Harmonisation of Regulatory Oversight in Biotechnology

Safety Assessment of Transgenic Organisms in the Environment, Volume 7

OECD CONSENSUS DOCUMENTS

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Foreword

From their first commercialisation in the mid-1990s, genetically engineered crops (also known as "transgenic" or "genetically modified" plants) have been approved for commercial release in an increasing number of countries, for planting, entering in the composition of foods and feeds, or use in industrial processing. Up to now, the majority of these agricultural productions remain for soybean, maize, cotton and rapeseed (canola) bearing pest resistance and/or herbicide tolerance traits aiming to improve yields and reduce the costs of production, as outlined in the OECD study *Farm Management Practices to Foster Green Growth*. Other engineered crops might gain importance and come into play in the short to medium term. Despite differences in total estimates, all analyses and statistics concur in underlining the general increasing trend in volumes produced and traded, and growth potential for agriculture productivity. For instance, the International Service for the Acquisition of Agri-biotech Applications reports in its annual *Global Status of Commercialized Biotech/GM Crops* survey that the surface area of transgenic crops worldwide has constantly increased since the first commercial planting in 1996 to reach 185.1 million hectares grown in 26 countries in 2016. To date, genetically engineered varieties of over 25 different plant species (including crops, flowers and trees), and more recently of two animal species, have received regulatory approval in OECD and non-OECD economies alike. Such approvals for release in the environment usually follow a science-based risk/safety assessment before being granted.

The five main producers of genetically engineered crops in 2016 were the United States, Brazil, Argentina, Canada and India, covering together almost 91% of the total area. Interestingly, developing countries grew more of global transgenic crops (54%) than industrial countries, at 46%. Among the 26 countries having planted those crops in 2016, only 9 of them were OECD countries, listed by decreasing area as follows: the United States, Canada, Australia, Spain, Mexico, Chile, Portugal, the Slovak Republic and the Czech Republic. This represents, however, a significant part of the total agricultural acreage in OECD countries: the *OECD Compendium of Agri-environmental Indicators* estimates that about 18% of the total OECD arable and permanent cropland area was sown to transgenic crops on average for the 2008-10 period. In addition, some countries do not grow genetically engineered plants but import commodities derived from them, for use in their feed industry in particular. This is the case in several European countries and some other economies worldwide: according to the ISAAA, from 1992 to 2016, a total of 40 countries gave approvals for such commodities to be used as food or feed, and/or cultivation.

Information on the transgenic crops approved for commercial release in at least one country for use in agriculture and/or foods and feeds processing can be found in the OECD *Biotrack Product Database* (https://biotrackproductdatabase.oecd.org). Each transgenic product and its Unique Identifier are described, with information on approvals in countries. To date, this database covers about 255 approved genetically engineered plant varieties, and will be extended in future years to include additional species and information from a larger group of countries.

Modern biotechnologies are applied to plants, but also to trees, animals and micro-organisms. The safety of the resulting genetically engineered organisms when released in the environment for their use in agriculture, the food and feed industry, as biofuel or for other applications represents a challenging issue. This is already true for transgenic crops, and will be even more critical in the future as applications of biotechnologies widen to new species and new areas: a growing number of novel organisms will have to be assessed before their possible use and market release. Recent examples include animal species: since 2014, genetically engineered mosquitos have been used in several areas to control insect populations and contribute to fighting the diseases transmitted by them; an Atlantic salmon strain, modified for fast growth, was approved in a few countries in 2015-16 for production in confined environments and commercial use.

Among biotechnology developments, some crops (maize, sugarcane) have been modified for adaptation features such as resistance to certain biotic/abiotic stresses including drought tolerance, leading to better resilience to climate change. "Bio-fortification" (applied to rice, tuber crops and other species) involves varieties with enhanced content in some of their food and feed components (e.g. vitamins or minerals). Plants with reduced lignine or with increased oil content are examples of products sought to facilitate industrial uses of the commodities and decrease the production costs. As highlighted in the proceedings of the OECD Conference "Biosafety and the Environmental Uses of Micro-organisms" held in 2012, a range of new species are being contemplated as potential biofuels to provide renewable energy. This includes algae such as photosynthetic cyanobacteria, which are of special interest as they can be cultivated year round on non-arable land, alleviating the pressure on agricultural areas and freshwater resources that would be exerted by crops grown for biofuel purposes. Other biotechnology developments, applied in particular to micro-organisms, might lead to other products including biofertiliser organisms living in symbiosis in crop roots and optimising the nitrogen fixation, or biocontrol agents acting as plant protection products to control disease and attack by insects or other invertebrates. Other exploratory fields may involve bioremediation by using living organisms for removing contaminants from the environment such as polluted land, or in the development of detergents containing micro-organisms.

The fast development and increasing use of a range of new breeding techniques, including "genome editing", will allow for quicker development of applications at lower cost. These techniques are being reviewed by regulators, risk assessors, researchers and plant developers, including at the OECD, for discussing their potential impact on risk assessment (see the report of the OECD "Workshop on Environmental Risk Assessment of Products Derived from New Plant Breeding Techniques" held in 2014).

Even if it is difficult to predict which of these biotechnology developments would lead to large applications in the medium term, it is expected that some of the products will have important impacts in their respective economic sectors. A scientifically sound approach to their risk assessment should inform biosafety regulators and support national decisions regarding their potential release. Genetically engineered products are rigorously assessed by their developers during their elaboration, and by governments when ready for commercial use, to ensure high safety standards for the environment, human food and animal feed. Such assessments are felt essential for healthy and sustainable agriculture, industry and trade.

An environmental safety/risk assessment of transgenic organisms is normally based on the information on the characteristics of the host organism, the introduced traits and the environment into which the organism is introduced. The interaction between these

elements and the intended application are also of importance. At its first session held in June 1995, the OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology decided to focus its work on identifying parts of this information which could be commonly used in countries for environmental safety/risk assessment, in order to encourage information sharing and prevent duplication of efforts. The biosafety consensus documents are one of the major outputs of its work.

The biosafety consensus documents constitute a "snapshot" of current information on a specific host organism or trait, for use during regulatory assessments. They are not intended to be a comprehensive source of information on everything that is known about a specific host or trait, but they do address the key or core set of issues that OECD member countries believe to be relevant to risk/safety assessment. Several non-member economies, as well as other international organisations, are associated with the work and share their expertise. The information collated in the consensus documents is said to be mutually acceptable among OECD countries and beyond in other juridictions wishing to use them during their assessment process.

As of November 2017, a total of 56 consensus and guidance documents on biosafety have been published by the Working Group. They include documents which address the biology of plants, animals (one species to date), trees and micro-organisms, as well as those dealing with specific traits that are used in genetically engineered crops. In addition, documents of broader nature aiming to facilitate harmonisation have been developed.

This volume contains a compilation of those biosafety consensus documents issued in 2016 and 2017. It also includes the "Introduction to the biosafety consensus documents" published earlier (and slightly updated from the previous volumes). The introduction explains the purpose of the documents and how they are relevant to risk/safety assessment. It also describes the process by which the documents are drafted, using a "lead country" approach.

Along with the previous six volumes, the present publication offers ready access to those consensus documents published on the OECD BioTrack website thus far. As such, Volume 7 should be of value to applicants for commercial uses of transgenic organisms, regulators in national authorities, breeders, risk assessors as well as the wider scientific community.

This biosafety work is complementary to the activities of the OECD programme on the safety of novel foods and feeds, in particular to the consensus documents developed on the composition of foods and feeds derived from transgenic organisms. These documents describe the key nutrients, anti-nutrients, toxicants and other constituents that can be used in a comparative approach. More information on this programme can be found in the introduction to this volume.

As each of the consensus documents may be updated in the future as new knowledge becomes available, users of this book are encouraged to provide any information or opinions regarding the contents of the consensus documents or indeed, the OECD's other harmonisation activities. Comments can be provided to: ehscont@oecd.org.

The published consensus documents are also available individually free of charge on the OECD's Biotrack website (www.oecd.org/biotrack). Please note, however, that there have been updates to some statistical production data and citations in the current edition.

Acknowledgements

This book is the result of the common effort of the participants of the OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology. Each chapter is composed of a "consensus document" which was prepared under the leadership of one or several countries and observer delegations, as listed at the end of this volume. During their successive draftings, valuable inputs and suggestions for the documents were provided by a number of delegates and experts from the working group, whether from OECD member countries, non-member economies or observer organisations.

Each consensus document was issued individually, as soon as it was finalised and agreed on declassification, by the OECD Environment, Health and Safety (EHS) Division in the Series on Harmonisation of Regulatory Oversight in Biotechnology. This volume, containing the 2016-17 consensus documents, was prepared by Jennifer Allain and edited by Bertrand Dagallier, under the supervision of Peter Kearns, at the EHS Division, OECD Environment Directorate.

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Acronyms and abbreviations

ABA	Abscisic acid
BGD	Bacterial gill disease
BKD	Bacterial kidney disease
CL	Conservation limit
CMS	Cytoplasmic male sterility
CMS	Cardio myopathy syndrome
cm	Centimetre
COS	Conserved ortholog set
DO	Dissolved oxygen
DU	Designatable unit
ERM	Enteric red-mouth disease
FCR	Feed conversion rate
GA	Gibberellin
HDPE	High-density polyethylene
ICES	International Council for the Exploration of the Sea
IPN	Infectious pancreatic necrosis
IPNV	Infectious pancreatic necrosis virus
kg	Kilogram
Km	Kilometre
lpm	Liter per minute
Mbp	Mega base pairs
mln t	Million tonnes
MSW	Multiple sea winters
mtDNA	Mitochondrial DNA
NASCO	North Atlantic Salmon Conservation Organization
NGO	Non-governmental organisation
PCR	Polymerase chain reaction
PIT	Passive integrated transponder

QTL	Quantitative trait locus
RAS	Recirculating aquaculture system
RFLP	Restriction fragment length polymorphisms
SNP	Single nucleotide polymorphism
UMG	Upper modal group
UV	Ultraviolet
WG-HROB	Working Group on Harmonisation of Regulatory Oversight in Biotechnology
WG-SNFF	Working Group for the Safety of Novel Foods and Feeds
WWW	World Wildlife Fund

Executive summary

This document constitutes the seventh volume of the OECD Series on Harmonisation of Regulatory Oversight in Biotechnology, which relates to the environmental risk/safety assessment of transgenic organisms, also called "biosafety". It is a compendium collating in a single volume the individual "consensus documents" published by the Working Group on the Harmonisation of Regulatory Oversight in Biotechnology. The six previous volumes of the series covered documents issued from 1996 to 2015. The current volume contains the consensus documents published in 2016-17.

Modern biotechnologies are applied to plants, as well as trees, animals and micro-organisms. The safety of the resulting transgenic organisms when released in the environment for their use in agriculture, the food and feed industry, or for other applications represents a challenging issue. This is true nowadays with the increasing cultivation of genetically engineered crops, and might become more crucial with future biotechnology developments widening to new species (such as animals or algae). In addition, new breeding objectives can lead to obtaining crops with new traits adapted to climate change, plants of improved composition (biofortification), products for easier processing, renewable biofuels, fast-growing fish, insects modified to fight diseases, biofertilisers and other applications. Genetically engineered products are rigorously assessed by their developers during their elaboration, and by governments when ready for release, to ensure high safety standards for the environment, human food and animal feed. Such assessments are felt essential for a healthy and sustainable agriculture, industry and trade. The growing number of novel organisms will also need to be assessed through a scientifically sound approach to risk assessment that will inform biosafety regulators and support the decision concerning their release.

The OECD Working Group on Harmonisation of Regulatory Oversight in Biotechnology was established in 1995. It gathers national authorities responsible for the environmental risk/safety assessment of products of modern biotechnology in OECD countries and in other economies which are key stakeholders in their production and use. International organisations and experts involved in biosafety are associated with this work. The primary goals of the working group are to promote international regulatory harmonisation, to ensure that methods used in the risk assessment of genetically engineered products are as similar as possible. This opens the way to possible recognition and even acceptance of information from the assessments of other countries. The benefits of harmonisation are multiple: it strengthens mutual understanding among countries, avoids duplication, saves resources and increases the efficiency of the risk assessment process. Overall, it improves safety while reducing unnecessary barriers to trade.

The consensus documents constitute working group's main deliverables. They offer practical tools which compile science-based information relevant to the risk/safety assessment of transgenic organisms intended for release in the environment. They are publicly available and considered worldwide as solid references for biosafety.

The introduction to the biosafety consensus documents presents the OECD working group, key background concepts, principles and common approach prevailing in risk/safety assessment of transgenic organisms. The purpose of the consensus documents are described as well as the process by which they are developed.

Chapter 1 deals with the biology of sorghum (*Sorghum bicolor*), an important crop used as staple food or as livestock feed and forage in many countries. This information can be a useful tool for the biosafety assessment of transgenic varieties. It contains elements of taxonomy; morphological characteristics; centre of domestication, geographic distribution and cultivation practices; reproductive biology; genetics; outcrossing and gene flow; ecology; common pests and pathogens; and biotechnological developments.

The biology of tomato (*Solanum lycopersicum*), another key cultivated plant which is consumed globally, is similarly considered in Chapter 2.

Chapter 3 addresses the biology of Atlantic salmon (*Salmo salar*). It is the first OECD publication of this series to deal with an animal species, in this case a commonly cultured, domesticated fish reared for food production but also present in oceans and rivers as undomesticated populations. The biology and ecology of wild Atlantic salmon is described including: classification; life stages; reproduction; centres of origin; geographical distribution; population dynamics; and interaction with other organisms. Similarly, aspects of the farmed form are considered such as: domestication; aquaculture rearing practices; biocontainment; and interactions with the external environment. It also provides elements of genetics and research on genetically engineered salmon, and suggests bibliographic resources for its risk assessment.

The set of science-based information and data contained in this volume, previously agreed by consensus and published by the OECD, constitute a solid reference recognised internationally. It is already widely used as part of biosafety assessments. As such, this publication should be of value to applicants for commercial uses of transgenic organisms, to risk assessors and regulators from national authorities responsible for granting approvals to their release in the environment, as well as the wider scientific community.

Introduction to the biosafety consensus documents

About the OECD's working group for biosafety

The OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology (the "WG-HROB") comprises delegates from the 35 member countries of the OECD and the European Commission. Typically, delegates are from those government ministries and agencies which have responsibility for the environmental risk/safety assessment of products of modern biotechnology. The WG-HROB also includes a number of observer delegations and invited experts who participate in its work, such as Argentina, the Russian Federation, the United Nations Environment Programme (UNEP), the Secretariat of the Convention on Biological Diversity (SCBD), the Food and Agriculture Organization of the United Nations (FAO), the United Nations Industrial Development Organisation (UNIDO), and the Business and Industry Advisory Committee to the OECD (BIAC).

In recent years, with the increasing use of biotech products in many regions of the world, together with the development of activities relating to tropical and subtropical species, participation was enlarged to other non-member economies including Brazil, Bangladesh, the People's Republic of China, Colombia, India, Indonesia, Kenya, Lithuania, Paraguay, the Philippines, South Africa and Viet Nam, as well as the African Biosafety Network of Expertise from the New Partnership for Africa's Development, a body of the African Union (AU-NEPAD-ABNE). From July 2011 to December 2014, a programme was jointly implemented by the World Bank, the ILSI Research Foundation – Center for Environmental Risk Assessment (ILSI-CERA) and the OECD in the framework of the "Partnership for Biosafety Risk Assessment and Regulation", which developed new links, enhanced collaboration and supported the participation of four non-member economies in the activities of the WG-HROB.

Regulatory harmonisation

The Working Group on Harmonisation of Regulatory Oversight in Biotechnology was established in 1995[1] at a time when the first commercial transgenic crops were being considered for regulatory approval in a number of OECD countries. From the beginning, one of the group's primary goals was to promote international regulatory harmonisation in biotechnology among members. Regulatory harmonisation is the attempt to ensure that the information used in risk/safety assessments, as well as the methods used to collect such information, is as similar as possible. This should lead to countries recognising or even accepting information from one anothers' assessments. The benefits of harmonisation are clear. It increases mutual understanding among countries, which avoids duplication, saves on scarce resources and increases the efficiency of the risk/safety assessment process. This, in turn, improves safety while reducing unnecessary barriers to trade (OECD, 2000).

The need for harmonisation activities at the OECD

The establishment of the WG-HROB and its programme of work followed a detailed analysis by member countries of whether there was a need to continue work on harmonisation in biotechnology at the OECD, and if so, what it should entail. This analysis was undertaken by the Ad Hoc Group for Environmental Aspects of Biotechnology (established by the Joint Meeting),[2] in 1994.

The Ad Hoc Group for Environmental Aspects of Biotechnology took into consideration, and built upon, the earlier work at the OECD which had begun in the mid-1980s. Initially, these OECD activities focused on the environmental and agricultural implications of field trials of transgenic organisms, but this was soon followed by a consideration of their large-scale use and commercialisation. (A summary of this extensive body of work can be found in the annex to this section.)

Key background concepts and principles

The Ad Hoc Group for Environmental Aspects of Biotechnology took into account previous work on risk analysis that is summarised in *Safety Considerations for Biotechnology: Scale-up of Crop Plants* (OECD, 1993). The following quote gives the flavour: "Risk/safety analysis is based on the characteristics of the organism, the introduced trait, the environment into which the organism is introduced, the interaction between these, and the intended application." This body of work has formed the basis for environmental risk/safety assessment that is now globally accepted. In considering the possibilities for harmonisation, the Ad Hoc Group paid attention to these characteristics and the information used by risk/safety assessors to address them.

This was reinforced by the concept of familiarity, also elaborated in the above-mentioned document (OECD, 1993). This concept "is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants whose biology is well understood ... Familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of plants and micro-organisms into the environment." For plants, familiarity takes account of a wide range of attributes including, for example, knowledge and experience with "the crop plant, including its flowering/reproductive characteristics, ecological requirements, and past breeding experiences" (OECD, 1993; see also the annex for a more detailed description). This illustrates the role of information related to the biology of the host organism as a part of an environmental risk/safety assessment.

The Ad Hoc Group for Environmental Aspects of Biotechnology also considered the document *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology* (OECD, 1993b), which focuses on host organisms. It presents information on an initial group of 17 different crop plants, which are used (or are likely to be used) in modern biotechnology. It includes sections on phytosanitary considerations in the movement of germplasm and on current uses of these crop plants. There is also a detailed section on current breeding practices.

A common approach to risk/safety assessment

An important aspect for the Ad Hoc Group for Environmental Aspects of Biotechnology was to identify the extent to which member countries address the same questions and issues during risk/safety assessment. Big differences would mean difficulties in working towards harmonisation, while a high level of similarity would suggest it is more feasible.

This point was resolved by two studies considered by the Ad Hoc Group: one covered crop plants (OECD, 1995a; 1995b) while the other concerned micro-organisms (OECD, 1995c; 1995d). Both studies involved a survey with national authorities responsible for risk/safety assessment. The aim was to identify the questions they address during the assessment process (as outlined in national laws/regulations/guidance texts) in order to establish the extent of similarity among national authorities. The studies used the information provided in the OECD's "Blue Book" on *Recombinant DNA Safety Considerations* (OECD, 1986) as a reference point, in particular the sections covering: 1) general scientific considerations; 2) human health considerations; and 3) environmental and agricultural considerations (Appendices B, C and D). Both studies showed a remarkably high degree of similarity among countries in the questions/issues addressed in risk/safety assessment.

The emergence of the concept of consensus documents

The Working Group on Harmonisation of Regulatory Oversight in Biotechnology was therefore established with the knowledge that national authorities have much in common in terms of the questions/issues addressed when undertaking risk/safety assessment. It also took into account those characteristics identified as part of the assessment (i.e. the organism, the introduced trait and the environment) around which harmonisation activities could focus.

It was further recognised that much of the information used in risk/safety assessment relating to the biology of host organisms (crop plants, trees, animals or micro-organisms) would be similar or virtually the same in all assessments involving the same organism. In other words, the questions addressed during risk/safety assessment which relate to the biology of the organism, for example the potential for gene transfer within the crop plant species, and among related species, as well as the potential for weediness, remain the same for each application involving the same host species. This also applies to some extent to information related to introduced traits.

Consequently, the WG-HROB put forth the idea of compiling information common to the risk/safety assessment of a number of transgenic products, and decided to focus on two specific categories: the biology of the host species and traits used in genetic modifications. The aim was to encourage information sharing and prevent duplication of effort among countries by avoiding the need to address the same common issues in applications involving the same organism or trait. It was recognised that biology and trait consensus documents could be agreed upon relatively quickly by member countries (within a few years). This compilation process was quickly formalised in the drafting of consensus documents.

The purpose of consensus documents

The consensus documents are not intended to be a substitute for a risk/safety assessment, because they address only a part of the necessary information. Nevertheless, they should make an important contribution to environmental risk/safety assessment.

Consensus documents are intended to be a "snapshot" of current information, for use during the regulatory assessment of products of biotechnology. They are not intended to be a comprehensive source of information covering the full knowledge about a specific host organism or trait; but they address – on a consensus basis – the key or core set of issues that countries believe to be relevant to risk/safety assessment.

The aim of the documents is to share information on these key components of an environmental safety review in order to prevent duplication of effort among countries. The documents are envisaged to be used: 1) by applicants as information to be given in applications to regulatory authorities; 2) by regulators as a general guide and reference source in their reviews; and 3) by governments for information sharing, research reference and public information.

Originally, it was said that the information in the consensus documents is intended to be mutually recognised or mutually acceptable among OECD member countries, though the precise meaning of these terms is still open for discussion. During the period of the Ad Hoc Group for Environmental Aspects of Biotechnology and the early days of the WG-HROB (1993-95), the phrase "mutual acceptance of data" was discussed. This concept, borrowed from OECD's Chemicals Programme, involves OECD Council decisions that have legally binding implications for member countries. In the case of the consensus documents, there has never been a legally binding commitment to use the information they contain, though the WG-HROB is interested in enhancing the commitment of countries to make use of the documents. Participation in the development of documents, and the intention by countries to use the information, is done in "good faith". It is expected, therefore, that reference will be made to relevant consensus documents during risk/safety assessments. As these documents are publicly available, they can be of interest for any country wishing to use them in national assessments.

The process through which consensus documents are initiated and brought to publication

There are a number of steps in the drafting of a specific consensus document. The first occurs when a delegation, in a formal meeting of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology, makes a proposal to draft a document on a new topic, typically a crop species or a trait. If the WG-HROB agrees to the proposal, a provisional draft is prepared by either a single country or two or more countries working together ("lead country approach"). Typically, the lead country(ies) has had experience with the concerned crop or trait and is able to draw on experts to prepare a provisional draft.

The provisional draft is first reviewed by the Bureau of the WG-HROB[3] to ensure that it addresses the range of issues normally covered by consensus documents and is of sufficiently high quality to merit consideration by the WG-HROB as a whole.

Based on the comments of the Bureau, a first draft is prepared for consideration by the full WG-HROB. This is the opportunity for each delegation to review the text and provide comments based on their national experiences. Inputs are incorporated in a second draft, which is again circulated to the WG-HROB. At this point, the WG-HROB may be asked to recommend that the document be declassified. Such a recommendation is only forthcoming when all delegations have come to a consensus that the document is complete and ready for publication. Sometimes, however, the text may need a third or even more discussions in the WG-HROB before a declassification can be contemplated.

Once the WG-HROB has agreed to recommend a document for declassification, it is forwarded to the supervisory committee, the Joint Meeting, which is invited to declassify the document. Following the agreement of the Joint Meeting, the document is then published.

It is important to note that the review of consensus documents is not limited to formal meetings of the WG-HROB. Much discussion also occurs through electronic means, especially via the protected website dedicated to the WG-HROB. This enables a range of experts to have input into drafts.

For a number of documents, it has also been necessary to include information from non-member countries. This wider share of expertise has become increasingly important in recent years with the development of activities relating to tropical and subtropical species. This has been particularly true in the case of crop plants where the centre of origin and diversity occurs in a non-member country(ies). In these cases, UNEP, UNIDO and the FAO have assisted in the preparation of documents by identifying experts from relevant countries. For example, this occurred with the consensus document on the biology of *Oryza sativa* (rice) published in 1999.

The full series of consensus documents developed by the WG-HROB is also published in compendium documents, as it is the case for this volume. Volumes 5 and 6 were published in 2016 (covering 2011-15), Volumes 3 and 4 in 2010 (covering 2007-10), while Volumes 1 and 2 were issued in 2006 (covering 1996-2006) (OECD, 2016a; 2016b; 2010a; 2010b; 2006a; 2006b).

Current and future trends in the Working Group on Harmonisation of Regulatory Oversight in Biotechnology

The WG-HROB continues its work on the preparation of specific consensus documents, and on the efficiency of the process by which they are developed. An increasingly large number of crops and other host species (trees, animals, micro-organisms) are being modified, for an increasing number of traits, and the WG-HROB aims to fulfil the current needs whilst preparing for emerging topics.

At the OECD Workshop on Consensus Documents and Future Work in Harmonisation, held in Washington, DC in October 2003, the WG-HROB considered how to set priorities for drafting future consensus documents among the large number of possibilities. The workshop also recognised that published consensus documents may be in need of review and updating from time to time, to ensure that they include the most up-to-date information. The WG-HROB considers these aspects on a regular basis when planning future work. For the preparation of future documents, the workshop identified the usefulness of developing a standardised structure of consensus documents. The WG-HROB contemplated developing, first, a guidance document on "Points to consider" for consensus documents on the biology of cultivated plants that was published in 2006, and then that of the trait documents. The "Points to consider' document, included in Volumes 3 and 4 of the compendia series, is currently being updated by the WG-HROB.

Among the important activities of the WG-HROB, a new document is being developed on the "Environmental considerations for the risk/safety assessment for the release of transgenic plants". Focused on the core of the biosafety work that is applied to crops and trees, and taking into account the most recent views from countries of all regions of the world, this document will constitute a key guidance tool for developers, assessors and regulatory authorities. It is expected to be published in 2019.

An important step was taken in 2017 with the publication of the first consensus biology document dedicated to an animal species, the Atlantic salmon (*Salmo salar*), which is included in the present volume. Another document is being prepared (publication expected in 2018) on the biology of the mosquito *Aedes aegypti*, for which some

genetically engineered strains have been used since 2014. This has taken place in limited areas to control the virus-vector insect population and participate in the fight against tropical diseases such as dengue fever and chikungunia that have been dramatically extending in many regions of the world over the last decade.

The WG-HROB is also considering projects on micro-organisms, therefore opening up to new areas, for instance, bioenergy, with the preparation of a document on eukaryotic micro-algae having started recently. The photosynthetic cyanobacteria are potential providers of renewable energy and are of special interest as they can be cultivated year round on non-arable areas, alleviating the pressure on farmland and freshwater resources that would be exerted by crops grown for biofuel purposes, as stated in the proceedings of the OECD Conference on Biosafety and the Environmental Uses of Micro-Organisms set up by the WG-HROB in 2012 (OECD, 2015a). Other biotechnology developments applied to micro-organisms might be considered to prepare future documents: updated review of biofertiliser organisms living in symbiosis in crop roots and optimising the nitrogen fixation, or biocontrol agents acting as plant protection products to control disease and attack by insects and other herbivores. Other exploratory fields may comprise bioremediation by using living organisms for removing contaminants from the environment such as polluted land, or the development of detergents containing micro-organisms.

In recent years, the WG-HROB has started to exchange knowledge and promote discussion on the new plant breeding techniques and their potential impact on biosafety assessment. An OECD workshop was organised on these matters by the WG-HROB in 2014; the key message from its report at the time was that "Experience to date indicates that current guidance and tools for environmental risk/safety assessment of transgenic plants are applicable to plants developed using [new plant breeding techniques]," where such assessment may be required (OECD, 2016c). Specific events on new plant breeding techniques are regularly organised at the OECD for increasing awareness and sharing information, including a conference on genome editing application to be organised in 2018. The subject will be kept under review.

The OECD Working Group for the Safety of Novel Foods and Feeds

The OECD Task Force for the Safety of Novel Foods and Feeds, established in 1999, addresses aspects of the assessment of human food and animal feed derived from genetically engineered crops. This body was renamed the Working Group for the Safety of Novel Foods and Feeds (WG-SNFF) from 1 January 2017. As with the WG-HROB, the main focus of the WG-SNFF work is to ensure that the types of information used in risk/safety assessment, as well as the methods to collect such information, are as similar as possible amongst countries. The approach is to compare transgenic crops and derived products with similar conventional ones that are already known and considered safe because of their history of safe use. Harmonised methods and the sharing of information are facilitated through the WG-SNFF's activities.

In a similar approach to the biosafety programme, the main outcome of the foods and feeds programme is the set of consensus documents on compositional considerations of new varieties of specific crops. The WG-SNFF documents compile a common base of scientific information on the major components of crop plants, such as key nutrients, toxicants, anti-nutrients and allergens. These documents constitute practical tools for regulators and risk/safety assessors dealing with these new varieties, with respect to foods and feeds. To date, 28 consensus documents have been published on major crops and on general considerations for facilitating harmonisation, including regular updates of the

oldest issues. They constitute the Series on the Safety of Novel Foods and Feeds which is also available on the OECD's website (www.oecd.org/biotrack).

The full series of consensus documents developed by the Task Force was published in 2015 in two compendium documents, Volume 1 covering 2002-08 and Volume 2 covering 2009-14 (OECD, 2015b; 2015c). Volume 3 is under preparation.

The two bodies (WG-HROB and WG-SNFF) are implementing closely related and complementary programmes, focused on environmental aspects for the first and on food and feed aspects for the second. Their co-operation on issues of common interest resulted in a document developed jointly by the two bodies, the "Consensus document on molecular characterisation of plants derived from modern biotechnology", published in 2010 (included in Volume 3 of the current series). The two bodies also refer to the same "Unique Identifiers" assigned to transgenic products approved for cultivation and/or for food and feed use, and they wish to keep this system defined by OECD (described in Volume 3 of the current series) always relevant and adapted to new types of products-new species.

Notes

1. The original title of the Working Group was the "Expert Group for the Harmonisation of Regulatory Oversight in Biotechnology". It became an OECD working group in 1998.
2. The Joint Meeting was the supervisory body of the Ad Hoc Group for Environmental Aspects of Biotechnology and, as a result of its findings, established the Working Group as a subsidiary body. Today, its full title is the Joint Meeting of the Chemicals Committee and the Working Party on Chemical, Pesticides and Biotechnology.
3. The Bureau comprises the Chair and Vice-Chairs of the working group. The Bureau is elected by the working group once per year. At the time of preparing this publication, the Chair is from the United States, and the Vice-Chairs from Australia, Belgium, Canada, Finland and Japan.

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Annex: OECD biosafety principles and concepts developed prior to the Working Group on Harmonisation of Regulatory Oversight in Biotechnology (1986-94)

Since the mid-1980s the OECD has been developing harmonised approaches to the risk/safety assessment of products of modern biotechnology. Prior to the establishment of the Working Group on Harmonisation of Regulatory Oversight in Biotechnology, the OECD published a number of reports on safety considerations, concepts and principles for risk/safety assessment as well as information on field releases of transgenic crops, and a consideration of traditional crop breeding practices. This annex notes some of the highlights of these achievements that were background considerations in the establishment of the working group and its development of consensus documents.

Underlying scientific principles

In 1986, the OECD published its first safety considerations for genetically engineered organisms (OECD, 1986). These included the issues relevant to human health, the environment and agriculture that might be considered in a risk/safety assessment. In its recommendations for agricultural and environmental applications, it suggested that risk/safety assessors:

- "Use the considerable data on the environmental and human health effects of living organisms to guide risk assessments.
- Ensure that recombinant DNA organisms are evaluated for potential risk, prior to application in agriculture and the environment by means of an independent review of potential risks on a case-by-case basis.
- Conduct the development of recombinant DNA organisms for agricultural and environmental applications in a stepwise fashion, moving, where appropriate, from the laboratory to the growth chamber and greenhouse, to limited field testing and finally to large-scale field testing. And,
- Encourage further research to improve the prediction, evaluation, and monitoring of the outcome of applications of recombinant DNA organisms."

The role of confinement in small-scale testing

In 1992, OECD published its *Good Developmental Principles* (OECD, 1992) for the design of small-scale field research involving transgenic plants and micro-organisms. It describes the use of confinement in field tests. Confinement includes measures to avoid the dissemination or establishment of organisms from a field trial, for example, the use of physical, temporal or biological isolation (such as the use of sterility).

Scale-up of crop-plants – "risk/safety analysis"

By 1993, the focus of attention had switched to the scale-up of crop plants as plant breeders began to move to larger scale production and commercialisation of transgenic plants. The OECD published general principles for scale-up, which reaffirmed that:

> … safety in biotechnology is achieved by the appropriate application of risk/safety analysis and risk management. Risk/safety analysis comprises hazard identification and, if a hazard has been identified, risk assessment. Risk/safety analysis is based on the characteristics of the organism, the introduced trait, the environment into which the organism is introduced, the interaction between these and the intended application. Risk/safety analysis is conducted prior to an intended action and is typically a routine component of research, development and testing of new organisms, whether performed in a laboratory or a field setting. Risk/safety analysis is a scientific procedure which does not imply or exclude regulatory oversight or imply that every case will necessarily be reviewed by a national or other authority. (OECD, 1993)

The role of familiarity in risk/safety assessment

The issue of scale-up also led to an important concept – familiarity – which is one key approach that has been used subsequently to address the environmental safety of transgenic plants.

The concept of familiarity is based on the fact that most genetically engineered organisms are developed from organisms such as crop plants, whose biology is well understood. It is not a risk/safety assessment in itself (US-NAS, 1989). However, the concept facilitates risk/safety assessments, because to be familiar means having enough information to be able to make a judgement of safety or risk (US-NAS, 1989). Familiarity can also be used to indicate appropriate management practices, including whether standard agricultural practices are adequate or whether other management practices are needed to manage the risk (OECD, 1993). Familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of plants and micro-organisms into the environment and this indicates appropriate management practices. As familiarity depends also on the knowledge about the environment and its interaction with introduced organisms, the risk/safety assessment in one country may not be applicable in another country. However, as field tests are performed, information will accumulate about the organisms involved, and their interactions with a number of environments.

Familiarity comes from the knowledge and experience available for conducting a risk/safety analysis prior to scale-up of any new plant line or crop cultivar in a particular environment. For plants, for example, familiarity takes account of, but need not be restricted to, knowledge and experience with the following:

- "The crop plant, including its flowering/reproductive characteristics, ecological requirements, and past breeding experiences
- the agricultural and surrounding environment of the trial site
- specific trait(s) transferred to the plant line(s)
- results from previous basic research including greenhouse/glasshouse and small-scale field research with the new plant line or with other plant lines having the same trait

- the scale-up of lines of the plant crop varieties developed by more traditional techniques of plant breeding
- the scale-up of other plant lines developed by the same technique
- the presence of related (and sexually compatible) plants in the surrounding natural environment, and knowledge of the potential for gene transfer between crop plant and the relative, and
- interactions between/among the crop plant, environment and trait." (OECD, 1993)

Risk/safety assessment and risk management

Risk/safety assessment involves the identification of potential environmental adverse effects or hazards, and determining, when a hazard is identified, the probability of it occurring. If a potential hazard or adverse effect is identified, measures may be taken to minimise or mitigate it. This is risk management. Absolute certainty, or "zero risk", in a safety assessment is not achievable, so uncertainty is an inescapable aspect of all risk assessment and risk management (OECD, 1993). For example, there is uncertainty in extrapolating the results of testing in one species to identify potential effects in another. Risk assessors and risk managers thus spend considerable effort to address uncertainty. Many of the activities in intergovernmental organisations, such as the OECD, address ways to handle uncertainty (OECD, 2000).

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Part I.

Biology of crops

Chapter 1.

Sorghum (*Sorghum bicolor*)

*This chapter deals with the biology of sorghum (*Sorghum bicolor*). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It includes elements of taxonomy, morphological characteristics, centre of domestication, current geographic distribution and cultivation practices, reproductive biology, genetics; outcrossing and gene flow, ecology, common pests and pathogens; and biotechnological developments.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with South Africa and the United States as the co-lead countries. It was initially issued in June 2016. Updates have been made on production and yield data from FAOSTAT, including Tables 1.2-1.4.

Taxonomy

Classification and nomenclature

The word "sorghum" typically refers to cultivated sorghum (*Sorghum bicolor* [L.] Moench subsp. *bicolor*), a member of the grass family Poaceae, tribe Andropogoneae, and subtribe Sorghinae (Clayton and Renovoize, 1986) that is grown for its grain (grain sorghum), its sugary sap (sweet sorghum) or as a forage (forage sorghum) (Figure 1.1). A variety of common names are used in different regions to refer to cultivated sorghum, including great millet, guinea corn, broomcorn, kaffir corn, durra, mtama, milo, jowar or kaoliang (FAO, 1995).

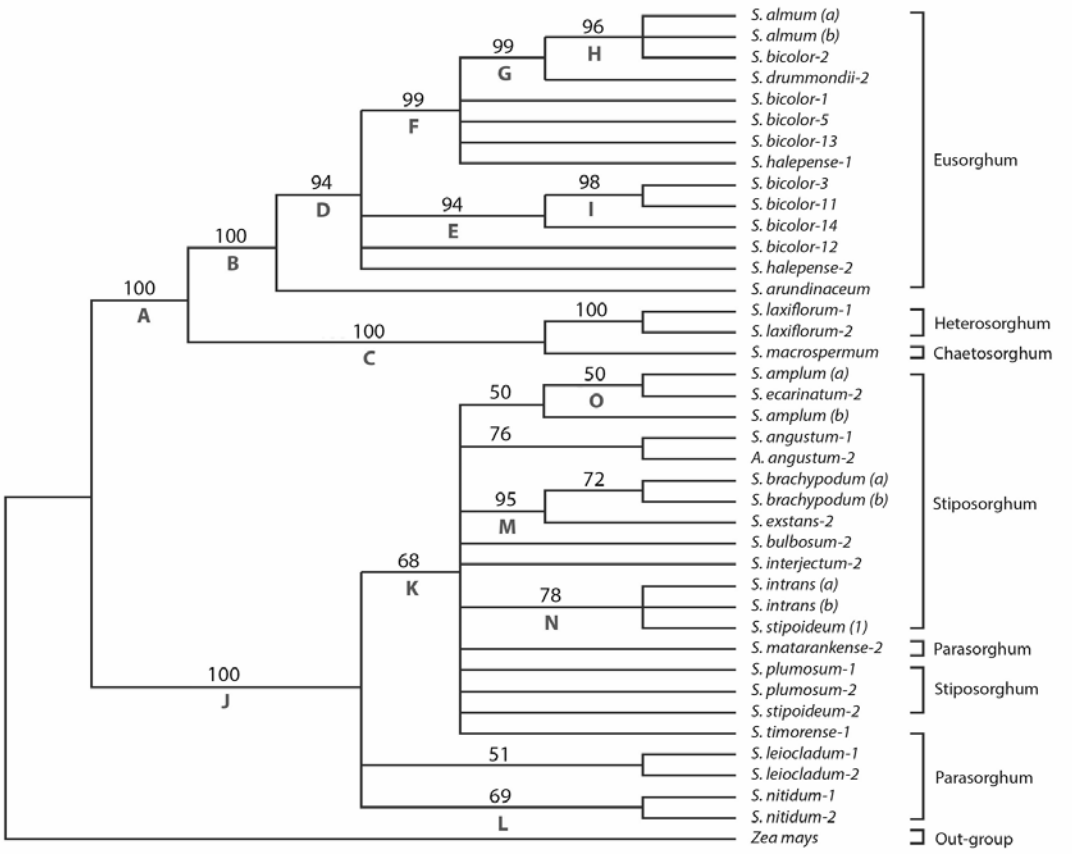
Figure 1.1. **Grain sorghum (upper-left), sweet sorghum (upper-right) and forage sorghum (bottom)**

Source: Upper-left: Daniel Georg Döhne, licensed under CC BY-SA 3.0. Upper-right: Judgefloro, licensed under CC BY-SA 4.0. Bottom: courtesy of Alex Stelzleni, University of Georgia College of Agricultural and Environmental Sciences.

Cultivated sorghum is only one member of the genus sorghum, made up of 25 species (USDA-ARS, 2012) and separated into five taxonomic sections: Chaetosorghum, Heterosorghum, Parasorghum, Stiposorghum and Eusorghum (Garber, 1950). Agronomically important Eusorghum species are listed in Table 1.1 and include cultivated sorghum, its wild progenitor (*Sorghum bicolor* [L.] Moench subsp. *verticilliflorum* [Steud.] de Wet ex Wiersema & J. Dahlb.), Sudan grass (*Sorghum bicolor* nothosubsp. *drummondii* [Steud.] de Wet ex Davidse), and weedy relatives such as Johnsongrass (*Sorghum halepense* [L.] Pers.), shattercane (a feral form of *Sorghum bicolor* nothosubsp. *drummondii* [Steud.] de Wet ex Davidse) and *S. propinquum* (Kunth) Hitchc.

Ng'uni et al. (2010) published a phylogenetic analysis showing the relationships between the taxonomic sections based on four regions of the chloroplast DNA (*trn*Y-*trn*D, *psb*Z-*trn*G, *trn*Y-*psb*M and *trn*T-*trn*L) and the internal transcribed spacer region of the 18S-5·8S-26S nuclear ribosomal DNA from 21 sorghum species. The results are shown in Figure 1.2. Germplasm accessions used in their study include wild sorghum species and several cultivated sorghums obtained from the Australian Tropical Crops Genetic Resource Centre, Biloela, Queensland, Australia; and the Zambian National Plant Genetic Resources Centre.

Figure 1.2. **Phylogenetic analysis of 21 sorghum species based on 4 regions of chloroplast DNA and internal transcribed spacers of nuclear ribosomal DNA**



* Clades are indicated by letters below the branches. Bootstrap values ≥ 50%, indicating the percentage likelihood that subgroups differ, are located above the branches.

Source: Ng'uni et al. (2010).

The division of cultivated sorghum into subspecies and races over the past century has been somewhat archaic, with many competing classifications that are not properly validated. Synonyms are often used even at the species level. One such example is the ongoing use of *Sorghum caffrorum* and *Sorghum vulgare* by agencies throughout the world to indicate *Sorghum bicolor*.

The nomenclature of cultivated sorghum and its wild and weedy relatives was thoroughly reviewed by Wiersema and Dahlberg (2007). Competing names and priorities were considered and three subspecies were validated for *S. bicolor*: *S. bicolor* subsp. *bicolor*, *S. bicolor* subsp. *verticilliflorum* and *S. bicolor* subsp. *drummondii*. *S. bicolor* subsp. *bicolor* comprises the cultivated sorghums; *S. bicolor* subsp. *verticilliflorum* comprises annual wild relatives of cultivated sorghum native to Africa, Madagascar, the Mascarenes, and introduced varieties to India, Australia and the Americas; *S.* subsp. *drummondii* comprises annual weedy derivatives arising from hybridisation of cultivated sorghum and *S. bicolor* subsp. *verticilliflorum*. A complete listing of the names of all known subspecies plus homotypic species names is provided in Wiersema and Dahlberg (2007).

Section Eusorghum also includes the rhizomatous taxa Johnsongrass and *S. propinquum* (de Wet, 1978). Although Johnsongrass is native to southern Eurasia and India, its introduction to temperate regions and introgression with cultivated sorghums has caused it to become a troublesome weed (de Wet, 1978). *S. propinquum* is generally restricted to Sri Lanka, southern India, and Burma east toward Southeast Asia (de Wet, 1978; Doggett, 1988). By natural crossing with cultivated sorghums in the Philippines, *S. propinquum* has also become a geographically isolated noxious weed (de Wet, 1978).

Of the 25 recognised species of *Sorghum*, 17 are native to Australia and Southeast Asia, of which 14 are endemic to Australia (Lazarides et al., 1991). Basic chromosome numbers vary from 10-40, and in some cases, such as within *S. timorense* (Kunth) Buse, there are multiple ploidy levels. These species are not within the Eusorghum section and previously were regarded as sufficiently distant to be sexually incompatible with cultivated sorghum. Recent studies have demonstrated that *S. bicolor* × *S. macrospermum* crosses are not only possible (Price et al., 2005), but that there is significant genomic introgression of the wild germplasm into the cultivated species after backcrossing the hybrids (Price et al., 2005; Kuhlman et al., 2010).

Description

Cultivated sorghum is a cane-like grass with diverse morphology (NRC, 2004). Plant height ranges from 0.5 metres (m) to 6 m. Culms (stalks) are erect and range from slender to stout. Tillers (adventitious stems originating from the plant base) can range in quantity from none to profuse. Leaf blades vary from linear to lanceolate, and can be smooth or hairy, measuring up to 100 centimetres (cm) long and 10 cm wide with smooth to thinly pilose sheaths. The inflorescence consists of a single panicle with many racemes. Panicles may be either compact or open up to 50 cm long and 30 cm wide; panicle branches are stiffly ascending or spreading and pendulous, with the bottom branch being almost half as long as the panicle. At maturity, racemes have one to eight nodes and can be either fragile or tough. Spikelets may be glabrous or hirsute, elliptic to obovate, and up to 6 mm long. Glumes (bracts) range from leathery to membranous, often with winged keels. Lower lemmas are approximately 6 mm long while upper lemmas are slightly shorter and often awned. Both upper and lower lemmas of sessile spikelets are somewhat ciliate and translucent (Doggett, 1988).

