COMPARATIVE GENETICS AND GENOMICS OF ATLANTIC SALMON (*SALMO SALAR* L.) POPULATIONS IN NORWAY AND IRAQ

ABDULLA, C. S.[1,2*] – PARMAKSIZ, A.[1]

[1]*Department of Biology, Faculty of Science and Art, Harran University, Sanlıurfa 63100, Turkey (e-mails: fef@harran.edu.tr, aprmksz@gmail.com)*

[2]*Department of Animal Source, College of Agricultural Engineering Science, Salahaddin University-Erbil, Kurdistan Region, Iraq*

**Corresponding author*
e-mail: friad10@hotmail.com

(Received 2nd May 2024; accepted 23rd Sep 2024)

Abstract. The aquaculture industry, spearheading the "blue revolution," relies significantly on Atlantic salmon (*Salmo salar* L.), a crucial economic and ecological asset. This study offers a comprehensive insight into the genetic and genomic landscape of Atlantic salmon populations in Norway and Iraq, crucial for sustainable aquaculture practices. Supporting recent advancements in genetics and genomics, we investigated key genes, sexually dimorphic on the Y-chromosome (sdY) and vestigial-like protein 3 (vgll3), essential for physiological maturation. Utilizing next-generation sequencing and genotyping techniques, we analyzed 20 samples each from Norwegian and Iraqi populations. We successfully amplified and sequenced sdY and vgll3 gene fragments, revealing significant genetic variations. Multiple sequence alignment and variant analysis uncovered notable differences in amino acid sequences between the two populations, shedding light on their evolutionary divergence. This research contributes to the understanding of salmon genetics and informs sustainable aquaculture practices globally. Furthermore, the data generated from this study have been deposited in GenBank for broader accessibility and future research endeavors.
Keywords: *Atlantic salmon, aquaculture, genetics, genomics, sustainable practices, population comparison, next-generation sequencing, GenBank*

Introduction

The aquaculture industry is at the forefront of global food production, catalyzing what has been termed the "blue revolution." Within this transformative landscape, Atlantic salmon (*Salmo salar* L.) stands as a cornerstone, occupying a pivotal economic role as a globally significant aquaculture product. Derived from the Latin word "Salmo," meaning "to leap," salmon's remarkable journey encompasses not only its iconic life cycle but also its profound economic, ecological, and nutritional importance. This paper provides a comprehensive overview of the salmon farming industry in both Norway and Iraq, shedding light on the genetic and genomic underpinnings of this economically vital species. We delve into the genetic intricacies of Atlantic salmon, investigating its relationship with various species, including trout, and the Pacific salmon, all of which play significant roles in the aquaculture sector. Our exploration is grounded in recent advancements in genetics research, fueled by state-of-the-art technologies such as next-generation sequencing and genotyping (Macqueen et al., 2017). Recent breakthroughs in genomic research, including the release of a high-quality reference genome for salmon by Lien et al. (2016), have paved the way for innovative approaches to breeding and advancing our understanding of salmon genetics,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

physiology, ecology, and evolution. Such progress is instrumental in addressing the environmental challenges associated with salmon farming. By selecting individuals with heightened disease resistance and adaptability to new feed sources, the industry aligns with environmentally conscious consumer preferences, thereby ensuring sustainable production practices (Wacker et al., 2021; Christensen et al., 2018b). The economic significance of salmon aquaculture cannot be overstated, with a market value of approximately GBP 8.5 billion. Beyond economic contributions, salmon farming bolsters food security and employment opportunities in several nations, notably Norway.

Nonetheless, the fishing industry in Iraq, especially in the northern region, has not performed up to pace due to a lack of supply. The need for fish as a nutritious food source has significantly expanded in Iraq's northern region. These requests are consistent with the fact that the country does not have the capacity to supply these demands for such a significant commodity. Furthermore, there is now little government support to safeguard the national product in order to progress actual agricultural output, even in the face of the availability of the components required to grow and improve fish production (Abdulhasan et al., 2019). As we navigate the dynamic intersection of genetics, genomics, and salmon production, we are witnessing a remarkable synergy between research investments and industry support, propelling the field forward at an unprecedented pace.

Aquaculture's ability to expand globally is frequently limited by the gradual loss of breeding germplasm quality brought about by improper fish husbandry, selection requirements in specific fish breeding schemes, and the repeated use of specific (appealing or higher yielding) brood stock. Disease incidence is also a contributing factor, particularly when production is increased in an effort to secure food security, increase incomes, and provide a living. Fish that are resistant to disease restrict infection by reducing the pathogen's ability to replicate within their bodies (Doeschl-Wilson et al., 2012). Disease resistance is a valuable characteristic in and of itself for fish, animal, or plant breeding programs because it restricts the unnecessary use of chemicals or medications, which have a wider range of effects, including on non-target organisms in the environment (Caipang et al., 2020). However, disease resistance may not be effective enough to reduce the frequency and severity of diseases. Similar to this, microbial resistance to medications or antibiotics is a genuine and significant issue in agricultural output which is made worse by global warming (Rodríguez-Verdugo et al., 2013). Consequently, it is preferable to use alternative, more economical, environmentally friendly, and sustainable methods of disease management in salmon farming. One of these tactics is the breeding of superior strains, which enhances the aquaculture enterprise's quality, productivity, profitability, and sustainability by taking advantage of natural genetic variation for disease resistance. The growing arsenal of genetic resources, particularly high throughput next generation sequencing technology, breeding for superior strains or varieties is becoming an extremely efficient procedure. As a result, breeding goals can now be centered on traits like growth, sex determination, or disease resistance (Robledo Sánchez et al., 2018). Since the 1990s, breeding for disease-resistant strains has been a top priority in the production of farmed salmon because it gives the fish a cumulative and long-lasting resistance to illnesses. Nonetheless, a population with adequate genetic variety for the characteristic is necessary for breeding for resistance. Several salmonid species have been found to have high levels of additive genetic variation for disease resistance suggesting that salmonids

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

may benefit from selective breeding for disease resistance. Genome-wide association study (GWAS) Study using a set of molecular markers (most often SNPs) covering the entire genome aiming to identify genetic variant(s) associated with a phenotypic trait.

In contrast to mammals, which share the master sex determination (SD) gene (SRY1), fish have at least five genes known to regulate SD: amhy, amhr2, dmY, gsdf, and sdY2. Although each functions differently, they all seem to share the ability to regulate the start and rate of cell division. Sexually dimorphic Y (sdY) chromosome is thought to be the genetic regulation of sex determination in salmonids, and it is believed to use a male heterogametic (XY) system3. Since sdY has not been linked to sexual development in other species, its mechanism of action is less known than that of other fish master SD genes (Lubieniecki et al., 2015). A portion of the diversity in Atlantic salmon maturation can be explained by vestigial-like protein 3 (vgll3). Vgll3 is found in the chr25 region of the genome and accounts for around 35% of maturation variance. The early (E) and late (L) maturation alleles of Vgll3 are dominant in male. Early maturation in females appears to be unaffected by the gene (Barson et al., 2015; Ayllon et al., 2015).

