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Scientific Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food and Environment

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Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. The project group consisted of 4 VKM members, 1 VKM staff and 4 external experts. Two referees commented on and reviewed the draft opinion. The Committee, by the Panel on Animal Health and Welfare, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work, and the authors have contributed as members of the project group and/or the VKM Panel on Animal Health and Welfare appointed specifically for the assignment.

Members of the project group (in alphabetical order after chair of the project group):

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Acknowledgement

VKM would like to thank the referees Tillmann Benfey (University of New Brunswick, Canada) and James Ferguson Turnbull (University of Stirling, Scotland) for their valuable comments through critical review of the draft opinion. VKM emphasises that the referees are not responsible for the content of the final opinion. VKM would also like to thank Angelika Agdestein, who was a member of the Panel on Animal Health and Welfare until August 2023, for her valuable input during the mandate discussion. VKM is grateful to Bente Mangschou and Kristin Opdal Seljetun at the secretariat staff, for their important contributions in the manuscript. In accordance with VKM's routines for approval of a risk assessment (VKM, 2018), VKM received their comments before evaluation and approval by VKM Panel on Animal Health and before the opinion was finalised for publication.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third-party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

Background and terms of reference

Farmed Atlantic salmon (*Salmo salar*) that escape into the wild could interbreed with native fish, posing a potential risk to the genetic diversity of wild Atlantic salmon populations. The Atlantic salmon in aquaculture are diploid, meaning the fish has two sets of chromosomes. To mitigate the genetic impact on wild populations, the concept of producing sterile triploid farmed Atlantic salmon has been suggested as a solution. However, it is important to ensure that the utilization of triploids in commercial farming aligns with the regulations set forth in the Norwegian Animal Welfare Act.

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food and Environment (VKM) to do an assessment about health- and welfare consequences in triploid Atlantic salmon under commercial farming conditions, as compared to diploid counterparts. VKM was also requested to describe the underlying physiological mechanisms concerning consequences of triploidy as well as address potential measures to reduce the negative impacts on the health and welfare of the fish.

Methods

A working group consisting of members with expertise in salmonid biology, aquaculture systems, veterinary medicine, fish health and welfare, virology, bacteriology, parasitology, breeding and genetics has drafted this opinion. To answer the Terms of Reference as mandated by the NFSA, the authors addressed fish health and welfare as a unified concept in this report. Two external experts have reviewed and provided their opinion before it was assessed and approved by the VKM's Panel on Animal Health and welfare.

The literature used in this work was peer-reviewed studies retrieved from a search in four databases as well as non peer-reviewed reports. Selection of studies was conducted independently by two members in the working group and based on predefined inclusion and exclusion criteria.

Conclusions

Under commercial farming conditions, triploid Atlantic salmon are often found to have lower standards of health and welfare compared to diploids. For example, field and experimental studies have found triploids to be more prone to skeletal and heart deformities, and cataracts, while field studies suggest that under commercial farming conditions they cope less well with handling and are more susceptible to skin ulcers. However, research has indicated that some of the effects of triploidy can be mitigated through specialized diets or environmental adjustments.

There is a noticeable tendency across farm studies and experimental trials for triploid salmon to be equal or larger in size at the end of freshwater phase, but equal or smaller in size at the end of the seawater phase.

Most publications conclude that within what is considered the optimal temperature range of diploids, oxygen consumption rate, oxygen binding capacity, and aerobic swimming capacity do not significantly differ between triploid and diploid Atlantic salmon. However, findings from experimental trials suggest a lower optimal temperature range for triploids, and data consistent across studies indicate that triploids possess lower tolerance to hypoxia at elevated temperatures. Triploid Atlantic salmon are less robust to higher water temperatures than diploid, and have other nutritional needs than diploids, especially regarding phosphorus, and histidine.

There are few studies on the susceptibility of triploid salmon to infectious agents and diseases. Field observations indicate that triploid fish are more susceptible to primary infectious salmon anaemia (ISA) outbreaks than diploids under commercial farming conditions at the level of the farm, and at cage level within farms that experience an ISA outbreak. A higher susceptibility to the ISA virus would potentially affect not only the health and welfare of the triploid fish at the farm with an outbreak but may potentially spread to other farms. Field data also suggests triploids are more susceptible to *Moritella viscosa*, and thus ulcer development under commercial farming conditions, than diploids. However, experimental challenge data of triploids for ISA an*d M. viscosa* is scarce, and relatively inconclusive.

Triploid salmon appear to have different requirements for diet, environment (e.g. water temperature) and are more sensitive to stress (e.g. handling and hypoxia at high temperature) and, therefore, require different operative conditions than diploids. Research shows that many of the investigated differences in health and welfare can be fully or partially mitigated if conditions are optimized for triploids. Still, some of the necessary adjustments are not feasible under the present commercial farming conditions.

Several other health and welfare parameters are evaluated in this report.

Uncertainties and data gaps

As several of the findings from field studies versus experimental studies are not consistent, and as well-designed studies comparative studies investigating triploid and diploid Atlantic salmon health and welfare aspects are scarce, there are uncertainties regarding some of the conclusions. However, the findings under present farming conditions are more consistent.

There are data gaps in understanding factors such as the impact of early environmental conditions, temperature tolerance, interactions between ploidy and genotype and other genetic parameters, susceptibility to diseases, stress responses, the use of less invasive techniques for triploid production, the influence of cell size on fish physiology, and smoltification protocols/the timing for transfer of triploids to sea.

Key words: Atlantic salmon, triploid, deformities, farming conditions, health and welfare, mitigation measures, stress coping, susceptibility to disease, temperature tolerance

Sammendrag på norsk

Bakgrunn og spørsmål

Laks (*Salmo salar*) i oppdrett kan rømme og formere seg med villaks og dermed utgjøre en risiko for det genetiske mangfoldet til ville laksebestander. Laks i oppdrett er per i dag diploid, det vil si at fisken har to sett av kromosomer. For å redusere risikoen for uheldig genetisk påvirkning av ville laksebestander har steril triploid oppdrettslaks blitt foreslått som en løsning. Det er imidlertid viktig å sikre at bruken av triploid laks i kommersielt oppdrett er i tråd med regelverket i dyrevelferdsloven.

Mattilsynet ba Vitenskapskomiteen for mat og miljø (VKM) om en vurdering av helse- og velferdskonsekvenser for triploid atlantisk laks ved bruk i kommersielt oppdrett. VKM ble også bedt om å beskrive de underliggende fysiologiske mekanismene for konsekvenser av triploidi, og peke på mulige tiltak for å redusere eventuelle negative påvirkninger som triploidi kan ha på fiskens helse og velferd.

Metoder

En prosjektgruppe bestående av medlemmer med kompetanse innen laksefiskbiologi, akvakultursystemer, veterinærmedisin, fiskehelse og velferd, virologi, bakteriologi, parasitologi, avl og genetikk har utarbeidet kunnskapsoppsummeringen. Forfatterne bak denne rapporten valgte å slå sammen dyrehelse og dyrevelferd til et felles begrep for å tydelig kunne svare på spørsmålene fra Mattilsynet. To eksterne eksperter har gjennomgått og gitt innspill før oppsummeringen ble vurdert og godkjent av VKMs faggruppe for dyrehelse og velferd.

Litteraturen som ble brukt i arbeidet var fagfellevurderte studier hentet fra et søk i fire databaser, samt ikke-fagfellevurderte rapporter. Utvalget av studier var basert på forhåndsdefinerte inklusjons- og eksklusjonskriterier.

Konklusjoner

I kommersielt oppdrett har man sett at triploid laks ofte har dårligere helse og velferd enn diploid laks. Eksperimentelle forsøk og feltobservasjoner har vist at triploid laks er mer utsatt for misdannelser i skjelett og hjerte og for katarakt (grå stær), og undersøkelser fra kommersielt oppdrett tyder på at triploid laks tåler håndtering dårligere og er mer utsatt for hudsår enn hva diploid laks er. Forskning har imidlertid indikert at noen av effektene av triploidi kan begrenses gjennom spesialiserte dietter eller miljøtilpasninger.

Både i felt- og eksperimentelle forsøk er det vist en tydelig tendens til at triploid laks har samme størrelse eller er større enn diploid laks ved slutten av ferskvannsfasen, mens den er like stor eller mindre enn diploid laks ved slutten av sjøvannsfasen.

De fleste vitenskapelige studier konkluderer med at oksygenforbruk, oksygenbindingskapasitet og aerob svømmekapasitet ikke er signifikant forskjellig mellom triploid og diploid laks i temperaturområdet som er optimalt for diploid laks. Basert på funn fra eksperimentelle forsøk har triploid laks derimot en tendens til å ha en lavere optimal temperatur. I tillegg indikerer data som er konsistente fra flere studier, at triploid laks har lavere toleranse for hypoksi, det vil si mangel på oksygen i blodet, hvis vanntemperaturen er høy. Triploid laks er mindre robust overfor høyere vanntemperaturer enn diploid laks, og har andre ernæringsbehov enn diploid laks, spesielt når det gjelder fosfor og histidin.

Det er gjort få studier av triploid laks mottagelighet for smittsomme agens og -sykdommer. Observasjoner under kommersielle oppdrettsforhold indikerer at oppdrettsanlegg med triploid laks er mer utsatt for primære utbrudd av infeksiøs lakseanemi (ILA) enn anlegg med bare diploid laks, og i oppdrettsanlegg som har et pågående ILA-utbrudd er merder med triploid laks mer utsatt for infeksjon med ILA-virus enn merder med bare diploid laks. Dette kan påvirke helse og velferd til triploid fisk ved et utbrudd, og infeksjonen kan potensielt spre seg til andre oppdrettsanlegg. Feltdata tyder også på at triploid laks er mer utsatt for sårinfeksjon med bakterien *Moritella viscosa* enn hva diploid laks er. Det er gjort svært få eksperimentelle forsøk med eksponering av triploid laks for ILA-virus og *M. viscosa,* og resultatene fra disse forsøkene er usikre.

Triploid laks har andre krav til kosthold og vanntemperatur, er mer følsom for stress som for eksempel håndtering, og er mer følsom for hypoksi ved høy temperatur enn hva diploid laks er. Triploid laks krever derfor andre oppdrettsbetingelser enn diploid laks. Forskning har vist at flere av forskjellene i helse og velferd mellom triploid og diploid laks kan begrenses dersom forholdene tilrettelegges for triploid laks. Enkelte av de nødvendige justeringene er imidlertid ikke mulig å gjennomføre under dagens kommersielle oppdrettsforhold.

Flere andre helse- og velferdsparametere er evaluert i rapporten.

Usikkerheter og datahull

Siden flere av funnene fra feltstudier og eksperimentelle studier ikke er konsistente, og ettersom det er få godt utformede studier av ulike helse- og velferdsaspekter hos triploid versus diploid laks, er noen av konklusjonene i kunnskapsoppsummeringen beheftet med usikkerhet. Funn som er gjort under dagens oppdrettsforhold er imidlertid mer konsistente.

Det er datahull i forståelsen av faktorer som effekten av tidlige miljøforhold, temperaturtoleranse, samspill mellom ploiditet og genotype og andre genetiske parametre, mottakelighet for sykdommer, stressresponser, bruk av mer skånsomme teknikker ved produksjon av triploid laks, hvilken påvirkning cellenes størrelse hos triploid laks har på fiskens fysiologi, og protokoller brukt for smoltifisering av fisken og tidspunkt for overføring av triploid laks til sjø.

Stikkord: laks, triploid, misdannelser, oppdrettsforhold, helse og velferd, avbøtende tiltak, stressmestring, mottakelighet for sykdom, temperaturtoleranse

Abbreviations and glossary

Abbreviations

AGD – Amoebic gill disease

DNA – deoxyribonucleic acid

dph – days post hatching

GMO – Genetically modified organism

IMR – The Institute of Marine Research

ISA – Infectious salmon anaemia

ISAV – Infectious salmon anaemia virus

mRNA – messenger ribonucleic acid

NFSA – The Norwegian Food Safety Authority

RAS – Recirculating aquaculture system

R&D-licences – Research and development licenses

SAV – Salmonid alphavirus

WOAH – World Organization for Animal Health

Glossary

Aerobic scope – The difference between the maximum aerobic metabolic rate and standard maintenance levels.

Fish health – Absence of disease or normal functioning and behaviour of the fish. See Fish health and welfare and chapter 2 "Fish health and welfare" for more information.

Fish welfare – Experiences, perceptions, and quality of life as perceived by the fish itself. See chapter 2 "Fish health and welfare" for more information.

Fish health and welfare – In this report, we have addressed fish health and welfare as a unified concept as these are highly intertwined.

Atlantic salmon family – In the context of a salmon breeding program and genetic studies, a family can include full siblings and (or) half siblings (one parent in common). Testing a large number of families of sufficient group size (number of siblings), allows for distinguishing between and estimating variation of additive genetic effects and variation of common environment and non-additive genetic effects.

Category F national diseases – National notifiable diseases which pose a serious threat to aquatic animals in Norway.

Dead end gene – Gene essential for the development of primordial germ cells.

Diploid – The natural ploidy state of an individual with two sets of chromosomes.

Experimental trials/studies – In general, this refers to studies conducted in a controlled environment, either in indoor tanks or outdoor cages in a research facility, in which external parameters can be manipulated. The sample size in these trials is usually smaller than studies conducted in sea cages under "commercial settings".

Field trials/studies – In general, these types of studies include large numbers of fish in realistic settings but lack the strict controls necessary for experimental studies. Some of the data may also be subjective in nature, and conclusions may be made without statistical analysis.

Green aquaculture licenses – Licenses aimed to reduce the negative environmental impact of salmon farming.

Handling and operational procedures – Handling includes all handling of fish (e.g. sorting, netting, vaccination, crowding, pumping). Operational procedures mean how fish is being farmed and does not necessarily involve handling. Examples are feeding protocols and water quality settings.

Monoamines – Neurotransmitters involved in the regulation of cognitive processes.

Performance – A measurement of productivity in aquaculture including survival rate, growth rate, and feed utilisation efficiency in the fish.

Polyploidy – Chromosome set manipulation/ an individual with more than two complete chromosome sets.

Primary outbreak – An outbreak where the source of the virus is unknown.

Secondary outbreak – An outbreak where the chain of transmission is known.

Second polar body – The maternal chromosome set that is normally ejected from the fertilised egg.

Smoltification – The process of physiological and behavioural changes where juvenile salmon adapts from living in freshwater to marine habitat.

Telencephalon – The cerebral hemispheres of the central nervous system (CNS).

Tetraploid – An individual with four complete chromosome sets.

Triploid – A sterile individual with three complete chromosome sets.

Welfare indicator – Observations or measurements that provide information about the extent to which the animal's welfare needs are met. Welfare Indicators can be animal based – observations made on or from the animal, or environment based – observation made on the environment. Animal based may be information about behaviour, feeding, growth, health, morphology, etc. used to assess the welfare of the fish.

Background as provided by the Norwegian Food Safety Authority

The use of sterile Atlantic salmon (*Salmo salar*) has been regarded as a potential risk-reducing measure in addressing environmental consequences associated with escapees from salmon farms, since sterile fish cannot interbreed and thereby influence the genetic integrity of wild populations. One approach to achieve sterility is through induced triploidisation, resulting in the eggs containing three sets of chromosomes instead of two (Stien et al., 2019).

The welfare and health consequences of production with triploid salmon need to be documented in accordance with the Norwegian Animal Welfare Act. Furthermore, this type of production method must protect the welfare of triploid salmon. The documentation needs to demonstrate that the production method promotes characteristics which yield robust animals which are acceptably functional and healthy.

