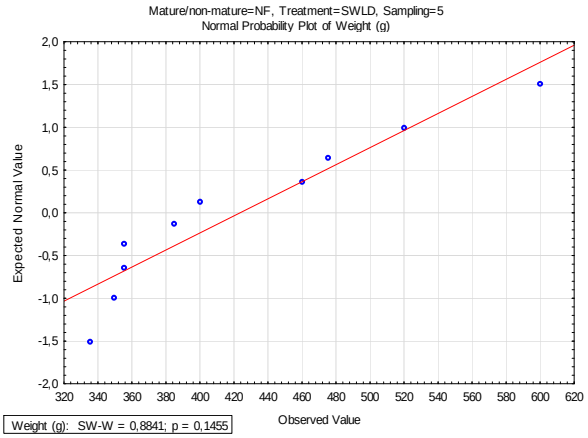


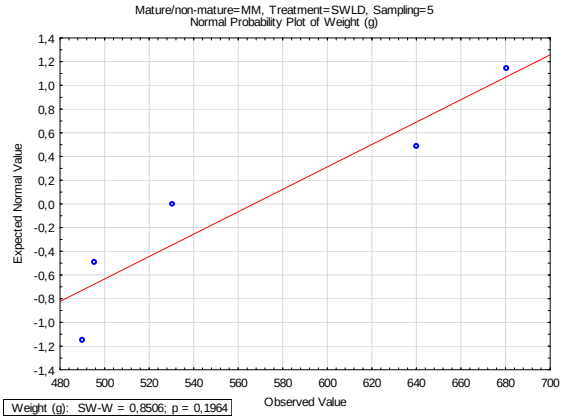


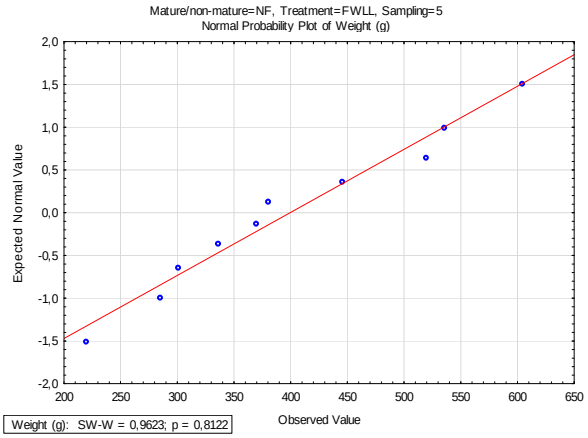
Normal probability plot of residual for Body length divided into samplings 5-8, treatment groups (SWLL, SWLD, FWLL and FWLD) and maturation category (NF and MM):

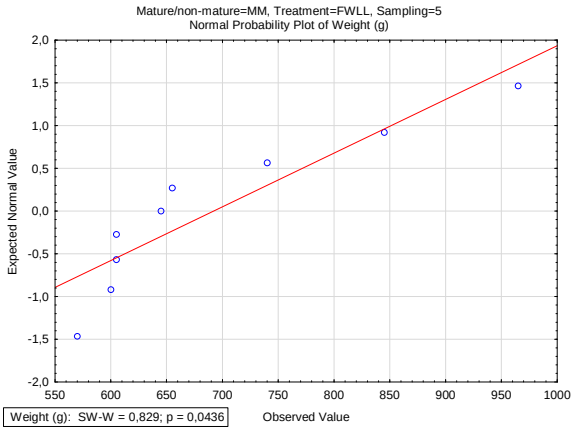


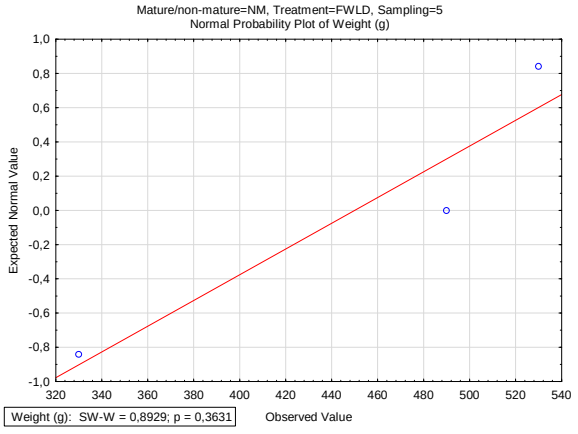


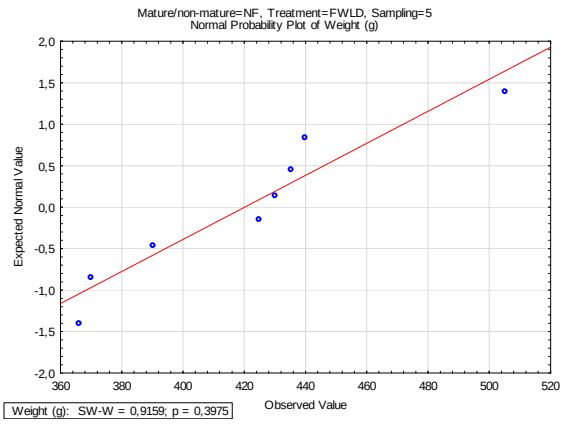

Mature/non-mature=NF, Treatment=SWLD, Sampling=5
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,8841; p = 0,1455

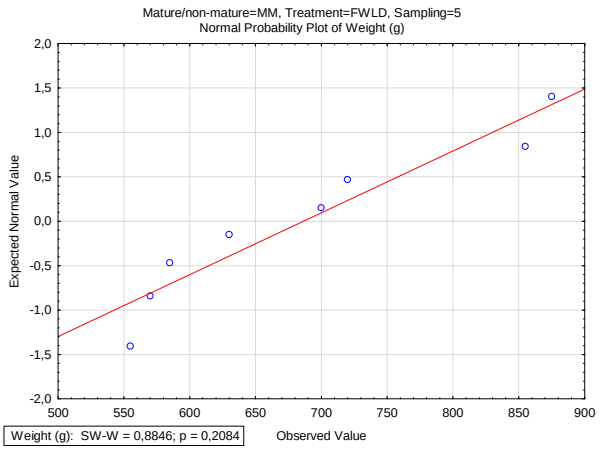

Mature/non-mature=MM, Treatment=SWLD, Sampling=5
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,8506; p = 0,1964

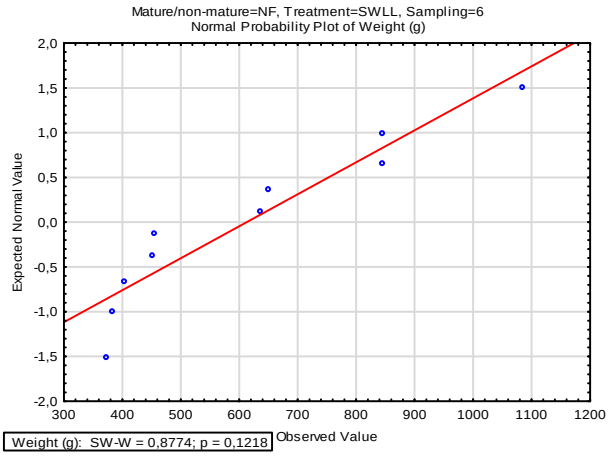

Mature/non-mature=NF, Treatment=FWLL, Sampling=5
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9623; p = 0,8122

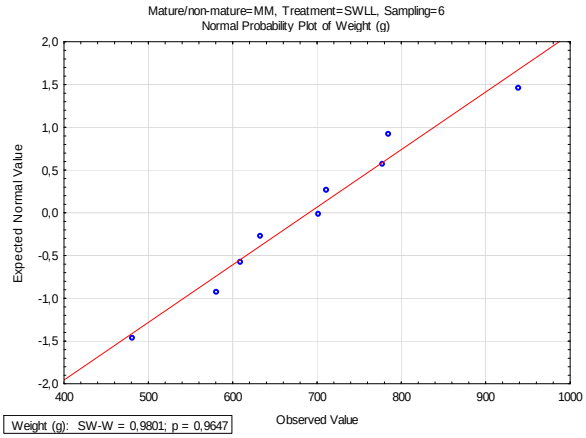

Mature/non-mature=MM, Treatment=FWLL, Sampling=5
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,829; p = 0,0436

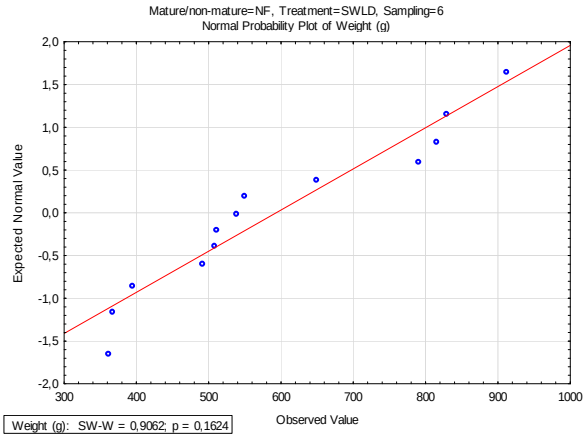

Mature/non-mature=NM, Treatment=FWLD, Sampling=5
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,8929; p = 0,3631

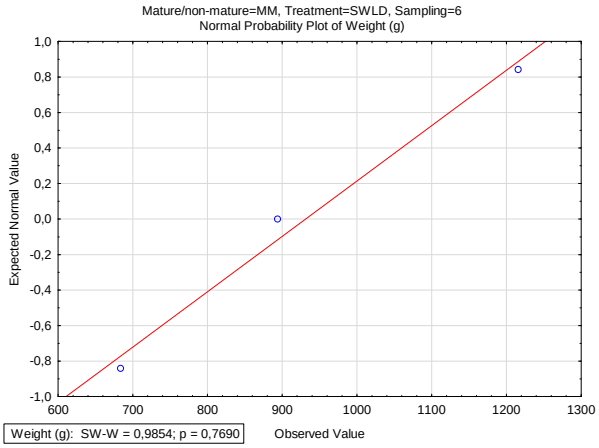

Mature/non-mature=NF, Treatment=FWLD, Sampling=5
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9159; p = 0,3975

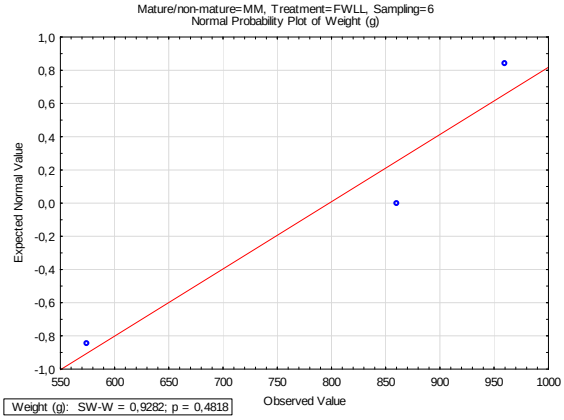

Mature/non-mature=MM, Treatment=FWLD, Sampling=5
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,8846; p = 0,2084

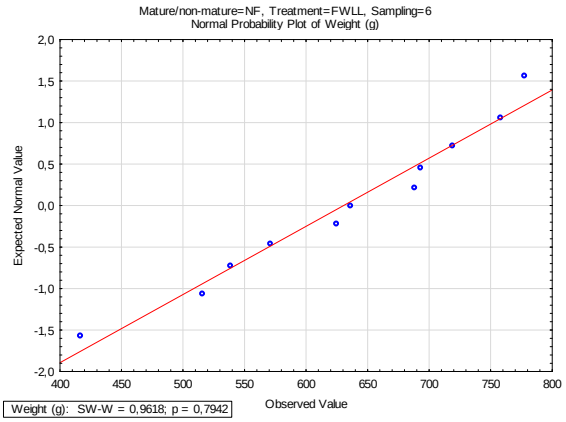

Mature/non-mature=NF, Treatment=SWLL, Sampling=6
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,8774; p = 0,1218

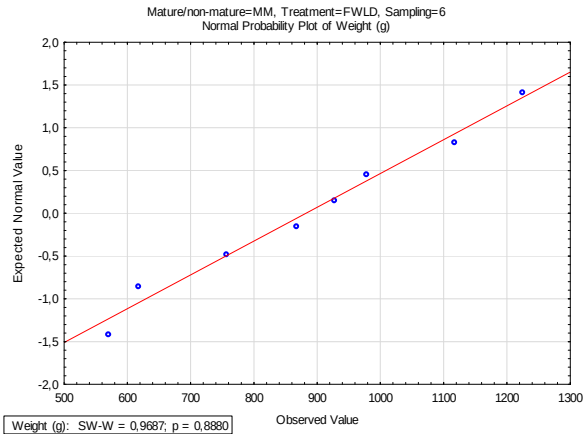

Mature/non-mature=MM, Treatment=SWLL, Sampling=6
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9801; p = 0,9647

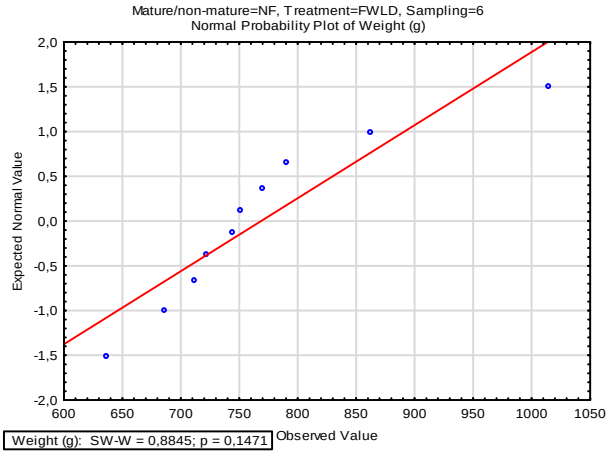

Mature/non-mature=NF, Treatment=SWLD, Sampling=6
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9062; p = 0,1624

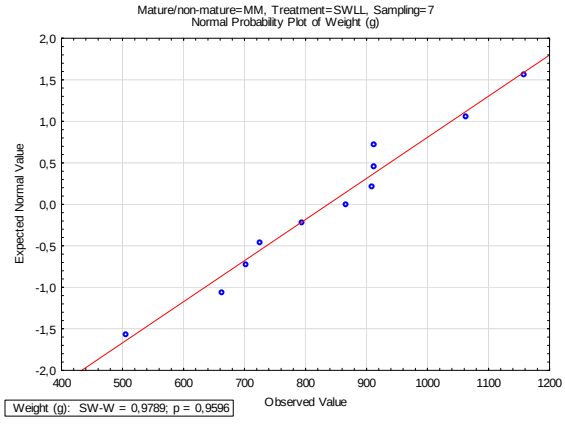

Mature/non-mature=MM, Treatment=SWLD, Sampling=6
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9854; p = 0,7690

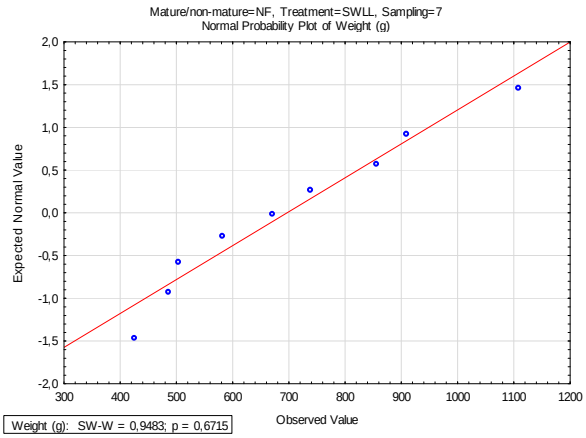

Mature/non-mature=MM, Treatment=FWLL, Sampling=6
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9282; p = 0,4818

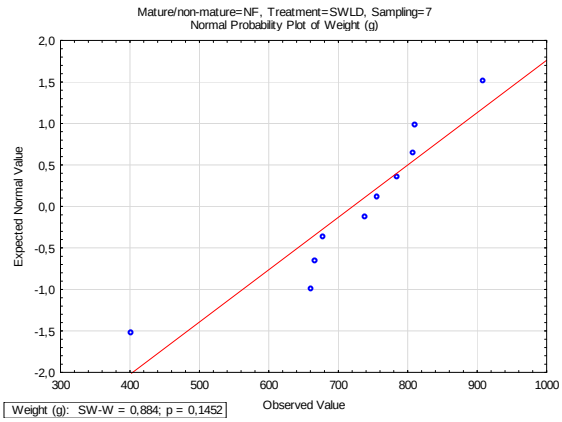

Mature/non-mature=NF, Treatment=FWLL, Sampling=6
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9618; p = 0,7942

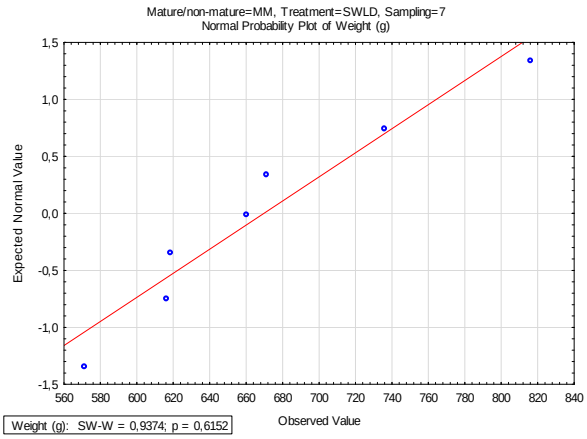

Mature/non-mature=MM, Treatment=FWLD, Sampling=6
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9687; p = 0,8880

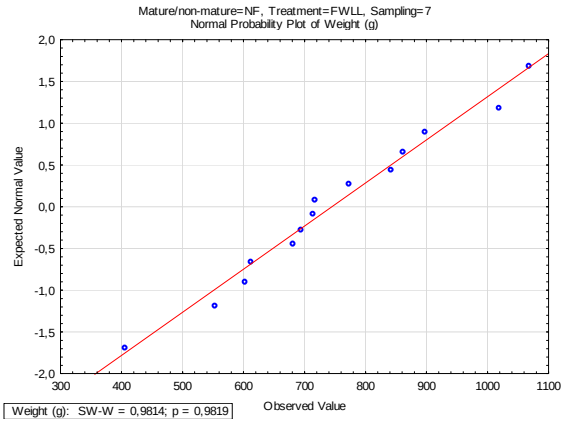

Mature/non-mature=NF, Treatment=FWLD, Sampling=6
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,8845; p = 0,1471

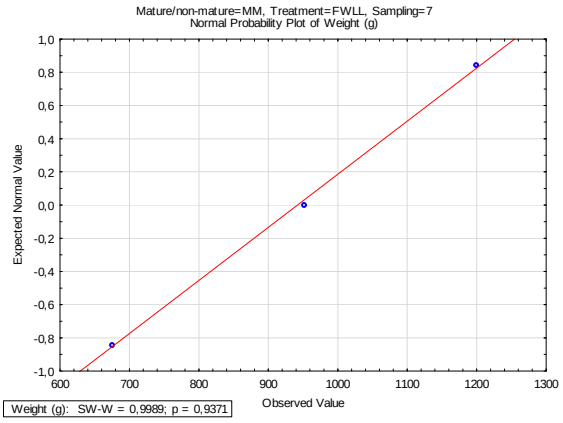

Mature/non-mature=MM, Treatment=SWLL, Sampling=7
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9789; p = 0,9596

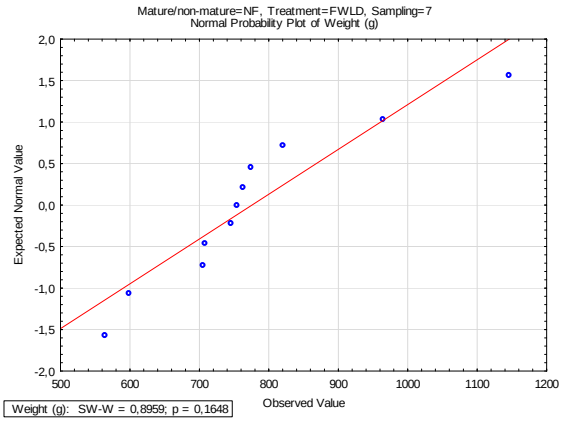

Mature/non-mature=NF, Treatment=SWLL, Sampling=7
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9483; p = 0,6715

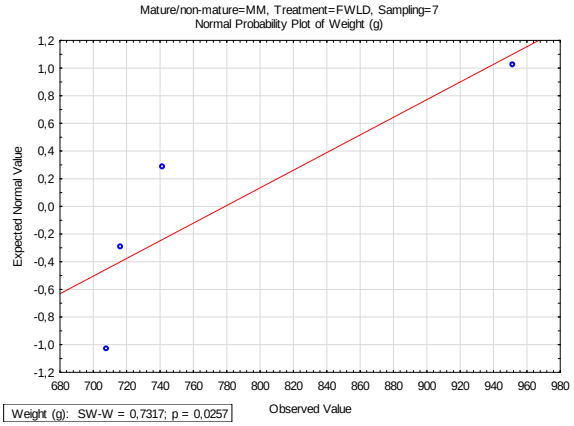

Mature/non-mature=NF, Treatment=SWLD, Sampling=7
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,884; p = 0,1452

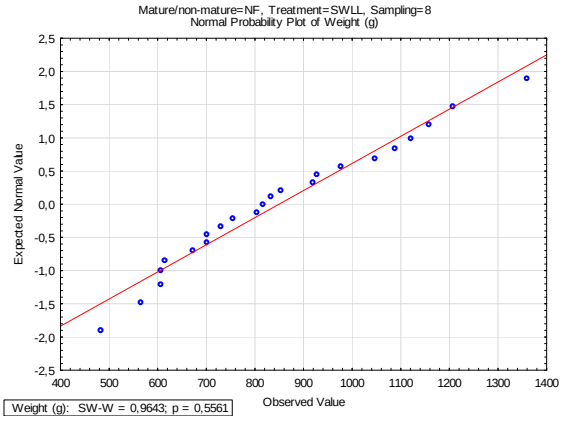

Mature/non-mature=MM, Treatment=SWLD, Sampling=7
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9374; p = 0,6152

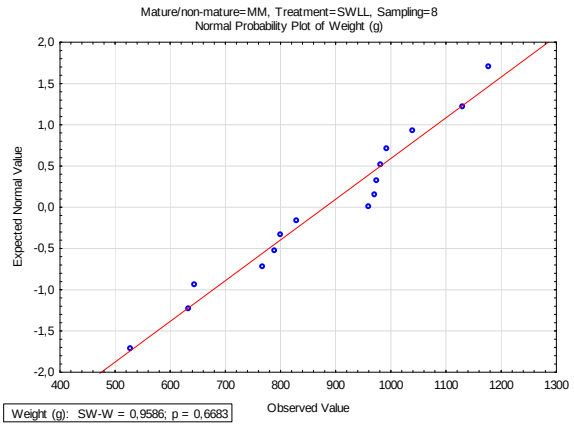

Mature/non-mature=NF, Treatment=FWLL, Sampling=7
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9814; p = 0,9819

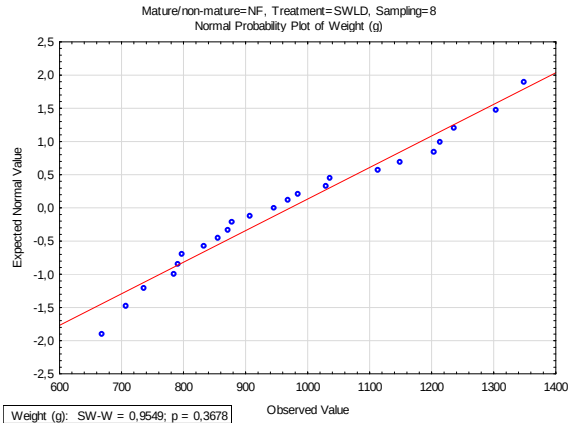

Mature/non-mature=MM, Treatment=FWLL, Sampling=7
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9989; p = 0,9371

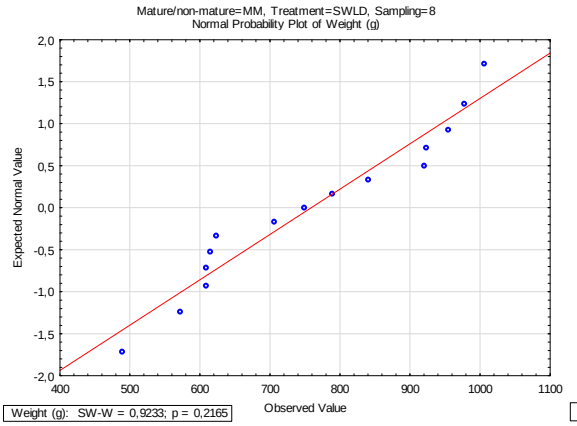

Mature/non-mature=NF, Treatment=FWLD, Sampling=7
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,8959; p = 0,1648

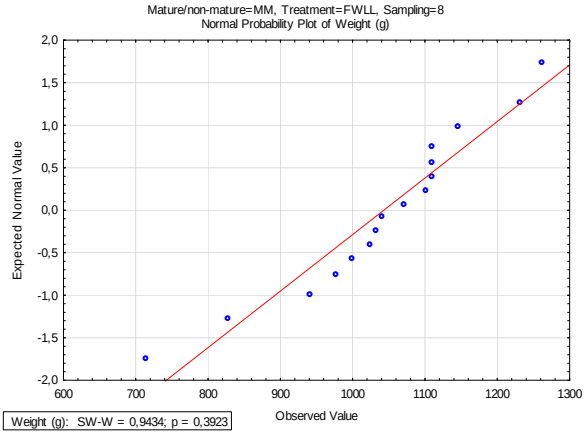

Mature/non-mature=MM, Treatment=FWLD, Sampling=7
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,7317; p = 0,0257

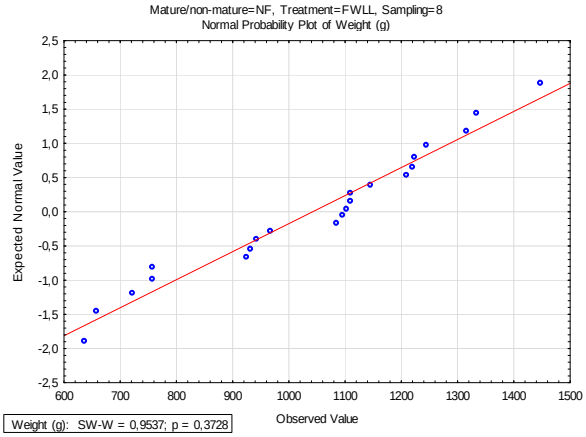

Mature/non-mature=NF, Treatment=SWLL, Sampling=8
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9643; p = 0,5561

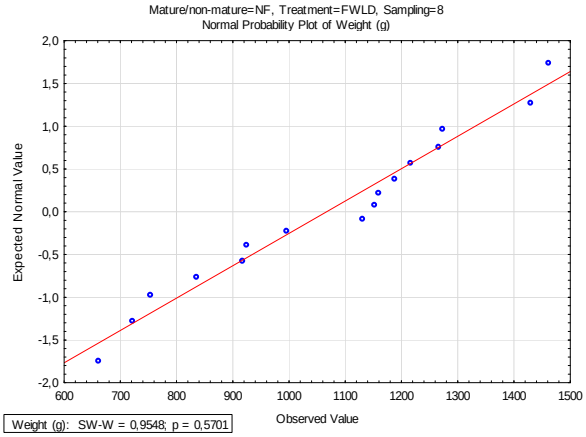

Mature/non-mature=MM, Treatment=SWLL, Sampling=8
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9586; p = 0,6683