Different environments clearly show the gene's varying potency. The effects of vgll3 are stronger in wild salmon than in farmed salmon (Ayllon et al., 2015). When fish receive less feed than when they are feed a complete feed ration to support growth, the effects of Vgll3 are stronger. There might be evidence linking lipid reserves, energy state, and size to vgll3. According to Kjaerner-Semb et al. (2018), homozygous EE fish may mature with a lower energy status than LL fish. Additional studies have discovered a connection between vgll3 and lipid reserves in mice's adipose tissue (Halperin et al., 2013). The gonads, heart, and gills express Vgll3 (Kjærner-Semb et al., 2018). Mature post-smolts, called jacks, are uncommon in the wild but common in aquaculture. Environmental conditions like high temperatures and continuous light (24L0D) are required in land-based facilities since they encourage growth. Growth circumstances are ideal when combined with an adequate diet. These elements also encourage maturation, therefore an increase in jacks in aquaculture would be expected (Fjelldal et al., 2011). This study examines the responses of several vgll3 genotypes to contemporary farming practices.

This study aims to provide a holistic understanding of the genetics and genomics of Atlantic salmon, emphasizing its role as a critical food source, an economically valuable commodity, and an emblematic sportfish. Our research further extends to the comparison of salmon populations in Iraq and Norway, seeking to uncover genetic variations and similarities through a comprehensive sequence analysis. By doing so, we contribute to the evolving narrative of sustainable salmon farming, aligning with the evolving demands of responsible aquaculture.

Materials and methods

Sample selection and gene investigation.

The selection of fish species for this study was based on local availability in food stores. It was crucial that the selected fish individuals were gutted without removing appendages, scales, and the skeleton since multiple tissue types were to be examined. Samples of farmed Atlantic salmon (*Salmo salar* L.) from Norway 20 samples and 20 samples from *Salmo salar* L. in Iraq, a total of 40 samples were collected.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

We aimed to investigate two genes with distinct transcriptional origins, both playing crucial roles in physiological maturation. The first gene, sexually dimorphic on the Y-chromosome (sdY), is a notable component of the Y-chromosome genomic sequence, exhibiting high conservation across all salmonid species. It stands out as a male-specific Y-chromosome gene in the majority of salmonid species (Yano et al., 2013).

The second gene of interest was the vestigial-like protein 3 (vgll3), which is situated on somatic chromosome number 25 (Kjærner-Semb et al., 2018).

DNA extraction

Genomic DNA extraction was performed on muscles tissue samples using the Beta Bayern Tissue DNA Preparation Kit, sourced from Beta Bayern GmbH in 90453 Bayern, Germany.

DNA quality and quantity

The quantity and purity of the extracted DNA were assessed using the Nanodrop (Nanodrop Spectro 117 432-UK), following which it was stored at -20°C. Subsequently, genomic DNA underwent additional analysis by electrophoresis, with 60 ng loaded onto a 1% agarose gel and run for 60 min at 80 V.

Amplification of sdY and vgll3 genes

In order to pinpoint specific regions of the genes under examination, we retrieved the DNA sequences for sdY and vgll3 from the National Center for Biotechnology Information (NCBI) using their respective accession numbers: KT223111 for sdY and NC_059466 for vgll3. The retrieved sequences were used to design a pair of primers for each gene using Primer3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). These primers were designed to anneal to precise positions within each gene.

For sdY, the selected primers were designed to amplify the segment spanning nucleotide positions 675 to 1009 (see *Fig. 1*; *Table 1*), whereas, for vgll3, the primers were designed to amplify the region from nucleotide positions 293 to 3514 that includes the fragment that produces transcription cofactor vestigial-like protein 3 (see *Fig. 2*; *Table 1*).

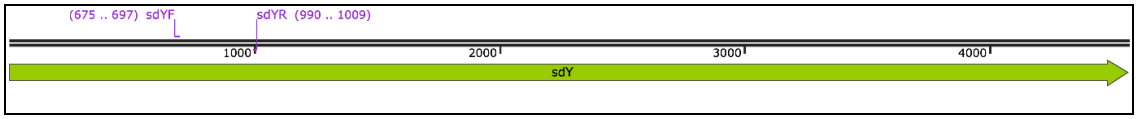

Figure 1. *Primer's binding sites on sdY gene. sdF and sdYR primes provide A 335 bp PCR amplicon*

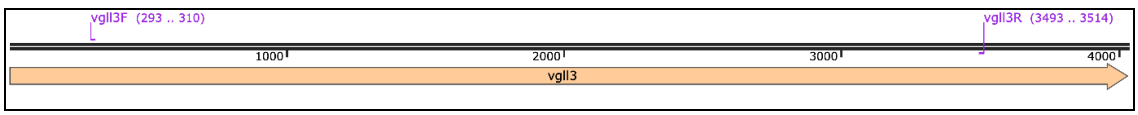

Figure 2. *Primer's binding sites on vgll3 gene. vgll3F and vgll3R primes provide a 3222-bp PCR amplicon*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

Table 1. *Names of genes, binding sites, sequences, and size of primes used in this study*

Primer name	Sequence 5' > > > > > > > > 3'	Target gene (accession number)	Product size (bp)	Annealing Tm
sdYF	CCAGCACTCTTTTCTTGTCTCAG	*sdY* (KT223111)	335	58
sdYR	GCATCACAGGATCCACATCA			
vgll3F	CTGCCAGCTTCGTCACAG	*vgll3* (NC_059466)	3222	59.5
vgll3R	CAGGAGGTTCTGTTAGAGCTGA			

An amplification of a precise segment of the sdY genes was conducted in a 20-µl PCR reaction mixture. This reaction mixture consisted of 10 µl of 2x Master Mix (obtained from AMPLIQON A/S, located at Stenhuggervej 22), 1 µl each of sdYF and sdYR primers at a concentration of 5 pmol, and 2 µl of the DNA template. The reaction volume was then adjusted by adding 6 µl of nuclease-free water (as specified in *Table 2*). The PCR was carried out using the Bioresearch PTC-200 Gradient thermocycler as follows: Initial denaturation at 95°C for 5 min, then 35 cycles of 40 s at 95°C for denaturing, 35 s for annealing at 57.5°C, 60 s for the extension at 72°C, and 5 min for the final extension at 72°C.

Table 2. *PCR reagents for amplifying a specific DNS segment of the sdY gene*

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	10
2	sdYF Primer	5 Pmol	1
3	sdYR Primer	5 Pmol	1
4	Nuclease-free water	-	6
5	DNA template	50 ng/µl	2
Total			20

A partial amplification of the vgll3 genes was conducted in a 20 µl PCR reaction mixture. This reaction mixture consisted of 10 µl of 2x Master Mix (obtained from AMPLIQON A/S, located at Stenhuggervej 22), 1.5 µl each of vgll3F and vgll3R primers at a concentration of 5 pmol, and 2 µl (equivalent to 100 ng) of the DNA template. The reaction volume was then adjusted by adding 5 µl of nuclease-free water (as specified in *Table 3*). The PCR was carried out using the Bioresearch PTC-200 Gradient thermocycler as follows: Initial denaturation at 95°C for 5 min, then 40 cycles of 45 s at 95°C for denaturing, 55 s for annealing at 59.5°C, 90 s for the extension at 72°C, and 5 min for the final extension at 72°C.

