Executive Summary:

The SALMOTRIP project was a full-scale feasibility study of the potential for the production of triploid salmon with the principal aim to provide information to support decision-making regarding future aquaculture policies and the use of triploidy within the Atlantic salmon industry. The project has been highly successful and significantly improved the state-of-the-art knowledge base on triploid production and physiology. Trials have indicated that successful triploid induction and comparable survival to diploids can be attained by using eggs of optimal quality, and that correct monitoring of ovulation is essential. Previous attempts at commercial up-scaling of triploidy may have been limited by improper consideration of these factors. Survival during freshwater has been comparable between ploidy, while triploids consistently demonstrate 20-30% faster growth during these stages with minimal visible deformity in both experimental and commercial conditions. A series of trials have also examined smoltification photoperiod regimes and demonstrated that out-of-season triploid smolts can be successful produced, which is imperative to ensure the year round supply to ensure a sustainable industry should triploidy be adopted. Furthermore, experiments have indicated differential smolt rates between ploidy, suggesting that poor saltwater transfer survival in previous studies may be in part related to considering triploid as diploid smolts. Seawater (SW) grow-out trials both at experimental and commercial level have revealed that triploids initially show rapid SW growth, however, the initial growth advantage is lost over time, with diploids often exceeding triploids in terms of weight at harvest. It would appear that the prevalence of deformity and increase in ocular cataract continues to have detrimental effects on triploid performance, although it is important to note that the prevalence of deformities observed was lower than in previous studies and is within commercially acceptable limits although animal welfare issues persist. However, we have demonstrated through dietary trials that conventional diploid diets (as used in all previous studies) are not sufficient to support the more rapid growth of triploids, and that specific triploid diets must be developed. Modification of key dietary components at certain key life stages have prevented the development of skeletal malformations, and arrest the development of cataracts. During all growth studies, by working in close collaboration with selective breeding companies, it has become apparent that the best performing diploid stocks also produce the best performing triploid stocks in terms of growth which should allow relatively easy incorporation into existing breeding programs. Grow-out trials have also been conducted across different environmental conditions and countries using the same sibling stocks to determine ploidy-genotype-environment interactions and we have determined in which conditions triploids should not be grown. This will also be important in future planning and policy development as this data can be used to determine what management strategies can be implemented on farm in order to avoid potentially compromising triploid stocks in culture and correct selection of farming sites. Finally, the project explored across Europe product acceptance, marketing strategies and communication messages to accompany the launch of triploid salmon products within the EC. In general knowledge of triploidy is limited within the market which may be a positive benefit as the correct information messaging can be developed. Importantly consumers prefer information on triploid salmon to come from the EU and the NGO's and not industry due to issues of trust. Finally, triploidy itself did not affect the majority of consumer acceptance in terms of quality satisfaction but will be important to determine how this may affect purchasing behavior. Overall, the SALMOTRIP results are

highly encouraging although certain problems still exist, these data and have paved the way for future research in aspects of triploid production. If approached correctly, capitalising on these findings may offer the industry and EC a means to improve sustainable aquaculture production whilst minimizing environmental risks of these operations.

Project Context and Objectives:

The use of triploid salmon has the potential to make an important contribution towards a more sustainable salmon farming by offering a substantial step forwards in the prevention of negative impacts of escapees on wild populations. Commercially viable sterile triploid fish would reduce the need for the energy intensive photoperiod control now widely used by the industry as well as reduce the welfare issues of disease and mortality still associated with maturation during the production cycle. However we can only realise these benefits if we understand and overcome some of the problems reported in earlier studies on triploid salmon. These included higher mortalities, poor growth performances, poor tolerance to sub-optimal environmental conditions and high incidence of morphological deformities. Because of these problems the industry did not see triploid salmon as a viable farming option. However, more recent results have suggested that triploid salmon have been shown to perform as well as diploid fish and even better in some cases.

Objectives

The overall aim of the SALMOTRIP project was therefore to enhance our knowledge on how triploid salmon should be reared and their potential performance. This will be achieved by testing and refining current production practices for triploid salmon as well as assessing how such a product would be perceived throughout the salmon food chain and could be marketed. The project is centred on a full scale feasibility study and market perception analysis. This will determine whether triploid salmon are suitable for farming as a means to minimize the impact of farmed fish on the environment (i.e. sterile escapees) while improving fish welfare and providing a consistent year long quality product. Triploid induction in salmonids has been the subject of many studies and projects over the last 20 years but there are considerable gaps in our understanding of the environmental requirements, the family effects and overall welfare of these fish. To support this work the project is organised around a number of deliverables to provide protocols and additional information which will both help ensure the optimal design and analysis of the feasibility study, but will thereafter be innovative resources by themselves which will be available to the industry for future development.

The overall objective of the project is to strengthen the European research Area for Atlantic salmon culture which mainly is located in northern Europe. More specifically, the project aims to:

1) Strengthen the scientific understanding of biological needs of triploid fish for successful cultivation by testing and refining current husbandry protocols

2) Improve triploid fish welfare through better understanding of rearing requirements and environmental sensitivity

3) Perform field validation of triploid production in commercial on-growing systems

4) Assess family effect on triploid induction and contribution to performance

5) Explore market and societal acceptance and possible added value of the production type.

It is only through the establishment of a strong trans-national collaboration supported by key players in the salmon industry that such a project could be undertaken. Therefore, the two EU leading salmon breeding SMEs within the EU (Landcatch and Aqua Gen) joined in this project along with salmon fish farming SMEs from three principle European countries where salmon is produced (UK, Norway and France) with the support of leading EU Research Institutes (University of Stirling, Institute of Marine Research and Wageningen University Research centre). Collaboration between SME's and R&D institutions is without any doubt the key to a successful feasibility study.