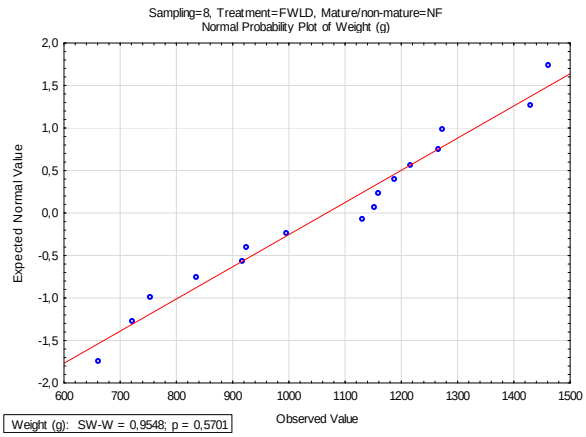

Mature/non-mature=NF, Treatment=SWLD, Sampling=8
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9549; p = 0,3678


Mature/non-mature=MM, Treatment=SWLD, Sampling=8
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9233; p = 0,2165

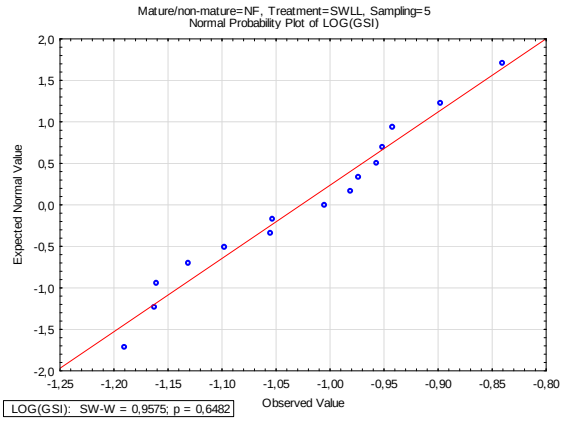

Mature/non-mature=MM, Treatment=FWLL, Sampling=8
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9434; p = 0,3923

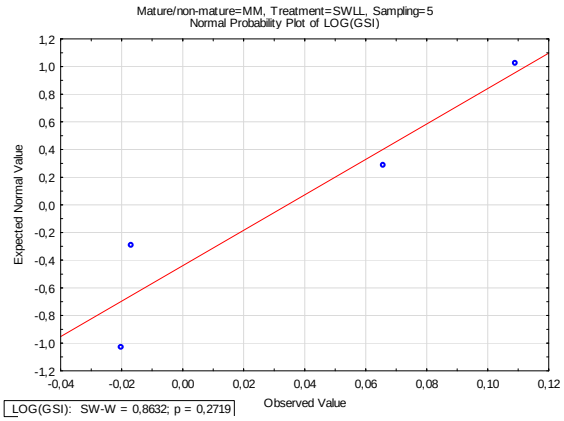

Mature/non-mature=NF, Treatment=FWLL, Sampling=8
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9537; p = 0,3728

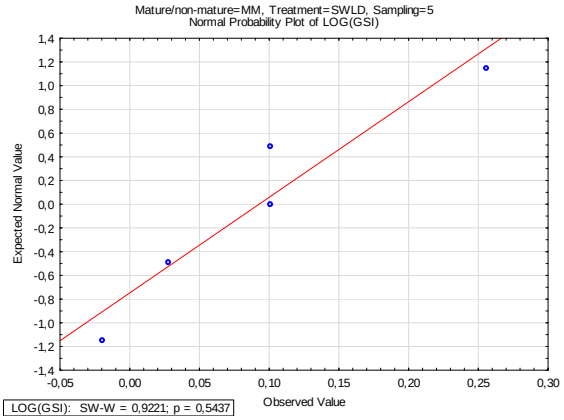

Mature/non-mature=NF, Treatment=FWLD, Sampling=8
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9548; p = 0,5701

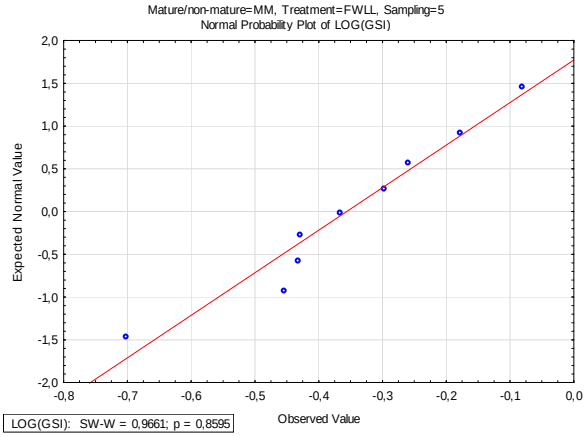

Sampling=8, Treatment=FWLD, Mature/non-mature=NF
Normal Probability Plot of Weight (g)
Expected Normal Value
Observed Value
Weight (g): SW-W = 0,9548; p = 0,5701

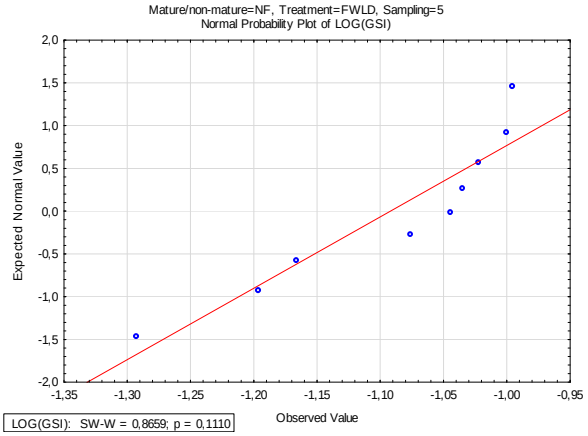
Normal probability plot of residual for Log GSI divided into samplings 5-8, treatment groups (SWLL, SWLD, FWLL and FWLD) and maturation category (NF and MM):

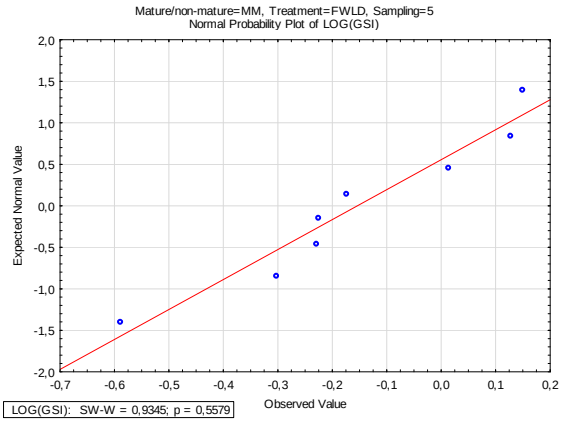


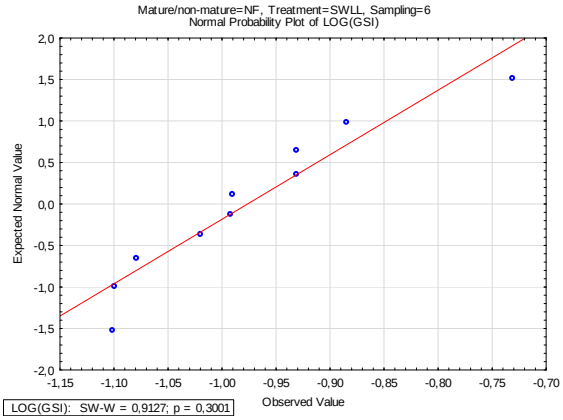


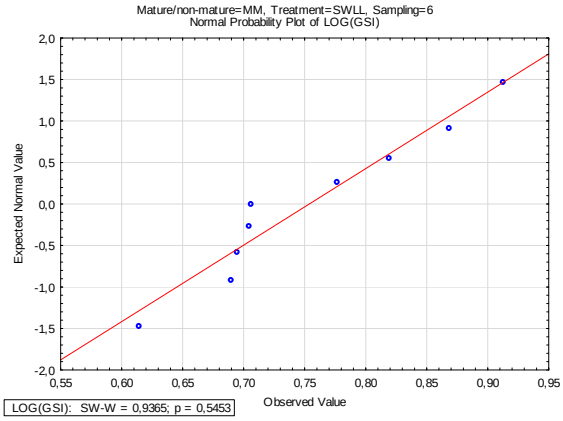


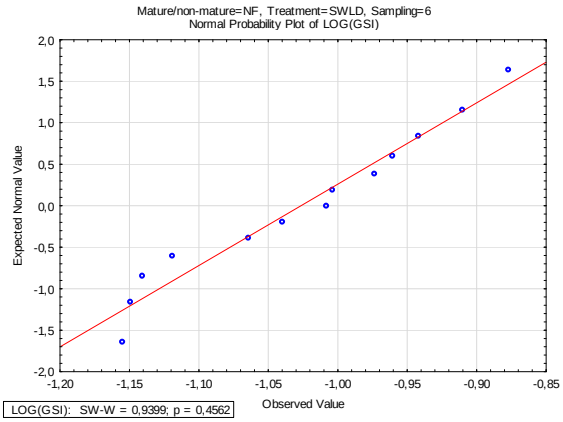


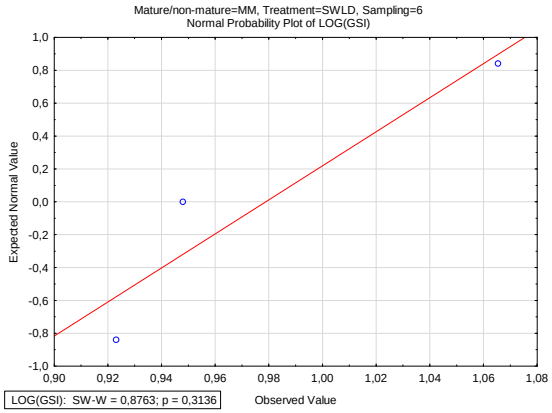


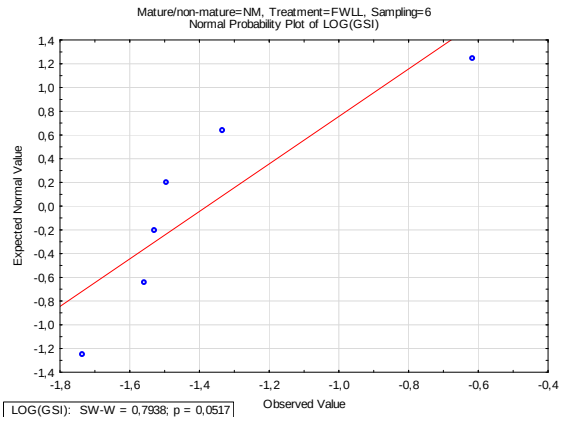


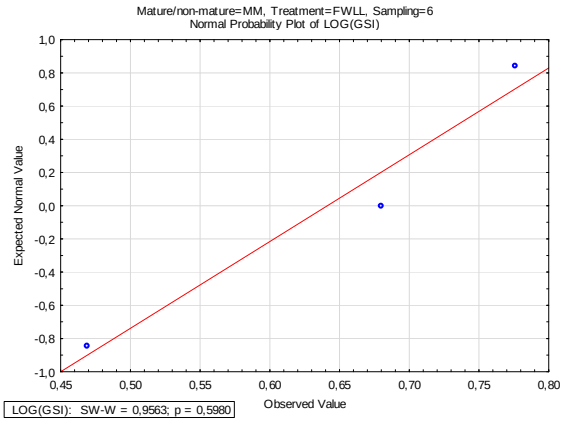

Mature/non-mature=NF, Treatment=SWLL, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9127; p = 0,3001

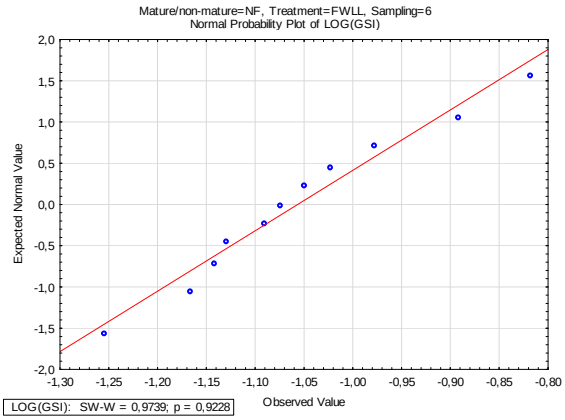

Mature/non-mature=MM, Treatment=SWLL, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9365; p = 0,5453

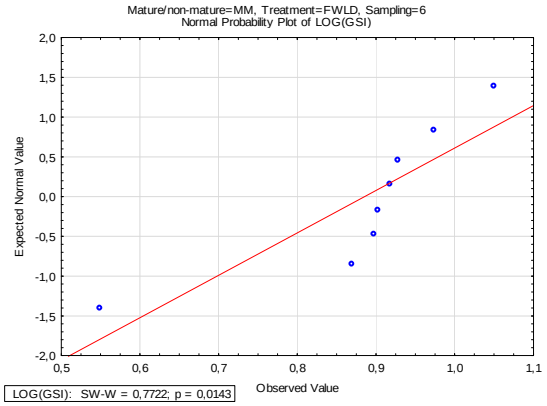

Mature/non-mature=NF, Treatment=SWLD, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9399; p = 0,4562

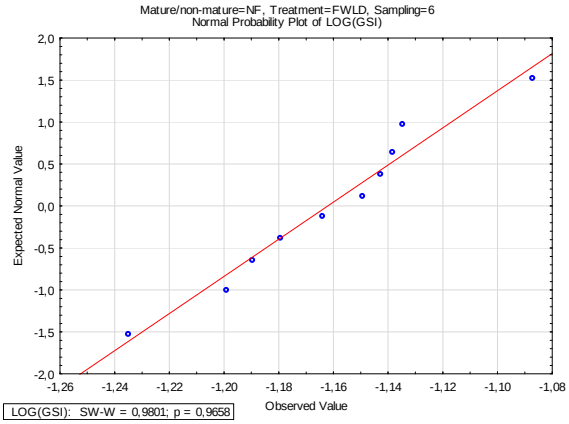

Mature/non-mature=MM, Treatment=SWLD, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,8763; p = 0,3136

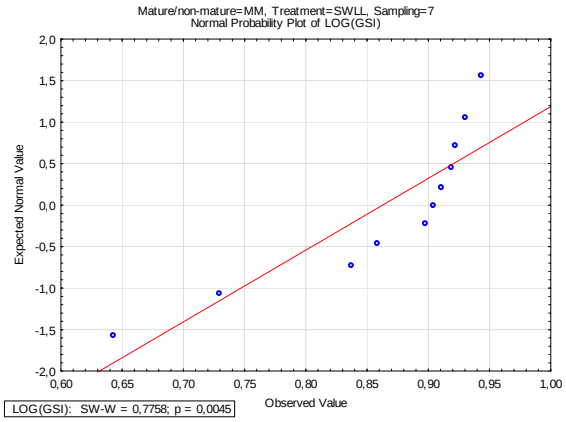

Mature/non-mature=NM, Treatment=FWLL, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,7938; p = 0,0517

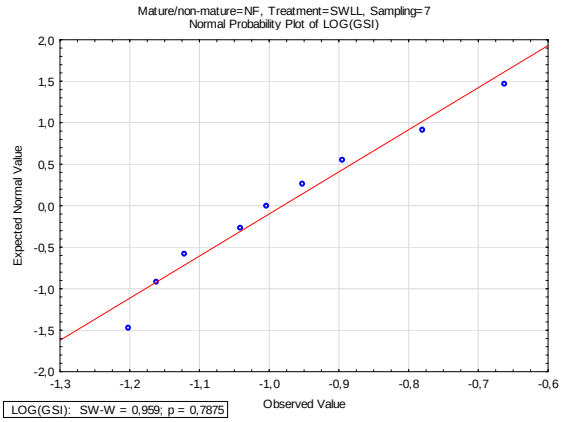

Mature/non-mature=MM, Treatment=FWLL, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9563; p = 0,5980

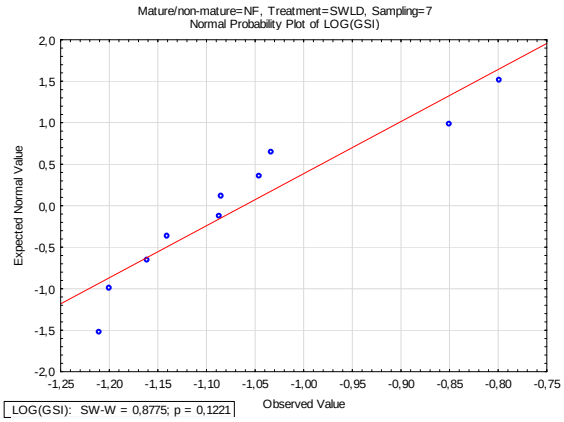

Mature/non-mature=NF, Treatment=FWLL, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9739; p = 0,9228

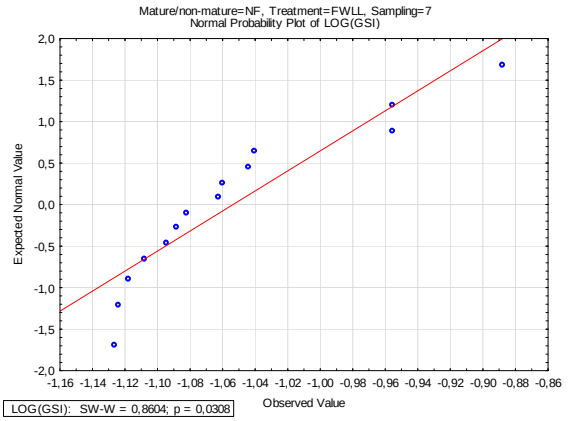

Mature/non-mature=MM, Treatment=FWLD, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,7722; p = 0,0143

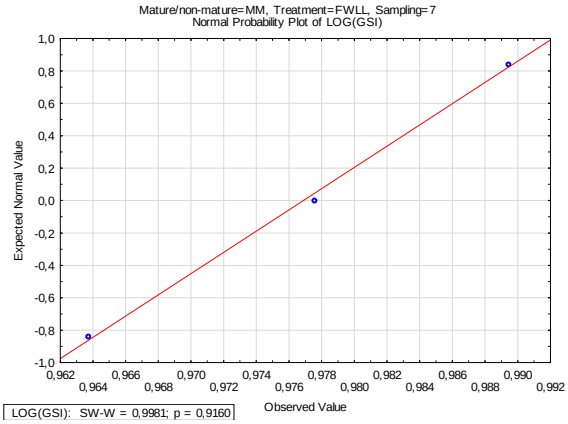

Mature/non-mature=NF, Treatment=FWLD, Sampling=6
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9801; p = 0,9658

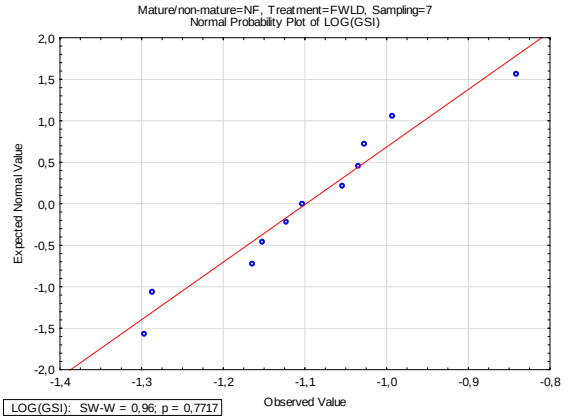

Mature/non-mature=MM, Treatment=SWLL, Sampling=7
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,7758; p = 0,0045

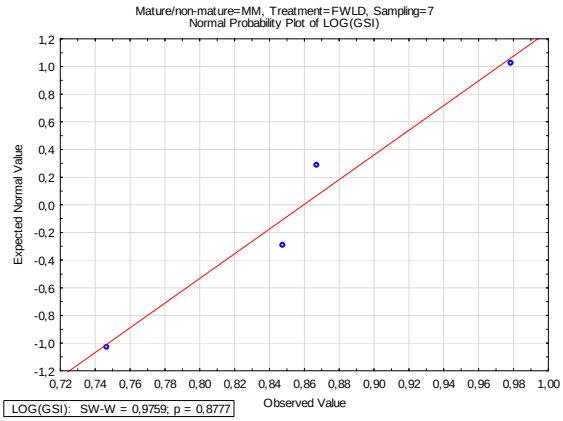

Mature/non-mature=NF, Treatment=SWLL, Sampling=7
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,959; p = 0,7875

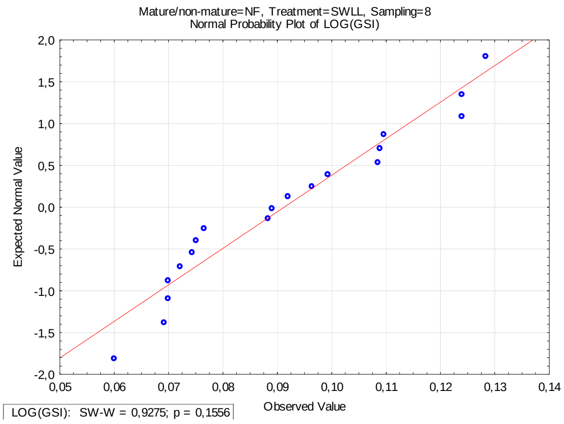

Mature/non-mature=NF, Treatment=SWLD, Sampling=7
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,8775; p = 0,1221

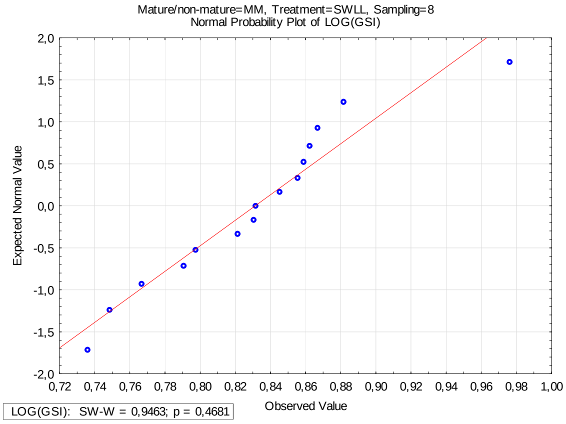

Mature/non-mature=NF, Treatment=FWLL, Sampling=7
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,8604; p = 0,0308

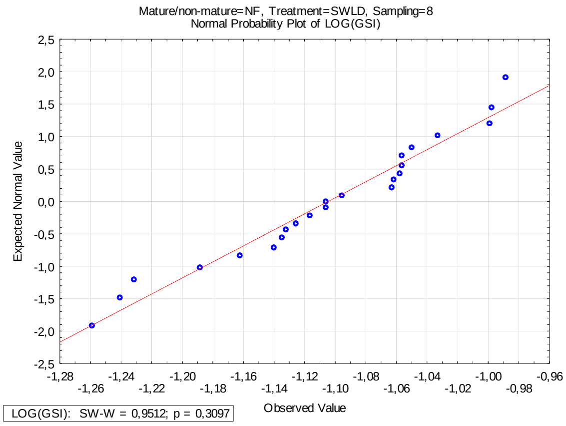

Mature/non-mature=MM, Treatment=FWLL, Sampling=7
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9981; p = 0,9160

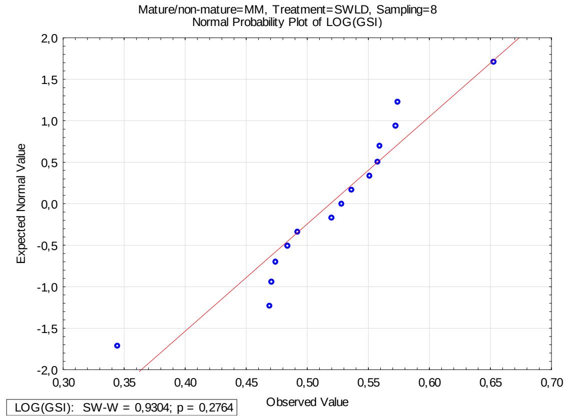

Mature/non-mature=NF, Treatment=FWLD, Sampling=7
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,96; p = 0,7717

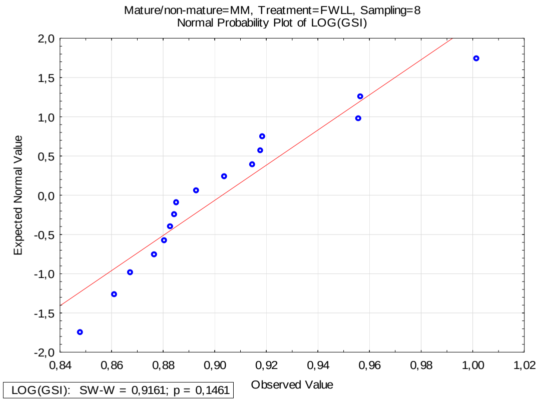

Mature/non-mature=MM, Treatment=FWLD, Sampling=7
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9759; p = 0,8777

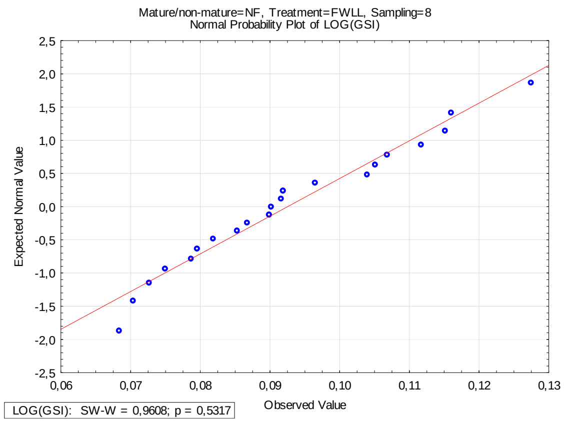

Mature/non-mature=NF, Treatment=SWLL, Sampling=8
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9275; p = 0,1556

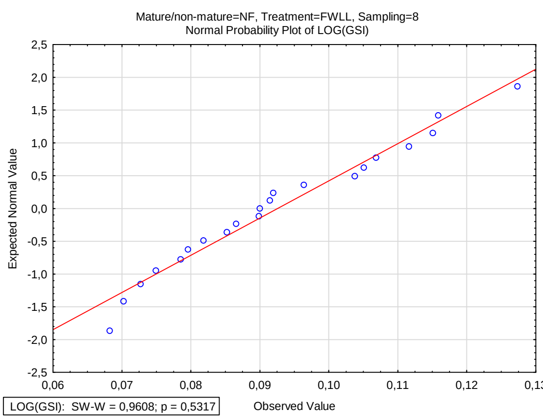

Mature/non-mature=MM, Treatment=SWLL, Sampling=8
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9463; p = 0,4681

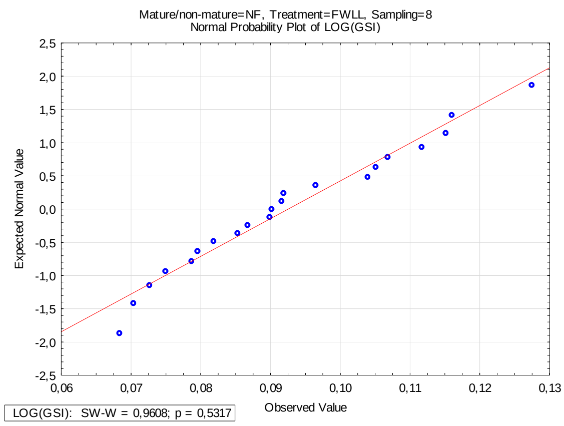

Mature/non-mature=NF, Treatment=SWLD, Sampling=8
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9512; p = 0,3097