Current Norwegian legislation

The purpose of the Animal Welfare Act is "to promote good animal welfare and respect for animals" (§1). The legislation applies to cultured fish in the same way as it does for terrestrial farmed animals (according to §2). It states that "Animals have an intrinsic value which is irrespective of the usable value they may have for man. Animals shall be treated well and be protected from danger of unnecessary strains and stress" (§3).

The Animal Welfare Act §24 further states that "The animal keeper shall ensure that the animal receives good supervision and care, including securing that: b) the animals are protected from injury, disease, parasites, and other dangers. Sick and injured animals shall be given appropriate treatment and be killed if necessary and c) spreading of infectious disease is limited".

The Norwegian Food Law also includes provisions for the promotion of acceptable animal health.

The Animal Welfare Act §8 states that "…industrial methods, equipment and technical solutions which are used for animals shall ensure that they are tested and found to be suitable, taking into account animal welfare."

The Aquaculture Operation Regulations §20 furthermore requires that methods, installations, and equipment may only be used in an aquaculture facility when the consequences for the welfare of fish is documented.

According to the Animal Welfare Act §25 "Breeding shall encourage characteristics which give robust animals which function well and have good health. Reproduction, including through methods of gene technology, shall not be carried out in such a way that it: a) changes genes in such a way that they influence the animals' physical or mental functions in a negative way, or passes on such genes, b) reduces the animals' ability to practise natural behaviour or c) cause general ethical reactions".

Paragraph §51, concerning breeding and reproduction, in Aquaculture Operation Regulations, states the following: Breeding programs shall focus on the production of healthy and robust fish. Domestication of fish shall be emphasised. No fish shall be kept under farming conditions unless the genotype or phenotype indicates that it is possible to ensure acceptable welfare and health. Natural or artificial fertilisation procedures that cause, or likely will cause, harm or unnecessary stress, shall not be used.

The Norwegian Food Safety Authority (NFSA) released updated guidelines for fish welfare and the development and use of methods, equipment, and technology in aquaculture in 2020 (NFSA, 2020).

Trials and documentation – triploidisation

The method of producing triploid salmonids is known from the past production of rainbow trout (*Oncorhynchus mykiss*). The Institute of Marine Research (IMR) conducted large scale trials on Atlantic salmon for many years, under the supervision of "SalmoTrip" between 2007-2012 (Stien et al., 2019[1]). The project's major findings were that triploid salmon have specific nutritional requirements that are needed to avoid the development of cataracts and skeletal deformities. Other findings were that mortality was elevated in triploid salmon throughout its entire life cycle, growth was impaired, and tolerance for stress and unfavourable environmental conditions was lower (Stien et al., 2019)[2].

From 2013, five aquaculture research and development (R&D)-licences were authorized for triploid salmon production. In the following year, eleven "green aquaculture licences" were authorised solely to produce sterile (limited to triploidisation during this time frame) salmon. The objective of the green licenses is to stimulate the development of novel technologies or production regimes that reduce the environmental impact of aquaculture. Both types of licences allowed for trials within commercial settings in Norway from 2013 onwards. These licences, which state the conditions for sterile (triploid) salmon, were given before the welfare consequences of triploid salmon production under commercial settings were sufficiently documented (for more information, see the assessment in the letter from the IMR on 7 July 2014) (Hansen et al., 2012).

As documentation concerning the consequences of triploid salmon production was considered insufficient, production was conducted in the form of trials, in order to generate documentation about the consequences on fish welfare in a full-scale commercial setting. IMR monitored the trials scientifically, whereas the NFSA was responsible for supervision.

Results from the subsequent investigations conducted by IMR indicate challenges related to fish health and welfare from the production of triploid salmon (Madaro et al., 2022; Stien et al., 2021a; Stien et al., 2021b; Stien et al., 2019). This coincides with experiences made through inspections by the NFSA, suggesting that triploid salmon cope less well with handling and are more prone to develop skin lesions and diseases than diploid salmon. The project, comprising trials and documentation, was initially expected to be finalized within a few years. However, the timeframe was gradually extended, to address the challenges for

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[1] Correction: Citation should be Hansen et al., 2012)

[2] Correction: In addition, it was found that triploids could show good growth and low mortality in seawater, although farmers should avoid producing them in areas with high temperatures and/or low oxygen where they seemed to struggle more than regular diploids (Hansen et al., 2012).

fish welfare by optimising operational conditions. In Norway, 30-35 million triploid salmon have been transferred to seawater since 2013, as part of the trials and documentation of producing triploid salmon under commercial settings. Currently, the final deadline for the project is set to 31 December 2023.

According to Norwegian regulations pertaining to animal health, welfare, and the environment, aquaculture production shall be performed within a responsible framework. Experiences from inspections by the NFSA and reports about triploid salmon production from the IMR illustrate the need for reviewing and summarising knowledge about this type of production method.

On this basis, the NFSA requests VKM to summarise knowledge about animal health and welfare consequences when farming triploid salmon. In addition, the NFSA asks VKM to describe potential risk-reducing measures, and to assess to what extent such actions may prevent or reduce negative effects on animal health and welfare.

In this assignment, knowledge about diploid salmon production will be used as a basis of comparison for the conditions triploid salmon will be exposed to.

As trials on triploid salmon production through green aquaculture licences are still ongoing until the end of 2023, it may be relevant for the NFSA to request an updated knowledge status of the topic as soon as results from the final trials are available.

The report by VKM will be used as a basis to assess whether production of triploid salmon is in accordance with the framework of the Animal Welfare Act and Norwegian Food Law.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority request VKM to:

1. Summarise knowledge about animal health and welfare in triploid salmon (*Salmo salar*) under commercial farming conditions, as compared with traditional diploid salmon in aquaculture, in various stages of salmon production, from hatchery to slaughter. This includes:
    - Robustness
    - Biological functionality
    - Mortality
    - Susceptibility to disease
    - Potential to transmit infections and disease

2. Describe the underlying physiological mechanisms concerning the consequences of triploidisation, using current, available knowledge.

3. Describe possible risk-mitigating measures and assess whether such actions may prevent or lower the probability of the occurrence of negative consequences for animal health and welfare.

Methodology and Data

Data and information gathering

The project group used the scientific literature as basis for information and data collection. A literature search was performed after key topics were identified and agreed upon, through a prefilled PICO-form. Each member also gathered information from articles through specific narrow searches and/or by scanning reference lists in relevant papers (i.e. snow-balling).

Literature search and selection

A literature search was conducted by the library staff at the National Public Health Institute on February 14th in 2023. Searches were done in the data bases Web of Science, MEDLINE, CAB Abstracts, and Embase. These databases were chosen to ensure comprehensive data retrieval. There were no restrictions on language or publication date if articles were deemed within the mandate. Both the pre-filled PICO-form, search strings and number of hits for each database are specified in Appendix I.

The main searches resulted in a total of 1098 records, corrected for duplicates. Screening of titles and abstracts was performed in a pairwise blinded manner using Rayyan, a web application for systematic reviews (Ouzzani et al., 2016), set against the inclusion and exclusion criteria (as determined by the Terms of Reference). After the first round of (primary) screening, the blinding was removed, and the reviewers discussed conflicting decisions. If the two reviewers were unable to reach an agreement, the paper in question was evaluated by a third reviewer. Abstracts that did not appear to fulfil the inclusion criteria were excluded from further analysis. In cases where relevancy was unclear, titles were retained for further screening. Publications that passed the primary screening were retained for a second screening and assessed for relevance and quality. The primary screening resulted in 309 titles, of which 77 passed the secondary screening (Figure A). Articles that fulfilled the inclusion criteria during the second screening were included in the opinion. To ensure that articles were not overlooked by the primary screenings, manual more targeted searches for papers or grey literature were also performed for relevancy by all members. This resulted in 66 articles that were also included. In total, 143 articles were cited in the opinion.

Inclusion criteria:

- Original articles, review papers, editorial chapters and meeting abstracts that address triploidy, both health and welfare aspects, in cultured salmonids. Other fish species in aquaculture were also included, if applicable within the mandate.
- Only publications in English or Norwegian were included, without any restrictions on date of publication.

Exclusion criteria:

- Original articles, review papers, editorial chapters and meeting abstracts that address naturally occurring triploidy in wild populations, effectiveness of triploidy induction or triploidy in aquaculture organisms other than fish.

- Publications that address ethical or socio-economical aspects of using triploids in aquaculture.

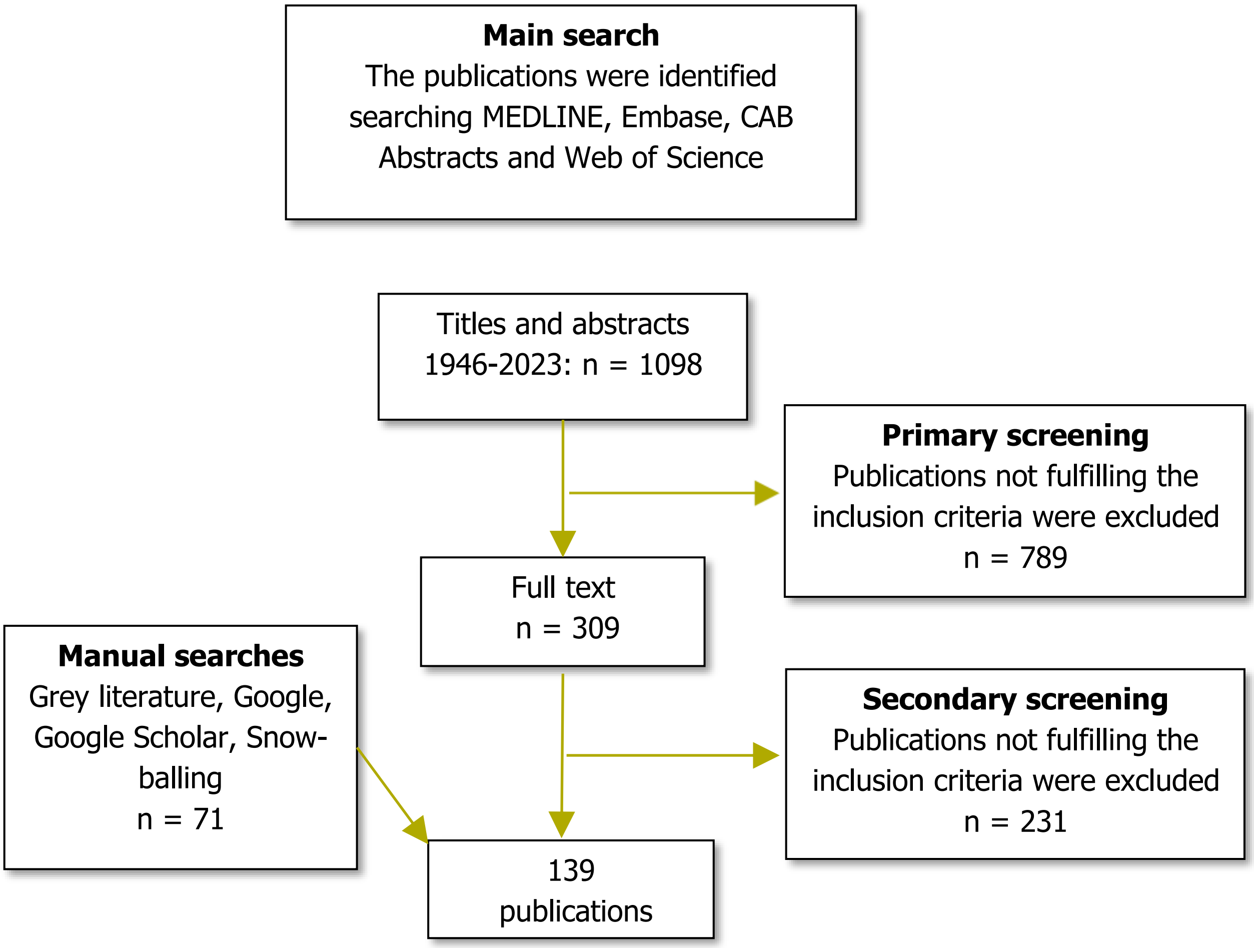

Figure A. Flow chart for the literature search and screening for cultured triploid salmonids.

1 Introduction

Norwegian rivers are home to the world's largest populations of wild Atlantic salmon (*Salmo salar*). However, the estimated number of returning adults has decreased significantly over the last 40 years from around 1.1 million individuals in the early 1980's to less than 500 000 in 2021 (Thorstad et al., 2021). Interbreeding between farm escapees and wild salmon is considered one of the primary threats to the wild salmon populations in Norway due to genetic introgression and resulting declined fitness (Thorstad et al., 2021; Skaala et al., 2019; Solberg et al., 2023; Solberg et al., 2020).

Norway is the world's largest producer of farmed Atlantic salmon (FAO, 2023). The industry uses salmon that have been genetically selected over 50 years (≥ 12 generations) to perform well in aquaculture. These selected strains grow much faster than wild salmon (Besnier et al., 2022; Bolstad et al., 2021), but escapees show poorer survival in a natural setting (Solberg et al., 2020; Wacker et al., 2021). Despite their relatively poor performance in the wild, farm escapees can be found on spawning grounds. Although the number of farm escapees has decreased significantly in Norway, due to different preventive actions since records began, there may still be large numbers of escaped salmon in some rivers. Since 1998, a total of 6.8 million farmed Atlantic salmon are known to have escaped, with over 1 million in the last decade alone (Figure 1-1). In 2020, 66.5% of Norwegian rivers surveyed (*n* = 239) showed genetic mixing between wild and farmed salmon (Figure 1-2) (Diserud et al., 2020). Farmed/wild hybrids have inferior survival and fitness to purebred wild salmon in the natural environment. Hence, natural selection will reduce these hybrids chances of successful breeding and viable offspring, but they can still use valuable resources that would otherwise be available to wild salmon (Glover et al., 2017).

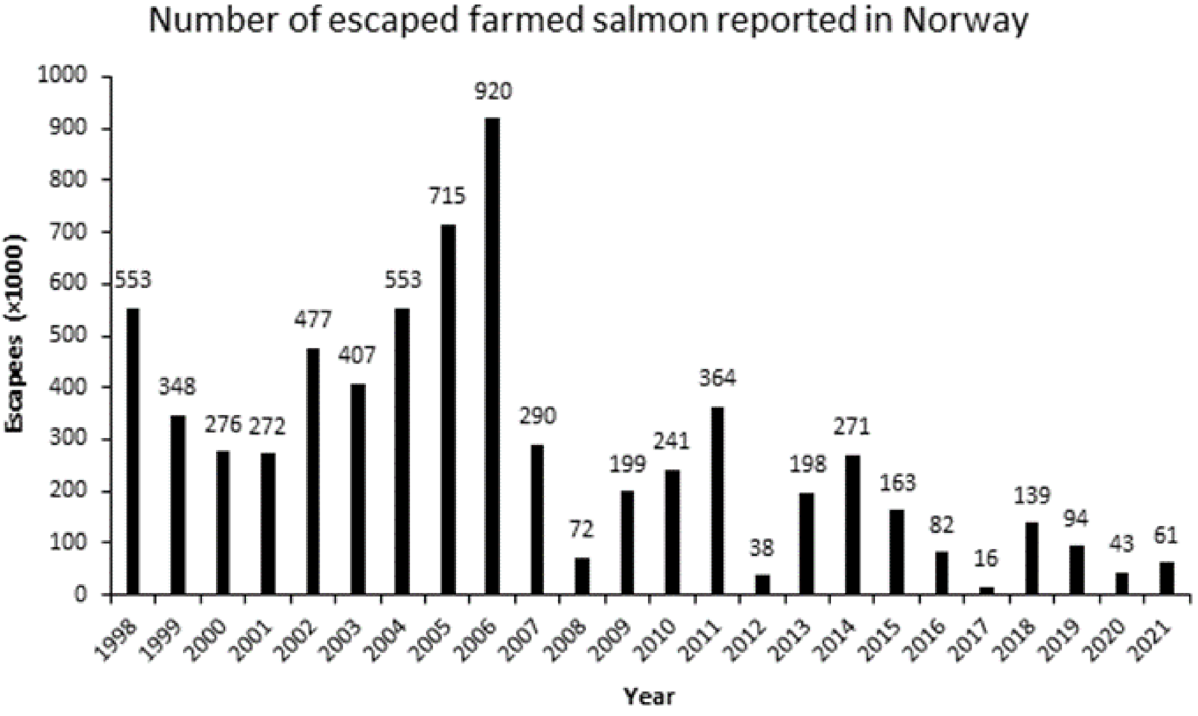

Figure 1-1. Reported number of escapees from Norwegian salmon farms. The data is provided by the Directorate of Fisheries (accessed 2023).