The specific aims for the SALMOTRIP project period were:

1) To refine and produce triploid salmon to be used for lab-based studies and commercial trials (WP1)

2) To test the efficiency of lighting regimes, currently used in diploids, to produce out of season triploid smolts (WP2)

3) To test the sensitivity of triploid salmon to sub-optimal environmental conditions and husbandry practices, specifically, to test the effects of oxygen saturation and temperature on growth survival and deformities on one hand and tress, feed intake and disease challenges on another hand, and test the dietary effects on early seawater performances of triploid and diploid S0 in cages (WP3)

4) To assess the viability of rearing triploid salmon at a commercial scale and assess the relative merits of triploid and diploid salmon (WP4)

5) To study consumer perception analysis of triploid salmon, specifically to report on Intrinsic and extrinsic associations and beliefs with respect to triploid salmon products and production, and produce an appropriate questionnaire (WP5)

Project Results:

The SALMOTRIP project has been highly successful in achieving its objectives, and has attained some highly significant results. The following section will identify and summarise the main S&T findings of the SALMOTRIP project. Recommendations for future areas for research will also be provided. The key results are summarized in the form of:

A) Triploid Egg Production

B) Triploid Performance

C) Environmental Tolerance

D) Selective Breeding

E) Market and consumer perception

F) Areas for future research

A) TRIPLOID PRODUCTION

A major objective of the SALMOTRIP project was in the production of triploid fish for experimental and commercial trials. The second objective was to examine family-ploidy interactions with respect to triploid induction. Throughout the project, four different year classes (2007, 08, 09 and 2010) have been produced by partners in Scotland (Uos-LNS) and (IMR-AG). For experimental trials (UoS & IMR) up to 10 families (full-sib crosses) have been utilised, while increased family numbers have been produced for commercial trials (26-90, full & half-sib crosses). Triploid induction rate itself did not differ between family, with 100% success rate in all families when a standardized a hydrostatic pressure shock of 9,500 PSI (655Bar) was applied 300˚min post-fertilisation for a duration of 50˚min. Survival was generally comparable between ploidy and family in all experiments performed. From observations we conclude that egg quality had a greater effect on survival than family or ploidy when inducing triploidy. This is a major result and significant improvement over many previous studies and highlighted to farmers the importance of using eggs of the "highest quality" when looking to produce triploid fish. Traditionally, triploid egg production has often been based on using the last few remaining egg batches from the stripping season which may be of substandard quality to tolerate the triploid induction process itself. This factor may have confounded earlier studies and hence compromised growth & development of triploids used in many previous studies.

Results from this area of research and all trials conducted identify 5 areas for thought in order to make triploid production a viable commercial option. The following sections summarises

the points for consideration if triploidy were to be implemented at a commercial scale in the Atlantic salmon industry.

1) Improving Triploid Egg Survival

It is evident that triploids can have comparable survival to diploids at experimental level (<10% mortality). However, under commercial operations, triploid survival was generally lower than diploids, although diploid survival was also lower. In all trials (both experimental and commercial) most of mortalities (up to 50%) occurred during the pre-eyed to eyed egg stages. Thereafter, triploids had comparable survivals to diploids during all FW and SW stages. These observations suggest that the feasibility of triploidy may in part hinge on the improvements of egg survival during the hatchery operation stages. From results obtained envisage that using the best quality eggs (which requires indicators of quality to be developed in future research) and regular broodstock inspection/stripping may significantly improve triploid yield. Furthermore, improved egg handling and modified operational procedures to incorporate the triploid induction process may also improve yield.

2) Market Pressure & Up-scaling

If customers/legislators demanded it, broodstock farmers were asked if they would we be able to introduce triploidy treatment into commercial hatchery practices, in which case, if it was demanded, then they it was felt that the process could be incorporated at a cost on a small scale. However, when trying to upscale if 100% triploid production was adopted, then it was felt that it would necessitate a dedicated team and facilities, and multiple vessels. The key to success in commercial up-scaling will be to improve triploid egg survival (see above, point 1). 50% survivals and below would not be not economically viable, where broodstock/egg suppliers typically aim for ≥ 80% survival. It will also be essential to ensure 100% triploid rate if it is to be commercially acceptable.

3) Incorporation to Selection Breeding Programs and Neomale Production

The SALMOTRIP project did not include All-female, and at present we do not know if the industry would prefer All-female or mixed sex. The growth spurt in prepubertal males may be eliminated in All-female production due to lack of steroid increase in the spring period prior to the autumn spawning window. This was evident in the commercial trials, where males were typically larger than female triploids. However, using mixed-sex triploids, could still be successful if photoperiod regimes are implemented to prevent maturation of triploid males as is current industry practice in mixed-sex diploid stocks.

Producing All female triploids would also pose extra challenges in the broodstock production cycle. Producing neomales from scratch each year is not a big problem per se, but based on what we know from rainbow trout the success rate in the production of All-female is not always 100%. Therefore it is common to establish All-female" lines" (in order to be sure that 100% neomales is produced). In some countries such "lines" are established through gynogenesis. Depending on what process is chosen, the production of All-female will have impacts on the realization of genetic progress in the population (i.e. increased genetic lag).

4) Biosecurity

The impact of the triploid production process on biosecurity control measures will be dependent on each individual hatchery operation. Hatcheries will still be able to maintain a separation between clean and unclean areas, and the eggs will be disinfected before the eggs are transferred from the clean to the unclean area. However, where individual incubators are used per hen (e.g. family systems), the process could massively impact operations if every bucket was to be triploided, and as such less hens would be stripped per day. Any equipment would have to be disinfected between batches or pools. Increasing pool sizes beyond individual buckets would increase the risk of losses if there are any positives. Disinfection of equipment will be time consuming, and the temperature bath needed to control the rate of cell division prior to triploidisation would have to be changed every time, which in theory could be managed but it isn't easy. These aspects would be central to logistical planning if production levels are to be increased. Increased number of dead eggs could exacerbate problems with fungus. Maintaining biosecurity on such a commercial scale could be challenging to say the least depending on hatchery protocol.