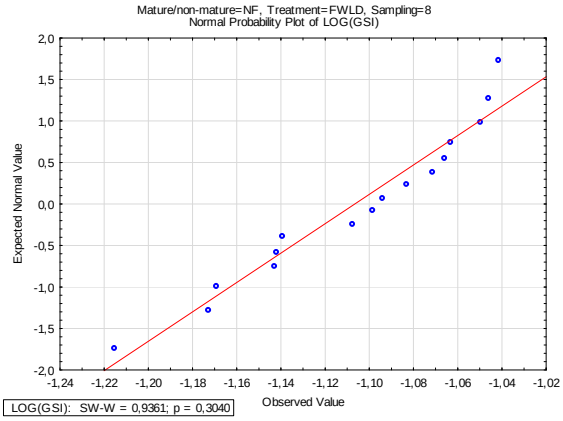

Mature/non-mature=MM, Treatment=SWLD, Sampling=8
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9304; p = 0,2764

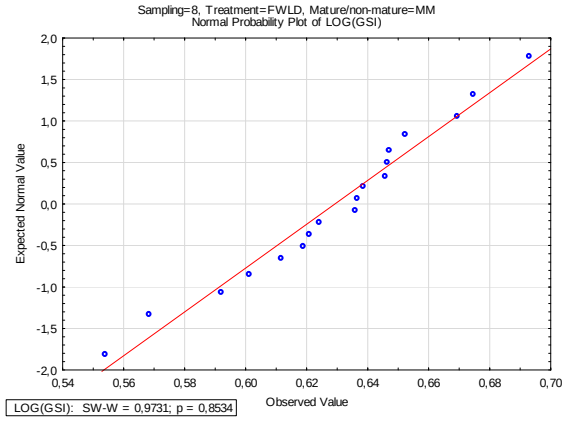

Mature/non-mature=MM, Treatment=FWLL, Sampling=8
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9161; p = 0,1461


Mature/non-mature=NF, Treatment=FWLL, Sampling=8
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9608; p = 0,5317

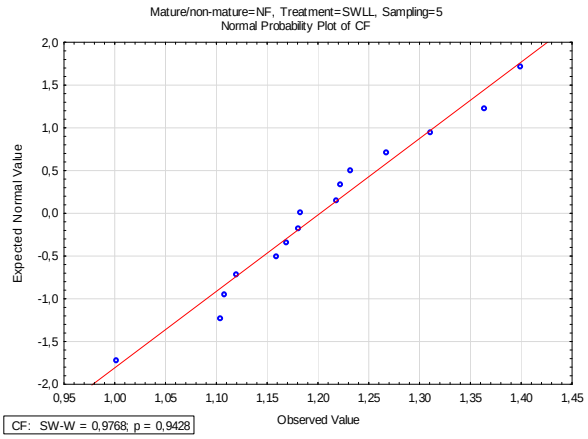

Mature/non-mature=NF, Treatment=FWLL, Sampling=8
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9608; p = 0,5317

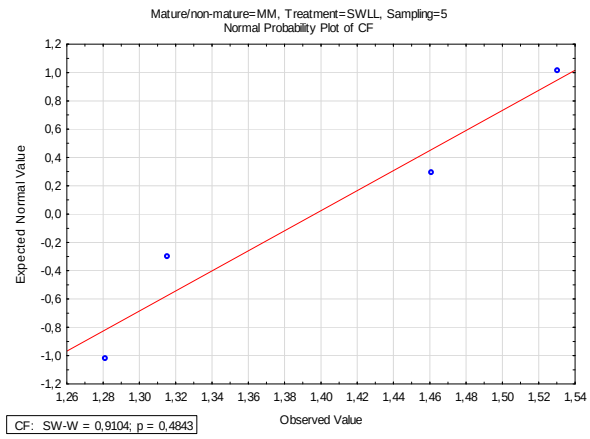

Mature/non-mature=NF, Treatment=FWLL, Sampling=8
Normal Probability Plot of LOG(GSI)
Expected Normal Value
Observed Value
LOG(GSI): SW-W = 0,9608; p = 0,5317

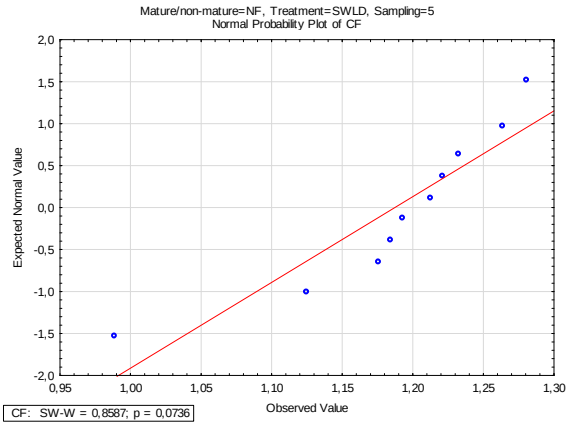


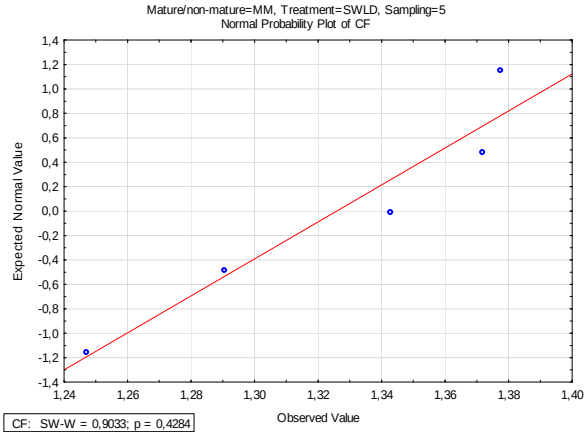


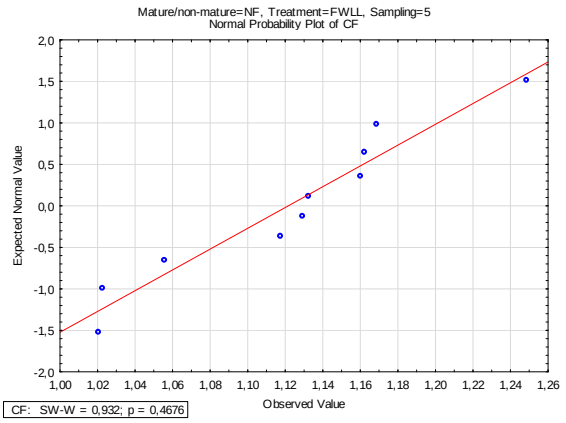
Normal probability plot of residual for Condition factor divided into samplings 5-8, treatment groups (SWLL, SWLD, FWLL and FWLD) and maturation category (NF and MM):

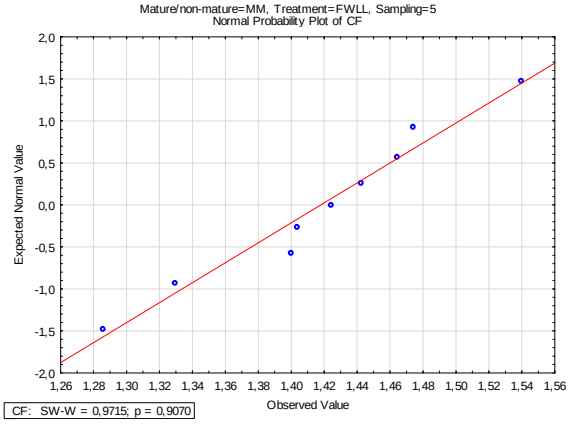


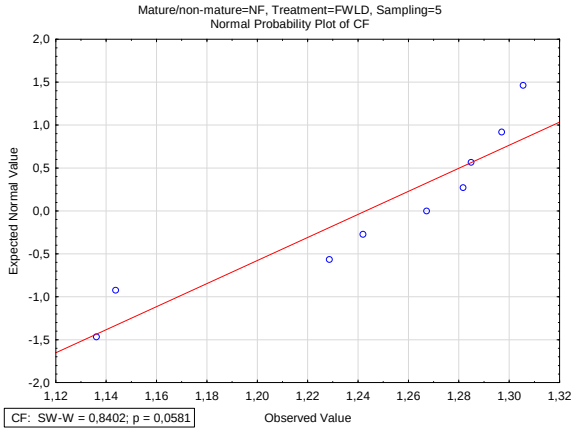


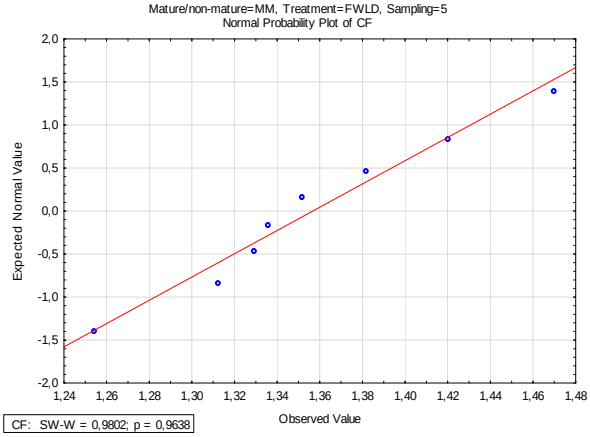


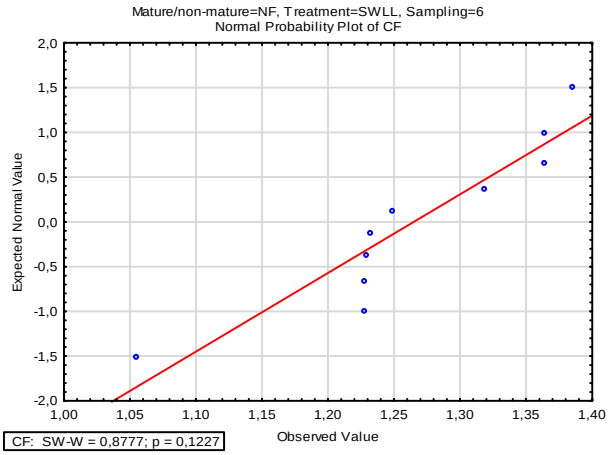


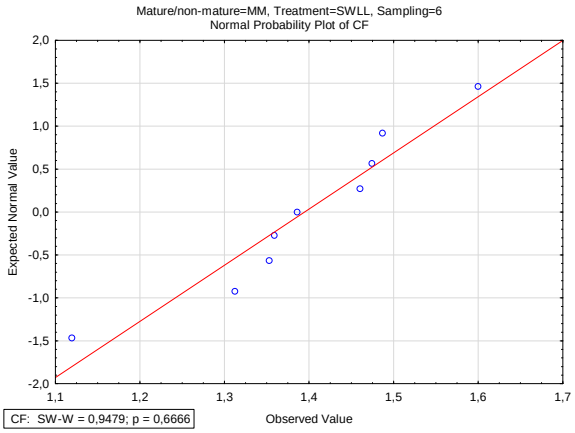

Mature/non-mature=NF, Treatment=FWLL, Sampling=5
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,932; p = 0,4676

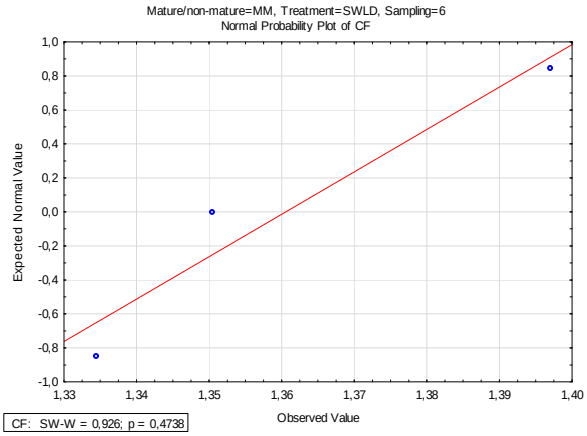

Mature/non-mature=MM, Treatment=FWLL, Sampling=5
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9715; p = 0,9070

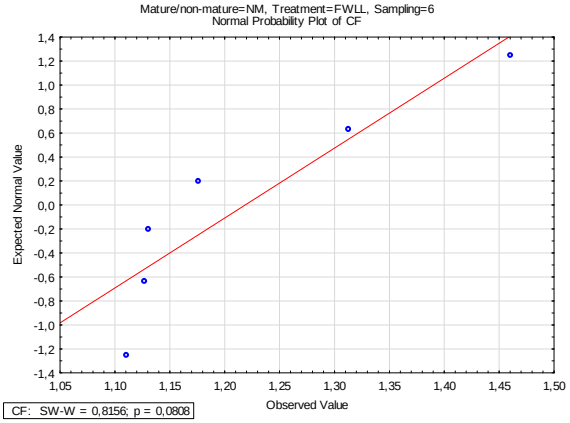

Mature/non-mature=NF, Treatment=FWLD, Sampling=5
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,8402; p = 0,0581

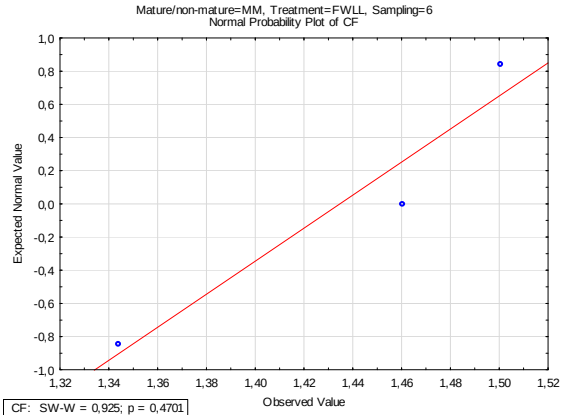

Mature/non-mature=MM, Treatment=FWLD, Sampling=5
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9802; p = 0,9638

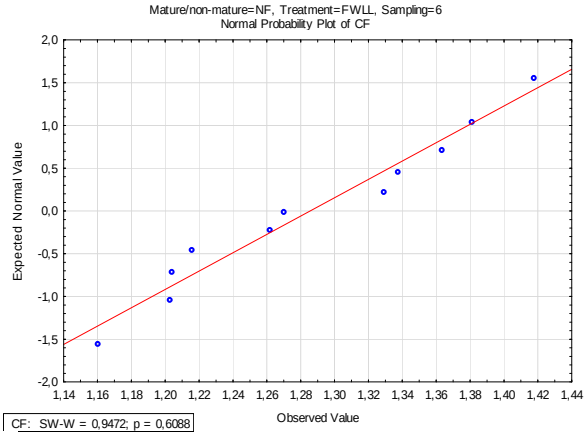

Mature/non-mature=NF, Treatment=SWLL, Sampling=6
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,8777; p = 0,1227

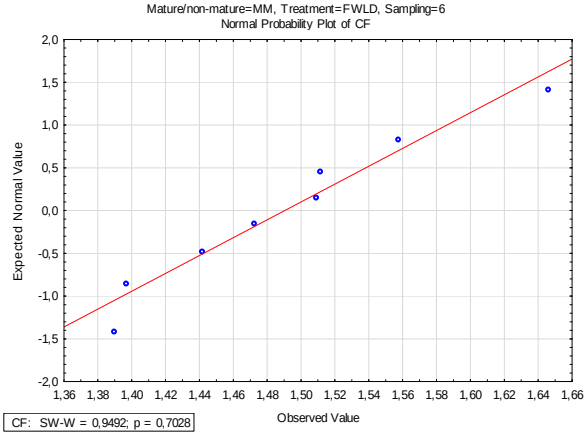

Mature/non-mature=MM, Treatment=SWLL, Sampling=6
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9479; p = 0,6666

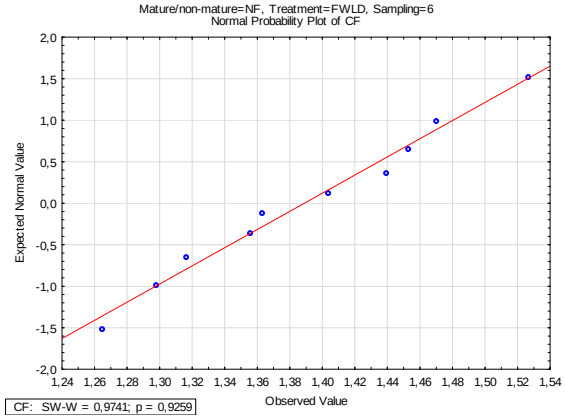

Mature/non-mature=MM, Treatment=SWLD, Sampling=6
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,926; p = 0,4738

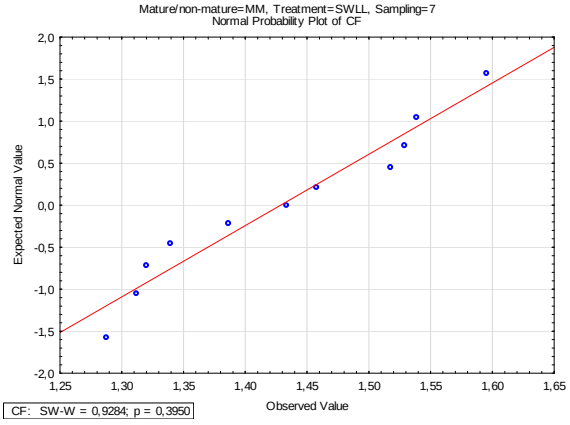

Mature/non-mature=NM, Treatment=FWLL, Sampling=6
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,8156; p = 0,0808

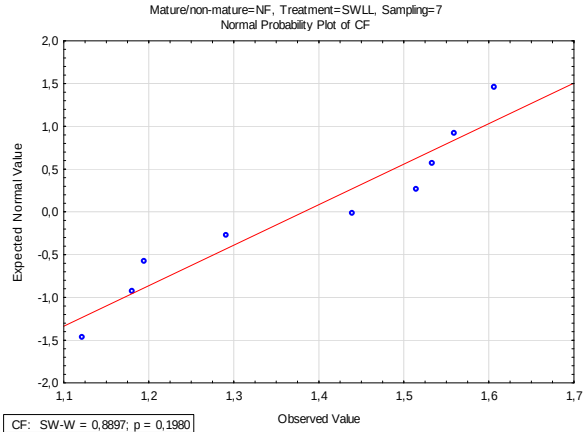

Mature/non-mature=MM, Treatment=FWLL, Sampling=6
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,925; p = 0,4701

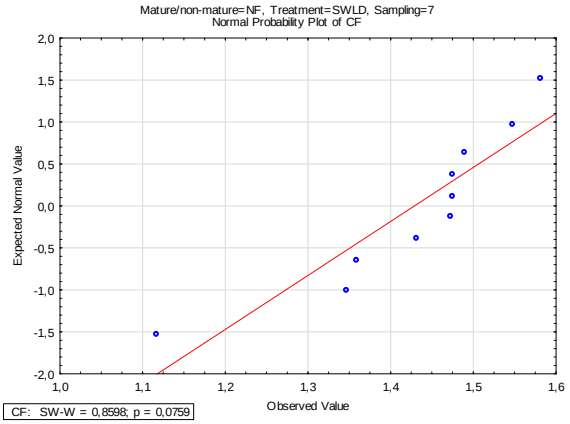

Mature/non-mature=NF, Treatment=FWLL, Sampling=6
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9472; p = 0,6088

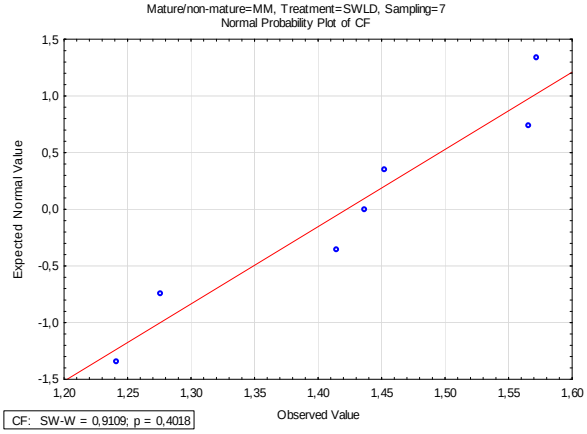

Mature/non-mature=MM, Treatment=FWLD, Sampling=6
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9492; p = 0,7028

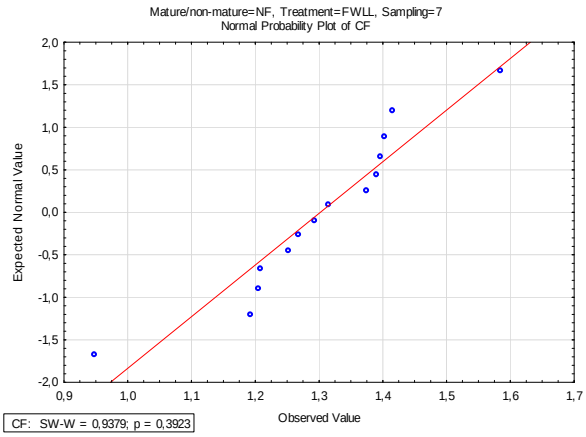

Mature/non-mature=NF, Treatment=FWLD, Sampling=6
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9741; p = 0,9259

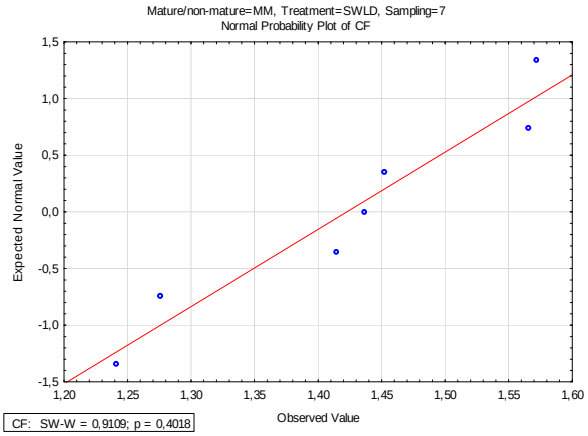

Mature/non-mature=MM, Treatment=SWLL, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9284; p = 0,3950

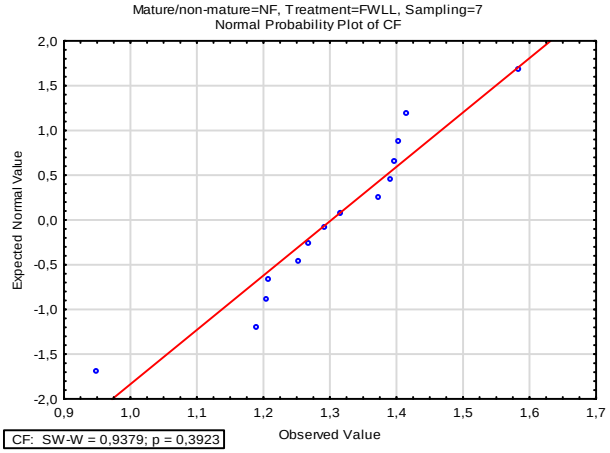

Mature/non-mature=NF, Treatment=SWLL, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,8897; p = 0,1980

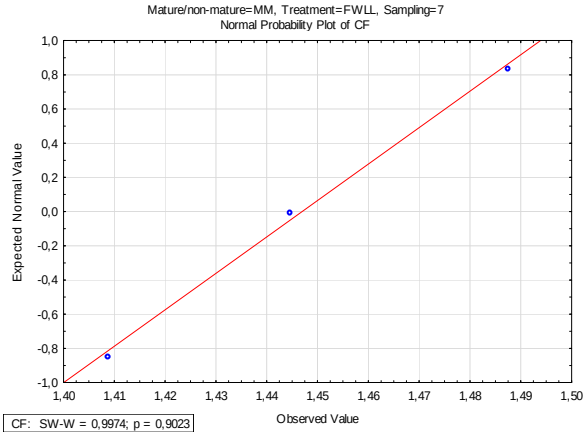

Mature/non-mature=NF, Treatment=SWLD, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,8598; p = 0,0759

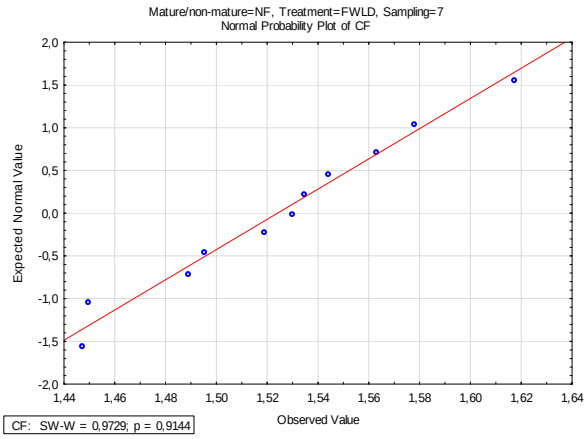

Mature/non-mature=MM, Treatment=SWLD, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9109; p = 0,4018

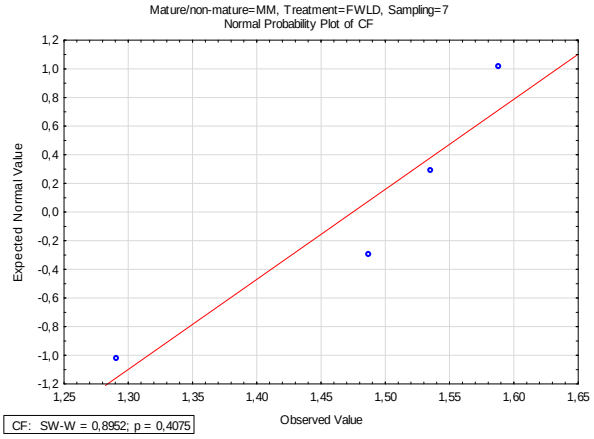

Mature/non-mature=NF, Treatment=FWLL, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9379; p = 0,3923

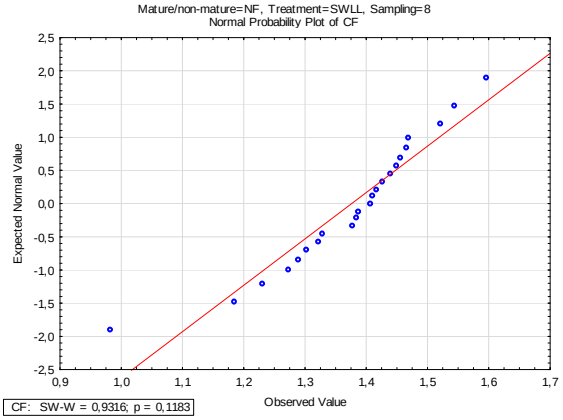

Mature/non-mature=MM, Treatment=SWLD, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9109; p = 0,4018

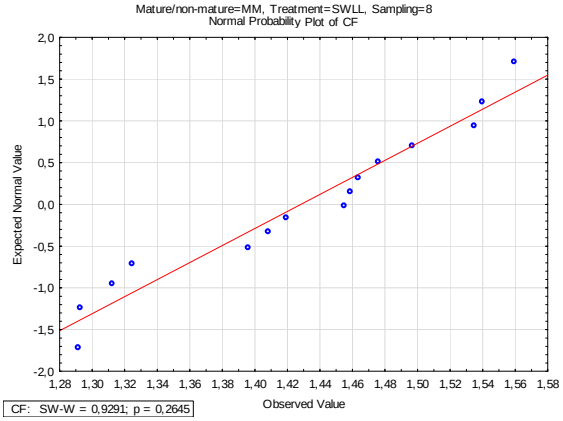

Mature/non-mature=NF, Treatment=FWLL, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9379; p = 0,3923