Table 3. *PCR reagents for amplifying a specific DNS segment of the vgll3 gene*

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	10
2	vgll3F Primer	5 Pmol	1.5
3	vgll3 R Primer	5 Pmol	1.5
4	Nuclease-free water	-	5
5	DNA template	50ng/µl	2
Total			20

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

Agarose gel electrophoresis

To verify the presence of the desired PCR amplicons, 2 µl of the PCR products were subjected to electrophoresis on a 1.5% agarose gel containing 0.07% ethidium bromide (EtBr). This gel was run alongside a 100 bp DNA ladder (Genedrix) in 1X Tris-Borate-EDTA (TBE) buffer at 80 V for a duration of 60 min. The aim was to confirm the accurate amplification of the target gene. Following the electrophoresis run, the DNA molecules were visualized and captured using a UV Gel Imager, specifically the SynGene 1409.

Sequencing of sdY and vgll3 PCR amplicon

The PCR amplicons, one measuring 335 bp for sdY and the other 3222 bp for vgll3, were sent to Macrogen Inc., a South Korean sequencing company. The sequencing protocol involved using the sdYR primer for sdY gene fragments and vgll3R for vgll3 gene fragments.

Quality of the sequenced genes

The DNA Baser Assembler program was used for sequence quality assessment, analysis, and editing purposes. Specifically, the program was used to trim the initial and final segments of the sequence to evaluate and enhance sequence quality.

The sequences analyses

We successfully met our criteria with 20 copies each of the sdY and vgll3 genes, evenly distributed between Iraqi *Salmo salar* and Norwegian *Salmo salar*, with 10 samples from each population meeting our requirements.

We conducted a thorough examination of sequence similarities among all DNA samples by employing the multiple sequence alignment technique through Muscle. Furthermore, we mapped the gene sequences to the reference genomes of salmon sdY (KT223111) and vgll3 (NC_059466) obtained from the NCBI using Bowtie.

Results and discussion

Genomic DNA quality evaluation

To evaluate the quality of the DNA extracted from the tissue samples, we employed gel electrophoresis in conjunction with Nanodrop analysis. Remarkably, *Figure 3* illustrates that no signs of DNA degradation were observed in any of the genomic DNA samples. This finding is further substantiated by the Nanodrop results, which reveal an average purity ratio (A260/A280) of 1.87 for these samples.

Amplification of sdY and vgll3 gene fragments

In this study, we achieved successful amplification of DNA fragments belonging to the sdY and vgll3 genes, with sizes of 335 bp and 3222 bp, respectively. These amplifications were consistently observed across all DNA templates extracted from *Salmo salar* samples collected from both Iraqi and Norwegian populations. Notably, no PCR products were detected in the negative controls, as shown in *Figures 4* and *5*. This robust amplification highlights the reliability of our experimental approach in confirming the presence of sdY and vgll3 gene fragments in the examined *Salmo salar* L. populations.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

Figure 3. *Agarose gel electrophoresis analysis of genomic DNA extracted from fish samples. A 1.5% agarose gel, infused with 0.07% ethidium bromide (EtBr), was subjected to electrophoresis at 80 V for 60 min. A 100-bp DNA ladder from fig3 served as the molecular weight marker. Lanes 1-10 correspond to samples from Iraqi Salmo salar L., while lanes 11-20 represent genomic DNA extracted from Norwegian Salmo salar L. Additionally, lane C served as a negative control, using distilled water instead of tissue in the DNA extraction process. Importantly, all samples showed no signs of DNA degradation. The negative control validated the successful completion of the DNA extraction process*

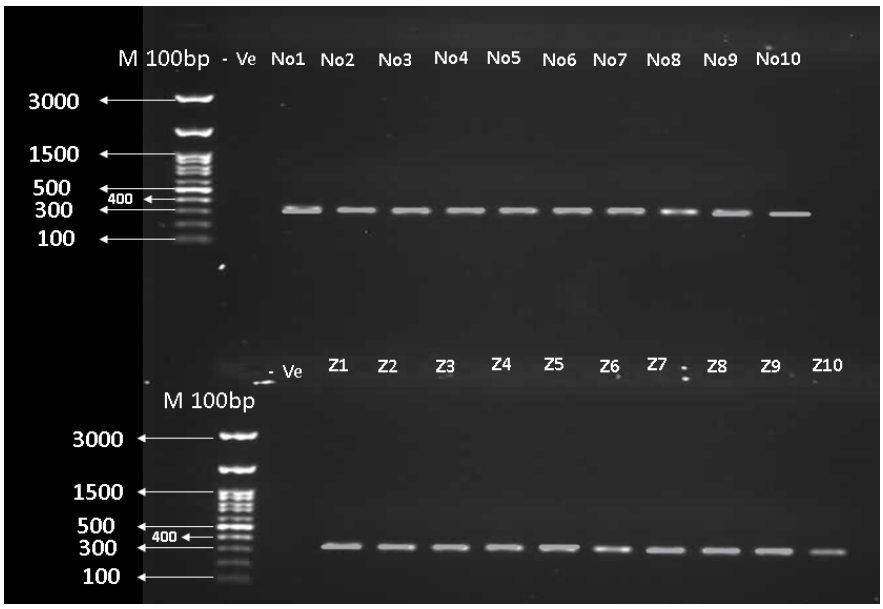

Figure 4. *PCR Amplification of sdY gene fragments in 20 Salmo salar L. samples. The lane annotations are as follows: Lane 1 (M) features a 100-bp DNA marker for reference, lanes (No1-No10) correspond to DNA samples extracted from Norwegian Salmo salar individuals., while lanes Z1-Z10 shows samples from Iraqi Salmo salar L. fish. Additionally, lane C serves as a negative control, representing a PCR reaction conducted without any DNA template*

Remarkably, the gel analysis reveals success of DNA fragment amplification, yielding a product of 335 base pairs in size across all tested samples. Notably, there is a complete absence of PCR product in the negative control (lane C), indicating the absence of contamination or non-specific amplification. The electrophoresis was

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

conducted on a 1% agarose gel, supplemented with 0.07% ethidium bromide (EtBr), and subjected to an 80 V electric field for a duration of 60 min. These optimized experimental conditions ensure clear and reliable visualization of the amplified DNA fragments.