5) Equipment, Logistics & Personnel

If triploidy was to be incorporated into procedures, it was felt that general hatchery operation would not be affected, although logistical considerations and planning would have to be implemented. If production of triploid eggs is to be up-scaled to constitute a major part of the total production (e.g. 100% triploid production), there have to be more than one line for shock treatment, depending on the size of the pressure chamber to keep apace with the stripping rate required from large numbers of broodstock utilised. Production of triploid eggs also implies challenges in the production process, primarily due to the accuracy in timing and temperature requirement. More personnel will be necessary to produce a certain amount of eggs. Production of triploid eggs must be reflected in higher price per egg, which also will be influenced by higher egg mortality. Depending on scale of operation there may be the need to add electrical infrastructure to maintain pumps, water and heaters etc. which may be beyond what most hatcheries currently have in place. Any impact on fertilization could consequently reduce survival due to fungal infection. Hatchery organisation could also be impacted if

mixed ploidy were produced within one hatchery, and control mechanisms would have to be put in place to keep dips and trips separate. Finally, the additional risk to wholesale losses through infrastructure failure would have to be factored in as a parameter in its own right (e.g. spare pressure chamber, water heaters etc.).

B) TRIPLOID PERFORMANCE

This area can be broken down in three parts with relevant key findings. These three areas were identified as Smolt Production, Growth Performance and Deformity Occurrence.

1) Smolt Production

Due to poor seawater performance of triploids previously reported in many studies, it was important to examine more closely the smoltification process in triploid fish. Secondly, it was important to establish whether out-of-season (S0+) triploid smolts could be produced, which is an important step to ensuring year round sustainability of production of Atlantic salmon. The SALMOTRIP results are the first demonstration that triploid out-of-season (S0+) smolts can be successfully produced using conventional square wave advancing photoperiods (LL-LD10:140LL). Triploids also smolted earlier than diploids under S0+ regimes (up to 4 week advance) suggesting different size thresholds for successful smoltification may exist. This remains to be confirmed in future studies. Saltwater adaptation mechanisms (i.e. gill Na+K+-ATPase profiles) are similar between ploidy with triploids showing typical smolt characteristics (fully silvered, low K factor) which is in contrast to previous literature. This should facilitate easier monitoring. Smoltification success was influenced by family, and is most likely linked to growth rate. Triploid survival during sea transfer and subsequent on-growing was excellent and is comparable to that of diploids, a significant improvement over previous reports. Overall, findings suggest that once malformation issues are resolved (see later) then triploidy may indeed be a commercially viable option.

2) Growth Performance

In both experimental and commercial trials, survival after first feeding has been comparable between ploidy in both fresh and saltwater. Furthermore, triploid growth rate was consistently 20-30% greater during freshwater rearing than their diploid siblings although this was affected by family in both experimental and commercial conditions. However, growth of triploids in seawater was either comparable or slower (up to -10%) than their diploid siblings in all trials, although initial triploid growth (3-6months) was higher. Despite the differences in growth between fresh and saltwater stages, actual growth rate of triploids from egg to table was the same as diploids in all trials. Growth was significantly affected by sex, with males

growing faster than females in both ploidy. Female diploid and female triploids however grew at a comparable rate. However at both commercial and experimental levels we observed that triploid growth rate in both fresh and saltwater was lower at higher water temperatures (>18˚C) than it was at cooler temperatures (<16˚C) in sibling stocks, and suggests rearing environment and site selection may need careful consideration (see section C-Environmental Tolerance). Production traits for growth exhibited good heritability estimates (h2 >0.2). Maturation rates were generally low (<5%) in all trials, although lowest in triploid (<0.2%). Diploid males exhibited the highest prevalence of grilsing. Interestingly triploid growth was also compromised by mixed ploidy rearing, and may explain poor growth results in some previous studies. Harvest quality was comparable between ploidy with similar grading scores. Down grading was generally due to melanin, deformed or under-sized fish. Flesh quality was comparable between ploidy, although triploids appeared to have a higher lipid content and warrants further research to determine optimum dietary levels and feeding rates.

3) Deformity Occurrence

Externally visible deformity was generally low and comparable between ploidy during freshwater rearing, but triploids appeared more prone to increased deformity and severity in seawater stages particularly jaw and vertebral anomalies. X-ray radiography also revealed that vertebral deformities are present in freshwater, but only manifest themselves in terms of severity during seawater stages (where growth is typically more rapid in salmon) and subsequently reduces growth performance. Diet supplementation of phosphorous during saltwater grow out did not influence or reduce deformity occurrence. In an additional study we have shown that the vertebral deformities that triploids typically develop in freshwater can be avoided by supplementing their diet with extra phosphorous. Deformity occurrence thus appears to be a multifaceted trait, and is a function of faster growth rates and nutritional deficiency in the diet and/or environmental sensitivity (e.g. increasing water temperature) in the saltwater environment. This requires further investigation to determine dietary inclusion levels for "triploid specific" diets. Cataract severity was significantly higher in triploids, and as with deformity, appeared to be a function of faster growth rate and increasing water temperature. Trials provisionally exploring higher dietary Histidine inclusion (an essential amino acid known to decrease prevalence in diploids) prevented the increase in cataract severity in triploids. This supports the hypothesis that triploids appear to have higher nutritional requirements to support their more rapid growth. Finally, there was an interaction between ploidy and deformity and/or cataract also suggesting that certain families in the "triploid state" may be more prone to malformation. Overall results suggest that dietary deficiency in triploid diets may in part account for occurrence of deformity and cataract, which then subsequently reduces growth. This nutritional aspect and interaction with environmental and growth must be addressed as a priority in future studies. Overall, reducing the incidence of deformity in triploids may be possible by addressing nutritional requirements of triploids during periods of rapid growth, and requires further research to develop "triploid specific" diets. We believe that success of viable triploid production will be based upon future research into determining their dietary requirements and developing triploid specific diets.