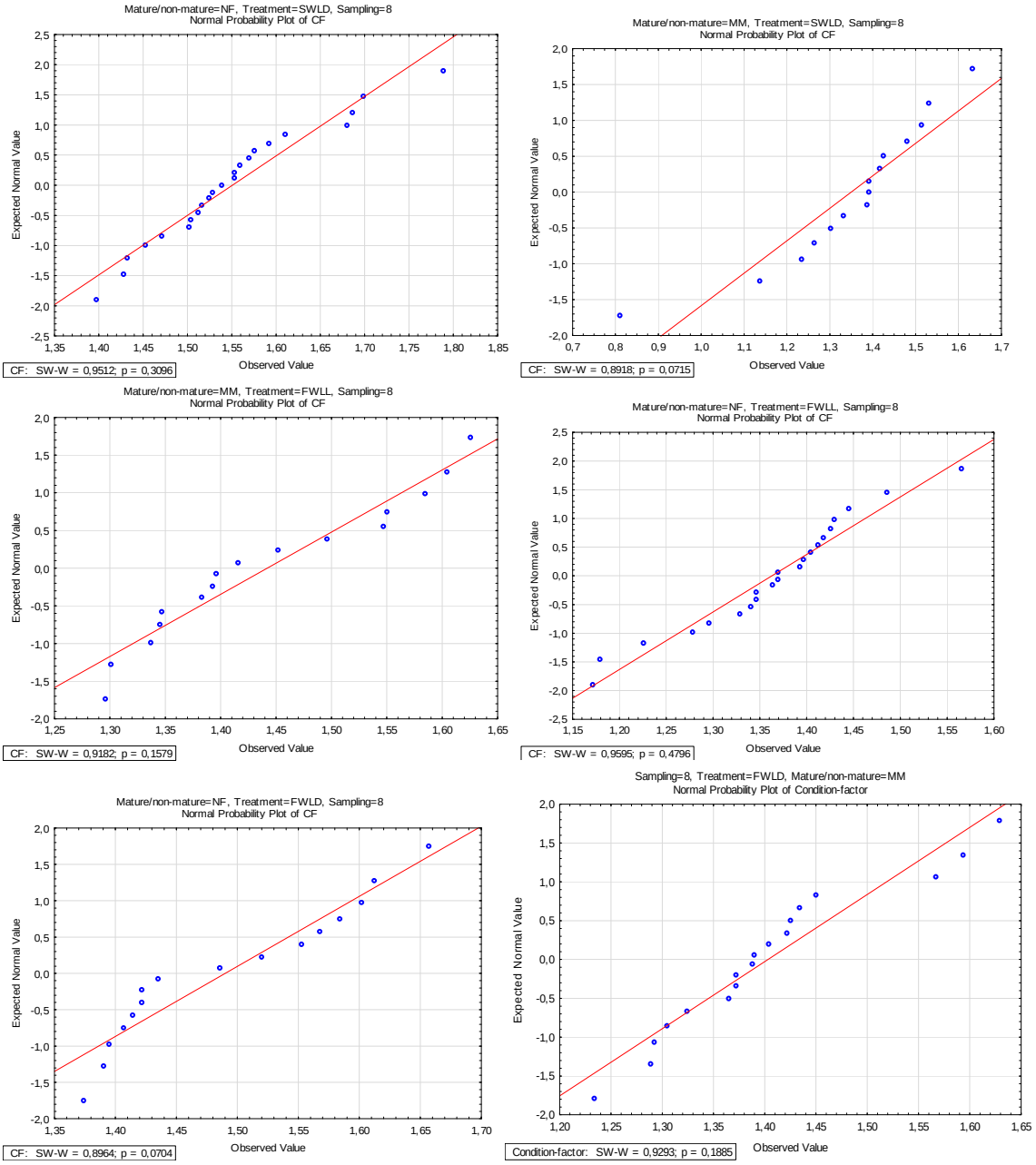

Mature/non-mature=MM, Treatment=FWLL, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9974; p = 0,9023


Mature/non-mature=NF, Treatment=FWLD, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9729; p = 0,9144

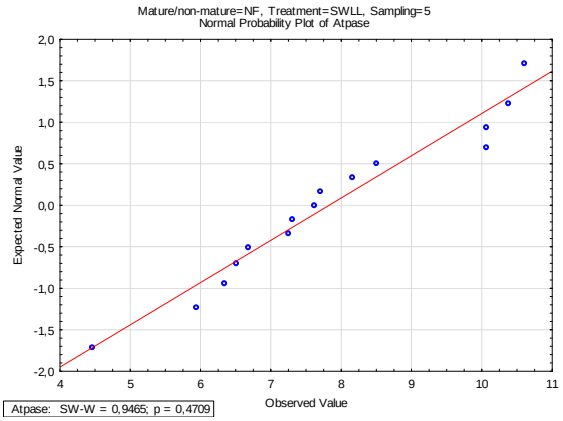

Mature/non-mature=MM, Treatment=FWLD, Sampling=7
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,8952; p = 0,4075

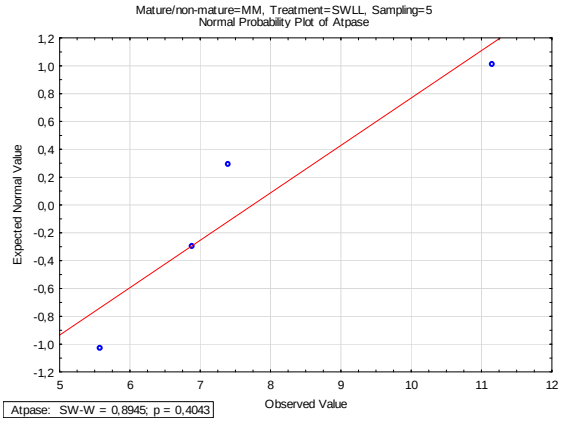

Mature/non-mature=NF, Treatment=SWLL, Sampling=8
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9316; p = 0,1183

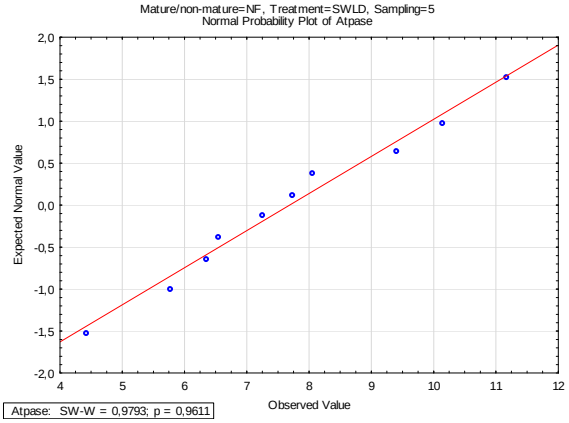

Mature/non-mature=MM, Treatment=SWLL, Sampling=8
Normal Probability Plot of CF
Expected Normal Value
Observed Value
CF: SW-W = 0,9291; p = 0,2645

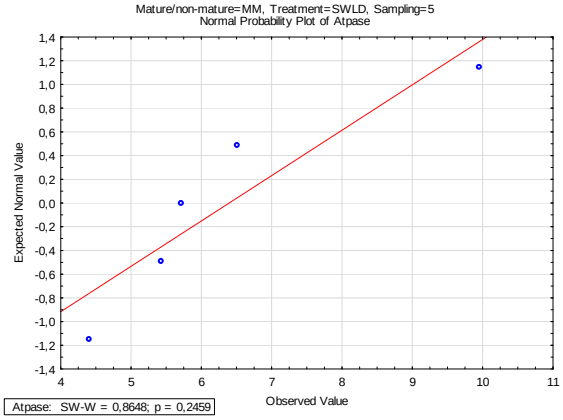


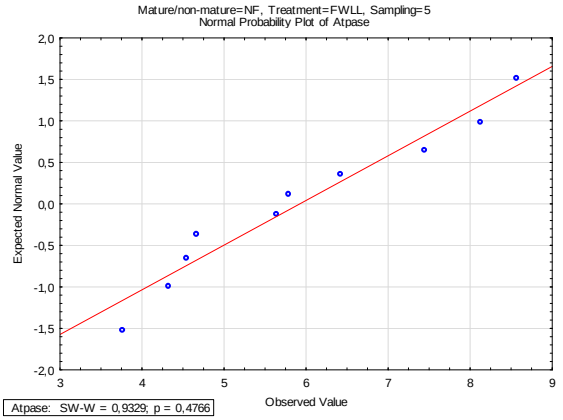
Normal probability plot of residual for NKA activity divided into samplings 5-8, treatment groups (SWLL, SWLD, FWLL and FWLD) and maturation category (NF and MM):

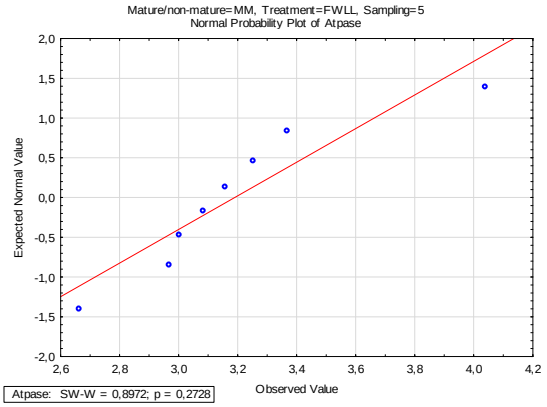

Mature/non-mature=NF, Treatment=SWLL, Sampling=5
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9465; p = 0,4709

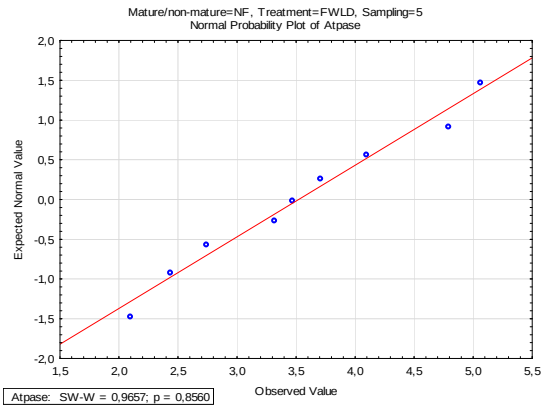

Mature/non-mature=MM, Treatment=SWLL, Sampling=5
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,8945; p = 0,4043

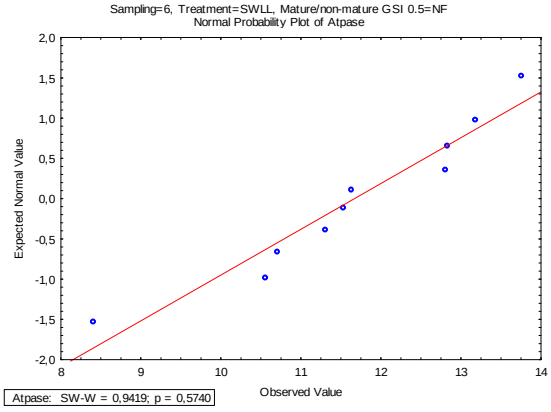

Mature/non-mature=NF, Treatment=SWLD, Sampling=5
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9793; p = 0,9611

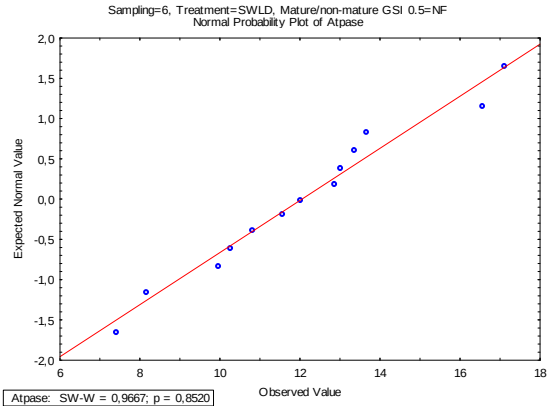

Mature/non-mature=MM, Treatment=SWLD, Sampling=5
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,8648; p = 0,2459


Mature/non-mature=NF, Treatment=FWLL, Sampling=5
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9329; p = 0,4766

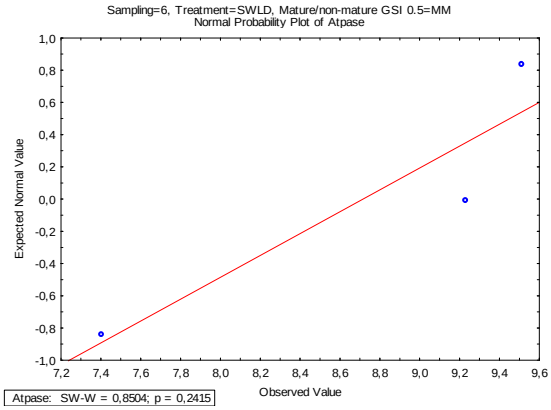

Mature/non-mature=MM, Treatment=FWLL, Sampling=5
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,8972; p = 0,2728


Mature/non-mature=NF, Treatment=FWLD, Sampling=5
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9657; p = 0,8560

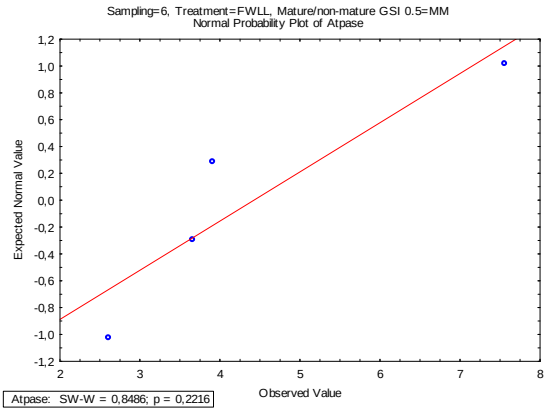

Sampling=6, Treatment=SWLL, Mature/non-mature GSI 0.5=NF
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9419; p = 0,5740



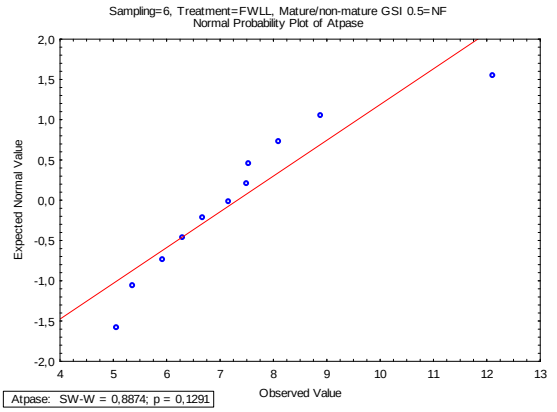
Atpase: SW-W = 0,9667; p = 0,8520



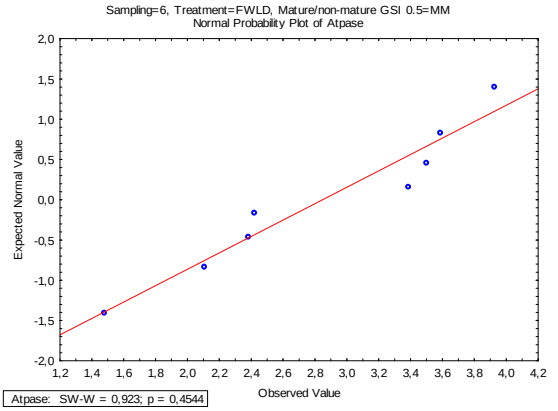
Atpase: SW-W = 0,8504; p = 0,2415



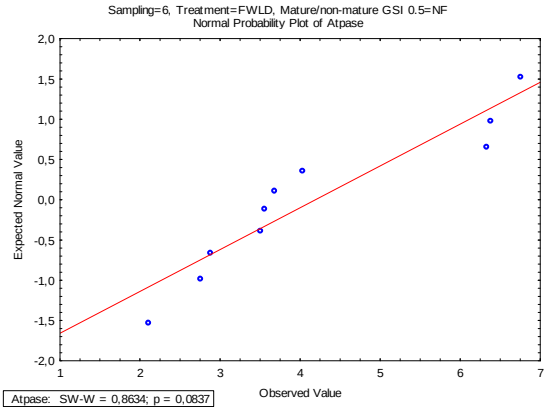
Atpase: SW-W = 0,8486; p = 0,2216



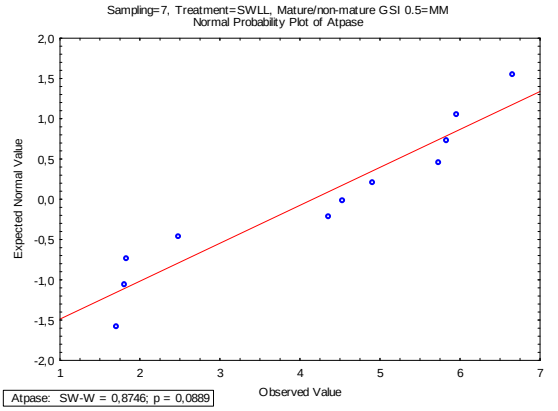
Atpase: SW-W = 0,8874; p = 0,1291



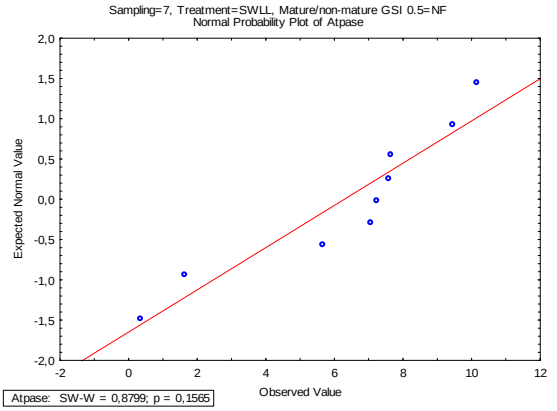
Atpase: SW-W = 0,923; p = 0,4544



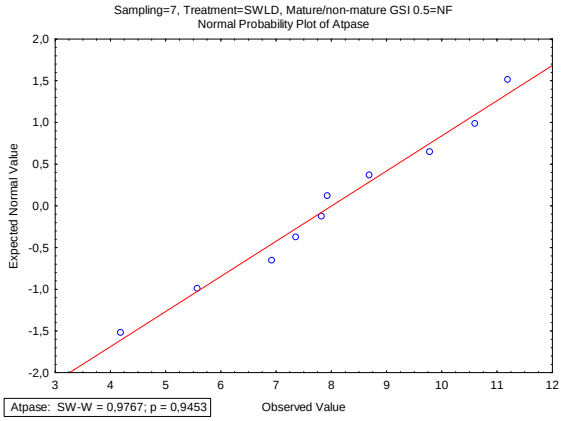
Atpase: SW-W = 0,8634; p = 0,0837

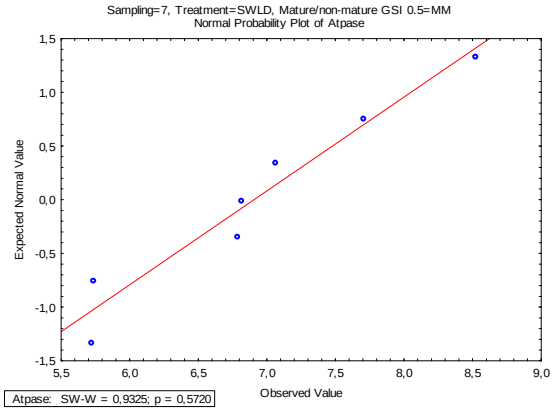


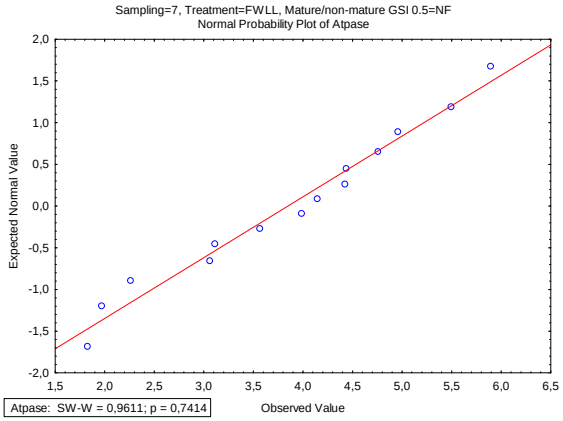
Atpase: SW-W = 0,8746; p = 0,0889

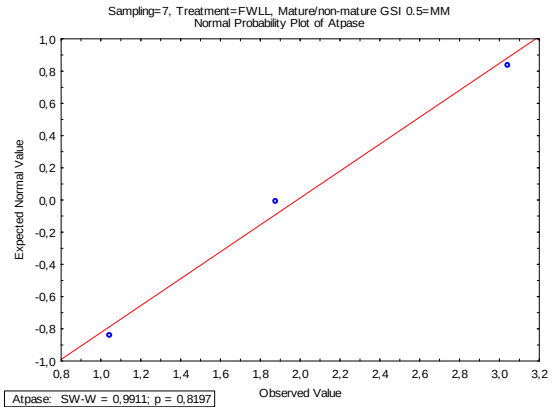


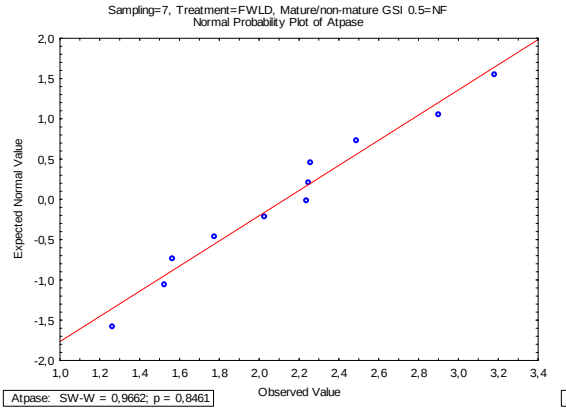
Atpase: SW-W = 0,8799; p = 0,1565

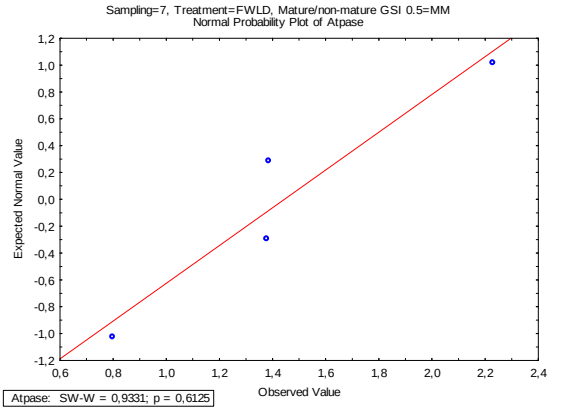

Sampling=7, Treatment=SWLD, Mature/non-mature GSI 0.5=NF
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9767; p = 0,9453

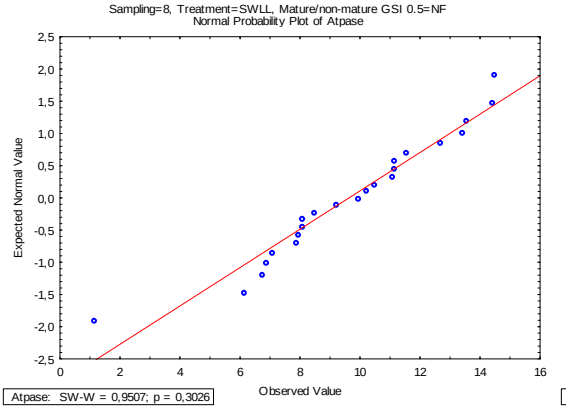

Sampling=7, Treatment=SWLD, Mature/non-mature GSI 0.5=MM
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9325; p = 0,5720

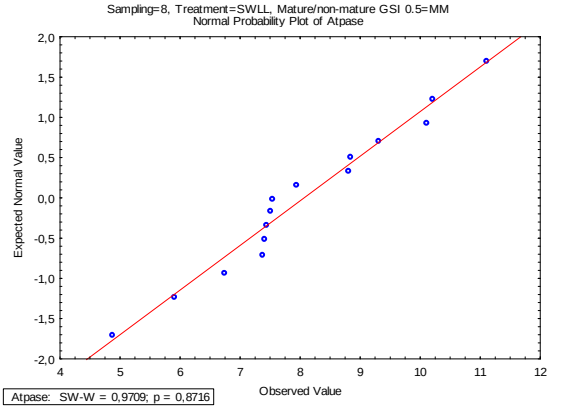

Sampling=7, Treatment=FWLL, Mature/non-mature GSI 0.5=NF
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9611; p = 0,7414

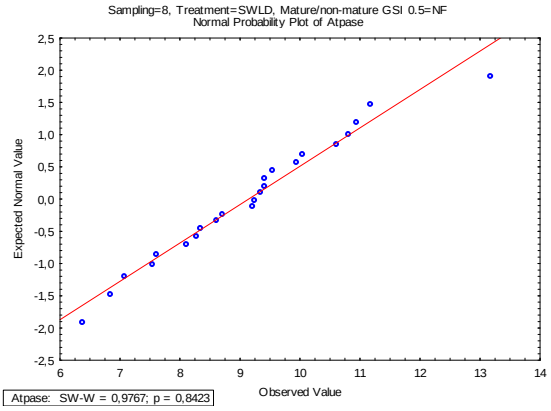

Sampling=7, Treatment=FWLL, Mature/non-mature GSI 0.5=MM
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9911; p = 0,8197

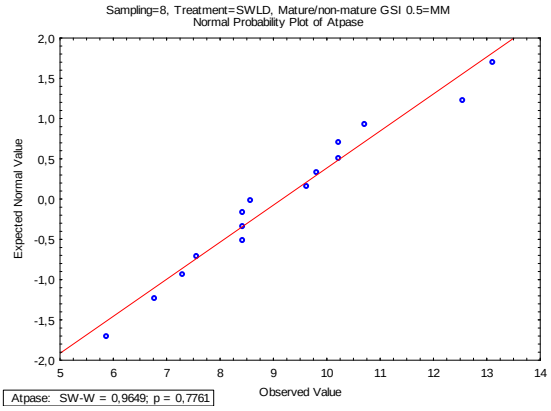

Sampling=7, Treatment=FWLD, Mature/non-mature GSI 0.5=NF
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9662; p = 0,8461

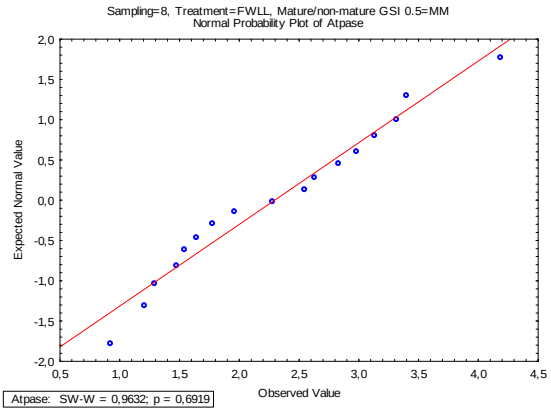

Sampling=7, Treatment=FWLD, Mature/non-mature GSI 0.5=MM
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9331; p = 0,6125

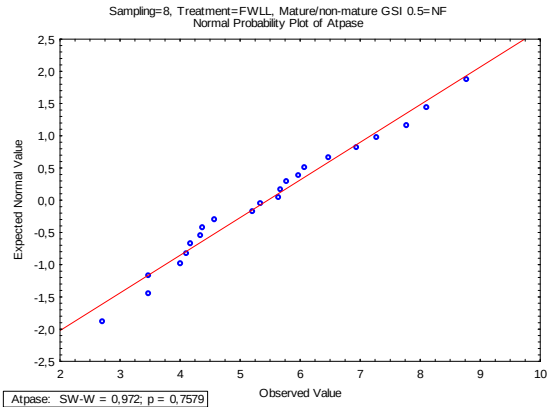

Sampling=8, Treatment=SWLL, Mature/non-mature GSI 0.5=NF
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9507; p = 0,3026