For many years, sorghum breeders have classified cultivated sorghum into races (Snowden, 1936) or working groups (Murty and Govil, 1967) according to morphological characteristics. De Wet et al. (1970) described the various groups of cultivated sorghum and identified their historical geographic distribution. A system was then developed dividing cultivated sorghum into five basic interfertile races (Bicolor, Kafir, Caudatum, Durra and Guinea) and ten intermediate races, based on floral morphology (Harlan and de Wet, 1972). This classification system was widely adopted. An integrated classification of cultivated sorghum was proposed by Dahlberg (2000) following the morphological guidelines outlined above and simplifies their classification systems by presenting working groups numerically. Brown et al. (2011) and Morris et al. (2013) provide molecular support for classification of the races. A more detailed description of the characteristics of each of the five main races of cultivated sorghum can be found in Table 1.1. Diagrams of spikelet and head types of the races are in Figure 1.3.

Table 1.1. **Cultivated sorghum race characteristics**

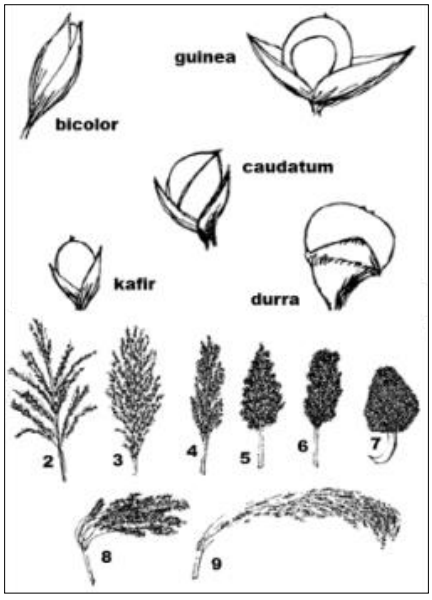
Race	Distinct characteristics
Bicolor	– Open inflorescences with pendulous branches – Long, clasping glumes – Elliptic grain
Kafir	– Moderately compact, cylindrical inflorescences – Elliptic spikelets – Tightly clasping, long glumes
Caudatum	– Compact to open inflorescences – Grains with one side flat, opposite side curved – Shorter glumes that expose grains
Durra	– Compact inflorescences – Flat, ovate shaped sessile spikelets – Middle-creased lower glume – Distinct texture on tip of lower glume
Guinea	– Large, open inflorescences with pendulous branches – Long, separated glumes that expose grains – Obliquely twisted grains

Source: Doggett (1988).

Sorghum grain is a staple food for millions of people in the semiarid regions of Africa and Asia where it is used to make food products such as tortillas, breads, cakes, noodles, couscous, beer and porridge (Rooney and Waniska, 2000). Sweet sorghum sap can be processed into sweeteners for the food industry or fermented into ethanol. Nearly all sorghum production (97%) in the western hemisphere is for livestock feed and forage because it is a lower cost alternative to maize and requires less water to grow (Hancock, 2000). Developing countries also use sorghum plant products for cooking fuel, construction materials, leather dyes and as physical support for vining crops like yams (NRC, 2004).

Cultivated sorghum ranks fifth in worldwide cereal crop production behind maize, rice, wheat and barley (FAOSTAT, 2017). It is a widely adapted species capable of growing in semiarid, subtropical, tropical and temperate climates. An extensive root system and the ability to become dormant during water stress make cultivated sorghum drought-resistant (Whiteman and Wilson, 1965), typically requiring only one-half to two-thirds the amount of rainfall as maize (Hancock, 2000). Plants are primarily self-pollinated, but some wind pollination occurs. Cultivated sorghum is physiologically a perennial that is typically grown as an annual. In some environments a second ratoon (resprouted) crop is produced from the unharvested roots and stolons of the first crop.

Figure 1.3. **Spikelet types of the five races of cultivated sorghum and their associated head types**



* Head type 1 (not shown) is reserved for wild races and is more diffuse than Type 2. Types 2, 3 and 4 have Bicolor and Guinea spikelets; Types 5, 6 and 7 have Kafir and Durra spikelets; many head types have Caudatum spikelets; and Broomcorn (Type 9) has Bicolor spikelets. Type 8 spikelets were not specified by the authors.

Source: Harlan and de Wet (1972).

In 2014, approximately 68.9 million tonnes (mln t) of sorghum was produced on almost 45 million hectares in 112 countries (FAOSTAT, 2017). In the same year, the leading sorghum-producing countries included the United States (11.0 mln t), Mexico (8.4 mln t), Nigeria (6.7 mln t), Sudan (8.4 mln t) and India (5.4 mln t) (Table 1.2). Africa is the world regional leader in total production of sorghum at 29.2 mln t (Table 1.3). Although Africa leads in total production, its average yield per hectare is the lowest, at 994 kg ha^{-1} (Table 1.4). This disparity may be attributed to the relative prevalence of subsistence agriculture in Africa as opposed to other regions.

Table 1.2. **Leading sorghum grain-producing countries, 2014**

Country	Total production (10^6 t)
United States	11.0
Mexico	8.4
Nigeria	6.7
Sudan	6.3
India	5.4
Ethiopia	4.3
Argentina	3.5
China (People's Republic of)	2.9
Brazil	2.3
Burkina Faso	1.7
Niger	1.4
Australia	1.3

Source: FAOSTAT (2017).

Table 1.3. **Sorghum grain production by region, 2014**

Region	Total production (10^6 t)
Africa	29.2
North America	11.0
Asia	9.7
Central America	8.8
South America	7.5
Europe	1.4
Oceania	1.3

Source: FAOSTAT (2017).

Table 1.4. **Average sorghum grain yield by region, 2014**

Region	Average yield (kg/ha^{-1})
Europe	3 525
North America	4 242
Central America	3 954
South America	3 308
Oceania	2 413
Asia	1 298
Africa	994
World average	1 533

Note: kg/ha^{-1} = kilograms per hectare.

Source: FAOSTAT (2017).

Geographic distribution, domestication and cultivation

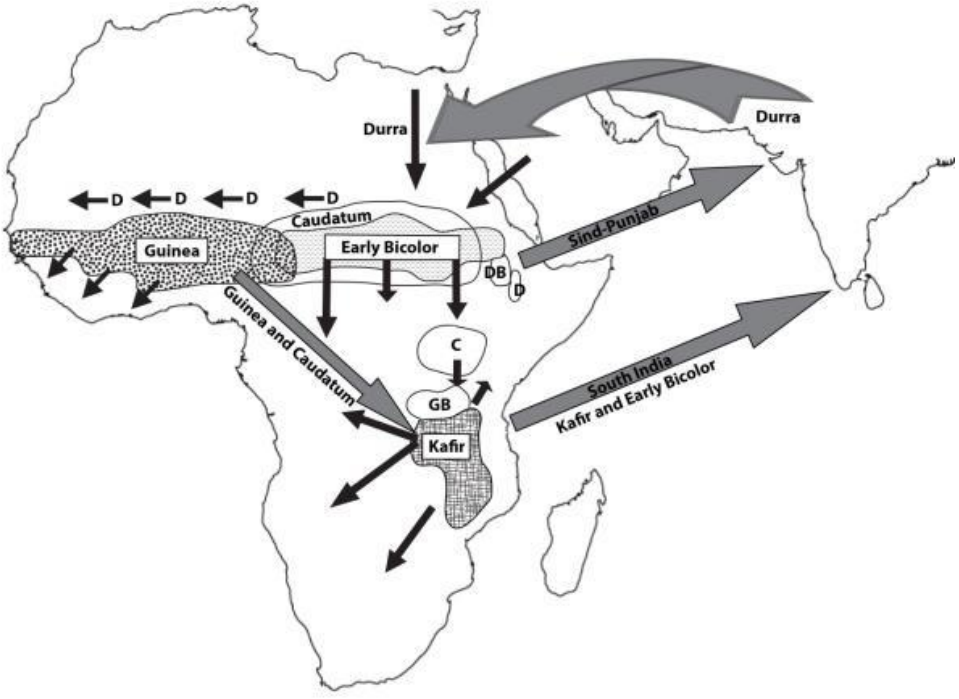
Centre of domestication and ancient geographic distribution

Sorghum's centre of domestication is likely the Ethiopia-Sudan region in north-east Africa because the greatest plant diversity and variation in ecological habitats occurs there (Doggett, 1988). Archaeological evidence suggests sorghum was originally cultivated around 5000 B.P. (Krzyzaniak, 1978). Studies comparing the morphology of ancient and modern grain (Dahlberg and Wasylikowa, 1996) and data from molecular markers (Deu et al., 1995) agree that the different races be classified as the same biological species. It is possible that a single domestication event occurred and that the various races were derived from it. Alternatively, multiple domestication events may have occurred, leading to the development of different races that subsequently anastomosed into the current, extant *S. bicolor* lineage. Regardless, distinct cytoplasmic markers have been identified in race Guinea (Deu et al., 1995), including alleles specific to the margaritiferum subrace (Deu et al., 2006; Figueiredo et al., 2008), whose grain is cooked and eaten like rice.

Following domestication in east Africa, humans moved cultivated sorghum across much of sub-Saharan Africa. The germplasm was diversified through selection and introgression with sympatric wild populations according to the needs of different ecological conditions and desired crop uses (Doggett, 1988). Grain size and the ability to withstand dry or wet conditions became important selection criteria leading to diversity within the germplasm. For example, race Guinea was bred for grain production in wetter conditions with open panicles that would prevent seed moulding. Conversely, race Durra was adapted to drier conditions by developing more compact panicles as humans

expanded the crop into the southern Sahara (Doggett, 1988). Smith and Frederiksen (2000) illustrated the movement and domestication of sorghum races in Figure 1.4.

Figure 1.4. **Origins and movements of the five races of *S. bicolor***



Source: Smith and Frederiksen (2000), Harlan and Stemler (1976), Harlan (1976), Doggett (1988), and Ehret (1988).

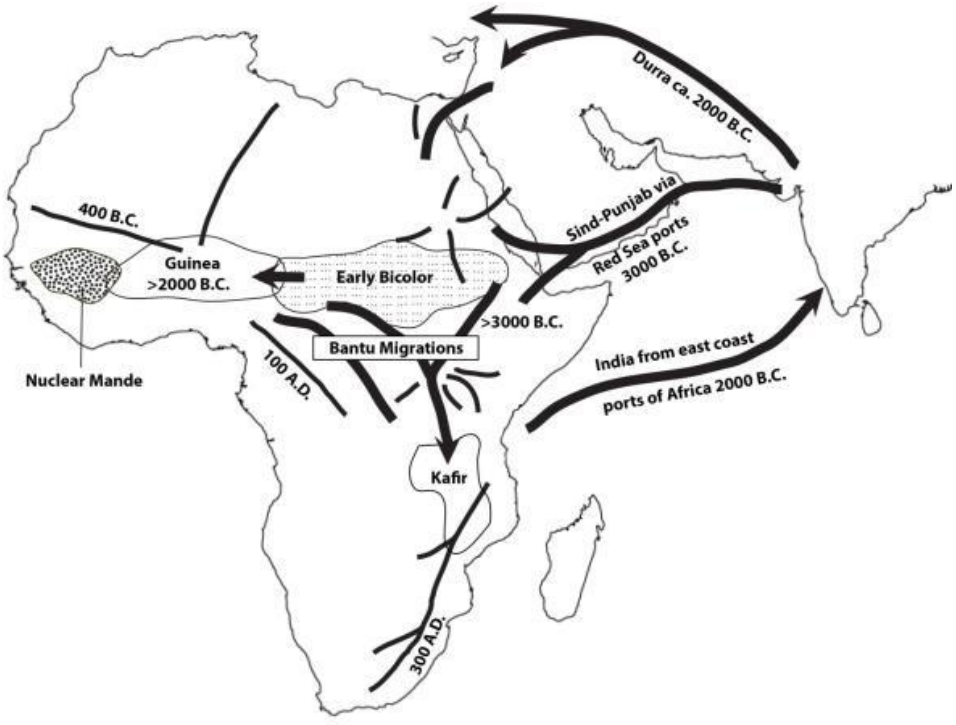
Cultivated sorghum was transported from Africa to India via trade routes over the Arabian Peninsula and the Indian Ocean (Figure 1.5). Durra varieties began emerging in India as the crop was adapted to the environmental conditions and needs of people. The earliest archaeological evidence of domesticated sorghum in India is dated around 4000 B.P. (Kimber, 2000). Domesticated sorghum continued to be spread from India to the People's Republic of China (hereafter "China") along overland trade routes. In China, the crop was adapted to tolerate temperate conditions and varieties known as the Kaoliangs were developed that are tolerant of cooler early season temperatures (Doggett, 1988). Sorghum came from Africa to America relatively recently through the slave trade. In the United States, the crop has been bred for commercial purposes since its introduction, resulting in the development of dwarf hybrids which are easier to cultivate on a commercial scale (Smith and Frederiksen, 2000).

Contemporary geographic distribution and methods of cultivation

Sorghum's adaptability to a range of environmental conditions allows it to be cultivated in multiple regions around the world with substantially varied climates (Figure 1.6). There are currently two main belts of cultivation in Africa. The northern belt ranges from the Ivory Coast north to the Sahara, and east towards Sudan and Ethiopia. The races Bicolor, Durra, Guinea and Caudatum are primarily grown in this belt. The second African sorghum belt runs north to south from Ethiopia to South Africa. Races grown

include Kafir, Bicolor and Caudatum. In India, sorghum is mainly cultivated on the Deccan Plateau, with only minor production in northern India. Sorghum is produced throughout China but the core of production is in the northern region, especially the areas north of the Qinling Mountains, and between the Yellow and Yangtze Rivers (de Wet et al., 1970; House, 1985; House et al., 2000).

Figure 1.5. **Early human migrations and associated diffusion of *S. bicolor* races**



* Wide arrows, postulated early sorghum routes; narrow arrows, diffusion of iron-making technologies; dots, earliest centres of iron making.

Source: Smith and Frederiksen (2000); Murdock (1959); Harlan et al. (1976); Doggett (1988); and Shillington (1989).

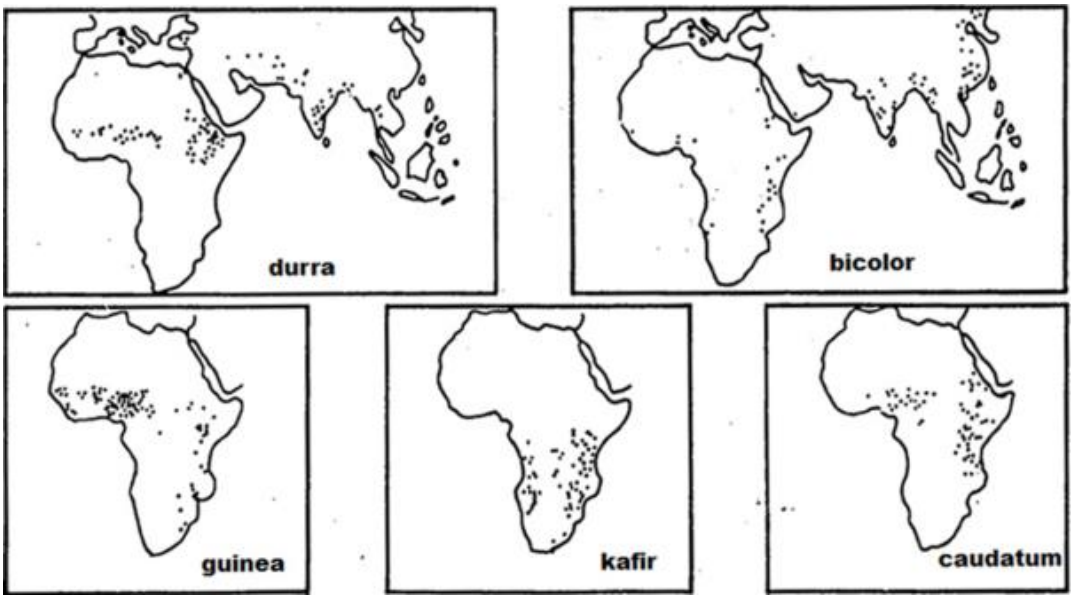
Regardless of where it is grown, required annual rainfall ranges from 400 mm to 750 mm, making it an important crop for areas too dry for maize. Although primarily known for its drought resistance, cultivated sorghum can also withstand temporary water logging. Altitudinal range is from sea level to 3 000 m, while latitudinal range is from 50°N in Germany to 40°S in Argentina. Favourable temperatures range from 10°C to 35°C for germination, with 30°C being optimal. Plant breeding has developed some cultivars to grow in lower temperatures. Tolerated soil types vary widely from heavy or cracked clay to light or deep sand. Soil acidity may range from pH 5.0 to 8.5. Cultivated sorghum possesses some tolerance to soil salinity.

Argentina, Brazil, Bolivia, Uruguay and the Bolivarian Republic of Venezuela lead sorghum production in South America. Cultivation in Central America is located in parts of Mexico, El Salvador, Nicaragua, Honduras, Guatemala, Belize and Panama (FAOSTAT, 2017). In the United States, an area extending from South Dakota to Texas and from Colorado to Mississippi is the primary sorghum-producing region (USDA-NASS, 2012). Most of Europe's sorghum production occurs in France, Italy, Ukraine and the

Russian Federation (FAOTAT, 2017). More than 95% of Australia's sorghum cultivation takes place in the states of Queensland and northern New South Wales (ABS, 2012).

Sorghum is a highly domesticated crop plant that does not generally survive outside of cultivation; however, its weedy relatives do survive in the wild. These weeds are feral in road ditches, stream banks, field margins or abandoned areas. Sorghum's progenitor, S. *bicolor* subsp. verticilliflorum, originated in Africa but has been introduced wherever sorghum is cultivated and occurs alongside the crop as an annual weed (Doggett, 1988). Johnsongrass has become a noxious weed since being naturalised in temperate regions. It is especially difficult to control due to its rhizomes (Warwick et al., 1984). Shattercane is a weed wherever it is sympatric with cultivated sorghum, and is a prolific self-sower due to its shattering panicle which facilitates seed dispersal (Doggett, 1988; Dahlberg, 2000; Hoffman and Buhler, 2002).

Figure 1.6. **Historical geographic distribution of the five basic races of cultivated sorghum in Africa, India and China**



Source: House (1985) and de Wet et al. (1970).

Cultivation and management practices of sorghum differ by region, particularly between the commercial-scale production in Australia, Europe and the United States and the smaller-scale production in Africa and Asia. In order to optimise yields, commercial producers consider soil quality, moisture content, nutrient availability and pest prevalence when developing plans for crop management. Most commercial cropping system strategies involve crop rotation for soil conservation or to reduce weed, pest and disease pressure (Cothren et al., 2000).

Land preparation in commercial-scale operations begins immediately following harvest by shredding the stalks of the harvested sorghum and disking or plowing the land. Mixing the soil and stalk residue aids decomposition, improves soil nutrient and water content, and reduces erosion. The land is then bedded and controlled for weeds through chemical herbicides, rotary hoeing or bed reshaping (Cothren et al., 2000). Insects may be controlled through insecticides, biological predators or cultural methods such as crop

rotation, variety selection and seed treatment (Teetes and Pendleton, 2000). Planting of the selected hybrid or cultivar is mechanised and the rows are evenly spaced, with spacing between rows determined by the moisture content (optimal yield requires adequate moisture, and if planting density is too high, there may not be enough moisture for each plant). Recommended plant populations range from 30 000 plants ha^{-1} in parts of Australia with marginal rainfall to 250 000 plants ha^{-1} under irrigation in the United States. Irrigation may be used to achieve higher yield despite sorghum's water efficiency characteristics. To achieve proper soil nutrient values, fertilisers are applied at planting and sometimes later as a broadcast application (Cothren et al., 2000).

The methods of subsistence food production in Africa and Asia vary according to climate, degree of mechanization, availability of improved varieties and fertilisers, methods of pest control, size of land holdings, moisture, and soil type. Land holdings are typically smaller with limited mechanization (House et al., 2000). Land is prepared by hand hoe, animal-drawn equipment, disks or plows. Planting is done by hand by placing several seeds in each hole to help plants emerge through the soil crust and resist wind. Row spacing is farther apart in relatively drier areas and closer together where more moisture is expected (House et al., 2000). In temperate climates, planting occurs in the spring or summer, and sowing early can help protect against pervasive pests like shoot flies or stem borers. In tropical climates planting is done in the wet season such that moisture is abundant during plant growth and restricted during grain harvest (Doggett, 1988; House et al., 2000). In some tropical climates, two cropping seasons are possible, depending on precipitation patterns. The second crop is seeded or ratooned. Manure is the primary fertiliser used, although phosphorous and potassium solutions are applied if available. Weed control is performed by hand with tools specialised for different soil types. Farmers usually do not have access to chemicals to control insects, weeds or diseases, due to cost and/or lack of availability (House et al., 2000). Few opportunities exist for surface irrigation.

Reproductive biology

Generation time and photoperiodism

A broad range of generation times, associated with high levels of genetic variance, has been introduced among cultivated sorghums through adaptation to different climates and cropping systems. The lifecycle is typically 90-120 days post-germination. Flowering begins at 60-90 days (Quinby, 1974) and lasts about a week (Stephens and Quinby, 1934). Photoperiod and temperature sensitivity play major roles in determining the time between sowing and panicle initiation for adapted genotypes. Thus, generation times may be considerably longer in parts of Africa and Asia where tall photoperiod-sensitive landraces are cultivated.

Photoperiod-insensitive cultivated sorghum hybrids are typically used in commercial agriculture to boost production through more consistent growth durations. Such systems include cultivars tailored to a particular duration of growing season, pest pressure and response to inputs like fertilisers, pesticides and opportunities for irrigation (Doggett, 1988; Folliard et al., 2004; Kouressy et al., 2008). However, photoperiod sensitivity is a crucial environmental adaptation for subsistence farmers in tropical climates who must wait until precipitation has softened the ground before land preparation can begin. This makes the time of sowing inconsistent and potentially spread out over several weeks. However, regardless of when seeds were sown, once the appropriate photoperiod threshold has been reached, floral initiation begins uniformly, coinciding with the most favourable time for seed production in varieties adapted to that climate (Doggett, 1988).

In West Africa, floral initiation occurs at the end of the rainy season, thus minimising the potential for damage caused by grain mould, insects or birds in early-maturing varieties; and by water shortage in late-maturing ones (Folliard et al., 2004; Kouressy et al., 2008). In the Ethiopian highlands, for example, the crop has been bred to grow through two wet seasons, with anthesis as late as 160-180 days (Shewayrga et al., 2008).

Plant growth (the vegetative phase) occurs primarily under decreasing day length, and floral development (the reproductive phase) typically requires photoperiod to fall below a specific day length to initiate (Folliard et al., 2004), although this threshold varies widely. Many traditional varieties require 12 or more hours of darkness to initiate flowering. Ellis et al. (1997) identified three developmental stages of sorghum undergone during the vegetative phase, and differentiated according to photoperiod and temperature responses. The first stage is photoperiod-insensitive, with a temperature-sensitive response that will delay floral initiation if the temperatures are too hot or too cold (optimum temperature is around 30°C). The second stage features a photoperiod-sensitive, temperature-insensitive response period where the stalk and leaves develop. The third stage occurs immediately prior to the reproductive phase and is insensitive to both photoperiod and temperature. The reproductive phase begins with panicle initiation, followed approximately 21 days later by anthesis (pollen shed) (Doggett, 1988). Grain development begins after fertilisation and maximum dry weight is reached approximately 25-55 days after anthesis. Harvest is often conducted 10-20 days after this point to reduce moisture content (Doggett, 1988).

Cultivated sorghum is typically produced as an annual crop with one generation per growing season, but some types are non-senescent and often associated with substantial seed production from tillers. In semi-arid areas of Australia and India, the "staygreen" trait has been introduced to improve post-anthesis drought tolerance (Borrell et al., 2000). Plants bearing this trait may be ratooned provided the climate and soil fertility are favourable. Harvests from the ratoon crop may be more substantial than harvests from the seeded crop in some areas. In Uganda, for example, multiple ratoons may be harvested from the same plants due to two rainy seasons each year (Downes, 1968; Escalada and Plucknett, 1975; Doggett, 1988).

Reproductive biology

Floral morphology and pollination

The many racemes and spikelets that compose the panicle develop about 21 days after the onset of the reproductive phase. A single panicle may bear 6 000 florets in total (Karper and Quinby, 1947). Spikelets along the racemes occur in sessile and pedicelled pairs. The sessile spikelets are fertile while the pedicelled spikelets are staminate and are formed on a short pedicel. The glumes of the sessile spikelets encase two florets. While the lower floret is sterile and consists of a lemma only, the upper floret is perfect, consisting of a lemma, two lodicules flanking the lemma, three stamens and a single-celled ovary with two plumose stigmas (Doggett, 1988).

Anthesis can begin as soon as the panicle begins to emerge from the culm, but usually begins several days later after the peduncle has reached its maximum growth. The first flower to bloom is the uppermost flower, and blooming continues down the panicle in a regular pattern. Sessile spikelets bloom first with pedicelled spikelets blooming two to four days later. Temperature, size of panicle and variety are the primary factors that determine duration of flowering, which is typically about one week, but varies from 2 to

15 days (Stephens and Quinby, 1934; Quinby, 1974). Reports in the literature on bloom time over a 24-hour cycle are inconsistent. Doggett (1988) stated that the time of blooming is affected by darkness and temperature, but generally occurs between 10 pm and 8 am. This is supported by observations in hot and dry Bellary, Karnataka, India (Ramanathan, 1924); Chillicothe, Texas (Stephens and Quinby, 1934); and at other subtropical locations (Ball, 1910; Robbins, 1917; Nafziger, 1918; Vinall, 1926). However, cool nights with heavy dews in more temperate latitudes are associated with delayed flowering, from 8 am to 4 pm (Graham, 1916; Patel and Patel, 1928; Ramanathan, 1924; Ayyangar and Rao, 1931). The flower opens in about ten minutes, allowing the stigmas and anthers to emerge. Pollen may dehisce from the anthers immediately upon emergence or may delay shortly depending upon environmental conditions. The time between the opening and closing of the glumes ranges from half an hour to four hours, but averages about two hours (Doggett, 1988).

Cultivated sorghum panicles may produce up to 24 million grains of pollen (Karper and Quinby, 1947), which is sensitive to desiccation (Lansac et al., 1994) and remains viable for only three to six hours (Stephens and Quinby, 1934; Doggett, 1988). However, in one study, pollen kept at 4°C and 75% relative humidity remained viable for 94 hours, and pollen stored in pollination bags in the shade in the field in Davis, California remained viable for over 20 hours (Sanchez and Smeltzer, 1965). While stigmas can be receptive two days before and up to a week after flowering, the optimal timeframe for pollination is within the first 72 hours (Ross and Webster, 1957; Doggett, 1988). Pollen germinates immediately upon reaching a receptive stigma and fertilisation of the egg cell occurs about two hours later to initiate seed development.

Cultivated sorghum is primarily self-pollinating; however, wind-mediated cross-pollination does occur. Schmidt and Bothma (2005) suggested that insect pollination may also occur, based upon their observations of honey bees, wild bees (sometimes known as solitary bees) and one species of beetle visiting several sorghum flowers consecutively. Upon collection of the insects, pollen grains identical to the grains collected from the sorghum anthers were found on all of the insects, with the honey bee carrying the most and the beetle carrying the least. However, no attempt was made to determine if insect movement resulted in cross-pollination.

Nunes-Silva et al. (2010) reported that the flower fly *Toxomerus politus* (Say) visits cultivated sorghum flowers to feed on pollen during the time that stigmas are receptive. The authors suggested that the flies may also contribute to pollination, but only in a minor way, since the relationship of *T. politus* with cultivated sorghum was similar to mutualisms between pollinators and their host plants wherein the larvae of pollinators also consume the reproductive organs of the host plants. Thus, the observation of insects associated with pollen is not necessarily indicative of the insect's efficiency as a pollinator.

More studies are called for to determine the extent of insect pollination in cultivated sorghum. Further information on outcrossing and gene flow can be found in the following section.

Seed dormancy, dispersal and viability of cultivated and weedy sorghum species

Pre-harvest sprouting due to low seed dormancy is an important challenge in cultivated sorghum, especially when grain maturation occurs under high humidity and rainfall conditions (Maiti et al., 1985); however, considerable variability in the level of seed dormancy exists among varieties (Lijavetzky et al., 2000; Rodríguez et al., 2012; Steinbach et al., 1995). In general, seed dormancy release is under the control of the

hormones abscisic acid (ABA) and gibberellin (GA) and varies greatly in how it is regulated within plant tissues (Finch-Savage and Leubner-Metzger, 2006). In a study of two inbred lines, Rodríguez et al. (2012) found that low seed dormancy was associated with a loss of embryo sensitivity to ABA and greater accumulation of GA, whereas greater seed dormancy was associated with increased embryo sensitivity to ABA and suppression of GA synthesis genes. Currently more than 130 forms of GA have been identified in plants and fungi. Thus, variations in genes affecting different GAs or their associated metabolic pathways, and the overall regulation of these pathways, may account for wide variations in pre-harvest sprouting susceptibility both between and within species. Furthermore, since ABA and GAs are associated with many physiological and developmental features of plants, including environmental sensing (de Lucas et al., 2008), breeding for reduced pre-harvest sprouting is challenging due to its polygenic nature and the hormones' systematic and environmentally modified effects within the plant.

The seed of cultivated sorghum does not shatter and must be transported by wind, water, animals or humans (Andersson and de Vicente, 2010). Seed viability was evaluated for 36 483 germplasm accessions stored under controlled conditions at the International Crops Research Institute for the Semi-Arid Tropics (Sastry et al., 2008). The reported storage period, which ranged from 5 to 21 years, demonstrated a greater than 85% viability, meeting the minimum standard for conservation in international gene banks (FAO, 2014). By contrast, in Sudan where there are few opportunities for controlled seed storage conditions, Ahmed and Alama (2010) reported viability under 85% after only one or two years in a brick warehouse, a corrugated iron warehouse or an underground pit. Evans et al. (1961) studied germination in ten cultivated sorghum genotypes and emphasised the significance of interactions among genotypes, soil moisture and germination temperature in modifying germination outcomes.

Shattercane, as its name implies, is able to disperse its seeds through shattering of the panicles. Its persistence in the seed bank is due to seed dormancy and seed longevity mechanisms (Burnside et al., 1977; Fellows and Roeth, 1992; Kegode and Pearce, 1998). Reports regarding the longevity of shattercane seeds in the soil range from 2 years (Teo-Sherrell and Mortensen, 2000) to 13 years (Burnside et al., 1977). Cold, wet soil conditions contributed to the two-year longevity estimate, such that 80% of seeds died in the first winter and virtually none survived the second (Teo-Sherrell and Mortensen, 2000). However, the authors point out that even a few survivors may be enough to ensure persistence of shattercane in the field due to its high rate of seed production.

Johnsongrass produces large numbers of shattering seeds that also may be carried by the wind, on animals or may remain dormant in the soil for up to 30 months (Holm et al., 1977) with some variability in the level of dormancy (Taylorson and McWhorter, 1969; Ghersa et al., 1992). Its seed does not survive as long at shallow soil depths, but large seed banks can be accumulated in the upper layer of soil by frequent seed input each year. Johnsongrass seeds are much more adapted to survival at depths greater than 22 cm, meaning persistent seed banks can accumulate when they are sufficiently buried (Andersson and de Vicente, 2010). While Johnsongrass primarily reproduces through seed, its invasiveness is also due to its rhizomes (Holm et al., 1977).

Genetics

Considerations for plant breeders

Gene pools

Cultivated sorghum is a genetically diverse diploid (2n = 2x = 20) with 200 classified phenotypic, genotypic and cytogenetic trait genes (Rooney, 2000). It is sexually compatible with some of its wild or weedy relatives, and the level of cross-compatibility determines its primary and secondary gene pools. The primary gene pool lies within section Eusorghum and includes the other diploid species *S. propinquum*, S. *bicolor* subsp. verticilliflorum, and shattercane. Crosses within this gene pool are fully interfertile. The high level of fertility and spontaneous outcrossing of the primary gene pool leads to frequent introgression when distributions overlap and conditions are favourable (Doggett and Majisu, 1968; Baker, 1972; Ejeta and Grenier, 2005).

The secondary gene pool consists of the tetraploid (2n = 4x = 40) members of Eusorghum: Columbus grass (*Sorghum almum* Parodi) and Johnsongrass. Domesticated sorghum is capable of outcrossing with members of the secondary gene pool despite ploidy level differences, producing either sterile triploids or somewhat fertile tetraploids (Arriola and Ellstrand, 1997, 1996; Morrell et al., 2005).

The tertiary gene pool includes species from other sections of sorghum. Outcrossing of cultivated sorghum with members of this gene pool is highly unlikely under natural conditions, and crosses produced through human intervention are anomalous, lethal or almost completely sterile (Ejeta and Grenier, 2005). However, crosses have been made with some of the Australian native sorghum species under controlled conditions using embryo rescue (Price et al., 2005).

The cultivated sorghum genome has been sequenced (Paterson et al., 2009). The haploid genome size is approximately 730 Mega base pairs (Mbp), larger than both *Arabidopsis* and rice (155 Mbp and 510 Mbp, respectively). However, these three plants have similar numbers of gene families. Molecular analysis of the genus has identified relatives of the species with novel traits, endosperm structure and composition that may be used to expand upon its currently known gene pool (Dillon et al., 2007).

Two traits, maturity and male sterility, are considered the most relevant when considering management of gene flow to wild or weedy relatives within cultivated sorghum's gene pools and vice versa. First, genes controlling maturity and their nuanced interaction with day length and temperature are critical for the timing of floral initiation in cultivated sorghum, and, consequently, reproductive success. Second, genes affecting male sterility can significantly modify the ability to cross-pollinate. Further examination of these traits and the potential for gene flow is given below. However, it is important to note that both traits are subject to modification by extremely high temperatures or drought such that flowering or early flowering may still occur under unusual circumstances in otherwise non- or late-flowering backgrounds, respectively; and self-pollination and seed set may occur in otherwise male-sterile backgrounds.

Traits affecting maturity

Time-to-maturity traits are polygenic with the Ma1, Ma2, Ma3 and Ma4 maturity loci containing 13, 13, 16 and 12 alleles, respectively, thus modulating a wide range of floral initiation dates (Rooney, 2000). Maturity is subject to significant genotype × environment (G × E) interactions. Numerous studies have reported a complex relationship between

maturity genotype, day length and temperature (Quinby, 1967; Miller et al., 1968; Caddel and Weibel, 1971; Hammer et al., 1989; Craufurd et al., 1999; Tarumoto, 2011; Tarumoto et al., 2003).

Quinby (1967) focused on 11 varieties of cultivated sorghum and their days to flowering according to genotype (Table 1.5), revealing substantial information about the role of each locus in determining the time from germination to flowering. Specifically, the use of various combinations of alleles may extend the time to flowering over long periods within a single environment.

Day length is a critical factor in the expression of maturity genes. Lane (1963) observed four varieties of cultivated sorghum under both 10-hour (short) and 14-hour (long) days (Table 1.6). "SM90" and "60M" are considered temperate varieties, while "80M" and "100M" are considered tropical varieties. The short day length hastened floral initiation in all varieties; however, the photoperiod-sensitive tropical varieties exhibited more delayed floral initiation than the less photoperiod-sensitive temperate varieties (Lane, 1963). The critical day length required to cause a delay in floral initiation was an additional indicator in determining how influential photoperiod is on floral initiation. For example, a difference of only one hour of day length between "SM90" and "100M" resulted in a dramatic delay of over 30 days to floral initiation (Lane, 1963).

Table 1.5. **Genotypes and time to flowering among 11 cultivated sorghum varieties in Plainview, Texas (United States), 1964**

Variety	Genotype	Time to flowering (days)
"100-day Milo (100M)"	Ma1Ma2Ma3Ma4	90
"90-day Milo (90M)"	Ma1Ma2ma3Ma4	82
"80-day Milo (80M)"	Ma1ma2Ma3Ma4	68
"60-day Milo (60M)"	Ma1ma2ma3Ma4	64
"Sooner Milo (SM100)"	ma1Ma2Ma3Ma4	56
"Sooner Milo (SM90)"	ma1Ma2ma3Ma4	56
"Sooner Milo (SM80)"	ma1ma2Ma3Ma4	60
"Sooner Milo (SM60)"	ma1ma2ma3Ma4	58
"44-day Milo (44M)"	Ma1ma2ma3 RMa4	48
"38-day Milo (38M)"	ma1ma2ma3 RMa4	44
"Hegari (H)"	Ma1Ma2Ma3ma4	70

Source: Quinby (1967).

Table 1.6. **Influence of photoperiod on four cultivated sorghum varieties**

Variety	Critical day length (hours)	Time to flowering (days)	
		10-hour days	14-hour days
"Sooner Milo" (SM90)	13.0	19	35
"60-day Milo" (60M)	12.5	19	38
"80-day Milo" (80M)	12.5	19	44
"100-day Milo" (100M)	12.0	19	70

Source: Lane (1963).

The influence of temperature on maturity can be observed when cultivated sorghum is grown at the same latitude to control for day length but at different elevations, where high elevation is associated with lower night-time temperatures. Quinby (1967) evaluated five varieties grown at both Chillicothe, Texas and Plainview, Texas. The Plainview location

was about 500 metres above the Chillicothe site, corresponding to approximately 2°C lower at night. The observed differences in time to flowering varied among varieties: three varieties were hastened, one was delayed and one hardly changed at the Plainview site (Table 1.7). These deviations indicate a maturity genotype × temperature interaction.

In addition to temperature and day length effects, specific combinations of alleles are used to delay or prevent the onset of the reproductive phase in temperate growing regions. For example, use of the Ma5/Ma6 genotype has recently been proposed for the production of late- or non-flowering cultivated sorghum hybrids (Mullet et al., 2010). Cropping systems using the non-flowering trait may focus solely on biomass or sugar production, as opposed to grain production, and have the potential to lower the likelihood of gene flow.

Table 1.7. **Influence of temperature on five cultivated sorghum varieties**

Variety	Time to flowering (days)		Influence of lower temperature on flowering
	Chillicothe	Plainview (2°C lower)	
"Hegari" (H)	78	68	Hastened
"Early Hegari" (EH)	71	62	Hastened
"100-day Milo" (100M)	100	90	Hastened
"60-day Milo" (60M)	66	66	Minor difference
"Sooner Milo" (SM60)	52	60	Delayed

Source: Quinby (1967).

Traits affecting male and female sterility

Cultivated sorghum possesses genes affecting both male and female sterility. Factors causing male sterility can be divided into two groups: nuclear male genetic sterility (commonly called genetic male sterility) and cytoplasmic-nuclear male sterility (commonly called cytoplasmic male sterility, or CMS). The genes ms1, ms2 and ms3 are associated with genetic male sterility due to the production of normal anthers but dysfunctional pollen (Ayyangar and Ponnaiya, 1937; Stephens, 1937; Webster, 1965). Other genetic male-sterile lines lack either pollen or anthers (Rooney, 2000). Genetic male sterility is used by some breeders to facilitate crossing, but since the genes are recessive, only the homozygous recessive individuals are male-sterile. Genetic male sterility systems are not used to produce commercial hybrids, and new varieties or lines generated using these systems to facilitate making crosses should be fixed back to the homozygous dominant (fertile) condition prior to deployment.

Female sterility is also a nuclear trait and has been observed in the dominant action of genes Fs1 and Fs2, which in the heterozygous condition result in viable pollen but only rudimentary development of stigmas, styles and ovaries, such that no seed set occurs (Casady et al., 1960).

CMS depends on the interaction of nuclear and cytoplasmic genes and renders the production of commercial F_1 cultivated sorghum hybrids economically viable (Stephens and Holland, 1954). The CMS system was first discovered in cultivated sorghum in a "Day" (race "Milo") × "Texas Blackhull" (race Kafir) cross and has been designated the A1 CMS system (Rooney, 2000). The genetics are reviewed in detail by Rooney (2000) and are more complex than summarised here. Briefly, three types of lines are involved: A-lines, B-lines and R-lines. A-lines contain cytoplasmic and nuclear genes that interact to produce male-sterile plants. B-lines contain the same nuclear genes, but not the

cytoplasmic genes for sterility, such that fully fertile plants are produced. A given B-line is backcrossed to an A-line and eventually the B-line is recovered in the A-line cytoplasm. This process yields an A/B-line pair that is essentially identical, except that the A-line is male-sterile and the B-line is male-fertile. The latter is used as a pollen source to "maintain" its A-line pair.

R-lines contain nuclear genes that override CMS and restore male fertility in the F_1 of an A-line × R-line cross. Other similarly functioning male sterility systems have been identified in alternate cytoplasms and are classified as the A2, A3 and A4 CMS systems (Schertz and Ritchey, 1978; Schertz, 1983; Kishan and Borikar, 1989). Most commercial F_1 hybrids of sorghum are currently produced using the A1 CMS system. Protocols utilising CMS to reduce the likelihood of gene flow through pollen were proposed by Pedersen et al. (2003).

Outcrossing

Cultivated sorghum is primarily self-pollinating; however, wind-mediated cross-pollination resulting in gene flow can occur in sorghum crop-weed complexes if the crop and wild or weedy relatives are sexually compatible, sympatric and flower simultaneously. This is often the case wherever cultivated sorghum is grown (de Wet and Harlan, 1971, 1975; Arriola and Ellstrand, 1996).

Outcrossing rates in cultivated sorghum are estimated at 5-30% under field conditions, based upon multiple methods of calculation (Ellstrand and Foster, 1983; Dogget, 1988; Pedersen et al., 1998; Djè et al., 2004). Significant variation exists between varieties and lines (Pedersen et al., 1998; Djè et al., 2004). The more compact panicles of race Durra, which is commonly used in commercial production, outcross at the lower end of the spectrum, about 7% (Djè et al., 2004).

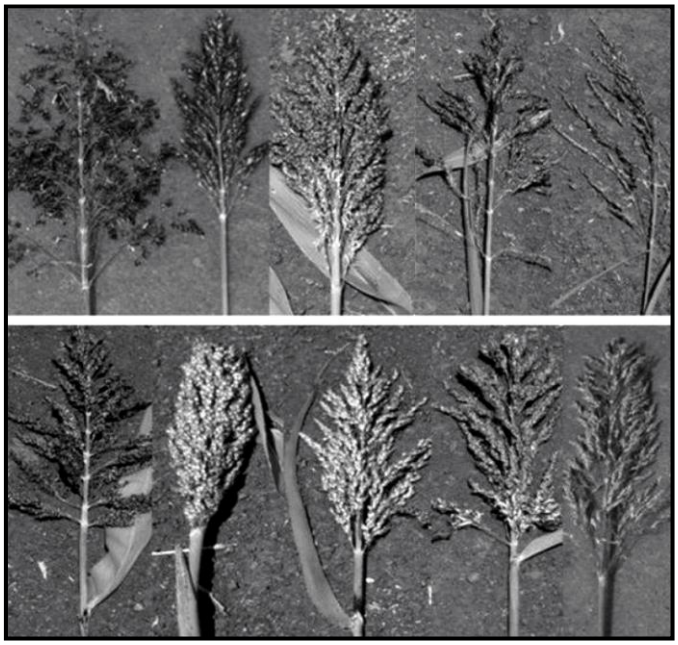
Interspecific crosses have likely occurred since cultivated sorghum's domestication. Crop-specific alleles have been found in samples of wild and weedy sorghum taxa that were genetically analysed for progeny segregation, allozymes and restriction fragment length polymorphisms (RFLP)s (Doggett and Majisu, 1968; Aldrich and Doebley, 1992; Aldrich et al., 1992; Paterson et al., 1995; Morrell et al., 2005). Morrell et al. (2005) surveyed RFLP allelic diversity in five Johnsongrass accessions from different parts of the United States. Among them, the frequency of individuals carrying at least one crop-specific allele ranged from 0.91 to 0.79 in Texas and Nebraska where cultivated sorghum is more frequently grown, and from 0.47 to 0.27 in New Jersey and Georgia where it is less frequently grown. These results suggest that when Johnsongrass is in close proximity to cultivated sorghum, higher rates of crop-to-weed gene flow are likely in the absence of management practices designed to avoid it, despite ploidy levels varying between the two species. Thus, the introduction of cultivated sorghum genes may persist in wild Johnsongrass populations if natural selection favours their presence in the genome. Factors affecting the fitness of crop alleles in wild sorghum populations are discussed in greater detail in the next section.

Phenotypic evidence of crop-to-weed introgression was observed by Tesso et al. (2008), who studied the geographic distribution of wild sorghum species in Ethiopia and Niger. A wide variety of phenotypic variation was observed within different regions, locations and individual fields (Figure 1.7), although the number of subspecies was not identified. The differing phenotypes varied according to habitat and proximity to cultivated sorghum: wild plants most similar in phenotype to cultivated sorghum occurred within crop habitats, while wild plants exhibiting smaller stature, thinner culms and very loose

panicles were found primarily in disturbed habitats, suggesting previous hybridisation and introgression events (Tesso et al., 2008).

Sudan grass is thought to be a hybrid between cultivated sorghum and *S. bicolor* subsp. *verticilliflorum*, and is found throughout Africa wherever cultivated sorghum is grown (Ejeta and Grenier, 2005; Andersson and de Vicente, 2010). Pedersen et al. (1998) investigated *in situ* crossing between Sudan grass individuals, with particular emphasis on the effects of flowering date combined with floret location on the panicle, and their influence on the rate of hybridisation. Two genotypes of Sudan grass were planted twice in two years in a checkerboard pattern and tagged to indicate the approximate date of pollination. The plants were harvested at maturity and the panicles were divided into three parts to be analysed: the upper, middle and bottom thirds. Pollination date was a major factor affecting the level of outcrossing, with the middle pollination date in both years having the highest rate of outcrossing (57.1% in 1991 and 38.9% in 1992). The middle date coincides with the time frame in which the most plants were observed to be entering anthesis resulting in the highest pollen density of the three time frames studied (early, middle and late). Conversely, the early pollination time period which generated the lowest pollen density exhibited the smallest amount of outcrossing in both years (36.0% in 1991 and 20.6% in 1992). Floret location on the panicle influenced outcrossing rates during the middle and later pollination dates in 1992; outcrossing occurred at higher levels in the upper third than in the lower third of the panicle with 48.7% and 30.3%, respectively, at the middle pollination date (Pedersen et al., 1998). Similar observations of outcrossing rate due to the location of florets on the panicle have been made in sorghum; additionally, the more compact grain sorghum panicles outcross at lower rates (10-15%) than the more open Sudan grass panicles (Maunder and Sharp, 1963; Ellstrand and Foster, 1983; Schmidt and Bothma, 2006).