C) TRIPLOID ENVIRONMENTAL TOLERANCE

The SALMOTRIP project specifically investigated occurrence of deformities with respect to vaccination, diet and environmental conditions. High temperatures reduced growth in both ploidies, while triploid growth was lower than diploid siblings at high temperature (19oC), but growth was not different at optimum temperature (15oC). Triploids also showed increased mortality at high temperature, but no differences were found between ploidy at lower temperature. Results indicate that there may be a thermal maximum for successful triploid tolerance, growth and survival when oxygen is limited to 90% saturation. Under commercial rearing due to the chosen site geographic locations, triploid stocks experienced a wide range of environmental conditions. Depending on rearing site environmental conditions (principally water temperature) again appeared to affect growth, deformity and mortality in both diploid and triploid sibling stocks. We confirmed that triploid growth rate in both FW and SW was lower at higher water temperatures (>18˚C) than it was at cooler temperatures (<16˚C) in sibling stocks. Triploids also had greater deformity (Jaw & Vertebral) prevalence at lower temperatures, than diploids, while no difference was observed at higher temperatures. These results indicate that higher growth rate is a significant factor influencing deformity occurrence in triploids. Lower oxygen saturation at high temperature reduced growth in both ploidy, but the effect was greatest in triploids. Triploids also showed increased mortality at reduced oxygen saturation (70%) under high temperature, but no differences were found between ploidy at 100% saturation. Results indicate that oxygen saturation in the culture environment should be maintained as close to 100% as possible for triploids, particularly during periods of high temperature. Triploids showed higher deformity and cataract than diploids at high temperature irrespective of oxygen saturation, suggesting temperature and possible interactions with higher growth rate are in part responsible for increased occurrence of deformity. Results from blood parameters suggest there may be some differences in metabolic homeostasis, and functional differences in energy budgets between ploidy particularly under oxygen deficit. This requires further studies looking at tissue/organ specific and physiological response to sub-optimal environmental conditions. Triploids grew faster than diploids both in freshwater and seawater and growth was also affected by vaccination so at the termination of the experiment the unvaccinated triploids were biggest and the vaccinated diploids were smallest.

Based on these findings the SALMOTRIP project has recommended that where triploid salmon are farmed that husbandry in general should not differ from that of diploids. However, considerable care should be taken during grading events, bath treatments, crowding, vaccination and anesthesia to avoid oxygen deficits (i.e. increased oxygenation/aeration) particularly under high water temperatures. Furthermore, careful evaluation of site selection for growing triploids should be given with respect to seasonal oxygen and temperature profiles.

D) SELECTIVE BREEDING OF TRIPLOIDS

Family had a strong effect on growth, deformity and cataract. Growth within a given family did not differ between ploidy suggesting, the best performing diploid stock could be used to create the best performing triploid stocks. Family also had a significant influence on the prevalence of deformity and cataract severity. FW ranking for traits of interest were not consistent among family other than survival & smolt rate, while SW ranking for traits of interest appears to be consistent and strong among ploidy for all families. Results are suggestive that selection traits, principally based on saltwater performance characteristics as used in conventional diploid broodstock selection could be equally applied to breeding programs where triploids wish to be produced, i.e. the best performing diploid parentage also make the best performing triploids. This is a significant advancement in the knowledge and shows the potential for triploid specific breeding programs exists, and could easily be incorporated into current breeding programs of Atlantic salmon. However, considerations presented in section A will need to be addressed to make it a viable success. For practical purposes, a genetic correlation of one is not necessary in order to select diploids for performance as triploids as long as the correlation is substantial and in the favourable direction (i.e. positive), as seen within the family evaluation assessments under the SALMOTRIP project. However, the rate of progress in a diploid-based selection program might not be expressed to the same extent in triploid stock derived from it. It would become a matter of personal judgement as to how far a genetic correlation would have to fall before initiating a testing regime based directly on triploid performance.

E) MARKETING AND CONSUMER PERCEPTION

The objective of WP5 was to create an overview of associated negative (risks) and positive (benefits) perceptions and beliefs held by consumers towards triploid salmon products and production in order to develop effective communication messages to be used by the industries. To test the effectiveness of the communication to improve value and avoid negative consumer images in realistic settings.

1) INTRINSIC AND EXTRINSIC ASSOCIATIONS AND BELIEFS WITH RESPECT TO TRIPLOID SALMON PRODUCTS AND PRODUCTION

Firstly we explored intrinsic and extrinsic associations and beliefs with respect to triploid salmon products and production. This was investigated in four main areas:

i) Legal issues and EU regulations on package labelling; in which animal welfare needs to be guaranteed: triploid salmon requires the same welfare conditions as regular salmon. Importantly, production methods do not need to be indicated on the package except for genetic modification (2009). There are currently (2009) no regulations on triploid salmon with regards to required information on the food packaging, although EU regulations on package labelling might change in the future.

ii) Internet discourse analysis: which information in triploid salmon is accessible by the lay public on the internet? We observed that triploidization is associated in a positive way with trout, referring to bigger fish that do not harm the environment, while triploidization is associated in a negative way with genetically modified organisms and farmed salmon. We suggest that positive communication on triploid salmon might be possible as it is also available on triploid trout. It is essential that communication on triploid salmon should aim at distinguishing it from GMO issues and negative associations with farmed salmon.

iii) NGOs opinions on triploid salmon; since triploid salmon is in such an early stage of development, some NGO's indicated that they held no formal opinion it. Others based their opinion on the worst case scenario. Six dimensions were important to the NGO's in forming an opinion on triploid salmon: farmed salmon, GMO, environment, health, animal welfare, and the public. The main risks in communication on triploid salmon will be GMO and the remaining problems of escapee salmon.

iv) Consumer opinions on triploid salmon; some consumers perceive triploid salmon as unnatural and therefore unwanted. On the contrary, some consumers perceive triploid salmon as advantageous as long as animal welfare, human health, the environment and the sensory aspects of the salmon did not suffer from it. In general consumers were not well aware of escapee problems. Information on escapee problems raised questions in the consumers on GMO and farmed salmon. Importantly consumers prefer information on triploid salmon to come from the EU and the NGO's.