There are two main strategies for preventing genetic interactions between farm escapees and wild salmon. The first is to stop domesticated strains from escaping in the first place. Although management practices have succeeded in this to some extent, large escape events still occur. For example, 61 000 farmed salmon were reported to have escaped in Norway in 2021 (Figure 1-1). The second option is to farm reproductively sterile fish. This ensures that if farmed fish do escape, they would not be able to breed and impact wild salmon genetically.

There are numerous methods for producing sterile fish. These include surgery, irradiation, hormonal treatments, vaccination, interspecific hybridisation, chromosome set manipulation (polyploidy), transgenics, gene-editing, or cell ablation technology, which either target primordial germ cells, interfere with gonad development, or directly remove the gonads (reviewed in Benfey (2016); Tveiten et al. (2022), and Wong and Zohar (2015)). Of all these, only triploidisation, a form of chromosome set manipulation, has so far been considered feasible for the large-scale production of reproductively sterile Atlantic salmon (Benfey, 2016). This method also comes with the bonus that each subject can be assessed for their ploidy status using tissue biopsies, without the need for terminal sampling (Benfey and Sutterlin, 1984; Jacq, 2021). Triploids are not classified as being genetically modified organisms (GMOs) in conventional farming, and so are not restricted by these regulations.

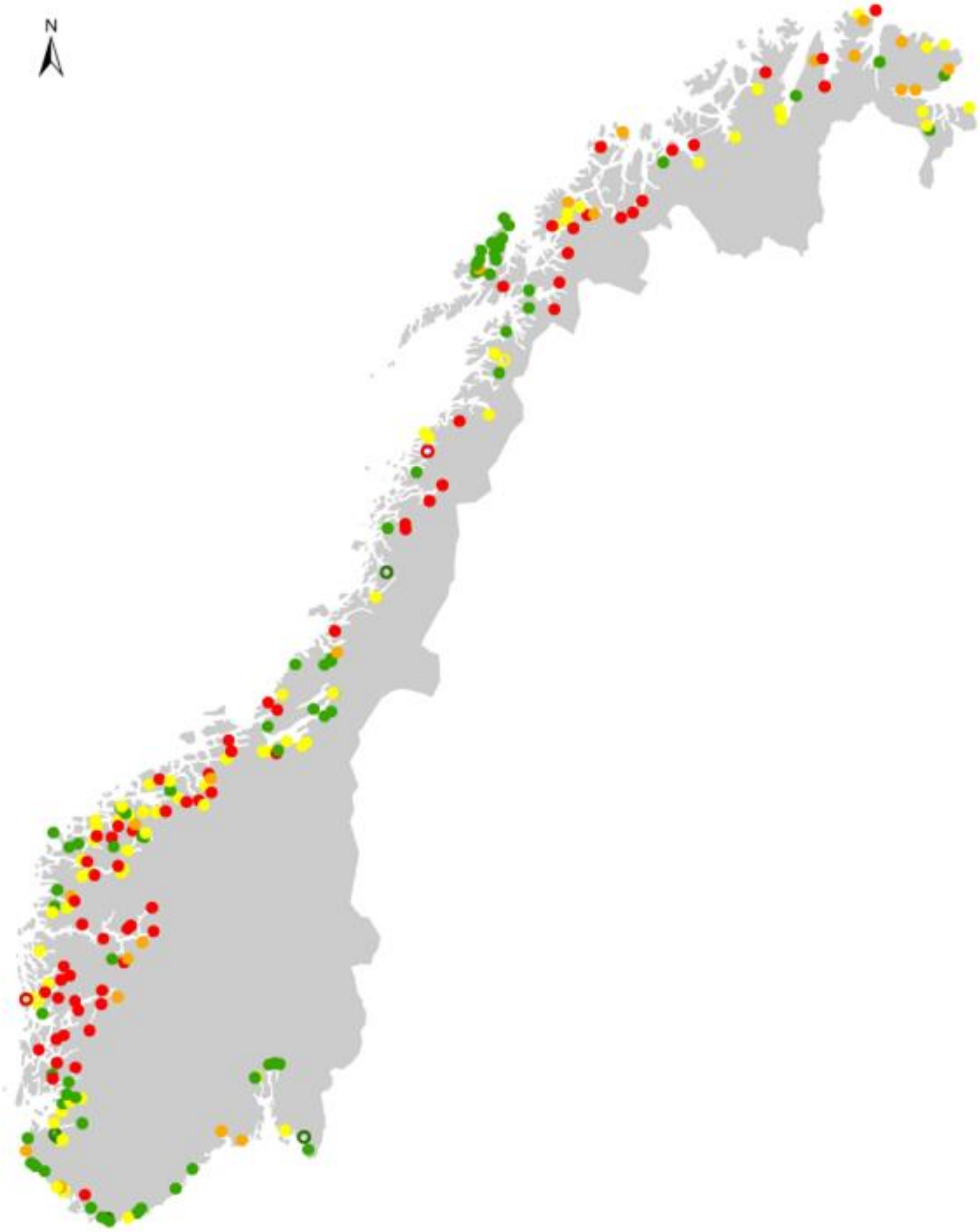

Figure 1-2. Genetic status in 239 salmon population in Norway based on a measure of genetic integrity. Eleven of the 239 populations are not defined as salmon populations, and they are shown with a coloured ring instead of a filled circle. The level of genetic introgression within each population has been determined as none (green), low (yellow), moderate (orange), or high (red). Figure adapted from Diserud et al. (2020).

Existing transgenic and gene editing technology could theoretically be scaled up to produce sterile fish. For example, *dead end* knockouts can already be mass produced (Kleppe et al., 2020; Wargelius et al., 2016) and used to make breeding lines (Güralp et al. 2020). They are regarded as GMOs in accordance with the regulations in Norway (statement from The

Norwegian Biotechnology Advisory Board, 2019) and EU and regulated as such. However, temporarily inactivating *dead end* through antisense (e.g. gene knock-down) technology has also been shown to be effective at preventing early male sexual maturation and later reproductive phenotypes in males and females (Andersen et al., 2022). Antisense technology does not come under GMO regulations as it is not a permanent change in the DNA, it only temporarily reduces the mRNA expression of the *dead end* gene during embryogenesis which prevents the development of primordial germ cells. Therefore, this technology is promising. However, there is limited data on the complete life cycle and the antisense technology currently requires each individual egg to be microinjected. Attempts at bathing the embryos in an antisense solution, as applied on zebrafish (*Danio rerio*) by Wong and Zohar (2015), and recently on rainbow trout and Atlantic salmon, resulted in high mortalities and low sterilisation success in Atlantic salmon (Andersen et al., 2022). This technique therefore requires further optimisation before successful upscaling is possible. Furthermore, individuals need to be sacrificed to assess sterility as it cannot be assessed by biopsy.

1.1 Using triploidy to produce sterile fish in Atlantic salmon aquaculture

A triploid is an individual which has three complete chromosome sets. The natural ploidy state in Atlantic salmon is diploidy, whereby an individual has two complete chromosome sets with one inherited from each parent. Theoretically, triploid Atlantic salmon can inherit their third chromosome set from either parent. Currently, the most common method used to produce them (pressure shock, see below) ensures the additional chromosome set comes from the maternal side.

Although triploidy could be described as a genetic modification, triploids are not classified as GMOs in Norwegian and EU regulations (statement from The Norwegian Biotechnology Advisory Board, 2019). The main arguments are that triploid Atlantic salmon are occasionally found in nature and that gene technology has not been applied in the production of triploidy (Jørgensen et al., 2018). Furthermore, other fish species have both natural diploid and triploid self-sustaining populations (Piferrer et al., 2009), and genome duplication has played an important role in salmon evolution. For instance, salmonids have undergone four rounds of whole genome duplications with the last occurring 80 million years ago. Subsequently, salmonids have undergone a process known as re-diploidisation (Houston and Macqueen, 2019). Simply, this implies that what we call a diploid today would have been classified as a polyploid (an individual with more than two complete chromosome sets) in the past. Nevertheless, some experts believe triploids should fall under the regulations for producing GMOs (statement from the Norwegian Biotechnology Advisory Board, 2019). Also, rearing polyploid organisms is excluded according to EU regulations of organic aquaculture (Busacca and Lembo, 2019).

1.2 Techniques to produce triploids

The most common way to produce triploids is to double up the maternal DNA (Piferrer et al., 2009). This is because the unfertilised salmon egg has both maternal sets of chromosomes. However, one maternal set is normally ejected from the fertilised egg in a structure known as the second polar body. In contrast, salmon sperm is haploid, containing only one of the paternal chromosome sets. As such, each individual salmon is technically triploid with two maternal chromosome sets immediately following fertilisation, but shortly after one of the maternal chromosome sets is ejected, resulting in a return to diploidy.

There are several methods available for preventing the removal of the second polar body from the fertilised egg. The most widely used are chemical, temperature, or pressure shock (reviewed in Piferrer et al., 2009). For commercial purposes, pressure shock is used, as it has been found to be highly efficient and can be done to large numbers of eggs simultaneously. If done correctly with high-quality eggs, it is possible to achieve a 100% triploidisation success rate in Atlantic salmon (Benfey, 2016).

Although not yet currently available in Atlantic salmon, an alternative method for producing triploids is to cross a tetraploid (with four chromosome sets) with a diploid. Tetraploidy has been achieved in several salmonid species including rainbow trout (Chourrout et al., 1986) and brook trout (*Salvelinus fontinalis*) (Weber et al., 2015). The advantage of this method is that the eggs do not need to be shocked after fertilisation. There is some suspicion that the pressure shock currently used to induce triploidy could have long-term negative consequences on the individual, although this has been poorly studied (Fraser et al., 2012b).

In Norway, about 30-35 million triploid salmon have been transferred to seawater since 2013 under commercial settings. There are several reports that indicate challenges related to fish health and welfare from this production (Stien et al., 2021a, b; Stien et al., 2019).

2 Fish health and welfare - "robustness"

This chapter elaborates on aspects related to fish health and welfare and aims at summarising knowledge about fish health and welfare in triploid Atlantic salmon (*Salmo salar*) as compared with traditional diploid salmon in aquaculture, in various stages of salmon production, from hatchery to slaughter. Literature referred to in this section and experience amongst farmers and fish health personnel strongly indicates differences between triploid and diploid salmon regarding welfare, where triploids are more sensitive to farming operations and environment. The lower tolerance towards higher rearing temperatures will affect the fish throughout their entire lifecycle. Fish that have reduced welfare may be less robust, however, this is a broad term and can interpreted in various ways. Robustness also includes other keywords from the Terms of Reference; biological functionality, mortality, susceptibility to disease, potential to transmit infections, and disease. Basically, robustness relates to an animal's ability to cope with environmental challenges and thus determining its tolerance, or adaptability. We have chosen this approach to address the Terms of Reference.

The concept of animal welfare includes physical, physiological, and mental states of individual animals. A range of indicators should be considered to provide high quality evaluation of welfare. Also, indicators of fish welfare should be species-specific, life stage-specific, validated, reliable, and feasible (EFSA, 2009).

The Norwegian Food Safety Authority's provided Terms of Reference, Question 1 for this risk assessment asks for: "Summarise knowledge about animal health and welfare in triploid salmon (*Salmo salar*) under commercial farming conditions". In this context, as the concepts of health and welfare are related, both have been combined into a singular entity, encompassed by the term "welfare".

Breeding and welfare of animals are regulated by the Norwegian Animal Welfare Act §25: "Breeding shall encourage characteristics which give robust animals which function well and have good health. Reproduction, including through methods of gene technology, shall not be carried out in such a way that it: a) changes genes in such a way that they influence the animals' physical or mental functions in a negative way, or passes on such genes, b) reduces the animals' ability to practise natural behaviour or c) cause general ethical reactions. Animals with a genetic constitution as cited in the second article shall not be used for subsequent breeding. The King may issue specific regulations regarding breeding of animals in conflict with the principles in this Section" (Animal Welfare Act. - regjeringen.no).

Animal welfare relates to the experiences, perceptions, and quality of life as perceived by the animal itself (Broom, 2022; Noble et al., 2018). As it is difficult to assess the true fish welfare, it is commonplace to use various practical welfare indicators to describe the state of the fish. These indicators will vary depending on the scientific focus and will never completely cover the welfare issue, but will provide valuable information to the researcher and reader. In commercial farming, the use of operational welfare indicators (OWI) is commonly used since they do not require complex analytical methods. Examples of OWIs are behaviour, feeding, growth, morphology, etc. (Noble et al., 2012). Also, the use of real-time data as opposite to historical data is often preferred as real-time data give information about fish welfare in real-time. The appropriate selection of welfare indicators depends on life stage, and what the fish are exposed to, e.g. production system and handling procedures. Therefore, in the current report addressing triploidy, the following indicators have been looked at in association with triploidy to assess differences in welfare between triploid and diploid Atlantic salmon: deformities, cataract, skin conditions, tolerance to handling, stress coping, and behaviour.

However, it is not within the scope of this assessment to provide an opinion on what qualifies as favourable or unfavourable welfare conditions other than triploidy for farmed Atlantic salmon in general.

2.1 The consequences of triploidy on health and welfare

2.1.1 Deformities

A variety of deformities have been reported in triploid fish, with those of the spine and lower jaw being generally consistent across studies. A few studies have also reported issues around shortened opercula and deformities in the gills and heart, but they are less consistently observed (Fjelldal and Hansen, 2010; Leclercq et al., 2011; Sadler et al., 2001; Taylor et al., 2013). As for gill deformities, these are expressed as the absence of primary gill filament (gill filament deformity syndrome) (Sadler et al., 2001). Amoroso et al. (2016) showed in one experiment with salmon at a size of 0.2-8 grams that ploidy had no effect on skeletal anomaly prevalence. In the same study, however, in a second experiment at a size of 8-60 grams, triploid fish had significantly more lower jaw deformities compared with diploids. In rainbow trout, the percentage of skeletal deformities in heat-shocked triploid larvae was significantly higher than that of the diploids (Benfey, 1996).