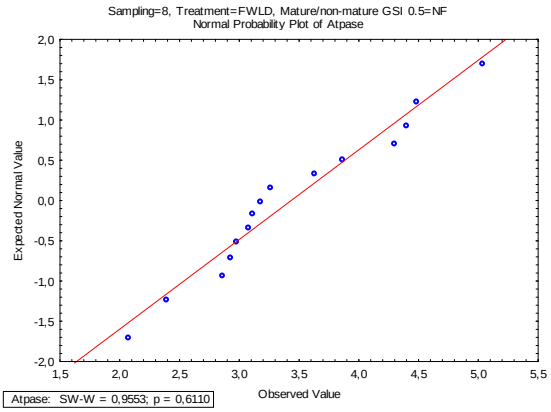

Sampling=8, Treatment=SWLL, Mature/non-mature GSI 0.5=MM
Normal Probability Plot of Atpase
Expected Normal Value
Observed Value
Atpase: SW-W = 0,9709; p = 0,8716

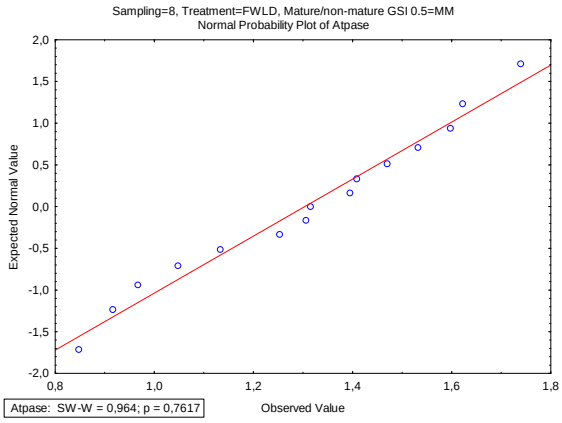




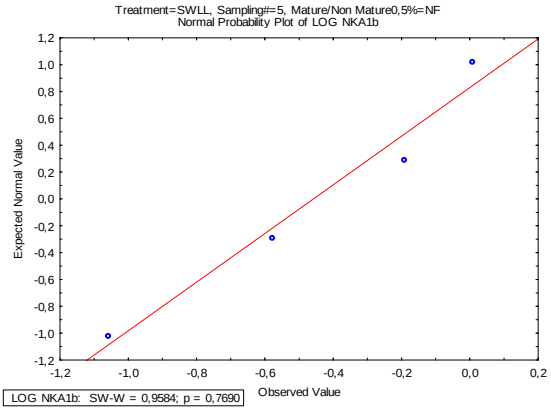


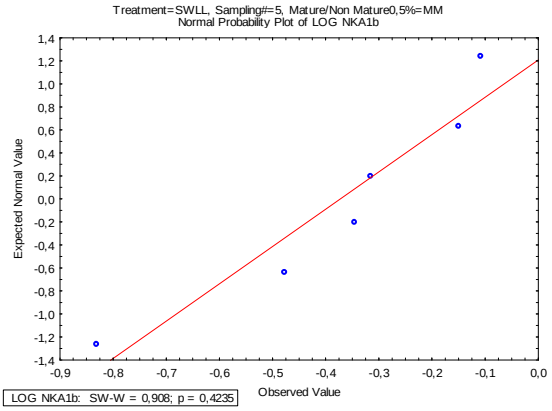


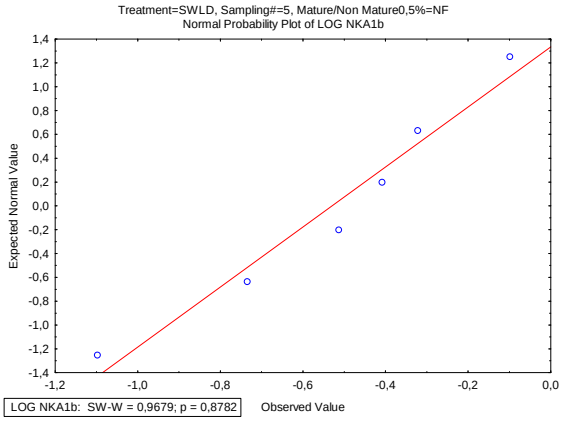


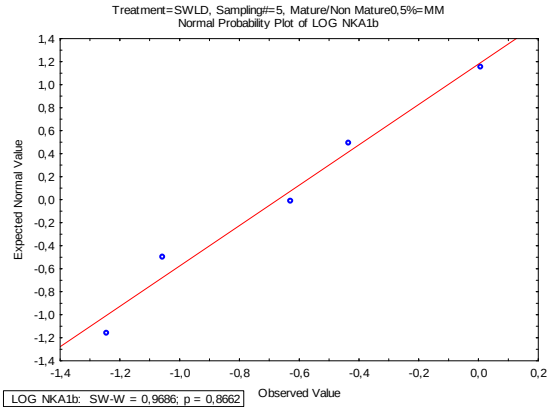


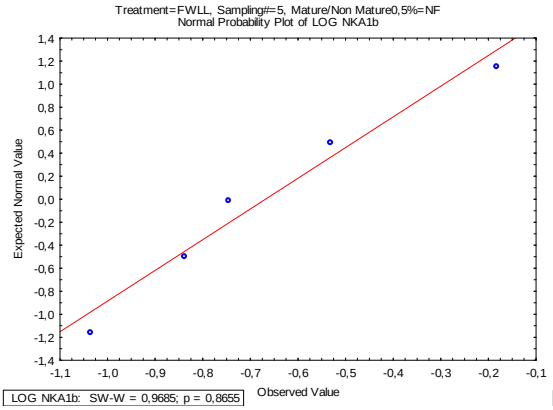
Normal probability plot of residual for Log NKA1b divided into samplings 5-8, treatment groups (SWLL, SWLD, FWLL and FWLD) and maturation category (NF and MM):

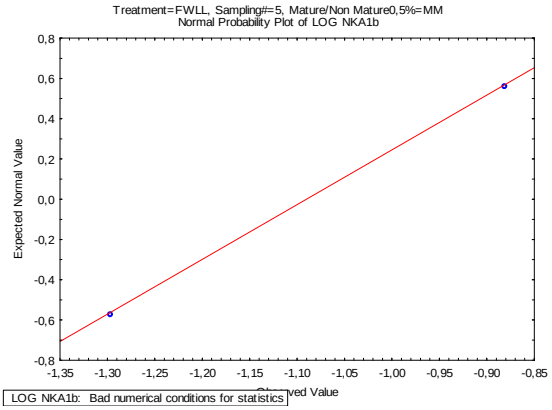


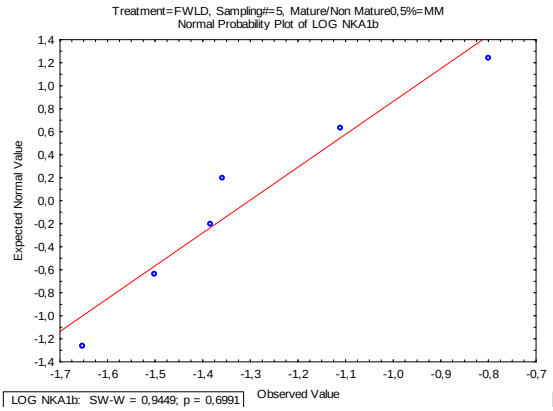


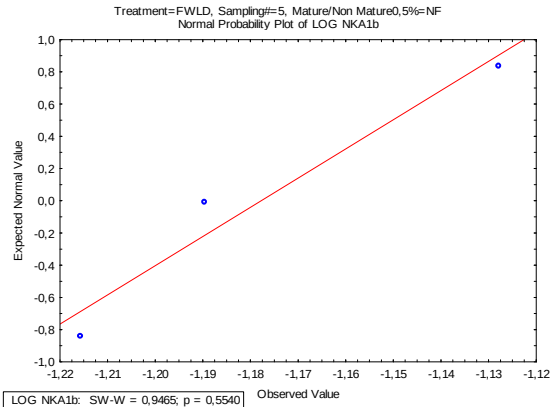

Treatment=SWLD, Sampling#=5, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9679; p = 0,8782

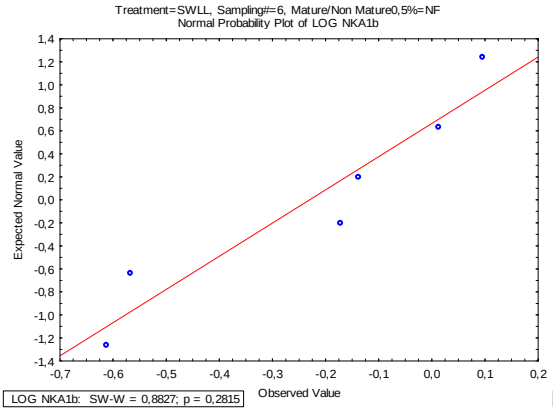

Treatment=SWLD, Sampling#=5, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9686; p = 0,8662

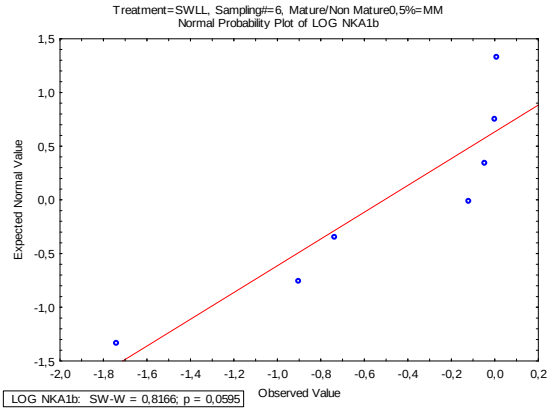

Treatment=FWLL, Sampling#=5, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9685; p = 0,8655

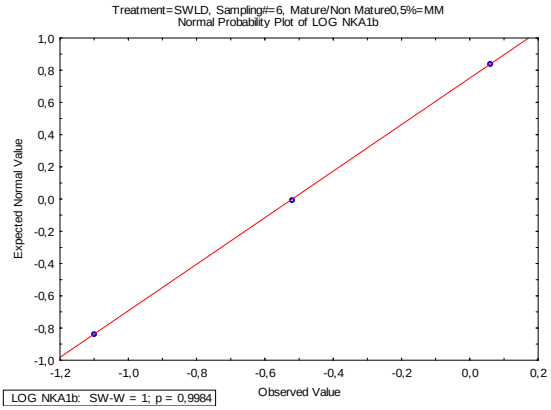

Treatment=FWLL, Sampling#=5, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
LOG NKA1b: Bad numerical conditions for statistics

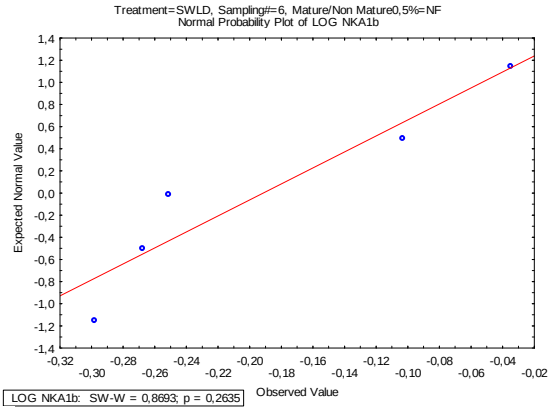

Treatment=FWLD, Sampling#=5, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9449; p = 0,6991

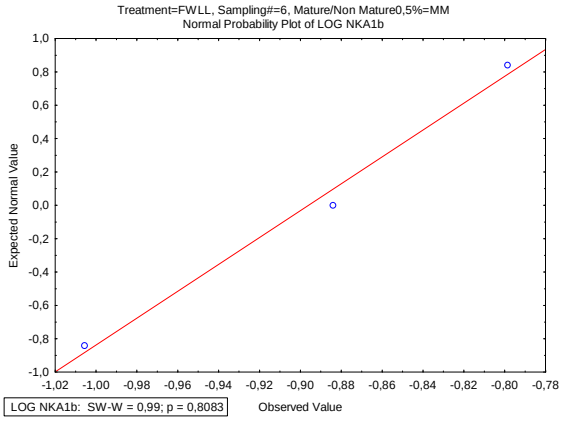

Treatment=FWLD, Sampling#=5, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9465; p = 0,5540

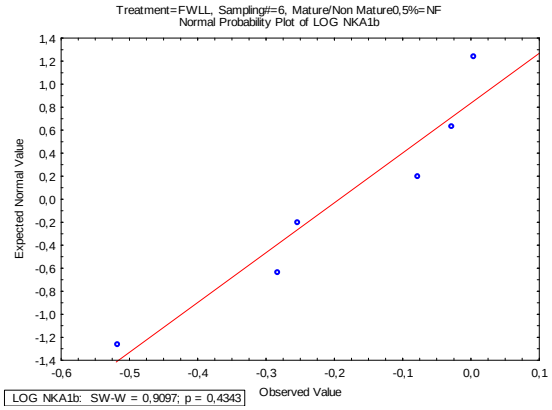

Treatment=SWLL, Sampling#=6, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,8827; p = 0,2815

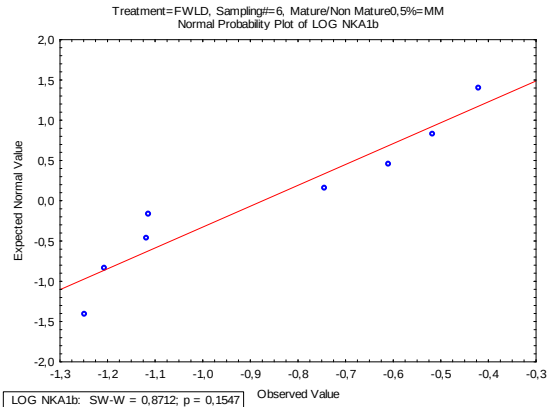

Treatment=SWLL, Sampling#=6, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,8166; p = 0,0595

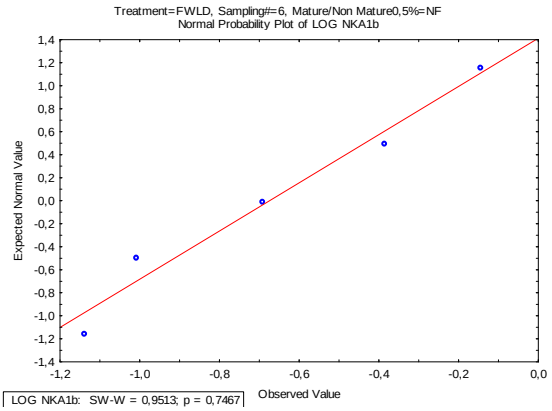

Treatment=SWLD, Sampling#=6, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 1; p = 0,9984

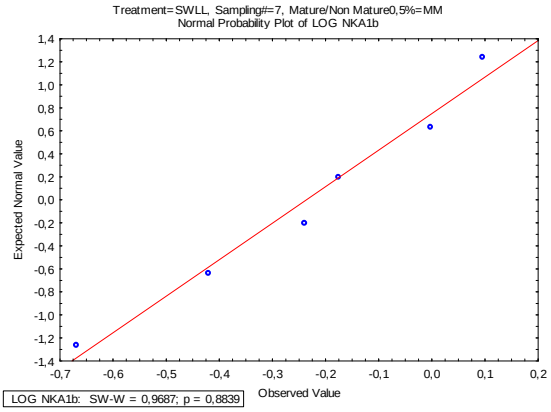

Treatment=SWLD, Sampling#=6, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,8693; p = 0,2635

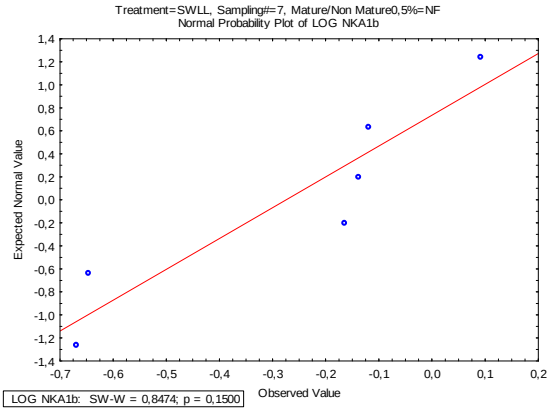

Treatment=FWLL, Sampling#=6, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,99; p = 0,8083

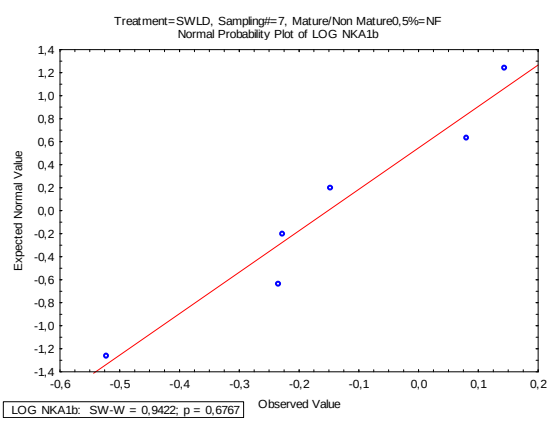

Treatment=FWLL, Sampling#=6, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9097; p = 0,4343

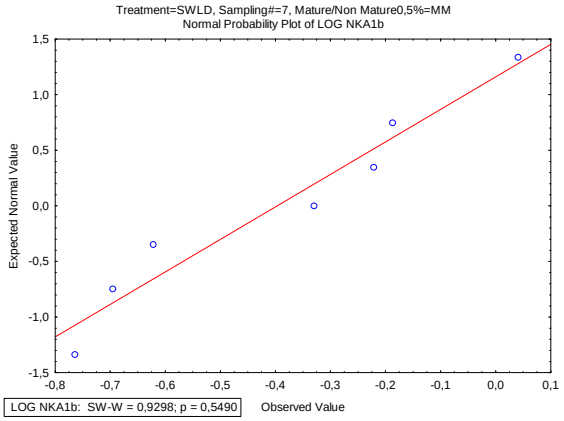

Treatment=FWLD, Sampling#=6, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,8712; p = 0,1547

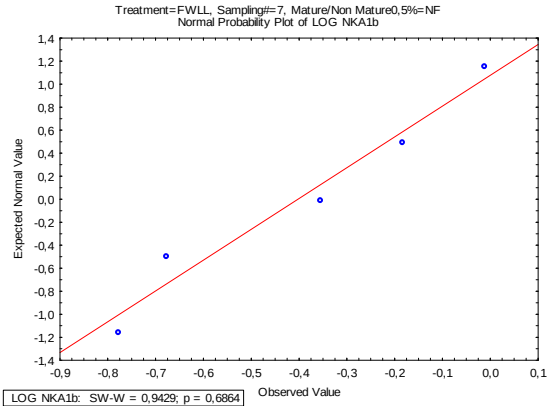

Treatment=FWLD, Sampling#=6, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9513; p = 0,7467

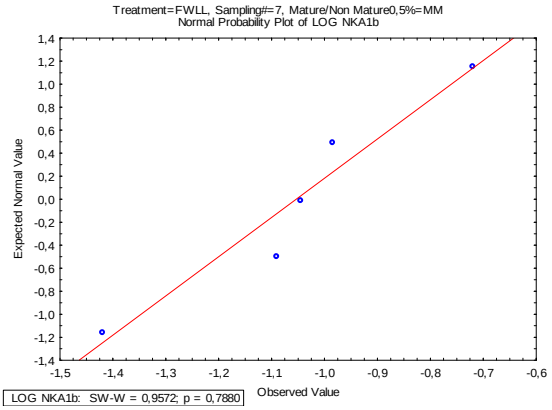

Treatment=SWLL, Sampling#=7, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9687; p = 0,8839

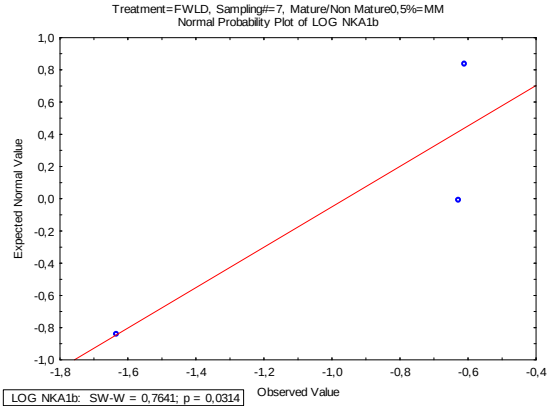

Treatment=SWLL, Sampling#=7, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,8474; p = 0,1500

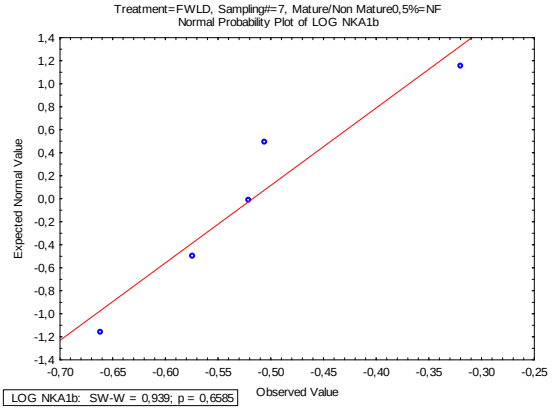

Treatment=SWLD, Sampling#=7, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9422; p = 0,6767

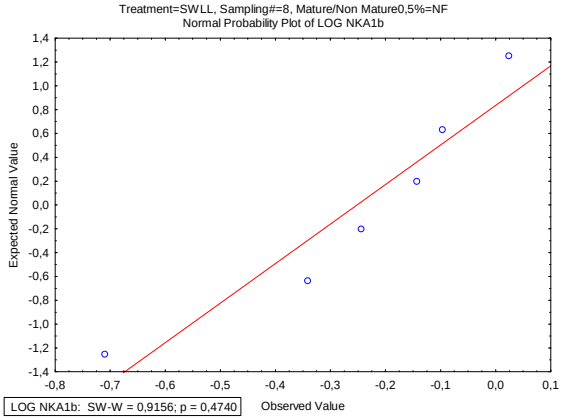

Treatment=SWLD, Sampling#=7, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9298; p = 0,5490

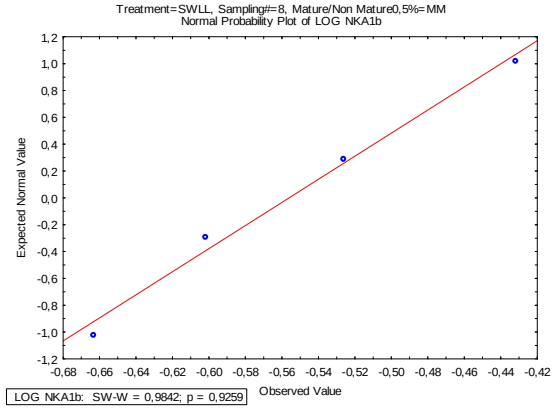

Treatment=FWLL, Sampling#=7, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9429; p = 0,6864

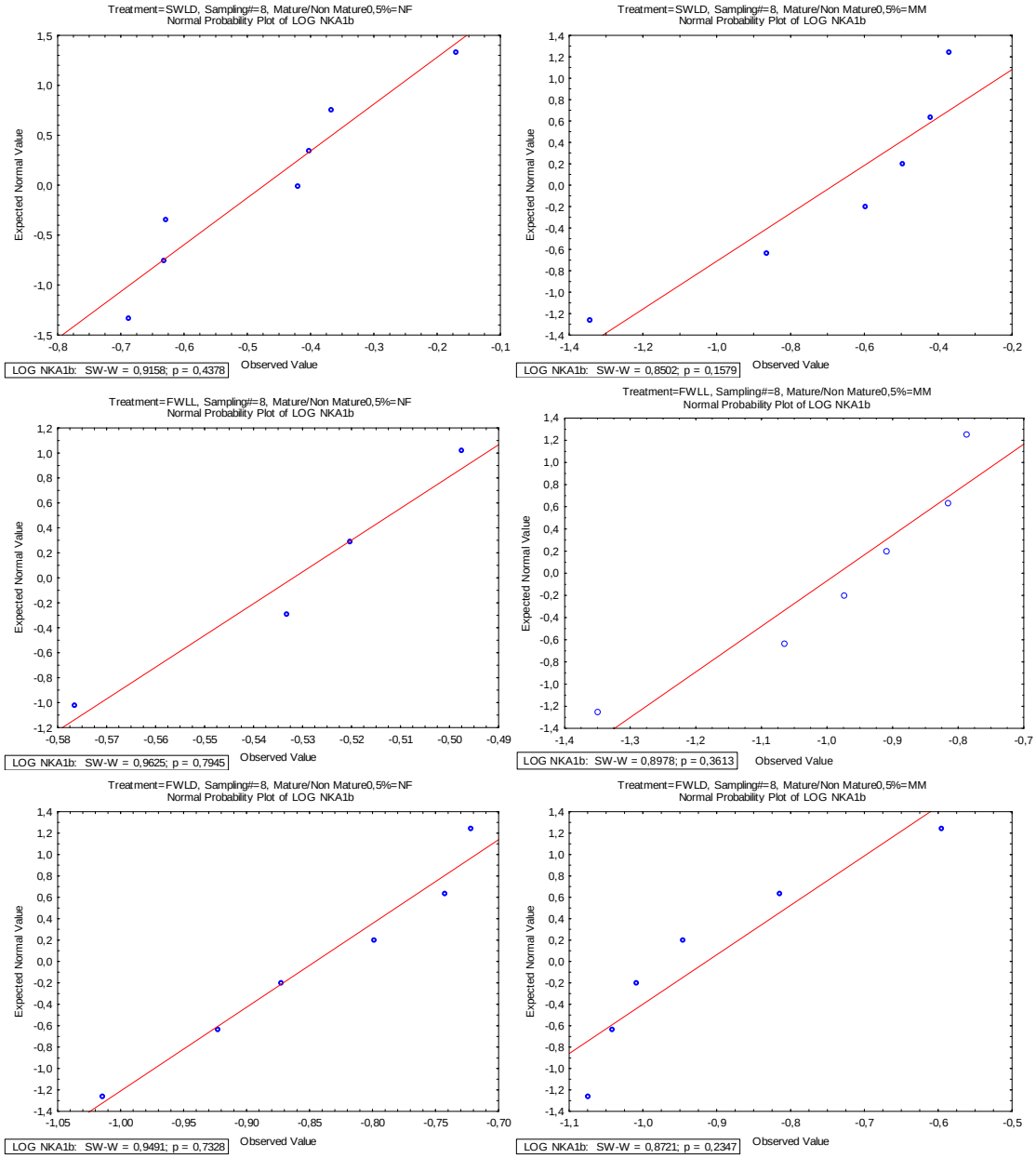

Treatment=FWLL, Sampling#=7, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9572; p = 0,7880


Treatment=FWLD, Sampling#=7, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,7641; p = 0,0314

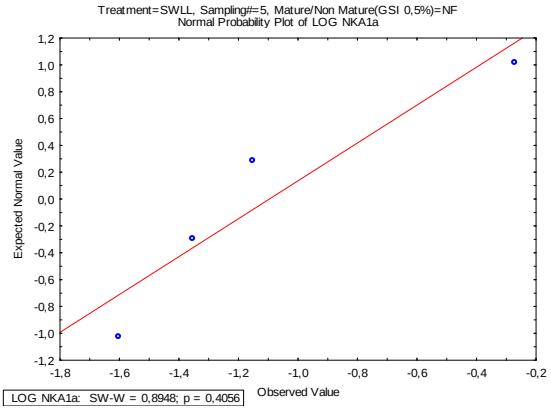

Treatment=FWLD, Sampling#=7, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,939; p = 0,6585

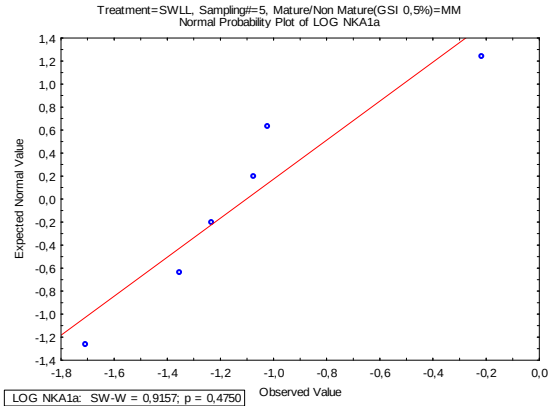

Treatment=SWLL, Sampling#=8, Mature/Non Mature0,5%=NF
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9156; p = 0,4740