2) QUANTIFIED RELATIONSHIPS BETWEEN THE DIFFERENT ASSOCIATIONS AND BELIEFS, AND ESTABLISH THEIR IMPORTANCE

Based on these information scenarios we developed a questionnaire in order to examine quantified relationships between the different associations and beliefs, and establish their importance. In total over 3000 participants from the UK, France and Germany completed the questionnaire. The main conclusions of the survey were that the impact of the communicator of information depends on the level of trust a consumer has in that communicator, especially if the communicator is from the industry. Explaining both the environmental and economic arguments helps to increase acceptance. Perceived benefits are equally accepted from NGO's and industry. Perceived risks are higher when communication is coming from industry - depending on the level of trust in industry. In industry communication, emphasis should be on reducing perceived risks and increasing trustworthiness. Explaining all related interests including the economic ones might serve that purpose. Three consumer segments were identified based on acceptation scores:

a) Progressive consumers (25%): positive associations with sterile farmed salmon;

b) Romantic consumers (23%): negative associations with sterile farmed salmon

c) Moderate consumers (52%): Mixed associations with sterile farmed salmon.

The distribution of these three consumer segment differs somewhat between the three countries: the progressive consumer group is relatively large in the UK, while the romantic consumer group is relatively large in France. Communication on triploid salmon should be aimed at the moderate consumer segment as this segment is most influenceable.

3) APPROPRIATE COMMUNICATION MEANS FOR TRIPLOID SALMON WITHIN THE EU SALMON INDUSTRY

General acceptation of triploid salmon was 3.5 on a 7-point scale, which is fairly low. The acceptation rate varies between 2.5 and 4.5. Similarly trust in salmon farmers was scored fairly low at 4 on a 7-point scale. Negative attitudes towards triploid salmon are associated with consumers that are either food technophobic or concerned about health and nature.

We observed that the response to additional information on triploid salmon divides the consumers in a persistent group (71%) and a reactive group (29%). This distribution is consistent throughout the three participating countries (France, UK, Germany).

* The persistent group (71%)

o is quite indifferent to the type of information provided on triploid salmon;

o does not change its mind much as more information becomes available on triploid salmon.

o responds more to price promotions compared to the reactive group.

o responds slightly positive to the recognition of triploidy as an already accepted technique in food production.

* The reactive group (29%), with an initial negative response to the idea that sterile products are already available, consists of:

o a negative reactive group (12%) which is unlikely to buy triploid salmon at this moment and with a negative response to information provision, reducing acceptability of triploid salmon even more;

- and a positive reactive group (17%) reacting positively to specific information on the positive effects of sterility, increasing acceptability of triploid salmon.

Taken collectively, the information on positive aspects of sterility might provide a useful information means to reach the persistent consumer group and the positive reactive consumer group. Furthermore, the lack of reaction of the persistent consumer group to any type of information can be perceived as promising from a producer standpoint, in the sense that they also do not tend to respond negatively to additional information. However, the lack of reaction of the persistent consumer group may also be considered a risk should negative associations be formed in this group. In this sense it is important to be aware of the perceived risk of sterility in this group. Of all consumer groups the positive reactive consumer group seems to be most sensitive to a combination of environmental and economic arguments in favour of triploid salmon as compared to only environmental or only economic arguments.

4) EFFICACY OF TRIPLOID COMMUNICATION MESSAGES IN REALISTIC SETTINGS

Finally we tested efficacy of triploid communication in realistic settings based on product labelling. In general the amount of information (smoked salmon, smoked triploid salmon, smoked triploid salmon + explanation) on packaged smoked salmon has little effect on the evaluation of characteristics of the salmon (e.g., colour, texture, fattiness) by consumers. In some cases, salmon labelled with less information was considered more salty than salmon labelled with more information. The amount of information on packaged smoked salmon has no effect on the evaluation of tastiness and other quality evaluations of the salmon. Tastiness was evaluated fairly positive regardless of the amount of information on the package label. It is currently not known if the amount of information on packaged smoked salmon will affect purchasing behaviour of triploid salmon.

In general we can conclude that the type and amount of information on triploid salmon does not affect the sensory evaluation of triploid salmon. This overall result is promising as potential negative associations with triploid salmon are not translated to the sensory evaluation of the salmon, regardless of the amount on information on the package. Associations with quality aspects of salmon are also not affected and overall healthiness ratings were still 5 or higher on a seven-point scale. These outcomes show that potential negative associations with triploidy or farmed salmon do not result in less or poorer evaluation of the product, regardless of the amount of information on the package.

F) SUMMARY & FUTURE AREAS OF RESEARCH

Based on the results generated within the SALMOTRIP project current expectations from triploid Atlantic salmon if they were to be cultured at present would be that:

Positive Expectations

* Show comparable survival to diploids from first feeding onwards

* Would successfully smolt under natural and out-of-season regimes

* Growth rate from egg to table is the same as diploids

* Harvest & flesh quality is comparable to diploids

* Explaining both the environmental and economic arguments helps to increase acceptance.

* In industry communication, emphasis should be on reducing perceived risks and increasing trustworthiness.

* Communication on triploid salmon should be aimed at the moderate consumer with mixed associations with triploid salmon (52% of the population) as this segment is most influence able.

* The recognition of triploidy as an already accepted technique in food production slightly increases the acceptance of triploid salmon in the majority of consumers (71%). Additional information on triploid salmon does not affect their opinion much.

Negative Expectations

* Increased prevalence of visible & internal deformity (severity highly variable)

* Increased occurrence of cataracts (severity highly variable)

* Some sensitivity to low oxygen conditions at high water temperature

* There is little knowledge on triploid salmon among the public and experts.

* Negative associations with triploid salmon mainly concern GMO and farmed salmon.

* Part of the NGO's and consumers are sensitive to the possible positive benefits of triploid salmon farming.

* Consumers that respond negatively (12%) to information on triploid salmon are not likely to buy triploid salmon at this moment. They have a strong desire for labelling of the salmon in order to be able to avoid it.

Given these observations we have identified critical areas which must be evaluated by further research studies before triploid salmon can become a commercial reality. These include:

* Address occurrence of malformation based on our findings

* Critical to define triploid dietary requirements & develop specific diets

* Continuation of family assessments

* Development of neomale broodstock lines

* Sea lice & disease challenge studies under optimal and sub-optimal conditions

* Consideration of communication messages to target different market and consumer groups

Potential Impact:

This section provides areas where key SALMOTRIP results have been or could be implemented to have impacts wider than R&D exploitation.