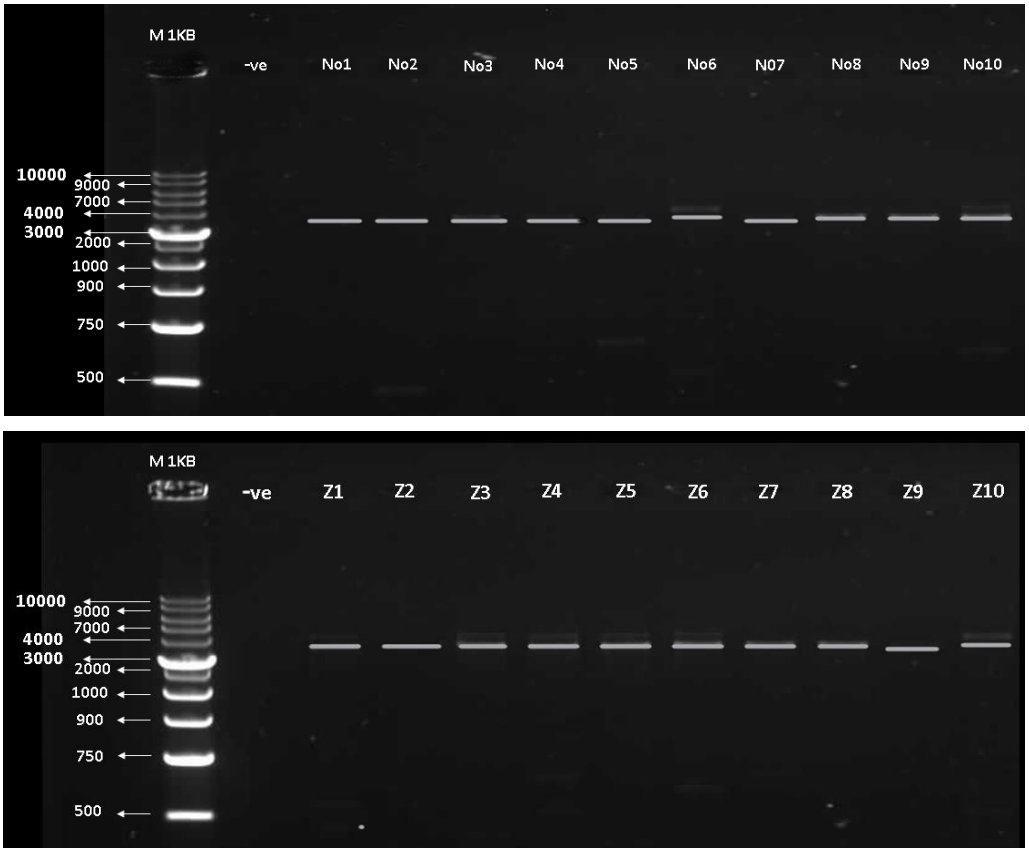

Figure 5. *PCR Amplification of vgll3 gene fragments in 20 Salmo salar L. samples. Lane 1: 100-bp DNA marker. Lanes No1-No10: Norwegian Salmo salar, Lanes Z1-Z10: Iraqi Salmo salar L. Lane C: Negative control (no DNA template). Successful 3222-bp fragment amplification observed in all samples, while the negative control showed no PCR product. Gel electrophoresis was conducted on 1% agarose gel with 0.07% EtBr at 80 V for 60 min*

The gel analysis uncovers the successful amplification of DNA fragments, resulting in a uniform product size of 3222 base pairs across all samples. Particularly, the absence of any PCR product in the negative control (lane C) signals the absence of contamination or unintended amplification.

Quality assessment of the sequenced DNA fragments

Following the initial DNA sequencing, we subjected all the samples under investigation to rigorous quality assessment. This involved assessing their Quality Values (QV), specifically considering those exceeding a threshold of 40. A representative example of a high-quality sequence is depicted in *Figure 6*.

Sequences failing to meet our established criteria (quality value below 40) underwent a second round of sequencing for improved accuracy. An illustration of an unsatisfactory sequence is available in *Figure 7* for reference.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

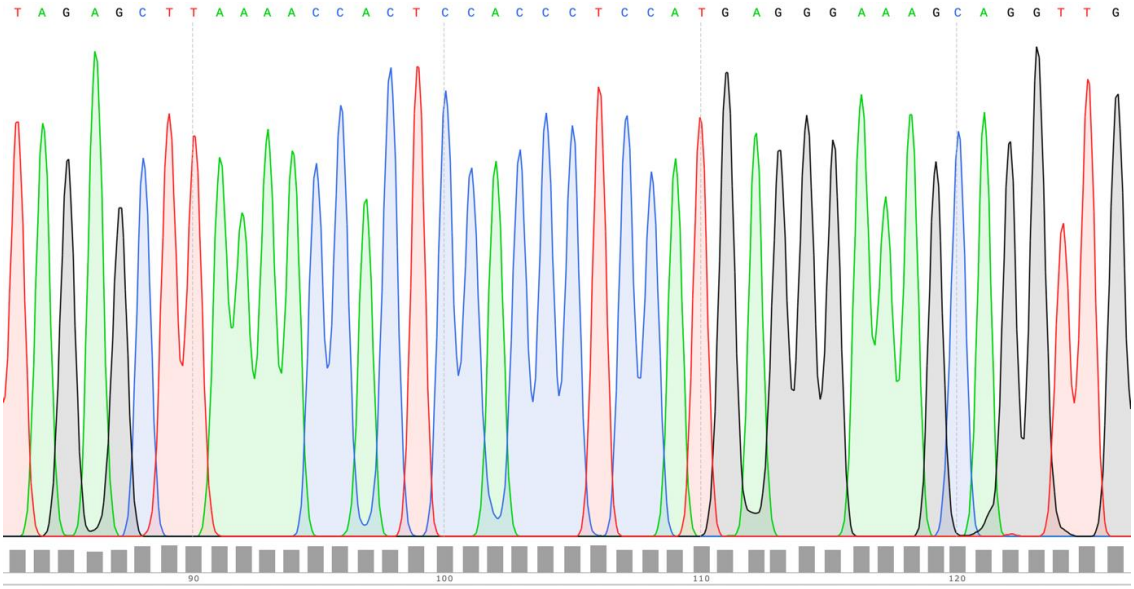

Figure 6. *A representative of good DNA sequence. The bottom gray bars represent a single base's quality value, which is more than 40 in our cases. There is no noise and the peaks are well and evenly spaced. The baseline is consistent and flat over the whole sequence chromatogram. The base is called precisely above each peak*

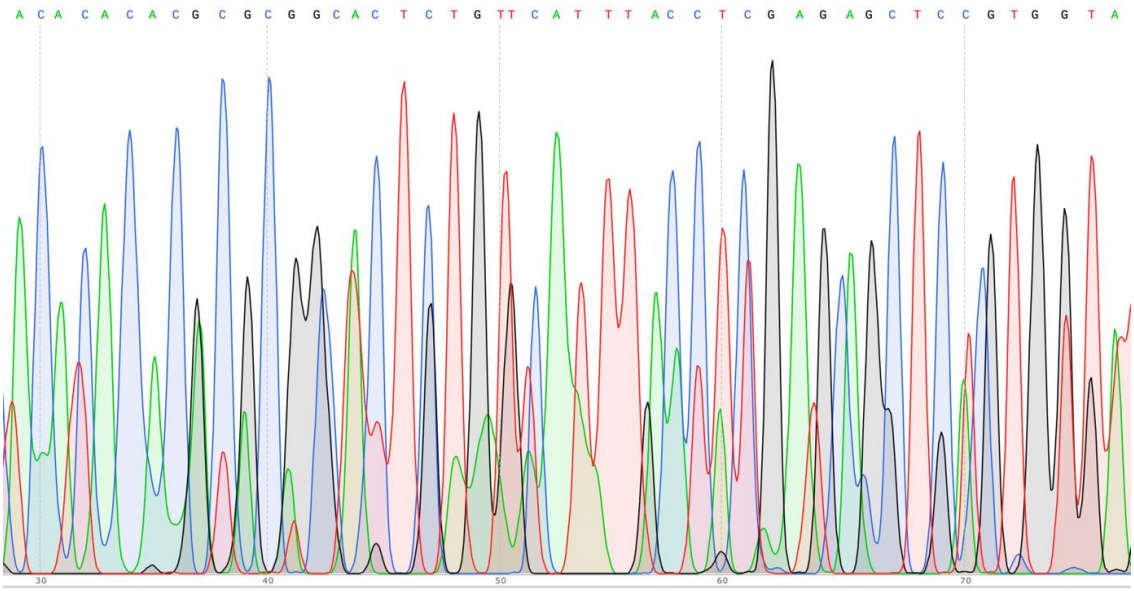

Figure 7. *A representative of bad DNA sequence. The bottom gray bars represent a single base's quality value, which is more than 40 in our cases. There is no noise and the peaks are well and evenly spaced. The baseline is consistent and flat over the whole sequence chromatogram. The base is called precisely above each peak*

Sequence alignment and submission gene to GenBank

The qualified sequences of both genes were uploaded to GenBank (*Table 4*). Homology of both genes including insertions - deletions, stop codons, and frameshifts were checked using NCBI- BLAST, the comparisons were carried out to find out

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

more similarity and nucleotide variation with other targets figure. The variation that were found with the sdY genes are shown in *Table 5* and with vgll3 were found in *Table 6*.