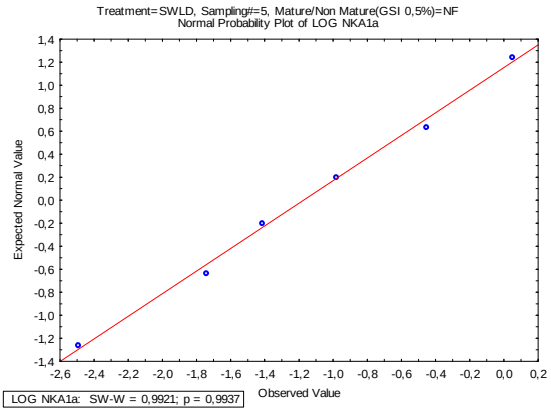

Treatment=SWLL, Sampling#=8, Mature/Non Mature0,5%=MM
Normal Probability Plot of LOG NKA1b
Expected Normal Value
Observed Value
LOG NKA1b: SW-W = 0,9842; p = 0,9259

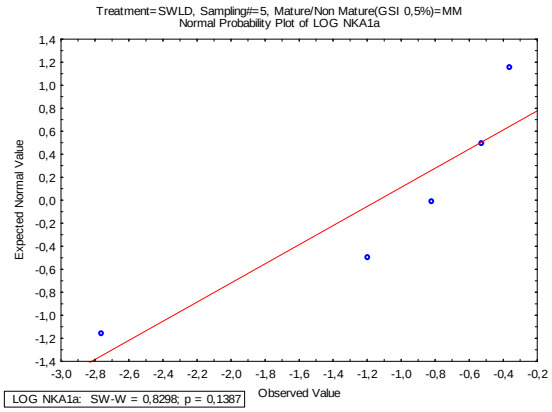


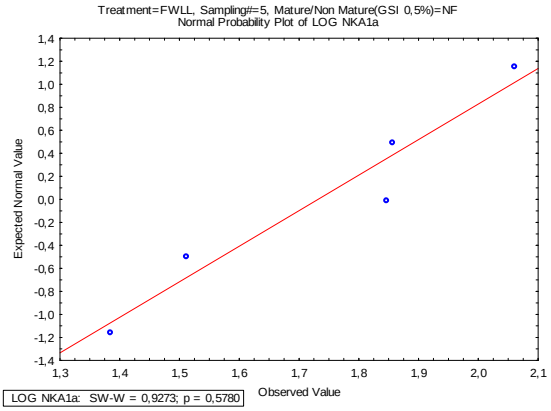
Normal probability plot of residual for Log NKA1a divided into samplings 5-8, treatment groups (SWLL, SWLD, FWLL and FWLD) and maturation category (NF and MM):

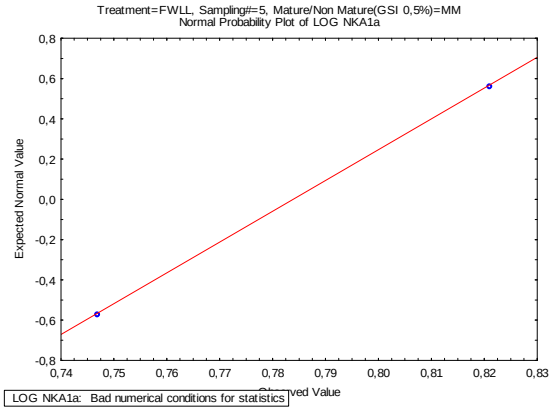

Treatment=SWLL, Sampling#=5, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8948; p = 0,4056

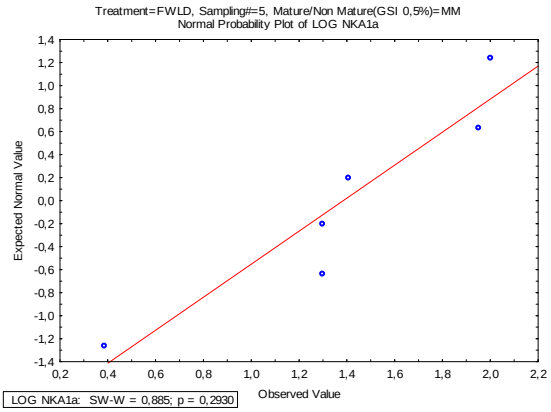

Treatment=SWLL, Sampling#=5, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9157; p = 0,4750

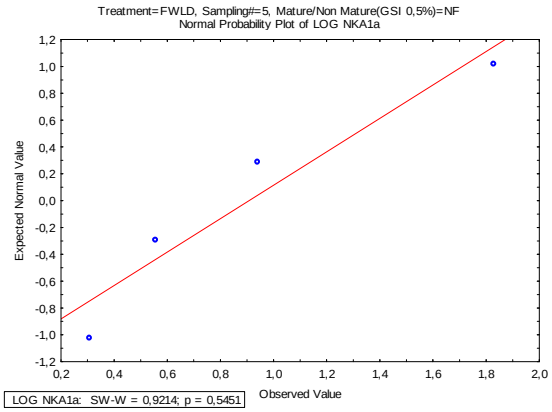

Treatment=SWLD, Sampling#=5, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9921; p = 0,9937

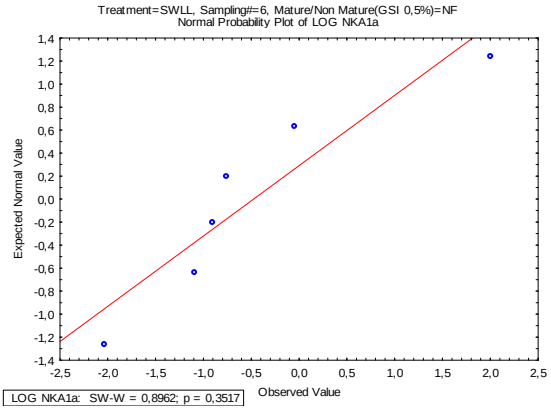

Treatment=SWLD, Sampling#=5, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8298; p = 0,1387

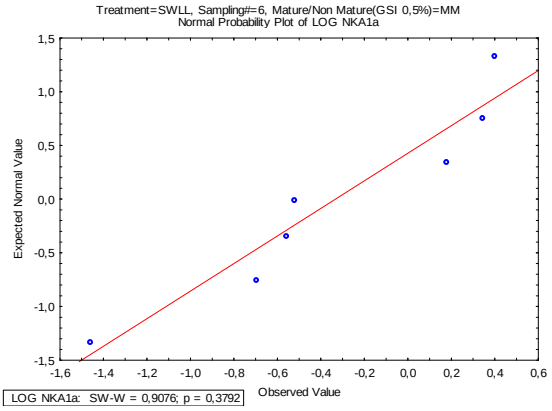

Treatment=FWLL, Sampling#=5, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9273; p = 0,5780

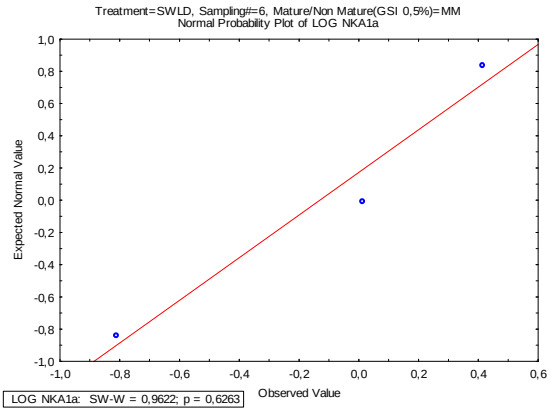

Treatment=FWLL, Sampling#=5, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
LOG NKA1a: Bad numerical conditions for statistics

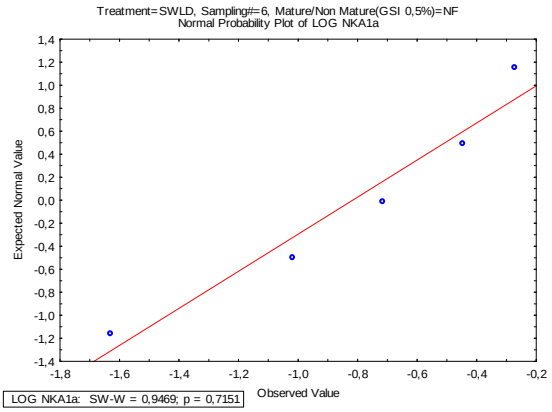

Treatment=FWLD, Sampling#=5, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,885; p = 0,2930

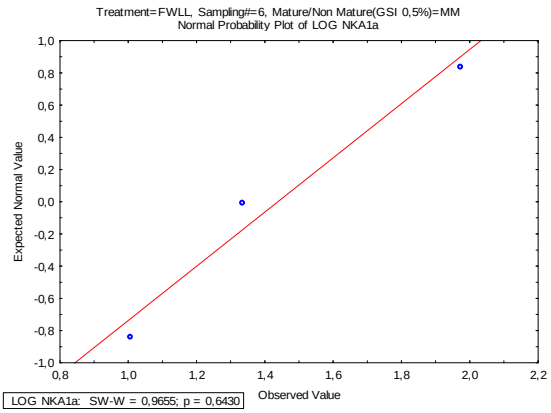

Treatment=FWLD, Sampling#=5, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9214; p = 0,5451

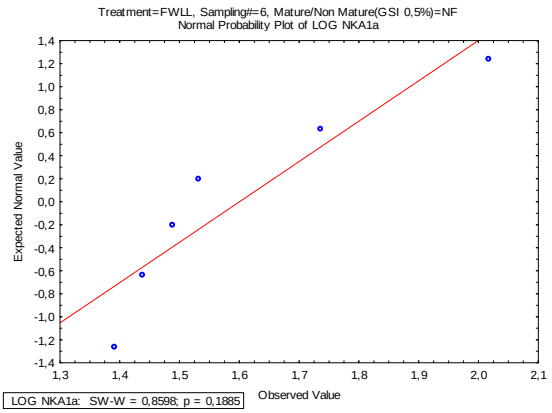

Treatment=SWLL, Sampling#=6, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8962; p = 0,3517

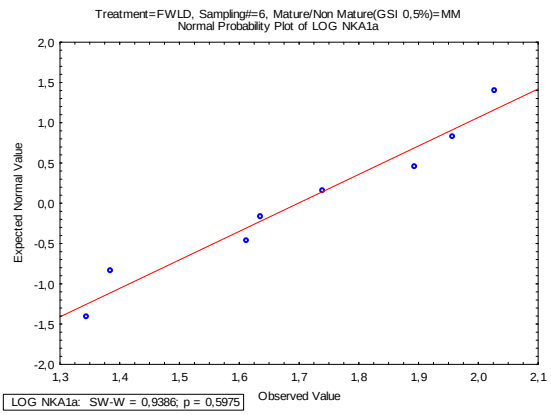

Treatment=SWLL, Sampling#=6, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9076; p = 0,3792

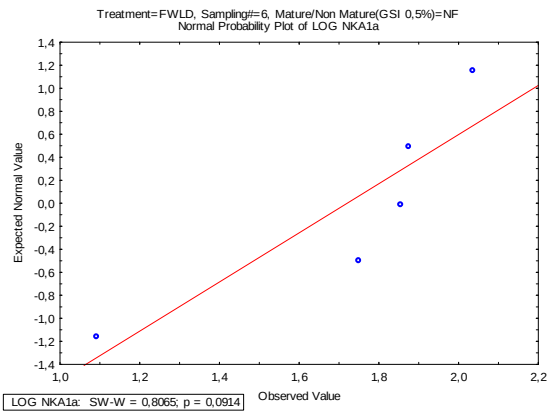

Treatment=SWLD, Sampling#=6, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9622; p = 0,6263

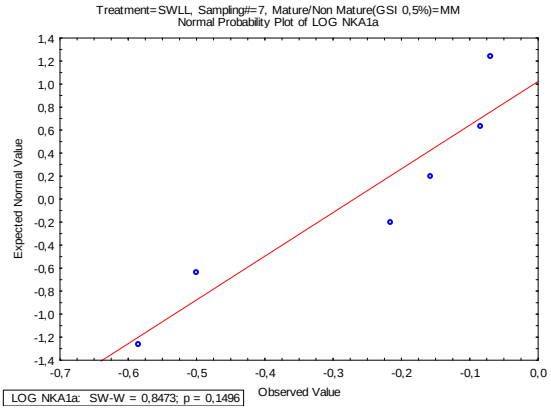

Treatment=SWLD, Sampling#=6, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9469; p = 0,7151

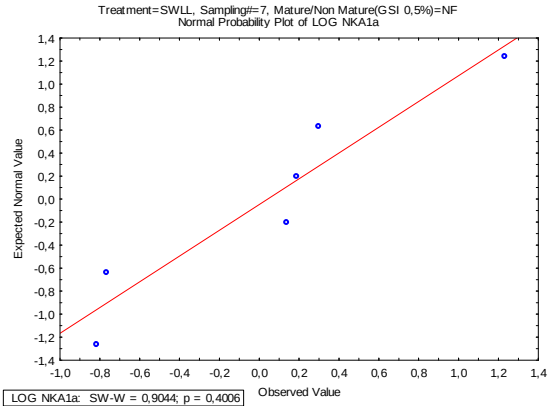

Treatment=FWLL, Sampling#=6, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9655; p = 0,6430

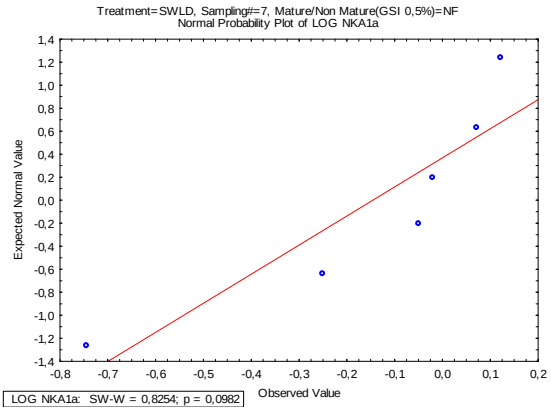

Treatment=FWLL, Sampling#=6, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8598; p = 0,1885

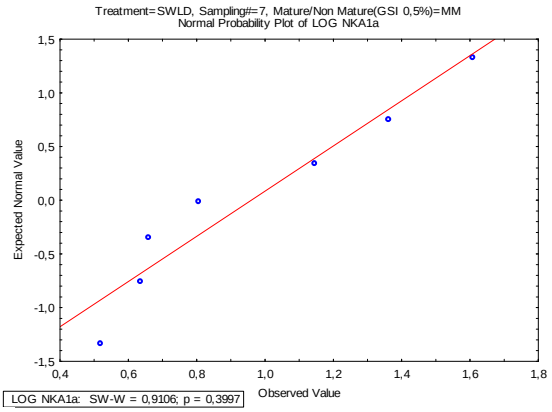

Treatment=FWLD, Sampling#=6, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9386; p = 0,5975

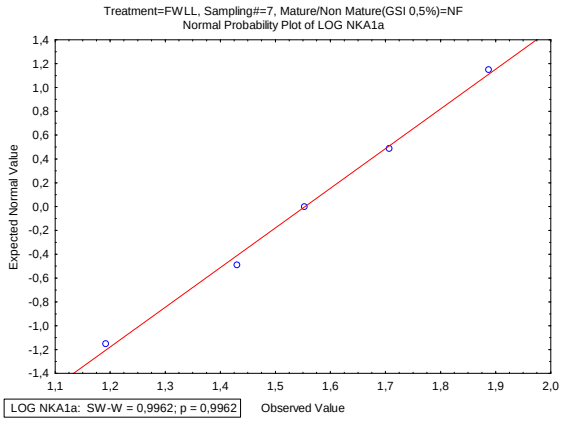

Treatment=FWLD, Sampling#=6, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8065; p = 0,0914

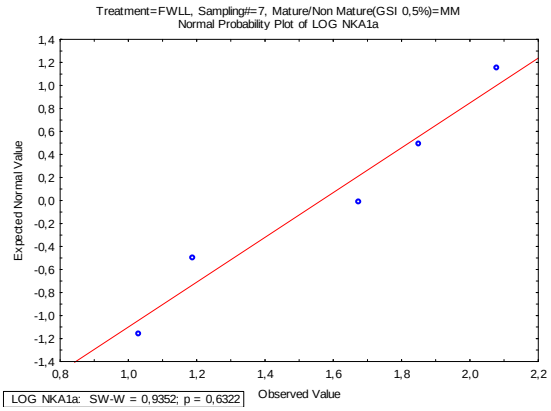

Treatment=SWLL, Sampling#=7, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8473; p = 0,1496

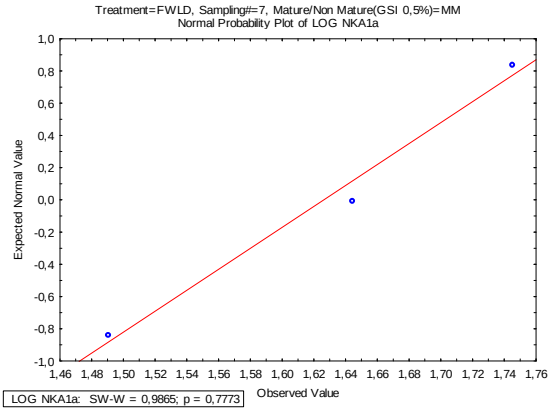

Treatment=SWLL, Sampling#=7, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9044; p = 0,4006

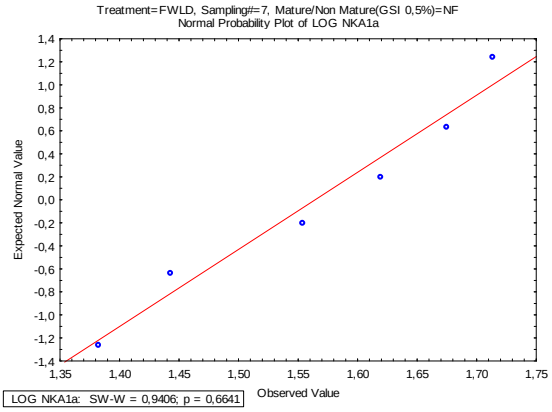

Treatment=SWLD, Sampling#=7, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8254; p = 0,0982

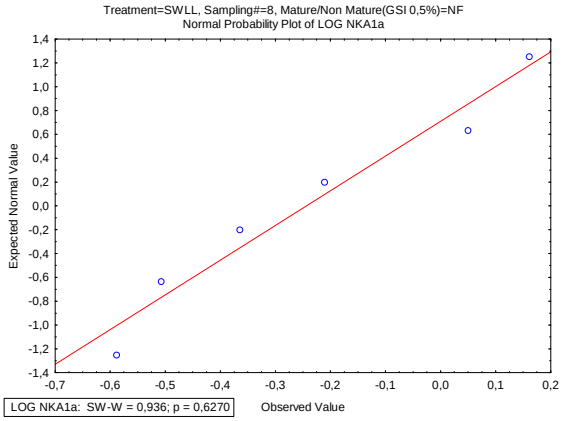

Treatment=SWLD, Sampling#=7, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9106; p = 0,3997

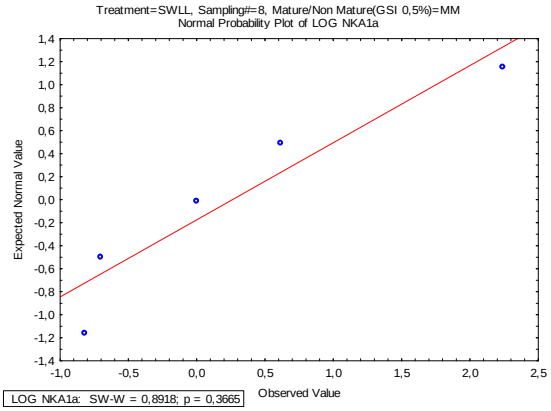

Treatment=FWLL, Sampling#=7, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9962; p = 0,9962

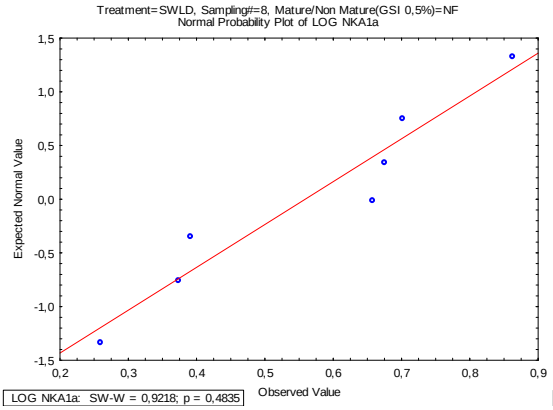

Treatment=FWLL, Sampling#=7, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9352; p = 0,6322

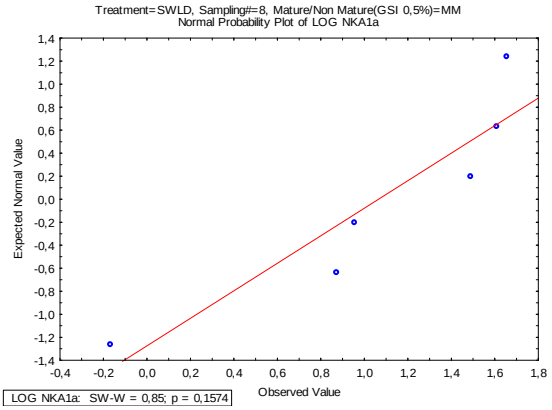

Treatment=FWLD, Sampling#=7, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9865; p = 0,7773

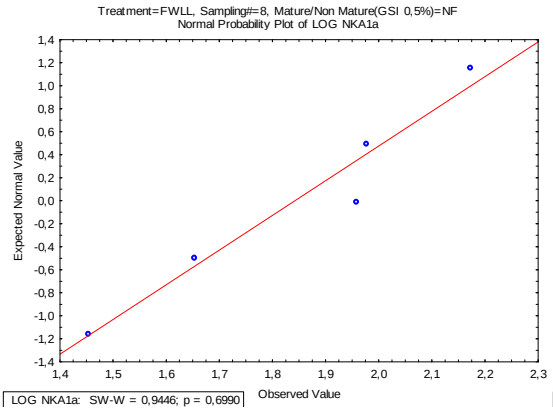

Treatment=FWLD, Sampling#=7, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9406; p = 0,6641

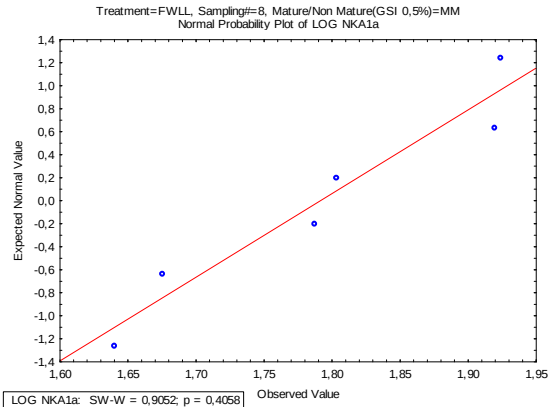

Treatment=SWLL, Sampling#=8, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,936; p = 0,6270

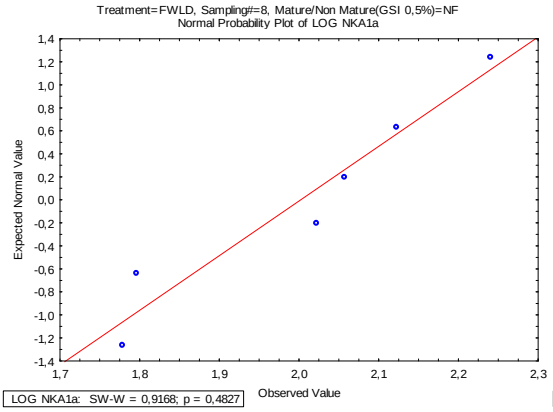

Treatment=SWLL, Sampling#=8, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8918; p = 0,3665

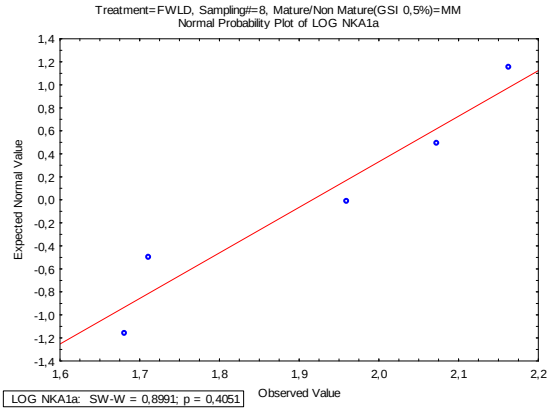

Treatment=SWLD, Sampling#=8, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9218; p = 0,4835


Treatment=SWLD, Sampling#=8, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,85; p = 0,1574


Treatment=FWLL, Sampling#=8, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9446; p = 0,6990


Treatment=FWLL, Sampling#=8, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9052; p = 0,4058


Treatment=FWLD, Sampling#=8, Mature/Non Mature(GSI 0,5%)=NF
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,9168; p = 0,4827


Treatment=FWLD, Sampling#=8, Mature/Non Mature(GSI 0,5%)=MM
Normal Probability Plot of LOG NKA1a
Expected Normal Value
Observed Value
LOG NKA1a: SW-W = 0,8991; p = 0,4051

Analysis of variance (ANOVA)

Fork length sampling 1-4

Table A. III. 29: One-way ANOVA of **Fork length** between sampled tanks in sampling 1-4. Significant values ($p<0.05$) are highlighted in bold.

		SS	Degr. of	MS	F	p
Sampling 1	Intercept	10192,61	1	10192,61	7840,471	**0,000000**
	Tank nr	0,34	3	0,11	0,087	0,966405
	Error	20,80	16	1,30		
Sampling 2	Intercept	13676,45	1	13676,45	6288,023	**0,000000**
	Tank nr	1,75	3	0,58	0,268	0,847353
	Error	34,80	16	2,18		
Sampling 3	Intercept	17731,01	1	17731,01	4246,949	**0,000000**
	Tank nr	25,94	3	8,65	2,071	0,144461
	Error	66,80	16	4,18		
Sampling 4	Intercept	19313,11	1	19313,11	1819,846	**0,000000**
	Tank nr	21,84	3	7,28	0,686	0,573631
	Error	169,80	16	10,61		

Table A. III. 30: Factorial ANOVA of **Fork length** for females and males during smoltification (Sampling 1-4). Significant values ($p<0.05$) are highlighted in bold.