Contribution at the European and/or International level

SALMOTRIP results have generated new innovative culture options and identified areas that must be addressed in which thereafter could significantly improve economic performance of the salmon industry in Europe while addressing environmental and farmed animal welfare concerns. Important concern of the European member states is the competitiveness of industries. SALMOTRIP has made a significant European scientific contribution to maintaining the license to produce by improving knowledge about issues of current societal concern, e.g. environment and sustainable development and animal welfare.

(1) SME Competitiveness

The competitiveness of the SME partners could be considerably improved if the developments are adopted and recommendations for future research followed. By using sterile fish, the impact of escapees on wild fish populations will be minimised, and ultimately result in the ability to market a more environmentally friendly product. Furthermore, by avoiding maturation through farming sterile fish the overall economic return from the culture operation can be drastically improved. Comparison can also be made between the different country locations to observe the effects of three different environments on estimated economic parameters. On this basis, information from the three different European regions (UK, Norway, France), results can be used to modulate protocols and advice for the European salmon culture in general. SALMOTRIP has developed cooperation between fish farms and scientific institutes by promoting active exchanges of knowledge and know-how ensuring the potential to rapidly implement the key results into farming practice. One of the key successes of the SALMOTRIP project has been the development of "The Triploid Salmon Farming handbook: Guide of Best Practice" which is available to the fish farming industry. Furthermore, the involvement of aquaculture producers and breeding organisations in SALMOTRIP (LNS and AG) will ensure the rapid implementation in Europe of all feasible solutions developed within the project. Furthermore, the commercial exploitation of producing sterile fish would be an additional advantage from an IPR perspective by preventing unauthorized propagation of domesticated strains. The information generated within the SALMOTRIP project is relevant not only to the species studied herein but also to other species of commercial interest to fish farming.

(2) Contribution to Policy Developments

The SALMOTRIP project has generated knowledge that could contribute to the development of current and future policies to preserve and/or enhance the environment. The rational development of sterile salmon farming with an efficient control of maturation and a higher feed efficiency (maturing fish do not feed) would help to preserve wild stocks by reducing pressure on wild fishmeal fisheries, in compliance with the recommendations of the Convention on Biological Diversity. It would also significantly contribute to the protection of the genetic integrity of any local populations. Even a very low percentage of surviving offspring or reproductively competent escapees could have a significant negative impact on discrete genetic populations by interacting and propagating with wild stocks. Furthermore, the ideas and technologies developed by the SALMOTRIP project will contribute to the implementation of EU and National Policies, particularly with regard to the Common Fisheries Policy and Codes of Best Farming Practice. If adopted by the salmon farming industry, triploidy could result in a more cost effective and therefore more sustainable aquaculture industry, with higher competitiveness and higher feed efficiency providing protection against crisis in a quite fragile sector. The circumvention of maturation can significantly shorten the production cycle meaning a reduction in waste production and feed requirements per production unit making the cycle more economically efficient and environmentally friendly. This should also result in the development of alternative employment possibilities for agricultural workers in rural areas.

SALMOTRIP can contribute to European policy in several ways:

☐ European Research Area: By networking major scientists in a wide range of disciplines with regard to improved farming methods and environmental impacts of aquaculture

☐ Sustainable Development: By improving aquaculture for the environment, taking into account technical, social and environmental aspects. 'Towards a Global Partnership for Sustainable Development'(2002), makes it clear that for the EU a priority objective is to ensure that the objectives of sustainable development are progressively integrated into European polices, with due respect to both their internal and external dimensions. To that end, the process of adapting the Common Fisheries Policy should be continued.

☐ Sustainable development: To meet the needs of the present generation without compromising those of future generations - is a fundamental objective under the Treaties of the European Community. The European Council invites industry to take part in the development and wider use of new environmentally friendly technologies - in 'SALMOTRIP' the relevant industries are integrated into the project. In this context the European Council stresses the importance of decoupling economic growth from resource use. The Union's Sustainable Development Strategy is based on the principle that the economic, social and environmental effects of all polices should be examined in a co-ordinated way and taken into account into decision-making (European Council meeting in Gothenburg, 2001). Among the

priorities identified is the management of natural resources in a more responsible way. Strong economic performance must go hand in hand with sustainable use of natural resources, lowering levels of waste, maintaining biodiversity, preserving ecosystems and avoiding desertification. To meet these challenges, the European Council agrees that the review of the Common Fisheries Policy will take this into account, and that biodiversity decline should be halted. The Council aims to finalise and further develop sector strategies for integrating environmental considerations into all relevant areas of Community policy. At the Brussels summit (2003), the EU committed itself to maintain its leading role in promoting sustainable development on a global scale by, among other measures, ensuring the protection of the marine environment and natural resources, including biodiversity. Exploitation of key 'SALMOTRIP' results will assist in achieving these goals.

☐ Common Fisheries Policies: By circumventing sexual maturation by using sterile triploid fish in culture the environmental impact of genetic pollution is taken into account. In the Green Paper on the Future of CFP (2001), it is stated that European aquaculture needs to confront effectively the challenges arising from environmental requirements. The SALMOTRIP project has provided significant knowledge on production techniques and requirements for potentially implementing the use of triploids as a viable culture option.

☐ Food Safety and Human Health: The SALMOTRIP project addresses these issues by working on improvements in production technologies, while maintaining quality assessment methodologies suitable for aquaculture salmon. This aspect will improve animal health and allow for a more sustainable food production and enhance the trust of consumers in responsible, safe food production. Increase of fish consumption by humans is desired to reduce risk of cardiac diseases. The development of products that will fit better with the demand of the consumers is of prime importance to improve European consumption habits. Moreover increase of aquaculture production is also required in order to respond to growing demands as markets from new member states develop.

☐ FP7 Food Quality and Safety: By improving salmon farming the aim is to achieve safe, qualitative food in sustainable circumstances are met.