Figure 1.7. **Phenotypic diversity of wild Sorghum species in a single field in Wollo, Ethiopia**



* At the time of peak cultivated sorghum flowering in 2005. The number of species or subspecies was not identified.

Source: Tesso et al. (2008).

Agriculturally important weedy relatives within cultivated sorghum's gene pool

Weedy *Sorghum* species exist either as rhizomatous perennials or as annuals resulting from hybridisation events with cultivated sorghum (Ejeta and Grenier, 2005). Johnsongrass and shattercane (Figure 1.8) are the primary weedy relatives of interest to agriculture due to their invasiveness and propensity to evolve resistance to herbicides (Holm et al., 1977; Heap, 2012). In parts of Southeast Asia, *S. propinquum* also readily crosses with cultivated sorghums (Ejeta and Grenier, 2005).

Rhizomatous relatives of cultivated sorghum are likely derived from the highly rhizomatous *S. propinquum*. Its classification in cultivated sorghum's primary gene pool indicates that it is fully interfertile (Andersson and de Vicente, 2010). In most instances, geographic isolation has prohibited cultivated sorghum from outcrossing with *S. propinquum* due to differing environmental adaptations (Dahlberg, 1995); however, that has not been the case in the Philippines, where frequent crosses with cultivated sorghum have produced progeny that have become noxious weeds (Ejeta and Grenier, 2005).

Figure 1.8. **Two agriculturally important weedy relatives of cultivated sorghum: Johnsongrass (left) and shattercane (right)**

Source: Courtesy of Pamela B. Trewatha, Missouri State University.

Johnsongrass is an aggressive, rhizomatous perennial grass recognised as one of the world's worst weeds (Holm et al., 1977). It is generally considered self-compatible with less than a 10% outcrossing rate (Warwick and Black, 1983; Burke et al., 2007). Regardless, the ability of Johnsongrass to cross with cultivated sorghum is well-documented (Arriola and Ellstrand, 1997, 1996). *S. almum*, also known as Columbus grass, is genetically similar to Johnsongrass. It grows taller and has larger stems and leaves than Johnsongrass, but it has shorter rhizomes and is less troublesome a weed (Magness et al., 1971). *S. almum* and Johnsongrass both belong to cultivated sorghum's secondary gene pool such that F_1 progeny from cultivated sorghum ($2n$) × Johnsongrass ($4n$) crosses are usually completely sterile triploids and progeny from cultivated sorghum ($2n$) × *S. almum* ($6n$) crosses are partially fertile tetraploids (Endrizzi, 1957; Warwick and Black, 1983; Sangduen and Hanna, 1984). However, reports of fertile tetraploid offspring

from cultivated sorghum × Johnsongrass crosses exist and are reviewed in Warwick and Black (1983). Further information about cultivated sorghum × Johnsongrass crosses and gene flow between these species is found in the next section.

Shattercane resembles cultivated sorghum but differs in that it grows taller because it has no dwarfing genes; it is able to disperse seeds through seed shattering, and its seeds exhibit greater dormancy and longevity in the soil (Quinby and Martin, 1954; Burnside et al., 1977; Fellows and Roeth, 1992).

Gene flow and fitness of crop × weed hybrids

In a study investigating crop-to-crop gene flow in race Kafir, Schmidt and Bothma (2006) observed that outcrossing rates among pollen receptors decreased as their distance increased from pollen donors. The experiment was laid out with the pollen donors (male-fertile B-line "Redlan") grown in a 30 × 30 metre block from which eight arms of the pollen receptors (male-sterile A-line "Redlan") radiated out at distances ranging from 13 metres to 158 metres. The average outcrossing rate, across directions, was 2.54% at 13 metres, less than 1% at or beyond 26 metres, and 0.06% at 158 metres. Mathematical models estimated maximum gene flow distance to be 200-700 metres. These values were in agreement with observations by cultivated sorghum breeders, who use isolation distances of 100 metres to achieve less than 1% gene flow from neighbouring fields. Distance and wind direction were found to be the primary factors determining the rate of gene flow. The authors suggested that outcrossing rates under natural conditions would be expected to be lower than what they observed because the use of male sterile receptors eliminated pollen competition and allowed the female flowers to remain receptive longer in the absence of pollination. Female flowers can remain receptive up to 16 days in the absence of pollination even though flowering is typically complete in 4-7 days (Schertz and Dalton, 1980). Under natural circumstances, fully fertile plants are about 70-95% self-pollinated (Ellstrand and Foster, 1983; Pedersen et al., 1998; Djè et al., 1999, 2004; Smith and Frederiksen, 2000).

Crop-to-weed gene flow has been observed between cultivated sorghum and Johnsongrass. Arriola and Ellstrand (1996) investigated the level of spontaneous hybridisation between Johnsongrass and cultivated sorghum at two test sites over a two-year period. They planted a central plot of sorghum (diploid pollen source) surrounded by pots of Johnsongrass (tetraploid maternal plants) at distances of 0.5, 5, 50 and 100 metres. Results indicated a trend toward decreased hybrid production as distance from the crop increased, but crop-to-weed hybrid seedlings were detected at the furthest distance at both sites. No weed-to-crop hybrid seedlings were detected. Measured rates of hybridisation ranged from 0% to 100% per plant, with hybridisation levels as high as 2% at a distance of 100 metres. Like Schmidt and Bothma (2006), an increase in relative pollen flow was needed to produce hybrids at further distances. The triploid hybrids generated in this experiment were capable of being pollinated by diploid sorghum to restore partial self-fertility. Arriola and Ellstrand (1996) concluded that hybrid formation between cultivated sorghum and Johnsongrass was highly variable and somewhat unpredictable, as the observed hybridisation rates in this study varied according to the distance between the weed and crop plants, the location of the study site, and the year the study was performed. The highly variable results were attributed to the large degree of morphological and genetic variation seen within Johnsongrass that influences the hybridisation abilities of different plants and their dynamics in differing systems. In summary, the study concluded that distance was the primary factor affecting relative gene flow, with many more hybrids being produced closer to the pollen source.

Sangduen and Hanna (1984) also evaluated cultivated sorghum × Johnsongrass hybrids. Although not used in cultivation, tetraploid *S. bicolor* has been experimentally produced and was used in their experiments. Two such tetraploid sorghum lines and a tetraploid Johnsongrass were used as both maternal and paternal parents in crosses. Hybrid seeds were produced by covering the flowering panicles with bags after being dusted with pollen. Seeds subsequently produced were then planted for observation. Results revealed that interspecific hybrids were produced at a higher frequency when Johnsongrass served as the female parent than when cultivated sorghum served as the female, with 71-83% of seeds being hybrid compared to 0-33%, respectively. This variation was likely due to specific responses to the crossing technique, cross-incompatibility or a mixture of both. The hybrid plants morphologically resembled Johnsongrass due to their perennial and rhizomatous growth, open inflorescence, seed shattering, seed shape, and seed colour. Stem thickness, number of rhizomes, leaf width and seed size were traits expressed as intermediate between both parents. Hybrid plants were more leafy and vigorous with longer and larger inflorescences than either parent. The high rate of outcrossing is not especially concerning from an agroecological viewpoint. Although tetraploid sorghum exists as an experimental tool, it is not cultivated.

Schmidt (2011) evaluated gene flow between cultivated sorghum and shattercane, using cultivated sorghum as a pollen source and shattercane as a pollen receptor. Cross-pollination ranged from 4% to 16% among shattercane plants placed directly within the area occupied by pollen donors, and decreased to nearly 0% at 200 metres downwind.

In a separate study, crosses between a single shattercane inbred line and cultivated sorghum were produced by Sahoo et al. (2010) in order to assess the fitness components of hybrids. Fitness components evaluated were temperature requirements for germination, rate of germination, dormancy, vegetative growth and seed production. For components of fitness affecting seeds, temperature was a strong modifier of the proportion of seeds able to germinate, their rate of germination and the length of dormancy prior to germination. Overall, the response of F_1 hybrids was similar to shattercane at lower temperatures and to cultivated sorghum at higher temperatures. This could be attributed to the position of the seed within the glumes of the F_1 hybrids; shattercane exhibits a great deal of dormancy and seed protection due to the seed's complete encapsulation by the glumes, whereas cultivated sorghum seeds are not encapsulated. The hybrids were morphologically intermediate with their seed only partially encapsulated by the glume, thus potentially weakening their protection to extreme heat and humidity.

For components of fitness affecting vegetative growth and seed production, Sahoo et al. (2010) observed that shattercane grew taller than cultivated sorghum and that F_1 hybrids exhibited hetorosis, growing taller and producing more biomass than both parents. Cultivated sorghum had the largest leaf area index and shattercane had the smallest, but the F_1 hybrid was intermediate and closer to sorghum. Leaf emergence was greater for sorghum and the hybrid than for shattercane, but seed size and production were more similar to shattercane, which produced many small seeds, than to cultivated sorghum, which produced fewer, larger seeds. When considering these traits together, F_1 hybrid fitness was similar to that of shattercane, suggesting that crop genes that are either neutral or beneficial to shattercane would persist in populations within agro-ecosystems.

Arriola and Ellstrand (1997) measured fitness components of Johnsongrass × cultivated sorghum hybrids relative to the Johnsongrass parents, including time to flowering, pollen viability, seed production, panicle production, tiller production and biomass. The only observed difference between genotypes was a slightly higher level of pollen sustainability

(an estimate of viability) in the hybrid plants; however, overall performance of the hybrids was indistinguishable from Johnsongrass. Therefore, it is expected that hybrid fitness in these crosses is equal to that of the weedy parent.

Methods to mitigate crop-to-weed gene flow

As the above studies show, distance is the primary factor mitigating crop-to-weed gene flow because greater distances are associated with a reduction in pollen density from the source (Arriola and Ellstrand, 1996; Schmidt and Bothma, 2006). Isolation distances for sorghum in OECD countries have been designated as 200, 300 or 400 metres, depending upon seed category and climatic conditions and in many cases have proved sufficient to reliably achieve less than 0.1% outcrossing; however, these distances may not be enough under all circumstances (Andersson and de Vicente, 2010). Gene flow may be substantially influenced by wind strength and direction, genotype, plant morphology and topography.

Where physical separation is not feasible, ensuring different flowering times is the most effective way to reduce opportunities for gene flow (Ellstrand, 2003). In a study of six cultivated lines of rice, the degree of outcrossing with wild relatives was shown to be the highest in the cultivar with the longest overlapping flowering period with the wild relatives (Langevin et al., 1990). However, it must be noted that extreme temperatures or drought may induce flowering among late- or non-flowering cultivated sorghum lines.

Population size and structure also influence pollen density, as does spatial arrangement: Ellstrand and Foster (1983) observed a higher rate of outcrossing in plants grown in a dispersed arrangement than plants grown in a stratified arrangement.

Sexual compatibility influences the possibility of gene flow, but does not prevent it completely. Cultivated sorghum is sexually compatible with the entire section Eusorghum (Ejeta and Grenier, 2005). Hybridisation across gene pools can produce sterility or reduced fertility. In the case of hybrids between tetraploid Johnsongrass and diploid cultivated sorghum, some reduced fertility was observed; however, through backcrossing with diploid parents, partial self-fertility was restored (Arriola and Ellstrand, 1996).

Other genetic barriers to outcrossing have also been proposed. Namely, genetic or cytoplasmic male sterility in cultivated sorghum could be used to create barriers to outcrossing as there is no viable pollen available to initiate spontaneous hybridisation (NRC, 2004). Pedersen et al. (2003) proposed a scheme to take advantage of this in sorghum by using a source of cytoplasmic male sterility with few known fertility restoring R-lines and including a low percentage of fertile pollinators in seedlots. However, pollen competition may be a confounding factor in such systems. Muraya et al. (2011) showed that self-pollination results in higher rates of seed set than cross-pollination, and suggested that the use of male-sterile bait plants in gene flow studies may overestimate gene flow rates and that pollen competition may be a significant factor in reproductive success. Furthermore, extreme temperatures or drought may cause otherwise sterile plants to regain fertility. A recent summary of current strategies to mitigate crop-to-weed gene flow, from crop management to molecular level, and those proposed for future deployment are outlined in detail in Oliver and Li (2012).

Ecology

Potential for increased weediness among wild sorghum species due to gene flow

Wild and weedy sorghum species have the ability to outcompete cultivated crops for nutrients and light, and are also carriers of harmful pests and diseases, such as sorghum ergot caused by the fungus *Claviceps africana* (Ejeta and Grenier, 2005). Mechanised farming practices do not involve the hand pulling of weeds, making it possible for the seeds that survive winter to spread uncontrolled (Ejeta and Grenier, 2005). Weeds rely on traits such as seed dormancy, variable germination, vegetative plasticity and increased fecundity to enhance their ecological fitness (Sahoo et al., 2010), whereas most crops are bred to remove these traits to enhance uniformity and control. If weedy relatives inherit crop traits intended to eliminate seed dormancy or reduce vegetative growth, the new traits are not expected to confer survival or invasiveness advantages (Linder and Schmitt, 1995). The above studies confirmed that cultivated sorghum × Johnsongrass and shattercane hybrids were no more problematic than their weedy parent (Arriola and Ellstrand, 1997; Sahoo et al., 2010). Crop traits expected to confer an advantage to weedy relatives, such as herbicide resistance, are of more concern (Arriola and Ellstrand, 1997, 1996; Schmidt and Bothma, 2006). Hokanson et al. (2010) outlined strategies to mitigate any potential risks that may be associated with the introduction of transgenic plants to Africa, although their suggestions are applicable to policy makers everywhere. Oliver and Li (2012) provide further discussion of the issue of containment.

Improved sorghum has been deployed throughout the world for over a century and many genotype interactions have been studied (Ejeta and Grenier, 2005). Evidence of domestic alleles that are present and persistent in wild populations suggests that crop-to-weed hybridisation is the rule rather than the exception (Ellstrand et al., 1999). These studies indicate that hybridisation with wild relatives has the potential for weed evolution and gene introgression, but little risk of extinction.

Hybridisation between crops and their wild and weedy relatives may confer neutral, detrimental or beneficial selective advantages. These modulate a hybrid's fitness, and consequently a gene's potential to introgress and persist in the environment. Outbreeding depression occurs when detrimental traits in the hybrid confer a selective disadvantage, potentially leading to extinction (Ellstrand et al., 1999). Genetic swamping occurs when continued introgression of neutral or beneficial traits causes hybrids and their progeny to assimilate into the dominant parent population. This is also a form of extinction (Levin et al., 1996). Both forms of extinction are of particular concern in Africa (Doggett, 1988; Schmidt and Bothma, 2006). Although sorghum readily hybridises with its wild and weedy relatives, so far there has been no evidence of genetic swamping or extinction amongst its wild relatives. The only known instances of genetic erosion have been due to habitat change (Ejeta and Grenier, 2005).

It is important to note that even beneficial alleles may not persist following crop-to-weed introgression because other genetic and environmental factors influence subsequent propagation. For example, volunteer cultivated sorghum plants do not typically survive winter in temperate regions (Andersson and de Vicente, 2010). Other genetic factors may also counterbalance a new beneficial allele. Keeler (1989) predicts that a single beneficial trait is unlikely to cause significant increased weediness or invasiveness; however, a single trait like herbicide resistance has obvious consequences in increased weed fitness (NRC, 2004). Nevertheless, a trait's potential to confer increased fitness must be evaluated in

combination with relevant environmental factors to be accurately assessed (Arriola and Ellstrand, 1997, 1996; Sahoo et al., 2010).

Interactions in natural and managed ecosystems

Weedy relatives can be carriers of diseases and pests that can cause significant damage to natural and agroecosystems alike. The potential for increased weediness due to crop-to-weed introgression of herbicide resistance further exacerbates this problem by increasing the number of surviving weeds (Ellstrand et al., 1999). Shootfly and sorghum midge are two notorious pests whose control relies in large part on the time of planting, since these pest populations decrease significantly in the absence of sorghum hosts during winter or the rainy season. Wild, weedy or cultivated sorghum volunteers serve as hosts for these pests between cropping seasons, such that pest populations accumulate (Doggett, 1988). *Claviceps africana* is an ergot-causing fungal parasite that lives only in the flowers of certain grasses and survives in wild sorghum species like Johnsongrass (Ejeta and Grenier, 2005). This pathogen has spread rapidly around the world, and concern exists that it could become endemic on Johnsongrass if it becomes established (Odvody et al., 1999). *Claviceps africana* is a threat to grain sorghum production, as it infects unfertilised ovaries of cultivated sorghum (Frederiksen, 2000). A list of sorghum's common pests and pathogens can be found in Annexes 1.A1 and 1.A2. Bailey (2007) in particular provides a review of pests specific to Australia.

Impact on animals in the environment

Certain factors can render sorghum forage toxic to grazing animals. Environment, genetics, plant part and growth stage are important modifiers of sorghum forage toxicity. Like other C4 forage plants, including maize and pearl millet, cultivated sorghum accumulates nitrates (Pedersen and Fritz, 2000), but at higher rates (Sidhu et al., 2011). Several factors can contribute to increased nitrates in sorghum forage, including environmental conditions, nitrogen fertiliser use, growth stage and plant part (Sidhu et al., 2011). Drought and frost severely interfere with the crop's normal growth, slowing development and allowing higher concentrations of nitrate to accumulate in plant tissues (Pedersen and Fritz, 2000; Sidhu et al., 2011). Young plants have a higher rate of nitrate uptake and generally contain higher levels than mature plants (Sidhu et al., 2011). Stems have the highest concentration of nitrate, followed by roots and leaves, and concentrations in flowers and grain are considered negligible (Sidhu et al., 2011). Excess nitrate in sorghum forage can be toxic to ruminants and other grazing animals through the production of methemoglobin (Wright and Davison, 1964).

Cyanogenic glycosides are secondary products that are produced in a range of plant species, including sorghum (Ganjewala et al., 2010). These compounds are believed to be largely involved in defence against predators, most particularly insects. Excess cyanogenic glycosides can be toxic to ruminants and other grazing animals through the production of cyanoglobin (Vough, 1978). When present, cyanogenic glycosides are mainly found in germinating seeds, sprouts and the leaves of immature sorghum plants. The most abundant of these is dhurrin, which may comprise 3-4% of the leaves of germinating seeds (Newton et al., 1980; Doggett, 1988). Cyanogenic glycosides may be converted in the rumen or nonruminant stomach into prussic acid (also known as hydrocyanic acid, HCN, the aqueous form of cyanide). Environmental stresses including drought and frost are major environmental conditions resulting in higher HCN levels (Pedersen and Fritz, 2000). Frost releases HCN quickly in frozen leaves and may kill the top of the plants, causing new shoots and leaves at the bottom to be high in prussic acid (Vough, 1978).

Drought stunts the growth of the plant preventing it from growing out of the young plant stage, which generally has higher levels of HCN (Vough, 1978). Cyanogenic glycosides are not found in mature grain. Modern screening methods based on near-infrared spectroscopy have been developed to monitor levels of cyanogenic glycosides where the technology exists (Fox et al., 2012).

Sorghum varieties developed specifically for grazing such as Sudan grass have reduced levels of cyanogenic glycosides.

OECD (2010) provides pertinent detailed information for the management of anti-nutrients and toxicants for food and feed.

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Annex 1.A1.
Common insect pests

Common insect pests of cultivated sorghum include:

1. Chinch bug – *Blissus* spp.
2. Corn leaf aphids – *Rhopalosiphum maidis* (Fitch)
3. Greenbugs – various species within order Homoptera, especially *Shizaphis graminum* (Rondani)
4. Soil cutworm – various species within family Noctuidae
5. Wireworms – various species within family Elateridae
6. Seedcorn maggot – *Delia platura* (Meigen)
7. Seedcorn beetle – *Stenolophus lecontei* (Chaudoir)
8. Sorghum midge – various species within family Cecidomyiidae, especially *Stenodiplosis sorghicola* (Coquillett)
9. Fall armyworm – *Spodoptera frugiperda* (J. E. Smith)
10. Stalk and stem borers – various species within order Lepidoptera, especially *Busseola fusca* (Fuller), *Chilo partellus* (Swinhoe), *C. orichalcociliellus* (Strand), *Sesamia calamistis* (Hampson), *Eldana saccharina* (Walker), *Diatraea saccharalis* (Fabricius), *D. lineolata* (Walker) and *D. grandiosella* (Dyar)
11. Shoot fly – *Atherigona soccata* (Rond.)
12. Lesser cornstalk borer – *Elasmoplapus lignosellus* (Zeller)
13. Corn earworm – *Helicoverpa zea* (Boddie)
14. Sorghum webworm – *Nola sorghiella* (Riley)
15. Stink bug – various species within Genera *Nezera*, *Euschistus* and *Oebalus*
16. Billbug – *Sphenophorus* spp.
17. Sugarcane beetle – *Euetheola humilis rugiceps* (LeConte)
18. Yellow sugarcane aphid
19. White grub – *Phyllophaga crinita* (Burmeister)

Annex 1.A2.
Common pathogens

Cultivated sorghum is susceptible to bacterial, fungal, nematode, plant, phytoplasma and viral diseases. Those of greatest agronomic importance are listed below. A complete list may be found in Frederiksen (2000).

1. Grain mould – *Fusarium thapsinum* and various other *Fusarium*, *Alternaria* and *Cochliobolus* spp.
2. Ergot – *Claviceps africana*
3. Sorghum downy mildew – *Peronosclerospora sorghi*
4. *Fusarium* stalk rot – *Fusarium proliferatum* and other *Fusarium* spp.
5. Bacterial stalk rot – *Erwinia chrysanthemi*
6. Charcoal rot – *Macrophomina phaseolina*
7. Anthracnose – *Colletotrichum sublineolum* and *C. graminicola*
8. Rust – *Puccinia purpurea*
9. Zonate leaf spot – *Gloeocercospora sorghi*
10. Head smut – *Sporisorium reilianum*
11. Sooty stripe – *Ramulispora sorghi*
12. Gray leaf spot – *Cercospora sorghi*
13. Sorghum mosaic virus
14. Witchweed – *Striga* spp.

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Annex 1.A3. Biotechnological developments

Sorghum has proven to be highly recalcitrant to genetic transformation (Andersson and de Vicente, 2010), but improvements have been achieved. *Agrobacterium*-mediated modification and particle bombardment are two proven methods for introducing transgenic traits to sorghum. Recent reports have indicated that in some genotypes, transformation efficiencies in excess of 20% are achievable (Liu and Godwin, 2012). There is currently no commercially available genetically modified sorghum, but research has progressed in the four following areas.

Biofortification

A major obstacle to cultivated sorghum use as food is its nutritional deficiency: it has low protein digestibility and lysine content. The Africa Biofortified Sorghum (ABS) Project aims to create nutritionally enhanced transgenic lines with increased lysine content, protein digestibility and bioavailability of iron and zinc (Zhao, 2007). Iron-deficiency anaemia in particular is a problem in many rural areas of Africa. Using *Agrobacterium*-mediated methods of genetic transformation, suppression of kafirin protein synthesis has resulted in compensatory synthesis of other proteins with higher lysine content and increased digestibility (Zhao, 2007; Taylor and Taylor, 2011). ABS #1, a first-generation line with 50% more lysine, was developed based on transgenes originally developed for maize (Zhao et al., 2003). Subsequently, creation of a second generation, ABS #2, was successful and has been crossed with African varieties. The second generation has improved protein quality and digestibility, as well as increased levels of iron, zinc, and vitamins A and E (AHBFI, 2007). Taylor and Taylor (2011) reported that transgenic cultivated sorghum had 52-115% more lysine and 23-102% greater protein digestibility. Furthermore, foods prepared from these grains had improved protein quality.

Insect resistance

Bacillus thuringiensis (*Bt*) genes have been deployed experimentally to confer Lepidopteran insect resistance. Girijashankar et al. (2005) created transgenic cultivated sorghum via particle bombardment of shoot apices with a synthetic *Cry1Ac Bt* gene controlled by *mpiC1*, a promoter from the maize protease inhibitor gene. The resulting transgenic plants were grown in a greenhouse and artificially infested with *Chilo partellus* larvae (spotted stem borer) to assess the degree of insect resistance. In non-transgenic control plants leaves, larvae consumed over 80% of the material within five days whereas transgenic plants showed less than 50% leaf damage, 40% larval mortality and a 36% reduction in surviving larval weight. Assays of shoots indicated no significant decrease in larval weight, which suggests a lower level of *Bt* transgene expression in stem tissue than in leaf tissue. These results document partial resistance in *Bt* sorghum (Girijashankar et al., 2005).

Disease resistance

Transformation of cultivated sorghum for resistance to anthracnose, a fungal disease caused by *Colletotrichum sublineolum*, and to stalk rot-causing fungi like *Fusarium thapsinum,* has achieved some success (Krishnaveni et al., 2001; Kosambo-Ayoo et al.,

2011). Genes encoding chitinase or chitosanase hydrolyse fungal cell walls, rendering them osmotically sensitive. Kosamboo-Ayoo et al. (2011) used particle bombardment to create lines that were significantly more tolerant to anthracnose than non-transgenic control plants. Krishnaveni et al. (2001) used biolistic transformation to introduce rice chitinase into cultivated sorghum. Five to 50% of transformed seedlings demonstrated moderate resistance to stalk rot caused by *F. thapsinum*, but transgene expression varied.

Bioenergy

Grain sorghum and sweet sorghum are excellent candidates for bioenergy use due to high biomass and sucrose production, and the ability to grow them in a wide range of environments with minimal inputs. Grain sorghum can be used in the production of grain ethanol, while sweet sorghum's high sucrose content can be used to produce ethanol from saccharine juice through fermentation (Saballos, 2008). Potential traits for improvement as a bioenergy crop include increased yield and biomass quality such that cultivated sorghum becomes even more cost-effective to process into usable energy (Saballos, 2008). Lignin modification is important to increase the bioenergy production efficiency of sorghum (Saballos, 2008; Basu et al., 2011). Producing ethanol requires the hydrolysis of cellulose polymers, but lignin hinders the enzymatic process and inhibits conversion of lignocellulose (Dien et al., 2009). Two lines of transgenic sorghum with altered lignin composition have been created through *Agrobacterium*-mediated transformation (Basu et al., 2011). These transgenic plants had 28% less total lignin with significant increases in cellulose content and soluble sugars, which would increase the efficiency of fermentation when processing sweet sorghum for bioenergy.

Wu et al. (2007) provide information about seed composition, seed structure and other physical features that either help or hinder conversion of sorghum grain to ethanol based on the analysis of 70 genotypes and elite hybrids. In particular, the authors observed that the major factors having a positive effect on the bioconversion of elite genotypes included high starch content, rapid liquefaction, low viscosity during liquefaction, high fermentation speed and high fermentation efficiency. Major adverse factors included tannin content, low protein digestibility, high mash viscosity and an elevated concentration of amylose-lipid complexes in the mash. A more detailed review of sorghum's potential for ethanol production may be found in Serna-Saldívar et al. (2012).

The United States Environmental Protection Agency announced in December 2012 that sorghum grain qualified as an advanced biofuel. The Environmental Protection Agency's analysis found that ethanol produced from grain sorghum has an estimated lifecycle greenhouse gas emissions reduction of 32% when produced at dry mill ethanol facilities that use natural gas, producing on average 92% wet distillers grains; and a reduction of 52% when produced at dry mill ethanol facilities that use only biogas for process energy and obtain from an off-site supplier 0.15 kWh of electricity per gallon of ethanol produced, compared to the baseline gasoline fuel it would replace. Therefore, grain sorghum ethanol produced at dry mill ethanol facilities using natural gas met the minimum 20% greenhouse gas emissions reduction threshold for conventional biofuels, and grain sorghum ethanol produced at plants using only biogas for process energy and obtain from an off-site supplier no more than 0.15 kWh of electricity per gallon of ethanol produced, and met the 50% greenhouse gas emissions reduction threshold for advanced biofuels as required by the Energy Independence and Security Act of 2007, accessible at: www.epa.gov/otaq/fuels/renewablefuels/documents/420f12078.pdf.

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Chapter 2.

Tomato (*Solanum lycopersicum*)

*This chapter deals with the biology of tomato (*Solanum lycopersicum*). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties intended to be grown in the environment (biosafety). It includes elements of taxonomy, centre of origin and distribution, crop production and cultivation practices, reproductive biology, genetics, hybridisation and introgression, interactions with other organisms (ecology), pests and diseases, and biotechnological developments.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with Spain and Mexico as the co-lead countries. It was initially issued in September 2016. Production data have been updated based on FAOSTAT.

Introduction

The cultivated tomato, *Solanum lycopersicum* L., is the world's most highly consumed vegetable due to its status as a basic ingredient in a large variety of raw, cooked or processed foods. It belongs to the family Solanaceae, which includes several other commercially important species. Tomato is grown worldwide for local use or as an export crop. In 2014, the global area cultivated with tomato was 5 million hectares with a production of 171 million tonnes, the major tomato-producing countries being the People's Republic of China (hereafter "China") and India (FAOSTAT, 2017). Tomato can be grown in a variety of geographical zones in open fields or greenhouses, and the fruit can be harvested by manual or mechanical means. Under certain conditions (e.g. rejuvenation pruning, weeding, irrigation, frost protection), this crop plant can be perennial or semi-perennial, but commercially it is considered an annual (Geisenberg and Stewart, 1986).

Although there are many types of growing systems for greenhouse tomatoes, the two principal cropping systems are two crops per year and one crop per year. Its importance lies not only in profit, but also in the income generated in local economies for farmers and agricultural workers (Villarreal, 1982; Coll-Hurtado and Godínez Calderón, 2003). Protected agriculture is a wide category of production methods providing some degree of control over various environmental factors. This category includes production technologies such as: greenhouses, glasshouses, tunnels and covered fields (Nieves-García, van der Valk and Elings, 2011). Although there is no quantitative data about the world's vegetable production in greenhouses, some calculations have been made. For example, in 2012, the greenhouse vegetable production was about 81 million kilograms (kg), of which 40 million kg was tomato, and 37 million kg was cucumber. More specifically, in 2012, the tomato production in greenhouses in North America accounted for the 52% of the market in Canada and the 22% of the market in the United States (Farm Credit Canada, 2012).

The commercially important tomato fruit can vary in colour, size and shape (Vaughan and Geissler, 1997). The fruit contains a large quantity of water, vitamins and minerals, low amounts of proteins and fats, and some carbohydrates. It also contains carotenes, such as lycopene (which gives the fruit its predominantly red colour) and *beta*-Carotene (which gives the fruit its orange colour). Modern tomato cultivars produce fruits that contain up to 3% sugar of fresh fruit weight. It also contains tomatine, an alkaloid with fungicidal properties. The concentration of tomatine decreases as the fruit matures and tomatine concentration contributes to determining the taxonomy of the species. Thus it can be useful in crop breeding for cultivated tomatoes (OECD, 2008; Spooner, Anderson and Jansen, 1993).

Cultivated tomato is related to wild tomatoes originating from Peru, Ecuador and other parts of South America including the Galapagos Islands. The centre of its domestication and diversification is Mexico (Rick, 1978; Jenkins, 1948; Peralta, Spooner and Knapp, 2008). Wild relatives of tomato and intermediate forms (landraces or creoles) harbour a wealth of genetic diversity and are important sources of genetic material in crop improvement and conservation programmes (Sánchez-Peña et al., 2004).

Tomato is one of the best studied cultivated dicotyledonous plants at the molecular level and has been used as a model species for research into gene mapping, gene characterisation (e.g. plant pathogen resistance genes) and gene transfer approaches. It is also useful to study other plant traits such as fruit ripening, hormone function and vitamin biosynthesis (Gebhardt et al., 1991; Chetelat and Ji, 2006; Ji and Scott, 2006).

The common name known all over the world, tomato, originates from a Spanish usage assigned to the Mexican word in Náhuatl "*xictomatl*" ("*xictli*": navel and "*tomatl*": tomato), meaning the tomato with a navel. This refers to the scar left on the fruit by the peduncle. In Mexico the plant is frequently called "*jitomate*".

General description and taxonomy

General description

Tomato is a perennial herbaceous plant but it is often grown as an annual crop even if biennial and perennial forms exist. Tomato is cultivated in tropical and temperate climates in open field or under greenhouse in temperate climate. Greenhouses are often used for large-scale production. In warm climate with the right light intensity for growth, around 45 days are necessary from germination to anthesis and 90-100 days to reach to beginning of fruit ripeness (Nuez, 2001). The end use of the crop, whether for the processing market or fresh market, will determine the cultivars sown, the time of harvest and harvest processes, which can be manual or mechanical (Nuez, 2001).

The growth habit of the plant varies from indeterminate to determinate and may reach up to 3 metres (m) in height. The primary root may grow several metres in length. The stem is angular and covered by hairy and glandular trichomes that confer a characteristic smell. Leaves are alternately arranged on the stem with a 137.5° phyllotaxy. Leaves range in shape from lobed to compound, with segments arranged pinnately. Compound leaves are typically comprised of five to nine leaflets. Leaflets are petiolated and dentated. All leaves are covered by glandular, hairy trichomes.

The tomato fruit is globular or ovoid. Botanically, the fruit exhibits all of the common characteristics of berries; a simple fleshy fruit that encloses its seed in the pulp. The outer skin is a thin and fleshy tissue that comprises the remainder of the fruit wall as well as the placenta. The colour of the fruit is derived from the cells within the fleshy tissue. Tomato fruits can be either bilocular or multilocular. Between 50 and 200 seeds are located inside the locular cavities and are enclosed in gelatinous membranes. On average, the seeds are small (5 x 4 x 2 mm) and lentil shaped. The seed contains the embryo and the endosperm and is covered by a strong seed coat, called the testa. The development of the fruit takes seven to nine weeks after fertilisation. The many end uses of tomato fruit, as well as food and feed safety considerations, including composition of key food and feed nutrients, anti-nutrients, allergens, and toxicants, are detailed in the "OECD consensus document on compositional considerations for new varieties of tomato" (OECD, 2008).

Taxonomy

The cultivated tomato is a member of the genus *Solanum* within the family Solanaceae. The Solanaceae, commonly known as the nightshade family, also includes other notable cultivated plants such as tobacco, chilli pepper, potato and eggplant.

Tomato classification has been the subject of much discussion and the diversity of the genus has led to reassessment of earlier taxonomic treatments. Tomato was originally named *Solanum lycopersicum* by Linnaeus in 1753; *Lycopersicon lycopersicum* (L.) Karsten has also been used (Valdes and Gray, 1998). Miller (1768) in *The Gardener's Dictionary* used *Lycopersicon esculentum*. Rick (1979) included nine species in the *Lycopersicon* genus. For a long time tomatoes were known as *L. esculentum*, but recent research has shown that they are part of the genus *Solanum* and are now again broadly referred to as *Solanum lycopersicum* (Spooner, Anderson and Jansen, 1993; Bohs and Olmstead, 1997; Olmstead and Palmer, 1997; Knapp, 2002; Spooner et al., 2005, 2003; Peralta et al., 2008).

The genus *Solanum* consists of approximately 1 500 species. The tomato clade (section *Lycopersicon*, formerly recognised as the genus *Lycopersicon*) includes the cultivated tomato (*Solanum lycopersicum*) and 12 wild relatives, all natives to western South America (Table 2.1). Tomato (*Solanum lycopersicum*) is derived from two wild ancestor species, *Solanum pimpinellifolium* and *Solanum cerasiforme*. Other wild species are useful for breeding disease resistance, colour improvement and desirable quality traits (Ranc et al., 2008). The 12 wild members of the *Lycopersicum* clade demonstrate a high level of phenotypic and genetic variation, including a great diversity in mating systems and reproductive biology (see the section on hybridisation and introgression and Bedinger [2011]). Peralta, Spooner and Knapp (2008) recognised 12 species of wild tomato; this was an increase on the 9 species of tomato recognised by Rick, Laterrot and Philouze (1990). Within these 12 species, informal species groupings were made: 4 closely related green-fruited species – *S. arcanum*, *S. huaylasense*, *S. peruvianum* and *S. corneliomulleri* – were grouped in the *S. peruvianum sensu lato* (*sensu lato* refers to a broad concept of a species). Another group of yellow to orange-fruited species contains two species endemic to the Galapagos Islands: *S. galapagense* and *S. cheesmaniae*.

Table 2.1 lists species belonging to the tomato clade, including the cultivated tomato (*S. lycopersicum*) and 12 wild tomato species, as well as 4 other closely affiliated *Solanum* species (Peralta, Spooner and Knapp, 2008). Table 2.2 lists tomato species for the genus *Solanum* subsect. *Lycopersicon* (USDA-ARS, 2009).

Table 2.1. **Taxonomy of the genus *Solanum* sect. *Lycopersicoides,* sect. *Juglandifolia*, sect. *Lycopersicon***

Species	Synonyms
S. lycopersicoides Dunal	*L. lycopersicoides* (Dunal) A. Child ex J.M.H. Shaw
S. sitiens I.M.Johnst	*L. sitiens* (I.M.Johnst) J.M.H. Shaw
S. juglandifolium Dunal	*L. juglandifolium* (Dunal) J.M.H. Shaw
S. ocharanthum Dunal	*L. ocharanthum* (Dunal) J.M.H. Shaw
S. pennellii Correl	*L. pennellii* (Correl) D'Arcy
S. habrochaites S.Knapp & D.M.Spooner	*L. hirsutum* Dunal
S. chilense (Dunal) Reiche	*L. chilense* Dunal
S. huaylasense Peralta	partly *L. peruvianum* (L.) Miller
S. peruvianum L.	*L. peruvianum* (L.) Miller
S. corneliomulleri J.F.Macbr.	partly *L. peruvianum* (L.) Miller
(1 geographic race Misti near Arequipa)	also known as *L. glandulosum* C.F.Müll
S. arcanum Peralta	partly *L. peruvianum* (L.) Miller
(4 geographic races humifusum, lomas, Marañon, Chotano-Yamaluc)	
S. chmielewskii (C.M.Rich et al.)	*L. chmielewskii* C.M.Rich et al. D.M.Spooner et al.
S. neorickii D.M.Spooner et al.	*L. parviflorum* C.M.Rich et al.
S. pimpinellifolium L.	*L. pimpinellifolium* (L.) Miller
S. lycopersicon L.	*L. esculentum* Miller
S. cheesmaniae (L. Riley) Fosberg	*L. cheesmaniae* L. Riley
S. galapagense S.C. Darwin & Peralta	Partly *L. chesmaniae* L. Riley

Source: Peralta, Spooner and Knapp (2008).

Geographic distribution, centre of origin and domestication, cultivation, and management practices

In the case of cultivated plants, in addition to the centre of biological origin, other areas exist where wild ancestors and other related forms in an incipient stage of domestication (e.g. weed forms and local landraces) co-exist. This area, known as the

centre of genetic diversity, contains an extraordinary diversity of forms. Harlan, de Wet and Price (1973) defined geographic areas different from the natural centre of distribution of the crop as secondary centres or centres of trans-domestication. These are the zones where the species is domesticated. Occasionally, both areas coincide. In the case of tomato, its centre of origin and its centre of diversity are different (Harlan, 1971).

Table 2.2. **Taxonomy of the genus *Solanum* sect. *Lycopersicoides***

1	*Solanum agrimoniifolium* (Dunal) J. F. Macbr. (subgroup. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) = *Solanum habrochaites* S. Knapp & D. M. Spooner
2	*Solanum arcanum* Peralta (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (=) *Lycopersicon peruvianum* var. *humifusum* C. H. Müll.
3	*Solanum caldasii* Dunal (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Juglandifolia*) = *Solanum ochranthum* Dunal
4	*Solanum cheesmaniae* (L. Riley) Fosberg (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (≡) *Lycopersicon cheesmaniae* L. Riley
5	*Solanum chilense* (Dunal) Reiche (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (≡) *Lycopersicon chilense* Dunal
6	*Solanum chmielewskii* (C.M. Rick et al.) D.M. Spooner et al. (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (≡) *Lycopersicon chmielewskii* C.M. Rick et al.
7	*Solanum corneliomulleri* J.F. Macbr. (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (≡) *Lycopersicon glandulosum* C.H. Müll.
8	*Solanum galapagense* S.C. Darwin & Peralta (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (=) *Lycopersicon cheesmaniae* var. *minor* (Hook. f.) D.M. Porter (=) *Lycopersicon cheesmaniae* f. *minor* (Hook. f.) C.H. Müll.
9	*Solanum habrochaites* S. Knapp & D.M. Spooner (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (=) *Lycopersicon agrimoniifolium* Dunal (≡) *Lycopersicon hirsutum* Dunal (=) *Lycopersicon hirsutum* f. *glabratum* C.H. Müll. (=) *Solanum agrimoniifolium* (Dunal) J.F. Macbr.
10	*Solanum huaylasense* Peralta (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*)
11	*Solanum juglandifolium* Dunal (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Juglandifolia*)
12	*Solanum lycopersicoides* Dunal (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Lycopersicoides*)
13	*Solanum lycopersicum* L. (subg. Potatoe sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*)
14	*Solanum lycopersicum* var. *cerasiforme* (Alef.) Fosberg (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (≡) *Lycopersicon esculentum* var. *cerasiforme* Alef. (≡) *Lycopersicon lycopersicum var. cerasiforme* (Alef.) M.R. Almeida
15	*Solanum lycopersicum* var. *lycopersicum* (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (≡) *Lycopersicon esculentum* Mill. (=) *Lycopersicon esculentum* var. *commune* L.H. Bailey (≡) *Lycopersicon esculentum* var. *esculentum* (=) *Lycopersicon esculentum* var. *grandifolium* L.H. Bailey (=) *Lycopersicon esculentum* f. *pyriforme* (Dunal) C.H. Müll. (=) *Lycopersicon esculentum* var. *pyriforme* (Dunal) Alef. (=) *Lycopersicon esculentum* var. *validum* L.H. Bailey ○ (≡) *Lycopersicon lycopersicum* (L.) H. Karst. ○ (=) *Lycopersicon lycopersicum* var. *pyriforme* auct. (=) *Lycopersicon pyriforme* Dunal

Table 2.2. **Taxonomy of the genus *Solanum* sect. *Lycopersicoides*** (*continued*)

16	*Solanum neorickii* D.M. Spooner et al. (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (=) *Lycopersicon parviflorum* C.M. Rick et al.
17	*Solanum ochranthum* Dunal (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Juglandifolia*) Synonyms: (=) *Solanum caldasii* Dunal
18	*Solanum pennellii* Correll (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (≡) *Lycopersicon pennellii* (Correll) D'Arcy (≡) *Lycopersicon pennellii* var. *pennellii* (≡) *Lycopersicon pennellii* var. *puberulum* (Correll) D'Arcy (=) *Solanum pennellii* var. *elachistus* Martic. & Quezada (=) *Solanum pennellii* var. *pennellii* (=) *Solanum pennellii* var. *puberulum* Correll
19	*Solanum pennellii* var. *elachistus* Martic. & Quezada (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) = *Solanum pennellii* Correll
20	*Solanum pennellii* var. *pennellii* (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) = *Solanum pennellii* Correll
21	*Solanum pennellii* var. *puberulum* Correll (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) = *Solanum pennellii* Correll
22	*Solanum peruvianum* L. (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (=) *Lycopersicon dentatum* Dunal (≡) *Lycopersicon peruvianum* (L.) Mill. (=) *Lycopersicon peruvianum* var. *dentatum* (Dunal) Dunal (≡) *Lycopersicon peruvianum* var. *peruvianum*
23	*Solanum pimpinellifolium* L. (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (≡) *Lycopersicon esculentum* subsp. *pimpinellifolium* (L.) Brezhnev (≡) *Lycopersicon esculentum* var. *racemigerum* (Lange) Brezhnev (≡) *Lycopersicon pimpinellifolium* (L.) Mill. (=) *Lycopersicon racemigerum* Lange
24	*Solanum rickii* Correll (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Lycopersicoides*) = *Solanum sitiens* I.M. Johnst.
25	*Solanum* sect. *lycopersicon* hybr. (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (=) *Lycopersicon* hybr. *Solanum* sect. *lycopersicon* spp. (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Neolycopersicon*) Synonyms: (=) *Lycopersicon* spp.
26	*Solanum sitiens* I.M. Johnst. (subg. *Potatoe* sect. *Petota* subsect. *Lycopersicon* ser. *Lycopersicoides* Synonyms: (=) *Solanum rickii* Correll

Source: USDA-ARS (2015).

Natural centre of origin

The natural geographic distribution or centre of origin of *Solanum lycopersicum*, (*S.* section *Lycopersicon*) has been localised in the narrow band between the Andes mountain ranges and the Pacific coast of western South America (WWF and IUCN, 1997). This extends from southern Ecuador to northern Chile, including the Galapagos Islands (Peralta, Spooner and Knapp, 2008; Nuez et al., 1996; Jenkins, 1948). This is based on the geographic distribution of the native wild ancestors of the genus between coordinates 0º-20º S and 64º-81º W where they grow spontaneously and sympatrically (Taylor, 1986).

Based on research from the Tomato Genome Consortium 2012, the three wild species most closely related to cultivated tomato include the red-fruited species *S. pimpinellifolium* and the orange-fruited species found on the Galapagos Islands, *S. galapagense* and *S. cheesmaniae* (Menda, Strickler and Mueller, 2013).