Fjelldal and Hansen (2010) showed that there was a significantly higher prevalence of triploid individuals classified with spinal deformities during palpation, and of individuals with one or more deformed vertebrae on radiographs compared with diploids. Also, Leclercq et al. (2011) showed that the incidence of external deformities, dominated by jaw malformation, was 12% in triploids while below 5% in diploids.

Increasing egg incubation temperatures higher than 6 °C (usually 8 °C is the maximum used by the industry) for triploid fish increased the number of fish showing one or more deformed vertebrae. Triploids also had an increased prevalence of lower jaw deformities with increased incubation temperature, whereas they were rare in diploids (Fraser et al., 2015a).

Under commercial conditions, total discards and downgrading were increased by triploidy due to deformities and triploids also had more discards categorized as "small fish", compared with diploids (Fraser et al., 2013). Radiology confirmed a higher proportion of triploids with one or more deformed vertebrae. In a long-term study by Taylor et al. (2013), triploids showed no visual deformity in freshwater but a significantly increased prevalence in seawater, mainly evident as jaw malformations and radiologically observed deformations of vertebrae. However, the severity of deformities was considerably lower than in previous studies.

High incubation temperature may increase the prevalence of defects or missing septum transversum of the heart, a diaphragm which separates the heart from the abdominal cavity, in triploids compared with diploids (Fraser et al., 2014a). Triploids showed an increasing prevalence from 6 to 10 °C, while it only occurred in diploids incubated at 10 °C and to a lower extent than in triploids (Fraser et al., 2014a). Large triploid salmon reared in sea cages had heavier hearts and less fat deposition in the epicardium than in diploid salmon (Fraser et al., 2015b; Fraser et al., 2013). Other trials have not shown such differences, but there are reports of altered heart morphology with a lower angle of the bulbus arteriosus in triploids compared to diploids (Fraser et al., 2013; Leclercq et al., 2011). The functional significance of these observations remains unclear, but in rainbow trout, triploids demonstrate an earlier onset of cardiac arrhythmia in response to increasing temperature, compared to diploids (Verhille et al., 2013).

Apart from the above-mentioned deformities, gut abnormalities have also been reported. In a study by (Peruzzi et al., 2015), the intestinal length, pyloric caeca number, and mass were significantly smaller in triploid versus diploid post-smolt salmon (132-135 grams), suggesting that morphological differences in the guts of diploids and triploids could influence digestive efficiency and play a role in determining subsequent growth of the fish. Larger triploids of around 3 kg were also found to have a relatively smaller gut mass (the combined weight of the intestines, liver, and adipose tissue) compared with diploid counterparts (Fraser et al., 2022).

Skeletal deformities and aplasia of the septum transversum in triploids are associated with either nutritional deficiency and/or inappropriate rearing temperatures. This is further discussed in detail in the chapter "Risk mitigation measures".

2.1.2 Cataract

Cataract is a well-documented disorder in triploid fish causing less functional eye lenses and vision. Triploid Atlantic salmon post-smolts are more prone to cataracts than diploids (Leclercq et al., 2011; Taylor et al., 2015; Wall and Richards, 1992). Leclercq et al. (2011) showed in a study that the most significant detrimental effect of triploidy was on the rate and severity of cataract (50% and 92% of diploids and triploids respectively) that appeared after 1-year in seawater. Also, Fonseka et al. (2022) found that triploids had a significantly higher prevalence of ocular cataracts (84 versus 98% in diploids and triploids, respectively) with a higher mean cataract score for triploids compared with diploids. Higher cataract scores among triploid salmon were also found by Sambraus et al. (2017a), where a severe cataract outbreak was recorded in freshwater in both ploidies reared at 16 °C. The cataract development at 10 °C in freshwater was on the contrary minor, while both diploids and triploids reared at 10 °C developed cataracts during the seawater period, with a higher severity score in triploids than diploids.

Cataract in triploids may be linked to nutritional deficiency of histidine. This is further discussed in detail in the chapter "Risk mitigation measures".

2.1.3 Skin conditions

There are some farm reports of increased susceptibility to skin ulcers in triploid salmon. Stien et al. (2019) showed that for the sea phase, triploid salmon in many cases developed ulcers during winter with subsequent high mortalities. In the report series from the Institute of Marine Research (IMR), where commercial farming of triploid salmon has been monitored over several years, the researchers generally observe more skin ulcers, especially on the snout, in triploid compared to diploid fish (Stien et al., 2021a).

2.1.4 Stress coping

There are reports of poor performance and welfare in triploid fish that include lower jaw deformities, increased mortality, poor growth, and reduced tolerance to chronic stress (Benfey, 1996). Also, Fraser et al. (2015c) studied the monoamine activity in diploid and triploid salmon. They found that both diploids and triploids showed an increase in serotonergic activity following stress, but the increase was significantly greater in the telencephalon of triploids compared with diploids. While telencephalic dopaminergic activity was significantly increased in diploids, there was no response in triploids following stress (Fraser et al., 2015c). These results suggest that triploids experience increased reactivity and monoaminergic dysregulation following stress that may impair fish welfare.

Results from experiments with rainbow and brook trout showed that resting blood cell (erythrocytes and leukocytes) concentrations were significantly lower in triploids than in diploids, but in other respects, triploids showed no significant differences from diploids prior to or after induced acute stress (Benfey and Biron, 2000).

Many publications report no differences in stress coping between triploid and diploid fish. Sadler et al. (2000a) compared the stress response after confinement stress in diploid and triploid salmon during the freshwater parr stage and four weeks after transfer to seawater and found no differences between diploid and triploid fish with regard to lactate and cortisol. Little or no effects of ploidity were observed in the recovery time from anaesthesia in salmon post smolts (Fraser et al., 2014c). In a study examining the responses to acute stressors, such as temporary hypoxia and netting, Cnaani et al. (2014) found little differences in blood parameters (i.e. cortisol, pH, pO_2, pCO_2, osmolality) between triploids and diploids. Chalmers et al. (2018) reported that triploid salmon did not differ from diploids in response to chemical treatment with H_2O_2.

Sadler et al. (2000b) also exposed salmon smolts to confinement stress and found similar stress responses between diploid and triploid smolts. The triploids displayed higher mean cell haemoglobin (MCH) simply because triploid red blood cells are larger, but have lower haemoglobin (Hb) concentrations compared with diploid smolts. The blood oxygen affinity was similar and the isohaemoglobin components were identical. It was concluded that despite having fewer, larger erythrocytes, triploids have very similar oxygen carrying capacity and haematological response to stress as diploids (Sadler et al., 2000b), however, there are some contradictory findings (Verhille et al., 2012; Graham et al., 1985). Finally, transported diploid and triploid rainbow trout expressed similar stress response, as levels of plasma cortisol, glucose, cellular hepatic glutathione (GSH), and heat shock protein 70 (Hsp70) were similar between ploidy groups, indicating that triploid fish respond to transportation in much the same way as diploid fish (Leggatt et al., 2006).

2.1.5 Tolerance to handling

There are some indications that triploid salmon show less tolerance towards some types of handling but not in others, compared with diploid salmon although other results referred above, showed similar responses to transportation. In the above-mentioned report series from IMR, they found that farmers and fish health personnel observed that handling and other disturbances result in panic reactions in triploid fish that are not observed in diploids. They also report more injuries and ulcers related to handling procedures in triploid fish cages. Ulcers may lead to bacterial infections and mortality (Stien et al., 2021a). Field studies suggest that triploid salmon are more vulnerable to handling than diploid salmon, although this data has never been quantified (Stien et al., 2021). These observations were supported by Madaro et al. (2022), who found that most of the triploid mortalities under commercial conditions seems to be correlated to delousing treatments, possibly indicating a lower tolerance to handling and stress.

2.1.6 Behaviour

Behavioural traits, like boldness in an open field and swimming activity in response to a stressor, e.g. changing lights, have been observed to be similar between triploid and diploid salmon kept at constantly low temperatures (Benhaim et al., 2020). Studies from other salmonids suggest that triploids are less aggressive than diploid fish. Triploid chinook salmon (*Oncorhynchus tshawytscha*) were significantly less aggressive during feeding than diploid fish. However, at the end of the trial, plasma cortisol levels were similar between the groups (Garner et al., 2008). Interestingly, the same authors documented decreased levels of cortisol in triploid fish in a subsequent trial while the growth rate remained the same for diploid and triploid fish (Garner et al., 2008).

Triploid brown trout (*Salmo trutta*) have also been reported to be less aggressive and display subordinate behaviour compared to diploids (Preston et al., 2014). The authors observed that pairs of diploid trout were more aggressive than triploid pairs. Regarding feeding behaviour, surface feeding was the same between ploidy groups, but triploids adopted a non-aggressive feeding strategy while diploids used more time defending territory. Even though aggression was lower in triploid compared to diploid pairs, a dominance hierarchy was also observed between individuals of the same ploidy as dominant triploid fish were more aggressive and consumed more feed items than subordinate triploid individuals. Dominant triploid fish, however, appeared to be more tolerant to subordinate individuals and did not display the same degree of aggression as seen in the diploid matchings.

Fraser et al. (2012a) found a significant effect of ploidy on the volume of the olfactory bulb, with it being 9% larger in diploids compared with triploids. The cerebellum and telencephalon, however, were significantly larger in triploids compared with diploids. Whether these differences are related to the behavioural differences described above, remain unknown.

2.1.7 Other health and welfare consequences

The previously mentioned Madaro et al. (2022), who studied diploid and triploid groups of the same genetic lineage farmed in western, mid, and northern Norway from seawater transfer until slaughter found a higher incidence of emaciated fish (approximately 5-10% more in triploid fish) and scored significantly lower quality rating (less superior fish and more production loss) during primary processing. The same result was found in an earlier experimental trial of 20 000 triploids grown from incubation until harvest in western Norway under identical conditions to diploid counterparts (Fraser et al., 2013). Farm data also suggests triploids generally have a lower quality rating than diploids when transferred to sea in the spring, but not in those transferred to sea in the autumn (Stien et al., 2023).

2.2 The consequences of triploidy on physiology, and susceptibility to disease

Triploidy increases the DNA content of every cell by 50% compared with the diploid counterparts. This leads to a 50% increase in the size of the cell nucleus. As triploid cells maintain the same nuclear to cytoplasmic ratio as diploids, this results in larger size of triploid cells (Small and Benfey, 1987). This is most obvious in cells with large nuclei such as red blood cells, which are generally 50% larger in triploids, but less obvious in cell types that have relatively small nuclei (Small and Benfey, 1987). For instance, there is little difference in muscle cell sizes between diploids and triploids (Johnston et al., 1999). Although triploid cells are larger, body, and organ sizes in aged-matched fish are relatively similar, suggesting triploids are composed of fewer cells (Small and Benfey, 1987).

The impact of cell size on fish physiology is poorly studied in general. However, it is notable that in many ecosystems, species with larger cell sizes generally live in colder environments (Hessen et al., 2013). This suggests that they either provide an advantage at lower temperatures or that they are selected against in warmer environments. The most prominent theory explaining this phenomenon is the negative correlation between cell size and mass-specific metabolic rate at a given temperature (Maciak et al., 2011). In other words, larger cells are expected to be less suited for warmer temperatures which elevate metabolic demands in ectotherms due to the acceleration of biochemical processes.

Triploidy leads to functional sterility in both sexes, but the degree of infertility differs. Macroscopically, the triploid immature testis looks identical to that of diploids. Triploid males can be sexually mature and behave as fertile males on the spawning grounds (Fjelldal et al., 2014) and can compete with diploid males, albeit they often produce less sperm, and the sperm produced is aneuploid with abnormal numbers of chromosomes (Benfey et al., 1986). If it is used to fertilise diploid eggs, the offspring die during early development (Murray et al., 2018). In females, triploidy blocks the very early stages of ovary development. This means they remain underdeveloped and fail to produce ripe eggs (Murray et al., 2018). Triploid ovaries can be easily distinguished visually, as they remain small and grey compared with the immature diploid ovary which is larger and yellow/orange in colour (Figure 2.2-1).

Figure 2.2-1. The immature triploid (left) and diploid (right) ovary from Atlantic salmon. Scale bar = 5mm. Reproduced from Fraser (2013).

Methods for producing triploids (see chapter 1) may affect performance and mortality. Mating of tetraploids and diploids creating interbreed triploid groups may be less invasive compared with pressure or shock treatment and has yielded promising results in terms of performance and mortality both in rainbow trout (Weber et al., 2014) and striped catfish (*Pangasianodon hypophthalmus*) (Carman et al., 2022). Information is however relatively scarce, but there are currently trials ongoing in Atlantic salmon at the IMR (personal communication, Alison Harvey).

2.2.1 Smoltification

The timing of seawater transfer will impact performance as smoltification measures may be different between the two ploidies (Fraser et al., 2022; Fraser et al., 2021). Leclercq et al. (2011) and Taylor et al. (2012) noted that Atlantic salmon triploids completed out-of-season smoltification as 0+/autumn smolts four weeks earlier than diploids based on body colouration. Subtle differences in the timing of smoltification based on gill-gene expression in out-of-season smolts between diploids and triploids have also been observed (Fonseka et al., 2022). If triploid physiology follows that of diploids, these results suggest triploids may be more susceptible to beginning the process of reversing smoltification earlier than diploids, which can impair performance and survival upon sea transfer. On the other hand, earlier out-of-season smoltification could be advantageous in an intensive aquaculture setting as fish could be moved to sea earlier. The timing of smoltification does not seem to differ between the two ploidies when producing 1+/spring smolts on natural photoperiods and no physiological challenges have been found in fish completing the transfer (Bortoletti et al., 2022).

Farm data suggests triploids performed better for many indicators (disease outcomes, growth, mortality) as spring compared to autumn smolts (Stien et al., 2023; 2021a,b; 2019). However, as the smolt status of the fish used in the farm data is unclear (no physiological data is available), it is unclear whether this is related to differences in smolt timing, the environmental conditions the fish enter into, or other reasons.

A study by Fonseka et al. (2022) suggested out-of-season triploids may have a lower salinity optimum than diploids as post-smolts, with triploids growing relatively better at 23 (brackish/low strength seawater) compared to 35 (full-strength seawater) ppt in comparison to diploids where the opposite occurred. No other study has assessed long-term salinity tolerance in triploid fish.

2.2.2 Growth

Freshwater phase

Although literature may vary, there is a general tendency of diploid Atlantic salmon to be larger and grow better than triploids until they reach the parr stage. Triploids then generally outperform diploids being larger when reaching the end of the normal freshwater stage (smolt) (Benhaim et al., 2020; Fraser et al., 2021; Fraser et al., 2012b; Nuez-Ortin et al., 2017; Taylor et al., 2013; Taylor et al., 2012; Taylor et al., 2011).

Seawater phase and large fish

In post-smolt salmonids and large fish, data are rather inconsistent and results have shown growth to be equal (Fraser et al., 2012b; Sacobie et al., 2012; Smedley et al., 2016), better (Fraser et al., 2022; Fraser et al., 2012b; O'Flynn et al., 1997; Oppedal et al., 2003), or poorer (Fraser et al., 2013; Fraser et al., 2012b; Madaro et al., 2022; Taylor et al., 2014; Taylor et al., 2013). This clearly suggests that there are elements of external confounding factors having a major impact on the growth rates.