Table 4. *GenBank accession no. of both gene SdY and vgll3 of Salmo salar L.*

Name	Accession No.	Gene name
Salmo salar (Iraq)	OQ737078	sdY
Salmo salar (Iraq)	OQ737079	sdY
Salmo salar (Iraq)	OQ737080	sdY
Salmo salar (Iraq)	OQ737081	sdY
Salmo salar (Iraq)	OQ737082	sdY
Salmo salar (Iraq)	OQ737083	sdY
Salmo salar (Iraq)	OQ737084	sdY
Salmo salar (Iraq)	OQ737085	sdY
Salmo salar (Iraq)	OQ737086	sdY
Salmo salar (Iraq)	OQ737087	sdY
Salmo salar (Norway)	OQ737088	sdY
Salmo salar (Norway)	OQ737089	sdY
Salmo salar (Norway)	OQ737090	sdY
Salmo salar (Norway)	OQ737091	sdY
Salmo salar (Norway)	OQ737092	sdY
Salmo salar (Norway)	OQ737093	sdY
Salmo salar (Norway)	OQ737094	sdY
Salmo salar (Norway)	OQ737095	sdY
Salmo salar (Norway)	OQ737096	sdY
Salmo salar (Norway)	OQ737097	sdY
Salmo salar (Iraq)	OR248659	Vgll3
Salmo salar (Iraq)	OR248660	Vgll3
Salmo salar (Iraq)	OR248661	Vgll3
Salmo salar (Iraq)	OR248662	Vgll3
Salmo salar (Iraq)	OR248663	Vgll3
Salmo salar (Iraq)	OR248664	Vgll3
Salmo salar (Iraq)	OR248665	Vgll3
Salmo salar (Iraq)	OR248666	Vgll3
Salmo salar (Iraq)	OR248667	Vgll3
Salmo salar (Iraq)	OR248668	Vgll3
Salmo salar (Norway)	OR248649	Vgll3
Salmo salar (Norway)	OR248650	Vgll3
Salmo salar (Norway)	OR248651	Vgll3
Salmo salar (Norway)	OR248652	Vgll3
Salmo salar (Norway)	OR248653	Vgll3
Salmo salar (Norway)	OR248654	Vgll3
Salmo salar (Norway)	OR248655	Vgll3
Salmo salar (Norway)	OR248656	Vgll3
Salmo salar (Norway)	OR248657	Vgll3
Salmo salar (Norway)	OR248658	Vgll3

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

Table 5. *Explain the numbers and variant position of nucleotides of each sample are changed with amino acid codons in the sdY gene of Salmo salar L.*

Sample position	Gene name and position sequences	Variant position	Nucleotide changed	Amino acid changed	Codon number changed	GenBank reference accession number
Iraq	sdY (First Exon) 674-1036	722 728 732 751, 752 875 935 998, 999 1001, 1002 1007	A A T A, G A C A, T A, T A	H = histidine H = histidine D = Aspartic acid R = Arginine K = Lysine P = proline D = Aspartic acid H = histidine K = Lysine	16(CAT) 18(CAT) 19(GAT) 26(AGA) 67(AAG) 87(CCA) 108(GAT) 109(CAT) 111(AAG)	KT223111.1
Norway	sdY (First Exon) 674-1036	722 728 732 751, 752 875 935 998, 999 1001, 1002 1007	C C G C, A T A C, C T, G T	P = proline P = proline E = Glutamic acid Q = Glutamine M = Methionine Q = Glutamine A = Alanine L = Leucine M = Methionine	16(CCT) 18(CCT) 19(GAG) 26(CAA) 67(ATG) 87(CAA) 108(GCC) 109(CTG) 111(ATG)	KT223111.1

Table 6. *The numbers and variant position of nucleotides of each sample are changed with amino acid codons in the vgll3 gene of Salmo salar L.*

Sample position	Gene name and position sequences	Variant position	Nucleotide variant	Amino acid variant	Codon number changed	GenBank reference accession number
Iraq	*vgll*3 181-1533	601 931, 933 936 1078 1148 1318	G G, T A G G G	E = Glutamic Acid A = Alanine E = Glutamic Acid A = Alanine E = Glutamic Acid A = Alanine	97 (GAG) 207(GCT) 208(GAA) 255(GCT) 293(GAG) 336(GCC)	XM_014173901
Norway	*vgll*3 181-1533	601 931, 933 936 1078 1148 1318	A C, A C C C C	K = Lysine P = Proline D = Aspartic acid P = proline Q = Glutamine P = proline	97(AAG) 207(CCA) 208(GAC) 255(CCT) 293(CAG) 332(CCC)	XM_014173901

Alignment and detection of variant amino acid sdY protein

Among 20 submitted sequences of sdY between two Iraqi and Norwegian *Salmo salar* L. nine different variations of amino acid appear according to alignment in the program of MEGA version of 11 (*Fig. 8*).

Alignment and detection of variant amino acid in Vgll3 protein

Among 20 submitted sequences of Vgll3 between two Iraqi and Norwegian *Salmo salar*, nine different variations of amino acid appear according to alignment in the program of MEGA version 11 (*Fig. 9*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

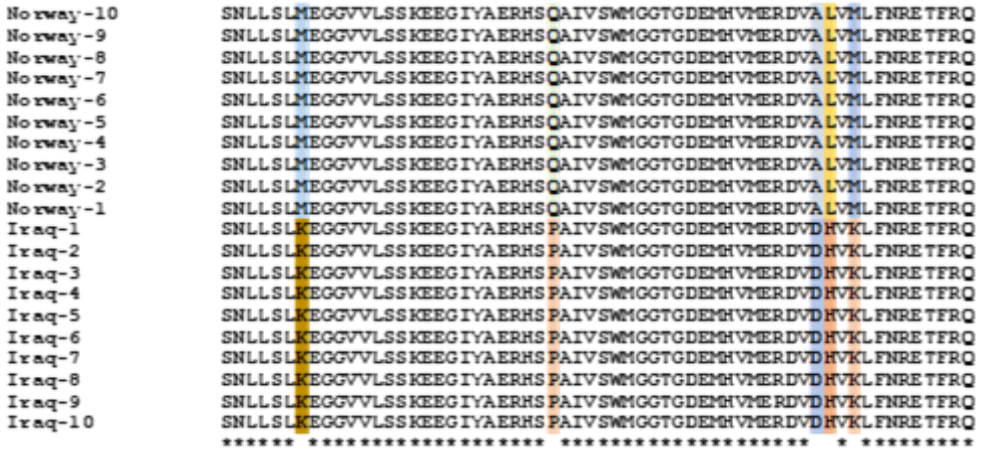

Figure 8. *Multiple protein sequence alignment analysis of sdY gene among 20 submitted sequences of sdY between two Iraqi and Norwegian Salmo salar L.*