☐ FP7 Research and Technological Development: SALMOTRIP has integrated the following cross-cutting issues of FP7: 1) Needs of SME's, 2) Ethical principles and requirements, 3) Women in science, 4) Socio-economic aspects, 5) Reporting to European Parliament and Council and 6) Promotion of innovation.

(3) Contribution to Community Societal Objectives

Education

As reported during the PROFET meetings in 2003 and 2004 all over Europe (FEAP), there is a lack of cooperation on transfer of technologies. In this respect, the SALMOTRIP project has strengthened cooperation within the European salmon industry by studying, applying and transferring current and new scientific knowledge generated during this project. The

SALMOTRIP project results have the ability to promote awareness and understanding of the benefits of farming triploid salmon to the public sector (see dissemination).

Employment

Aquaculture is carried out in more remote parts of Europe and thus provides much needed employment in these areas. The social-economic impact of salmon farming is especially important in the rural community in UK, France and Norway. Exploitation and implementation of SALMOTRIP results could contribute indirectly to the employment of aquaculture SME's (ensuring their 'licence to produce' from society), and to a rational debate among producers, consumers, and policy makers and fro decision-making based on technological, economical and societal information on this issue.

Training and an adequate human resources policy are of equal importance, since both help the labor force to adapt to changed skill demands. The availability of a skilled labor force offers greater productivity, facilitates the dissemination of new knowledge and techniques, and consolidates those exposed to international competition. By favoring exchange of expertise and skills inside the project, and particularly towards the industrial partners, SALMOTRIP will contribute to improve human resources. In the medium to long term, this potential will help create new jobs in existing or to-be-created hatcheries, thus offering new opportunities for students in fish farming. Atlantic salmon is also one of the model species for numerous scientific studies, and as in the past, R&D development on this species could also benefit other aspects of aquaculture.

Contributions to Standards

The SALMOTRIP project will investigate alternative culture options and provide improved husbandry protocols for triploid salmon farming within Europe. SALMOTRIP results provide a reference voluntary standard for viable and sustainable aquaculture production of salmon. Improved growth, quality and welfare could have implications not only for the economic outcomes, but also how the public perceive the industry. The market perception analysis conducted within the project contribute to the acceptance of voluntary reference standards in the trade of aquaculture salmon. This information will provide marketers and consumers with valuable information about the characteristics of different products that are available on the market.

Raising Public Participation and Awareness

The main risk perceived by society regarding salmon aquaculture relate to the environment and sustainable resource utilisation, which was addressed by the SALMOTRIP project by developing methods to avoid maturation and minimize impacts of escapees of salmon in culture. The consortium will ensure that discussions about this in various scientific circles as well as in the media will be monitored during the project, and communication actions will be taken as appropriate to inform relevant stakeholders about the possible benefits to be derived from the SALMOTRIP project, and how it aims to reduce the impact of escapees. Also improvement of feed efficiency in a longer on-growth period will help to reduce water pollution of the fish farms which will reduce potential negative impacts of this industry through environmental enrichment. Furthermore, the welfare, quality parameters and nutritional value of aquaculture salmon are of interest to consumers, and communications about such results from the project were included in presentations about the SALMOTRIP project to enhance confidence and trust in the food supply from salmon culture.

MAIN DISSEMINATION ACTIVITIES AND EXPLOITATION OF RESULTS

The SALMOTRIP project identifies the following key results as areas of exploitation for SME partners and for future dissemination. In some instances active uptake of this knowledge has already occurred, while other areas require further research in order to make these a viable option should the production of triploids within the industry be proposed or enforced through legislation changes. The following sections summarise the key areas of actual or potential exploitation.

* Transfer of triploid induction technology to SMEs

RTD partners (UoS and IMR) have provided full training in triploid induction techniques to all SME partners and staff within the respective facilities, and have provided recommendations and refinements on working protocols. Breeding partners (LNS and AG) benefited from such training as it has allowed them to identify areas of current hatchery operation that need to be modified in order to initiate effective triploid egg production. It was also viewed highly important that this technology could be further utilized by these companies to protect their IPR of selected strains by having the ability to produce and sell sterile eggs/fish and thus prevent unauthorised propagation. Such technological knowledge could be implemented across the whole EU salmon production sector in the future. At present both breeding companies have received order enquiries for triploid eggs from the farming sector wishing to trial triploid salmon within their own on-growing facilities.

* Refined husbandry protocols for triploid smolt production & smolt monitoring

Although industry protocols for out-of-season diploid smolts currently exist, the SALMOTRIP project has explored and successfully demonstrated for the first time in both experimental and commercial trials the potential to produce out-of-season triploid smolts, which is critical to ensure that SME on-growers can provide a year round product should triploidy be adopted within industry. This information will allow the establishment of a standardised industry working protocol through knowledge transfer exchange within the sector.

An essential part to successful smolt transfer is that of the monitoring of the smolt process, and we have determined that diploids and triploids show differential patterns of smoltification. The major implication of this is that triploids cannot be regarded as the "same" as diploids and have different characteristics. Importantly, from a scientific viewpoint, these findings could in part explain high mortalities in earlier published work where triploids were subjected to conventional diploid rearing methods with little thought to their physiological differences. Overall, these results have benefited RTD scientific knowledge and further the understanding of smolt physiology, which has ultimately allowed knowledge transfer from RTDs to SMEs to advise on the most appropriate times for smolt manipulation and how best to monitor these changes to ensure optimum success rate. SMEs (SDF, CAC) and other enterprises (MH) will therefore be able to adapt and enhance their current practices, and have benefited by having access to and training in smolt monitoring tools/techniques provided by RTDs. This advancement in knowledge is not only applicable to triploid salmon but may also further enhance the understanding of standard commercial diploid smolt production and monitoring.