It has been observed that triploids perform better under experimental trials in land-based tanks and early seawater phase (Fraser et al., 2021), but there are other studies that have not found this (Taylor et al., 2014, Crouse et al., 2021). There is also a general trend that triploids perform less than diploids in sea pens. Overall, the general trend is that when triploids are grown to slaughter size, overall growth performance is worse in both Atlantic salmon (Crouse et al., 2021; Taylor et al., 2013) and rainbow trout (Janhunen et al., 2019). Additionally, there were higher numbers of emaciated fish in the triploid groups along with lower quality rating at slaughter. Similar data were found when salmon were reared up to 3 kg in open net pens where triploid groups had more small, emaciated fish and reduced percentage of superior quality fish (Fraser et al., 2013).

It appears that part of the challenge of optimising triploid performance may be related to the method of triploidisation. When rainbow trout interbreeds (tetraploid-diploid mating) were maintained in tanks to 1.5 kg and compared with diploids and conventional triploids, Weber et al. (2014) found that interbred triploids generally performed similar to diploids and better than conventional triploids during most of the growth phase. At the end of trial, interbred groups performed better than the other groups. Deformities of the interbred group were at the level of diploids and much lower than the groups produced by standard protocols for triploidisation. There were no effects of ploidy (diploid/triploid) on feed intake.

2.2.3 Coping at different ambient temperatures

Two of the main factors affecting welfare of triploids are water temperature and oxygen levels. Triploid salmonids do not, with some exceptions (Atkins and Benfey, 2008), seem to differ from diploids in their oxygen consumption rate, oxygen binding capacity, or aerobic swimming capacity (Bernier et al., 2004; Riseth et al., 2020; Scott et al., 2015; Verhille and Farrell, 2012). Some differences in aerobic scope or basal metabolism have been suggested, but data are variable and indicatory at best. A lower aerobic scope in triploids would indicate that more of the energy is spent on maintenance, and the fish would, therefore, be more susceptible to oxygen deficiencies at higher temperatures. Reduced aerobic scope at high temperatures has been suggested in small salmon in freshwater (Bowden et al., 2018), triploid brook trout (*Salvelinus fontinalis*) (O'Donnell et al., 2017), and chinook salmon (Bernier et al., 2004). Higher feed intake in triploids at higher temperatures (19 °C) points in the same direction (Preston et al., 2017). However, altered feed intake and changes in aerobic scope (10-18 °C) are usually not observed in Atlantic salmon (Bowden et al., 2018; Sambraus et al., 2018; Sambraus et al., 2017b).

On the other hand, triploid Atlantic salmon and brook trout are reported to have higher metabolic rates compared with diploids at 12 °C and lower at 15 °C, suggesting the possibility of lower temperature optima for triploids (Atkins and Benfey, 2008). Polymeropoulos et al. (2014) showed that triploid Atlantic salmon alevins had elevated metabolic rates during both normoxia and acute hypoxia conditions at 8 °C. Other studies in brown trout have shown opposite effects where oxygen consumption is lower in triploids at both 9 and 18 °C, but with a much higher gill ventilation rate (Lahnsteiner et al., 2019). It is possible that some of these variations are due to species differences.

It is a general assumption that ectothermic animals with smaller cells are less vulnerable to oxygen limitations at higher ambient temperatures. This appears to be valid for most ectothermic animals and is well documented in several fish species like zebrafish (van de Pol et al., 2021). Large triploid Atlantic salmon (> 2 kg) in particular, appear to grow better than diploids at temperatures up to 9 °C, where they also have higher feed intake and oxygen consumption (Sambraus et al., 2018). At 12 °C, feed intake was similar between ploidies and at 15 °C feed intake dropped in both ploidies but more in triploids. Similar findings, but less pronounced, were made in triploid Atlantic salmon of around 350 g when kept at 19 °C where feed intake reduction in triploids was more pronounced than diploids (Hansen et al., 2015). Triploids also ram ventilated, i.e. swimming with open mouth to reduce energetic cost of respiration. In post-smolts exposed to temperatures of 3 – 18 °C, Sambraus et al. (2017b) found that feed intake was better in triploids up to 9 °C, but lower at 15 °C with feed intake peaking at 12 °C for triploids and 15 °C for diploids. A maximum temperature for triploids was suggested to be around 15 °C.

2.2.4 Coping at different oxygen levels

Oxygen saturations at the level of 70-100% at low temperatures often has little effect on both diploid and triploid large salmon in tanks (Sambraus et al., 2018). One of the more consistent data of triploids is that they have lower hypoxia tolerance at high temperatures. In triploid brook trout (Jensen and Benfey, 2022) and rainbow trout (Benfey and Devlin, 2018) hypoxia tolerance was consistently lower than in diploids based on measures, such as PO_2 at loss of equilibrium and time to loss of equilibrium. However, the magnitude of these effects was rather small. Similarly, when fish were kept at 19 °C and exposed to 70% O_2-saturation, appetite dropped significantly, and more in triploids compared with diploids, while feed conversion ratios and mortality increased (Hansen et al., 2015). Mortality also increased in triploid post-smolts when exposed to 60% oxygen saturation at 18 °C, but not at 6 °C (Sambraus et al., 2017b). Using large salmon > 2 kg, Sambraus et al. (2018) found less response to hypoxia with little or no effects on mortality on either ploidies, at 18 °C.

Similar results were seen in triploid brook trout when exposed to hypoxia at high temperatures (18 °C). They had inferior tolerance compared with diploids, but both ploidies had improved tolerance when kept at high temperatures (15 °C) prior to exposure (Jensen and Benfey, 2022). In triploid rainbow trout, juveniles have lower hypoxia tolerance than larger fish (Scott et al., 2015).

2.2.5 Mortality

Studies of mortality in different ploidies of Atlantic salmon have shown lower, higher, or no difference. Egg mortality has been reported not to be affected by ploidy if “good quality eggs” are used (Taylor et al., 2011), or higher in triploid Atlantic salmon (Fraser et al., 2014a). However, there are studies where no ploidy effect on egg mortality was found (Taylor et al., 2011). For larger fish, most intermediate to long -term studies report low mortalities in both ploidies (Fraser et al., 2013; Fraser et al., 2012a; Leclercq et al., 2011;

Taylor et al., 2013; Taylor et al., 2011), and in some cases lower in triploids than diploids. Again, environment appears to be an important determinant of mortality. The effect of high temperature and hypoxia has already been discussed above. On the other end, rearing triploids at low temperature (8 °C) appeared to improve survival (Benhaim et al., 2020).

Following Atlantic salmon through a whole production cycle in commercial farms, Madaro et al. (2022) reported a trend towards higher mortality of triploids in some farms, but not in others, and the variation between farms was higher than the ploidy effect within farm. There were also higher numbers of emaciated fish in the triploid groups along with lower quality ratings at slaughter. Similar data were found when salmon were reared up to 3 kg in open net pens where triploid groups had more small, emaciated fish and a reduced percentage of superior quality fish (Fraser et al., 2013). Reduced survival was also noted in large (> 1 kg) freshwater cage reared triploid rainbow trout at increasing water temperatures, compared to diploids (Karayucel et al., 2018).

2.2.6 Infectious disease and triploidy

The extent to which triploidisation influences the susceptibility of Atlantic salmon to develop infectious diseases remains relatively poorly investigated. These are nevertheless highly relevant topics to consider, as increased pathogen susceptibility in triploids would likely increase mortality and constitute an elevated biosecurity risk towards both farmed and wild salmon. This could result in significant fish health, welfare, and economic consequences, and not only restricted to the triploid fish.

Following injection of lipopolysaccharides (LPS) from Gram-negative bacteria, which are known to readily interact with immune systems of vertebrates, one study found that triploids need longer time to recover complement activity, and have a slower onset of the hypoferraemic response, suggesting that they may be at a disadvantage compared with their diploid siblings in their defence against bacterial infections (Langston et al., 2001).

While some lab-based challenge trials (see separate sections below) involving commercially important salmon pathogens have been conducted, it is important to be aware that such trials may not necessarily be indicative of disease susceptibility under field conditions in salmon farms. Environment, operational procedures, and various other factors may significantly impact the risk of disease outbreaks, e.g. due to stress or physical injury, skewing the overall balance of the epidemiological triad in favour of increased disease incidence. Knowledge regarding triploids’ (versus diploids’) resilience towards such external impacts would thus be informative for evaluating differing disease risks. A comprehensive comparison of triploid versus diploid susceptibility to infection and disease development under field conditions would likely require large-scale longitudinal monitoring studies with extensive epidemiological recordings. Ideally, both field studies and lab-based challenge studies should be available.

Comparison of susceptibility to infectious salmon anaemia and “winter ulcer” during the sea-phase in triploid versus diploid fish are considered the most important conditions/diseases for answering the terms of reference, and these sections are therefore more detailed and thoroughly discussed than the other sections.

Viruses

There are few available studies on the relative susceptibility to viral diseases for triploid versus diploid Atlantic salmon.

Salmonid alphavirus (SAV), the causative agent of pancreas disease (PD)

Using a bath challenge model, the susceptibility to SAV3 shortly after seawater transfer was studied for triploid versus diploid salmon (Moore et al., 2017). Neither copy number of SAV3 RNA in heart tissue nor histopathological changes typical of pancreas disease differed between the groups. The prevalence of SAV3 infection increased more slowly in the triploid group, but both groups reached 100% prevalence by the end of the 35-day experimental period. The authors concluded in that study that triploid salmon were not more susceptible to SAV3 than diploids (Moore et al., 2017). In a study where SAV1 was administered to both diploid and triploid fry, it was found that both were susceptible to infection. A lower virus load found in the triploids was suggested to be related to differences in cell metabolism (Herath et al., 2017).

Infectious salmon anaemia

There are field data that indicate increased susceptibility of triploid Atlantic salmon to infectious salmon anaemia (ISA). The clinical and macroscopic changes of ISA can vary from low and minor to episodes of acute high mortality and the difference in severity is influenced by the virus strain, management, and genetics of the fish (Mjaaland et al., 2002). ISA is caused by infectious salmon anaemia virus (ISAV). ISAV comes in two different genetic strains; ISAV HPRΔ that causes the disease ISA; and ISAV HPR0 that is considered as non-pathogenic (Rimstad and Markussen, 2020). ISAV HPRΔ is listed and notifiable in Norway, in the EU, and by the World Organization of Animal Health (WOAH), while infection with either ISAV HPRΔ or ISAV HPR0 also is listed and notifiable by WOAH. Outbreaks of ISA in Norway are regulated with strict measures with removal of fish and restricted zones, typically 5–10 km radius, around a locality with ISA. An outbreak where the source of the virus is unknown is called a primary outbreak, while outbreaks where the chain of transmission is known are called secondary outbreaks.

Only one paper from the literature search compared susceptibility of triploid versus diploid salmon to ISA. This paper described the study of a commercial farming company where production of triploid fish was a large part of total production, with 12 confirmed and two suspected ISA outbreaks in the period 2015–2020. The company had 57 sea transfers into a total of 428 net cages in the period 2015–2019, where diploid fish made up 71.3% and triploid 28.7% of stocked cages (Aunsmo et al., 2022). There was an increased risk of primary field outbreaks of ISA in triploids compared with diploids at a site level (odds ratio 9.8). At a cage level, there was also an increased risk of ISA in triploids (odds ratio 3.4) in sites which experienced primary ISA outbreaks. This suggests that triploid fish have an increased risk compared with diploid fish for primary outbreak at the site and increased susceptibility to ISA within the infected site (Aunsmo et al., 2022). In Stien et al. (2023), a larger dataset was used, which encompassed that used by Aunsmo et al. (2022) with additional data from other farms, and the ploidy effect on the susceptibility to ISA was reduced and was not found to be statistically significant. However, there was still a trend for triploids to be more susceptible to ISA.

The field study of Aunsmo et al. (2022) described examples of production sites where ISA had been diagnosed in the cages with triploid fish but not in the cages with diploid fish. The lack of detection of ISAV-HPRΔ in diploid fish in neighbouring cages to infected triploid fish, indicated a lower susceptibility to the infection in diploids compared with triploids (Aunsmo et al., 2022).

An experimental study failed to find strong ploidy effects on the susceptibility to ISA in Atlantic salmon post-smolts, but there were some indications that triploids were more susceptible (Aunsmo et al., 2022). In favour of a ploidy effect, i) survival was lower in non-ISA-vaccinated triploids compared with diploids counterparts, although there was no ploidy effect in ISA-vaccinated fish, and ii) triploid non-ISA-vaccinated fish were found to harbour a higher viral RNA load in the heart and kidney in one out of two trials, but there was no ploidy effect in ISA-vaccinated fish. In the second replicate which had some minor methodologic differences, there was no ploidy effect on viral RNA loads in the heart or kidney. A shortcoming to this study would, however, be that the ploidy status of the fish was not confirmed. It is also unknown whether both ploidy shared the same environment/diet prior to the experiment. Further, triploid smoltification was assumed to have progressed at the same rate as diploids even though an out-of-season smoltification protocol was used in which triploids have been suggested to desmoltify earlier than diploids (see section 12.2.1 above), and both ploidy shared the same tank which has been suggested to impair triploid growth performance (Taylor et al., 2013).

The findings of increased susceptibility to primary outbreaks with ISA in the field were only partly reproduced in experimental infections of triploid Atlantic salmon (Aunsmo et al., 2022). However, primary field outbreaks are assumed to be due to emergence of a virulent ISAV variant while experimental infections use a known virulent variant of the virus and therefore do not mirror very well the emergence of a primary ISA field outbreaks. ISAV replicates in the cell nucleus and the virus replication are predatory on the cell's transcription machinery and ISAV utilises this machinery both in triploid as in diploid cells.

Bacteria

Several previously impactful bacterial diseases in Norwegian salmon aquaculture have been efficiently controlled by the use of targeted oil-based injection vaccines over the last three decades. For some bacterial salmon diseases, however, vaccines are still not available, or they do not provide full protection. A worrying trend in recent years is an increase in the number of bacterial disease outbreaks in farmed salmon in Norway, as recorded annually by the Norwegian Veterinary Institute (Sommerset et al., 2023). One apparent contributor to this is the stress and mechanical injury commonly arising from the increasingly frequent mechanical delousing operations (Sommerset et al., 2023). As mentioned, it remains uncertain how triploid salmon would cope under such conditions, and if the associated risk of secondary disease outbreaks would be affected.

"Winter ulcer" during the sea-phase was diagnosed in at least 433 salmon farms in Norway in 2022 (Sommerset et al., 2023). Commonly involved bacteria are *Moritella viscosa* and *Tenacibaculum* spp. No peer-reviewed papers addressing this topic were identified, but one relevant non-peer reviewed report from the Norwegian Veterinary Institute, was identified. Here, a series of challenge trials with *M. viscosa* and *Tenacibaculum* spp. were performed in diploid and triploid salmon (Sindre et al., 2018). Although confounding factors complicated result interpretations, two challenge trials found indications of triploid salmon, in comparison to diploids, being more susceptible to *M. viscosa* infection, while a third trial suggested better performance in triploids compared to diploids. The same study found no differences regarding *Tenacibaculum* spp. infection susceptibility, but a slightly faster onset of mortality and perhaps higher susceptibility to cohabitant challenge in triploids, was registered. Importantly, however, the authors state that no firm conclusions could be drawn due to problems with the challenge models and partially conflicting results. Also, the ploidy status of the fish was not documented, and the triploids and diploids were fed different diets. In addition to this, the report series from the Institute of Marine Research (Stien et al., 2023; Stien et al., 2021a; Stien et al., 2021b; Stien et al., 2019) monitored commercially sea-farmed triploid salmon over several years. Overall, their findings indicated a relatively high incidence during the winter season of ulcers and ulcer-related bacteria (*M. viscosa* and *Tenacibaculum* spp.) among triploid groups transferred to sea from October to December. Post-handling related bacterial ulcers leading to mortality were also more frequently observed in triploid groups. In general, triploid groups experienced considerably larger problems with ulcers and ulcer-related bacteria compared to the diploid reference groups. Furunculosis, caused by *Aeromona salmonicida* subsp. *salmonicida*, is a fish disease primarily affecting salmonids, and is notifiable (category F; national diseases) in Norway. Due to effective vaccines, the disease is rarely detected in farmed salmon in Norway today, but it remains endemic amongst wild Atlantic salmon in a few regions. Studies have revealed similar susceptibility of triploids and diploids to *A. salmonicida* subsp. *salmonicida* challenge, and responses (antibody production and challenge resistance) following vaccination were also similar, although higher post-vaccination adhesion and/or melanin scores were recorded in triploids (Chalmers et al., 2020; Chalmers et al., 2016).