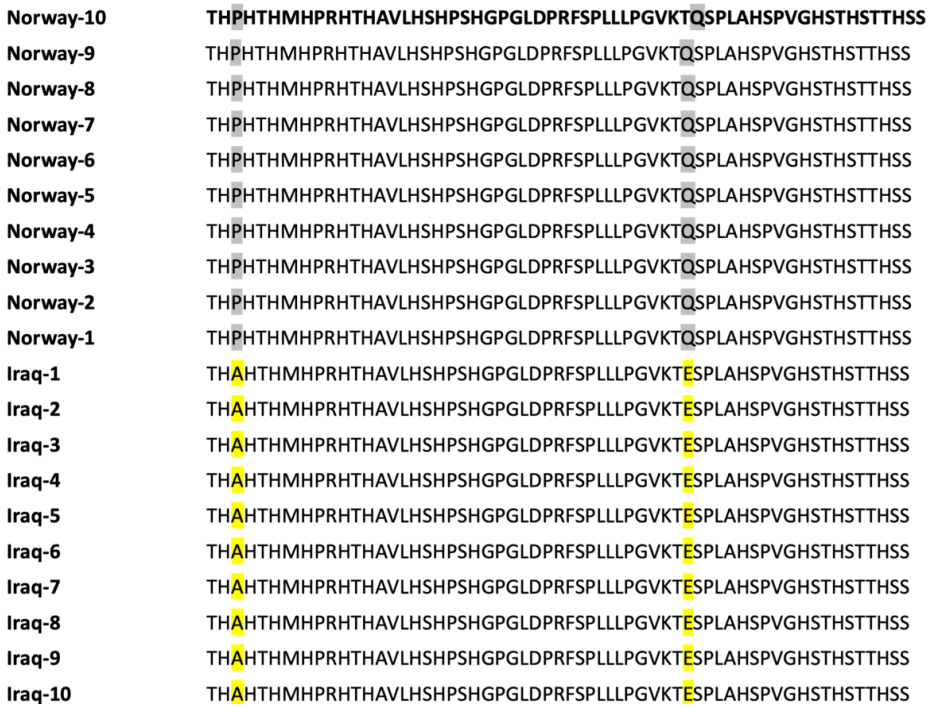

Figure 9. *Multiple protein sequence alignment analysis of vg113 gene among 20 submitted sequences of vg113 between two Iraqi and Norwegian Salmo salar L.*

The sdY (SRY-box containing protein 9) gene is a sex-determining gene that is involved in the determination of the male phenotype in fish. To determine if there are any significant differences in the sdY gene between *Salmo salar* L. fishes in Iraq and Norway, genetic studies have been conducted. Genetic studies can involve analyzing the DNA sequences of the sdY gene from both populations and comparing the nucleotide and amino acid sequences. If there are differences in the DNA sequence of the gene between the two populations, this could indicate that the gene has evolved differently in each population due to genetic drift, natural selection, or other evolutionary forces.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

Analyzing the variant position, nucleotide, and amino acid change sequences, and codon number changes in the sdY gene can provide additional insights into the nature of the observed differences and how they might be functionally relevant. Overall, our research findings suggest that there are significant genetic differences between *Salmo salar* L. fishes in Iraq and Norway, and these differences may have implications for muscle development and growth in these populations. Salmonids have demonstrated that the regulation of muscle growth mechanisms by the MSTN is contingent upon several factors such as age, muscle type, developmental stage, and nutritional state (Churova et al., 2029). Fisher's exact test was used to compare the frequency of different nucleotide or amino acid variants between the two populations is appropriate in this context.

Our research findings indicate that there are significant differences only in amino acid variants in the sdY gene (*Fig. 10*) between *Salmo salar* L. fishes in Iraq and Norway. The significant differences in amino acid variants suggest that there are differences in the sequence of the protein that is produced from the gene (p-value = 0.0065).

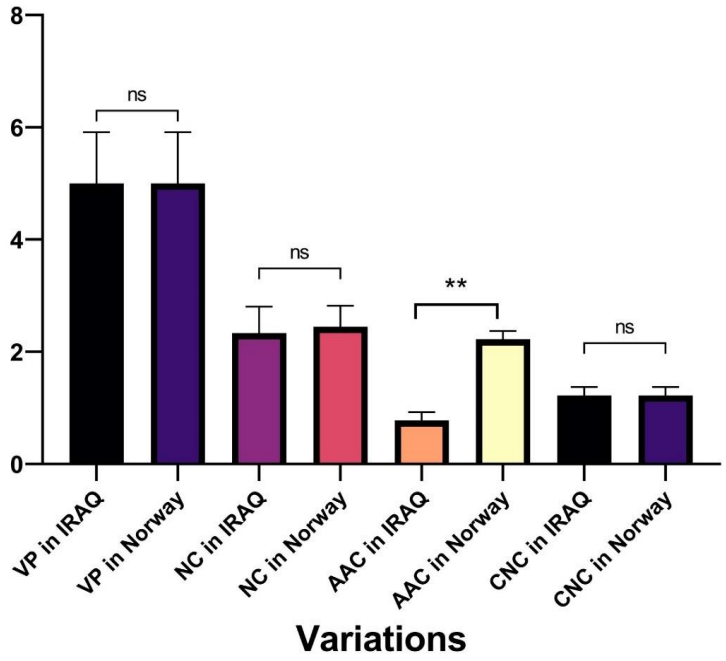

Figure 10. *Genetic variation in the sdY gene between the fish population in Iraq and Norway*

The vgll3 gene is a protein-coding gene that plays a role in muscle development and growth. It has been studied in various fish species, including the Atlantic salmon *(Salmo salar* L.). To determine if there are any associations or significant differences in the vgll3 gene between *Salmo salar* L. fishes in Iraq and Norway, in our research, genetic studies were conducted. This study typically involved collecting tissue samples from the two fish populations and analyzing the DNA sequences of the vgll3 gene.

Genetic studies can involve analyzing the DNA sequences of the vgll3 gene from both fish populations and comparing the variant position, nucleotide and amino acid variant sequences, and codon number changes. Nucleotide variants refer to differences

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

in the DNA sequence of the gene, while amino acid variants refer to differences in the sequence of the protein that is produced from the gene. By comparing the variant position, nucleotide and amino acid variant sequences, and codon number changes, we identify the significant differences between the two populations and assess whether these differences are statistically significant. For example, we use Fisher's exact tests to compare the frequency of different nucleotide or amino acid variants between the two populations, and there is a significant difference in nucleotide variants (p-value = 0.0476) and amino acid variants (p-value = 0.0079) between the fish population in Iraq and Norway and revealed in *Figure 11*.