	SS	MS	F	p
Intercept	58558,04	58558,04	13093,05	**0,000000**
Sampling	828,89	276,30	61,78	**0,000000**
Sex	2,46	2,46	0,55	0,460945
Sampling*Sex	17,72	5,91	1,32	0,274307
Error	322,02	4,47		
Total	1221,85			

Fork length sampling 5-8

Table A. III. 31: Factorial ANOVA of **Fork length** for NF and MM in treatment group SWLL during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

SWLL	SS	Degr. of	MS	F	p
Intercept	107455,2	1	107455,2	12973,60	**0,000000**
Sampling	329,0	3	109,7	13,24	**0,000000**
Mature/non-mature	22,4	1	22,4	2,70	0,103768
Sampling*Mature/non-mature	19,9	3	6,6	0,80	0,497532
Error	728,9	88	8,3		

Table A. III. 32: Factorial ANOVA of **Fork length** for NF and MM in treatment group SWLD during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

SWLD	SS	Degr. of	MS	F	p
Intercept	80902,92	1	80902,92	12843,35	**0,000000**
Sampling	229,80	3	76,60	12,16	**0,000001**
Mature/immature	19,31	1	19,31	3,07	0,083938
Sampling*Mature/immature	116,39	3	38,80	6,16	**0,000828**

Error	485,04	77	6,30		

Table A. III. 33: Factorial ANOVA of **Fork length** for NF and MM in treatment group FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLD	SS	Degr. of	MS	F	p
Intercept	95917,68	1	95917,68	15314,18	**0,000000**
Sampling	286,09	3	95,36	15,23	**0,000000**
Mature/immature	7,20	1	7,20	1,15	0,287093
Sampling*Mature/immature	167,62	3	55,87	8,92	**0,000041**
Error	463,49	74	6,26		

Table A. III. 34: Factorial ANOVA of **Fork length** for NF and MM in treatment group FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLL	SS	Degr. of	MS	F	p
Intercept	88488,08	1	88488,08	11998,19	**0,000000**
Sampling	754,73	3	251,58	34,11	**0,000000**
Mature/immature	47,77	1	47,77	6,48	**0,012803**
Sampling*Mature/immature	60,21	3	20,07	2,72	**0,049671**
Error	604,76	82	7,38		

Body weight sampling 1-4

Table A. III. 35: One-way ANOVA of **Body weigh** between sampled tanks in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

		SS	Degr. of	MS	F	p
Sampling 1	Intercept	94,67522	1	94,67522	28273,81	**0,000000**
	Tank nr	0,00309	3	0,00103	0,31	0,819752
	Error	0,05358	16	0,00335		
Sampling 2	Intercept	111,8142	1	111,8142	21860,86	**0,000000**
	Tank nr	0,0014	3	0,0005	0,09	0,963388
	Error	0,0818	16	0,0051		
Sampling 3	Intercept	129,7340	1	129,7340	17556,57	**0,000000**
	Tank nr	0,0601	3	0,0200	2,71	0,079624
	Error	0,1182	16	0,0074		
Sampling 4	Intercept	130,3256	1	130,3256	5815,040	**0,000000**
	Tank nr	0,0542	3	0,0181	0,807	0,508355
	Error	0,3586	16	0,0224		

Table A. III. 36: Factorial ANOVA of **Body weight** for females and males during smoltification (Sampling 1-4). Significant values (p<0.05) are highlighted in bold.

	SS	MS	F	p
Intercept	450,3882	450,3882	46626,95	**0,000000**
Sampling	1,8270	0,6090	63,05	**0,000000**
Sex	0,0059	0,0059	0,61	0,436554
Sampling*Sex	0,0384	0,0128	1,32	0,273321
Error	0,6858	0,0097		
Total	2,6419			

Body weight sampling 5-8

Table A. III. 37: Factorial ANOVA of **body weight** for NF and MM in treatment group SWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

SWLL	SS	Degr. of	MS	F	p
Intercept	40107037	1	40107037	1054,760	**0,000000**
Sampling	1381969	3	460656	12,115	**0,000001**
Mature/immature	232196	1	232196	6,106	**0,015373**
Sampling*Mature/immature	74995	3	24998	0,657	0,580411
Error	3384208	89	38025		

Table A. III. 38: Factorial ANOVA of **body weight** for NF and MM in treatment group SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

SWLD	SS	Degr. of	MS	F	p
Intercept	29872733	1	29872733	1088,465	**0,000000**
Sampling	1398538	3	466179	16,986	**0,000000**
Mature/immature	44537	1	44537	1,623	0,206485
Sampling*Mature/immature	733346	3	244449	8,907	**0,000038**
Error	2140696	78	27445		

Table A. III. 39: Factorial ANOVA of **body weight** for NF and MM in treatment group FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLD	SS	Degr. of	MS	F	p
Intercept	40523772	1	40523772	1379,150	**0,000000**
Sampling	1508239	3	502746	17,110	**0,000000**
Mature/immature	10664	1	10664	0,363	0,548732
Sampling*Mature/immature	972517	3	324172	11,033	**0,000005**
Error	2174353	74	29383		

Table A. III. 40: Factorial ANOVA of **body weight** for NF and MM in treatment group FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLL	SS	Degr. of	MS	F	p
Intercept	37959606	1	37959606	1217,841	**0,000000**
Sampling	3488972	3	1162991	37,312	**0,000000**
Mature/immature	477922	1	477922	15,333	**0,000186**
Sampling*Mature/immature	264990	3	88330	2,834	**0,043248**
Error	2555906	82	31170		

Gonadosomatic Index sampling 1-4

Table A. III. 41: One-way ANOVA of **GSI** between sampled tanks in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

		SS	Degr. of	MS	F	p
Sampling 1	Intercept	0,050413	1	0,050413	75,40977	**0,000000**
	Tank nr	0,000728	3	0,000243	0,36313	0,780498
	Error	0,010696	16	0,000669		
Sampling 2	Intercept	0,082643	1	0,082643	139,9939	**0,000000**

	Tank nr	0,003259	3	0,001086	1,8399	0,180568
	Error	0,009445	16	0,000590		
Sampling 3	Intercept	0,081913	1	0,081913	204,8639	**0,000000**
	Tank nr	0,001836	3	0,000612	1,5303	0,245048
	Error	0,006397	16	0,000400		
Sampling 4	Intercept	0,095040	1	0,095040	171,0170	**0,000000**
	Tank nr	0,001101	3	0,000367	0,6604	0,588278
	Error	0,008892	16	0,000556		

Table A. III. 42: Factorial ANOVA of **GSI** for females and males during smoltification (Sampling 1- 4). Significant values (p<0.05) are highlighted in bold.

	SS	MS	F	p
Intercept	0,302986	0,302986	1699,569	**0,000000**
Sampling	0,001530	0,000510	2,860	**0,042774**
Sex	0,028664	0,028664	160,785	**0,000000**
Sampling*Sex	0,000808	0,000269	1,511	0,218884
Error	0,012836	0,000178		
Total	0,046281			

Gonadosomatic Index sampling 5-8

Table A. III. 43: Factorial ANOVA of **Log GSI** for MM in all treatment groups during maturation (Sampling 5-8). Significant values (p<0.05) are highlighted in bold.

MM all treatments	SS	Degr. of	MS	F	p
Intercept	30,36356	1	30,36356	861,5085	**0,000000**
Sampling	14,69447	3	4,89816	138,9760	**0,000000**
Treatment	0,39157	3	0,13052	3,7034	**0,013571**
Sampling*Treatment	2,70013	9	0,30001	8,5124	**0,000000**
Error	4,37034	124	0,03524		

Condition factor sampling 1-4

Table A. III. 44: One-way ANOVA of **Condition factor** between sampled tanks in sampling 1-4. Significant values (p<0.05) are highlighted in bold.

		SS	Degr. of	MS	F	p
Sampling 1	Intercept	34,27399	1	34,27399	5267,440	**0,000000**
	Tank nr	0,01252	3	0,00417	0,642	0,599302
	Error	0,10411	16	0,00651		
Sampling 2	Intercept	33,86392	1	33,86392	9902,888	**0,000000**
	Tank nr	0,02864	3	0,00955	2,792	0,074024
	Error	0,05471	16	0,00342		
Sampling 3	Intercept	36,31222	1	36,31222	5192,248	**0,000000**
	Tank nr	0,01485	3	0,00495	0,708	0,561237
	Error	0,11190	16	0,00699		
Sampling 4	Intercept	29,38360	1	29,38360	2826,962	**0,000000**
	Tank nr	0,04593	3	0,01531	1,473	0,259507
	Error	0,16630	16	0,01039		

Table A. III. 45: Factorial ANOVA of **Condition factor** for females and males during smoltification (Sampling 1- 4). Significant values (p<0.05) are highlighted in bold.

	SS	MS	F	p
Intercept	129,9070	129,9070	17457,70	**0,000000**
Sampling	0,1846	0,0615	8,27	**0,000086**
Sex	0,0001	0,0001	0,02	0,899982
Sampling*Sex	0,0104	0,0035	0,47	0,705872
Error	0,5283	0,0074		
Total	0,7277			

Condition factor sampling 5-8

Table A. III. 46: Factorial ANOVA of **Condition factor** for NF and MM in treatment group SWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

SWLL	SS	Degr. of	MS	F	p
Intercept	149,4509	1	149,4509	10142,02	**0,000000**
Sampling	0,1763	3	0,0588	3,99	**0,010260**
Mature/immature	0,2178	1	0,2178	14,78	**0,000227**
Sampling*Mature/immature	0,0649	3	0,0216	1,47	0,228774
Error	1,3115	89	0,0147		

Table A. III. 47: Factorial ANOVA of **Condition factor** for NF and MM in treatment group SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

SWLD	SS	Degr. of	MS	F	p
Intercept	111,8603	1	111,8603	7219,434	**0,000000**
Sampling	0,4358	3	0,1453	9,376	**0,000023**
Mature/immature	0,0001	1	0,0001	0,008	0,929111
Sampling*Mature/immature	0,3711	3	0,1237	7,985	**0,000104**
Error	1,2086	78	0,0155		

Table A. III. 48: Factorial ANOVA of **Condition factor** for NF and MM in treatment group FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLL	SS	Degr. of	MS	F	p
Intercept	102,2706	1	102,2706	9867,940	**0,000000**
Sampling	0,2328	3	0,0776	7,488	**0,000174**
Mature/immature	0,3969	1	0,3969	38,294	**0,000000**
Sampling*Mature/immature	0,1438	3	0,0479	4,624	**0,004896**
Error	0,8395	81	0,0104		

Table A. III. 49: Factorial ANOVA of **Condition factor** for NF and MM in treatment group FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLD	SS	Degr. of	MS	F	p
Intercept	111,8603	1	111,8603	7219,434	**0,000000**
Sampling	0,4358	3	0,1453	9,376	**0,000023**
Mature/immature	0,0001	1	0,0001	0,008	0,929111
Sampling*Mature/immature	0,3711	3	0,1237	7,985	**0,000104**
Error	1,2086	78	0,0155		

NKA activity sampling 1-4

Table A. III. 50: One-way ANOVA of **Condition factor** between sampled tanks in sampling 1-4. Significant values ($p<0.05$) are highlighted in bold.

NKA		SS	Degr. of	MS	F	p
Sampling 1	Intercept	1725,056	1	1725,056	577,0208	**0,000000**
	Tank nr	36,724	3	12,241	4,0946	**0,024648**
	Error	47,833	16	2,990		
Sampling 2	Intercept	1860,375	1	1860,375	1292,914	**0,000000**
	Tank nr	17,043	3	5,681	3,948	**0,027713**
	Error	23,022	16	1,439		
Sampling 3	Intercept	424,4526	1	424,4526	221,3468	**0,000000**
	Tank nr	5,5899	3	1,8633	0,9717	0,430405
	Error	30,6814	16	1,9176		
Sampling 4	Intercept	1496,007	1	1496,007	363,1472	**0,000000**
	Tank nr	11,107	3	3,702	0,8987	0,464770
	Error	61,793	15	4,120		

Table A. III. 51: Factorial ANOVA of **NKA activity** for females and males during smoltification (Sampling 1-4). Significant values ($p<0.05$) are highlighted in bold

	SS	MS	F	p
Intercept	5168,506	5168,506	1736,794	**0,000000**
Sampling	336,972	112,324	37,745	**0,000000**
Sex	10,462	10,462	3,516	0,064902
Sampling*Sex	12,538	4,179	1,404	0,248649
Error	211,288	2,976		
Total	567,895			

NKA activity sampling 5-8

Table A. III. 52: Factorial ANOVA of **Log NK1b** for NF and MM in treatment group SWLL during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

SWLL	SS	Degr. of	MS	F	p
Intercept	5069,330	1	5069,330	962,2044	**0,000000**
Sampling	230,631	3	76,877	14,5919	**0,000000**
Mature/non-mature GSI 0.5	72,430	1	72,430	13,7479	**0,000363**
Sampling*Mature/non-mature GSI 0.5	38,511	3	12,837	2,4366	0,069882
Error	468,892	89	5,268		

Table A. III. 53: Factorial ANOVA of **Log NK1b** for NF and MM in treatment group SWLD during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

SWLD	SS	Degr. of	MS	F	p
Intercept	4356,296	1	4356,296	1066,476	**0,000000**
Sampling	96,041	3	32,014	7,837	**0,000123**
Mature/non-mature GSI 0.5	30,544	1	30,544	7,478	**0,007729**
Sampling*Mature/non-mature GSI 0.5	22,817	3	7,606	1,862	0,142939
Error	318,611	78	4,085		

Table A. III. 54: Factorial ANOVA of **Log NK1b** for NF and MM in treatment group FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLD	SS	Degr. of	MS	F	p
Intercept	486,6613	1	486,6613	527,2418	**0,000000**
Sampling	22,9681	3	7,6560	8,2944	**0,000079**
Mature/non-mature GSI 0.5	30,3987	1	30,3987	32,9335	**0,000000**
Sampling*Mature/non-mature GSI 0.5	2,9647	3	0,9882	1,0707	0,366841
Error	68,3044	74	0,9230		

Table A. III. 55: Factorial ANOVA of **Log NK1b** for NF and MM in treatment group FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLL	SS	Degr. of	MS	F	p
Intercept	1103,906	1	1103,906	523,9387	**0,000000**
Sampling	55,303	3	18,434	8,7493	**0,000043**
Mature/non-mature GSI 0.5	105,793	1	105,793	50,2119	**0,000000**
Sampling*Mature/non-mature GSI 0.5	3,439	3	1,146	0,5441	0,653530
Error	170,662	81	2,107		

NKAα1b gene expression sampling 1-4

Table A. III. 56: Factorial ANOVA of **NKA1b gene expression** for females and males during smoltification (Sampling 1- 4). Significant values (p<0.05) are highlighted in bold

	SS	MS	F	p
Intercept	0,60542	0,605423	1,461868	0,233912
Sampling	6,56246	2,187488	5,281956	**0,003738**
Gender	1,16964	1,169640	2,824239	0,100845
Sampling*Gender	0,07843	0,026143	0,063124	0,978980
Error	16,15159	0,414143		

NKAα1b gene expression sampling 5-8

Table A. III. 57: Factorial ANOVA of **Log NK1b** for NF and MM in treatment group SWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

SWLL	SS	Degr. of	MS	F	p
Intercept	5,646870	1	5,646870	39,60549	**0,000000**
Sampling	0,179311	3	0,059770	0,41921	0,740271
Mature/Non Mature	0,142265	1	0,142265	0,99780	0,324331
Sampling*Mature/Non Mature	0,338604	3	0,112868	0,79162	0,506349
Error	5,275384	37	0,142578		

Table A. III. 58: Factorial ANOVA of **Log NK1b** for NF and MM in treatment group SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	SS	Degr. of	MS	F	p
Intercept	8,614652	1	8,614652	78,59564	**0,000000**
Mature/Non Mature	0,565545	1	0,565545	5,15974	**0,029025**
Sampling	0,921695	3	0,307232	2,80302	0,053183
Mature/Non Mature*Sampling	0,040787	3	0,013596	0,12404	0,945315
Error	4,055468	37	0,109607		

Table A. III. 59: Factorial ANOVA of **Log NK1b** for NF and MM in treatment group FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	SS	Degr. of	MS	F	p
Intercept	31,02641	1	31,02641	318,8047	**0,000000**
Sampling	0,93291	3	0,31097	3,1953	**0,035275**
Mature/Non Mature	0,66750	1	0,66750	6,8587	**0,012951**
Sampling*Mature/Non Mature	0,18830	3	0,06277	0,6449	0,591356
Error	3,40624	35	0,09732		

Table A. III. 60: Factorial ANOVA of **Log NK1b** for NF and MM in treatment group FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

FWLL	SS	Degr. of	MS	F	p
Intercept	16,78453	1	16,78453	291,3217	**0,000000**
Sampling	0,40552	3	0,13517	2,3462	0,094221
Mature/Non Mature	2,46206	1	2,46206	42,7330	**0,000000**
Sampling*Mature/Non Mature	0,11696	3	0,03899	0,6767	0,573626
Error	1,61322	28	0,05762		

NKAα1a gene expression sampling 1-4

Table A. III. 61: Factorial ANOVA of **NKA1a** for females and males during smoltification (Sampling 1- 4). Significant values (p<0.05) are highlighted in bold

	SS	MS	F	p
Intercept	1701,090	1701,090	139,0569	**0,000000**
Sampling	632,306	210,769	17,2294	**0,000000**
Gender	5,081	5,081	0,4154	0,523131
Sampling*Gender	8,779	2,926	0,2392	0,868453
Error	464,856	12,233		

NKAα1a gene expression sampling 5-8

Table A. III. 62: Factorial ANOVA of **Log NKA1a** for NF and MM in treatment group SWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	SS	Degr. of	MS	F	p
Intercept	7,25294	1	7,252940	11,38486	**0,001716**
Sampling	7,47312	3	2,491038	3,91016	**0,015790**
Mature/Non Mature	0,07606	1	0,076059	0,11939	0,731604
Sampling*Mature/Non Mature	0,98110	3	0,327034	0,51334	0,675543
Error	24,20862	38	0,637069		

Table A. III. 63: Factorial ANOVA of **Log NKA1a** for NF and MM in treatment group SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	SS	Degr. of	MS	F	p
Intercept	0,43952	1	0,439519	1,16294	0,287838
Sampling	26,88120	3	8,960401	23,70858	**0,000000**
Mature/Non Mature	3,59910	1	3,599099	9,52296	**0,003830**
Sampling*Mature/Non Mature	1,72602	3	0,575339	1,52230	0,224814
Error	13,98375	37	0,377939		

Table A. III. 64: Factorial ANOVA of **Log NK1a** for NF and MM in treatment group FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	SS	Degr. of	MS	F	p
Intercept	102,1935	1	102,1935	793,7278	**0,000000**
Sampling	3,5130	3	1,1710	9,0950	**0,000137**
Mature/Non Mature	0,1161	1	0,1161	0,9014	0,348907
Sampling*Mature/Non Mature	0,4840	3	0,1613	1,2532	0,305407
Error	4,5063	35	0,1288		

Table A. III. 65: Factorial ANOVA of **Log NK1a** for NF and MM in treatment group FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	SS	Degr. of	MS	F	p
Intercept	76,98966	1	76,98966	880,9135	**0,000000**
Sampling	1,23873	3	0,41291	4,7245	**0,008369**
Mature/Non Mature	0,67332	1	0,67332	7,7041	**0,009546**
Sampling*Mature/Non Mature	0,97317	3	0,32439	3,7116	**0,022502**
Error	2,53453	29	0,08740		

Post hoc tests

Dunnett test

Table A. III. 66: Dunnets test of difference in **Gill NKA activity** between tanks in sampling 1. Significant values (p<0.05) are highlighted in bold.

	Tank nr	{1}
1	2	
2	11	0,999990
3	5	0,973088
4	9	**0,023632**

Table A. III. 67: Dunnets test of difference in **Gill NKA activity** between tanks in sampling 2. Significant values (p<0.05) are highlighted in bold.

	Tank nr	{1}
1	13	
2	9	0,352780
3	15	0,108654
4	16	**0,010644**

Newman-Keuls tests

Fork length

Table A. III. 68: Newman-Keuls test of difference in **Fork length** between samplings and gender during smoltification (Sampling 1- 4). Significant values (p<0.05) are highlighted in bold.

	Sampling	Gender	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	1	F		0,728367	**0,001126**	**0,000625**	**0,000124**	**0,000126**	**0,000123**	**0,000127**
2	1	M	0,728367		**0,001750**	**0,000688**	**0,000150**	**0,000127**	**0,000126**	**0,000124**
3	2	F	**0,001126**	**0,001750**		1,000000	**0,000585**	**0,001218**	**0,000124**	**0,000725**
4	2	M	**0,000625**	**0,000688**	1,000000		**0,001451**	**0,001918**	**0,000128**	**0,001318**
5	3	F	**0,000124**	**0,000150**	**0,000585**	**0,001451**		0,963076	0,101162	0,814625
6	3	M	**0,000126**	**0,000127**	**0,001218**	**0,001918**	0,963076		**0,042436**	0,979300
7	4	F	**0,000123**	**0,000126**	**0,000124**	**0,000128**	0,101162	**0,042436**		0,098506
8	4	M	**0,000127**	**0,000124**	**0,000725**	**0,001318**	0,814625	0,979300	0,098506	

Table A. III. 69: Newman-Keuls test of difference in **Fork length** between sampling and gender in SWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,007251**	0,064171	0,132832	0,101442	**0,004584**	**0,001630**	**0,001689**
2	5	MM	**0,007251**		0,387791	0,284126	0,253840	0,794060	0,781148	0,882955
3	6	NF	0,064171	0,387791		0,946176	0,896998	0,349289	0,220343	0,243032
4	6	MM	0,132832	0,284126	0,946176		0,707437	0,286379	0,191076	0,222009
5	7	NF	0,101442	0,253840	0,896998	0,707437		0,339871	0,271321	0,334068
6	7	MM	**0,004584**	0,794060	0,349289	0,286379	0,339871		0,683879	0,884404
7	8	NF	**0,001630**	0,781148	0,220343	0,191076	0,271321	0,683879		0,949303
8	8	MM	**0,001689**	0,882955	0,243032	0,222009	0,334068	0,884404	0,949303	

Table A. III. 70: Newman-Keuls test of difference in **Fork length** between sampling and gender in SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,109600	0,094214	**0,000122**	**0,023119**	0,056405	**0,000149**	**0,006201**
2	5	MM	0,109600		0,624032	**0,000669**	0,463512	0,605351	**0,007338**	0,260728
3	6	NF	0,094214	0,624032		**0,002367**	0,596297	0,642626	**0,021831**	0,419011
4	6	MM	**0,000122**	**0,000669**	**0,002367**		**0,019753**	**0,007123**	0,398497	**0,047242**
5	7	NF	**0,023119**	0,463512	0,596297	**0,019753**		0,613921	0,090108	0,572279
6	7	MM	0,056405	0,605351	0,642626	**0,007123**	0,613921		**0,048520**	0,533089
7	8	NF	**0,000149**	**0,007338**	**0,021831**	0,398497	0,090108	**0,048520**		0,121823
8	8	MM	**0,006201**	0,260728	0,419011	**0,047242**	0,572279	0,533089	0,121823	

Table A. III. 71: Newman-Keuls test of difference in **Fork length** between sampling and gender in FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,005880**	**0,014125**	**0,000942**	**0,000695**	**0,000151**	**0,000119**	**0,000123**
2	5	MM	**0,005880**		0,967505	0,487989	0,512635	0,170810	**0,001117**	**0,003492**
3	6	NF	**0,014125**	0,967505		0,271852	0,375483	0,128395	**0,000954**	**0,002787**
4	6	MM	**0,000942**	0,487989	0,271852		0,813276	0,512925	**0,020694**	**0,044027**
5	7	NF	**0,000695**	0,512635	0,375483	0,813276		0,387333	**0,025621**	**0,044397**
6	7	MM	**0,000151**	0,170810	0,128395	0,512925	0,387333		0,115878	0,120961
7	8	NF	**0,000119**	**0,001117**	**0,000954**	**0,020694**	**0,025621**	0,115878		0,658138
8	8	MM	**0,000123**	**0,003492**	**0,002787**	**0,044027**	**0,044397**	0,120961	0,658138	

Table A. III. 72: Newman-Keuls test of difference in **Fork length** between sampling and gender in FWLD during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,000523**	**0,000305**	**0,000149**	**0,001174**	**0,000523**	**0,000121**	**0,000313**
2	5	MM	**0,000523**		0,869730	0,639387	0,977831	0,865020	**0,009272**	0,838580
3	6	NF	**0,000305**	0,869730		0,566963	0,784828	0,893746	**0,025981**	0,895130
4	6	MM	**0,000149**	0,639387	0,566963		0,557590	0,734157	**0,041019**	0,759818
5	7	NF	**0,001174**	0,977831	0,784828	0,557590		0,628795	**0,007523**	0,701073
6	7	MM	**0,000523**	0,865020	0,893746	0,734157	0,628795		**0,021836**	0,750308
7	8	NF	**0,000121**	**0,009272**	**0,025981**	**0,041019**	**0,007523**	**0,021836**		**0,033384**
8	8	MM	**0,000313**	0,838580	0,895130	0,759818	0,701073	0,750308	**0,033384**	

Body weight

Table A. III. 73: Newman-Keuls test of difference in **Body weight** between samplings and gender during smoltification (Sampling 1- 4). Significant values ($p<0.05$) are highlighted in bold.

Sampling	Gender	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	F		0,852473	**0,000342**	**0,000234**	**0,000127**	**0,000126**	**0,000123**	**0,000124**
1	M	0,852473		**0,000307**	**0,000198**	**0,000124**	**0,000127**	**0,000126**	**0,000150**
2	F	**0,000342**	**0,000307**		0,952762	**0,000778**	**0,000428**	**0,000154**	**0,003507**
2	M	**0,000234**	**0,000198**	0,952762		**0,001186**	**0,000512**	**0,000164**	**0,007959**
3	F	**0,000127**	**0,000124**	**0,000778**	**0,001186**		0,669073	0,495125	0,403542
3	M	**0,000126**	**0,000127**	**0,000428**	**0,000512**	0,669073		0,481972	0,416773
4	F	**0,000123**	**0,000126**	**0,000154**	**0,000164**	0,495125	0,481972		0,206360
4	M	**0,000124**	**0,000150**	**0,003507**	**0,007959**	0,403542	0,416773	0,206360	

Table A. III. 74: Newman-Keuls test of difference in **Body weight** between sampling and gender in SWLL during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,016061**	0,094576	**0,041545**	0,063105	**0,002513**	**0,002255**	**0,001056**
2	5	MM	**0,016061**		0,435250	0,740127	0,500476	0,477826	0,681422	0,664978
3	6	NF	0,094576	0,435250		0,443079	0,682723	0,180684	0,186724	0,123370
4	6	MM	**0,041545**	0,740127	0,443079		0,949344	0,469884	0,515923	0,420061
5	7	NF	0,063105	0,500476	0,682723	0,949344		0,350726	0,434011	0,369889
6	7	MM	**0,002513**	0,477826	0,180684	0,469884	0,350726		0,902032	0,903914
7	8	NF	**0,002255**	0,681422	0,186724	0,515923	0,434011	0,902032		0,761270

8	8	MM	**0,001056**	0,664978	0,123370	0,420061	0,369889	0,903914	0,761270	

Table A. III.75: Newman-Keuls test of difference in **Body weight** between sampling and gender in SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,097063	0,122675	**0,000125**	**0,007274**	**0,025775**	**0,000121**	**0,002585**
2	5	MM	0,097063		0,761073	**0,000872**	0,281450	0,455319	**0,000305**	0,174788
3	6	NF	0,122675	0,761073		**0,001635**	0,299363	0,372467	**0,000533**	0,220626
4	6	MM	**0,000125**	**0,000872**	**0,001635**		**0,041592**	**0,015697**	0,636884	**0,046746**
5	7	NF	**0,007274**	0,281450	0,299363	**0,041592**		0,552396	**0,022055**	0,658024
6	7	MM	**0,025775**	0,455319	0,372467	**0,015697**	0,552396		**0,006105**	0,553248
7	8	NF	**0,000121**	**0,000305**	**0,000533**	0,636884	**0,022055**	**0,006105**		**0,038676**
8	8	MM	**0,002585**	0,174788	0,220626	**0,046746**	0,658024	0,553248	**0,038676**	

Table A. III. 76: Newman-Keuls test of difference in **Body weight** between samplings and gender in FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,003461**	**0,009784**	**0,000267**	**0,000988**	**0,000127**	**0,000123**	**0,000119**
2	5	MM	**0,003461**		0,479901	0,448475	0,544887	0,117729	**0,001305**	**0,001813**
3	6	NF	**0,009784**	0,479901		0,226259	0,389375	**0,031712**	**0,000252**	**0,000297**
4	6	MM	**0,000267**	0,448475	0,226259		0,546450	0,301608	**0,017383**	**0,030853**
5	7	NF	**0,000988**	0,544887	0,389375	0,546450		0,232673	**0,005616**	**0,008618**
6	7	MM	**0,000127**	0,117729	**0,031712**	0,301608	0,232673		0,082005	0,185507
7	8	NF	**0,000123**	**0,001305**	**0,000252**	**0,017383**	**0,005616**	0,082005		0,991799
8	8	MM	**0,000119**	**0,001813**	**0,000297**	**0,030853**	**0,008618**	0,185507	0,991799	

Table A. III. 77: Newman-Keuls test of difference in **Body weight** between sampling and gender in FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,002198**	**0,000323**	**0,000131**	**0,000641**	**0,000790**	**0,000121**	**0,000531**
2	5	MM	**0,002198**		0,326134	0,185996	0,705654	0,799003	**0,000479**	0,572499
3	6	NF	**0,000323**	0,326134		0,651168	0,995948	0,999372	**0,007804**	0,982489
4	6	MM	**0,000131**	0,185996	0,651168		0,411323	0,218541	**0,029894**	0,538399
5	7	NF	**0,000641**	0,705654	0,995948	0,411323		0,969744	**0,004465**	0,948819
6	7	MM	**0,000790**	0,799003	0,999372	0,218541	0,969744		**0,002667**	0,994280
7	8	NF	**0,000121**	**0,000479**	**0,007804**	**0,029894**	**0,004465**	**0,002667**		**0,005813**
8	8	MM	**0,000531**	0,572499	0,982489	0,538399	0,948819	0,994280	**0,005813**	

Gonadosomatic index

Table A. III. 78: Newman-Keuls test of difference in **GSI** between samplings and gender during smoltification (Sampling 1- 4). Significant values (p<0.05) are highlighted in bold.