Mexico is presumed to be the most probable region of domestication, with Peru as the centre of diversity for wild relatives (Larry and Joanne, 2007). *Solanum lycopersicum cerasiforme* is thought to be the ancestor of cultivated tomato, based on its wide presence in Central America and the presence of a shorter style length in the flower (Cox, 2000).

Centre of domestication

During prehispanic times, various useful plants were introduced and domesticated in Mesoamerica from South America. The original South American tomato fruit became a synanthrophyte, a plant species brought indirectly to Mexico through trade between prehispanic cultures. The characteristics of this wild fruit were different from the cultivated fruit: small size (1-2 cm diameter), bilocular and acid taste (Jenkins, 1948). Upon its arrival in Mesoamerica, its similar morphology with the green tomato (*Physalis*) facilitated its adoption and adaptation by Mexican cultures. Since those times, the use and diversification in morphotypes, dimensions, forms and colours of the fruits used as food by Mexican indigenous cultures were extraordinary (de Sahagún, 1979). As such, Mexico, together with the Andes zone, houses the largest morphological variability in tomato (Rick, 1978; Jenkins, 1948) and is considered the centre of diversity and domestication of *S. lycopersicum* (Larry and Joanne, 2007; Nuez et al., 1996; Rick, 1990; Jenkins, 1948).

Crop migration

Historical records allow the reconstruction of the arrival of tomatoes in the Old World, following European contact. The Spanish navigators brought seeds to Europe in the 16th century and friars sent some of these to their brothers. The tomato first arrived in Andalusia (via the Canary Islands) and was dispersed throughout Spain. The Spanish and the Italians were the first to accept this "exotic" fruit. According to Mattioli (Nuez et al., 1996; Rick, 1978), it was consumed with oil, salt and pepper in Italy. In other European countries acceptance was slow and the tomato long remained an ornamental plant because of the fear of poisoning or "the curse of the dulcamara" (Long Towell, 2001). This belief was associated with the toxic, hallucinogenic and aphrodisiac properties of other members of the Solanaceae, such as Belladona (*Belladona*) and Mandragora (*Mandragora*), which have detrimental effects on health caused by some alkaloids (OECD, 2008).

The first mention of tomato in England was by the botanist Gerard in 1597. Besler (1613), a German naturalist, first showed engravings of tomato plants present at the Eichstätt Garden in Germany. Considering the size of the fruit shown in the engravings, it is assumed that they depict plants already domesticated as ornamentals. In 1760, tomato was represented as an ornamental in the Andrieux-Vilmorin catalogue in France (Fournier, 1948).

Tomato returned to the Americas in the 18th century, according to reports of its cultivation in the West Indies and the Caribbean. Tomato was also transported to North America in the 18th century by European colonists arriving at commercial harbours in New Jersey, the United States. The first written account dates from 1710, when it was registered as an ornamental plant by William Salmon. However, it was not trusted as a foodstuff in the United States until the beginning of the 20th century because of its similarity to certain poisonous fruits (Rick, 1978).

Knowledge of the tomato's nutritional importance increased from the end of the 19th century to the beginning of the 20th century (Rick, 1978). The first improved tomatoes were developed by Italian breeders in the 17th or early 18th century, who converted the small, wrinkled and hard tomato into the red coloured, smooth and juicy varieties known today (Atherton and Rudich, 1986; Rick, 1976). Starting from these cultivars, the United States began in 1867 the production of various cultivars and nine commercial varieties (Early Smooth, The Cook's Favorite, Tildem, Powells Early, FEUE, Large Red, Large Yellow, Tree Tomato Red and Yellow Plum) (Atherton and Rudich, 1986).

Tomato is now a cosmopolitan crop with major production in temperate regions, even though its origins lay in tropical regions.

Evolution of cultivation

Tomato has been cultivated since prehispanic times with the earliest agricultural techniques, and its cultivation and production keep improving and evolving. This depends on several factors, such as the organoleptic properties of the fruits, farming system, soil type, environmental conditions, the crop variety used, degree of technological development and capital available, as well as the goal of the production.

The first methods of cultivation developed within the Mesoamerican farming system of milpa, a polyculture association of maize (*Zea mays*), beans (*Phaseolus* spp.) and squash (*Cucurbita* spp.). This trilogy is an important source of carbohydrates, proteins and fats. Moreover, other species found in milpa, such as chili pepper and tomato, provide vitamins and minerals, so that this production system satisfies nearly all nutritional necessities. The milpa was the first home of tomato; it arrived in Mexico as a synanthrophyte and incorporated itself in the local production systems. It underwent the same selection processes as other useful weeds: collected, tolerated (a weedy variant of tomato called "*tomate de culebra*" [snake tomato] is still tolerated), and/or protected within cultivation (Casas, 2001). Subsequently, by attracting human attention as an edible plant, it was subject to a more intensive selection process in combination with a habitat change. Cultivation and management practices provided it with better environmental conditions which induced better production (Zizumbo, 1986).

Traditional farming systems are also a reservoir of available genetic resources. Cultivating plants, while allowing their co-existence with their wild or weedy relatives, can conserve crop genetic resources.

Crop production

About 171 million tonnes of tomatoes are harvested annually from plantings of 5 million hectares. Almost 60% of world production comes from Asia, 11.1% from Africa, 13.3% from Europe, 11.3% from Africa, 8.7% from North America, and 6.6% from Central America and South America (FAOSTAT, 2017). According to FAOSTAT (2017), the world's top five greatest producers of tomato in 2014 were China, India, the United States, Turkey and Egypt. Tomato is considered to be one of the most important vegetables produced in commercial agriculture because it is cultivated in temperate and warm regions of the world and it generates cash as an export crop.

Climate

Tomatoes require a warm climate for growth and do not tolerate frost. The usual life cycle in cultivation spans one spring and summer. Its optimum temperature is around 26ºC (day) and 12ºC (night). Plants require minimum temperatures above 18ºC for

vegetative growth, but can survive at lower temperatures (12ºC). Temperatures above 31ºC reduce the rates of flower fertilisation, plant development and fruit ripening. Table 2.3, adopted from Geisenberg and Stewart (1986), lists the optimal temperature ranges required at different stages of tomato development.

Table 2.3. **Temperature ranges**

Stages of plant development	Temperature (°C)		
	Minimum	Optimum	Maximum
Germination	11	15-30	30
Vegetative growth	18	20-24	30
Fruit set night	10	14-20	24
Fruit set day	18	20-24	30
Red colour development	10	20-24	30

Source: Garza and Molina (2008); Nieves-García, van der Valk and Elings (2011).

Air relative humidity between 55-60% is important for effective pollen production and pollination.

Soil

Tomatoes grow well on most mineral soils, but they prefer deep, well-drained sandy loams. Deep tillage enables adequate root penetration in heavy clay-type soils, thus allowing the production of tomato. Tomato is moderately tolerant to a wide range of pH. Worley (1976) showed that tomato yield was higher in soils with a pH between 6.5 and 6.9 compared with that obtained in acidic soils. Soils with acidic pH or salinity lead to a decrease in the size of the fruit (Doss, Evans and Turner, 1977; Papadopoulos and Rendig, 1983).

Soil preparation

This practice plays a role in the establishment of the crop, either by direct seed sowing or transplantation.

Nutrient requirements

Nutrient requirements of the tomato crop depend on variety, yield and cultural practices (for instance, see Sainju, Dris and Singh [2003]). Soil and tissue analyses should be taken throughout the growing and production season to ensure essential nutrients are present in their proper amounts and ratios. We can consider the following nutrient requirements as average: 30 t/ha organic matter; 50 kg/ha nitrogen (N); 80-100 kg/ha phosphorus (P); 200-250 kg/ha potassium (K). Under greenhouse conditions the nutrient doses can be higher to increase the yield. Fertiliser use is limited in organic production and it may also be limited in conventional production in some countries due to the cost.

Seedling nursery

This practice is aimed to obtain vigorous, healthy and uniformly growing seedlings, optimal for transplantation and assuring 100% survival in the field or greenhouse. Overseeding (relative to the number of transplants needed) is required to compensate for a lack of germination or emergence and seedling death. It is also important to have additional transplants in order to select a vigorous, uniform group for transplanting. The per cent of seedlings lost for reasons listed above vary by operation and situation.

In general, lack of germination (10%) or emergence (10%) and seedling death (5%) may require overseeding by up to 25%.

Transplantation

The field which will receive the seedlings must be humid and holes must be made in order to deposit the seedlings. These must be removed from the seedbed avoiding physiological damage to the roots. The periods of transplantation are generally from May to June in the northern hemisphere. However, there are cultivated varieties which are planted from November till February. Planting distance is 25-50 cm between seedlings and 1.50-1.80 m between rows.

Planting distance changes with production goal: for fresh consumption, 22 000- 25 000 plants/ha; and for industry, 40 000-60 000 plants/ha.

Fertilisation

In general, fertiliser is applied during three stages: first, before transplantation; second, 60 days afterwards; and third, after 100 days. Fertilisation is limited in organic production; and in some countries (e.g. the Netherlands) also in conventional production.

Irrigation

Tomato requires frequent irrigation to delay maturity and prolong plant productivity. Irrigation also helps to reduce salinisation. Some authors suggest that soil moisture levels should never exceed 0.2 bars, whereas other authors suggest a maximum of 2 bars (see irrigation chapter in Nuez [2001]). The recommended soil moisture level varies with cultivation method, variety and climate (Castilla, 1995). Erratic moisture conditions can cause radial and concentric cracking on the fruit (Peet and Willits, 1995). This is a serious physiological disorder that leaves the affected tomatoes unmarketable and leads to quick deterioration. Moisture requirements vary with crop variety, prevailing climate and soil characteristics.

Tomatoes in fields or glasshouses can be grown in polyethylene-mulched beds with drip irrigation which allows for close monitoring of nutrients. The plastic mulch helps to maintain a high efficiency in the use of water and fertiliser (Jensen, Kimball and Ricketson, 1989). Drip irrigation for tomatoes has gained popularity as it increases water use efficiency and also allows the application of fertilisers mixed in the irrigation water. With drip irrigation it is possible to closely synchronise weekly water and nutrient application rates with the corresponding stage of crop development.

Pruning and guidance of the plants

Through pruning, shoots appearing in leaf axils are removed to create a plant architecture which facilitates management. The advantages of pruning are: stimulation of plant development, more efficient phytosanitary control and achievement of higher quantitative and qualitative yield. Pruning of leaves is necessary for phytosanitary control, and a vegetative balance and generative control. Plants may be supported by a trellis, e.g. 2-metre posts (sunk to 50 cm) positioned at regular intervals of 3-5 metre support cotton threads or galvanised metal wire to lift and support the plant and facilitate access for crop management and pest control.

Earthing-up

Earthing-up consists of massing up earth at the plant base with the aim to assure the growth of adventitious roots providing better anchorage. The first earthing-up occurs between the first and second week after transplantation and is repeated between the fourth and fifth week. On occasion, this practice is also performed during weeding.

Harvest

The level of maturity at which fruits are harvested depends on the final production goal. The harvest interval may continue up to seven months.

Table 2.4. **Harvest indicator**

Production goal	Harvest indicator
Local consumption	Turgid fruits with intense red colour.
Regional consumption	Pink fruits.
Export	"Green-mature". Because of semi- and long life varieties, it is now common in Europe to export tomato with red colour making use of colour tables or instruments.
Industry	Physiologic maturity; there is no explicit colour.

Source: Garza and Molina (2008); Nieves-García, van der Valk and Elings (2011).

Farming practices described above may be adapted for the open field, as well as for protected cultivation and intensive farming systems. Agricultural techniques for the cultivation of tomato are an integral part of culture and should be applied according to the type of crop that will be sown. As such, they will be listed below in a general manner. Beside this, one of the most important factors affecting yield and quality obtained in tomato production is the occurrence and effects of pests and diseases. Major tomato pests and diseases are listed in Annexes 2.A1 and 2.A2 respectively.

Production in modern and intensive systems

With the help of advances in modern technology, tomato can now be cultivated in both tropical and temperate zones in the open field, in home gardens, in small-scale agricultural patches, or as large-scale urban market production or agro-industry. It can be found in traditional farming systems (shifting cultivation) as well as in modern and intensive systems using acclimatised greenhouses, plastic cover nurseries, hydroponics and fertigation. This vegetable species is adapted to grow under different environmental and cultural conditions (OECD, 2008).

Due to climatic variations, like low temperatures in North America and Europe during a large period of the year, as well as cloudiness and high precipitation in tropical and subtropical regions of the world, it has been necessary to search for alternative protected production systems. Greenhouse production is an alternative intensive production system. Its objective is to obtain higher production levels per area unit by controlling nutrition, temperature and light among other conditions that affect plant growth.

Adding up to the structural characteristics and energy input of the greenhouse, the crop's morphological characteristics, its physiological requirements and cultivation practices must be considered (Berenguer, 2003; Castilla, 2003). As such, sowing density,

pruning techniques, fertiliser concentrations, etc., will depend largely on the sown crop variety.

This concept is sustained within new proposals of tomato production systems. As an alternative of the general protected cultivation concept through improvement of plants' environmental conditions in order to augment yield, there exist the Mediterranean greenhouses, based on a minimal or almost zero energy input inducing minimal modifications in the microclimate (Enoch, 1986; Monteiro, 1990).

A result of the use of micro environmental modifications is the use of the so-called "biospaces", the combined effort of efficient agronomic practices and micro environmental modification (mesh, cloth and pipes) in zones with high radiation and temperature and low relative humidity in order to favour growth and development of fruity vegetables (Bustamante, 2003).

A cultivation technique which can be combined with the biospace is the plastic cover nursery. This involves complete ground cover around the crop with plastic in order to detain weed growth, diminish humidity loss and improve fertility. These advantages are the result of the temperature increase under the plastic and the retained humidity, which stimulates processes of nitrification and solubility of salts, thereby causing germinating weeds to be killed.

Nowadays, the intensive greenhouse production of tomato involves the use of hydroponics, a production system in which the roots are irrigated with water containing a mix of essential nutritional elements while sustained in a substrate of inert material or the same solution instead of soil (Sánchez del Castillo and Escalante Rebolledo, 1981).

Vegetable grafting is gaining interest in open-field and high tunnel tomato production. There are a variety of grafting techniques, but the most widely adopted method worldwide for grafted tomato production is tube grafting. Resistant rootstocks are available for tomato, and can be used to manage economically important soil-borne pathogens such as *Ralstonia solanacearum* and root-knot nematodes (*Meloidogyne* spp.) or *Sclerotium rolfsii* (Rivard, Peet and Louws, 2010).

A new production alternative is ecological agriculture, or the production of healthy and innocuous products while conserving basic natural resources like water, soil and biodiversity (García and Hernández, 2004).

An organic production system of tomatoes does not use chemical products, applies integrated pest management, occupies ten times less area and achieves prices ten times higher than conventional cultivation (Navejas et al., 2002).

Reproductive biology

Floral biology

Although some tomato wild species of the genus *Solanum* are allogamous, all commercial tomato cultivars are considered to be mainly self-compatible and inbreeding, i.e. autogamous (Rick, 1979; Taylor, 1986). Tomato flowers are perfect, regular and hypogynous and are borne on inflorescences that may be either determinate (cymose) or indeterminate (racemose), depending on the species. The flower is connected to the axis by a pedicel that includes the abscission point. The first flower appears when the plant has three leaves and, frequently, the first and the last bud of an inflorescence are aborted. The timing of floral landmarks for *S. pimpinellifolium* is described in detail in Buzgo et al. (2004).

The number of flowers produced by an inflorescence is dependent upon environmental factors. A plant growing at 16°C produces four times more flowers than a plant growing at 24°C. Temperatures below 10°C, or less than 12 hours of light, reduce yield by causing premature flower abscission. As flowers form sequentially, buds, flowers and fruits can co-exist in an inflorescence (Chamarro, 1995). The flowers are yellow and generally less than 2.5 cm in diameter when in full bloom. They possess four helically arranged whorls of organs; green sepals form the outer whorl or calyx, at least five yellow petals are present in the corolla, stamens alternate with petal position and are fused to form an anther cone and a whorl of two or more fused carpels form the pistil at the centre of the flower. The number of carpels found in the pistil varies between species and relates to the number of locules present in the resulting fruit.

Pollination, pollen dispersal, pollen viability

For some varieties, flowers have the style shorter than the tip of the anther cone, while for other varieties the style is longer than the anther cone. The stigma is receptive from one to two days before to four to eight days after its own flower releases pollen, thus cross-pollination is possible. The first meiosis during pollen production occurs when the anthers reach one-third of their final length. The optimal temperature range for pollen production is 10-35°C and the number of pollen grains formed in an anther is genetically determined. Anther dehiscence delivers thousands of pollen grains into the channel formed by the hairs. However, as anthers release pollen inwardly towards the style, vibration-assisted self-pollination is usual, especially in short-style varieties. In long-style varieties, the downward posture of the flower allows self-pollination by gravity. The anther cone releases pollen around the stigma at the slightest vibration. Wind and insects provide the vibrating action necessary for self-pollination under field conditions. Under greenhouse conditions, mechanical vibrating devices or insects are used. Optimal conditions for pollination are temperatures of 17-24°C and humidity above 70%. High humidity and low temperatures favour outcrossing (Nuez, 2001).

As is the case for most self-pollinating plants, the viability of exposed tomato pollen is limited. Pollen viability and the number of pollen grains are reduced by high temperatures above 32/26°C day/night. The effect of temperature is associated with alterations in carbohydrate metabolism during another development (Pressman, Peet and Pharr, 2002; Firon et al., 2006). Natural cross-pollination rates among commercial varieties range from 0.07% to 12% (Richardson and Alvarez, 1957; Groenewegen, King and George, 1994; Accotto et al., 2005). The rate of crossing quickly decreases as the distance from the pollen source increases (Currence and Jenkins, 1942) and little viable pollen is transferred beyond 30 m (~95 feet) from its source (Quiros and Marcias, 1978). The distance required between foundation seed fields in United States is ~61 m (200 feet) which, in practical terms, is considered the security isolation distance that assures that a pollen grain cannot pollinate under field conditions (Rick et al., 1976).

Although tomato is generally self-fertile, cross-pollination between species is possible (discussed further in the section on "Hybridisation and Introgression") and fruit set is similar in self- or cross-pollinated plants (Free, 1993). Male sterility exists in tomato and, as this condition precludes self-fertilisation, such plants can be used to produce hybrid seed. Cross-pollination of male-sterile flowers is achieved by insect activity, rather than by wind or mechanical vibrators as employed for self-fertilisation (McGregor, 1976). Despite an extensive history of use (see Section *General description and taxonomy*), a search of the relevant literature yields a surprising lack of data relating to basic biological characteristics of the domesticated tomato plant. In particular, it is difficult to find

information that contributes to an understanding of the potential for gene flow (including pollen and seed dispersal) and data on seed viability or dormancy. These characteristics are generally understood to contribute to the potential weediness of a species. For tomato, the scarcity of information relating to such characteristics may be because it is not widely regarded as weedy (Randall, 2012). Keeler (1989) included tomato as a comparator (non-weedy) crop plant in a study on the potential for crop species to acquire weedy characteristics, noting a similar difficulty in acquiring information for non-weedy species.

Seed production and dormancy

The tomato seed matures 35-50 days after pollination, during which seeds become germinable, desiccation tolerance is induced and water content decreases. Fruit is red and ripe by 60 days after pollination. There are three stages of tomato seed development: morphogenesis, maturation and seed quiescence (DeCastro and Hilhorst, 2000). Primary dormancy occurs in tomatoes, where seeds become dormant during development. It is considered to assist plants to survive in periods of unfavourable growth conditions. Primary dormancy is often removed by exposure of dry seed to high temperatures or of imbibed seed to low temperatures and abscisic acid (ABA) is thought to play a part in breaking primary dormancy (Hillhorst and Downie, 1995). However, tomato seed development appears to be independent of ABA (DeCastro and Hilhorst, 2000).

Genetics

The new taxonomy adopted (Peralta, Spooner and Knapp, 2008) that include the former genus *Lycopersicon* under the genus *Solanum*, has also created and eliminated various species and modified certain sections of the genus. To be accurate with the work of the different authors in this section, the name used by them was maintained.

Genetics

Tomato is often used as a model system for diploid plant research into classical genetics, cytogenetics, molecular genetics and molecular biology. The advantages of using tomato for research have been reviewed by Ji and Scott (2006) and are summarised here as follows:

Genome size

Tomato has a relatively small genome size (around 950 Mb). About 30% of the genome is composed of repetitive sequences which are mainly located in heterochromatin regions (Van der Hoeven et al., 2002). Tomato and its wild relatives have 12 chromosomes (2n=2x=24). The 12 tomato chromosomes were first identified by Barton (1950).

Genetic mutation

Mutation has played an important role in tomato genetics. Spontaneous mutation is an important source of genetic variation (Chetelat and Ji, 2006). One spontaneous mutation, providing plants with determinate growth habit, has revolutionised tomato production (Atherton and Harris, 1986). Other mutations have been identified that confer male sterility (Stevens and Rick, 1986) or cause aneuploidy (Ji, Pertuzé and Chetelat, 2004). In addition, the use of artificial mutagenesis has led to the production of around 1 200 mutant lines that can be used for scientific research. Around 1 000 mutant loci have been characterised, 400 of which have been assigned to specific chromosomes (Chetelat, 2002; Chetelat and

Ji, 2006). Monogenic mutants, markers, disease resistance genes and other types of stocks are maintained by the Tomato Genetic Resources Center (http://tgrc.ucdavis.edu). The Solanaceae Genome network (SGN) maintains 13 000 M2 characterised families derived from tomato mutagenesis (http://zamir.sgn.cornell.edu/mutants).

Chromosomal rearrangements

Variations have been produced at the chromosomal level, with tomato euploids, haploids, triploids and tetraploids reported. Euploids have arisen spontaneously or have been produced by crosses between genotypes with different ploidy level. Haploids and triploids are meiotically instable and generally have low fertility, but tetraploids are meiotically stable and can be reproduced by seeds. Aneuploid variation of tomato at diploid level can occur one of two ways: the deletion of a chromosome that produces monosomic lines that carry the monosome only in half of the gametes (Gill, 1983) and the addition of chromosomes that produces trisomics or alien addition lines. Complete sets of primary trisomics, and other types of trisomics derived from them, have been generated (Lesley, 1928; Khush and Rick, 1968). A complete set of tomato addition and substitution lines has been produced in the *S. lycopersicoides* background (Chetelat et al., 1998; Ji and Chetelat, 2003). Addition and substitution lines have also been produced using *S. sitiens* (Pertuzé, Ji and Chetelat, 2002). Chromosomal structural alterations have been identified in tomato (Gill, 1983; Khush and Rick, 1968). These chromosomal alterations have allowed the assignment of genes and markers (ex: quantitative trait locus, or QTLs) to specific chromosomes and have facilitated the establishment and orientation of genetic linkage maps.

Markers can be associated with chromosomes, parts of it, traits, genes, etc. by studying its co-segregation with the chromosome, chromosomal fragment, trait or gene in question. When tightly linked to a gene or trait, markers can assist in breeding, and particularly for traits such as quantitative ones which need long and complex evaluation under field conditions.

Introgression lines

Introgression lines that contain chromosome segments from alien relatives in the background of the cultivated tomato greatly increase the genetic diversity available for improvement. They can also be advantageous for QTL mapping and gene identification studies (Gur and Zamir, 2004) and have been used to develop numerous high-density molecular linkage maps, genomic databases and DNA libraries. One series of 98 introgression lines has been obtained in which at least 85% of the genome of *S. habrochaites* f. *typicum* is represented in the background of *S. lycopersicum* (Monforte and Tanksley, 2000). The segments introgressed from *S. habrochaites* were identified by molecular markers and most of the lines were reasonably fertile. However, several lines were partially sterile, prompting a study of hybrid incompatibility that used QTL associated with pollen fertility and seed viability to identify loci that control fertility in interspecific crosses (Moyle and Graham, 2005). In another study, physical and genetic maps surrounding a major fruit weight QTL have been developed from isogenic lines derived from a *S. lycopersicum* × *S. pennellii* cross (Alpert and Tanksley, 1997; Frary et al., 2005). These maps may lead to a better understanding of the molecular biology of fruit development and to the genetic engineering of fruit size characteristics (Alpert and Tanksley, 1997; Frary et al., 2005). Introgression libraries are also being developed for *S. chmielewskii* and *S. lycopersicoides* (Chetelat and Meglic, 2000; Canady, Meglic and Chetelat, 2005). QTL analysis strategies have found wide application in tomato studies by using breeding populations involving *S. pimpinellifolium*, *S. peruvianum*, *S. hirsutum* and *S. pennellii* (Ji and Scott, 2006).

Genetic linkage maps

Chetelat and Ji (2006) reviewed the genetic linkage maps available for tomato. The first linkage map developed for tomato consisted of classical morphological and isozyme markers (Stevens and Rick, 1986) and has since been revised by many authors. The first molecular linkage map was published by Tanksley et al. (1992) and has since been followed by numerous other maps. Fulton et al. (2002) used conserved ortholog set (COS) markers (markers derived from single- or low-copy genes conserved in two or more species that share common ancestry) to develop a new molecular linkage map. COS markers allow the development of linkage maps of plant genomes through comparative genetic maps, especially for species belonging to the same family, allow understanding of genome structure, and comparison of closely and distantly related species. The ability to detect single-copy orthologous genes among plant genomes has permitted comparative plant genomics to advance (for a review, see Paterson et al. [2000]) (Barone et al., 2009). Additional polymerase chaine reaction (PCR)-based anchor markers have been developed by Frary et al. (2005) that can facilitate mapping studies in tomato and related species.

The SGN website houses map and marker data for Solanaceae species as well as other genetic and mutagenesis in tomato populations (https://solgenomics.net). Peralta, Spooner and Knapp (2008) and Bedinger (2011) review the taxonomy, genetics, interspecific crossing barriers and breeding of tomatoes.

COS markers are genes that are conserved throughout evolution in both sequence and copy number (usually single or low copy) identified by comparative genomic studies involving two divergent species (tomato and *Arabidopsis*; Fulton et al., 2002). These genes may play roles that are essential to all plant species and can be used for comparative mapping, synteny and phylogenetic studies across the plant taxa. COS genes were further analysed and shortlisted to generate COSII markers which are PCR-based markers developed from single-copy, orthologous genes conserved across multiple species (tomato, potato, pepper, coffee and *Arabodopsis*; Wu et al., 2006). A list of these markers and universal primers designed based on sequences of COSII genes are available on the SGN website.

Hybridisation and introgression

Breeding tomato

Tomato (*Solanum lycopersicon*) has undergone intensive breeding for decades. Breeding and selection have been based on traits desirable for the processing or the fresh market. The processed market often involves growing tomatoes in open fields requiring simultaneous fruit ripening and machinery harvesting. In addition, traits such as high sugar and total soluble solids content are required for the processed market. In the case of fresh market tomatoes, traits such as large fruit size, uniform fruit shape, uniform colour, long shelf life and fruit firmness are important (Menda, Strickler and Mueller, 2013; Rick, 1978).

Over the last century, breeding and selection of tomatoes have resulted in numerous hybrids and cultivars. During the 1950s, hybrid tomatoes were developed to obtain higher yields and improve fruit quality and disease resistance. Hybrids accounted for more than 50% of production both in protected cultivation and in the open area. The production of hybrid tomatoes requires emasculation of flowers prior to cross-pollination. However, 40 male-sterile mutants have been identified in tomato (Stevens and Rick, 1986) that can facilitate hybrid seed production. Marker-assisted selection is now a major instrument in

conventional breeding. Markers linked to characteristics/traits of interest for breeding have been identified and developed for tomato (Ji and Scott, 2006).

In vitro culture and somatic hybridisation were also used in tomato breeding. Although all forms of *S. esculentum* var. *esculentum* are self-compatible and mainly inbreeding, the wild cherry tomato types have a tendency to outcross due to exsertion of the stigma beyond the anther cone at anthesis (Rick, 1950; McGuire and Rick, 1954). This may also happen to some degree in other *S. esculentum* forms through genetic control (Currence, 1944), resulting in changes to floral morphology (Rick, 1950) or adaptation to environmental conditions such as temperature (Howlett, 1939; Rick, 1950) and nutrients (Howlett, 1939). The domestication of the wild cherry tomato types (*S. esculentum* var. *cerasiforme*) in Mexico (Jenkins, 1948) eventually spread to Europe and by selection led to larger fruited varieties. It is believed that this selection also led to progressive shortening of the style and withdrawal of the stigma into the anther cone (Rick, 1950). This gave rise to the large-fruited self-compatible inbreeding varieties cultivated today (Rick, 1950). For this reason it is relatively easy to maintain a "true-to-type variety" by saving their seed while not having to worry too much about outcrossing with other varieties of tomato. The several botanical varieties of tomato can be easily crossed with each other to produce viable offspring.

High fruit total soluble solids (TSS) in tomatoes is a key component of fruit quality. TSS is a proxy for sugar content. Higher TSS increases consumer fruit likeability. Genetic, molecular and biochemical characterisation of wild tomato species with high fruit TSS (10-15% compared with 4-6% in cultivars) can be exploited in breeding programmes (Beckles et al., 2012). Nevertheless, wild species with high TSS have low yield. An example of breeding a variety with both high TSS and yield is *Solara*.

Decades of breeding have resulted in a loss in genetic diversity. The challenges for breeders today include reintroducing the complex trait of flavour and breeding for novel disease resistance genes, that on average are effective for five years until the pathogen overcomes resistance (Menda, Strickler and Mueller, 2013). The wild species are the most valuable source of such traits.

Interspecific crosses

The nomenclature used in this section of the document is the original that appears in each paper mentioned. The new names are not directly comparable with the previous ones (Tables 2.1 and 2.2). The genus *Lycopersicon* has been divided into two subgenera based on their ability to cross with cultivated tomato. The *S. esculentum*-complex contains seven species that are easily crossed with cultivated tomato and these have served as a source of genetic variability for the improvement of tomato varieties (Rick, 1979). In contrast, the *L. peruvianum*-complex contains two species that are crossed with considerable difficulty (Stevens and Rick, 1986; Taylor, 1986), thus limiting the use of these species for tomato improvement. Nevertheless, gene flow between *L. peruvianum* and *L. chilense* has taken place to a limited extent and hybrids between species can be generated by grafting, if required (Städler, Roselius and Stephan, 2005). Hybridisation between these two subgenera usually leads to early embryo breakdown, which results in seed that is not viable. This problem can be circumvented by embryo culture and other laboratory techniques, albeit at great effort. Bedinger (2011) reviews interspecific reproductive barriers in the tomato clade. Table 2.5 summarises the breeding potential of *Lycopersicon.*

Table 2.5. **Breeding potential of *Lycopersicon***

Species	Mating system	Crossability with *L. esculentum*	Breeding use
***L. esculentum* complex**			
L. esculentum	Autogamous	Reciprocally compatible	Minor
L. pimpinellifolium	Mostly autogamous	Reciprocally compatible	Disease resistance Pest resistance Lycopene content
L. cheesmanii f. *cheesmanii*	Autogamous	Reciprocally compatible	Jointless pedicels High *beta*-Carotene Higher dry matter
L. cheesmanii f. *minor*	Autogamous	Reciprocally compatible	Salt and drought tolerance Disease resistance
L. parviflorum	Autogamous	Reciprocally compatible	Disease resistance
L. chmielewskii	Allogamous Self-compatible	Reciprocally compatible	Sucrose accumulation Disease tolerance
L. hirsutum f. *typicum*	Allogamous Self-incompatible	Unilaterally compatible	Cold tolerance
L. hirsutum f. *glabratum*	Self-compatible	Reciprocally compatible	Insect resistance Disease resistance Sucrose content
L. pennellii (*L. pennellii*)	Allogamous Self-compatible and self-incompatible biotypes	Unilaterally compatible	Drought tolerance Insect resistance Disease resistance
***L. peruvianum* complex**			
L. chilense	Allogamous Self-incompatible	Difficult Embryo rescue	Disease resistance Nematode resistance
L. peruvianum (L.) Mill.	Allogamous Self-incompatible	Very difficult Embryo rescue Bridge lines	Insect resistance Disease resistance Nematode resistance High sucrose content

Source: Taylor (1986) and Jones et al. (1993) with modifications.

L. pimpinellifolium, now S. pimpinellifolium

Some populations of this species differ considerably in morphology whereas others are highly uniform. Some populations are exclusively autogamous (self-pollinating) whereas others allow some outbreeding. This is due to exserted stigmas that project well beyond the anther cone (Rick, 1950). This species tends to readily cross as male parent with *L. esculentum* and is the only species to have exhibited a natural introgression with *L. esculentum*. In fact, it is probable that both species evolved from a common ancestor (Rick, 1950).

L. cheesmanii, now S. cheesmaniae

All forms of *L. cheesmanii* are self-compatible and are exclusively inbreeding. They can be hybridised with the cultivated tomato (*L. esculentum*).

L. parviflorum, now S. neorichii

This species is self-compatible and, due to floral morphology, is highly autogamous. *L. parviflorum* has an extremely small flower and the stigmas rarely protrude out of the anther cone. As a result, populations tend to be highly homozygous (Rick et al., 1976).

L. chmielewskii, now S. chmielewskii

This species is self-compatible. The flowers are large and very showy with long exerted stigmas. This seems to encourage outbreeding and, as a result, much variability is present in its population.

L. hirsutum, now S. habrochaites

L. hirsutum f. *typicum* is a strong outbreeder with a very long, exerted stigma. Most plant introductions are self-incompatible. Those that do self-fertilise produce weak progeny that suffer greatly from inbreeding depression. This form does not readily cross with *L. esculentum*. The other form, *L. hirsutum* f. *glabratum*, readily self-fertilises and progeny do not suffer from inbreeding depression. The latter form is also capable of crossing with *L. esculentum*. One of the first hybrid crosses was performed by Sawant (1958) to determine the relationships between *L. esculentum* and the two forms of *L. hirsutum*.

L. pennellii, now S. pennellii

This species can be readily crossed to *L. esculentum*. Both self-compatible and self-incompatible types exist. *L. pennellii* hybridises readily with cultivated forms and can also be crossed with *L. pimpinellifolium*, *L. cheesmanii*, *L. parviflorum* and *L. hirsutum* but not with members of the *peruvianum* complex.

L. chilense, now S. chilense

This species is an obligate outbreeder. The first *L. chilense* x *L. esculentum* cross was performed and described by Holmes (1939). Crossing this species with the cultivated tomato is extremely difficult due to several barriers. The stigma of *L. chilense* will not accept pollen from the cultivated tomato and almost always leads to the abortion of the flower. The reciprocal cross, pollen from *L. chilense* applied to the stigma of *L. esculentum*, can result in the formation of fruit but few seeds are viable. However, some of the seeds do contain embryos of sufficient size to facilitate embryo rescue.

L. peruvianum, now S. peruvianum and S. corneliomulleri and S. arcanum

This species is exclusively an outbreeder. Crossing *L. peruvianum* with *L. esculentum* is rarely successful and attempts to cross these two species frequently result in embryo or flower abortion, even after the use of embryo rescue techniques (Hogenboom, 1972; Demirel and Seniz, 1997). To overcome this problem, Lanzhuang and Adachi (1996) developed an embryo culture method to obtain hybrid plants. Fortunately, these hybrids are capable of backcrossing to an *L. esculentum* parent (Kamal et al., 2001). Another method that has been successful at overcoming the incompatibility between the cultivated tomato and *L. peruvianum* is the use of *L. chilense* as a bridge species (*L. peruvianum* is crossed to *L. chilense* and that progeny is crossed to *L. esculentum*) (Poysa, 1990). Unfortunately this method often fails, but it does yield better results than a direct cross. A third method for crossing *L. peruvianum* and *L. esculentum* is the production of fertile somatic hybrids, with which backcrossing is possible (Kinsara et al., 1986).

Other species

The closest genetic relatives of tomato, *S. rickii* (Rick, 1988; DeVerna et al., 1990), *S. ochranthum* (Stommel, 2001), *S. juglandifolium* (Rick, 1988), *S. lycopersicoides* (Rick, 1951) and *S. sitiens* (Ji, Pertuzé and Chetelat, 2004), are also crossable to *S. esculentum*.

Chromosomal regions of *S. lycopersicoides and S. sitiens* have been introgressed into tomato (Pertuzé, Ji and Chetelat, 2003; Canady, Meglic and Chetelat, 2005).

Conservation of genetic diversity

The majority of the known improved varieties are related to the original fruit domesticated in Mesoamerica more than 500 years ago. The most important changes introduced by the domestication process are: reduction of the gene pool, modification of the reproductive system and increase of fruit size. The gene pool characterising *Solanum lycopersicum* as a species has constantly been under human management. If this selection process diminishing genetic diversity continues, there is a risk of losing the genetic diversity that once gave rise to the original fruit.

As such, the breeding possibilities offered by using the knowledge of wild relatives of cultivated tomato are very diverse. At present, some characteristics of agricultural importance of tomato have been adapted based on the gene diversity present in wild relatives (Sánchez-Peña et al., 2004). As a result, diagnostic investigations and distribution studies of wild and weedy relatives present at the moment are a priority because of the high levels of genetic diversity they still preserve (Sim et al., 2012).

Bai and Lindhout (2007) report on the genetic diversity collections in the Germplasm banks. This information is mentioned in order to promote the conservation of the genetic diversity of tomato:

- Germplasm Resources Information Network: https://www.ars-grin.gov
- Tomato Genetics Resource Center, Davis, California: http://tgrc.ucdavis.edu
- Botanical and Experimental Garden: www.ru.nl/bgard
- Solanaceae Genome Network: http://zamir.sgn.cornell.edu/mutants.

The contribution of Mesoamerica and the Andes area to the world not only apply to the domesticated fruit, but also includes the amount of genetic information sheltered in the country's rural zones where domesticated crops, landraces and wild relatives co-exist.

General interactions with other organisms (ecology)

Tomato plants compete with plant species or weed species for nutrients and resources. The broadleaf weeds and their control are the most important in tomato production. Examples of common problem weeds include velvetleaf (*Abutilon theophrasti*), redroot pigweed (*Amaranthus retroflexus*) and common lambsquarters (*Chenopodium album*). Weed management involves the use of herbicides and inter-row cultivation (Robinson et al., 2006).

Weed species can also act as hosts for viruses and viral vectors. Two common weed species, lambsquarters (*Chenopodium album)* and cheeseweed (*Malva parviflora*) serve as hosts for both, an insect vector – the western flower thrip (*Frankliniella occidentalis*) – and the tospovirus (tomato spotted wilt virus) it carries (Kahn, Walgenbach and Kennedy, 2005).

Tomato plants are subject to attack by a variety of arthropods (listed in Annex 2.A1) and this can result in yield losses. Tomato defence mechanisms against arthropod attack involve many factors, such as the chemical defenses of glandular trichomes and constituitive and wound-induced defences associated with leaf lamella (Kennedy, 2003). Glandular and non-glandular trichomes are found on the foliage and stems of *Lycopersicon* spp. Some varieties that utilise trichome–mediated defences, for example the wild species *L. hirsutum*

and *L. pennelli*, are more resistant than others to insect attack. Certain glandular trichomes exude acylsugars that are toxic to several common tomato arthropod pests, including whiteflies, aphids, fruitworm, beet armyworm and agromyzid leafminer (Kennedy, 2003). In tomatoes, the jasmonic acid signal molecule is thought to represent an inducible plant defence to herbivory. Application of jasmonic acid induces proteinase inhibitors and polyphenol oxidases and decreases the abundance of many common herbivores, such as thrips, noctuid caterpillars and aphids (Thaler, 1999). Foliar tomato (*L. esculentum*) proteins, such as polyphenol oxidase, proteinase inhibitors and peroxidases, are differentially induced in response to herbivore attack (Stout, Workman and Duffey, 1994). In plants, the jasmonic acid and salicylic acid signalling pathways can provide resistance to herbivore and pathogen attack and sometimes these pathways can interact. Interaction of these pathways in tomatoes results in reduced resistance of tomatoes to the herbivore *Spodoptera exigua* and does not affect the bacterial pathogen, *Psuedomonas syringae pv. Tomato*. However, increased resistance to the bacterial pathogen is associated with salicylic acid-activated responses (Thaler, Fidanstef and Bostock, 2002).

There are many micro-organisms (bacteria, fungi and viruses) associated with tomato crops; some are beneficial while many represent pathogens of the tomato plant. Microbial pathogens are listed in Annex 2.A2. The interactions between micro-organisms, viruses, plants, and indeed, insect vectors, is complex. For example, the bacterial endosymbiont (*Rickettsia* spp.) infects the sweet potato whitefly (*Bemisia tabaci*) and increases the transmission efficiency of the tomato yellow leaf curl virus that the whitefly carries (Kliot et al., 2014).

Mycorrhizal fungi are ubiquitous soil microbes that form a symbiotic relationship with most terrestrial plants, and the largest group associated with most plant species are the vesicular-arbuscular mycorrhizal (VAM) fungi. VAM fungi interact with other micro-organisms such as plant-growth-promoting rhizobacteria. VAM colonised roots of tomato plants were found to attract higher levels of the rhizobacteria – *Azotobacter* and *Psuedomonas flourescens* – in comparison to non-VAM tomato roots (Sood, 2003). Other beneficial plant growth-promoting bacteria and fungi include *Pseudamonas flourescens* and *Glomus mosseae*, which increase plant mineral nutrition by increasing leaf phosphorus content (Gamalero et al., 2004). Some plant growth-promoting rhizobacteria can exhibit antagonism towards some of the most common soil-borne root pathogens of tomato such as *Fusarium oxysporum* f. sp. *radices-lycopersici*, *Pythium ultimum*, *Rhizoctonia solani* and *Pyrenochaeta lycopersici*. In particular, antagonism is associated with siderophore producers (De Brito, Gagne and Antoun, 1995).

Micro-organisms isolated from the rhizosphere of tomato plants were examined and two species, a bacterial species (*Pseudamonas putida*) and a fungal species (*Tricoderma viride*), demonstrated plant growth-promoting activity on greenhouse tomato plants grown under a hydroponic system. Plant growth promotion is thought to be mediated through the production of indole acetic acid by the micro-organisms (Gravel, Antoun and Tweddell, 2007).

Human health and biosafety

Tomato is widely consumed worldwide. It is a popular species preferred in gastronomy for its characteristic flavour. It is used in several traditional dishes because of its compatibility with other food ingredients and high nutritional value (OECD, 2008). The many end uses of tomato fruit, as well as food and feed safety considerations (including composition of key food and feed nutrients, anti-nutrients, allergens, and toxicants) are detailed in the OECD consensus document on tomato composition (OECD, 2008).

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Annex 2.A1.
Tomato pests

Table 2.A1.1. **Most important insect pests of tomato**

Scientific name	Common name	Virus transmitted*
Bemisia argentifolii Bellows and Perring	Silverleaf whitefly	TYLCV
Circulifer tenellus Baker	Beet leafhopper	CTV, BLTVA
Epitrix hirtipennis Melsheimer	Flea beetles	None
Frankliniella bispinosa Morgan	Florida flower thrip	TSWV, TSV
Frankliniella fusca Hinds	Tobacco thrip	TSWV, TSV
Frankliniella occidentalis Pergande	Western flower thrip	TSWV, TSV
Frankliniella shultzei Trybom	Common blossom thrip	TSWV, TSV
Heliothis armigera Hübner/*H. zea* Boddie	Fruit worms	None
Keiferia lycopersicella Wallshingham	Tomato pinworm	None
Leptinotarse decemlineata Say	Colorado potato beetle	None
Lygus ssp. Hahn	Lygus bugs	None
Lyriomyza trifolii Burguess	Vegetable leafminer	None
Manduca sexta L./*M. quinquemaculata* Havorth	Tobacco and tomato hornworms	None
Nezara viridula L.	Southern stink green bug	None
Peridroma saucia Hübner *Agrotis ipsilon* Hufnagel	Variegated cutworm Black cutworm	None
Phthorimaea operculella Zeller	Potato tuberworm	None
Scutigerella immaculate ssp. Newport	Garden symphlyans	None
Spodoptera exigua Hübner	Beet armyworm	None
Thrips tabaci Lindeman	Onion thrip	TSWV, TSV
Trialeurodes vaporariorum Westwood	Greenhouse whitefly	Tomato infectious chlorosis virus
Trichoplusia ni Hübner *Trioza* spp. *Tuta absoluta* Meyrick	Cabbage looper Tomato psyllid Micro lepidoptiron moth	None
Various species	Aphids	AMV, CMV, TEV

* See Annex 2.A2 for a list of the viruses.

Table 2.A1.2. **Most important mite pests of tomato**

Scientific name	Common name	Virus transmitted
Aculops lycopersici Massee	Tomato russet mite	None
Polyphagotarsonemus latus Banks	Broad mite	None

Table 2.A1.3. **Most important nematodes of tomato**

Scientific name	Common name	Virus transmitted
Meloidogyne ssp. Göldi *Meloidogyne enterolobii*	Root knot nematodes	None
Xiphinema americanum Cobb	American dagger nematode	TRSV
Rotylenchulus reniformis Lindfor and Oliveira	Reniform nematode	None

Annex 2.A2.
Tomato diseases

The following lists include the most relevant diseases in terms of economic losses.