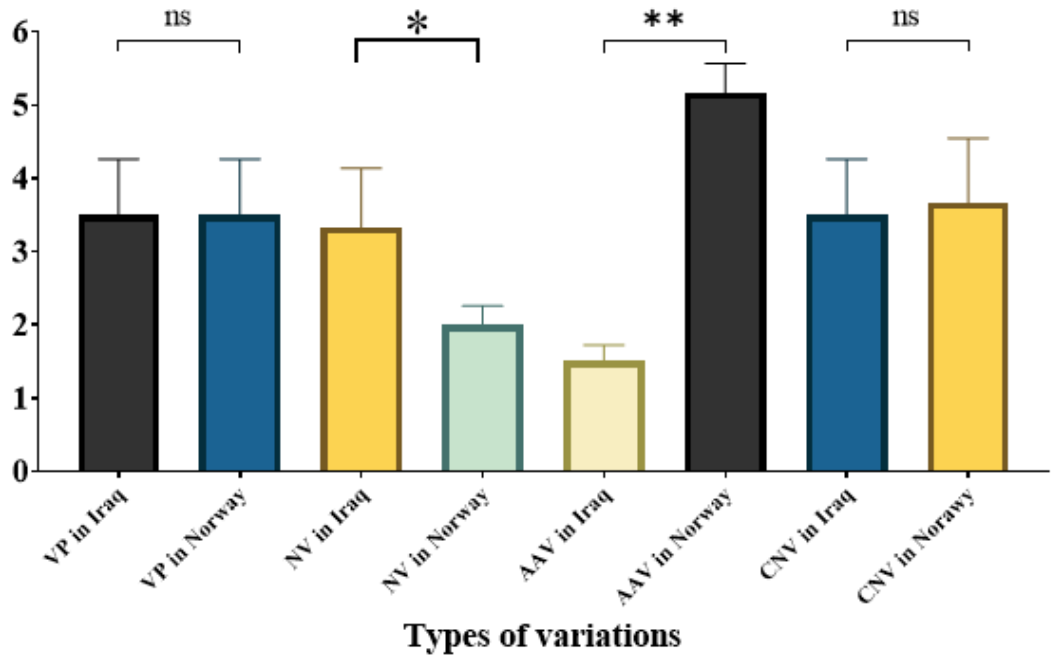

Figure 11. *Genetic variation in the vgll3 gene between the fish population in Iraq and Norway*

Discussion

The genetic and genomic analysis conducted in this study provides valuable insights into the Atlantic salmon (*Salmo salar* L.). populations in Norway and Iraq, with implications for both aquaculture management and evolutionary biology. By focusing on key genes associated with physiological maturation, such as sexually dimorphic on the Y-chromosome (sdY) and vestigial-like protein 3 (vgll3), we have uncovered significant genetic variations between the two populations (Barson et al., 2015).

Significant genetic variety is found in the Norwegian and Iraqi Atlantic salmon populations as well as between them, according to the study (Glover et al., 2017). Given the many environmental and geographical circumstances, this diversity is expected. The inhabitants of Iraq dwell in warmer, less nutrient-dense habitats, while the populations in Norway inhabit cold, nutrient-rich waters. The observed genetic divergence is the product of unique selection pressures created by these variations, which promote local adaption.

In order to shape a species' genomic architecture, local adaptation is essential. It enables populations to tailor their reproductive and physiological characteristics to their unique circumstances. The significance of local adaptation is highlighted by the genetic

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

differences found in the sdY and vgll3 genes between the populations of salmon from Iraq and Norway. These differences most likely represent responses to various environmental stresses, including temperature, water flow, and food availability, which affect growth rates, the timing of reproduction, and general fitness.

In Norway, for example, the shorter growing season and lower water temperatures may favor genetic variations that favor rapid development and early maturation. These variants may also be selected for these populations. Conversely, populations of salmon in Iraq may possess genetic features that enable them to withstand higher temperatures and less consistent food supplies, which could result in slower growth rates but increased resistance to heat stress. The populations' ability to survive and procreate in their particular habitats depends on these local adaptations.

According to Barson et al. (2015), the sdY and vgll3 genes are important for sex determination and maturation, which are important parts of the reproductive biology of Atlantic salmon. Population dynamics and the success of reproduction can be significantly impacted by variations in these genes. For instance, variations in sex ratios among communities may result from variations in the sdY gene, which determines male sex. Comparably, differences in vgll3, which are linked to the time of maturation, may affect the age at which salmon attain sexual maturity, hence affecting their reproductive cycles and survival rates. There are various theories regarding how these variants affect the physiology of maturation at the molecular level, most of which center on the vgll3 gene. In vertebrates, Vgll3 regulates adiposity. The relationship between the fat reserve level and the age of maturation in Atlantic salmon appears to be straightforward since it is thought to be a crucial factor in regulating the onset of maturation [39]. Two missense variants at amino acids 54 and 323 in the vgll3 gene are highly correlated with age at maturation. The amino acids threonine (Thr) and lysine (Lys) are encoded by the haplotype linked to late maturation (e.g., three sea winters), whereas aspartic acid (Asp) and methionine (Met) are encoded by the haplotype linked to early maturation (Rivera et al., 2021).

Comprehending these genetic foundations is crucial for managing aquaculture sustainably. Aquaculture operations have the potential to improve the resilience and reproductive success of farmed salmon populations by identifying and maybe selecting for advantageous genetic features. The long-term survival of salmon stocks depends on maintaining genetic variety, lowering the risk of inbreeding, and optimizing breeding programs (Glover et al., 2017).

In aquaculture settings, for instance, choosing genetic variations that encourage faster maturation may shorten the time to market and increase economic efficiency. This must be weighed against the requirement to preserve genetic diversity and prevent any unfavorable effects on health and fitness, though. In order to manage wild populations and prevent breeding programs from unintentionally favoring features that could lower fitness in natural contexts, it is also helpful to understand the genetic basis of maturation and sex determination.

Our findings reveal substantial genetic diversity within and between the Norwegian and Iraqi Atlantic salmon (*Salmo salar* L.). populations. This diversity is not unexpected given the distinct geographical locations and environmental pressures experienced by these populations. The genetic differences observed highlight the importance of local adaptation and evolutionary processes in shaping the genomic architecture of Atlantic salmon (Glover et al., 2017).

The observed genetic variations in the sdY and vgll3 gene fragments between the Norwegian and Iraqi populations may have important implications for their

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

reproductive biology and population dynamics. These genes play crucial roles in regulating sex determination and maturation, and variations in their sequences could influence reproductive success and population fitness. Understanding the genetic basis of these traits is essential for the sustainable management of Atlantic salmon (*Salmo salar* L.). populations and the development of effective breeding programs in aquaculture.

Furthermore, our study contributes to the broader understanding of salmonid genetics and evolutionary biology. The comparative analysis of Atlantic salmon (*Salmo salar* L.). populations from geographically distant regions provides valuable insights into the processes driving genetic divergence and speciation in aquatic organisms (Lien et al., 2011). By elucidating the genetic mechanisms underlying population differentiation, we can better predict the responses of salmon populations to environmental changes and human-induced pressures, such as climate change and habitat degradation.

It is important to note that this study has some limitations. The sample size was relatively small, and the analysis focused on a limited number of genetic markers. Future research employing larger sample sizes and whole-genome sequencing approaches could provide a more comprehensive understanding of the genetic diversity and population structure of Atlantic salmon (*Salmo salar* L.). populations worldwide.

Conclusion

In conclusion, our study highlights the significant genetic differences between Atlantic salmon (*Salmo salar* L.). populations from Norway and Iraq, particularly in genes related to sex determination and maturation. These findings emphasize the importance of considering local adaptation in the management of salmon populations.