	Sampling	Gender	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	1	F		**0,000124**	0,293999	**0,000150**	0,482396	**0,000131**	0,704003	**0,000168**
2	1	M	**0,000124**		**0,000123**	0,152341	**0,000127**	**0,046870**	**0,000126**	**0,021969**

3	2	F	0,293999	**0,000123**		**0,000126**	0,536828	**0,000127**	0,333411	**0,000124**
4	2	M	**0,000150**	0,152341	**0,000126**		**0,000124**	0,333511	**0,000127**	0,296410
5	3	F	0,482396	**0,000127**	0,536828	**0,000124**		**0,000152**	0,925338	**0,000121**
6	3	M	**0,000131**	**0,046870**	**0,000127**	0,333511	**0,000152**		**0,000126**	0,599570
7	4	F	0,704003	**0,000126**	0,333411	**0,000127**	0,925338	**0,000126**		**0,000163**
8	4	M	**0,000168**	**0,021969**	**0,000124**	0,296410	**0,000121**	0,599570	**0,000163**	

Table A. III. 79: Newman-Keuls test of difference in **Log GSI** between samplings and gender in SWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,000119**	0,753466	**0,000123**	0,510211	**0,000118**	0,885902	**0,000122**
2	5	MM	**0,000119**		**0,000107**	**0,000114**	**0,000145**	**0,000145**	**0,000114**	**0,000107**
3	6	NF	0,753466	**0,000107**		**0,000145**	0,954966	**0,000123**	0,992709	**0,000119**
4	6	MM	**0,000123**	**0,000114**	**0,000145**		**0,000119**	0,272448	**0,000107**	0,272353
5	7	NF	0,510211	**0,000145**	0,954966	**0,000119**		**0,000122**	0,997663	**0,000123**
6	7	MM	**0,000118**	**0,000145**	**0,000123**	0,272448	**0,000122**		**0,000119**	0,656520
7	8	NF	0,885902	**0,000114**	0,992709	**0,000107**	0,997663	**0,000119**		**0,000145**
8	8	MM	**0,000122**	**0,000107**	**0,000119**	0,272353	**0,000123**	0,656520	**0,000145**	

Table A. III. 80: Newman-Keuls test of difference in **Log GSI** between samplings and gender in SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,000113**	0,991817	**0,000122**	0,747188	**0,000147**	0,378905	**0,000109**
2	5	MM	**0,000113**		**0,000109**	**0,000147**	**0,000147**	**0,000109**	**0,000122**	**0,000113**
3	6	NF	0,991817	**0,000109**		**0,000125**	0,474711	**0,000122**	0,251820	**0,000147**
4	6	MM	**0,000122**	**0,000147**	**0,000125**		**0,000124**	**0,000115**	**0,000121**	**0,000109**
5	7	NF	0,747188	**0,000147**	0,474711	**0,000124**		**0,000125**	0,380911	**0,000122**
6	7	MM	**0,000147**	**0,000109**	**0,000122**	**0,000115**	**0,000125**		**0,000124**	**0,000150**
7	8	NF	0,378905	**0,000122**	0,251820	**0,000121**	0,380911	**0,000124**		**0,000125**
8	8	MM	**0,000109**	**0,000113**	**0,000147**	**0,000109**	**0,000122**	**0,000150**	**0,000125**	

Table A. III. 81: Newman-Keuls test of difference in **Log GSI** between samplings and gender in FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,000108**	0,903312	**0,000146**	0,704991	**0,000124**	0,813400	**0,000121**
2	5	MM	**0,000108**		**0,000121**	**0,000115**	**0,000146**	**0,000146**	**0,000115**	**0,000108**
3	6	NF	0,903312	**0,000121**		**0,000124**	0,960452	**0,000119**	0,909317	**0,000123**
4	6	MM	**0,000146**	**0,000115**	**0,000124**		**0,000121**	**0,000108**	**0,000108**	**0,000125**
5	7	NF	0,704991	**0,000146**	0,960452	**0,000121**		**0,000123**	0,811313	**0,000124**
6	7	MM	**0,000124**	**0,000146**	**0,000119**	**0,000108**	**0,000123**		**0,000121**	0,150858
7	8	NF	0,813400	**0,000115**	0,909317	**0,000108**	0,811313	**0,000121**		**0,000146**
8	8	MM	**0,000121**	**0,000108**	**0,000123**	**0,000125**	**0,000124**	0,150858	**0,000146**	

Table A. III. 82: Newman-Keuls test of difference in **Log GSI** between sampling and gender in FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,000114**	0,554626	**0,000123**	0,902649	**0,000148**	0,959840	**0,000109**
2	5	MM	**0,000114**		**0,000123**	**0,000148**	**0,000109**	**0,000109**	**0,000148**	**0,000114**
3	6	NF	0,554626	**0,000123**		**0,000121**	0,459882	**0,000125**	0,299524	**0,000126**
4	6	MM	**0,000123**	**0,000148**	**0,000121**		**0,000126**	0,629585	**0,000125**	**0,000127**
5	7	NF	0,902649	**0,000109**	0,459882	**0,000126**		**0,000123**	0,881106	**0,000148**
6	7	MM	**0,000148**	**0,000109**	**0,000125**	0,629585	**0,000123**		**0,000126**	**0,000154**
7	8	NF	0,959840	**0,000148**	0,299524	**0,000125**	0,881106	**0,000126**		**0,000123**
8	8	MM	**0,000109**	**0,000114**	**0,000126**	**0,000127**	**0,000148**	**0,000154**	**0,000123**	

Condition factor

Table A. III. 83: Newman-Keuls test of difference in **Condition factor** between samplings during smoltification (Sampling 1- 4). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Gender	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	1	F		0,764971	0,612874	0,882269	0,905514	0,564136	**0,037395**	0,056039
2	1	M	0,764971		0,849526	0,970466	0,845777	0,426563	0,098494	0,106889
3	2	F	0,612874	0,849526		0,971875	0,805562	0,423674	0,095116	0,121134
4	2	M	0,882269	0,970466	0,971875		0,912065	0,497421	0,061798	**0,047508**
5	3	F	0,905514	0,845777	0,805562	0,912065		0,368571	**0,035871**	0,057668
6	3	M	0,564136	0,426563	0,423674	0,497421	0,368571		**0,003005**	**0,005811**
7	4	F	**0,037395**	0,098494	0,095116	0,061798	**0,035871**	**0,003005**		0,775689
8	4	M	0,056039	0,106889	0,121134	**0,047508**	0,057668	**0,005811**	0,775689	

Table A. III. 84: Newman-Keuls test of difference in **Condition factor** between samplings and gender in SWLL during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,026364**	0,328197	**0,038454**	**0,045893**	**0,014115**	**0,038211**	**0,011112**
2	5	MM	**0,026364**		0,101123	0,982228	0,982525	0,943900	0,932248	0,895197
3	6	NF	0,328197	0,101123		0,260332	0,263346	0,150256	0,195290	0,118631
4	6	MM	**0,038454**	0,982228	0,260332		0,846850	0,855218	0,955491	0,597936
5	7	NF	**0,045893**	0,982525	0,263346	0,846850		0,886005	0,925498	0,750421
6	7	MM	**0,014115**	0,943900	0,150256	0,855218	0,886005		0,923489	0,996996
7	8	NF	**0,038211**	0,932248	0,195290	0,955491	0,925498	0,923489		0,846227
8	8	MM	**0,011112**	0,895197	0,118631	0,597936	0,750421	0,996996	0,846227	

Table A. III. 85: Newman-Keuls test of difference in **Condition factor** between samplings and gender in SWLD during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,084827	0,149261	0,062957	**0,005876**	**0,005900**	**0,000125**	0,062625
2	5	MM	0,084827		0,484552	0,851114	0,501285	0,438788	**0,009685**	0,709452
3	6	NF	0,149261	0,484552		0,601002	0,205176	0,187430	**0,001397**	0,531398
4	6	MM	0,062957	0,851114	0,601002		0,542021	0,337840	**0,020735**	0,867526
5	7	NF	**0,005876**	0,501285	0,205176	0,542021		0,924753	0,060807	0,611814
6	7	MM	**0,005900**	0,438788	0,187430	0,337840	0,924753		0,119524	0,497442
7	8	NF	**0,000125**	**0,009685**	**0,001397**	**0,020735**	0,060807	0,119524		**0,020271**
8	8	MM	0,062625	0,709452	0,531398	0,867526	0,611814	0,497442	**0,020271**	

Table A. III. 86: Newman-Keuls test of difference in **Condition factor** between samplings and gender in FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,000121**	**0,002057**	**0,000123**	**0,002017**	**0,000125**	**0,000194**	**0,000120**
2	5	MM	**0,000121**		0,054302	0,942692	0,067020	0,894698	0,284562	0,965436
3	6	NF	**0,002057**	0,054302		0,050193	0,744566	0,059251	0,290528	**0,045342**
4	6	MM	**0,000123**	0,942692	0,050193		0,081670	0,846216	0,499975	0,886949
5	7	NF	**0,002017**	0,067020	0,744566	0,081670		0,086007	0,239407	0,079703
6	7	MM	**0,000125**	0,894698	0,059251	0,846216	0,086007		0,450609	0,939307
7	8	NF	**0,000194**	0,284562	0,290528	0,499975	0,239407	0,450609		0,534979
8	8	MM	**0,000120**	0,965436	**0,045342**	0,886949	0,079703	0,939307	0,534979	

Table A. III. 87: Newman-Keuls test of difference in **Condition factor** between sampling and gender in FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,008259**	**0,002415**	**0,000126**	**0,000121**	**0,000125**	**0,000127**	**0,001595**
2	5	MM	**0,008259**		0,444280	**0,024543**	**0,002804**	**0,030740**	**0,017649**	0,512538
3	6	NF	**0,002415**	0,444280		0,121327	**0,022044**	0,107678	0,083387	0,736251
4	6	MM	**0,000126**	**0,024543**	0,121327		0,422168	0,927391	0,994540	0,168647
5	7	NF	**0,000121**	**0,002804**	**0,022044**	0,422168		0,642896	0,695454	**0,039316**
6	7	MM	**0,000125**	**0,030740**	0,107678	0,927391	0,642896		0,717595	0,091291
7	8	NF	**0,000127**	**0,017649**	0,083387	0,994540	0,695454	0,717595		0,102140
8	8	MM	**0,001595**	0,512538	0,736251	0,168647	**0,039316**	0,091291	0,102140	

NKA activity

Table A. III. 88: Newman-Keuls test of difference in **NKA activity** between samplings and gender during smoltification (Sampling 1- 4). Significant values (p<0.05) are highlighted in bold.

	Sampling	Gender	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	1	F		0,780698	0,882553	0,865414	**0,000150**	**0,000115**	0,198232	0,750128
2	1	M	0,780698		0,892026	0,816382	**0,000125**	**0,000152**	0,261560	0,792919
3	2	F	0,882553	0,892026		0,824561	**0,000126**	**0,000129**	0,260570	0,642231
4	2	M	0,865414	0,816382	0,824561		**0,000128**	**0,000126**	0,276434	0,770510
5	3	F	**0,000150**	**0,000125**	**0,000126**	**0,000128**		0,237380	**0,000117**	**0,000123**
6	3	M	**0,000115**	**0,000152**	**0,000129**	**0,000126**	0,237380		**0,000313**	**0,000126**
7	4	F	0,198232	0,261560	0,260570	0,276434	**0,000117**	**0,000313**		0,137681
8	4	M	0,750128	0,792919	0,642231	0,770510	**0,000123**	**0,000126**	0,137681	

Table A. III. 89: Newman-Keuls test of difference in **NKA activity** between samplings and gender in SWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,877470	**0,002814**	0,857965	0,291958	**0,002996**	0,295366	0,970437
2	5	MM	0,877470		**0,002899**	0,940367	0,349308	**0,002910**	0,245232	0,937351
3	6	NF	**0,002814**	**0,002899**		**0,002246**	**0,000137**	**0,000118**	**0,049058**	**0,001975**
4	6	MM	0,857965	0,940367	**0,002246**		0,187402	**0,002798**	0,300102	0,976108
5	7	NF	0,291958	0,349308	**0,000137**	0,187402		**0,039455**	**0,018240**	0,413966

6	7	MM	**0,002996**	**0,002910**	**0,000118**	**0,002798**	**0,039455**		**0,000132**	**0,003224**
7	8	NF	0,295366	0,245232	**0,049058**	0,300102	**0,018240**	**0,000132**		0,128470
8	8	MM	0,970437	0,937351	**0,001975**	0,976108	0,413966	**0,003224**	0,128470	

Table A. III. 90: Newman-Keuls test of difference in **NKA activity** between samplings and gender in SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,434546	**0,001110**	0,586560	0,761849	0,456943	0,504840	0,617881
2	5	MM	0,434546		**0,000134**	0,181017	0,417499	0,625352	0,100971	0,123674
3	6	NF	**0,001110**	**0,000134**		**0,010605**	**0,002073**	**0,000213**	**0,018073**	**0,007137**
4	6	MM	0,586560	0,181017	**0,010605**		0,496184	0,312374	0,683630	0,903226
5	7	NF	0,761849	0,417499	**0,002073**	0,496184		0,546614	0,521247	0,682055
6	7	MM	0,456943	0,625352	**0,000213**	0,312374	0,546614		0,211879	0,265473
7	8	NF	0,504840	0,100971	**0,018073**	0,683630	0,521247	0,211879		0,983439
8	8	MM	0,617881	0,123674	**0,007137**	0,903226	0,682055	0,265473	0,983439	

Table A. III. 91: Newman-Keuls test of difference in **NKA activity** between samplings and gender in FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,021502**	0,152386	0,316913	**0,018562**	**0,000610**	0,850276	**0,001237**
2	5	MM	**0,021502**		**0,000400**	0,229189	0,915499	0,358657	**0,023277**	0,296668
3	6	NF	0,152386	**0,000400**		**0,024554**	**0,000382**	**0,000123**	0,237184	**0,000130**
4	6	MM	0,316913	0,229189	**0,024554**		0,125707	**0,026960**	0,208577	**0,041155**
5	7	NF	**0,018562**	0,915499	**0,000382**	0,125707		0,453090	**0,016961**	0,482087
6	7	MM	**0,000610**	0,358657	**0,000123**	**0,026960**	0,453090		**0,000817**	0,746333
7	8	NF	0,850276	**0,023277**	0,237184	0,208577	**0,016961**	**0,000817**		**0,001576**
8	8	MM	**0,001237**	0,296668	**0,000130**	**0,041155**	0,482087	0,746333	**0,001576**	

Table A. III. 92: Newman-Keuls test of difference in **NKA activity** between samplings and gender in FWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/non-mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,004470**	0,066552	0,120269	**0,035645**	**0,000145**	0,542832	**0,000209**
2	5	MM	**0,004470**		**0,000129**	0,235867	0,384557	0,250960	**0,017513**	0,237991
3	6	NF	0,066552	**0,000129**		**0,001405**	**0,000245**	**0,000120**	**0,040824**	**0,000123**
4	6	MM	0,120269	0,235867	**0,001405**		0,447655	**0,014680**	0,170640	**0,029709**
5	7	NF	**0,035645**	0,384557	**0,000245**	0,447655		0,071473	0,087114	0,104036
6	7	MM	**0,000145**	0,250960	**0,000120**	**0,014680**	0,071473		**0,000316**	0,681224
7	8	NF	0,542832	**0,017513**	**0,040824**	0,170640	0,087114	**0,000316**		**0,000723**
8	8	MM	**0,000209**	0,237991	**0,000123**	**0,029709**	0,104036	0,681224	**0,000723**	

Relative NKA1b gene expression

Table A. III. 93: Newman-Keuls test of difference in **Log NKA1b** between samplings and gender during smoltification (Sampling 1- 4). Significant values (p<0.05) are highlighted in bold.

Sampling	Gender	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}

1	1	F		0,421081	0,360246	0,340329	0,105542	**0,018749**	0,401891	0,126031
2	1	M	0,421081		0,573173	0,706547	0,359010	0,106643	0,725818	0,426798
3	2	F	0,360246	0,573173		0,868516	0,577843	0,257519	0,844354	0,683238
4	2	M	0,340329	0,706547	0,868516		0,441309	0,337069	0,759034	0,699839
5	3	F	0,105542	0,359010	0,577843	0,441309		0,629414	0,527637	0,975687
6	3	M	**0,018749**	0,106643	0,257519	0,337069	0,629414		0,280439	0,378036
7	4	F	0,401891	0,725818	0,844354	0,759034	0,527637	0,280439		0,680823
8	4	M	0,126031	0,426798	0,683238	0,699839	0,975687	0,378036	0,680823	

Table A. III. 94: Newman-Keuls test of difference in **Log NKA1b** between samplings and gender in SWLL during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/Non Mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,712022	0,917609	0,827248	0,706528	0,868577	0,806413	0,904014
2	5	MM	0,712022		0,971022	0,825275	0,672668	0,933369	0,859708	0,853660
3	6	NF	0,917609	0,971022		0,885071	0,997435	0,979474	0,995122	0,841920
4	6	MM	0,827248	0,825275	0,885071		0,740027	0,840678	0,796592	0,835942
5	7	NF	0,706528	0,672668	0,997435	0,740027		0,984674	0,921823	0,736046
6	7	MM	0,868577	0,933369	0,979474	0,840678	0,984674		0,945511	0,801111
7	8	NF	0,806413	0,859708	0,995122	0,796592	0,921823	0,945511		0,769087
8	8	MM	0,904014	0,853660	0,841920	0,835942	0,736046	0,801111	0,769087	

Table A. III. 95: Newman-Keuls test of difference in **Log NKA1b** between samplings and gender in SWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Mature/Non Mature	Sampling	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	NF	5		0,472167	0,446847	0,958705	0,488476	0,964726	0,915493	0,735351
2	NF	6	0,472167		0,846753	0,361719	0,198130	0,384458	0,319873	0,224405
3	NF	7	0,446847	0,846753		0,405215	0,170935	0,385070	0,458843	0,187574
4	NF	8	0,958705	0,361719	0,405215		0,763321	0,817625	0,712609	0,841112
5	MM	5	0,488476	0,198130	0,170935	0,763321		0,738789	0,663477	0,960163
6	MM	6	0,964726	0,384458	0,385070	0,817625	0,738789		0,819024	0,856285
7	MM	7	0,915493	0,319873	0,458843	0,712609	0,663477	0,819024		0,727460
8	MM	8	0,735351	0,224405	0,187574	0,841112	0,960163	0,856285	0,727460	

Table A. III. 96: Newman-Keuls test of difference in **Log NKA1b** between samplings and gender in FWLD during maturation (Sampling 5- 8). Significant values (p<0.05) are highlighted in bold.

	Sampling	Mature/Non Mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,108919	0,649752	0,953921	0,262385	0,934927	0,963001	0,945786
2	5	MM	0,108919		**0,044836**	0,216764	**0,007474**	0,209275	0,217867	0,220981
3	6	NF	0,649752	**0,044836**		0,577105	0,431991	0,603917	0,393606	0,625712
4	6	MM	0,953921	0,216764	0,577105		0,289192	0,900403	0,886743	0,842029
5	7	NF	0,262385	**0,007474**	0,431991	0,289192		0,246027	0,235136	0,285419
6	7	MM	0,934927	0,209275	0,603917	0,900403	0,246027		0,937292	0,814792
7	8	NF	0,963001	0,217867	0,393606	0,886743	0,235136	0,937292		0,936860
8	8	MM	0,945786	0,220981	0,625712	0,842029	0,285419	0,814792	0,936860	

Table A. III. 97: Newman-Keuls test of difference in **Log NKA1b** between samplings and gender in FWLL during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/Non Mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,125294	**0,043936**	0,191930	0,279068	0,132080	0,430818	0,172177
2	5	MM	0,125294		**0,000440**	0,669591	**0,006304**	0,831006	**0,030360**	0,807538
3	6	NF	**0,043936**	**0,000440**		**0,002656**	0,229453	**0,000563**	0,133414	**0,001062**
4	6	MM	0,191930	0,669591	**0,002656**		**0,034383**	0,630844	0,100543	0,612278
5	7	NF	0,279068	**0,006304**	0,229453	**0,034383**		**0,008106**	0,453029	**0,015770**
6	7	MM	0,132080	0,831006	**0,000563**	0,630844	**0,008106**		**0,036034**	0,685116
7	8	NF	0,430818	**0,030360**	0,133414	0,100543	0,453029	**0,036034**		0,059679
8	8	MM	0,172177	0,807538	**0,001062**	0,612278	**0,015770**	0,685116	0,059679	

Relative NKA1a gene expression

Table A. III. 98: Newman-Keuls test of difference in **Log NKA1a** between sampling and gender during Smoltification (Sampling 1- 4). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Gender	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	1	F		0,918728	0,850899	0,901797	**0,000559**	**0,001847**	0,578477	0,924462
2	1	M	0,918728		0,954163	0,949607	**0,000527**	**0,001857**	0,611536	0,949463
3	2	F	0,850899	0,954163		0,808331	**0,000693**	**0,002188**	0,571608	0,902312
4	2	M	0,901797	0,949607	0,808331		**0,000928**	**0,002734**	0,550031	0,851836
5	3	F	**0,000559**	**0,000527**	**0,000693**	**0,000928**		0,573574	**0,005453**	**0,000997**
6	3	M	**0,001847**	**0,001857**	**0,002188**	**0,002734**	0,573574		**0,008866**	**0,002455**
7	4	F	0,578477	0,611536	0,571608	0,550031	**0,005453**	**0,008866**		0,393883
8	4	M	0,924462	0,949463	0,902312	0,851836	**0,000997**	**0,002455**	0,393883	

Table A. III. 99: Newman-Keuls test of difference in **Log NKA1a** between sampling SWLL during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/Non Mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,989999	0,200256	0,257037	0,186066	0,320292	0,394552	0,091850
2	5	MM	0,989999		0,395007	0,382481	0,226228	0,418205	0,476597	0,110123
3	6	NF	0,200256	0,395007		0,766848	0,813317	0,902885	0,961904	0,638997
4	6	MM	0,257037	0,382481	0,766848		0,860487	0,895409	0,981031	0,725406
5	7	NF	0,186066	0,226228	0,813317	0,860487		0,791711	0,552840	0,648737
6	7	MM	0,320292	0,418205	0,902885	0,895409	0,791711		0,957076	0,684151
7	8	NF	0,394552	0,476597	0,961904	0,981031	0,552840	0,957076		0,545437
8	8	MM	0,091850	0,110123	0,638997	0,725406	0,648737	0,684151	0,545437	

Table A. III. 100: Newman-Keuls test of difference in **Log NKA1a** between sampling in SWLD during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/Non Mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		0,918686	0,618596	0,065042	**0,047505**	**0,000165**	**0,000811**	**0,000146**
2	5	MM	0,918686		0,407804	0,054825	**0,034054**	**0,000160**	**0,000761**	**0,000151**
3	6	NF	0,618596	0,407804		0,179533	0,084668	**0,000458**	**0,004604**	**0,000320**
4	6	MM	0,065042	0,054825	0,179533		0,968156	**0,017838**	0,076985	**0,016067**
5	7	NF	**0,047505**	**0,034054**	0,084668	0,968156		**0,029190**	0,164701	**0,022412**
6	7	MM	**0,000165**	**0,000160**	**0,000458**	**0,017838**	**0,029190**		0,297095	0,781035

7	8	NF	**0,000811**	**0,000761**	**0,004604**	0,076985	0,164701	0,297095		0,383687
8	8	MM	**0,000146**	**0,000151**	**0,000320**	**0,016067**	**0,022412**	0,781035	0,383687	

Table A. III. 101: Newman-Keuls test of difference in **Log NKA1a** between sampling in FWLD during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/Non Mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,043284**	**0,012577**	**0,011383**	**0,017876**	**0,016497**	**0,000793**	**0,001668**
2	5	MM	**0,043284**		0,586203	0,521141	0,436134	0,544366	0,125097	0,207528
3	6	NF	**0,012577**	0,586203		0,924971	0,902158	0,910871	0,438503	0,394472
4	6	MM	**0,011383**	0,521141	0,924971		0,826375	0,753033	0,547904	0,608271
5	7	NF	**0,017876**	0,436134	0,902158	0,826375		0,786723	0,404965	0,539785
6	7	MM	**0,016497**	0,544366	0,910871	0,753033	0,786723		0,475903	0,584801
7	8	NF	**0,000793**	0,125097	0,438503	0,547904	0,404965	0,475903		0,708554
8	8	MM	**0,001668**	0,207528	0,394472	0,608271	0,539785	0,584801	0,708554	

Table A. III. 102: Newman-Keuls test of difference in **Log NKA1a** between sampling in FWLL during maturation (Sampling 5- 8). Significant values ($p<0.05$) are highlighted in bold.

	Sampling	Mature/Non Mature	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1	5	NF		**0,001178**	0,530396	0,620754	0,825508	0,700932	0,856299	0,776223
2	5	MM	**0,001178**		**0,004115**	**0,003895**	**0,002511**	**0,004079**	**0,000561**	**0,000787**
3	6	NF	0,530396	**0,004115**		0,861159	0,972768	0,863775	0,651745	0,630901
4	6	MM	0,620754	**0,003895**	0,861159		0,580057	0,815496	0,465060	0,538845
5	7	NF	0,825508	**0,002511**	0,972768	0,580057		0,959791	0,731551	0,780754
6	7	MM	0,700932	**0,004079**	0,863775	0,815496	0,959791		0,668885	0,695110
7	8	NF	0,856299	**0,000561**	0,651745	0,465060	0,731551	0,668885		0,808197
8	8	MM	0,776223	**0,000787**	0,630901	0,538845	0,780754	0,695110	0,808197	