Bacteria

Scientific name	Common name
Clavibacter michiganense Smith	Bacterial canker
Pseudomonas corrugate Roberts & Scarlett	Tomato pith necrosis
Ralstonia (Pseudomonas) solanacearum Smith	Bacterial wilt
Pseudomonas syringae van Hall pv. *tomato*	Bacterial speck
Xanthomonas campestris Pammel	Bacterial spot

Oomycetes and fungi

Scientific name	Common name
Alternaria alternate (Fries) Keissler	Tomato black mould
Alternaria alternata f. sp.l*ycopersici* Grogan et al.	Alternaria stem canker
Alternaria solani (Ell.& Mart.) Jones & Grout.	Early blight
Botrytis cinerea	Gray mould
Cladosporium fulvum	Leaf mould
Colletotrichum Ssp. Cordá	Anthracnose
Fusarium oxysporum f. sp.l*ycopersici* Vawdrey & Peterson	Fusarium wilt
Fusarium oxysporum f. sp. *radicis-lycopersici* Jarvis & Shoemaker	Tomato Fusarium crown and root rot
Fusarium solani (Mart.) Sacc.	Tomato Fusarium foot rot
Leveillula taurica (Lev.) Arnaud syn. *Oidiopsis taurica* Salmon	Tomato powdery mildew
Phytium ultimum Trow	Tomato water mould
Phytophthora parasitica Dastur and *P. capsici* Leonian	Tomato Phytophthora root rot
Phytophtora infestans (Mont.) de Bary	Late blight
Pyrenochaeta lycopersici Schneider & Gerlach	Tomato corky root rot
Sclerotinia sclerotiorum (Lib.) de Bary	Tomato white mould
Sclerotium rolfsii Sacc.	Tomato southern blight
Verticillium albo-atrum Reinke & Berthold and *V. dahlia* Kleb.	Verticillium wilt

Viruses

Common name	Acronym
Alfalfa mosaic virus	AMV
Cucumber mosaic virus	CMV
Potato virus Y	PVY
Tobacco etch virus	TEV
Tobacco mosaic virus	TMV
Tobacco streak virus	TSV
Tomato big bud or Beet leafhopper transmitted viresence agent	BLTVA
Tomato bushy stunt virus	TBSV
Tomato infectious chlorosis virus	
Tomato mosaic virus	ToMV
Tomato or Beet curly top virus	CTV
Tomato ringspot virus	TRSV
Tomato spotted wilt virus	TSWV
Tomato yellow leaf curl virus	TYLCV
Tomato golden mosaic begomovirus	TGM
Tomato pepper huasteco begomovirus	TPH
Peanut bud necrosis tospovirus	PBN

Annex 2.A3.
Biotechnological developments

At present, great efforts of biotechnology in tomatoes have focused on the resistance against diseases caused by fungi, bacteria and viruses as well as on the tolerance to stress and pesticide exposure. In some cases, tomato plants are bred for development of varieties with increased nutritional or health benefits (Herbers, 2003).

The wealth of molecular biology research for tomato and the availability of efficient transformation protocols have made this crop species a highly attractive target for genetic manipulation. Indeed, the first product from a transgenic plant released and approved for human consumption was a transgenic tomato line called "Flavr Savr", which had delayed ripening properties. Flavr Savr was developed in 1994 by Calgene Company (Herrera and Martínez-Trujillo, 2005; Llop-Tous, Barry and Grierson, 2000; Bird et al., 1988), but was later withdrawn from the market due to the poorly adapted germplasm used at the early stage of biotech development. Subsequently, in 1995, a genetically modified tomato was produced by Zeneca with similar properties. This product is available nowadays on the market as a processed product, tomato purée (Herrera and Martínez-Trujillo, 2005). In addition to fruit-ripening characteristics, other potential targets of tomato gene manipulation are as follows:

- **Fruit quality:** Fruit ripening research discovered that the enzyme polygalacturonase (PG) is responsible for the degradation of pectin (which maintains the unity of the cell walls), causing subsequent softening of the fruit. The PG gene synthesising this enzyme was identified in order to block or delay its production without altering other ripening mechanisms and to extend shelf life of the fruit (Herrera and Martínez-Trujillo, 2005; Bird et al., 1988). Both Flavr Savr and the variety developed by Zeneca manipulated this gene. Another development was the suppression of the formation of the ripening hormone ethylene, by suppressing the enzymes (ACC synthase and ACC oxidase) involved in ethylene production or by metabolising ethylene precursors, SAM and ACC, by expressing enzymes like SAM hydrolase and ACC deaminase.
- **Virus resistance:** Disease resistance is one of the most thoroughly explored branches of genetic engineering. In the case of tomato, viruses are devastating phytopathogenic agents. For example, the Pepino mosaic virus is a major disease of tomatoes grown in greenhouses worldwide (Cottilon, Girard and Docouret, 2002; French et al., 2001; Hanssen, Lapidot and Thomma, 2010; Ling, 2007; Ling and Scott, 2007; Maroon-Lango et al., 2005; Mumford and Metcalfe, 2001; Pagán et al., 2006; Van der Vlugt et al., 2000). At present, plant lines resistant to Tobacco mosaic virus have been developed by adding the gene *Tm*. Several aspects regarding the interaction of a resistance gene product and a viral-encoded protein have been identified as well. This is particularly the case for recessive resistance genes operating against potyviruses, although the exact mechanism which inhibits virus infection is still not clear (Palukaitis and Carr, 2008; Palukaitis et al., 2008; Piron et al., 2010).
- **Disease resistance:** Fungi cause great losses in tomato cultivation. Transgenic tomatoes resistant to *Fusarium* attacks were developed by the identification of two genes which code for enzymes that degrade the most important components

of fungal cell walls (chitin and beta-1-3-glucan) (Tameling et al., 2002). The tomato *Pto* gene confers resistance to races of *Pseudomonas syringae* pv. *tomato* that carry the *avrPto* gene (Martin et al., 1993; Hammond-Kosack and Jones, 1997). Resistance to the leaf mould pathogen *Cladosporium fulvum* is conferred by distinct *Cf* genes, which have been introgressed from various wild *Solanum* species or landraces into cultivated tomato *S. lycopersicum* (Dixon et al., 1996). The gene *Mi*, which confers resistance to several species of root-knot nematode, is present in many modern tomato cultivars (Jacquet et al., 2005; Sorribás et al., 2005; Williamson, 1998).

- **Insect resistance**: Open field grown tomatoes suffer from Lepidopteran attacks. Genes from *Bacillus thuringiensis* (Bt) bacteria have been used to create plants resistant to those attacks. Certain Bt genes encode crystalline enzymes with insecticidal effect (delta endotoxins). As their activity is taxon specific (for example, the "Cry lll" protein only affect beetles), specialised transgenic plants have been generated that are resistant to specific insect attacks (Collinge, Lund and Thordal, 2007; Herrera and Martínez-Trujillo, 2005; Fillatti et al., 1987). The expression of δ-endotoxins in transgenic plants has provided a very effective means to control economically important insect pests in order to overcome the instability and degradation of *Cry* proteins when exposed to ultraviolet radiation and short persistence on the plant.
- **Resistance/tolerance to abiotic stress:** In order to increase the geographical range in which tomato can be grown, research is being undertaken to produce transgenic tomato lines resistant to drought, low temperatures and salinity. This would allow the transgenic plants to grow, flower and produce fruits in habitats with high levels of salinity. Moreover, they also preserve fruit quality with low sodium content (Herrera and Martínez-Trujillo, 2005, Goel et al., 2010).
- **Vaccine production:** Tomato has been used as a host system to produce a number of vaccines: plague, SARS, *E. coli*, Hepatitis, HIV, Alzheimers, enterovirus 71, RSV, malaria and cholera. A number of these transgenic fruits have been tested on laboratory animals shown to induce an immune response, indicating a potential for the development of human vaccines (Youm et al., 2008; Denis et al., 2007; Alvarez et al., 2006). The results indicate that tomato plants may provide a useful system for the production of human Ab antigen (Youm et al., 2008; Lou et al., 2007).
- **Anthocyanin accumulation in tomato:** In view of the presumed beneficial effect of plants' antioxidants to human health, several research groups have investigated the possibility of increasing the antioxidant levels in tomato fruit through transgenic approaches. Positive results have been obtained for carotenoides (Davuluri et al., 2005; Fraser et al., 2007), phenylpropanoids and especially polyphenols (Muir et al., 2001; Bovy et al., 2002; Verhoeyen et al., 2002; Davuluri et al., 2005; Schijlen et al., 2006; Butelli, Titta and Giorgio, 2008)

Table 2.A3.1. **Approved genetically modified events for modified product quality in tomato**

Genetically modified traits	Event	Name	Developer
Events with delayed ripening/senescence	1345-4		DNA Plant Technology Corporation (United States)
Events with delayed ripening/senescence	35-1-N		Agritope, Inc. (United States)
Events with delayed ripening/senescence	8338	CGN-89322-3	Monsanto Company
Events with delayed ripening/senescence	Huafan-1		Huazhong Agricultural University (China)
Lepidopteran insect resistance	5345		Monsanto Company
Antibiotic resistance	B	SYN-0000B6	Zeneca Plant Science and Petoseed Company
Antibiotic resistance	DA Dong No.9	SYN-000DA-9	Zeneca Plant Science and Petoseed Company
Antibiotic resistance	F (1401F, h38F, 11013F, 7913F)	SYN-0000F-1	Zeneca Plant Science and Petoseed Company
Events with delayed ripening/antibiotic resistance	FLAVR SAVR	CGN-89564-2	Monsanto Company
Viral disease resistance	PK-TM8805R		Beijing University
Novel tomato flavour		*Del Ros1*	Butelli, Titta and Giorgio

Source: ISAAA, *GM Approval Database*, www.isaaa.org/gmapprovaldatabase.

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Part II.

Biology of animals

Chapter 3.

Atlantic salmon (*Salmo salar*)

*This chapter deals with the biology of Atlantic salmon (*Salmo salar*). It contains information for use during the environmental risk/safety regulatory assessment of genetically engineered salmon. It is the first OECD biosafety publication to address an animal species, in this case a commonly cultured, domesticated fish reared for food production but also present in the wild in undomesticated populations. The chapter describes the biology and ecology of wild Atlantic salmon (including classification, life stages, reproduction, centres of origin, geographical distribution, population dynamics, interaction with other organisms) and of the farmed form (domestication, aquaculture rearing practices, biocontainment, interactions with the external environment). It also provides elements of genetics, research on genetically engineered salmon and bibliographic resources for risk assessment.*

This chapter was prepared by the OECD Working Group on the Harmonisation of Regulatory Oversight in Biotechnology, with Finland, Norway and the United States as the co-lead countries. It was initially issued in May 2017.

Introduction

The environmental risk/safety assessments of transgenic organisms are normally based on the information on the characteristics of the host organism, the introduced traits, the environment into which the organism is introduced, the interaction between these, and the intended application. The OECD's Working Group on Harmonisation of Regulatory Oversight in Biotechnology focus its work on identifying parts of this information, which could be commonly used in countries for environmental safety/risk assessment to encourage information sharing and prevent duplication of effort among countries. Biosafety consensus documents are one of the major outputs of its work.

Biosafety consensus documents are intended to be a "snapshot" of current information on a specific host organism or trait, for use during regulatory assessments. They are not intended to be a comprehensive source of information on everything that is known about a specific host or trait; but they do address the key or core set of issues that member countries believe are relevant to risk/safety assessment. This information is said to be mutually acceptable among member countries.

To date, 54 biosafety consensus documents have been published. They address the biology of crops, trees and micro-organisms as well as specific traits which are used in transgenic crops. This is the first biosafety consensus document to specifically address an animal other than a micro-organism, in this case a commonly cultured, domesticated fish that is reared for food production, Atlantic salmon, but which also occurs in the wild in undomesticated form, often in the very same geographical region. Thus in this document the biology and ecology of wild Atlantic salmon are described in addition to that of the domesticated form. Currently, used production and rearing practices are also described at length for domesticated Atlantic salmon because these practices may influence the ability of, and locations where, wild and domesticated forms of Atlantic salmon might interact in the environment and the types of interactions that may occur if they co-occur. This information is intended to benefit potential risk assessors that may need to consider these potential interactions and their effects, and in assessing the risks that they might pose.

In reading the biosafety consensus documents, it may be useful to consult two additional texts. The first, entitled "An introduction to the biosafety consensus document of OECD's Working Group for Harmonisation in Biotechnology", explains the purpose of the biosafety consensus documents and how they are relevant to risk/safety assessment. The second text is "Points to consider for consensus documents on the biology of cultivated plants". Although this document is specifically for cultivated plants, it contains a structured checklist of "points to consider" for authors when drafting or for those reviewing a consensus document relevant to the biology of domesticated animals used in agriculture. Amongst other things, this text describes how each point is relevant to risk/safety assessment.

The biosafety consensus documents are of value to applicants for commercial uses of transgenic organisms, regulators in national authorities as well as the wider scientific community. The consensus documents are not intended to be a substitute for a risk/safety assessment, because they address only a part of the necessary information. Nevertheless, they should make an important contribution to environmental risk/safety assessment.

As each of the documents may be updated in the future as new knowledge becomes available, users of consensus documents are encouraged to provide any information or opinions regarding the contents of this document or indeed, the OECD's other harmonisation activities.

Biology and ecology of wild Atlantic salmon

Classification and nomenclature

Scientific name of Atlantic salmon: Salmo salar *Linnaeus, 1758*

English: Atlantic salmon

French: *Saumon atlantique*

Spanish: *Salmón Atlántico*

Russian: *Semga*

German: *Atlantischer Lachs*, *Salm*

Phylum: Chordata

- Class: Osteichthyes (bony fishes)
  - Order: Salmoniformes
    - Family: Salmonidae (salmon and trout)
      - Subfamily: Salmoninae
        - Species: *Salmo salar* Linneaus

Atlantic salmon belongs to the family *Salmonidae* (Teleosts) (Nelson, 1984). The family comprises seven genera:

1. Salmo (includes *Salmo salar* – Atlantic salmon and *Salmo trutta* – brown trout and other endemic trout species)
2. Hucho (taimen)
3. Oncorhynchus (Pacific salmon)
4. Salvelinus (charr)
5. Salmothymus (endemic Balkan/Adriatic)
6. Brachymystax (lenok)
7. Salvethymus (S. svetovidovi).

The most closely related species to Atlantic salmon is the brown trout, *Salmo trutta*. Low rates of hybridisation between the two species are common in the wild throughout Europe, where they are native (Youngson et al., 1993); the F_1 generation can be fertile. Brown trout is not native to North America and where it has been introduced it is known to hybridise with Atlantic salmon (Verspoor, 1988a).

Atlantic salmon occurs naturally only in the northern hemisphere and can be divided genetically into two major lineages, a North American (west-Atlantic race) and a European one. Both the North American and European lineages again can be separated into smaller regional groupings. For example, in North America, there is regional distinction among the populations of the Gulf of Maine, the Outer Bay of Fundy, the Inner Bay of Fundy, Labrador/Ungava, the Gulf of St. Lawrence, the Southern Uplands of Nova Scotia and Newfoundland (excluding Gulf Rivers; Verspoor, 2005). In Europe, there is a major division between Atlantic salmon of the Baltic and other European populations, as well as a division between the east Atlantic and north Atlantic in the Barents Sea region.

- Atlantic salmon is a genetically substructured species, even at the inter- and intra-watershed scales.
- Genetic differentiation is based on homing to natal rivers (isolation of populations).
- Genetic differences have been demonstrated between populations in protein-coding genes, nuclear and mitochondrial DNA markers and genetically based performance traits.

Genetic markers

Identification of Atlantic salmon can be made through the use of genetic markers: chromosome numbers, allozymes, DNA analyses (see the section on "Genetics of Atlantic salmon").

Table 3.1. **Terminology: Stages in the life cycle of Atlantic salmon**

Stage	Definition
Alevin	Hatched fish still dependent on the yolk sac nutrition
Fry	Short transitional stage where the fish emerge from the redd and start to feed exogenously and disperse
Parr	Stage between full absorption of the yolk sac and smoltification
Precocious parr	*Sexually mature parr (mostly males)*
Smolt	Stage when seaward (landlocked: lakeward) migration occurs
Post-smolt	Stage from departure from the river (usually in spring/early summer) to the end of the first winter in the sea (sea-winter) or lake
Adult	Fish after the end of the first winter in the sea/mature fish which returns to river to spawn
Grilse (1SW)	A fish that returns to breed after one sea-winter
MSW (or multi SW)	A fish that returns to breed after multiple sea-winters
Kelt	Adult fish after spawning (spent), until it reaches the sea

Life history and characterisation

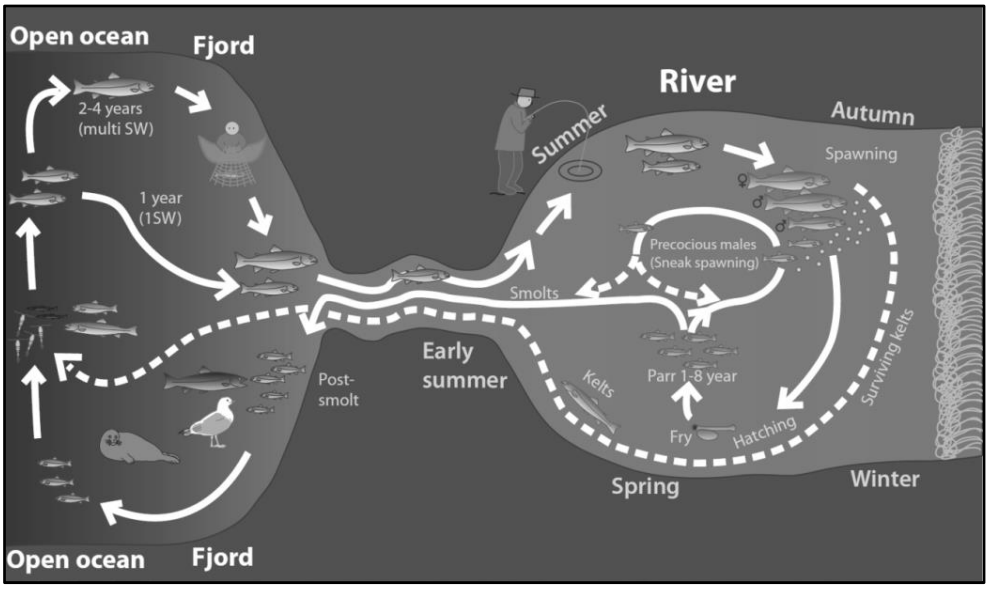
Characterisation

Atlantic salmon is anadromous typically: the young migrate from the river to the sea for feeding and at sexual maturation return to their natal river to spawn as adults (Figure 3.1). There are, however, populations, particularly in North America but also in Northern Europe, that complete their entire life cycle in fresh water and are known as landlocked. Freshwater resident salmon populations are more often separated by some geographical barrier from anadromous salmon populations (Klemetsen et al., 2003; Sandlund et al., 2014), but they also exist in sympatry with anadromous salmon in North America (Hutchings and Myers, 1985; Verspoor and Cole, 1989). Landlocked populations generally have lower genetic variation within populations, but larger genetic differences between populations, compared to anadromous populations (Bourret et al., 2013a; Sandlund et al., 2014). This is because of lower effective population size in some populations, and a lack of gene flow between populations because of geographical barriers. Resident salmon populations in sympatry with anadromous populations have been shown to be genetically different from anadromous populations in some watersheds but not in others (Adams, Cote and Hutchings, 2016).

Atlantic salmon requires a freshwater environment for spawning and the development of the early life stages. Smolts leave the rivers in spring and/or early summer. As they prepare to do so, they undergo physiological (e.g. increase Na+K+ ATPase production), morphological (e.g. become more streamlined and take on a silvery body colouration) and behavioural

changes for salt water. (Some landlocked populations may have abandoned key elements of the parr-smolt transformation associated with marine life [Nilsen, Ebbesson and Stefansson, 2003].)

Figure 3.1. **Schematic outline of the anadromous Atlantic salmon life cycle**



Source: Courtesy of Audun Rikardsen.

The main feeding grounds of anadromous Atlantic salmon is the Northern Atlantic, with European populations being found principally on the eastern side and North American populations on the western side. However, the two groups frequently overlap off Greenland. Baltic populations restrict their migrations to the Baltic Sea.

Once the returning salmon have entered the river, they migrate upstream until a suitable spawning site is reached. Their homing instinct brings the salmon back not only to their natal river, but also potentially to the same river section where they were born. This allows for geographic and genetic isolation, which are the key factors leading to river-specific adaptation (genetic differentiation) and ecological variability. Substantial genetic structuring is found both between and within watercourses throughout the species' distribution range.

Identification

All salmonids possess an adipose fin and an axillary process at the base of each pelvic fin. Wild anadromous Atlantic salmon have a body shaped like a torpedo, but old males can have a rather deep body. There are few spots below the lateral line and two to four spots on the operculum. The pectoral fins are long in comparison with other salmonids. The upper jaw reaches only the posterior of the eye, unlike that of the brown trout, which extends further. The caudal peduncle is also rather narrow and the caudal fin shallowly forked (V-shaped) in comparison with brown trout.

Atlantic salmon have a silvery colouration during ocean life and turn brownish during maturation, with males also developing reddish hues. Anadromous males develop a characteristic hooked jaw (kype) that is thought to be important during breeding competition.

Farmed Atlantic salmon have a plump body form with numerous spots and scales may be missing. Fins are commonly worn and may be crippled. Sometimes fin rays grow together or fuse, particularly in the dorsal and pectoral fins. Scales, which are a good indicator of age and growth, can be used to distinguish wild from farmed salmon. The cales of wild salmon demonstrate the characteristic narrow annuli of the freshwater phase and wide bands representing the fast marine growth. Farmed salmon have a rather steady growth and lack the clear difference between freshwater and marine phase annuli.

Juveniles in the parr stage have 7-13 dark "parr marks" on each side. Red spots occur mainly along the lateral line. The adipose fin is grey. Smolts turn silver as a subcutaneous deposit of guanin is laid down, concealing the parr marks, and the pectoral and caudal fins turn black. At this stage the juveniles establish the characteristic torpedo shape.

Smoltification (i.e. the process of preparing for the transition from fresh water to salt water) is size-dependent and may occur from *ca.* 10 cm fork length. This, however, varies among populations and among individuals within populations, with fast-growing parr smolting at younger ages and smaller sizes than slower growing parr (Jonsson and Jonsson, 2011). Older smolts can reach up to 22-25 cm. The maximum size of adults depends strongly on the time spent at sea. Female Atlantic salmon reach a length of 120 cm and males of up to 150 cm. Maximum weight is 40 kg. Very large fish are commonly repeat spawners in their second or third migration. Survival to repeat breed is generally low (11%, ranging from <1% to 43%), and more so for males than females (Fleming,1998).

The scale count between the base of the adipose fin and the lateral line and the length of the upper jaw bone or maxilla in relation to the eye are two of the most reliable external features for distinguishing *Salmo salar* and the trout *Salmo trutta*.

Table 3.2. **Distinguishing features of species of the genus Salmo and the genus Oncorhynchus**

	Salmo salar	*Salmo trutta*	*Oncorhynchus kisutch*	*Oncorhynchus gorbuscha*
Upper jaw bone	Extends to the level of the rear of the eye	Extends well beyond the level of the eye	Extends beyond the level of the eye	Extends beyond the level of the eye
Scale count between base of adipose fin and lateral line	10-13	13-16	(x)	(x)
Number of dorsal fin rays	10-12	12-14	9-12	10-15
Number of anal fin rays	8-11	10-12	12-17	13-19
Number of gill rakers on first arch	15-20 (slender)	14-17 (short and stubby)	18-25 (coarsely toothed)	24-35
Number of scales in the lateral line	(x)	(x)	121-148 (scales moderately large)	147-205 (scales small)
Other distinguishing features	Caudal peduncle narrow; caudal fin shallowly forked	Caudal peduncle deep and rather flat; caudal fin square-cut or slightly concave to slightly convex	A long scaly process in axil of pelvic fin; adipose fin well developed	Breeding males have a pronounced humpback

Source: Mills (1991).

Life stages and generation time

General aspects

The full life cycle of Atlantic salmon ranges from 3 to 12 years. The generation time in wild and domesticated Atlantic salmon strains has a genetic component, but it is strongly modified by environmental factors such as temperature, food abundance (cultivation: feeding regime) and density. Environmental determinants (temperature and flow regimes, predation,

and food availability) also provide the potential for population-specific adaptation of juvenile salmon to natal streams. The combination of genetic and environmental determinants allows for the wide diversity found in naturally occurring Atlantic salmon populations throughout their native range.

Figure 3.2. **Image of Atlantic salmon (*Salmo salar*)**

Source: Pål Thomas Sundhell.

The generation time varies among rivers and even between river sections, mostly as a result of variation in the mean age at smolting. Variation in growth rate results in variation in size and age at smolting and an association between an index of growth potential (combination of degree-days and day length) and mean age at smolting has been shown for Atlantic salmon throughout its range (Metcalfe and Thorpe, 1990).

Incubation

A spawning nest known as a redd is formed by the female in the gravel at the bottom of the river. Hatching usually occurs after 400-450 day-degrees (i.e. the sum of daily temperatures [^{o}C]), but this can be shorter for populations in the northern range of the distribution, where temperatures are consistently low for long periods. Incubation time correlates closely with climate, i.e. water temperature during incubation. In warmer regions (southern range of the distribution) it may be only eight weeks, with spawning occurring late (December-February) and emergence of the fry occurring early (March-April). In cold rivers (northern range), the incubation period can be up to eight months, with spawning occurring early (September-October) and emergence occurring as late as June due to the slow incubation process. The optimal temperature for incubation is 1-10°C; below and above this range incubation success is generally reduced. Survival during this period can correlate positively with egg size, particularly if oxygen conditions in the gravel nest become stressful for the embryos (Einum, Hendry and Fleming, 2002).

Alevin stage

After hatching, alevins live off their yolk sac, which is attached to their under surface. At this stage, which takes place in and around the gravel of the redd (15-30 cm below the river bottom), alevins become increasingly mobile. During the first days, alevins are positively geotactic and negatively phototactic, but as yolk sac absorption progresses they become negatively geotactic (movement towards the surface of the redd) and positively phototactic and rheotactic. The duration of the alevin stage depends on temperature and ranges from *ca.* 20 to 120 days. The time from fertilisation to emergence also varies with egg size, but the effect is small with large eggs taking a few days (*ca.* 3 days) longer than small eggs (Einum and Fleming, 2000a).

Fry stage

As yolk sac absorption nears completion, the fry stage begins with emergence of fry from the gravel and the start of active feeding (mostly on invertebrates). Fry then begin to disperse from the area around the redd. Most of the fry disperse downstream (though the highest concentrations often remain near the spawning grounds), with pools being avoided. The fry stage ends when the fish settle and establish small territories, which they defend against conspecifics of the same year-class. Evidence suggests that both earlier emergence (prior residency) and body size can afford fry success in competing to establish a territory (Brännäs, 1995; Cutts, Metcalfe and Taylor, 1999; Harwood et al., 2003) and in subsequent survival (Einum and Fleming, 2000a; 2000b).

Parr stage and age at smoltification

Effective foraging and anti-predator behaviours correlate positively with survival (Einum and Fleming, 2001). The duration of the parr stage again depends on productivity, temperature, density and on the social status of the individual (i.e. dominant individuals often attain faster growth than subordinate individuals; Harwood et al., 2003; Nislow, Armstrong and Grant, 2011). Faster growing juveniles generally go to sea at a younger age. Smoltification normally occurs at a minimum size of 10 cm fork length, but this varies among populations. As part of the population continues to grow through winter, juvenile salmon develop a bimodal length distribution. The upper modal group (UMG) is composed of larger fish likely to smolt the following spring. The lower modal group is composed of smaller fish likely to spend at least one more year before smolting; older smolts are usually larger at smolting than UMG smolts. In productive rivers older smolts, which spend one or more extra years in fresh water, can reach a size of more than 20 cm. Bimodality in length frequencies is a phenomenon mainly observed in wild populations that produce one-year old smolts.

Because smoltification is mainly size dependent, the proportion of early smolts correlates with temperature, density and productivity of the stream. Thus there is a general tendency for smolt age to increase with latitude, though the pattern differs between the European and North American continents (Metcalfe and Thorpe, 1990). Smolt age can vary between one and eight years. In the southern range, the vast majority of a year class reaches the threshold size of 10 cm in the first year and most smolts are one-year old. Smolts older than two years seldom occur. Generation time is therefore short. In central Europe (e.g. Rhine, Loire), and in the other regions with a moderate climate like Ireland and the southern British Isles, around 50% of the parr reach 10 cm after one year and emigrate as smolts. Age-One year old smolts measure usually 10-15 cm. Two-year old smolts reach 12-22 cm. Three-year smolts are rare (<1%). In the Gulf of Maine and Canadian Maritimes, Scotland and southern Scandinavia, the majority of smolts are two and three years old; one-year and four-year smolts are rare. In central Scandinavia, parts of the Russian Federation and parts of Canada (e.g. Quebec and Newfoundland), smolt age varies between two and five years. There are essentially no one-year smolts produced naturally. In northern Scandinavia and northern Canada (Labrador and Ungava Bay) parr commonly need four to eight years to reach the critical size for smoltification. Smolts of ages one and two rarely occur in these regions.

Atlantic salmon smolts emigrate from freshwater nursery areas during spring and early summer (March-August) to feed and grow in the North Atlantic Ocean. The timing of the transition from the freshwater ("parr") stage to the migratory stage ("smolt") is cued by photoperiod and water temperature, with photoperiod as the dominant cue

(McCormick et al., 1998; 2002). For Atlantic salmon, the timing of the smolt migration has an important role in determining smolt survival in the marine environment, and it is believed that Atlantic salmon smolts are adapted to use environmental cues in rivers that may predict favourable ocean conditions for them to initiate downstream migration (Hvidsten, Heggberget and Jensen, 1998, Thorstad et al., 2012, Otero et al., 2014). Hence, the timing of the freshwater emigration has likely evolved to meet environmental conditions in the ocean as these affect growth and survival of the post-smolts.

A recent analysis of spatio-temporal variations in the dates of downstream smolt migration in 67 rivers throughout the North Atlantic show that in addition to a latitudinal cline, with southern populations migrating earlier than northern ones (e.g. Hvidsten, Heggberget and Jensen, 1998), the timing of migration differed strongly between the east and west Atlantic, with western populations migrating to sea at later dates than eastern populations at corresponding latitudes (Otero et al., 2014). After accounting for this spatial effect, the initiation of the downstream migration among rivers was positively associated with freshwater temperatures, up to about 10°C and levelling off at higher values, and the timing was also positive associated with sea-surface temperatures. Earlier migration occurred when river discharge levels were low but increasing (Otero et al., 2014). On average, the initiation of the smolt seaward migration has occurred 2.5 days earlier per decade throughout the basin of the North Atlantic. This shift in phenology matches changes in air, river and ocean temperatures, suggesting that Atlantic salmon emigration is responding to the current global climate changes (Otero et al., 2014).

Post-smolt stage and sea age

Atlantic salmon spend one, two, three and sometimes four winters at sea. Those that spend more than one are known as multiple sea winters (MSW) fish. Five-sea-winter (5SW) fish are very rare, as are 0SW fish. Individual fish within a population that grow faster prior to smoltification tend to have younger sea ages than those with slower pre-smolt growth rates (Einum, Thorstad and Næsje, 2002). The duration at sea is also stock-dependent and, in part, genetically controlled. Recently, a study of 1SW and MSW Atlantic salmon populations from the three phylogeographic lineages of Atlantic salmon in Europe, based on the use of a 220 000 SNP-chip, has identified a gene that strongly affects sea age at maturity in salmon (Barson et al., 2015). Some populations consist mainly of grilse (1SW fish), others are dominated by 2SW fish (e.g. most populations in the river Rhine; French Loire-Allier; Danish Skjern; and Gulf of Maine; and many Norwegian, Scottish and Canadian Maritime populations). In some populations, 3SW fish are lacking completely (e.g. many Irish populations). Long/large rivers frequently have MSW populations and shorter/smaller rivers less frequently so (Jonsson, Hansen and Jonsson, 1991). Grilse populations dominate in small rivers, though there are exceptions. Thus migration distance alone does not explain the variability observed among populations. There are suggestions that changes in the grilse/MSW ratio can be explained by changes in oceanic temperature or feeding conditions at sea (Gudjonsson et al., 1995; Otero et al., 2012).

Growth in the marine environment is rapid. After one sea-winter, adult Atlantic salmon usually reach 50-75 cm total length. After two sea-winters, the fish reach 65-90 cm. 3SW salmon can measure more than 100 cm.

Lake age

The duration of feeding in lakes is also stock-dependent and growth tends to relate to the size of the lake (being faster in larger lakes). Atlantic salmon spend from one to nine winters in lakes (Klemetsen et al., 2003). Post-spawning mortality is low among resident

individuals (dwarf forms are an exception) and repeat spawning common (one individual had five "spawning marks"; Smirnov, 1979).

Age at sexual maturity

Sexual maturity is variable between sexes. In productive streams, males frequently reach sexual maturity at the parr stage during their second or third autumn. Maturity may be reached even during the first year at a size of less than 7 cm fork length. The frequency of males maturing as parr varies widely (range 2-100%), and appears to decrease with latitude (reviewed in Fleming [1998]). Females rarely mature as parr in anadromous populations. Females in landlocked/ouananiche populations, however, frequently mature at sizes smaller than that found in anadromous populations, and occasionally at sizes similar to that of mature male parr of anadromous populations.

In anadromous salmon populations having MSW fish, males often tend to mature at younger ages than females (i.e. males dominant numerically within the grilse component). However, some of the oldest and largest fish in these same populations may be males.

The persistence of a single year class in a stream (or the maximum age of a salmon) is generally shorter in the southern range than in the northern range. Repeat spawners are generally infrequent; however, in some populations over 40% of the spawners may be repeat breeders (e.g. historically Inner Bay of Fundy). The maximum life span in the wild is around 15 years.

Age structure:

- In all salmonids, the life span of fish of northern populations is longer than in southern populations.
- Males and females differ in the rate of sexual maturation; the early maturing age classes mostly comprise males.
- Atlantic salmon males can reach sexual maturity in fresh water at the parr stage (as early as 0+ fish); some may subsequently stay in fresh water as resident parr, while others may migrate to the ocean to undertake an anadromous life cycle.
- The age structure of different populations is not identical; the freshwater phase can last one to two years in southern populations and up to eight years in far northern populations.
- In the same population, age structure varies slightly from one generation to another, but one or two age groups usually remain dominant.

Reproduction

Salmon are known for their ability to return to the rivers in which they were born (usually more than 94-97% accuracy; Stabell, 1984; Jonsson, Jonsson and Hansen, 2003), a trait that segregates populations and leads to a variety of local adaptations. The low level of straying that does occur to neighbouring rivers may in some cases result in a metapopulation structure (i.e. a set of local breeding populations connected by exchange of some individuals). The resulting network of local populations provides a balance between local adaptation and the evolutionary flexibility that results from exchange of genetic material among populations (NRC, 2004).

The return to fresh water to breed may occur from spring until fall, and in some cases as much as a year in advance of spawning (e.g. in Ungava Bay, northern Norway and the Russian Federation). Migration timing is a characteristic of individual populations

(e.g. MSW fish entering before grilse) and environments (e.g. hydrological and temperature regimes, and length and physical difficulty of ascent). Upon entry, Atlantic salmon in some river systems remain for several months in the lower reaches of the river before ascending to the spawning grounds. In large river systems, it appears that those that enter earliest migrate the furthest upstream to spawn.

Spawning time, which is heritable, appears to be an adaptation to favourable conditions for spawning, embryo incubation, and juvenile emergence and subsequent feeding (Jensen, Johnson and Heggberget, 1991). A female's spawning time will dictate the thermal regime her embryos experience during development and to a large extent their hatching and emergence time from their gravel nest. Peak spawning times vary among populations from September to February (the most common period is late fall), reflecting differences in water temperature regimes during incubation (Heggberget, 1988). Thus, northern populations frequently spawn before more southerly populations, and upstream populations may spawn significantly earlier than downstream populations in the same river system (Webb and McLay, 1996). Spawning within populations spans several weeks and can last as long as ten weeks.

On the spawning grounds, the behaviour of the two sexes differs markedly, with female behaviour being shaped principally by natural selection for offspring production and survival, and male behaviour by sexual selection for access to matings (Fleming, 1996; Fleming and Einum, 2011). Females choose spawning locations within the river that provide favourable incubation environments for eggs (i.e. often the upstream end of riffles, having low concentration of fines and high oxygen permeability). She deposits her eggs within dedds that she creates by digging actions with her caudal fin. Once fertilised, the female covers the eggs immediately with gravel and begins preparation of the next nest. A female will construct 5-8 nests typically, and up to as many as 14, to deposit all her eggs in. The number of nests constructed increases with female size, as does their depth. Nest depth reduces susceptibility to destruction from superimposition by later spawning females, gravel shifts caused by flooding, and freezing. Once females have completed nesting, which takes a median of five to six days from start to finish, they do not overtly defend their site(s) in contrast to Pacific salmon, which do so until death. After spawning, female Atlantic salmon simply descend from the spawning grounds to a nearby pool or back to the mainstem river.

Female fecundity varies considerably both within and among salmon populations, as both egg number and size increase with body size (reviewed in Fleming [1996]). However, relative fecundity (i.e. eggs per kilogram body weight) varies much less (typically 1 200-2 000) and inversely with fish size (i.e. smaller fish have more eggs per kg than larger fish).

Male Atlantic salmon do not participate in nest acquisition or construction, but rather seek out and compete for access to spawning females. As a consequence, males spend considerably more time on the spawning grounds than females seeking breeding opportunities. While absolute sex ratio of anadromous fish (i.e. excluding mature parr) within spawning populations may vary from 20% to over 90% female (Fleming, 1998), the operational sex ratio on the spawning grounds (i.e. the number of sexually active males to females at any one time) is frequently male biased. This is because each female breeds for only five to six days, while each male has the capability to spawn rapidly and repeatedly over the several weeks of the spawning season. As a consequence, there is intense competition among males for access to spawning females, which has resulted in the evolution of specialised breeding traits in males, such as the hooked jaw or kype and an alternative male reproductive strategy (see next paragraph).

The intense competition among large anadromous males for access to breeding females (i.e. courting and fighting, with large size being advantageous), provided the opportunity for an alternative reproductive strategy to evolve. A proportion of males (2-100%) may mature precociously as parr, at less than a hundredth the weight of the anadromous males, and use their small size to sneak access to spawning females (i.e. rather than court and fight). The expression of the alternative strategy appears to have both a genetic and environmental (i.e. growth rate) component. Such males may also subsequently migrate to sea and return as a large anadromous male.

The larger size of anadromous males, and consequently greater ejaculate volume of sperm, typically affords them greater individual fertilisation success during spawning (averages 9-70% of the eggs) than mature parr (averages 3-14%; Fleming and Reynolds, 2004). However, mature male parr, as a group, can be responsible for fertilising a considerable proportion of the eggs within populations (11-65%; Fleming and Reynolds, 2004). As a result, they can increase the genetically effective size of the population and partly compensate for low returns of anadromous fish (males). The younger age at maturity compared to anadromous males also means that mature parr shorten generation times. However, they can also be vehicles for promoting genetic introgression (e.g. by escaped farm salmon; Garant et al., 2003), because they breed prior to migration to sea, where mortality can be high and selective.

Hybrids of Atlantic salmon and brown trout are found in all the regions where Atlantic salmon and brown trout are sympatric. The main factors contributing to the inter-specific hybridisation are thought to include sneaking by mature male parr, natural breeding by escaped and released cultured salmon, unstable river discharges, and overfishing. Experimental crosses suggest that the survival of F_1 hybrids can vary widely from little or no viability to full viability. The usual consequence of post-F_1 hybridisation is the wastage of gametes.

Centres of origin and geographical distribution

Centres of origin

There is conclusive palaeontological evidence that the existing salmonid species appeared in the late Tertiary period, in the Pliocene, and became widespread in the Pleistocene, i.e. several million years ago. Most studies agree that salmonids originated in fresh water, but the evidence is sparse. There is some morphological and genetic data suggesting that Atlantic salmon evolved from brown trout (Dorofeeva, 1998), which would imply that the species appeared first in Europe where brown trout is widespread. Modern populations of the British Isles have the highest mitochondrial DNA (mtDNA) haplotype diversity (Verspoor et al., 1999), with the Kola peninsula being a secondary centre of genetic diversity. The latter is an area of post-glacial mixing of Atlantic salmon from three refuges: "British", "Baltic" and "North American" (Makhrov et al., 2005).

Geographical distribution of native populations

The native range of Atlantic salmon extends along both sides of the North Atlantic Ocean (Figure 3.3). In North America, it occurs from the Connecticut River in the south (a re-introduced population, completely dependent on artificial supplementation) to Sango Brook (55°53'N) in Labrador and into Ungava Bay (58°N). Historically, Atlantic salmon were likely present as far south as the Hudson River, but have since been extirpated from watersheds south of Maine. Similarly, Atlantic salmon once extended as

far west as Lake Ontario, but were extirpated in the late 1800s, and are now confined to areas east from the Jacques Cartier River (71°45'W) near Quebec City. The Atlantic salmon distribution extends eastwards to Greenland (one population), Iceland, the British Isles and continental Europe. In Europe, Atlantic salmon occur from the Kara River (Kara Sea, Russian Federation) to the Douro River (northern Portugal) and into the Baltic Sea basin. The northern distribution limit in Europe extends to just above 70°N.

Figure 3.3. **Geographic marine distribution of the Atlantic salmon in the North Atlantic Ocean**

Source: Courtesy of Eva Thorstad and Kari Sivertsen.

Naturalised populations

Introduced and free-living (i.e. self-sustaining) anadromous populations of Atlantic salmon are known only within their broad native range (i.e. North Atlantic). Most such introductions have involved the removal of migratory barriers (e.g. impassable waterfalls) that restricted access to watersheds or river sections within watersheds. However, in the 1950s Atlantic salmon were introduced to the Faro Islands and have since become established in five rivers. Attempts to introduce Atlantic salmon to west Greenland rivers failed because of the low water temperatures. Greenland has only one river system in the south with an indigenous Atlantic salmon population.

Introduction efforts outside the natural distribution area

During the early 1900s attempts were made to introduce Atlantic salmon to some British Columbia (Canadian Pacific coast) watersheds in a deliberate attempt to establish runs for sport fishing. Nearly 200 introductions were made into 52 different water bodies and a total of 13.9 million eggs, alevins, fry or smolts were introduced. None of these

introductions was successful in terms of establishing runs of Atlantic salmon on the British Columbia coast. In the United States there have been at least 170 attempts in 34 different states where Atlantic salmon were not native, including Washington, Oregon and California (MacCrimmon and Gots, 1979). None of these efforts was successful. For example, in Washington state, attempts were made from 1904 to 1991 by US agencies to introduce and establish Atlantic salmon and not a single self-sustaining population was established.

Similar results have occurred with Atlantic salmon introductions in Australia, Chile, New Zealand, South Africa and many other countries. There has never been a documented successful introduction (i.e. resulting in a self-sustainable population) of sea run Atlantic salmon outside of their natural territory where other native salmon species were present. There is a successful introduction reported from New Zealand, although the Atlantic salmon releases resulted only in non-migratory populations (Waiau system). However, incipient feral Atlantic salmon populations (i.e. presence of juveniles from natural spawnings) have been reported from rivers in British Columbia, South America and the sub-Antarctic Kerguelen Islands (MacCrimmon and Gots, 1979; Ayllon et al., 2004).

Habitats, migration and ecological niche

The physical habitat requirements of the Atlantic salmon vary from life stage to life stage. Three major stages can be identified: 1) spawning and incubation; 2) juveniles in fresh water; and 3) post-smolts and pre-adults at sea.

Spawning habitat

Habitat requirements for spawning and incubation can vary among regions and populations. The major requirement for adult salmon is an accessible spawning area, which is of adequate size for digging nests and provides a safe location for these large fish. Shelter nearby is also important for salmon as they wait to spawn (e.g. undercut banks, overhanging and submerged vegetation, submerged objects like logs and rocks, floating debris, deep water, turbulence and turbidity; Bjorn and Reiser, 1991). The gradient of spawning rivers usually is 3% or less. The preferred spawning site is a transitional area between pool and riffle where the flow is accelerating and the depth decreasing, and where gravel of a certain coarseness is present (Petersen, 1978; Bjorn and Reiser, 1991). In such a location, downwelling water fluxes through the gravel are typical, providing a certain level of dissolved oxygen in the immediate vicinity of the eggs. However, wide ranges of water flow and depths are reported. In the Russian river Varzuga, Atlantic salmon spawn at depths of 2 m. Minimum depth seems to be 10-15 cm (depending on the size of the spawning fish). Areas with upwelling groundwater may also be selected as spawning sites. Spawning by Atlantic salmon in lakes is rare, but has been documented in the non-anadromous ouananiche (Cowan and Baggs, 1988).

The particle size distribution of the sediments at the spawning sites is normally dominated by gravel in the 32-128 mm range but varies within and between rivers, dependent on local- and catchment-scale characteristics (Petersen, 1978; Greig, Sear and Carling, 2005; Miller, Burnett and Benda, 2008). During the incubation of ova and the emergence of fry, the intergravel physio-chemical environment is critical, and adequate flow of water through the gravel is especially important. The proportion of fine sediment/sand in the gravel must, therefore, be low, i.e. <10-20% by weight (Petersen, 1978; Chapman, 1988; Crisp and Carling, 1989) to facilitate oxygen availability (Greig, Sear and Carling, 2007). Petersen (1978) found that if the content of sand (i.e. grain size less than 2 mm) exceeded 20% by weight, the permeability was reduced to zero. Other authors state that productive, good quality spawning gravel contains less than 5% fines (grain size less than 0.8 mm)

while unproductive gravel sites are characterised by more than 30% fines (reviewed in Fleming [1996]).