Our research contributes to the broader understanding of salmon genetics and evolutionary biology, providing insights crucial for sustainable management and conservation efforts. Moving forward, further research integrating genetic, genomic, and environmental data is needed to enhance our ability to predict and mitigate the impacts of human activities on salmon populations.

Overall, our study underscores the necessity for interdisciplinary collaboration to ensure the long-term survival of Atlantic salmon (*Salmo salar* L.) amid ongoing environmental challenges.

Conflict of interest. The author has declared that there is no conflict of interests regarding the publication of this article.

REFERENCES

[1] Abdulhasan, G. A., Alattar, N. S., Jaddoa, N. T. (2019): Comparative study of some virulence factors and analysis of phylogenetic tree by 16S rDNA Sequencing of Aeromonas hydrophila isolated from clinical and environmental samples. – Iraqi Journal of Science 2390-2397.

[2] Ayllon, F., Kjaerner-Semb, E., Furmanek, T., Wennevik, V., Solberg, M. F., Dahle, G., Taranger, G. L., Glover, K. A., Almen, M. S., Rubin, C. J., Edvardsen, R. B., Wargelius, A. (2015): The vgll3 locus controls age at maturity in wild and domesticated Atlantic salmon (Salmo salar L.) Males. – PLoS Genet 11: e1005628.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

[3] Barson, N. J., Aykanat, T., Hindar, K., Baranski, M., Bolstad, G. H., Fiske, P., Jacq, C., Jensen, A. J., Johnston, S. E., Karlsson, S., Kent, M., Moen, T., Niemela, E., Nome, T., Naesje, T. F., Orell, P., Romakkaniemi, A., Saegrov, H., Urdal, K., Erkinaro, J., Lien, S., Primmer, C. R. (2015): Sex-dependent dominance at a single locus maintains variation in age at maturity in salmon. – Nature 528: 405-8.

[4] Caipang, C. M. A., Suharman, I., Avillanosa, A. L., Bargoyo, V. T. (2020): Host-derived probiotics for finfish aquaculture. – IOP Conference Series: Earth and Environmental Science 430(1): 012026.

[5] Christensen, K. A., Rondeau, E. B., Minkley, D. R., Leong, J. S., Nugent, C. M., Danzmann, R. G., Ferguson, M. M., Stadnik, A., Devlin, R. H., Muzzerall, R., Edwards, M., Davidson, W. S., Koop, B. F. (2018): The Arctic charr (Salvelinus alpinus) genome and transcriptome assembly. – PloS ONE 13(9): e0204076.

[6] Churova, M. V., Shulgina, N., Kuritsyn, A., Krupnova, M. Y., Nemova, N. N. (2020): Muscle-specific gene expression and metabolic enzyme activities in Atlantic salmon Salmo salar L. fry reared under different photoperiod regimes. – Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 239: 110330.

[7] Doeschl-Wilson, A. B., Villanueva, B., Kyriazakis, I. (2012): The first step toward genetic selection for host tolerance to infectious pathogens: obtaining the tolerance phenotype through group estimates. – Frontiers in Genetics 3: 265.

[8] Fjelldal, P. G., Hansen, T., Huang, T.-S. (2011): Continuous light and elevated temperature can trigger maturation both during and immediately after smoltification in male Atlantic salmon (Salmo salar). – Aquaculture 321: 93-100.

[9] Glover, K. A., Solberg, M. F., McGinnity, P., Hindar, K., Verspoor, E., Coulson, M. W., Hansen, M. M., Araki, H., Skaala, Ø., Svåsand, T. (2017): Half a century of genetic interaction between farmed and wild Atlantic salmon: status of knowledge and unanswered questions. – Fish and Fisheries 18(5): 890-927.

[10] Halperin, D. S., Pan, C., Lusis, A. J., Tontonoz, P. (2013): Vestigial-like 3 is an inhibitor of adipocyte differentiation. – J Lipid Res 54: 473-81.

[11] Kjærner-Semb, E., Ayllon, F., Kleppe, L., Sørhus, E., Skaftnesmo, K., Furmanek, T., Segafredo, F. T., Thorsen, A., Fjelldal, P. G., Hansen, T., Taranger, G. L., Andersson, E., Schulz, R. W., Wargelius, A., Edvardsen, R. B. (2018): Vgll3 and the Hippo pathway are regulated in Sertoli cells upon entry and during puberty in Atlantic salmon testis. – Scientific Reports 8: 1912.

[12] Lien S., Koop B. F., Sandve S. R., Miller, J. R., Kent, M. P., Nome, T., Hvidsten, T. R., Leong, J. S., Minkley, D. R., Zimin, A., Grammes, F., Grove, H., Gjuvsland, A., Walenz, B., Hermansen, R. A., von Schalburg, K., Rondeau, E. B., Di Genova, A., Samy, J. K. A., Olav Vik, J., Vigeland, M. D., Caler, L., Grimholt, U., Jentoft, S., Inge Våge, D., de Jong, P., Moen, T., Baranski, M., Palti, Y., Smith, D. R., Yorke, J. A., Nederbragt, A. J., Tooming-Klunderud, A., Jakobsen, K. S., Jiang, X., Fan, D., Hu, Y., Liberles, D. A., Vidal, R., Iturra, P., Jones, S. J. M., Jonassen, I., Maass, A., Omholt, S. W., & Davidson, W. S. (2016): The Atlantic salmon genome provides insights into rediploidization. – Nature 533: 200-205.

[13] Macqueen, D. J., Primmer, C. R., Houston, R. D., Nowak, B. F., Bernatchez, L., Bergseth, S., Davidson, W. S., Gallardo-Escarate, C., Goldammer, T., Guiguen, Y., Iturra, P., Kijas, J. W., Koop, B. F., Lien, S., Maass, A., Martin, S. A. M., McGinnity, P., Montecino, M., Naish, K. A., Nichols, K. M., Olafsson, K., Omholt, S. W., Palti, Y., Plastow, G. S., Rexroa, C. E., Rise, M. L., Ritchie, R. J., Sandve, S. R., Schulte, P. M., Tello, A., Vidal, R., Vik, J. O., Wargelius, A., Yanez, J. M., The FAASG Consortium (2017): Functional Annotation of All Salmonid Genomes (FAASG): an international initiative supporting future salmonid research, conservation and aquaculture. – BMC Genomics 18: 1-9.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040

[14] Rivera, P., Gallardo, J., Araneda, C., Vasemägi, A. (2021): Sexual Maturation in Farmed Atlantic Salmon (Salmo Salar): A Review. – In: Lu, Q. (ed.) Salmon Aquaculture. IntechOpen, London.
[15] Wacker, S., Aronsen, T., Karlsson, S., Ugedal, O., Diserud, O. H., Ulvan, E. M., Hindar, K., Næsje, T. F. (2021): Selection against individuals from genetic introgression of escaped farmed salmon in a natural population of Atlantic salmon. – Evolutionary Applications 14(5): 1450-1460.
[16] Yano, A., Nicol, B., Jouanno, E., Quillet, E., Fostier, A., Guyomard, R., Guiguen, Y. (2013): The sexually dimorphic on the Y-chromosome gene (sdY) is a conserved male-specific Y-chromosome sequence in many salmonids. – Evolutionary Applications 6(3): 486-496.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2301_023040