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is another notifiable (category F) disease of salmonids in Norway, where some wild Atlantic salmon populations are regarded as endemically infected. The bacterium is capable of vertical transmission and rigorous biosecurity measures, especially in broodstock farms, are the current primary preventive tool available. One study found no significant differences regarding the susceptibility of triploid and diploid salmon to *R. salmoninarum* infection (Bruno and Johnstone, 1990).

When comparing the effect of Atlantic salmon ploidy upon their microbiomes, Brown et al. (2021) found that during a natural outbreak involving the potential gill pathogen *Candidatus* Branchiomonas, this bacterium became significantly more dominant in the gill microbiome of diploids, compared with triploids. Ploidy-related differences in bacterial pathogen susceptibility have also been investigated in a few other salmonid fish species. Specifically, triploid rainbow trout and coho salmon (*Oncorhynchus kisutch)*, respectively challenged with *Flavobacterium psychrophilum* (Weber et al., 2013) and *Vibrio anguillarum* (Jhingan et al., 2003), displayed slightly lower resistance towards disease development compared with their diploid counterparts. Due to effective vaccines and host specificity however, neither of these two agents are currently regarded as pathogens of high concern for Atlantic salmon farming in Norway.

Two other bacterial pathogens causing significant problems in Norwegian Atlantic salmon farming today are *Pasteurella atlantica* and *Yersinia ruckeri* (Sommerset et al., 2023), but no published studies exist yet addressing the relative susceptibility of triploids and diploids to these agents.

Parasites

Very few studies have compared the parasite infections in diploid and triploid Atlantic salmon, or even for other fish species. The few studies have mostly involved external parasites on the fish, which in general are the most problematic parasites on farmed fish. Frenzl et al. (2014) compared the susceptibility of diploid and triploid salmon to infection with salmon lice (*Lepeophtheirus salmonis*). Following a single infection challenge, results indicated a significant correlation between fish size and the number of attached salmon lice. Triploid fish were larger than diploids at the smolt stage. In the tank trials, no difference was found between infection levels on diploid and triploid salmon after a single infection challenge. The authors concluded that triploid Atlantic salmon are not more susceptible to salmon louse infection than diploid salmon (Frenzl et al., 2014).

Powell et al. (2008) found a reduced survival in triploid Atlantic salmon when challenged with *Neoparamoeba perurans* (syn. *Paramoeba perurans*), the causative agent of amoebic gill disease (AGD), but fewer gill lesions and no ploidy difference in immune responses. In a more recent study, Chalmers et al. (2017) compared disease susceptibility and innate immune responses between diploid and triploid Atlantic salmon following experimental infection with *N. perurans*. The results indicated that ploidy had no significant effect on gross gill score (how much of the total gill area is visually affected by anything abnormal) or gill filaments affected, while infection and time had significant effects. Ploidy, infection, and time did not affect complement or anti-protease activities. Ploidy had a significant effect on lysozyme activity at 21 days post-infection (while infection and time did not), although activity was within the ranges previously recorded for salmonids. The stock of the fish did not significantly affect any of the parameters measured. The study results suggest that ploidy does not affect the manifestation or severity of AGD pathology, or the serum innate immune responses (Chalmers et al., 2017). In contrast to Powell et al. (2008), Chalmers et al. (2017) found no significant effect of ploidy on salmon mortality.

In three reports, Stien et al. (2021a, b; 2019) studied the welfare of triploid Atlantic salmon, using the Salmon Welfare Index Model (SWIM) system, in several farms in Northern Norway. SWIM does not include infections as welfare indicators but in the reports, it is concluded that infections with the Myxozoan parasite *Parvicapsula pseudobranchicola* and parvicapsulosis constitutes one of the biggest hazards for triploid Atlantic salmon. However, the three reports present no parasitological data such as prevalence, intensity, abundance or mortality data from both triploids and diploid to support the conclusions.

Side effects of vaccinations

Lastly, responses to vaccines and vaccine side effects may also differ between diploid and triploid fish. Chalmers et al. (2020) tested the response of triploid and diploid Atlantic salmon to vaccination with commercially available vaccines. As mentioned, antibody responses to *Aeromonas salmonicida* vaccination were similar in both ploidies. However, at harvest, triploids were statistically more likely to have side effects, exhibiting higher scores of adhesions and melanin than diploids. Triploid out-of-season smolts were also found to have greater vaccine-induced abdominal adhesion scores than diploids, but there was no ploidy difference in spring smolts (Fraser et al., 2014b). The authors speculated this could be because the out-of-season smolts were vaccinated at a higher temperature, which is generally known to increase adhesion scores in salmon, compared with the spring smolts.

3 Risk mitigation measures

Much of the literature cited here suggests that triploid fish face significant challenges with welfare and with susceptibility to a limited number of infectious diseases for triploid fish. However, since triploidisation could be a promising method to protect wild salmon populations against interbreeding with farmed salmon, mitigation actions may be one way out from some negative effects on the triploids. Consequently, in the recent years researchers have focused on finding mitigation actions to compensate for impaired welfare. As triploid fish have different nutritional and environmental requirements than diploid fish, it raises the need for ploidy-specific rearing protocols. Taylor et al. (2013) suggested a potential for superior triploid growth and the possibility to establish viable triploid salmon aquaculture, with the use of triploid specific diets.

Dietary adjustments

Regarding increased risk for cataract in triploid fish, results from Taylor et al. (2015), indicated that triploid Atlantic salmon appear to have a higher dietary histidine requirement, when testing 12.6 versus 17.4 g/kg, than diploids and that preventive measures can be taken to mitigate further cataract development. Sambraus et al. (2017a), tested 10.4 versus 13.1 g/kg, and found that both diploid and triploid salmon reared at 10 °C developed cataracts during the seawater period, with higher severities in triploids and a reduced severity in the fish fed diet with higher amount of histidine.

Phosphorous has been shown to reduce skeletal deformities (Fjelldal et al., 2016). In the study by Fjelldal et al. (2016), dietary supplementary phosphorus reduced deformities in both triploid and diploid salmon at seawater transfer. However, triploids fed a low phosphorous diet (7.1 g/kg), and medium phosphorous (9.4 g/kg) diet had more deformities than diploids fed the respective diets, while there were no ploidy effects observed for fish fed a high phosphorous (16.3 g/kg) level. The results on mortality, growth, bone mineralization, and development of skeletal deformities demonstrate that triploids have a higher phosphorous requirement than diploids in freshwater. Also, Sambraus et al. (2020) suggested higher phosphorus requirement in the freshwater phase as their results concluded with that triploids with body size 3-30 g required more dietary phosphorus than diploids in order to maintain similar vertebral ash content. Additionally, Smedley et al. (2018) stated that triploids fed a low phosphorous (4.9 g/kg) diet had the highest prevalence of jaw and vertebral malformations as well as the highest number of deformed vertebrae in the central caudal vertebral region, which was more pronounced at parr than at smolt. Dietary phosphorous is also essential after sea transfer, as the progression of spinal deformity beyond that at sea transfer can be stabilised by increasing dietary phosphorous during the marine phase (Smedley et al., 2016). The form of phosphorus, i.e. its bioavailability, is important and has implications on the amount needed in the diet. It is worth mentioning that not all publications point in the direction of different dietary needs, as Bortoletti et al. (2022) documented similar performance between diploid and triploid salmon during parr-smolt transformation (3 months, covering the period 2454-3044 day-degrees post start-feeding), reared at low temperatures (10 °C) and fed either a diet enriched with phosphorous or a standard commercial diet from start feeding. According to the authors, the comparable growth and expression of growth-related biomarkers in both triploids and diploids during parr-smolt transformation points to the suitability of the feeding and rearing conditions in the study. They suggest that the water temperature and photoperiod applied (8L:16D) meet the requirements for rearing triploid salmon and the inclusion of phosphorus and hydrolysed fish proteins in the feeds may reduce any differences between animals of different ploidy. One possible interpretation of this discrepancy between triploids and diploids is that the higher dietary phosphorous requirement of triploids may be limited to the early freshwater phase (3-30 gram) (Sambraus et al., 2020).

As triploid salmon are more prone to skin lesions (Stien et al., 2021), there are some prophylactic measures available. Since skin ulcers take longer to heal at low temperatures, the lower tolerance of triploid salmon to higher water temperatures underscores the importance of dietary mitigation of such lesions. Dietary supplementations of zinc, selenium, and iron are of particular importance with a preference towards organic mineral species of significantly higher bioavailability than their inorganic forms increasing the risk of deficiency at a given regulatory maximum dietary inclusion level (Kousoulaki et al., 2021). Other minerals, such as zinc have also been shown to have skin healing properties (e.g. Jensen et al. (2015)), however it is important to be aware that EU has regulated zinc content in aquatic feeds to 180 mg zinc per kg complete feed (EU, 2016). Studies on rainbow trout suggest that triploids may have higher requirement for zinc (Meiler et al., 2021; Meiler and Kumar, 2021).

Management aspects

Besides nutritional needs, there are studies that suggest that triploid fish require lower rearing temperatures, especially during early life stages. Also, it has been suggested that the combination of low rearing temperature and phosphorus-rich diets minimises the risk of skeletal deformities in triploids (Peruzzi et al., 2018). Clarkson et al. (2021), and Fraser et al. (2015a) concluded that triploids require lower incubation temperature than the current industry standard of 8 °C to promote better overall performance. Temperature management include management of oxygen requirements as well because they are fundamentally linked. Benhaim et al. (2020) showed that when reared at cold and constant temperature (8 °C), juvenile triploid and diploid fish showed similar growth from 81 to 165 days post hatching (dph) but a difference occurred at 221 dph, when triploids started to grow faster. Triploid fish had higher survival rates and the prevalence of deformities was low in both ploidy groups. Triploid and diploid fish displayed similar swimming activity, and boldness traits and gut microbiome were similar. The results from Benhaim and colleagues suggest that when raised at low temperatures, triploid and diploid fish performed equally well, with even a better survival rate for triploids towards the end of the experiment, suggesting the need for developing different rearing protocols for diploid and triploid Atlantic salmon (Benhaim et al., 2020). Also, it is suggested that bigger fish require lower rearing temperatures, as Sambraus et al. (2018) found that large (2.5 kg) triploid Atlantic salmon perform better at colder water temperatures compared with diploids and differed in some physiological expression at increasing and high temperatures. The authors further saw that triploids fed more than diploids at 3 and 9 °C, had similar levels at 12 °C, but lower at 15 and 18 (Sambraus et al., 2018).

Welfare of triploid salmon may also depend on the time at seawater transfer as shown by Stien et al. (2019) who followed the life cycles, including seawater transfer of 24 triploid populations in the period 2014-2017. Skin wounds were more prominent in triploid salmon that was transferred to the sea in late autumn (November – December), and infection with

Parvicapsula pseudobranchicola was one of the major risk factors for impaired welfare among fish transferred during July – October. However, triploid salmon that were transferred in the spring (May – June) performed better and had similar mortality to diploid reference groups (Stien et al., 2019). The authors expressed the need for research that led to the improvement of skin health and robustness of triploid fish.

As mentioned above, ulcer healing is also linked to temperature (Jensen et al., 2015), where lower water temperature prolongs the healing process. Thus, it is more important to adopt management strategies that minimise the occurrence of ulcers for triploids compared with diploids who tolerate higher temperatures better. Examples of this would be preference towards treatments against sea-lice infestations that minimises stressors and crowding leading to ulcers. Lower fish density in general would be favourable to triploids, as density is shown to delay healing of ulcers (Sveen et al., 2018). This implies that other aspects of husbandry should take care to reduce crowding (e.g. distributing the feed widely across the net pen while feeding), and contact (e.g. avoid structures inside the net).

Breeding

Should triploid Atlantic salmon become an integral part of future Norwegian aquaculture production, one possible mitigation measure concerns the development of a breeding program parallel to that of diploid salmon. One must assume that not all desired breeding traits for diploids will be equally beneficial for triploids, and that the collective target traits of the ongoing diploid salmon breeding programs might not be optimal for triploid salmon. Heritability will vary for different traits affected negatively by triploidy, from temperature tolerance to susceptibility to chronic stress. Still, it can be a relevant ambition to monitor key performance indices of farmed triploid salmon, in order to identify the most suitable brood stock for next generation for inducing triploidy.

Separate breeding programs with specific objectives could alleviate some of the welfare and disease problems seen in triploids. However, we lack knowledge on the heritability of many traits of concern in triploids, and the level of interactions between ploidy and genotype for the same traits to conclude on the efficiency of selection programs.

If triploids do require their own breeding lines to improve their health and welfare, additional breeding programs will need to be established and maintained, and one would need to manage and improve specific breeding populations for this purpose.

The additional chromosome set of triploids could be complicating genetic selection, because their RNA expression levels are equal to that of diploids. This suggests triploids silence the expression of one of their three alleles (Glover et al., 2020). Furthermore, in one recent study, a low frequency of triploids was found to have chromosome aberrations. This included missing alleles, with unknown implications on their health and welfare (Glover et al., 2020).

Vaccines

The effectiveness of vaccinations administered according to established protocols and using existing vaccines seems to be similar in triploid and diploid salmon. This conclusion is drawn from the absence of any documented rise in the incidence of previously common bacterial diseases like vibriosis or cold water vibriosis, which are targeted by vaccination, among the

30-35 million triploid salmon, all vaccinated, that have been transferred to seawater since 2013.

There are indications that triploids are more susceptible to ISA (Aunsmo et al., 2022). ISA is a notifiable disease and its detection may lead to administrative consequences for the production facilities. A mandatory ISA vaccination regimen for triploid could possibly mitigate this. The vaccination efficiency in triploids in general is adequate as other common salmon pathogens covered by vaccination are not reported to occur more frequently in triploids than in diploids. No ploidy effect on survival was reported in a small-scale trial with ISA vaccinated fish (Aunsmo et al., 2022). However, the commercially available vaccines against ISA that do reduce disease and mortality (Aunsmo et al., 2022) and probably reduces the virus load, do not stop the fish from being infected by ISA virus.

Farm management

Several of the factors in which triploid salmon exhibit lower performance, i.e. deformities, cataract, skin ulcers, tolerance to handling, etc., compared with diploid salmon can be mitigated by employing distinct operational procedures for these two groups. This encompasses factors like rearing temperatures, particularly in the initial life stages, time of sea transfer, specialised diets, vaccine requirements, and differences in genetic lineages as discussed above.

4 Uncertainties

In an ideal scenario, we would expect consistent and replicable findings from both field studies and experimental challenge studies to draw reasonably certain conclusions. Nonetheless, the available evidence for certain aspects of the terms of reference is inadequate, leading to uncertainty in some of the conclusions reached.