* Identification of environmental and husbandry sensitivities

The different environmental "challenge" studies conducted with the SALMOTRIP project, in many ways confirm earlier research and observations, and have defined environmental conditions and husbandry procedures and/or combinations thereof that are detrimental to triploid welfare. Triploids do not perform well under high temperatures and hypoxic conditions. Our results also show that they are more susceptible to hypoxic conditions than temperature and that they perform acceptably under high temperatures for short periods and especially if given normoxic conditions. They also have a clear tendency to be more susceptible to developing deformities and cataracts and may in part be induced by more rapid growth. However, we have shown in the additional study that the most common deformity in triploids, a shortening of the vertebra in the trunk region of the vertebral column, can be prevented if the fish is given a diet with a higher availability of phosphorous. It is known that cataracts in diploid can be prevented with increasing levels of histidine in the diet. It is possible that triploids need a higher inclusion of also histidine, again suggesting the need for the development of triploid specific diets.

Large parts of the salmon production in the world is conducted in regions where high temperatures is not a problem. Triploid production should be an acceptable alternative to diploid production in these areas. However, the limitations in the temperature and hypoxic tolerance of the triploids should be investigated further. Together with historical environmental data, this information could be exploited for use in a feasibility study to make recommendations to where triploid could be produced without detrimental effects on their welfare. These findings will also aid the generation of standard operating conditions for SMEs in which triploids should not be cultured. Furthermore, SME partners will also be able to identify potential risk areas or times of year when sub-optimal conditions may be encountered. As such, specific risk planning measures may thereafter be developed by SMEs to account for different operating procedures. Should triploidy be adopted or enforced at the EU level, these results could be made commercially available given consortium agreement. This information will also be essential in legislative decision making should a step towards the adoption of triploidy be considered prior to implementation.

* Development of a deformity assessment scheme and transfer to SMEs

RTD partners provided full training in deformity assessment to SME partners and developed a standardized guide for reporting malformations with correct terminology. Causal effects and contingency procedures were also identified and remediation options explored. Results may be exploited to develop a standardised deformity assessment scheme applicable across the industry. Using a standardized scheme brings greater cohesion between cross-comparison of data and farming practices and will allow clearer identification and modeling of causal effects of specific malformations within triploid and diploid salmon stocks globally. On validation, such a tool may be exploited by other end-users within the salmon industry following agreement by the consortium on how this should be achieved and/or licensed.

* Selective Breeding of Triploid Salmon

As triploid salmon do not reproduce it is essential to determine how future generations can be produced and/or improved through selection of existing diploid broodstocks. LNS currently have Parentage Assignment Panel (PAP) software for diploid fish to determine parentage contribution to performance based on 2 chromosome sets. However, triploids carry an additional chromosome set and thus the current model cannot predict parentage. Family trails in the SALMOTRIP project were used to modify this software in conjunction with the expertise of UoS to develop a model for use in triploids based on a 3 chromosome construct. The outcome has been highly successful. Although this software will remain the IPR of LNS the knowledge of construction will be available to all consortium partners.

Results are suggestive that selection traits, principally based on saltwater performance characteristics as used in conventional diploid broodstock selection could be equally applied

to breeding programs where triploids wish to be produced, i.e. the best performing diploid parentage also make the best performing triploids. This is a significant advancement in the knowledge and shows the potential for triploid specific breeding programs exists, and could easily be incorporated into current breeding programs of Atlantic salmon. These findings provide options to SMEs to allow the correct selection of broodstock to optimize diploid production as well as producing triploids should the market and legislation dictate a requirement.

* Triploid Farming Handbook

Collectively, the results from the SALMOTRIP project have been used to develop a "Triploid Salmon Farming Handbook: Guide to Best Practice". At present this document has been circulated within all consortium partners and within their own organizations. At present as triploid production has not been enforced the Handbook will remain within the consortium, however, the document will be made publically available. At present the handbook defines current expectations of triploids based on the SALMOTRIP experiences, and provides areas of recommendation to potentially avoid problems. However, for full implementation considerable research is required within triploid nutrition and a greater understanding of their environmental and physiological tolerances. On such knowledge generation the Handbook will be updated and can be circulated within the sector accordingly. Promotion of the Handbook and the major SALMOTRIP project results formed the basis of the project workshop held at the EAS Conference, Porto, 2010.

* Defining a marketing strategy

Results generated by WUR in WP5 could be deployed to aid market communication if triploid salmon became available for marketing. This information will be available for all SMEs to develop and meet their own specific marketing strategy and needs. In some instances some consumers perceive triploid salmon as unnatural and therefore unwanted and will important to address these fears with the correct message. Importantly some consumers perceive triploid salmon as advantageous as long as animal welfare, human health, the environment and the sensory aspects of the salmon did not suffer from it. In general consumers were not well aware of escapee problems. Information on escapee problems raised questions in the consumers on GMO and farmed salmon. However, of significant importance is that any information messages conveyed to consumers would be preferred to come from the EU and the NGO's and not the industry itself principally to do with issues of trust. As anticipated the distribution of these consumer segment differs somewhat between countries, however, ccommunication on triploid salmon should be aimed at the moderate consumer segment (Mixed associations with sterile farmed salmon) as this segment is most influence able. However, formulating consumer segments based on their reactivity to additional information on triploid salmon might provide more insights. Finally, with respect to product labeling the overall conclusion that type and amount of information on salmon has little or no

effect on the evaluation of the salmon is very promising as potential negative associations with triploid and farmed salmon are not translated to the sensory evaluation of the salmon, regardless of the amount on information on the package. It is currently not known if the amount of information on packaged smoked salmon will affect purchasing behaviour of triploid salmon. Collectively the findings of the marketing research could be further assimilated within the EU market to show for example, how retailers should approach their customers when offering a new product.

List of Websites:

During the project a publically accessible website (http://www.salmotrip.stir.ac.uk) was established to promote the main aims of the project research (Fig. 1). The website was also used for promotional events including the project workshop held at the EAS Conference, Porto, 2010 (Fig. 2). The website will be updated at the end of the project to convey the major research findings obtained from the research conducted and will also provide open access to the Triploid Salmon Farming Handbook (Fig. 3). For additional promotional material at other conferences and networking events a brochure was also produced (Fig. 4). Finally, a list of all beneficiaries within the project are detailed in Table 1. with contact person for each organization.