Incubation, hatching and absorption of the yolk sac takes place some 10-30 cm deep in the gravel (De Vries, 1997). Under normal conditions, mortality at this stage is low (<20%) but there is a risk of additional mortality through scour and dewatering. When absorption of the yolk sac is almost complete, the fry emerges from the gravel bed to start feeding. Mortality rates are very high (68-88% in the first 17-28 days; Einum and Fleming, 2000b) due to displacement, starvation and predation. Emergence from the gravel and first-feeding are thus periods of intense selection.

Juvenile freshwater habitat

For the interpretation of spatio-temporal distribution patterns of juvenile Atlantic salmon within fresh water, it is necessary to distinguish between habitat preference, which is based on the habitat requirements of the individual (looking for its optimal micro-habitat), and habitat utilisation, which is a compromise (trade-off) between the innate requirements and how these can be met by availability within the habitat.

Freshwater habitat use includes fluvial, lacustrine and estuarial environments. Often individual fish will utilise several habitat types during thier freshwater residency. For example, parr may use small tributaries to spawning rivers as feeding areas during their first summer of life and as they get older move to the mainstem river or even into small lakes. The highest population densities are frequently associated with rivers that have moderate temperatures and flows. Such rivers contain riffle, run and pool sections in lower stream orders (i.e. tributaries and smaller rivers) and are dominated by moderate size "cobble" stones. Parr are highly territorial and territory size depends on food abundance, substrate coarseness (instream cover, visual isolation) and social status. Heggenes (1990) considered water depth, water velocity and streambed sub-stratum cover to be the principal physical variables for juvenile salmon *in situ*. Most relevant studies refer to one or more of these variables in discussions of habitat characteristics. Connectivity between a variety of habitats will also be important for providing alternative shelter/feeding opportunities seasonally and for providing a conduit to pass from one habitat to another (e.g. rearing stream to estuary).

Atlantic salmon inhabits cool temperature streams and can tolerate freshwater temperatures ranging from 0°C to 28°C. Under laboratory conditions (given food in excess), summer acclimatised juvenile salmon generally show positive growth from 5-7°C to 24-26°C and grow fastest at 16-20°C. The thermal range for growth declines with reduced food consumption, whereas the temperature for maximum growth appears not to change. Winter acclimatised salmon can under laboratory conditions, however, obtain positive growth in temperatures at least as low as 1°C (Forseth, Letcher and Johansen, 2011). Feeding and growth rates are highest in spring and early summer. Feeding rates decrease with falling temperatures in autumn, but juveniles also feed during winter (Johansen et al., 2011). At high temperatures, juveniles may cease feeding and seek refuge from thermal stress. Temperature, food availability, river discharge, season and density are the factors correlated most strongly with growth of juvenile salmon in fresh water. Growth is also state-dependent, with growth being accelerated or depressed according to physiological needs or life-history stages (Forseth, Letcher and Johansen, 2011).

Atlantic salmon have a minimum pH tolerance level between 5.0-5.4 depending on other river variables (e.g. aluminium levels); pH tolerance may be population-specific (Donaghy and Verspoor, 1997).

Fry and parr densities vary considerably in natural streams. The availability of suitable habitat is often considered the limiting factor. Mean salmon densities (m^2) for a number of river systems in Great Britain and Ireland have been reported to range from 0.036 to 2.06 for young-of-the-year and 0.027 to 0.334 for one-year-old parr (Kennedy, 1988). The highest reported density of fry was more than 30 per m^2, whereas the corresponding density of parr after the first summer can be 4-5 per m^2 (Veselov and Kalyuzhin, 2001). These values come from salmon habitat of high quality. Conversely, poor habitats support fewer fish.

Fry and underyearling parr have been found to occupy locations other than those occupied by older and larger parr. For some areas, significant differences between summer and winter microhabitats have been reported (Cunjak, 1988). Juvenile salmon have been observed in water flow velocities from 0 cm/s to 80 cm/s, with the highest densities in areas of 10-75 cm/s velocity. Pebbly riffles without boulders are considered to be prime nursery habitat for salmon less than 7 cm long (reviewed by Gibson [1993]).

The proportion of 0+ to 1+ age-group parr decreases as depth increases between 20 cm and 40 cm; yearling or older parr are rarely observed in riffles of less than 20 cm depth and without boulders (particle size >256 mm) (Heggenes, 1990; Gibson, 1993; Schneider, 1998).

Experiments indicate that as parr grow there is an increasing preference for deeper and swifter parts of riffles. At 8-9 cm in length, 80-90% of underyearlings prefer cobble/boulder habitats (particle size >6.4 cm) of more than 30 cm depth. In general, juvenile salmon occupy shallow, fast-flowing water with a moderately coarse substrate combined with overhead cover provided by surface turbulence. In summer, fry occupy shallower and faster flowing sections of rivers with slightly smaller sized gravel than that selected by parr (reviewed by Heggenes [1990]; Gibson, 1993).

Most studies on the microhabitats of juvenile salmon describe the distribution and location of the fish during the summer months. However, the habitat utilisation changes when the water temperature falls in the autumn. In Scottish rivers, juvenile salmon tend to leave the shallow riffle habitats during the autumn and move to deeper water in pools, reappearing in the shallow water when the temperature rises to 6-7°C in spring (Mills, 1989). Generally, salmonids prefer shelter and low water velocities during winter and movement out of summer habitats may not occur in autumn if summer habitats provide appropriate overwintering conditions (reviewed by Huusko et al. [2007]).

Summary

- Underyearling parr (<7 cm total length) are most common in shallow (<15 cm) pebbly riffles with broken water surface.
- Larger parr prefer riffles deeper than 20 cm with coarse substrate and some will migrate to lacustrine habitats (a niche shift commonly observed in some regions, e.g. Newfoundland).
- Depth preference and preference for coarse substrate increase with body size.

- Below temperatures of 6-7°C small parr shelter among coarse substrate or move to pools.

(review by Gibson [1993]).

Marine habitat

The transition from freshwater to marine environments for Atlantic salmon can be a critical period affecting survival. It is generally believed that water temperature is the main proximate variable controlling the onset of smoltification (i.e. process of preparing for the transition from fresh water to salt water), though photoperiod is also considered important. While the time spent in the estuary or inshore areas near the natal river is thought to be brief (hours to a few days), it can be critical for post-smolt survival.

In Europe, the fish appear to leave their natal rivers and head northwards with the shelf edge current towards the Norwegian Sea, where they appear to be distributed over large areas. Evidence suggests that a relatively large proportion of the European MSW salmon move into the west Atlantic. Grilse spend the winter mostly in the Norwegian Sea east of Iceland. Populations from northern Europe may move as far north as Spitsbergen and far eastwards into the Barents Sea.

Baltic populations are restricted to the Baltic Sea, where they live in brackish waters and an environment very different from the oceanic conditions of the North Atlantic.

Western Atlantic populations tend to stay in the western Atlantic. In late summer and autumn, non-maturing salmon are found inshore along the north-east Newfoundland and Labrador coasts, at West Greenland, in the Labrador Sea and in the Irminger Sea including the east Greenland coast. Most salmon destined to be MSW fish range over much of the north-west Atlantic, while those 1SW (grilse) salmon do not, staying closer to home. In Greenland, for instance, only salmon that would mature as 2SW and older are caught.

The distribution of Atlantic salmon in the sea appears to reflect environmental factors such as surface temperature and currents, and food availability. The marine environment can have a strong influence on survival and thus recruitment to, and the dynamics of, Atlantic salmon populations.

Migration

Smolt migration

Salmon are flexible and variable in their migration patterns – temperature and season (spawning time) seem to be the governing factors.

After the onset of smoltification when young salmon start their seaward migration, their displacement in the rivers is largely nocturnal at low water temperatures and affected by factors influencing water currents. At higher temperatures and at high latitudes with 24 hours of daylight, smolts may migrate at all times of the day (Davidsen et al., 2005; Ibbotson et al., 2006). The downstream migration was previously believed to result from passive transportation by the currents, but several studies have now documented that active migration also occurs, with smolts swimming faster than the currents. Progression rates of smolts in fresh water may vary considerably with reported speeds of 0.2-60 km/day (Thorstad et al., 2012). The seaward migration often starts in cool temperatures in spring, but the temperature varies among populations and also among years in the same river (Jonsson and Jonsson, 2011). Depending on the geographical factors (temperature,

day-length, discharge, feeding opportunity), smolt migration can take place between March (southern range) and August (northern range). Timing of seaward migration appears adapted to favourable temperature and feeding conditions at sea (Hvidsten, Heggberget and Jensen, 1998) and smolts from northern rivers generally migrate later than smolts from southern ones.

Post-smolt migration

In contrast to the relative uniformity of the riverine environment, the post-smolts encounter a complexity of environmental conditions in the estuaries, fjords and coastal waters, where the tides and/or winds influence the speed and directions of the surface currents, as well as the distribution of different water layers and any fronts that may evolve between these waters. The Atlantic salmon post-smolt migration is an active process with an overall seaward vector, but the migration pattern shows great individual variability, with some post-smolts taking a direct route towards the sea whereas others show more irregular movement patterns. Progression rates (how fast the post-smolt travels between two points on its route) vary among sites, years and groups of fish studied (Thorstad et al., 2012). Progression rates of wild Atlantic salmon post-smolt in coastal areas range from less than 2 km/day up to more than 30 km/day. True swimming speeds are usually higher as post-smolts do not always take the shortest possible route. Progression rates may also depend on the movements of the water currents. In Norwegian fjords (which are up to 200 kilometres long) most fish may spend from less than one week and up to four weeks before they enter the open ocean (Thorstad et al., 2012), whereas the residence period in the 230-kilometre long Bay of Fundy in Canada may be more than a month (Lacroix, 2008). Migration of post-smolt Atlantic salmon in coastal waters occurs during both day and night. Post-smolts usually swim close to the surface during the early marine migration (0-3 m depth), but make irregular dives down to about 6.5 m depth. They have been shown to swim closer to the surface at night than during the day (Thorstad et al., 2012).

Post-smolts have the capacity to travel rapidly over long distances. Ocean recaptures of post-smolts that were individually tagged leaving their rivers as smolts show minimum progression rates of 6-26 km/day (Shelton et al., 1997; Holm et al., 2003).

Europe: The observed distribution of post-smolts considered in relation to the prevailing hydrographic regime suggests a close correlation between strong northerly or north-easterly surface currents, temperature, salinity and post-smolt migrations in the north-east Atlantic (reviewed by Holm et al. [2003] and Hansen et al. [2003]). Also, tidal streams are used. The general patterns indicate that the use of currents enables the post-smolts to reach their northern feeding grounds with the least expenditure of energy. These currents may act as a "food-stream" as well, with a high concentration of potential prey (sand eels and invertebrates in coastal areas; herring, blue whiting, amphipods and other pelagic species in oceanic areas), which post-smolts feed on opportunistically. European Atlantic-going Atlantic salmon migrate north along the Norwegian coast. Atlantic salmon of Iberian, French and German origin have been recaptured in Irish coastal waters. There is evidence that post-smolts from southern Europe (Iberian peninsula, Denmark, France, Germany and the British Isles) use a migration route along the Faroe-Shetland Channel and western sector of the Norwegian Sea. A larger proportion of post-smolts from northern Europe (principally Norway) migrate through the eastern sector of the Norwegian Sea. Far north populations (Norway, Russian Federation) migrate westwards through the Barents Sea, or may use the Barents Sea as a rearing area.

Baltic Sea: Baltic populations are restricted to the Baltic Sea and rarely migrate into the Atlantic ocean. The main feeding areas of Baltic salmon are the Baltic Main Basin and the Gulf of Finland in the south and the Bothnian Sea in the north.

The United States and Canada: Post-smolts in the north-west Atlantic Ocean tend to move up into the Labrador Sea during their first year at sea for feeding. An exception is thought to be salmon of the Inner Bay of Fundy, which may remain within the bay and surrounding area. Because many post-smolt salmon are found in the Labrador Sea within four months of leaving their home rivers, this area is thought to be an important nursery habitat for salmon during their early marine life. Water temperatures during this period have been shown to influence post-smolt survival and growth, through effects on the salmon themselves and on the ecosystem they inhabit. Salmon in this region are found most abundantly in regions where sea surface temperatures range from 4°C to 10°C. They also tend to inhabit mostly the upper part of the water column, but do make deep dives probably in search of prey.

Spawning migration

The salmon's homing ability is the basis for the classification of the populations. Over the generations these populations have developed different inherited characteristics and have thus become adapted to their watercourse through natural selection.

- The time of entry of the main runs of salmon varies from river to river and runs peak at different times in different rivers.
- The spawning migration peak may correlate with mean monthly sea and river temperatures during spring: salmon arrive earlier when temperatures are higher and later when temperatures are lower (Dahl et al., 2004).
- MSW fish often enter rivers in spring. Grilse (1SW) runs are often recorded in summer and autumn.
- Some fish enter rivers up to 13 months before spawning (reasons unclear).
- Particularly in large river systems (e.g. Connecticut, Loire, Rhine), salmon enter all year round, but all-year return patterns are reported from many small rivers as well.
- In Arctic regions (Canada, Russian Federation) constraints to movement of salmon are imposed by sea and river temperatures (=> peak run in late summer).
- Few fish enter rivers for overwintering, without spawning (Berg, 1964).

The upriver spawning migration of wild Atlantic salmon takes place in three phases: 1) a migratory phase consisting of direct or step-wise movement to or close to the position that will be held at spawning; 2) a (short) search phase with repeated movements both upstream and downstream at or close to the position held at spawning; and 3) a holding phase with little or no movement until the spawning. After spawning the fish move down into pools of the river, where they hold before exiting the river that fall or more often, the following spring.

Farmed salmon escapees are "homeless" and usually stray to rivers nearby. In the eastern Atlantic, escaped smolts are usually transported north by marine currents, so straying normally occurs north of the escape sites.

Ecological niche (limiting environmental conditions)

Phenotypic plasticity bespeaks the great ability of this species to adapt to variable conditions and rigorous environments that are characteristic of northern latitudes. The life history of a local Atlantic salmon population can vary dependent upon water temperatures, photoperiod length, stream productivity, ocean productivity and a host of other environmental factors. One genotype may display a variety of phenotypic life histories, depending on environmental conditions (reviewed in Hutchings [2011]). Additionally, there is evidence that Atlantic salmon populations have evolved local and regional adaptations that are genetically based, due to the relative breeding isolation of populations returning to home streams or even stream segments for mating (reviewed in King et al. [2007]).

Atlantic salmon may be exposed to widely differing environmental conditions across the species range. At some point, one or more physical, chemical or biological factors likely become limiting and adversely affect a critical fitness trait such as survival, growth or reproduction. As discussed earlier, optimal and limiting environmental conditions may differ considerably with lifestage and/or life history phase, geographic location and habitat (e.g. such as whether the immediate environment is a freshwater river or the open ocean), and season.

Potentially limiting environmental conditions may be of a physical, chemical or biological nature. Examples of these include:

- physical: water temperature, turbidity, substrate type, flow
- chemical: water chemistry (e.g. pH, dissolved oxygen, salinity) and contaminants
- biological: food availability, competition, predators, pathogens.

Water temperature is perhaps the most important single factor controlling the overall natural distribution of Atlantic salmon and affecting this species' life history either directly or indirectly. Water temperature affects embryo development, fish growth and survival directly, but may also influence migratory behaviour (e.g. emigration of smolts), habitat utilisation and other aspects of life history which may indirectly affect growth, reproduction and survival. For example, water temperature may indirectly affect salmon growth and survival by influencing the distribution of plankton assemblages and the prey associated with them, which in turn influences food availability for the salmon.

In Atlantic salmon, like other salmonid fishes, the efficiency of the conversion of yolk to body tissue is temperature dependent (Heming, 1982; Petersen and Martin-Robichaud, 1995) and declines noticeably at temperatures of 12°C and above (Gunnes, 1979; Beacham and Murray, 1990). The optimum temperature for Atlantic salmon embryo development is near 6°C (Petersen, Spinney and Sreedharan, 1977) and the upper thermal limit near 16°C (Ojanguren, Reyes-Gavilán and Munõz, 1999). As noted previously, Atlantic salmon can tolerate temperature extremes from 0°C to 28°C, but depending on the lifestage, the optimal temperature can be much narrower. Upper lethal temperatures in Atlantic salmon may vary by as much as 3°C among individuals (Elliott, 1991) and the upper temperature limit for feeding in fresh water by juveniles is 22.5°C ± 0.3°C (Elliott, 2006). Maximum growth occurs at 16-20°C (Elliott, 1991, 2006; Forseth, Letcher and Johansen, 2011). Low flow conditions caused by summer droughts in combination with high water temperatures may be particularly limiting. In northern regions, low temperature may be the limiting environmental factor, with a cessation of growth normally below 4-7°C in juveniles (Jonsson et al., 2001; Elliott, 2006). In the marine environment, post-smolts seem to prefer a range of 9-11°C (Todd et al., 2011) and can achieve high growth rates at 10-18°C (Handeland, Imsland and Stefansson, 2008). There is widespread evidence that

the marine distribution of Atlantic salmon is dependent on temperature (Reddin and Shearer, 1987) and that marine mortality is temperature-related (Hansen et al., 2003); however, as pointed out by Potter and Crozier (2000), none of the studies to date has demonstrated a clear causal relationship.

The concentration of dissolved oxygen in water is inversely related to temperature, and as such is an additional stress that may be associated with high temperature conditions. The incipient oxygen level where juvenile Atlantic salmon begin to show stress affecting swimming ability is 4.5 mg/L O_2 (Davis, 1975). It is suggested that oxygen concentrations not fall below a single-day mean of 8 mg/L for spawning fish, while levels of 5.0-6.5 mg/L are acceptable for adult fish when not spawning (Binkley and Brown, 1993). For embryos, critical levels to meet O_2 demands depend on temperature and life stage, ranging from *ca.* 0.8 to 7.0 mg/L, with higher demands during the later stages of embryo development just prior to hatching (Davis, 1975). Survival during embryogenesis and during the hatching period appear to be limited primarily by oxygen supply and secondarily by water exchange, both having highly significant effects (Hamor and Garside, 1976). Availability of oxygen for embryos is tied directly to water flow through the incubation gravel and as such is affected by the presence of fines in the gravel (Petersen, 1978; reviewed in Fleming [1996]).

Embryos and alevins are highly sensitive to acidification and are affected detrimentally by pH lower than 5.5 and cannot tolerate a pH of much less than 4.5 (Petersen, Daye and Metcalfe, 1980; Lacroix, 1985). Increased acidity increases the mobility of toxic metals, particularly aluminium and as such is affected by local geology. Older freshwater life stages are also quite susceptible. For instance, low pH during the smoltification process can have subsequent detrimental effects resulting in mortality during the ocean migration (Magee et al., 2003; Rosseland and Kroglund, 2011). Little is known about the effects of pH in marine waters (7.9-8.3 in open ocean surface waters), which are typically much higher than those in fresh water, though pH has been decreasing (ocean acidification) in recent decades.

Salinity tolerance in Atlantic salmon is size dependent and the capability of tolerating full strength marine waters does not occur until after smoltification (physiological preparation) from *ca.* 10 cm in body length. Earlier life stages, however, can tolerate brackish waters (Cunjak, 1992).

Salmon are susceptible to deteriorating water quality as a result of both direct point-source discharges and diffuse or non-point-source pollution such as heavy metals and organic chemicals arising from land-use practices or industrialisation (reviewed by Hendry and Cragg-Hine [2003]).

Salmon can be affected by prey availability at all life stages where they feed exogenously, and prey availability will be affected by environmental conditions, such as temperature, water chemistry and photoperiod, and both intra- and interspecific competition for such resources.

Similarly, predators (other fishes, birds and mammals; reviewed in Ward and Hvidsten [2011]), parasites (e.g. sea lice and *Gyrodactulus salaris*; reviewed in Finstad et al. [2011]; Harris, Bachmann and Bakke, 2011) and pathogens (bacterial and viral; reviewed in Harris, Bachmann and Bakke [2011]) have considerable potential to affect Atlantic salmon populations. Their effects will be modulated by environmental conditions, both directly and indirectly, through any associated stress the fish may be under.

When this document was initially conceived it was assumed that the ecological niche of locally adapted wild Atlantic salmon could be defined, at least in broad terms. The genetic basis for the phenotypic traits of locally adapted wild Atlantic salmon is elucidated through the genome projects (see the section on “Genetics of Atlantic salmon”), and this

body of data and information may provide a basis for comparing wild Atlantic salmon to genetically engineered lines of Atlantic salmon.

Despite the extensive current and growing body of knowledge on Atlantic salmon, there is still insufficient information to adequately describe the critical or limiting environmental conditions controlling the survival and distribution of this species. In addition, the underlying genetics that allow for phenotypic adaptations to those limiting environmental conditions has not been adequately characterised.

Clear correlations of adaptive phenotypes with specific genes do not yet exist and may be available only partially in the future, due to the complications of the genetic heterozygosity and resultant phenotypic plasticity present and essential in wild populations of this species (see the section on "Genetics of Atlantic salmon"). See Devlin, Sundström and Muir (2006) for a discussion of environmental risk assessment of transgenic fish with a recognition of these complications. It is extremely difficult to make a convincing case that specific genes are "for" a given, relatively well-defined, trait (Kaplan and Pigliucci, 2001). In addition, determining the genetic underpinnings of many traits may be difficult, if not impossible, because some of the variation among individuals, populations and species is traceable to a certain number of regulatory elements (generically defined as any gene producing a product whose function is to turn on or off the action of other genes), or to the regulatory regions upstream of genes known to play important roles in development (Pigliucci, 2003).

Population dynamics

Populations of Atlantic salmon vary in size over time, and year-to-year variation in environmental conditions is likely to be causing variation in survival rates both in fresh water and at sea (Hutchings and Jones, 1998; Einum and Nislow, 2011). Causes for temporal and spatial variation in population abundance are commonly divided into two categories: density-dependent and density-independent processes. In the most common form of density-dependence, population growth rates will decrease with increasing population density of Atlantic salmon, and such compensatory mechanisms exert a strong regulatory effect on populations (Einum and Nislow, 2011). Competition among individuals for limited resources such as food or space (e.g. for access to feeding territories or shelters) increases with fish density. Such competition can influence fish survival either directly (by increasing fish mortality) or indirectly due to density effects on growth rates and thus fish size-at-age. However, several environmental factors may also influence population growth rates directly through density-independent mortality. Such factors have constant *per capita* effects, and operate independent of the population density. For example, large-scale climate oscillations in the marine environment appear to have such strong but density-independent effects on adult Atlantic salmon stock size (Todd et al., 2011).

There is considerable evidence for density dependence in the freshwater life stages of Atlantic salmon (Milner et al., 2003), which implies that there is an upper limit to the number of smolts produced in a given river system (Einum and Nislow, 2011; Hindar et al., 2011). Results from a number of recent studies support the idea that competition for food and space among similar aged fish, especially age-0 fish, is an important mechanism underlying population dynamics and population regulation in Atlantic salmon (Nislow, Armstrong and Grant, 2011). Studies from Canada indicate that the timing of population regulation varies among populations (Gibson, 2006). Density-dependent effects appear to be manifested rapidly in single age-classes in some populations, but to extend over multiple age-classes in other populations. The reasons for these differences among populations is poorly understood.

Density dependence in Atlantic salmon populations in the marine environment is relatively unstudied, but thought not to be strong, if it exists at all. Density-dependent mortality at sea is not likely because the population density is assumed to be far below the assumed carrying capacity for Atlantic salmon in that habitat (Jonsson and Jonsson, 2004), an assumption that is supported by empirical evidence from some populations (e.g. Jonsson, Jonsson and Hansen, 1998). Other density-dependent effects are, however, possible, such as density-dependent predation on migrating smolts in estuaries or adults prior to upstream migration for spawning.

Variability in freshwater survival may appear to be less than that in marine survival because of a compensatory process in fresh water that can potentially buffer some of the variability (Milner et al., 2003). That is, decreased survival at certain freshwater life stages can result in increased survival at others due to density-dependent processes. Compensatory survival in fresh water results from competition for limited resources, including food and space. Thus, populations are regulated more strongly by density-dependent processes in fresh water than in marine environments and variability in marine survival (due to density-independent factors) appears to be more important for determining overall population size.

Egg-to-smolt survival rates in Atlantic salmon have been observed to range from as low as 0.1% to as high as 6.5% (Klemetsen et al., 2003). Estimates of survival during the marine phase have often been more difficult to obtain because adults are enumerated back to the river and have been exposed to both natural and fishing mortality factors. There are a limited number of stocks for which the return rates of smolts to adults have been measured (Chaput, 2012). In a few instances, the return rates can be inferred to represent survival rates at sea, because the adults are almost entirely 1SW maturing salmon. In all other cases, where there are two or more ages at maturity, the return rates of smolts to 1SW are the product of the proportion of the smolts destined to mature as 1SW salmon and the first year survival at sea. In the North Atlantic, return rates of 1SW salmon are generally higher than those of 2SW salmon (Chaput, 2012). The highest measured return rates of 1SW salmon in predominantly 1SW stocks are generally in the range of 6-12%, whereas in MSW salmon stocks return rates of 1SW salmon are in the range of 1-6% and for 2SW salmon in the range of 1-3%. The return rates of European stocks are generally higher than for North American stocks, with return rates to the coast for smolts from the River Bush (1SW stock) being as high as 35% (Crozier and Kennedy, 1994) and return rates to the coast for 1SW fish from other stocks generally being >10%. There is evidence from hatchery smolts that body size is an important determinant of survival, but its influence for wild smolts has been poorly studied and patterns appear equivocal, with evidence for a role in some populations but not others (Friedland et al., 2009; reviewed in Todd et al. [2011]).

Population status and trends

Status of populations (by country)

The status of Atlantic salmon worldwide was assessed by Parrish et al. (1998) and by the World Wildlife Fund (WWF) (2001). Parrish et al. (1998) reviewed available information on the status of wild anadromous salmon based on numbers of adults returning to rivers to obtain patterns of salmon status across broad geographical areas. Generally, stable populations (no consistent decline in returns) were found in northern areas of the distribution range, whereas more southerly populations showed declining trends or were extirpated (no returns for at least ten years). The WWF (2001) collated information on 2 600 rivers from national representatives in all countries holding self-reproducing populations of wild

salmon. Information was considered sufficient for a rough classification in 2 005 rivers. Atlantic salmon populations are considered extinct from 309 rivers worldwide (15%), and from the following countries: Belgium, the Czech Republic, Germany, the Netherlands, the Slovak Republic and Switzerland. They are considered endangered in Estonia, Poland, Portugal and the United States. On the other hand, Atlantic salmon populations are considered healthy in 867 rivers (43%), most of which are located in Iceland, Ireland, Norway and Scotland (WWF, 2001). The WWF classification may, however, provide misleading information at smaller scales (Hindar et al., 2011) as the proportion of rivers with unknown status in this survey was rather large in Canada and the Russian Federation.

The North Atlantic Salmon Conservation Organization (NASCO) has defined the conservation limit in Atlantic salmon fishery management as the spawning stock level below which recruitment starts to decline significantly (NASCO, 1998, see Hindar et al. [2011]). The precautionary approach then dictates that the populations should be maintained above the conservation limit by use of a management or spawning target, that is the spawning stock level that ensures population viability. Such conservation limits are regularly applied in assessing status of Atlantic salmon by the International Council for the Exploration of the Sea (ICES) and national authorities. The assessments differ in detail from assessments of whole stock-complexes down to assesments for stocks in individual rivers, and are mainly used as a basis for catch advice for mixed-stock marine fisheries and catches in individual rivers.

The ICES performs yearly assessments of several stock-complexes in the north-east Atlantic Ocean that form the basis for catch advice for mixed-stock marine fisheries (ICES, 2012a). For each stock-complex, assessments are made for both 1SW and MSW salmon. In the latest assessment, the number of spawners of 1SW and MSW salmon from the northern north-east Atlantic stock-complex (populations from Finland, north and east Iceland, Norway, the west coast of Sweden and the Russian Federation,) are considered to be at full reproductive capacity and so is MSW salmon from the southern north-east Atlantic stock-complex (populations from France, Ireland, south and west Iceland, and the United Kingdom), while 1SW salmon from the southern north-east Atlantic stock-complex is considered to be at risk of suffering reduced reproductive capacity. Assessment at the stock-complex level can, however, mask the regional and river-specific situations of Atlantic salmon populations (Chaput, 2012). In some parts of the North Atlantic, the abundance of Atlantic salmon has declined by much greater amounts than suggested by stock-complex assessments, and the abundance of spawners is much lower than interpreted by such (Chaput, 2012). This poses particular threats to stocks that are at low abundance and subject to other threats unrelated to fishing, such as freshwater habitat degradation.

The ICES also provides updated status for salmon stocks at the national level and/or compliance with river-specific conservation limits for individual river stocks for the countries where such limits are established. In 2011, Iceland, Norway (for 2010), the Russian Federation and the United Kingdom (Northern Ireland and Scotland) met national conservation limits (CLs) for both 1SW and MSW salmon (ICES, 2012a). Ireland and the United Kingdom (England and Wales) were below national CLs for MSW and 1SW salmon, respectively, whereas France, Finland/Norway (the large River Teno/Tana) and Sweden did not meet such national CLs for either 1SW or MSW salmon.

Assessment for individual rivers in the north-east Atlantic showed that salmon in seven out of eight (88%) rivers in the Russian Federation met their river-specific CL in 2011. The figures for other countries were: 162 of 211 (77%) rivers in Norway (for 2010), 11 of 28 (39%) in France, 58 of 141 (41%) in Ireland, 2 of 7 (29%) in the United Kingdom (Northern Ireland) and 41 of 64 (64%) in the United Kingdom (England and Wales).

In North America, the ICES assesses the status of populations in six regions (Labrador, Newfoundland, Quebec, Gulf of St. Lawrence, Scotia-Fundy and the United States) and within each region individual river stocks are also assessed. The latest assessment showed that 2SW salmon spawner estimates were above their conservation limits in Newfoundland and the Gulf of St. Lawrence, marginally below in Quebec, and below the CL for the other three regions, as well as overall for the North American stock-complex (ICES, 2012a). The latest assessment was somewhat higher than prevous years' assessments. To date, 1 082 rivers have been identified in eastern Canada and 21 in the eastern United States, where Atlantic salmon are or were present within the last half century. Assessments were reported for 74 of these rivers in 2011 and 45 of the rivers (61%) exceeded their river-specific CL (estimated egg deposition by all sea ages combined), whereas 15 of the rivers (20%) reached less than 50% of their CLs. Individual river stocks which are failing to meet CLs were found in four of the regions, but particularly in the southern areas (Scotia-Fundy and the United States).

The status of Baltic salmon is assessed by evaluating the probability that individual salmon rivers have reached 50% and 75% of the potential smolt production (ICES, 2012b). In the Gulf of Bothnia and Baltic Main Basin the large, northernmost stocks have likely or very likely reached the 50% objective, but only three rivers have likely reached the 75% objective. Southern stocks and a few small northern stocks have variable and, on the average, much poorer stocks. In the Gulf of Finland, salmon stocks show indication of some recovery, but the status of most stocks is still poor.

In some countries the conservation status of stock-complexes or individual stocks is also characterised with respect to possible future status of the stocks.

The Committee on the Status of Endangerd Wildlife in Canada identifies and assigns conservation status of 16 distinct designatable units (DUs) for Atlantic salmon in Canada (COSEWIC, 2010). A DU represents discrete and evolutionary significant units of the species that are important to its evolutionary legacy as a whole and if lost would likely not be replaced through natural dispersion. Of 15 anadromous DUs, 5 were classified as endangered (facing imminient extirpation or extinction), 1 as threatened (likely to become threatened by extirpation or extinction if no action is taken), 4 as of special concern (may become threatened), 1 as data deficient and 4 as not at risk. The five DUs classified as endangered are located in the southern part of Canada (Inner and Outer Bay of Fundy, Nova Scotia Southern Upland, Eastern Cape Breton and Anticosti Island). In addition, the freshwater living Lake Ontario DU was classified as extinct.

The Norwegian Directorate for Nature Management identifies threats and assesses the status of wild salmon stocks in Norway.[1] The most recent update (from 2012) gives the status for 481 rivers where salmon are or were originally present. The status is based on assessments of how different human impacts affect the production of salmon with respect to the viability of the stock and its capacity to produce a harvestable surplus. In addition, the genetic status is assessed with respect to possible impacts on the viability of the stock from introgression of escaped farmed salmon. Of the 481 stocks, 54 were classified as critically endangered or lost; 44 as threatened, facing exctinction if the impacts continue or increase; 126 stocks were classified as vulnerable, potentially becoming threatened if the impacts continue or increase; 241 stocks as moderately affected with significant reductions in harvestable surplus; 16 stocks had good status; while no stocks were classified to have very good status.

Trends in abundance

Atlantic salmon abundance in the North Atlantic Ocean has declined the latest decades. Estimates by the ICES on the development of salmon abundance in the period 1970-2009 suggest that pre-fishery abundance (defined as number of fish on 1 January of their first winter at sea) was the highest in the early 1970s at some 10 million fish (Chaput, 2012). By the mid-1990s abundance had declined considerably and has, with some variation, remained low since. In the most recent five-year period, total pre-fishery abundance was estimated at about 3.5 million fish. The decline in abundance has generally been larger for MSW salmon than for 1SW salmon. The decline in the abundance of MSW salmon has been larger in the north-west Atlantic and in the southern part of the north-east Atlantic than in the northern part of the north-east Atlantic. In the period 1970-2009, the catches of Atlantic salmon declined considerably, especially in marine commercial fisheries. The reduction in marine exploitation (fishing) is achieved through great reductions in effort or in some cases complete bans. As a result, the estimated number of MSW spawners remained rather unchanged in the north-west Atlantic and in the northern part of the north-east Atlantic, while the number of 1SW spawners increased in these two areas during the same period. The estimated number of spawners decreased over the time period for both 1SW and MSW salmon in the southern part of the north-east Atlantic (Chaput, 2012).

Factors affecting abundance

Three main factors affect the abundance of adult wild Atlantic salmon: smolt production in fresh water, natural mortality in the marine environment, and exploitation in commercial (mostly marine) and recreational fisheries (mostly riverine).

Historically, many of the declines and extirpations of Atlantic salmon can be more or less directly attributed to human activities affecting freshwater production of salmon, such as dams, pollution (including acid rain) and dewatering of streams (Parrish et al., 1998). Today, populations are recovering in parts of the salmon distributional range due to stronger legal measures to control and reduce pollution from industry and sewage systems (*cf.* Mawle and Milner, 2003). For example, reduced acid depositions combined with extensive liming of rivers affected by acid rain have led to re-establishment of several salmon populations in south-western Norway that were extirpated or severely reduced (Hesthagen and Larsen, 2003). However, many salmon stocks worldwide still suffer reduced smolt production due to different human impacts.

The reasons for the more recent decline in the abundance of Atlantic salmon at the global scale are not always as obvious and a mix of interdependent factors is probably involved (Parrish et al., 1998). Over the past 30 years, post-smolt survival has declined in the entire North Atlantic (Chaput, 2012), and the coherence observed in the patterns of declining adult recruitment of salmon over large geographic areas suggests that recent changes in mortality have been dominated by factors operating in the marine environment. The ocean climate of the North Atlantic has undergone marked changes over the period of declining salmon abundance (Beaugrand, 2009; Beaugrand and Reid, 2012). There appears to be a close relationship between the growth, maturation, survival and distribution of salmon at sea and ocean climate as reflected in sea temperature (Friedland, Chaput and MacLean, 2005; Todd et al., 2008). Water temperature and other abiotic environmental factors acting indirectly to cause changes in the production and availability of suitable food items reflecting large-scale ecological changes in the marine ecosystem may be the primary cause of changes in the abundance of salmon, as well as other species

(*cf.* Friedland et al., 2009). It has been suggested that different factors may govern the successful return of Atlantic salmon to rivers in Europe and North America, and that survival of European Atlantic salmon is linked to growth and feeding conditions whereas survival of North American Atlantic salmon may be more linked to predation (Friedland, Chaput and MacLean, 2005; Friedland et al., 2012).

Marine mortality of salmon does not necessarily operate independently of factors acting in fresh water. Over recent decades, biological characteristics of Atlantic salmon smolts have changed in many rivers (Russell et al., 2012). Juvenile salmon have grown faster and migrated to sea at a younger age, so have been smaller typically than they were earlier. Over the same period, smolt run-timing across the geographic range has been earlier, at an average rate of almost three days per decade. How such changes in smolt characteristics and migration timing influence mortality at sea is unknown. Moreover, acidification, contaminants and other factors operating in fresh water may also impact smolt quality with adverse consequences for sea-survival of Atlantic salmon (Rosseland and Kroglund, 2011).

The survival of wild and hatchery-reared Atlantic salmon post-smolts during their first year at sea declined in the Baltic Sea from 25-40% in the late 1980s and early 1990s to 5-15% in the period 2005-10 (ICES, 2011). The open-sea ecosystems in the Baltic have experienced pronounced changes over the past two decades, characterised by shifts in species composition across several trophic levels (Möllmann et al., 2009; Diekmann and Möllmann, 2010). These changes in the ecosystem have affected the abundance of both the prey (herring and sprat) and the potential predators (grey seals) of Atlantic salmon. A recent analysis showed that the declining trend in post-smolt survival could be explained by the increased number of grey seals, whereas the annual variation in survival coincides with variation in the recruitment of Bothnian Sea herring (Mäntyniemi et al., 2012). Hence, both food availability and predation could contribute in regulating post-smolt survival. However, it remains uncertain whether the observed correlations arise from direct causalities or other mechanisms (Mäntyniemi et al., 2012).

Threats to salmon populations

Widespread declines and extirpations of Atlantic salmon populations have occurred in Europe and North America, particularly in southern portions of the range. Many of these declines or extirpations can be attributed to human impacts, such as dams, pollution (including acid rain), dewatering of streams and overfishing. The threats, however, are often multi-factorial involving both human impacts in concert with environmental change (e.g. changing ocean conditions). In an effort to evaluate the possible factors contributing to the decline of salmon, the Department of Fisheries and Oceans Canada convened an expert panel, which in the end identified 63 such factors (Cairns, 2001). The threats were often region-, or even river-specific, though some were broader, such as ocean conditions.

The major threats to wild Atlantic salmon populations include:

- Overfishing in the sea, estuaries and rivers that reduces population sizes to below a critical level.
- Hydropower dams and other man-made river obstructions that form severe obstacles to upstream and downstream migration of salmon, inhibiting access to habitats.
- River engineering schemes (e.g. for flood defence or navigation) result in direct habitat loss (e.g. through channel deepening) and disconnection of the main river from the complex of floodplain habitats (e.g. oxbow lakes, channels and islands).

Habitat degradation also occurs through the resulting changes in ecological processes such as nutrient cycling, sedimentation and flooding.

- Pollution (from industry, urban settlements and agriculture) resulting in acid rain, inputs of excessive nutrients and upstream sediments, heavy metals and other toxic substances, including endocrine disrupters. These pollutants degrade the salmon habitats and some have direct impacts on species mortality and behaviour.
- Erosion/homogenisation of the natural gene pool through interbreeding with salmon aquaculture escapes and thus disruption of local adaptations and evolutionary potential of wild stocks. Diseases and parasites (e.g. sea lice) transferred from caged salmon to wild salmon can represent a further hazard.

Conservation measures

The conservation and restoration of Atlantic salmon is a daunting task because of the complex and dynamic nature of the freshwater and marine ecosystems that the species exploits. It requires the identification of the units of conservation (e.g. evolutionary significant units as in the US Endangered Species Act or DUs in the Canadian Species at Risk Act) and then a well-documented action plan. Such an approach is being employed with the endangered Atlantic salmon of the state of Maine and the inner Bay of Fundy, Canada.

In the European Union (EU), Atlantic salmon is listed under Annex II in the Flora-Fauna-Habitats Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora). The 1992 Habitats Directive aims to protect wildlife species and habitats. Each member state is required to identify sites of European importance and to put in place a special management plan to protect them (in special areas of conservation), combining long-term conservation with economic and social activities, as part of a sustainable development strategy. The EU-Water Framework Directive (2000/60/E) requires that all inland and coastal waters within defined river basin districts reach at least good status by 2015 and defines how this should be achieved through the establishment of environmental objectives and ecological targets for surface waters. Anadromous species like Atlantic salmon presumably will act as indicators and should benefit from the activities in the member states.

NASCO is an international organisation established under the Convention for the Conservation of Salmon in the North Atlantic Ocean, which entered into force on 1 October 1983. All governments throughout the Atlantic salmon's native range are NASCO member countries. NASCO's main objective is to contribute to the conservation, restoration, enhancement and management of Atlantic salmon. Through the NASCO Convention, parties agreed to co-operate in the management of fisheries that exploit Atlantic salmon originating in rivers of other parties. The two principal fisheries that are regulated are the West Greenland fishery and the Faroese fishery. Both fisheries exploit a mix of salmon populations originating from broad geographical areas.

The North Atlantic Salmon Fund, as a non-governmental organisation (NGO), mobilises international support and persuades commercial fishermen to give up fishing for salmon, either permanently or for a period long enough to allow stocks to recover. The organisation has raised nearly USD 30 million to buy out netting rights to reduce excessive commercial exploitation. Another international NGO is the Atlantic Salmon Federation, which is based in North America and promotes the conservation and wise management of wild Atlantic salmon and its environment. The Atlantic Salmon Trust is a UK-based NGO. It

addresses the decline of salmon stocks, as well as the need for practical research into the problems regarding the decline of salmon populations.

Within the natural range of salmon the following management tools contribute to the conservation efforts:

- Habitat improvement and restoration:
  1. re-establishing migration corridors by building fish-passes at obstructions (weirs, sluices, dams), regulation of hydroelectric development or catch and transport of salmon to inaccessible spawning habitats (trap and truck)
  2. rehabilitation of spawning/nursing habitats
  3. reduction of pollution (including effects of acid rain by liming of rivers)
  4. regulation of land and water use.
- In-river management of populations:
  1. living and frozen gene banks and hatchery supplementation programmes, see NASCO[2] for more information about cultivation and stock enhancement
  2. fishing regulations (catch and release, biological reference points for setting allowable removal rates or escapement levels)
  3. legislation
  4. control of poaching.
- Regulating marine exploitation:
  1. fishing regulations, quotas (NASCO)
  2. buy-out of licenses/netting rights (North Atlantic Salmon Fund).
- Salmon farming, estuary management.

In order to reduce the possible impacts of fish farming, in some countries (such as Iceland and Norway) it was decided to protect (some) wild salmon stocks by establishing fish farm exclusion zones in the coastal marine/estuary environment.

Interactions with other organisms

Salmon as prey

Atlantic salmon are vulnerable to predators at every stage of their life cycle (Mather, 1998; Ward and Hvidsten, 2011). The impacts of predation on salmon populations are likely to be particularly severe at older life stages and for populations already suppressed by other factors (Ward and Hvidsten, 2011).

Predation on juvenile salmon and smolts in fresh water include:

- birds: heron, sawbill ducks, cormorant, gulls, belted kingfisher, merganser, goosander
- fish: salmon, several native and introduced trout species, charr, bull head, burbot, chub, eels, pike, pikeperch, perch, grayling, catfish, smallmouth bass, striped bass
- reptiles: water snakes
- mammals: otter, mink.

Predation on post-smolts in estuaries, coastal waters and sea include:

- birds: cormorant, gannets, terns, gulls, murres
- fish: gadoids, sea trout, eels, ling, sharks
- mammals: otter, grey seal, harbour seal, harp seal, harbour porpoise, bottlenose dolphin, beluga whale (Middlemas, Armstrong and Thompson, 2003).

In the Baltic sea, the number and abundance of potential predators on young salmon is low compared to that in the North Atlantic Ocean.

Salmon as predators

Atlantic salmon are generalist predators that feed on available prey. They are gape-limited so that prey-size usually increases as the fish grow in length. Juvenile salmon are able to feed successfully in different habitats ranging from small streams to large lakes (Johansen et al., 2011). They also feed under different light conditions and seasons. In running water, the salmon can feed on invertebrates drifting either on the surface or in the water column, as well as on invertebrates living on the streambed surface. Prey of salmon in fresh water:

- juveniles are opportunistic predators of aquatic invertebrates, especially those difting at the surface or in the water column (e.g. Ephemeroptera, Plecoptera, Trichoptera, Chironomiidae and Coleoptera)
- larger parr are also piscivorous, feeding on smaller trout and salmon juveniles and eggs
- adult Atlantic salmon are generally believed to cease feeding upon entry into fresh water.

Lakes can serve as nursery habitat for juvenile anadromous salmon and are the main rearing habitat for most land-locked or resident Atlantic salmon populations (Klemetsen et al., 2003). Prey of salmon in lakes include:

- invertebrates; fishes, mostly smelt, vendace and stickleback (Smirnov, 1979).

Atlantic salmon are opportunistic feeders, utilising a wide variety of available prey while feeding at sea (Rikardsen and Dempson, 2011). The first few months at sea are often regarded as an important feeding period in order for young salmon to rapidly enhance their size and reduce their risk of predation. Prey of salmon in marine waters:

- Post-smolts are primarily pelagic and mid-water feeders and their diet includes sand lance and other small fish, euphausiids, amphipods, copepods and crab larvae.
- Piscivory is the main feeding mode for post-smolts $\geq$25 cm in the north-west Atlantic Ocean and the diet frequently contains capelin, sand lance and herring.
- In the north-east Atlantic, invertebrates play a greater role in the salmon diet, which includes amphipods, euphausiids, herring, capelin, redfish larvae, blue whiting, lanternfish, sprat, cod and smelt.
- In the Baltic Sea, salmon feed on marine fish species such as herring and sprat. In the northern parts (Bothnian Sea) only herring is abundant in the diet.