The main uncertainties are highlighted below:

In general, triploid growth performance varies across studies, from better, to equal, to worse, but the factors explaining these inconsistencies remain relatively elusive. Most notably, genetics, nutrition, and environmental conditions vary considerably across studies and likely interact with ploidy. Older studies examining the effectiveness of triploidy may be outdated and not comparable to more recent research, adding to the uncertainty.

Of the individual organs, heart, brain, gill, liver, and gut size/morphology have been assessed in Atlantic salmon. Ploidy effects were found in all, but functional studies are generally lacking so the relevance of these differences remains unknown.

Numerous studies have assessed metabolic traits in triploid salmonids as larger cell sizes are expected to be more energetically costly to maintain. Evidence in Atlantic salmon is mixed, generally finding indicators of equal or impaired performance in triploids. Impaired performance is usually found when using relatively high temperatures for this species, suggesting ploidy effects depend on the environment.

Although a large body of data has been collected and reported from Norwegian farm trials (Stein et al., 2023; 2021a,b; 2019) and trends have been identified regarding aspects such as triploids increased susceptibility to stress, mechanical injury, and disease, these trials contained no controls for an extensive list of variables. This includes (but is not restricted to) genetics (between breeding companies, and strains within a given company), hatchery origin, farm location/connectivity, diet, water quality, vaccines, handling protocols, smoltification regimes, size and timing of sea transfer, cage design, and delousing methods. This has made it difficult to draw definitive conclusions, especially as variability across farm performance was generally high, irrespective of the ploidy status of the fish.

Norwegian farm trials suggest triploids are less capable of handling stressful scenarios than diploids but controlled experimental tank studies focusing on single stressors generally find no ploidy effects on stress physiology. The factors leading to this inconsistency have yet to be elucidated. The farm trials are highly applied but lack controls, whereas the current experimental trials are relatively small in scale and lack realistic environmental scenarios.

The extent to which triploidisation influences the susceptibility of Atlantic salmon to develop infectious diseases remains relatively poorly investigated. Most of the literature is either relatively old, non-peer reviewed publications or from unpublished sources. Data from the Norwegian farm trials suggests triploids are more prone to skin ulcers, e.g. caused by *M. viscosa,* than diploids. The only experimental study into ISA was relatively small (2 replicates) and found evidence of equal or more severe infections in triploids than diploids which depended on the vaccination status of the fish. The only experimental study into *M. viscosa* was a small-scale trial which suffered with a poorly functioning infection model, producing inconclusive and partially conflicting results.

5 Conclusions (with answers to the terms of reference)

1. Summarise knowledge about animal health and welfare in triploid salmon (*Salmo salar*) under commercial farming conditions, as compared with traditional diploid salmon in aquaculture, in various stages of salmon production, from hatchery to slaughter. This includes: Robustness, biological functionality, mortality, susceptibility of disease, and potential to transmit infections and disease

One conclusion of this review is that triploid Atlantic salmon in many aspects have reduced health and welfare compared with diploid Atlantic salmon under commercial farming conditions. However, previous and ongoing research shows that some of the differences in health and welfare can be fully or partially mitigated if the environmental and nutritional requirements are optimized for triploids. Some differences though, may be significant in that adjustments under commercial farming conditions are not feasible.

The literature concerning the health and welfare of triploid salmon exhibits considerable disparity across various studies, which adds uncertainty to conclusions. The variation in the triploid salmon's performance can partially be attributed to differences in the operating procedures used for production of triploid fish and in the fish's genetic lineage. This will affect the published results of the experiments and studies, and the resulting performance of farmed triploid salmon in Norway. There has been progress in tailored diets and rearing conditions for triploid fish, which could contribute positively to their health and welfare.

Robustness

See chapter 2.1 "The consequences of triploidy on health and welfare", i.e. sub-headings: Deformities, Cataract, Skin condition, Stress-coping, Tolerance to handling, and Behaviour.

Under experimental and commercial farming conditions, skeletal deformities have emerged as major reported side effects in triploid fish leading to a greater occurrence of discards and downgrading at slaughter compared to diploids. They are mostly attributed to triploids requiring more dietary phosphorus and lower incubation temperatures than diploids.

Under experimental and commercial farming conditions, cataracts have been extensively documented as a more prevalent disorder in triploid, compared with diploid Atlantic salmon. Cataracts are linked to poor vision and reduced feeding. This result has consistently been linked to insufficient dietary histidine in experimental trials and can generally be mitigated by using diets with increased histidine.

There are reports of altered heart morphology and deformities in triploid compared with diploid salmon. Heart deformities related to incubation temperature are generally not present if the triploids eggs have been incubated at a maximum of 6 °C. However, it is unclear why triploid heart morphology deviates from diploids and the functional consequences are unknown.

There are some farm reports of higher susceptibility to skin ulcers in triploid compared to diploid salmon, especially in fish transferred to sea in the autumn. This might be related to the indications that triploid salmon are more susceptible than diploids to *M. viscosa* infection.

There are some farm reports suggesting that triploid salmon, compared with diploid salmon, show less tolerance towards handling, with more panic reactions, injuries, and ulcers. However, this contrasts with experimental studies which find few to no differences in stress physiology between triploid and diploid salmon.

Several observations show divergence in behaviour between triploid and diploid salmon. Studies involving other salmonid fish species suggest that triploids are less aggressive than diploid fish. This may have implications in experimental studies conducted in common garden (i.e. when diploids and triploids share the same tank), as triploids may occupy a lower position in social hierarchies and be subject to greater aggression from diploid counterparts. However, its impact on the available literature is unclear.

Biological functionality/physiology

See chapter 2.2 "The consequences of triploidy on physiology, and susceptibility to disease" i.e. sub-headings: Smoltification, Growth, Coping at different ambient temperatures, and Coping at different oxygen levels.

Due to a 50% increase in DNA content compared with diploid counterparts, triploid cells exhibit larger cell nuclei and subsequently, larger cell sizes in general. However, our understanding of the influence of cell size on fish physiology remains insufficient for conclusion on the effects. Relative organ sizes among age-matched fish seem to be similar for triploids and diploids, indicating that triploids organs have fewer cells.

Although triploidy leads to functional sterility in both sexes, the triploid immature testis looks identical to that of diploids. Triploid males show secondary sexual characteristics and can behave as fertile males on the spawning grounds, but this leads to infertile spawnings. Female triploids do not show any ovarian development or secondary sexually characteristics.

The process of smoltification appears consistent between the two ploidy types under ambient conditions and natural daylengths but may differ in manipulated environments. This may impact the optimal time to move triploids to seawater.

There is a noticeable tendency across farm and experimental trials, for triploid salmon to be equal or larger in size at the end of freshwater phase, but equal or smaller in size at the end of the seawater phase.

Most publications conclude that within what is considered the optimal temperature range of diploids, oxygen consumption rate, oxygen binding capacity, and aerobic swimming capacity do not significantly differ between triploid and diploid salmonids. However, for several experimental endpoints triploids tend to have a lower optimal temperature, and data consistent across studies indicate that they possess lower tolerance to hypoxia at elevated temperatures.

The only study on optima for triploid post-smolts would suggest a lower salinity optimum than for diploids. However, it is unclear whether this was a methodological artifact or a general ploidy effect, and how this may impact farm performance.

Mortality

See chapter 2.2.5 "Mortality".

Studies of mortality in triploid and diploid salmon vary in results and have shown lower, higher or no differences in mortality for triploid salmon compared with diploid salmon.

Susceptibility to disease

See chapters 2.1.3 "Skin condition", and 2.2.6 "Infectious diseases and triploidy", i.e. sub-headings: Viruses, Bacteria, and Parasites.

The influence of triploidy on the susceptibility of Atlantic salmon to infectious diseases has received limited attention. Numerous factors can influence the development of disease outbreaks. The studies of the susceptibility of triploid Atlantic salmon to infectious diseases have either been under commercial farming conditions or experimental studies where the triploids have been reared and handled using diploid protocols.

In an experimental infection study, it was concluded that triploid Atlantic salmon were not more susceptible to SAV3 and development of PD than diploid salmon.

Field studies indicate that triploid fish have an elevated risk compared with diploids to primary outbreak of ISA under commercial farming conditions and being more susceptible to ISA virus infection in farms that experience an ISA outbreak. The latter was concluded as it was found that triploids in separate cages in farms with ISA were more susceptible to ISA-virus infection than diploids in other cages in the same farms. However, the finding that triploid salmon are more susceptible to ISA could not be replicated in an experimental infection study, although in one out of two replicates, experimentally exposed triploid salmon had higher loads of ISA-virus than diploids at peak of infection.

Farm reports, i.e. commercial farming conditions, have indicated an increased susceptibility of triploid fish to develop ulcers. In a non-peer reviewed report, two challenge trials found indications of triploid salmon, in comparison with diploids, being more susceptible to *M. viscosa* infection, while a third trial suggested better performance in triploids compared with diploids. The same study found no differences regarding infection susceptibility to *Tenacibaculum* spp., a pathogen also related to ulcers. The authors could not draw firm conclusions from the data.

Very few studies have directly compared parasite infections between diploid and triploid Atlantic salmon. Among the studies conducted, authors have concluded that triploid Atlantic salmon do not display a different susceptibility to salmon louse infection than diploid salmon. Furthermore, ploidy does not seem to influence the appearance or severity of AGD pathology.

However, triploid salmon seem to be more stressed during handling such as parasite treatments, which potentially may have various consequences.

Potential to transmit infections and disease

Regarding the potential for transmitting infections and diseases, quantifying the shedding of infectious agents proves challenging. Field data suggests that triploids are at an elevated risk for primary ISA outbreaks, as well as an increased susceptibility to ISA at the cage level. Farm reports also possibly indicate that triploid salmon might have a higher susceptibility to *M. viscosa* infection. Higher susceptibilities to ISAV and *M. viscosa* infections in triploids compared with diploids suggest an increased potential for transmitting these infections.

However, this has not been verified experimentally. Notably, ISA is an internationally notifiable disease.

2. Describe the underlying physiological mechanisms concerning consequences of triploidisation, using current, available knowledge.

See chapter 2.2 "The consequences of triploidy on physiology and functionality" i.e. sub-headings: Smoltification, Growth, Coping at different ambient temperatures, and Coping at different oxygen levels.

This question is also addressed under the question 1 – Biological functionality/physiology.

Triploids have different dietary and environmental requirements than diploids which may predispose them to unfavourable conditions such as stress when kept under commercial farming operational protocols developed for diploids. Due to a 50% increase in DNA content compared with diploid counterparts, triploid cells exhibit larger cell nuclei and subsequently, larger cell sizes. However, the influence of cell size on fish physiology remains unclear. In terms of body and organ sizes among age-matched fish, triploids and diploids perform relatively similarly.

Triploids tend to have a lower optimal temperature, and consistent data indicate that they possess lower tolerance to hypoxia at elevated temperatures.

3. Describe possible risk-mitigating measures and assess whether such actions may prevent or lower the probability of the occurrence of negative consequences for animal health and welfare.

See chapter 3 'Risk-mitigating measures" i.e. sub-headings: Dietary adjustments, Management aspects, Breeding, and Vaccines.

As mentioned under questions 1 and 2, triploid salmon appear to have different requirements for diet and environment (e.g. water temperature) and are more sensitive to stress (e.g. handling and hypoxia at high temperature) and therefore require different operative conditions than diploids. This produces suboptimal conditions for triploids when farmed according to the protocols for diploids, i.e. under current commercial farming conditions. This may be a predisposing factor for conditions such as viral and bacterial infections.

The major concerns surrounding triploid salmon production are susceptibility to disease, skeletal deformities, cataracts, and coping with stress. Below, we identify mitigation strategies that can be employed to address these concerns.

Susceptibility to disease: Vaccination efficiency using existing protocols and vaccines appears to be comparable for triploids to that of diploids, i.e. there are no reports of increased occurrence of previously common bacterial diseases covered by vaccination in the 30-35 million triploid salmon that have been transferred to seawater since 2013. Implementation of mandatory ISA vaccination regimen for triploid fish could possibly mitigate the increased susceptibility to ISA, but it is important to note that existing commercial vaccines do not prevent ISAV infection and are suboptimal to protect against disease.

There is currently no efficient mitigation strategy for infectious diseases that cannot be adequately controlled by vaccines. ISA is a notifiable disease, and its detection may lead to administrative consequences for the production facilities. It is not clear whether optimizing triploid production protocols (diet formulations, handling procedures, environmental conditions) can impact their susceptibility to infectious disease.

Diet: Compared with diploids, triploid Atlantic salmon have a higher dietary phosphorous requirement under commercial farming conditions to prevent skeletal deformities.

Triploid salmon have a higher dietary histidine requirement under commercial farming conditions to prevent cataract development than diploids. Cataracts can be alleviated through dietary supplementation.

However, these diets are generally more expensive and phosphorus waste can have negative consequences on the local environment.

Environment: Indications are that triploids have environmental requirements that can manifest into welfare issues during certain life stages. Temperature appears the most notable, with lower optima during egg incubation and a reduced hypoxia tolerance at relatively high temperatures. On land, it may be possible to control optimal temperatures, but in sea-cages temperature control is not feasible. Many other environmental factors important for salmon performance, such as day length, salinity, or stocking densities, have been poorly studied in triploids.

Genetics: Indications are that family ranking of performance indicators is generally similar between different ploidies, suggesting current breeding programs for diploids can also be beneficial for triploids. However, knowledge on the genetic parameters of welfare traits of concern in triploids are missing, and maternal effects may be more important for triploid performance.

Breeding: Using high quality eggs is known to improve the performance of triploids.

Handling protocols: Various strategies used to limit stress in diploids could be employed to reduce handling stress in triploids, but none have been experimentally tested. These include dietary intervention, feed restriction prior to stressful procedures, less stressful delousing methods, or alterations in cage structure to prevent ulcers and improve water quality.

As stated under questions 1 and 2, many of the health, welfare, and robustness factors in which triploid salmon exhibit lower performance compared with diploid salmon could be mitigated by employing distinct operational procedures for these two groups. This encompasses factors like breeding program tailored for triploid seed, rearing temperatures, particularly in the initial life stages, specialized diets. However, the potential significant health and welfare benefits from genetics gains for triploids will require generations of selection before realization.

6 Data gaps

In this report, we have found that triploid salmon have distinct needs from those of diploids and that this can partly be mitigated by developing specific production systems for triploids. There are data gaps regarding the determination of the factors necessary for effective production of triploids. Ideally, these factors should be established through consistent findings derived from both field observations and controlled experimental studies, which would ensure a high degree of confidence in the conclusions drawn. Additionally, to 48inimize confounding genetic variables, it is advisable to use sibling diploid and triploid fish in such comparative studies.

We have gathered available data, but there are still gaps in our understanding regarding factors such as: the impact of early environmental conditions, temperature tolerance, interactions between ploidy and genotype, susceptibility to diseases, stress responses, the use of less invasive techniques for triploid production (e.g. tetraploid-diploid mating), the influence of cell size on fish physiology and timing for transfer of triploids to sea.

It is now widely acknowledged that triploid fish exhibit distinct dietary requirements for phosphorus and histidine, although comprehensive testing of other various dietary components is still lacking. Similarly, there has been limited research into the relative susceptibility of triploid versus diploid Atlantic salmon to viral and bacterial diseases. To provide a comprehensive comparison of infection susceptibility and disease development between triploids and diploids, both field investigations and laboratory-based challenge studies with extensive epidemiological data collection are essential.