Competition

Juvenile Atlantic salmon may compete amongst themselves and with other species for critical resources such as food and space (Nislow, Armstrong and Grant, 2011). The fish assemblages of salmon river systems in the North Atlantic is generally species-poor, and salmon co-exist most frequently with resident and anadromous forms of other salmonids, such as brown trout (Europe; has been introduced into North America), brook charr (North America), Arctic charr (Europe and North America) and grayling (Europe). Non-salmonid species that co-occur with juvenile Atlantic salmon include cyprinids (frequently demersal species), cottids (bullheads), anguillids (eels) and lampreys. For the most part, these species tend to be habitat and trophic generalists and may therefore be potential competitors for food with Atlantic salmon (Fausch, 1998; Nislow, Armstrong and Grant, 2011). In some river systems, specialised piscivores such as pike and percids also co-occur with Atlantic salmon.

Competition for food and space

The large majority of studies of interspecific interactions in Atlantic salmon ecology involve salmonid species (review in Nislow, Armstrong and Grant [2011]). Due to the general reliance of stream salmonids on aquatic invertebrates, there is opportunity for prey resource competition between Atlantic salmon and co-occurring salmonid species. Moreover, competition for space is also expected as other stream salmonids may have more or less overlapping habitat requirements with Atlantic salmon. Studies of habitat use in rivers show that Atlantic salmon are often strongly associated with riffle habitats, whereas brown trout, Arctic charr and brook charr tend to use slower flowing areas more extensively. Atlantic salmon are particularly well-adapted to fast-flowing water due to their large pectoral fins, which may be used as hydrofoils to hold station in such environments. However, Atlantic salmon appear to prefer pool habitat both as fry and parr. Thus, their extensive use of riffles might be seen as a displacement due to competition with other stream salmonids (Nislow, Armstrong and Grant, 2011).

There is evidence that brown trout tend to be more aggressive than, and socially dominant to, Atlantic salmon of similar size. However, in competition for shelter during winter, dominance depended solely on the size and not the species. Both intrinsic dominance (often related directly to relative size) and prior residence in a patch of streambed are important factors in establishing outcomes of competition between pairs of fish. Such behavioural mechanisms may be important for the outcome of inter-specific competition of Atlantic salmon and other salmonids. However, even in situations where the strength and direction for pairwise interactions can be tested, the consequences of these interactions for habitat use and population dynamics can be complex and difficult to predict (reviewed by Nislow, Armstrong and Grant [2011]).

Impacts of interactions between Atlantic salmon and brown trout or brook charr are thought to be highest during the first year of life when density-dependent processes are most intense (Milner et al., 2003). A combination of studies involving behavioural ecology, habitat associations and fish distributions showed that interspecific interactions between Atlantic salmon and brown trout must be viewed in the context of scale (reviewed by Westley, Ings and Fleming [2011]). At fine spatial scales, brown trout may out-compete Atlantic salmon for many habitats, except those with relatively high water velocity. At large spatial and temporal scales, segregation of Atlantic salmon and brown trout among habitats may be apparent.

Quantitative studies on the effects of non-salmonid fishes on juvenile Atlantic salmon are rare. Ward, Nislow and Folt (2008) found no evidence of competition between reintroduced juvenile salmon and the native fish fauna in tributaries of the Connecticut River (United States). Studies in the laboratory and field surveys, however, indicate that juvenile salmon may influence both the presence and abundance of non-salmonid species at least in certain habitat types (review by Nislow, Armstrong and Grant [2011]). At present, it is still unclear whether the species assemblages of salmon rivers are best thought of as a community of strong interactors or as a collection of species responding independently to their environment (Nislow, Armstrong and Grant, 2011).

Competition for spawning sites

Superimposition of Atlantic salmon redds by brown trout or vice versa may occur when spawning is not segregated spatially. Atlantic salmon and brown trout tend to select similar spawning habitats, which contributes to redd superimposition. Both species were found to construct redds in areas with similar water depths, water velocities and distance to stream banks and there was a large overlap in gravel size (Heggberget et al., 1988; Louhi, Mäki-Petäys and Erkinaro, 2008). Brown trout tend to spawn earlier than Atlantic salmon, but overlap in spawning times can be considerable. Overlap in spawning times is also evident as hybrids between Atlantic salmon and brown trout commonly occur, but usually in low frequency, in nature (review in Westley, Ings and Fleming [2011]).

Other types of competition

Marine competition: It has been difficult to determine the extent of competitive interactions faced by Atlantic salmon in the marine environment because of the vast scale of the habitat exploited.

Pathogens

The total number of species of infectious agents reported from wild and domesticated (ranched/hatchery) Atlantic salmon in both marine and freshwater habitats is 225 (Table 3.3) (Bakke and Harris, 1998).

Few pathogens have caused significant disease epidemics in the wild, and although parasites of returning adults are well-documented, diseases among freshwater stages (parr; e.g. *Gyrodactylus salaris*) seem to be most important, in addition to infestations by the salmon louse (*Lepeophtheirus salmonis*) in sea water.

Gyrodactylus salaris is a freshwater parasite that does not occur naturally in Norway. It was probably introduced in Norwegian rivers in the 1970s by infected hatchery-reared salmon. An epizootic of this species was reported in Norway in 1975 (Johnsen and Jensen, 2003). The entire lifecycle of the parasite is in fresh water, the majority of it spent on young fish. It is less than 0.5 mm in length and attaches by hooklets to the scales and fins of the fish. *G. salaris* has a significant negative influence on the Atlantic salmon. Most often it will kill more than 90% of the young salmon in the river after being introduced. The monogenean *G. salaris* naturally occurs in the Baltic Sea drainage. The parasite was found in White Sea drainage in 1992 (Keret River) and 2002 (Pista River). The introduction in Norway has initiated an extensive programme for eradication of the parasite, mainly by use of the piscicide rotenone. This strategy has also made it necessary to keep affected salmonid populations in gene banks until they can be safely returned to the treated rivers.[3]

Table 3.3. **Total number of species of infectious agents in Atlantic salmon**

Group	Number of species
Virus	9
Monera	21
Protoctista	27
Animalia	
Hirudinea	3
Helminths	
Monogenea	11
Digenea	41
Cestoda	35
Nematoda	29
Acanthocephala	20
Crustacea	13
Mollusca	3
Acarina	2
Fungi	11
Total number	**225**

Source: Bakke and Harris (1998).

Many viruses infect salmon within aquaculture facilities, but there are no reports of disease epidemics due to viruses in wild salmon populations. However, there is increasing evidence for transfer of infectious pancreatic necrosis virus (IPNV) from farmed to wild salmon populations. (reviewed by Johansen et al. [2011]). It is also likely that the newly discovered piscine reovirus is transferred between wild and farmed fish. It is highly prevalent in both wild and escaped farmed salmon (Garseth et al., 2013).

Numerous bacterial pathogens may cause serious epidemics: *Renibacterium salmoninarum* causes bacterial kidney disease (BKD), a fatal systemic infection of both farmed and wild salmonids. Furunculosis, caused by *Aeromonas salmonicida*, is one of the most important diseases of wild and farmed salmonids, and most outbreaks occur when the water temperature is above 10°C.

Crustaceans such as sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) can be a problem for the Atlantic salmon farming industry and there are concerns about impacts on wild salmonids as well. Marine rearing pens may function as pathogen culture facilities at the crossroads for migrant salmonids moving between fresh water and salt water. A recent study demonstrates that sea lice infestations may have had a large effect on wild salmon in the north-east Atlantic Ocean (Krkošek et al., 2013), but a similar study focusing on Ireland only concludes that sea lice has a minor contribution to marine mortality of salmon (Jackson et al., 2013). Apparently, this is still a controversial issue.

Pearl mussel *Margaritifera margaritifera L.* larvae are a parasite of Atlantic salmon (some specialists [Ziuganov et al., 1994] speculated about salmon-pearl mussel symbiosis). The number of pearl mussel populations is decreasing. They are currently listed in the European Habitat & Species Directive Annexes II and V and in the Bern Convention Annex 3 (Geist, 2005).

Biology and rearing of domesticated farmed Atlantic salmon

Domestication

Directed domestication for the commercial production environment began with Atlantic salmon gametes obtained from wild stocks as the founder population. Individuals having desired phenotypic traits were retained for the next generation of commercial production. The first pedigreed broodstock programme for Atlantic salmon began in Norway in 1971 (Gjedrem, 2010). See the section "Broodstock rearing and breeding" for more information on selective breeding.

Intensive aquaculture production

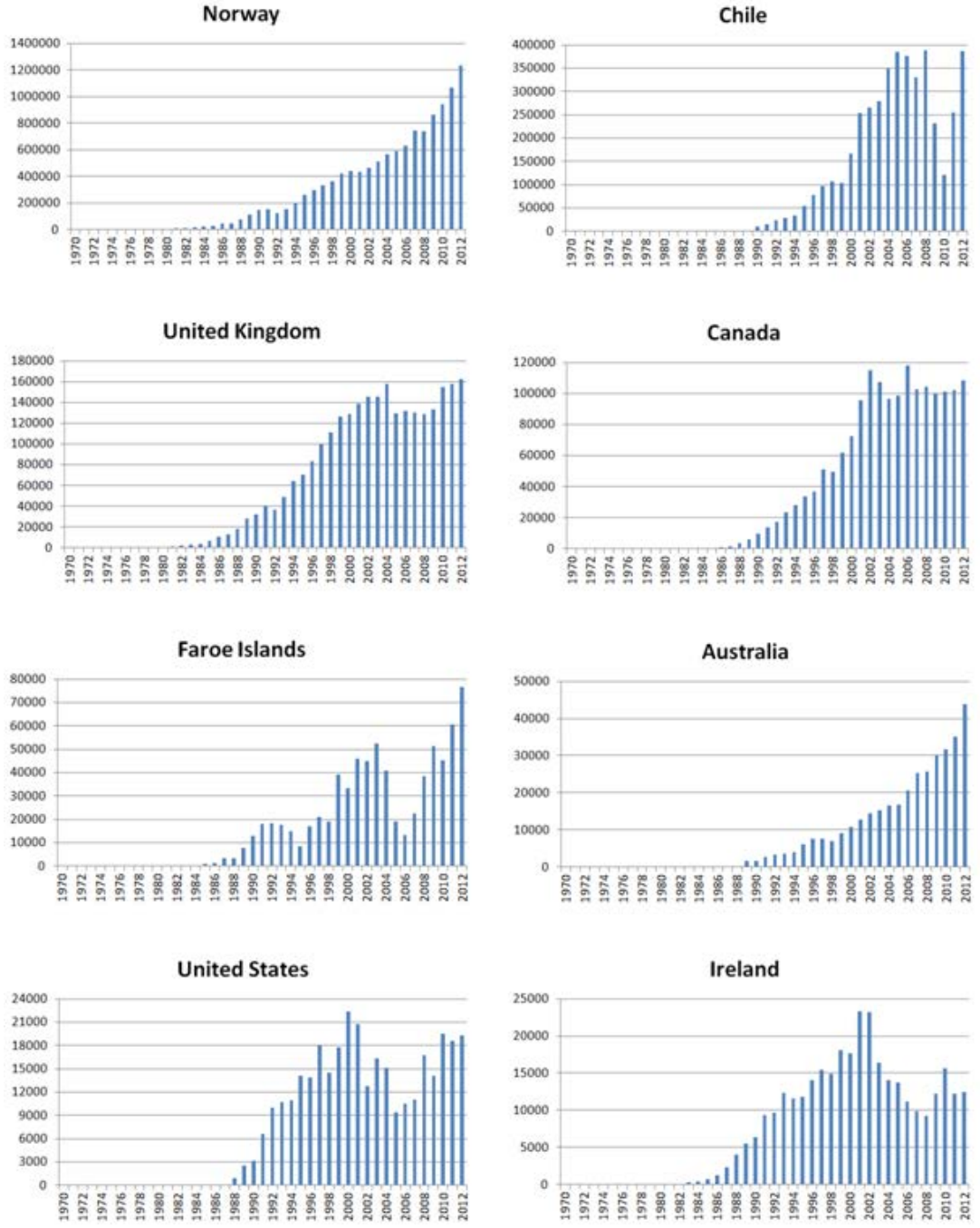
Culture of Atlantic salmon in sea cages was first trialled in the 1960s in Norway as a means to raise Atlantic salmon to marketable size. These early successes in Norway prompted Atlantic salmon culture development in Australia (Tasmania), Canada, Chile, the Faroe Islands, Ireland, Scotland and the north-eastern seaboard of the United States. Minor production also occurs in France, New Zealand and Spain. The major production areas for Atlantic salmon farming lie within latitudes 40-70° in the northern hemisphere and 40-50° in the southern hemisphere (FAO, 2014). Global production of Atlantic salmon exceeded 2.0 million tonnes in 2012 with Norway (1 232 095 tonnes), Chile (386 607 tonnes), the United Kingdom (162 600 tonnes), Canada (108 118 tonnes), the Faroe Islands (76 564 tonnes), Australia (43 785 tonnes), the United States (19 295 tonnes) and Ireland (12 440 tonnes) as the top eight producers (Figure 3.4). Specific details on the production of Atlantic salmon are described in subsequent sections.

Differentiation from wild stocks

Cultured fish may be distinguished from their wild counterparts by differences in external morphology (Lund, Hansen and Järvi, 1989; Fleming and Reynolds, 2004), growth patterns in scales and otoliths (Lund and Hansen, 1991; Hindar and L'Abée-Lund, 1992), pigmentation (Lura and Sægrov 1991a; 1991b) (for a comprehensive review, see Fiske, Lund and Hansen [2005]). However, the longer the fish have been in the wild, the more difficult it is to use such characters to distinguish them from wild fish. In some instances, fin-clipping and external or internal tags have been used to identify fish of cultured origin. Genetic differences between cultured and wild fish may also be used as a basis for separation of the two groups and their offspring (Skaala et al., 2004). For further information see the section on "Genetics of Atlantic salmon".

Figure 3.4. **Marine volume of Atlantic salmon production for the top eight salmon-producing countries, 1970-2012**

Metric tonnes



Note: Vertical axis production volumes vary by country.

Source: Numbers for graphs obtained from FAO (2014a).

Culture and rearing practices for commercial aquaculture

Broodstock rearing and breeding

Atlantic salmon broodstock programmes began in 1971 in Norway with spawning of the first wild Atlantic salmon from 18 rivers/strains. A total of 41 rivers were included to produce the first 4-year classes. The number of full sibling families ranged from 120-240 families produced (66-149 families tagged) for a total of 721 families (442 families tagged) (Gjedrem, 2010). Despite this effort, most of the genetic variation was actually found between and within families of river strains (Gjøen and Bentsen, 1997). Since these early efforts, other breeding programmes have begun worldwide using broodstock that are either indigenous to a specific area or comprise a mixture of strains/rivers and possibly from very different geographic areas. There is no accepted standard method to run each programme and these decisions are often a result of space availability and cost of rearing and maintaining fish. Typically, year classes are initially created independently using different wild or possibly formerly mass-selected broodstock. Over time, year classes are combined generally for two reasons: 1) to genetically link the year classes so all data can be combined and analysed together; and 2) to prevent inbreeding. Genetic diversity is lost over time from the complete removal of extremely poor performing families or potential loss of families during production (e.g. very poor survival).

The original Norwegian programme that began in 1971 eventually merged all year classes into a single breeding kernel (from 2005 onwards) comprising over 600 families that were evaluated for 22 traits including production/efficiency traits (e.g. fast growth; known as the effective group) or health/robustness traits (e.g. disease resistance; known as the robust group). Broodstock from these two groups could either be crossed to create families that are effective only (E x E), robust only (R x R) or a combination of effective with robust (E x R) depending on the desire of the company planning to grow the fish (Aquagen, n.d.). Other broodstock programmes producing and maintaining fewer families per generation might attempt to improve their desired traits simultaneously within each year class or choose specific traits to focus on within specific year classes (e.g. disease resistance in even years). If the latter approach is used, then introducing other technologies such as cryopreservation may be beneficial to link year classes and create families improved for all traits each year.

Primarily four-year old (three-year old from 2005) Atlantic salmon (male and female) are used as broodstock globally; however, individuals may be used in the breeding nucleus as young as two and as old as seven. In addition, Atlantic salmon can be reconditioned and spawned one or more times to contribute to the breeding programme. A common practice in breeding programmes is to use fish in their fifth year that did not mature as four-year olds (referred to as "silvers") to provide an additional means to link year classes. However, use of fish older than four years of age is usually kept to a minimum as older broodstock become very large with associated handling difficulty during spawning and cost considerably more given the additional time to feed and maintain.

No two breeding programmes appear to be alike in the approach taken to identify the best-performing individuals in the breeding nucleus and subsequently create the next generation crosses or families. To generalise, most breeding programmes use selection methodology that has been adopted and adapted from the livestock industry where each generation of data is added to the previous generation (e.g. a fish is selected based on its individual performance, family performance, parental performance, etc.). To this end, a combined selection method is typical and considers the merits offered by both the individual and the family to estimate a breeding value for a particular trait. Traditionally, some traits

could not be evaluated directly on the individual if basing selection from phenotypes, such as resistance to a particular disease that requires sacrifice of the challenged individual. In this case, the use of family information is necessary. It is now also possible to select an individual for resistance to a particular disease based on that individual's genotype or genetic makeup. However, identifying quantitative trait loci (QTLs) and using marker-assisted selection or genome-wide assisted selection is not yet available for all desired traits. Many breeding programmes are either working to obtain genetic markers for selection or continue to use phenotypic selection unable to afford these new technologies. Regardless of the approach or methods used, all breeding programmes strive to maintain as much genetic diversity as possible within the broodstock in their breeding nucleus/nuclei.

The sex of future broodstock is typically identified one or more years prior to spawning either by use of ultrasound or a molecular marker. Broodstock are anesthetised before being stripped manually, bled and then gonads removed, or compressed air may be used with females (this allows eggs to flow freely when ripe). Broodstock are often culled either before or after gametes are obtained for fish health sampling to occur. Broodstock are health tested throughout production, but health testing individual fish after using their eggs or milt (seminal fluid) allows for confirmation of negative results for specific pathogens or diseases. Sometimes broodstock are reconditioned after spawning for future use. Crosses are made to create families after gametes are obtained, either fresh or previously acquired milt that was cryopreserved and thawed for use. Crossing for the breeding nucleus will typically include some level of relatedness that aides in the removal of environmental effects during data analysis. It is more common for a male to be used with more than one female to create half sibling links. However, various strategies based on this general method of crossing are used globally. See Gjedrem (2000; 2010), Lutz (2001) and Gjedrem and Baranski (2009) for additional information on selective breeding including reference to marker usage. See Liu (2011) and Saroglia and Liu (2012) for specific reference to the use of sequencing and genomics in aquaculture.

Physical environment (tanks, nets, cages, etc.) and containment conditions

Broodstock are maintained in various different environments. Some broodstock are held in freshwater tanks throughout their entire life cycle or may be smolted and maintained in saltwater tanks (less common). Regardless of whether tanks use fresh water or salt water, the water is more often being recycled in reuse or recirculating systems. The same families are typically also stocked in saltwater cages where the breeding nucleus is held on land in tanks to compare family performance either between fresh water and salt water and/or between tanks and sea cages. The broodstock nucleus may also be reared in sea cages using standard commercial conditions in various broodstock programmes globally. Broodstock maintained in sea cages are typically transferred back to fresh water anytime from several months to a week prior to spawning. In the past, broodstock may have been selected and stripped directly from sea cages, but this now occurs to a lesser extent.

Rearing environment (water flow, DO, temperature, lighting/photoperiod)

Following broodstock stripping, crosses or families that have been created in a pedigreed breeding programme are either:

- Maintained separately prior to individual fry tagging with a passive integrated transponder (PIT tag) then followed by mixing and communal rearing.
- Fertilised eyed eggs are mixed in equal numbers to create a breeding nucleus/nuclei that are communally reared from that point forward. These unmarked communally

reared progeny must be later PIT tagged and fin clipped to identify parentage using markers.

Using the former strategy, PIT tagging generally occurs in individuals that are 5-20 grammes in size. PIT tagging and fin clipping of individuals in communal rearing situations may occur more than a year after production began, but sometime prior to spawning as family assignment is key for progeny evaluation. Herbinger et al. (1999) discuss performance variation between single family tanks versus mixed family tanks. Sonesson, Meuwissen and Goddard (2010) discuss the potential for use of communal rearing of families and DNA pooling in genomic selection schemes.

Broodstock located in tanks or cages are maintained similarly to Atlantic salmon that are grown for production (see associated sections below). However, broodstock will likely experience additional handling as assessments occur throughout the growing period. Future broodstock (progeny in a breeding nucleus) may undergo a numerical standardisation, for example, once they are on dry feed to better ensure a similar environment across all families as populations remaining within individual family tanks may vary based on initial survival. These fish will also be PIT tagged and/or fin clipped at some point as previously mentioned. In addition, measurements are expected to occur throughout production/growth. Pathogen challenges might occur based on the programme traits of interest. In such a challenge, a portion of Atlantic salmon from all or a subset of families are either directly injected intraperitoneally with a pathogen (e.g. *Renibacterium salmoninarum*) or passively exposed to the pathogen in the environment (e.g. *Renibacterium salmoninarum* co-habitation model, sea lice). Many pathogen challenges will occur in a biocontainment facility and these salmon cannot subsequently be used as broodstock after testing. Sometimes the salmon broodstock may be exposed to a pathogen in the rearing environment and the associated mortality data might also be useful to the broodstock programme if the families are known.

Photo (light) and thermal manipulation is common when attempting to either advance or delay spawning (see below). Exact details on photo and thermal manipulation are specific to individual companies and are, to some extent, a refined approach over time. Broodstock are photo-advanced to supply eggs earlier to growers than would be available on a natural cycle or photoperiod. Photo-delaying broodstock helps to produce eggs later than would normally be available. Advancing production, spawning naturally and delaying production is completed for various reasons. This can allow an egg producer to produce eggs almost continuously for variable desires of growers. Altering spawning time can also help a hatchery that might have limited egg incubation space.

In general, salmonids are annual autumn/winter spawners (Billard, Reinaud and Le Brenn, 1981) and mainly rely on seasonal cues to entrain the gamete maturation and spawning cycle. Photoperiod is the main driving factor, but when coupled with temperature the three main phases of reproduction become synchronised: induction of oogenesis, vitellogenesis and the concluding stages of maturation (ovulation and gamete release) (Wang et al., 2010). While most work on photoperiod manipulation of spawning salmonids has been completed on rainbow trout, it has proven to be a useful model for Atlantic salmon (Bromage, Porter and Randall, 2001; Taranger et al., 1998). In general, salmonids are induced by an increasing photoperiod, but it is the timing and the relative change in daylight hours (i.e. increasing or decreasing from a previous history) that is more important than amplitude and rate of change (Randall and Bromage, 1998). A decrease in the photoperiod following induction affects the rate of gametogenesis and synchronisation (Duston and Bromage, 1998; Taranger et al., 1998; Bromage, Porter and Randall, 2001; Davies and Bromage, 2002). Without the decrease in day length at the appropriate time,

small numbers of females will actually spawn in advance and most may delay spawning. Advanced photoperiod reduces the time available for gametogenesis as long as water temperature does not inhibit the process, resulting in a reduced time in which eggs can sequester yolk and in a lower oocyte developmental competence (Migaud et al., 2013). For rainbow trout, photoperiod advanced spawning can induce significant egg quality defects with transcriptome analysis identifying six genes significantly less abundant in photoperiod manipulated eggs than control eggs (Bonnet et al., 2007). In males, spermatogenesis and sperm quality are also affected, but to a lesser extent than egg quality.

Temperature does not play a role in the induction of oogenesis for salmonids but does have critical importance in the subsequent stages of gametogenesis (Wang et al., 2010). Temperature is acknowledged as an important environmental parameter affecting the reproductive development and the timing of spawning of fish. In salmonids, both low (Nakari, Soivio and Pesonen, 1988) and high temperatures (Taranger and Hansen, 1993; Pankhurst et al., 1996; Pankhurst and Thomas, 1998; King et al., 2003) have been observed to restrict or inhibit aspects of reproductive development. Temperature exerts fine tuning as cue to spawn and timing of spawning, compensating for temperature differences year to year, preventing spawning at high water temperatures, and consequently at a time when food would be scarce for alevins in the wild (Taranger and Hansen, 1993). Warm temperatures in late summer and early autumn can therefore be the environmental bottleneck to achieving forward phase shifts in the reproduction of Atlantic salmon (Taranger and Hansen, 1993; Taranger et al., 1998; Bromage, Porter and Randall, 2001).

As temperature is known to affect reproductive development and timing of spawning, temperature alterations just prior to spawning are typical and routinely completed when possible in a tank setting regardless of photoperiod manipulation, with the thought that the changes accelerate ovulation and sperm release. Taranger et al. (2000) reported that exposure to reduced water temperatures (approx 5°C below natural) both synchronised and advanced ovulation in Atlantic salmon. One methodology, for example, is to drop the ambient water temperature to 6-8°C approximately 4-6 weeks prior to the expected start of spawning.

Hormone stimulation is a useful management tool to enhance or synchronise ovulation or spermiation of a group of broodstock to provide the ability to manage large egg batches over the spawning season (e.g. stimulating those broodstock which might spawn later to spawn earlier during a peak) or when conditions such as temperature are suboptimal (King and Pankhurst, 2007; Taranger et al., 2003). Gonadotropin releasing hormone analogue (GnRHa) is a common hormone studied and the active ingredient of commercially available Ovaplant® or Ovaprim® (Syndel Laboratories Ltd.). Sustained release implants have been used to synchronise and advance ovulation in Atlantic salmon (Crim and Glebe, 1984; Mylonas et al., 1995; Taranger et al., 2003). The successful dose for Atlantic salmon is reported to be 50 μg per kg (Taranger et al., 2003). Male broodstock generally respond to hormone implants with increased sperm production. However, female broodstock results can be more variable.

The vast majority of Atlantic salmon produced globally are diploid mixed gender. Research has been conducted on the production of all female broodstock and triploid progeny (see the section on "Biocontainment"). The primary reason to produce triploid all female progeny for commercial production is to eliminate the opportunity for maturing production fish prior to harvest. Other methods are available to successfully manage early maturation, such as using lights in sea cages (see section below) and through trait selection within a broodstock programme.

Fish sizes, densities, growth rates

Individual Atlantic salmon within a broodstock programme are reared similarly to those in production tanks or cages; however, whenever possible, densities are kept lower compared with production fish. A production hatchery might allow its density to be as high as 80-100 kg/m^3 but the density for a future broodstock tank will likely be lower. As the fish grow, the broodstock density will likely be maintained as low as 25 kg/m^3 and as high as 40 kg/m^3 (depending on the broodstock programme, tank sizes and conditions, etc.). Density data varies between companies farming Atlantic salmon and is often not information that is publically shared or available.

The size at smolting Atlantic salmon, growth rates and feed conversion rates are all company information that is proprietary. However, feed itself makes up more than 60% of the cost of production (Gjedrem, 2010). Feed conversion rate (FCR) is difficult to accurately assess and is also very expensive to measure directly on individual fish or indirectly on family groups, but it is widely known that carnivorous fish, such as Atlantic salmon, are quite efficient in converting energy and protein to edible food for humans with a FCR of approximately 1-2 and yielding about 57 g of edible meat per 100 kg of feed (Marine Harvest, 2014). The genetic correlation between growth and feed conversion ratio in Atlantic salmon is documented to be high, ranging from 0.60 (Kolstad, Grisdale-Helland and Gjerde, 2004) to 0.90 (Thodesen et al., 1999) and likely falls somewhere in between these two values. This genetic correlation means that as the salmon are selected to grow faster (improvements expected of 10-15% in growth per generation; Gjedrem, 2010), they should also be achieving better FCRs, which means the fish population will consume less feed per kilogram of fish produced.

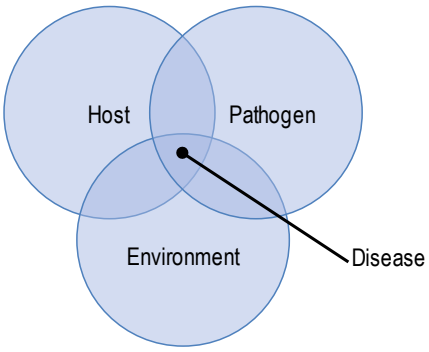
Feeds (types, sources, composition) and feeding (rates, methods)

Atlantic salmon nutritional requirements are well known following years of research resulting in all stages of production being fed a balanced diet of formulated extruded feed. Broodstock typically consume all the same diets as production fish (see other sections for more details) until a minimum of one year prior to maturation, when the diet switches from a production diet to one formulated to maximise the quality of developing gametes. Broodstock diets have increased levels of protein and fat, but oil content and make-up may be lower (e.g. only fish oil as the lipid source). The broodstock diet also has elevated quantities of astaxanthin, selenium, vitamins C and E, and Beta-glucans to activate macrophages to generally combat infection.

Diseases, pathogens and parasites – General concepts for all life stages

In any discussion of diseases, it is very important to remember that the presence of a parasite or pathogen does not equal disease. A disease is a physiological condition of an organism that can be directly (or indirectly) caused by a pathogen, parasite, genetic anomaly or an environmental factor (e.g. low oxygen). It is incorrect to say "a disease was transferred from one organism to another..." A pathogen was transferred and a disease may or may not develop. Consider the Venn diagram below, disease could occur in the small region where pathogen, host and environment all overlap. The environment acts on host and pathogen, but only when conditions favour the pathogen and debilitate the host, could a disease occur.

Figure 3.5. **Disease triangle**



The disease triangle is a conceptual model that shows the interactions between the environment, the host and an infectious (or abiotic) agent. This model can be used to predict epidemiological outcomes in plant, animal and public health. Disruption or change in one or more of the three elements may impact the outcome of the interaction.

Another important aspect to consider in any discussion of disease is diagnostics. Diagnosis usually begins with observation (e.g. abnormal colour, behaviour), then microscopy is used, followed by a combination of culture techniques (e.g. bacteria media plates), serological techniques (indirect fluorescent antibody technique, enzyme linked immunosorbent assay) and genetic testing (polymerase chain reaction [PCR], quantitative-PCR). One caveat is to never make a diagnosis on visible symptoms alone. Fish exhibit many similar symptoms for a variety of pathogens. It is essential to properly and accurately diagnose which pathogen is present, especially with regards to regulatory implications (e.g. fish transfer, local therapeutic protocols, etc.). For example, many viral and bacterial pathogens can be detected in a fish by the use of a standard molecular technique such as PCR. However, a PCR analysis only gives a genetic signal that the pathogen is present, it does not indicate if it is viable. Similarly, indirect fluorescent antibody technique and enzyme-linked immunosorbent assay methods use antibodies labelled with a fluorescent dye to detect presence of an antigen signal (e.g. part of a bacterium cell wall). Theoretically, PCR and antibody-based testing could give a "positive" result of a pathogen in a sample even if there were dead copies of the cell (or virus particle) present. For example, *Renibacterium salmoninarum* antigen can persist for several months following vaccination with a killed preparation (Pascho, Goodrich and McKibben, 1997). For some pathogens, it is also important that specific diagnostic tests distinguish between pathogenic and non-pathogenic strains in a particular host. For instance, the HPR0 strain of the infectious salmon anemia virus is considered non-pathogenic (Christiansen et al., 2011) and various subspecies of bacteria (e.g. *Aeromonas salmonicida* subsp *achromogenes* vs. *A.s*. subsp *salmonicida*) cause different pathologies in the host (Austin and Austin, 2007). Specific identification of a pathogen by either direct (culture) or indirect (e.g. PCR or antibody-based) methods indicates that the pathogen (or part of the pathogen) is present; however, this is not sufficient evidence to conclude a disease condition exists among a population since a pathogen can be present in the absence of disease.

As a result of variations in reliability of diagnostic tests, many countries follow international diagnostic standards of the World Organization for Animal Health for any regulatory diagnoses. Standardisation ensures all compliant labs are following the same protocols such that diagnostic results can be accurately compared among countries. For most pathogens and some parasites, part of the standard diagnosis involves culturing the organism to demonstrate its viability.

Diseases, pathogens and parasites of any type are of special concern for broodstock as they can result in loss of individual broodstock prior to spawning, which reduces the ability to produce the next generation. Pathogens may also result in the loss of entire families,

thereby reducing the genetic variability and potential genetic contribution to future generations. Positive results with either the dam or sire from health testing post-spawning may also result in the removal of progeny. A disease requiring eradication of a group of salmon (e.g. tank, sea cage, year class) can be particularly devastating if/when it occurs in the breeding nucleus, where top-performing individuals in a year class are maintained. These risks provide sufficient reason to maintain multiple breeding nuclei in different locations and possibly in different environments (i.e. land-based tanks may be better at keeping pathogens out through disinfection, but sea cages are easier to treat and remove pathogens after they are introduced).

There are two methods in which pathogens can be transferred between broodstock individuals and from broodstock to the progeny – vertical transmission and horizontal transmission.

- **Vertical transmission** is transfer of the pathogen from broodstock to progeny through the egg or milt. Vertically transmitted diseases are of special concern as such agents may spread quickly through the entire industry if they are introduced into broodstock. Vertical transmission is minimised through strict health testing regulations globally. Broodstock populations may be terminated for testing positive or a broodstock fish might be removed prior to egg fertilisation or fertilised eggs may be removed from a facility prior to hatching.
- **Horizontal transmission** is the transfer of a pathogen or parasite through the environment or water column. Horizontal transmission can be minimised by using strict biosecurity measures (e.g. on land use of footbaths, handwashes, no shared equipment between year classes or even tanks, year classes on different sites if broodstock are located in sea cages, etc.).

The transmission mode of pathogens and parasites is very important. The Norwegian Scientific Committee for Food Safety has considered the possibility of vertical transmission (from egg or milt to progeny) of some important diseases. True intra-ovum vertical transmission is well-documented for *Renibacterium salmoninarum* (causative agent of bacterial kidney disease in salmon) (Evelyn, Ketcheson and Prosperi-Porta, 1984). For infectious salmon anaemia virus, it was concluded that vertical transmission cannot be excluded, but is of little importance for spread of infectious salmon anaemia virus. Pancreas disease is not considered a vertically transmittable disease, and for heart and skeletal muscle inflammation there is insufficient information to form the basis for an assessment. IPNV is considered to transmit vertically. There is indirect evidence for vertical transmission of IPNV in Atlantic salmon, and this occurs in several other species of salmonids, with current disinfection procedures insufficient to prevent this.

Once a closed population has been identified as being free of particular pathogens, there is no risk of vertical or horizontal transfer within that population. However, pathogens are often present within the host watershed or water body and pumped into a land-based facility or the water passes through a sea cage, respectively. Pathogens in the water column are typically easier to remove with broodstock on land as water is likely mechanically filtered and disinfected using ultraviolet (UV) irradiation and/or ozone. Pathogens in the water column passing through sea cages are impossible to remove, but still might not cause an issue. If a cage of fish is healthy and located in favourable environmental conditions in a stress-free, low-density setting, then the likelihood of horizontal transfer from the environment decreases. Various diseases are also included in selective breeding programmes that will also decrease the likelihood for an outbreak of a particular disease in a sea cage. An excellent example of using selective breeding to

reduce mortality resulting from disease is that of infectious pancreatic necrosis (IPN). When farms chose IPN-resistant QTL-selected (Moen et al., 2009) stock, the number of IPN-diagnoses was reduced from 47% in 2009 to 7% in 2010 while IPN outbreaks were not reduced in those year classes on sites using non-QTL-selected stocks – 36% outbreaks in 2009 with 43% outbreaks on the same sites in 2010 (Aquagen, n.d.). Similarly, several reports document attempts at using breeding selection of Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon to improve resistance to BKD (Beacham and Evelyn, 1992; Withler and Evelyn, 1990).

Not all pathogens and parasites affecting Atlantic salmon production are an issue globally. However, transfer of any pathogen or parasite is of global concern (Table 3.4).

Table 3.4. **Pathogens and parasites recorded in Atlantic salmon**

Common name	Scientific name, causative agent	Major route of transmission	Present	Country
Amoebic gill disease (AGD)	*Neoparamoeba*/ *Paramoeba perurans*	H	SW	Australia, Chile, France, Ireland, New Zealand, Scotland, Spain, United States
Bacterial coldwater disease (BCWD)	*Flavobacterium psychrophilum*	H, V	FW, SW	Australia, British Isles, Canada, Chile, Norway, United States
Bacterial kidney disease (BKD)	*Renibacterium salmoninarum*	H, V	FW, SW	Global
Ceratomyxosis	*Ceratonova (Ceratomyxa) shasta*	H	FW	Canada, United States (west coast)
Cardiomyopathy syndrome (CMS)	Piscine myocarditis virus (PMCV)	H	SW	Ireland, Norway
Columnaris disease	*Flavobacterium columnare*	H	FW	Global
Enteric redmouth disease; yersiniosis	*Yersinia ruckeri*	H	FW	Canada, Norway, United States (west coast)
Erythrocytic inclusion body syndrome (EIBS) virus	Unknown	H	FW, SW	British Isles, Ireland, Norway, Scotland, United States (west coast)
Furunculosis	*Aeromonas salmonicida salmonicida*	H	FW, SW	Global
Furunculosis, atypical	Atypical *Aeromonas salmonicida*	H	FW, SW	Canada (Newfoundland), Chile, Iceland
Gyrodactylosis	*Grydactylus salaris*	H	FW	Norway, Northern Europe
Heart and skeletal muscle inflammation (HSMI)	Associated with *Piscine orthoreovirus* (PRV)	Likely H	FW, SW	Norway, Scotland
Ichthyobodosis	*Ichthyobodo necator* (stricto) (s.s.)	H	FW	Global
Ichthyobodosis	*Ichthyobodo salmonis* (sp. II)	H	FW, SW	Global
Infectious hematopoietic necrosis (IHN)	Infectious hematopoietic necrosis virus (IHNV)	H	FW, SW	Canada (west coast), Europe, United States (west coast)
Infectious pancreatic necrosis (IPN)	Infectious pancreatic necrosis virus (IPNV)	H, V	FW, SW	Canada, Chile, Norway, Scotland, United States
Infectious salmon anaemia (ISA)	Infectious salmon anaemia virus (ISAV)	H	FW, SW	Canada (east coast), Chile, Norway, United States (east coast)
Microsporidosis	*Loma salmonae*/ *Desmozoon lepeophtherii*/ *Paranucleospora theridion*	H	SW	Canada (*Loma*), Norway, Scotland
Mycotic nephritis (fungus)	*Exophiala* spp.	H		Norway
Myxosporean (parasite), kudoa disease	*Kudoa thyrsites*	H	SW	Canada (west coast), Ireland, Spain

Table 3.4. **Pathogens and parasites recorded in Atlantic salmon** (*continued*)

Common name	Scientific name, causative agent	Major route of transmission	Present	Country
Parvicapsulosis	*Parvicapsula pseudobranchicola*	H	SW	Canada, Chile, Norway, Scotland
Pancreas disease (PD)	Salmonid alphavirus (SAV)	H	SW	Europe
Piscirickettsiosis, salmonid rickettsialc septicaemia (SRS)	*Piscirickettsia salmonis*	H	FW, SW	Canada, Chile, Ireland, Norway
Phagocytolytic syndrome (PCLS)	Unknown; viral aetiology suspected	H	FW, SW	Ireland, Scotland
Proliferative kidney disease (PKD, formerly PKX)	*Tetracapsuloides bryosalmonae*	H	FW	North America, Western Europe, mainly affects rainbow trout
Pseudomonas fluorescens	*Pseudomonas fluorescens*	H	FW	Norway
Saprolegniasis, fungus	*Saprolegnia* spp.	H	FW	Global
Sea lice	*Lepeophtheirus salmonis*, *Caligus elongatus, C. clemensi*	H	SW	Canada, Ireland, Norway, Scotland, United States
Sea lice	*Caligus rogercresseyi*	H	SW	Chile
Tapeworm (parasite)	*Eubothrium spp.*	H		Canada (east coast), Norway
Varracalbmi	Bacterium related to *Pasteurella skyensis*	H	SW	Norway, Scotland
Vibriosis	*Vibrio anguillarum*, *V. ordali*	H	SW	Global
Vibriosis, coldwater	*Vibrio salmonicida*	H	SW	Global
Viral hemorrhagic septicaemia (VHS)	Viral hemorrhagic septicaemia virus (VHSV)	H	FW, SW	Global, identified in >80 fish species
Whirling disease	*Myxobolus cerebralis*	H	FW	United States (Alaska, west coast, central and east), Europe
Winter ulcer	*Moritella viscosa*	H	SW	Canada, Norway, United States
Mouth rot, fin rot	*Tenacibaculum maritimum*	H	SW	Canada, Norway, United States
Other bacterial infections (typically opportunist)	*Vibrio, Photobacterium*, *Alteromonas*, *Pseudoalteromonas*, *Phychrobacter*, *Polaribacter*			Global

Notes: There are various methods of transfer: horizontal (H), from wild to farmed fish, from farmed to farmed fish, etc.; or vertical (V), from broodstock to progeny. In some cases, the exact method of transfer might be unknown (U). Pathogens and parasites may also be present in only a freshwater (FW) or saltwater (SW) environment.

Sources: Information from personal communications with industry individuals and see also: Aamelfot, Dale and Falk (2014); Brown (1994); Declercq et al. (2013); Fryer and Hedrick (2003); Garseth et al. (2013); Graham et al. (2002); Isaksen et al. (2011); Kent and Poppe (1998a); Kent, Dawe and Speare (1995); Kibenge et al. (2004); Marty et al. (2014); McLoughlin and Graham (2007); Merck (2015); Meyers (2007); Nash (2001); Nematollahi et al. (2003); Nilsen et al. (2011); Olsen et al. (2011); Shaw et al. (2000); Tobback et al. (2007); Veterinærinstituttet (2013); Woo (2006; 2010); Woo and Bruno (2011); Woo, Bruno and Lim (2002).

Hatchery rearing of eggs and fry

Physical environment (tanks, nets, cages, etc.) and containment conditions

Fertilised eggs are very fragile and must not be handled until the eyed egg stage, at approximately 220-250 degree days, when the eyes of the larvae can be seen through the eggshell. Eyed eggs can tolerate handling and dead eggs are sorted from live eggs at this stage. This is typically completed using an automated egg picker for production; however, if the eyed eggs are part of the broodstock programme, this might be completed by hand to ensure no mixing of eggs between families.

Rearing systems for the egg stages vary according to the stage at which the Atlantic salmon will be transferred to another system for further grow-out. There are many types of egg incubators. A commonly used style is the vertical egg incubator, which stacks numerous trays vertically to use a relatively small amount of floor space (e.g. Marisource and CompHatch; Figures 3.6 and 3.7). These commercially available units are designed to maximise the number of eggs per square foot of space with high water quality, lower levels of fungus and cleaner hatching environments. Hatching jars have been used for decades, but recently modified to accommodate a higher number of eggs in commercial settings. Hatching jars can be used individually (Figure 3.8) or as a system and the design allows gentle rotation of incubating eggs without concussion. Salmon can hatch in jars, but must be moved before first feeding. See also reviews by Saunders (1995), Robson (2006) and Anderson (2007) for additional information and illustrations.

Figure 3.6. **Marisource 8-tray vertical incubator for salmon**

Source: Marisource, https://www.marisource.com/featured/marisource-8-tray-vertical-incubator-for-salmon.html.

Figure 3.7. **CompHatch hatching system illustration demonstrating the inclusion of a work lift**

Source: Alvestad, http://alvestad.com/Engelsk/Comphatch.html.

Figure 3.8. **Picture of a hatching jar sold by Pentair**

Source: Pentairaes, http://pentairaes.com/hatching-jar-and-hanger.html.

Combi tanks are a complete hatchery system designed for all stages: hatching eggs, first feeding fry and juvenile stages (Figure 3.9). This tank system initially uses a shallow insert with a square egg tray. Inflow can be at the surface or bottom of the tank, but water can only exit through a centre standpipe after upwelling through the eggs. As fish grow, the shallow insert can be removed to give the salmon more volume to occupy. This system is available in different sizes, with perhaps the most common size having a 1 m diameter tank.

Figure 3.9. **Combi tank designed for fertilised and hatching eggs, first feeding fry and juvenile stages of Atlantic salmon**

Source: Pentairaes, http://pentairaes.com/.

Less commonly used are hatchery troughs (Figure 3.10). They allow an easy survey of eggs, but require a larger footprint per number of eggs/fry. The trough is five metres long and houses up to seven baskets. These can be used until the first feeding fry stage

when fry are moved to feeding tanks thereby allowing less-developed alevins more time to absorb the yolk sac in clean water. First feeding can occur in the trough.

Figure 3.10. **Standard hatching trough that may be used for Atlantic salmon egg to first feeding fry stage**

Source: Aquaculture, www.aquaculture-com.net/bilder/brutrinnen.jpg.

The most common environment for fry rearing after the first feeding stage is circular fibreglass tanks. Every farm or hatchery will have different demands for tank size and first feeding tanks can range from 0.1 m^3 to 10 m^3 in volume. Juvenile rearing can occur in tanks up to 50 m^3 but using larger tanks is not typical practice for these early life stages. This is due to the flow index, which refers to the relationship of the fish weight and size to water inflow (Piper et al., 1986). Salmon must be able to handle the required turnover rates for the size of the tank. Large rearing units require high incoming water amounts and small fish cannot handle the velocity that is required to keep the oxygen levels and water exchanges at standard levels.

Although most hatchery facilities currently use flow-through water systems, there is increasing use of recirculating aquaculture systems (RAS) for rearing of Atlantic salmon, particularly early life stages (i.e. eggs, fry and pre-smolt). Recirculating systems are those that typically recycle or reuse most of the water used within the system, typically 90% or more, and sometimes even more than 99% of the water. There are several potential advantages for use of RAS in fish culture, including: 1) water conservation (reduced water requirements); 2) enhanced environmental control (particularly temperature, oxygen and pH); 3) enhanced biosecurity and disease control; 4) reduced land needs (due to higher stocking densities); and 5) greater site selection flexibility (independence from water source). Use of RAS also reduces the potential for eggs and fish to escape because most of the water within the system is routed internally through biofilters and other equipment used to remove solids and ammonia.