Very few studies have compared the susceptibility to infectious agents and diseases in triploid versus diploid Atlantic salmon. In some studies, triploids have been found to be more susceptible than diploids, however, this should be verified both in laboratory and field studies.

Separate breeding programs with specific objectives could alleviate some of the welfare and disease problems seen in triploids, given that they are heritable. So far, we lack knowledge on the heritability of many traits of concern in triploids, and on the level of interactions between ploidy and genotype for the same traits to conclude on the efficiency and feasibility of such genetic selection programs. Further complexity arrives as we don’t know the inherent genotype variability caused by the industrial induction of triploidy.

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8 Appendix I

8.1 PICO-form and results from the literature search strings

Hvilket spørsmål skal litteratursøket besvare?			
Are there differences in health and welfare between triploid and diploid salmon/Er det forskjell i helse og velferd mellom triploid og diploid laks?			
Spørsmålet i PICO-format			
Population **(pasient)**	**Intervention** **(tiltak)**	**Comparison** **(sammenligning)**	**Outcome** **(utfall)**
Triploid fish Atlantic salmon, Pacific salmon, Rainbow trout, Arctic char, Salmonids, Sea bass, Zebrafish, Tilapia, Sea bream, Turbot, Sturgeon, Catfish, Carp, Halibut, Atlantic cod, Sole, smolt	Breeding, selection, genetics for triploid performance, Feed, Vaccines, Management delousing *ikke søkt på*	Diploid salmon	Mortality, winter ulcer, skin health, disease resistance, stress, side-effects, fitness, cell-size, cataract, deformities, smoltification, gill problem, heart constitution, cardiac, vaccine, survival, emaciation, runts, infection, infestation, extern welfare, handling, ploidy, *ikke søkt på*
Kjente relevante studier			
Madaro et al.,2021: A comparison of triploid and diploid Atlantic salmon (*Salmo salar*) performance and welfare under commercial farming conditions in Norway . Journal of Applied Aquaculture Riseth et al., 2020. Is it advantageous for Atlantic salmon to be triploid at lower temperatures? Journal of Thermal Biology Aunsmo et al., 2022. Triploid Atlantic salmon (*Salmo salar*) may have increased risk of primary field outbreaks of infectious salmon anaemia. Journal of Fish Diseases Fraser et al., 2012. The effect of triploidy and vaccination on neutrophils and B-cells in the peripheral blood and head kidney of 0+ and 1+ Atlantic salmon (*Salmo salar L.*) post-smolts. Fish and Shellfish Immunology			

Database: **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions <1946 to February 10, 2023>**

Dato: 14.02.2023

Antall treff: 256

#	Searches	Results

1	*Salmo salar*/ or *Oncorhynchus mykiss*/ or Salmonidae/ or Bass/ or Zebrafish/ or Tilapia/ or Sea Bream/ or Flatfishes/ or Catfishes/ or Carps/ or Flounder/ or *Gadus morhua*/	81883
2	((pacific or atlantic) adj4 salmon?).tw,kf.	5819
3	((Salmo adj (brevipes or caerulescens or goedenii or gracilis or hamatus or hardinii or nobilis or ocla or renatus or rilla or salar or salmo or salmulus or gairdneri? or mykis? or kamloops or irideus)) or txid8030).tw,kf.	5770
4	((*Oncorhynchus* adj (mykis? or gairdneri? or kamloops)) or ((Rainbow or Redband or Kamchatka or Kamloops) adj trout) or steelhead? or txid8022 or (*Trutta* adj (relicta or salar or iridea))).tw,kf.	12602
5	(Arctic char? or *Salvelinus alpinus*).tw,kf.	615
6	(grayling? or salmonid? or salmonidae or thymallus or whitefish or txid504569 or txid8015).tw,kf.	6137
7	(sea bass* or temperate bass* or dicentrarchus or micropterus or morone or moronidae or serranidae or white perch or txid13488 or txid27705 or txid30871 or txid34815 or txid42148 or txid46260).tw,kf.	3810
8	(zebrafish* or (*zebra* adj (fish* or danio?)) or ((danio or brachydanio or Cyprinus) adj rerio) or *Danio frankei* or "b. rerio" or "d. rerio" or "d. rerios" or txid7955).tw,kf.	46931
9	(tilapia? or oreochromis mossambicus or txid8126 or txid8127).tw,kf.	6192
10	(((gilthead or sea) adj bream?) or seabream? or S*parus aurat** or porgy or txid8175).tw,kf.	3224
11	(flatfishes or pleuronectiformes or *Psetta maxima* or *Scophthalmus maximus* or solea or turbot? or txid28828 or txid52904 or txid8252).tw,kf.	2115
12	(Sturgeon* or Acipenseridae).tw,kf.	1970
13	(catfish* or arius or *Eremophilus mutisii* or heteropneustes or plotosus or siluriformes or ictaluridae or ictalurus or ameiurus or noturus or pylodict* or txid2023056 or txid243561 or txid75285 or txid7995 or txid93620 or txid50384 or txid61322 or txid75287 or txid7996 or txid7997 or txid7998).tw,kf.	7951
14	(carp or carps or "*Carassius carassius*" or *Ctenopharyngodon idellus* or cyprinus or hypophthalmichthys molitrix or txid13095 or txid217509 or txid7959 or txid7961 or txid7962).tw,kf.	13053
15	(flounder? or halibut or plaice or platichthys or pleuronectes or pseudopleuronectes or Hippoglossus or txid8259 or txid8261 or txid8262 or txid8264).tw,kf.	4115

16	(((atlantic or baltic) adj3 cod) or (gadus adj (morhua or callarias)) or txid8049).tw,kf.	2069
17	(Sole or smolt or smolts).tw,kf.	63212
18	or/1-17	178262
19	Triploidy/ or triploid?.tw,kf.	4445
20	Diploidy/ or diploid?.tw,kf.	36359
21	18 and 19 and 20	256

Database: **Embase <1974 to 2023 February 10>**

Dato: 14.02.2023

Antall treff: 271

#	Searches	Results
1	*Salmo salar*/ or exp *Oncorhynchus mykiss*/ or *Salvelinus alpinus*/ or salmonid/ or bass/ or European sea bass/ or zebra fish/ or Tilapia/ or sea bream/ or turbot/ or sturgeon/ or catfish/ or channel catfish/ or carp/ or exp halibut/ or Atlantic cod/ or smolt/	85036
2	((pacific or atlantic) adj4 salmon?).tw,kf.	5949
3	((*Salmo* adj (brevipes or caerulescens or goedenii or gracilis or hamatus or hardinii or nobilis or ocla or renatus or rilla or salar or salmo or salmulus or gairdneri? or mykis? or kamloops or irideus)) or txid8030).tw,kf.	6092
4	((*Oncorhynchus* adj (mykis? or gairdneri? or kamloops)) or ((Rainbow or Redband or Kamchatka or Kamloops) adj trout) or steelhead? or txid8022 or (Trutta adj (relicta or salar or iridea))).tw,kf.	13896
5	(Arctic char? or *Salvelinus alpinus*).tw,kf.	622
6	(grayling? or salmonid? or salmonidae or thymallus or whitefish or txid504569 or txid8015).tw,kf.	6378
7	(sea bass* or temperate bass* or dicentrarchus or micropterus or morone or moronidae or serranidae or white perch or txid13488 or txid27705 or txid30871 or txid34815 or txid42148 or txid46260).tw,kf.	4020
8	(zebrafish* or (zebra adj (fish* or danio?)) or ((danio or brachydanio or Cyprinus) adj rerio) or *Danio frankei* or "b. rerio" or "d. rerio" or "d. rerios" or txid7955).tw,kf.	56792
9	(tilapia? or *Oreochromis mossambicus* or txid8126 or txid8127).tw,kf.	6922
10	(((gilthead or sea) adj bream?) or seabream? or *Sparus aurat** or porgy or txid8175).tw,kf.	3415
11	(flatfishes or pleuronectiformes or *Psetta maxima* or *Scophthalmus maximus* or solea or turbot? or txid28828 or txid52904 or txid8252).tw,kf.	2248
12	(Sturgeon* or Acipenseridae).tw,kf.	2018
13	(catfish* or arius or eremophilus mutisii or heteropneustes or plotosus or siluriformes or ictaluridae or ictalurus or ameiurus or noturus or pylodict* or txid2023056 or txid243561 or txid75285 or txid7995 or txid93620 or txid50384 or txid61322 or txid75287 or txid7996 or txid7997 or txid7998).tw,kf.	8547

14	(carp or carps or "*Carassius carassius*" or *Ctenopharyngodon idellus* or cyprinus or *Hypophthalmichthys molitrix* or txid13095 or txid217509 or txid7959 or txid7961 or txid7962).tw,kf.	14324
15	(flounder? or halibut or plaice or platichthys or pleuronectes or pseudopleuronectes or Hippoglossus or txid8259 or txid8261 or txid8262 or txid8264).tw,kf.	4401
16	(((atlantic or baltic) adj3 cod) or (gadus adj (morhua or callarias)) or txid8049).tw,kf.	2133
17	(Sole or smolt or smolts).tw,kf.	77831
18	or/1-17	206551
19	triploidy/ or triploid?.tw,kf.	5338
20	diploidy/ or diploid?.tw,kf.	35552
21	18 and 19 and 20	271

Database: **CAB Abstracts <1973 to 2023 Week 06>**

Dato: 14.02.2023

Antall treff: 721

#	Searches	Results
1	Atlantic salmon/ or rainbow trout/ or Salvelinus alpinus/ or Salmonidae/ or sea bass/ or exp Tilapia/ or sea bream/ or turbot/ or sturgeons/ or sea catfish/ or freshwater catfishes/ or carp/ or halibut/ or Gadus morhua/	79574
2	((pacific or atlantic) adj4 salmon?).tw.	11278
3	((*Salmo* adj (brevipes or caerulescens or goedenii or gracilis or hamatus or hardinii or nobilis or ocla or renatus or rilla or salar or salmo or salmulus or gairdneri? or mykis? or kamloops or irideus)) or txid8030).tw.	12321
4	((Oncorhynchus adj (mykis? or gairdneri? or kamloops)) or ((Rainbow or Redband or Kamchatka or Kamloops) adj trout) or steelhead? or txid8022 or (Trutta adj (relicta or salar or iridea))).tw.	19894
5	(Arctic char? or Salvelinus alpinus).tw.	1264
6	(grayling? or salmonid? or salmonidae or thymallus or whitefish or txid504569 or txid8015).tw.	48720

7	(sea bass* or temperate bass* or dicentrarchus or micropterus or morone or moronidae or serranidae or white perch or txid13488 or txid27705 or txid30871 or txid34815 or txid42148 or txid46260).tw.	11110
8	(zebrafish* or (zebra adj (fish* or danio?)) or ((danio or brachydanio or Cyprinus) adj rerio) or Danio frankei or "b. rerio" or "d. rerio" or "d. rerios" or txid7955).tw.	11008
9	(tilapia? or oreochromis mossambicus or txid8126 or txid8127).tw.	18594
10	(((gilthead or sea) adj bream?) or seabream? or sparus aurat* or porgy or txid8175).tw.	6089
11	(flatfishes or pleuronectiformes or psetta maxima or scophthalmus maximus or solea or turbot? or txid28828 or txid52904 or txid8252).tw.	9259
12	(Sturgeon* or Acipenseridae).tw.	4341
13	(catfish* or arius or eremophilus mutisii or heteropneustes or plotosus or siluriformes or ictaluridae or ictalurus or ameiurus or noturus or pylodict* or txid2023056 or txid243561 or txid75285 or txid7995 or txid93620 or txid50384 or txid61322 or txid75287 or txid7996 or txid7997 or txid7998).tw.	19379
14	(carp or carps or "carassius carassius" or ctenopharyngodon idellus or cyprinus or hypophthalmichthys molitrix or txid13095 or txid217509 or txid7959 or txid7961 or txid7962).tw.	27503
15	(flounder? or halibut or plaice or platichthys or pleuronectes or pseudopleuronectes or Hippoglossus or txid8259 or txid8261 or txid8262 or txid8264).tw.	5581
16	(((atlantic or baltic) adj3 cod) or (gadus adj (morhua or callarias)) or txid8049).tw.	2929
17	(Sole or smolt or smolts).tw.	37636
18	or/1-17	181234
19	triploidy/ or triploid?.tw.	8653
20	diploidy/ or diploid?.tw.	33626
21	18 and 19 and 20	723

Database: **Web of Science**

Dato: 15.02.2023

Antall treff: 849

Kommentar: Exact search er brukt

#	Query	Results
1	TS=((pacific or atlantic) NEAR/3 salmon$)	26951
2	TS=((Salmo NEAR/0 (brevipes or caerulescens or goedenii or gracilis or hamatus or hardinii or nobilis or ocla or renatus or rilla or salar or salmo or salmulus or gairdneri$ or mykis$ or kamloops or irideus)) or txid8030)	29464
3:	TS=((Oncorhynchus NEAR/0 (mykis$ or gairdneri$ or kamloops)) or ((Rainbow or Redband or Kamchatka or Kamloops) NEAR/0 trout) or steelhead$ or txid8022 or (Trutta NEAR/0 (relicta or salar or iridea)))	55992
4	TS=("Arctic char$" or "Salvelinus alpinus")	3649
5	TS=(grayling$ or salmonid$ or salmonidae or thymallus or whitefish or txid504569 or txid8015)	16078
6	TS=("sea bass*" or "temperate bass*" or dicentrarchus or micropterus or morone or moronidae or serranidae or "white perch" or txid13488 or txid27705 or txid30871 or txid34815 or txid42148 or txid46260)	17592
7	TS=(zebrafish* or (zebra NEAR/0 (fish* or danio$)) or ((danio or brachydanio or Cyprinus) NEAR/0 rerio) or "Danio frankei" or "b. rerio" or "d. rerio" or "d. rerios" or txid7955)	63215
8	TS=(tilapia$ or "oreochromis mossambicus" or txid8126 or txid8127)	19472
9	TS=(((gilthead or sea) NEAR/0 bream$) or seabream$ or "sparus aurat*" or porgy or txid8175)	13321
10	TS=(flatfishes or pleuronectiformes or "psetta maxima" or "scophthalmus maximus" or solea or turbot$ or txid28828 or txid52904 or txid8252)	7597
11	TS=(Sturgeon* or Acipenseridae)	6279
12	TS=(catfish* or arius or "eremophilus mutisii" or heteropneustes or plotosus or siluriformes or ictaluridae or ictalurus or ameiurus or noturus or pylodict* or txid2023056 or txid243561 or txid75285 or txid7995 or txid93620 or txid50384 or txid61322 or txid75287 or txid7996 or txid7997 or txid7998)	23469
13	TS=(carp or carps or "carassius carassius" or "ctenopharyngodon idellus" or cyprinus or "hypophthalmichthys molitrix" or txid13095 or txid217509 or txid7959 or txid7961 or txid7962)	31817

14	TS=(flounder$ or halibut or plaice or platichthys or pleuronectes or pseudopleuronectes or Hippoglossus or txid8259 or txid8261 or txid8262 or txid8264)	14699
15	TS=(((atlantic or baltic) NEAR/2 cod) or (gadus NEAR/0 (morhua or callarias)) or txid8049)	11900
16	TS=(Sole or smolt or smolts)	86768
17	#16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1	317273
18	TS=(triploid$)	7104
19	TS=(diploid$)	35332
20	#17 AND #18 AND #19	849