Rearing environment (water flow, DO, temperature, lighting/photoperiod)

Optimal water flow is based on life stage and rearing environment. Vertical egg incubators typically require 4-25 liters per minute (lpm) per stack, with 4-10 lpm adequate up to the eyed egg stage. If the incubators are used for hatching, then water flow should be increased to a minimum of 8-15 lpm after 400 degree days and up to 25 lpm. The precise flow chosen for each incubator will depend on the required turnover for the density of the stack of trays. Jar incubators require different flows depending on the weight of eggs included. The minimum flow is 3.78 lpm, but this can be increased up to 15 lpm as required. Combi tanks start at 2 lpm and slowly increase as fish grow or

there is an increase in oxygen demand. Flow should increase to 4 lpm for alevin and 6-8 lpm when salmon are free swimming and actively eating. The target water flow for combi tanks when the shallow insert is removed is to have water turnover every 45-60 minutes. Troughs range 10-25 lpm, with flow increased as more baskets are added.

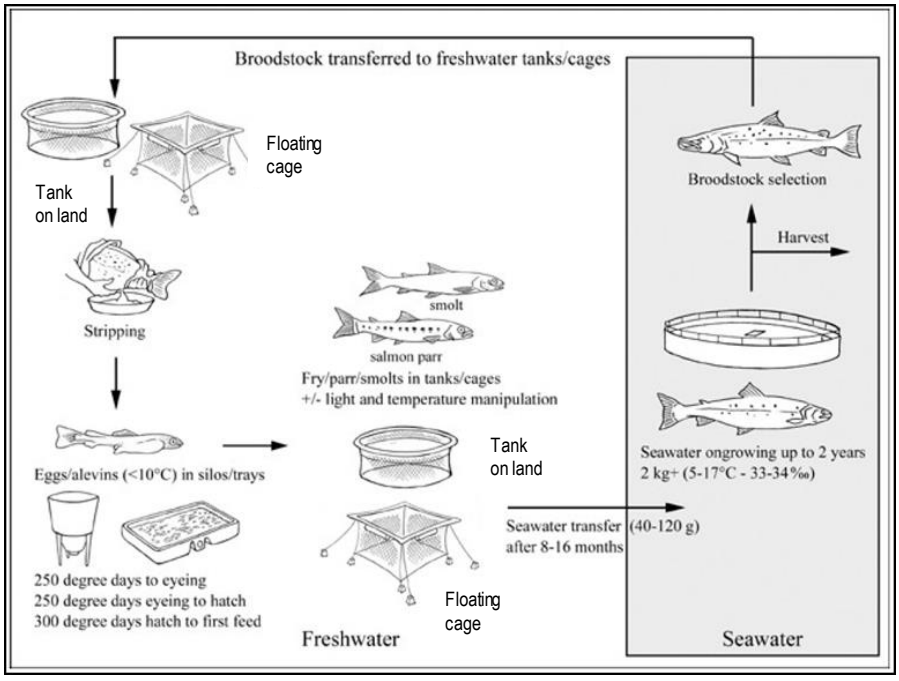
Oxygen demand is very low during egg incubation and increases as salmon grow. Oxygen uptake across the fish gills depends on the gradient of numerous parameters between the internal fish and external environments. Oxygen tension depends not only on the concentration of O_2 in the water, but also on other physical and chemical properties such as temperature, atmospheric pressure and salinity (Pennell and Barton, 1996). The saturation level will determine the ability of the water to hold oxygen and influence the total gas pressure. The per cent saturation determines the ease of transfer through the gills into the bloodstream and changes with temperature. As temperature increases, the ability of water to retain O_2 decreases. At high temperatures oxygen levels are kept at the lower end of the optimal concentration level to keep saturation levels under 130%. If saturation levels increase then total gas pressure will reach detrimental conditions. Optimal O_2 concentration for Atlantic salmon is 8.5-11 mg/l. Salmon will survive in oxygen concentrations of 6-8.5 mg/l, but metabolic response will decrease and overall health can be compromised. Under 6 mg/l is not advised and acute mortality is apparent at dissolved O_2 concentrations between 1 and 3 mg/l (Piper et al., 1986).

Water temperature will vary depending on the season or system. This parameter is one of the most important controlling factors for food conversion rates, growth and metabolite production. The optimal temperature range for egg rearing is 6-8°C, for the alevins this can be increased to 12°C, and after Atlantic salmon are free swimming the temperature can be safely set up to 15°C (discussion of thermal tolerance can be found in Elliott [1991]; see also Saunders [1995]). However, it is very important to monitor water quality closely at high temperatures as changes in organic loading can rapidly affect fish health. Egg development is the easiest to manipulate by controlling water temperature as the total time required to hatch is based on degree days (approximately 450), allowing easy prediction as water temperature is controlled. In this manner, egg development may be delayed to hatch by incubating at lower water temperatures. When water temperature is manipulated, it is best to raise or lower by 1°C per day. If it is necessary to adjust the water temperature quickly, doing so at a rate of no more than 1°C per hour is permissible for eggs, fry and juveniles. Total gas pressure should always be monitored closely when water is heated. High water temperature and heat shock are both contributing factors to increase the incidence of deformities in Atlantic salmon (Wargelius, Fjelldal and Hansen, 2005; Takle et al., 2005). For industry, start of first feeding is often between 900 and 1 000 degree days, but can be as low as 800-850 degree days. Alevin stage is from hatch to no yolk, or approximately 450-900 degree days. Fry stage is considered when salmon are swimming freely and the swimbladder is filled or >900 degree days to parr/smolt (Figure 3.11). All degree days discussed are in Celsius. Temperatures considered optimal and variations in degree days will vary to some extent based on citation, hatchery, etc.

Water quality parameters will vary depending on the degree of water reuse (i.e. flow-through or recirculation) and source of the incoming water (i.e. whether from a lake, well or municipal source). Incoming water can have lethal or supply subpar water chemistry and may need to be treated before entering a rearing tank. Suboptimal freshwater pH is common with incoming water supplies, and this may cause issues such as delayed egg hatching in low pH (Petersen, Daye and Metcalfe, 1980). Ideally pH should remain near 7.0, but eggs can handle a range of 6.5-8.0. If the incoming water is low or high in pH then a buffer may be required and a dosing system may be used to provide the optimal pH

levels. Regardless of the method used, it is important to maintain stable pH levels when buffering the water for proper egg development. A slightly high or low pH level that is consistent is preferable to one that frequently fluctuates.

Figure 3.11. **Example of a production cycle for Atlantic salmon including both freshwater and seawater phases**



Source: FAO (2014b).

Control of light intensity and photoperiod varies as Atlantic salmon develop. There is no photoperiod from fertilisation to the eyed egg stage. Eyed eggs and hatched yolk-sac alevins are typically maintained in little to no light. However, older alevin or fry at about 900 degree days are transferred to rearing tanks and photoperiod is switched to 24 hours of dim lighting during first feeding. Light intensity increases at approximately 1 200 degree days after "pin-heading" is no longer considered a potential issue (see additional information below). Use of 24-hour photoperiod can continue until the winter period, typically January to March in the northern hemisphere, at which time an 8- or 12-hour dark period is introduced. The winter period needs to be a minimum of 6 weeks before the salmon can return to 24-hour lighting or remain on ambient photoperiod. The exact light regime experienced after the required winter period will depend on the timing of and amount of weight gain required before smoltification.

Fish sizes, densities, growth rates

Eyed egg densities range per system. Vertical incubators hold approximately 10 000-360 000 eggs through to hatch per tray depending on the model used. The total number of eggs possible in a specific footprint is scaled based on the number of trays stacked per unit (up to 2.8 million eggs per unit). A 6-litre hatching jar will hold up to 80 000 eggs to the eyed egg stage while a typical basket of a trough will hold up to 20 000 eggs, but numerous baskets will be present within each trough. Combi tanks hold the fewest number of eggs for the footprint (i.e. lowest density), with a 1-metre tank holding

approximately 40 000 eggs. Eggs should be loaded in each of the systems at a greatly reduced total number if the operator plans to also hatch the eggs within the chosen incubation system (for instance, a 1-metre combi tank should be stocked with only 20 000 eggs per shallow insert if hatching in the tank is desirable). Stocking density will affect the growth rates and overall health of Atlantic salmon as they begin to feed. Optimal conditions for fry are to maintain a stocking density of 30 kg/m^3, but this is often pushed to 50 kg/m^3. Stocking density should be high enough to ensure competition to stimulate feed response, because fry that are stocked at low densities, such as less than 5 kg/m^3, lose interest in food.

Atlantic salmon egg size may vary but generally range from 0.1-0.4 g. Alevins grow as they absorb nutrients from the yolk sac. After the yolk sac is absorbed and "buttoning up" occurs, the alevin become fry and must start feeding to survive. Fry mortality rates significantly decrease after first feeding is successful and fry reach 1 g. Ongoing growth rates depend on many factors such as diet, husbandry, water quality and temperature. There are numerous growth models for Atlantic salmon at various life stages (Piper et al., 1986; Aunsmo et al., 2014) that are often available from commercial feed suppliers that will also take various factors into consideration, such as desired size by a specific date and/or budgetary constraints. Atlantic salmon fry will transition to the fresh water grow-out stage after 10 g. See Piper et al. (1986); Heen, Monahan and Utter (1993); and Stead and Laird (2002) for additional information.

Feeds (types, sources, composition) and feeding (rates, methods)

Feeding is the single most important component and greatest cost item in commercial aquaculture operations. When salmon hatch they start to absorb their yolk sac, but the fry must begin to consume outside feed sources as the yolk sac diminishes to survive. It is important to start the fish on a dry manufactured diet as the yolk sac reaches 3-5% of the total body weight. If this is delayed, then "pinheading" of the fry and eventual mortality may result. Feed rate starts at 6% body weight per day spread out over hourly feeding events during the 24-hour photoperiod if possible. Waste feed should be visibly apparent in each tank to ensure that all fry have access to feed but not large quantities of wasted feed that may deteriorate water quality conditions. After salmon fry reach a size of 1 g, the feed rate will decrease to 4% body weight per day and this rate will continue to decrease as the salmon grow larger.

Atlantic salmon hatcheries rarely use feed that is not a properly balanced diet manufactured by a professional feed mill. These manufactured diets start at a 0.3 mm crumble and increase in extruded pellet up to a 12 mm pellet. Fry start with the 0.3 mm crumble as the yolk-sac salmon begin to swim up and display an interest in foraging for food. At this time, the surface of the tank is lightly dusted to stimulate fry appetite. These starter feed diets are high in protein (52-58%) and (18-20%) lipids.

Commercial Atlantic salmon diets vary to some extent based on the physiological needs of the salmon at different stages of the production cycle. Basic information on these diets can be obtained from the feed companies directly (e.g. Skretting[4] or local representatives supplying the feed). There also might be some variations in diets by country as well. Several books deal with the specifics of fish nutrition and specifically Atlantic salmon, such as Lovell (1989) and Halver and Hardy (2002). Holt (2011) specifically references larval nutritional needs.

Diseases, pathogens and parasites

There is always concern for potential disease impacts throughout the production cycle. Although many of the same pathogens are of concern regardless of life stage, some diseases are more prevalent in the early stages. For example, in Norway, fungal infections (mainly *Saprolegnia* spp.) on eggs and fry (Bruno and Stamps, 1987; Woo, 2010) are the primary concern in the early stages of production. However, different types of gill pathologies with bacterial or environmental causes are not uncommon. In Canada, regional issues with viral hemorrhagic septicaemia virus, *Yersinia ruckeri* (enteric redmouth disease) and *Saprolegnia* spp. are of particular concern for egg incubation and fry production. In Chile, only *Saprolegnia* spp. is reported to be of importance.

Saprolegnia fungus (actually classified as a protozoan) is the most prevalent issue when incubating eggs and has an appearance of cotton mould that begins growing on dead eggs. The fungus is able to grow and reproduce fast under optimal conditions (high water temperature, elevated stocking density, dead and decaying eggs present), but can be controlled through good husbandry practices. Regular treatments typically with iodine-based disinfectants (e.g. Wescodyne™, Ovadine™) or diluted formalin are required after *Saprolegnia* is visible from the tank surface (Brown, 1994). *Saprolegnia* can continue to be an issue throughout early life stages, but only causes low level mortality when properly dealt with to prevent escalation of the issue.

Bacterial gill disease (BGD) describes infections to fish gills caused by several different species of bacteria, with the principle etiologic agent in Atlantic salmon being *Flavobacterium branchiophilum*. Salmon affected with BGD tend to orient themselves upstream toward the tank inlet as the gill lamellae are being suffocated. Gill covers are flared and can be seen from the surface and a heavy mucus layer will cover the gills. Salmon affected by BGD will be lethargic and often found lying on the tank bottom. However, as mentioned previously, a diagnosis should never be made based on visible symptoms alone. BGD is generally a result of inadequate husbandry, leaving the tanks with excess organic matter. This is largely the result of poor water conditions from overfeeding. Acute mortality will occur without proper care and treatment.

Gas bubble disease results from supersaturation of water and falls under the category of "non-infectious diseases" (Kent and Poppe, 1998a; Woo, 2010). The dissolved gas may leave the bloodstream and form air bubbles in the skin, organs, eyes or gills. This may be the result of sudden temperature gradients, especially warming the rearing water, or air entraining into the water typically from leaks in pumps or pipes. It can occur quickly in the larval salmon stages and salmon can darken as a result or become blind. If this condition is not addressed, acute mortality can occur. The first step to treating gas bubble disease is to immediately find and fix the issue causing supersaturation. Degassing within the production system to ensure that a normal level of total gas pressure (under 102%) is maintained will prevent gas bubble disease.

Furunculosis, the disease caused by the Gram-negative bacteria *Aeromonas salmonicida*, can also occur during these early life stages (Woo and Bruno, 2011; Austin and Austin, 2007; Brown, 1994). Furunculosis is exacerbated by stress, low oxygen levels and high densities. Outbreaks occur most often at temperatures above 10°C, the disease is highly infectious and can cause acute infections with rapid onset of mortality. Disinfection of fertilised eggs is the most important intervention against furunculosis in hatcheries and this treatment is obligatory in Norway. Effective vaccines exist to prevent this disease, thus it is no longer considered a major problem to salmon farming.

Enteric red-mouth disease (ERM) is caused by the highly pathogenic bacterium *Yersinia ruckeri* (Woo and Bruno, 2011; Austin and Austin, 2007; Brown, 1994). This disease is also often a result of poor water quality. It can be evident as a chronic or acute infection as salmon seem to be able to withstand high numbers of the bacteria without developing the disease. However, if stress is introduced the salmon may show signs of reddening around the throat and mouth areas and will be lethargic and lose interest in feed. Fry are most susceptible to ERM.

Drug treatments, vaccines, and usage of chemicals for cleaning and disinfection

No injectable vaccines are used on fish below 10 g. However, there are a variety of dip vaccines available that are administered as static baths based on biomass and administered to salmon as small as 2 g. Dip vaccines require that the fry are placed into the dosed bath water for 15-60 minutes depending on the specific vaccine requirements. Commonly used dip vaccines treat against ERM and furunculosis infections as well as many others including various forms of *Vibrio* (Brudeseth et al., 2013).

Egg surface disinfection one to three hours post-fertilisation is a common practice prior to laying the eggs down in their incubation environment. Ovadine™, containing 10% povidone-iodine, is frequently used as a surface disinfectant of fertilised eggs. Treatment may be completed during or after water hardening with the concentration and duration both adjusting the dose depending on the specific time of treatment. Egg surface disinfection delays the growth of fungus during the sensitive egg development stage when no other handling can be completed. These treatments, coupled with removal of unfertilised dead eggs from the incubator, should sufficiently retard fungal growth until the eggs have eyed, allowing another treatment to occur.

Bath treatments of formalin, an aqueous formaldehyde solution, are the most common treatment to control fungus from the eyed egg stage onwards. Formalin baths may be used several times a week with the dose changing based on a static (more diluted for a longer duration) or flow-through (more concentrated for a shorter duration) treatment (Brown, 1994). Formalin treatments can start at 110 degree days and continue until the first alevin is seen and may then continue again after first feeding. Formalin may also be cautiously used as a diluted bath to treat fungus in larger salmon. Use of formalin is expected to be phased out in the near future, resulting in considerable research to find an alternative, less toxic solution for use in hatchery environments. One alternative to formalin is the use of salt in a static bath or flow-through application multiple times each week.

Hydrogen peroxide is another alternative to treat fungus and BGD. Eggs can be treated using 500-1 000 mg/l for 15 minutes every day if required until salmon hatch. When required for fry or juveniles, a 100 mg/l dose can be used for 30 minutes for 3 treatments every other day. Hydrogen peroxide is considered an environmentally friendly alternative as it slowly breaks down into its constituent parts of oxygen and water. Use of hydrogen peroxide comes with some risk as it can be highly reactive to organics in the water so tanks should be cleaned before applying this treatment. In addition, it can be highly toxic to fish gills at temperatures of 14°C and higher (Bruno and Raynard, 1994; Roth, Richards and Sommerville, 1993).

In-feed or static bath antibiotic treatments (usually via veterinary prescription) can be administered if needed for most bacterial infections. However, BGD is not typically treated with an antibiotic, rather alternative bath treatments are more often used, such as hydrogen peroxide or Chloramine T at a supplier recommended dose.

Additional information can be found in Brown (1994); Scarfe, Lee and O'Bryen (2006); Austin and Austin (2007); Woo and Bruno (2011); and Gudding, Lillehaug and Evensen (2014).

Disposition of waste and carcasses

Most jurisdictions have developed regulations and policies outlining how and where hatcheries may dispose of effluent wastewater and carcasses. Certainly the majority of new hatcheries use technology to recirculate the rearing water to manage water budgets and reduce the level of effluent leaving the hatchery. These systems often use mechanical filtration, such as a drum filter or settling pond, to remove solids from the water leaving the hatchery production system. Some facilities also use new polymer technology as an option to bind and collect phosphorus before it exits the hatchery and enters the receiving ecosystem.

Jurisdictions often establish and certify specific facilities to dispose of diseased and contaminated animals. Regular non-diseased mortalities are often sent to a landfill for disposal. Some hatchery facilities will opt to incinerate all dead fish removed from the facility regardless of the presence of an infectious pathogen.

Freshwater grow-out and smoltification

Physical environment (tanks, nets, cages) and containment conditions

Larger salmon have different rearing requirements that will dictate the type of containment. Major considerations are cost and utilisation of volume that will allow the salmon to have appropriate water velocity, velocity distribution, removal of solids and numbers of exchanges (Pennell and Barton, 1996). The source of water available will determine whether flow-through, reuse or recirculation systems are best.

Salmon hatcheries and farms most commonly use circular fibreglass tanks. The main advantages of circular tanks are their self-cleaning capabilities and the fact that the velocity of the flow can be set independently of the incoming flow level. Raceways are long rectangular basins primarily constructed with reinforced concrete or polyester resin, but earthen raceways can also be made using plastic liners. Raceways are certainly less frequently used to culture salmon, but can provide a good use of space in some situations. Raceways require a high level of water flow to remove solids and distribute oxygenated water the entire length of the raceway. Baffles may be added along the length of the raceway to allow some self-cleaning capability, but may add difficulty when distributing feed to the entire stock. In Chile, it is common to transfer fry to cages in freshwater lakes for further grow-out until smoltification. However, this practice is less favourable owing to the disease situation in the lakes, therefore, more and more of the smolt production has been moved to large recirculation farms. The Scottish industry also uses cages in lochs for smolt production, although to a lesser extent than in Chile. It is not permitted to produce smolt in open cages in fresh waters in Norway.

Rearing environment (water flow, DO, temperature, lighting/photoperiod)

Smolt production facilities vary in the technology present to limit the amount of new water entering the hatchery for production. Flow-through facilities use water once as it enters the system, passes through the tanks on a single pass then exits the system returning to the receiving environment. As the name suggests, a reuse system will reuse a portion of the production water (typically up to 50%) to offset some of the total facility

water demand. Reuse facilities will filter the portion of effluent to be sent back to the production tanks prior to reuse, but will not integrate a biofilter given the high volume of new water entering the system. Recirculation systems reuse the majority of the system water volume and this can be as high at 99%. This strategy requires the effluent water to be fully filtered to remove particulate waste, stripped of gases such as carbon dioxide and nitrified to remove toxic levels of ammonia using many options for filtration, sterilisation, degassing and biofiltration. Water quality monitoring in flow-through systems is primarily concerned with water oxygen levels and temperature. Recirculation or high-level reuse systems can present significant risks to the fish stock if comprehensive water quality monitoring is not implemented, including measurement of total ammonia, ammonia nitrate, ammonia nitrite, CO_2, alkalinity and pH. Various buffers may be added to the water filtration systems as necessary to maintain these parameters within acceptable limits. Kolarevic et al. (2014) compare the performance of Atlantic salmon reared in flow-through and recirculation systems.

In all cases, facilities are likely to integrate filtration and disinfection strategies (e.g. bead or drum filters and UV disinfection) on the new incoming water as necessary to ensure that the quality of the water meets production requirements and is disease-free. Likewise, regulatory requirements generally require some filtration of effluent water regardless of the volume involved from smolt production facilities to minimise the effects on the receiving ecosystem.

Hatchery operators must be aware of dissolved organic material even after particulates are filtered from the reused or recirculated water. Ammonia is toxic to Atlantic salmon and must be converted to less toxic compounds before the reused water can be sent back to the hatchery tanks, especially in a high recirculation system. The first step in this process converts ammonia to a nitrite (NO2-) by several genera of bacteria, including *Nitrosospira* and *Nitrosomonas*, within a biofilter. Nitrite is still toxic to fish and is the cause for brown blood disease if nitrite levels are not kept below 0.1 mg/l. A second step in the conversion process involves *Nitrospira* and *Nitrobacter* bacteria within the same biofilter to convert toxic nitrite to nitrate (NO3-), which is not harmful to salmon under 250 mg/l. These nitrifying bacteria require oxygen and alkalinity to grow and reproduce in a biofilter. It is therefore important to keep pH between 6.8 and 7.5 and CO_2 levels under 12 mg/l. Buffering of recirculation systems is nearly always done to balance the water pH and maintain alkalinity above 70 ppm as the bacteria will not thrive below this level. There are many fish-safe products available to serve as a buffer and the specific type used will depend on the specifics of the system. Small systems needing a slight boost can add sodium bicarbonate. A dosing system may be used that continually drips in a buffer such as soda ash or caustic soda. Soda ash is a slow-release buffer and does not react quickly to raise the pH levels whereas caustic soda, liquid or bead form, can rapidly regulate the system.

It is imperative for fish health and growth to have the proper water flow for the rearing containment or type of system. Stocking density will change the degradation of water quality due to salmon faecal production and respiration, including oxygen consumption and CO_2 and ammonia production. These parameters will determine the number of exchanges of water required in the holding tank in a particular period of time or the turnover rate. Depending on tank size, water can typically be provided to allow a calculated turnover every 30 minutes to 3 hours. Incoming water flow may enter directly from an open pipe or pass through a spray bar or upweller depending on the system requirements to also add oxygen or strip gases at the time of water entry. Water velocity in the tank or raceway is another important consideration to ensure the fish have

adequate current to require constant swimming without exhaustion or being pushed backward when resting. Velocity can be set independently of incoming water flow in a circular tank by changing the angle and placement of incoming water, thereby creating a vortex effect. Water velocity in a raceway is dependant on the amount of incoming water flow, but can be assisted using baffles or weirs to change the flow dynamic. Water velocities of 0.5-2.0 times fish body length per minute are optimal for maintaining fish health, muscle tone and respiration (Losordo and Westers, 1994). The resulting spinning effect of the water in a circular tank also helps to remove solids from the tank with some additional cleaning required, but husbandry time should be decreased.

Oxygen parameters are the same as discussed for fry. Almost all rearing systems for grow-out require O_2 supplementation and may allow an increase in the tank-carrying capacity. In circular tanks the oxygen level will generally remain consistent throughout the volume of water. In raceways, oxygen levels become depleted the further they are from the source of incoming water.

Temperature of fresh water can range from 0-18°C depending on the season, fresh water source and system design. Warmer temperatures allow for higher food conversion rates and faster growth, but only to about 16°C. Higher water temperature can be detrimental to the stock health and performance. If recirculation systems are used then bacteria health within the biofilter must be a factor as the bacterial population can die if water temperatures fall below 4°C.

A 24-hour photoperiod can be used from fry into juvenile stages to optimise feeding and growth, but must be eventually reduced to ensure normal development of the light-brain-pituitary axis that is vital for smoltification (Ebbesson et al., 2007). A winter photoperiod, an 8- or 12-hour dark phase every 24 hours, should be started in late December or January in the northern hemisphere for a period of at least 6 weeks (this is approximately a year after production). The photoperiod can be returned to ambient for the time of year after this required winter period is completed. The increasing length of day experienced under ambient photoperiod for this time of year will trigger the physiological responses required to handle the stress associated with the transition to full salinity water during smoltification. Growth will be stimulated in the spring and a 24-hour photoperiod can be used to increase feeding, weight gain and therefore condition factors before the salmon are transferred from the hatchery as smolts into the marine environment for continued grow-out.

Commercial producers often size grade fry and subsequently divert individuals into two different production strategies around mid-summer in the first year of production – namely production of S0 or S1 smolts that are transferred to seawater at different times of the year. The fish that are chosen for S0 (0+ or underyearling smolts) production are the larger individuals in the size grading and are manipulated by photoperiod to smolt earlier than the later S1 smolt. The S0 smolt photoperiod is started approximately 12 weeks before the planned seawater transfer, and is initially a 6-week rearing period under short day lengths (12 hours or shorter) followed by 6 weeks on continuous light (Hansen et al., 1998a, 1998b; Bjørnsson et al., 2000). However, there is considerable variation in the industry on the specifics of this generally recommended photoperiod regime. The S0 smolt is transferred to seawater at the end of this intensified light regime in late summer/autumn and their age is less than one year from fertilisation. The size of an S0 smolt is 40-90 grams.

The smaller fry at the time of sizing grading remain in the hatchery for S1 smolt production. These individuals are normally transferred from continuous 24-hour light to an ambient photoperiod sometime between mid-summer and October. They are reared

under this ambient photoperiod until smoltification in the spring, 14-17 months after fertilisation. However, this light regime can also be varied with 24-hour light continued until approximately December, when a winter photoperiod of 8- or 12-hour dark phase every 24 hours is necessary in the northern hemisphere for 6 weeks. After this winter period, the photoperiod can be returned to ambient and the increasing length of day will trigger the physiological responses required to handle the stress associated with the transition to full salinity water during smoltification. As stated, growth will be stimulated into spring and a 24-hour photoperiod can be used to increase feeding, weight gain and therefore condition factors before leaving the hatchery as smolts to enter the marine environment for continued grow-out. The S1 smolt is transferred to seawater in early to late spring. The size of an S1 smolt is 60-200 grams.

Fish sizes, densities, growth rates

Although salmon range somewhat in size and condition factor before smoltification, it is imperative that all individuals have sufficient fat stores available before going in salt water to survive the stress associated with transitioning between fresh water and salt water and the resulting period before fish begin to feed again post-transfer. Ideally smolts should be a minimum of 60 g with a 1.2 condition factor as calculated based on Anderson and Neumann (1996) as follows:

$$Condition\ Factor = \frac{Weight\ (g)}{Length^3\ (cm)} x\ 100$$

Growth rate parameters in smolts are similar to fry with regards to parameters such as per cent feed per day (see above).

Stocking density is a balance between maximising use of tank volume while maintaining high growth rates while not compromising fish health and welfare. Stocking density of 30 kg/m^3 is optimal but 30-60 kg/m^3 is typical as specific system considerations allow a much higher density. The specific stocking density will be unique to the farm and ultimately is dependent on water quality, water turnover rates, temperature and dissolved oxygen demand.

Growth variations in individuals of the same age can cause issues in managing hatchery production. A large range of sizes in the same tank will result in considerable competition amongst individuals, use of inaccurate pellet sizes for feeding and can compromise the overall health of the population. Size grading separates salmon into similar size groups and allows an opportunity to cull the smallest grade of salmon for economic and fish health concerns. The grading process and type of grader used will vary depending on the size of the farm. Smaller facilities may use bar graders to grade a single tank or raceway. Bar graders are labour-intensive, requiring the fish to be poured into a hopper and the small fish subsequently swim and/or are pushed between bars while the large fish stay on the bar surfaces and move to another tank. Roller graders are presently the most common type used in larger hatchery facilities and use a series of continually rolling aluminium bars that direct the salmon to move through at a particular area. The rollers are spaced apart by specific distances such that small distances are positioned towards the front of the grader and this distance increases further along the length of the unit. Some hatcheries may still use belt graders that use a similar strategy, but involving two belts that widen as the fish move along the belt length and allow the fish to pass through depending on its girth, thereby grouping individuals of a common size range into a single tank. Each smolt production facility will have different growth rates and size separation of individuals that

will determine the frequency that size grading is required. Fish size grading can be completed at almost any stage of the hatchery life cycle past first feeding.

Feeds (types, sources, composition) and feeding (rates, methods)

Freshwater grow-out feed is most commonly a properly balanced diet of manufactured extruded pellet. Protein levels in most diets are 46-48% and lipid levels are 26-30%. Pellet size will depend on the weight of the salmon. Transitioning to larger feed sizes should be completed slowly, with gradual increases of the larger size in a mix that typically begins as a 50:50 blend of the two sizes involved in the transition. Transitioning salmon to the next largest pellet size is important when possible as less energy will be expended to consume a single larger pellet compared to multiple smaller pellets, thus resulting in higher food conversion rates.

Temperature and photoperiod are the two most important considerations when determining the total per cent body weight in feed to offer the population each day, but this will range from 0.1% to 3% body weight per day. Atlantic salmon held at temperatures above 4°C can be fed daily, but below 4°C feeding events should diminish to one to five times per week. The presentation of feed will also change as salmon grow. Fry initially require feed to be presented continually throughout the photoperiod, but this practice changes to discrete meals during grow-out. Meals are presented to feed the population to satiation 2-12 times per day using automatic or hand feeding. Periodic size grading will help to eliminate the size range in the tank; however, not all salmon will aggressively feed requiring the presentation of numerous meals and feeding to satiation. Extending each meal over a period of time will allow the highly competitive individuals within the population to ingest the first part of the feed presented then provide the less aggressive individuals feed during the latter part of the meal. Hand feeding some of these meals each day is highly recommended to allow an opportunity for hatchery staff to monitor the behaviour of the population to assess overall fish health. See general nutrition references above for additional information.

Diseases, pathogens and parasites

All fish health issues and treatments for fry are applicable throughout the entire freshwater grow-out phase. However, there are other pathogens to consider with larger salmon in fresh water.

Viral and bacterial diseases of salmonids have received much attention owing to the severe pathology associated with most infections and the ubiquity of these pathogens (see reviews in Kent and Poppe [1998b]; Woo, Bruno and Lim [2002]; Toranzo, Magariños and Romalde [2005]; Austin and Austin [2007]; Woo and Bruno [2011]).

Bacterial kidney disease (BKD, caused by *Renibacterium salmoninarum*) is a slow-growing Gram-positive pathogen that is present in many watersheds and shown to be vertically transmitted from the female. However, there is discussion as to whether males may also be involved in transmission, so hatcheries often also inject males with erythromycin prior to spawning. Infected fish can take months to show symptoms of BKD, but the disease can result in considerable losses of stock. Screening programmes for broodstock have helped to control the vertical transmission of the pathogen across generations. The infection rate of BKD in cool water tends to be slow, but it increases in warm water. Symptoms of BKD can include pale gills and distended abdomen with greyish white nodules on the spleen, liver and kidney (Roberts, 2012; Austin and Austin, 2007; Brown, 1994); however, these should be not used as the sole method of diagnosis.

Columnaris disease is caused by the Gram-negative *Flavobacterium columnare* bacteria. It can cause acute or chronic mortality. Typically, infection can be seen on the gills, skin and fins. It has a white patchy appearance, with yellow pigment, and, if caught early, can be treated externally. It is sometimes referred to as "saddleback" disease as often the infection will be localised at the base of the dorsal fin.

Tenacibaculum maritimum was previously referred to as *Flexibacter maritimus*, but changed classification in 2001 (Plumb and Hanson, 2011). It is one of the most prevalent health issues in salmon culture and a major cause of mouth rot and fin erosion in cultured salmon held in salt water. It is taxonomically related to *Flavobacterium*, thus results in similar features and symptoms; however, *Flavobacterium* is common in fresh water. The rate of infection can be controlled with bath treatments or antibiotics. *Tenacibaculum* sp. can occur in hatcheries with saltwater intake.

Whirling disease (caused by *Myxobolus cerebralis*) has been of great concern among trout hatcheries in the United States, such that there have been many regional initiatives to encourage research collaboration among states, promote public awareness and mitigate spread of the parasite.

Of similar concern, the parasitic monogene fluke, *Gyrodactylus salaris*, has been frequently used as a "worst case example" of what could happen to wild fish populations after release of hatchery-reared fish into streams. During the 1970s, *G. salaris* originating from a Baltic stock in Sweden was imported to aquaculture programmes in Norway and later spread through stocking of Atlantic salmon from a few infected hatcheries to several Norwegian rivers (Johnsen and Jensen, 1991; Bakke et al., 2004; Jansen, Matthews and Toft, 2007; Buchmann and Bresciani, 2006). The parasite also spread to neighbouring native wild populations of Atlantic salmon, resulting in catastrophic losses (average *ca*. 85%) in over 40 Norwegian rivers.

Parasites do not normally cause such extensive harm to their host to the point of becoming lethal. Part of a common theme with the above problematic parasites is that a parasite has encountered a "new host", thus there has been no period of co-evolution of host and parasite. The parasite (*M. cerebralis*) that causes whirling disease was first introduced into the United States via transfer of brown trout (*Salmo trutta*) from Europe. Brown trout are carriers or reservoir hosts, whereas rainbow trout (*Oncorhynchus mykiss*) are highly susceptible, thus the rapid spread. Sometimes the new host is not a new species of fish but rather a different strain or phylogeographic unit (e.g. the accidental transfer of *G. salaris* into Norwegian rivers), thus further emphasising the importance of understanding salmon broodstock and population genetics.

Ichthyophthirius multifiliis, commonly referred to as ich, is a singled celled protozoan that has three life stages, only one of which is treatable. These parasites are mostly found in facilities that receive incoming water from surface water sources. Infections are most often an issue in hatcheries that use outdoor rearing ponds or have no filtration. Salmon infected with ich become agitated, hyperactive and rub their gills against surfaces, referred to as flashing (Woo and Buchmann, 2012). Ich can cause acute mortality if left unchecked without treatment.

Costia (*Ichthyobodo* spp.) is a single-celled, flagellated parasite that lives on the skin and gills. Salmon can handle low-level infections and symptoms include flashing and rubbing, lethargy and laboured breathing.

Brown blood disease is caused from toxic levels of nitrite in the production water. This non-infectious disease changes the gills from red to a brown colour because the oxygen in the blood haemoglobin is bound, thereby starving the cells of O_2. Proper care and monitoring of water quality helps to prevent this disease from occurring. Feeding of the fish population should immediately be reduced when nitrite levels begin to escalate to give the bacteria present in the biofilter an opportunity to respond and begin converting the nitrite into the less harmful desirable nitrate. Addition of salt can prevent this condition (Woo, 2010; Brown, 1994).

Infectious pancreatic necrosis (IPN) is caused by a virus (infectious pancreatic necrosis virus, IPNV). Salmon with IPNV may have a swollen abdomen or eyes (exopthalmia), darkening of the skin, exhibit spiral swimming and faecal casts trailing from the vent. Internally, IPNV may be characterised by pancreatic necrosis, catarrhal exudates in the intestine and haemorrhages in the visceral organs. These symptoms are similar for most viral (and some bacterial) infections, further emphasising the importance of not using visible symptoms alone for diagnosis. IPN is managed through health testing (broodstock screening) and biosecurity measures. Several vaccines exist for IPN and a published DNA marker is available if its use is desired for screening broodstock (Moen et al., 2009).

In any aquaculture operation it is essential to minimise stress in the fish because stress causes release of cortisol and other steroids into the bloodstream which block or suppress various pathways of the immune system (Roberts, 2012; Brown, 1994). Similarly, when salmon are undergoing smoltification, their immune system is compromised due to the high levels of hormones in the blood and dynamic physiological state (Roberts, 2012). Each aquaculture operation will use various techniques to minimise stress when handling fish (e.g. use of anaesthetics, no feed prior to and after handling).

Drug treatments, vaccines, and use of chemicals for cleaning and disinfection

One of the largest economic impacts on the aquaculture industry is loss of animals from disease. A conservative estimate of 5% loss means the finfish aquaculture industry loses USD 1 billion annually on a global scale (Dixon, 2012). Intraperitoneal (IP) or intramuscular (IM) injectable vaccines are available during the pre-smolt stage that will protect salmon from a variety of pathogens upon entry into salt water. Pharmaceutical companies have different products that will target specific pathogens with numerous specific pathogens typically covered within a single injection of vaccine. Most commonly used multivalent vaccines treat against the outbreak of vibrio, furunculous, IPN, BKD, infectious salmon anemia and *Moritella*. Injection vaccines have a number of advantages providing a longer duration of protection and allowing multiple antigens to be mixed in one dose. IP vaccinations are usually completed on fish over 30 g and often require at least 500 degree days before transfer to seawater to ensure the development of sufficient disease resistance and to achieve proper efficacy. All salmon are vaccinated prior to transfer to salt water as a standard, often mandatory, practice. For example, in Norway it is mandatory to at least vaccinate against furunculosis, vibriosis and cold water vibriosis.[5] Vaccination at high temperatures can have undesirable side effects (Berg et al., 2006) and it is recommended to vaccinate at temperatures below 15°C. An opportunity is present during vaccination handling to remove and cull all sexually mature male parr and malformed individuals.

All internal bacterial infections may require an in-feed antibiotic treatment to control the effects of disease and transmission. The veterinarian will determine which antibiotic is best to use and the product will be released and controlled via a prescription. External bacteria or parasites can be treated or controlled using bath antibiotic or therapeutant treatments.

Following strict biosecurity procedures as part of a good fish health management plan will help prevent spread of pathogens and will increase the overall health of animals. Common practices to implement include detailed record keeping, the use of footbaths, hand washes and clean gear (e.g. nets, scrub brushes, etc.) not shared between buildings, year classes or species (if multiple species are housed on a single site). Numerous chlorine and iodine-based disinfectants are available commercially to kill most types of bacteria and viruses on contact or following an established duration of contact. These disinfectants are commonly used in footbaths and tank cleaning. Ethanol 70% may also be useful for cleaning small areas and instruments. Thorough cleaning and disinfection should be completed when entire systems are empty and before the next population of fish are added to the tanks. A combination of products can be deployed to better clear all biofilm, especially in areas that are difficult to manually scrub. Caustic soda may be used to raise the pH of the cleaning water and causing all pipes and surface areas to shed material. Bleach, Virkon™ or iodine can be used as a secondary sterilisation agent. A dry period should always follow cleaning and disinfection procedures. Water passing through cleaned and disinfected systems should be tested to ensure pH and chlorine are within normal limits before adding fish stock to the tanks because most of these cleaning agents are highly toxic to fish.

Disposition of waste and carcasses

Disposition of waste and carcasses is the same as the hatchery phase.

Marine grow-out

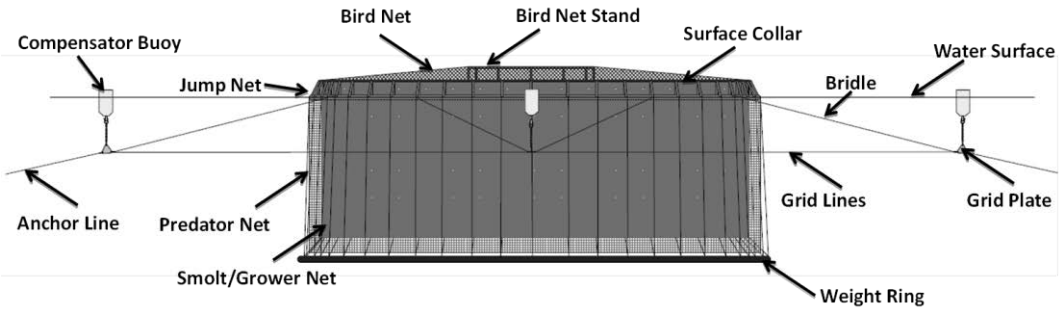
Physical environment (tanks, nets, cages) and containment conditions

Atlantic salmon marine grow-out sites exist in a wide variety of oceanographic conditions, but the vast majority of farm operations are sited near the coast in protected or relatively low energy environments. However, more and more new farm sites are now being developed in more exposed locations where use conflicts might be less, but the oceanographic energy is dramatically higher. Design and installation of marine grow-out sites are fairly consistent at a high level globally and generally well-known for those involved in the industry with some modifications expected on a site-by-site basis. General discussions related to marine set-up and operations may be reviewed in Beveridge (1996), Bridger and Costa-Pierce (2003), Costa-Pierce (2002), Landau (1992), Stickney and McVey (2002), and Willoughby (1999).

Atlantic salmon are raised in net pens through the marine phase of the grow-out cycle. The vast majority of grow-out cages used globally are classified as "gravity cages" as these net pens hang a net within the water column and rely on the force of "gravity" to maintain shape and volume (Figure 3.12; Loverich and Gace, 1998). Other cage types have been offered to the industry by commercial suppliers, especially for higher energy environments, which are more rigid in design. However, the industry has been slow to adopt new cage designs given simplicity in design, ease of operations and the lower cost per cubic metre of growing fish in gravity cages compared to other cage designs. Maintaining complete integrity of each net pen is essential to ensuring full containment of the Atlantic salmon stock.

Gravity cages have a structural floating surface collar that provides the required surface buoyancy from which the containment nets are hung to retain the stock of fish within a defined volume of the water column. Surface collars are primarily manufactured using

Figure 3.12. **Typical gravity net pen arrangement used predominately throughout the global Atlantic salmon farming industry**



Source: Bridger, Bridger and Jensen (2015).

high-density polyethylene (HDPE) pipe although wooden and steel surface collars are also sometimes used in some jurisdictions globally, but in environments that are much more benign from a surface wave perspective. The majority of HDPE collars comprise two separate concentric pipe rings that are connected using a series of stiff uprights or stanchions. Some HDPE collars may have three rings to provide a wider work platform and more robustness in higher energy environments. Regardless of the number of collar rings or the size of pipe used, the total buoyancy provided by the entire surface collar must offset the downward forces experienced from the weight of the series of nets with expected biofouling, weight ring and downward forces from the mooring system. Redundant buoyancy is provided in the surface collar rings by filling them with Styrofoam plugs in the unlikely case the structural integrity of the surface pipes is compromised (from structural fatigue and damage, poor workmanship, or vandalism) allowing water to enter.

The primary containment net hangs from the surface collar and is comprised of a twine mesh that is typically sized based on government requirement, stock insurance policy or fish farm experience to prevent fish escape. At least two containment nets are used throughout the grow-out cycle, including a smaller mesh smolt net used immediately when smolt are entered and a larger mesh grower net that replaces the smaller mesh when the smolt net is fouled and fish are large enough to not escape. The containment net is tied to the internal collar float pipe and has an upper jump net portion (typically about one-metre high) that extends from the water surface to the collar handrail. The jump net prevents the escape of farmed fish as they frequently jump out of the water. Various materials have been used for the containment net depending on the objectives of the fish farm operators. Today, most containment nets favour knotless nets, primarily of nylon or polyamide material, to decrease total material required to manufacture a net and therefore decrease the total net weight and associated costs of a net having knots.

Two other nets are typically deployed within a complete net pen system with both having primary roles to keep predators away from the target fish stock:

- Bird nets spread across the entire open surface area of individual net pens and serve as a deterrent to predatory or scavenging bird species. Bird nets are typically held up from the water surface using a bird net stand that is manufactured of HDPE pipe and positioned in the middle of the collar circumference. Bird nets tend to be deployed as a permanent part of the net pen system; however,

these nets are sometimes removed when the contained Atlantic salmon reach the target harvest size and the operator is preparing to harvest.

- Predator nets are deployed in specific jurisdictions and often seasonally as necessary (based on regulatory or insurance requirements) to protect the contained fish from aggressive large fish, marine mammal or shark predatory attacks. Predator nets are tied at the water level to the outer collar floatation pipe and extend down into the water column beyond the depth of the containment net and often completely encircling the containment. A shark guard net is often deployed in areas having a high shark population and attached outside of the bottom of the containment net. Predator nets serve absolutely no purpose to the containment of the Atlantic salmon stock and therefore use net mesh that can be several times larger than the biggest containment net mesh.

Net bagging and other distortions to the cylindrical shape of the containment net must be avoided to provide optimal growing conditions to the contained Atlantic salmon stock within each net pen. This is easy to achieve in locations having no (or very low) current. As current is introduced, the flexible net hanging from the surface collar will follow passing water current and result in considerable net bagging and loss of grow-out volume. Net bagging will increase fish stress, fish mortality and product downgrades through exterior abrasion of the fish on the bagging containment net and overstocked populations. In low current, tying small individual weights to soft eyes integrated in the intersection between the side and bottom net panels will hold the net pen volume through gravity. In higher current velocity, gravity cages may be tied directly to a continuous weight ring (or sinker tube) made from HDPE pipe filled with sand, concrete or steel wire cable/chain in the same general location to maintain the net shape and volume.

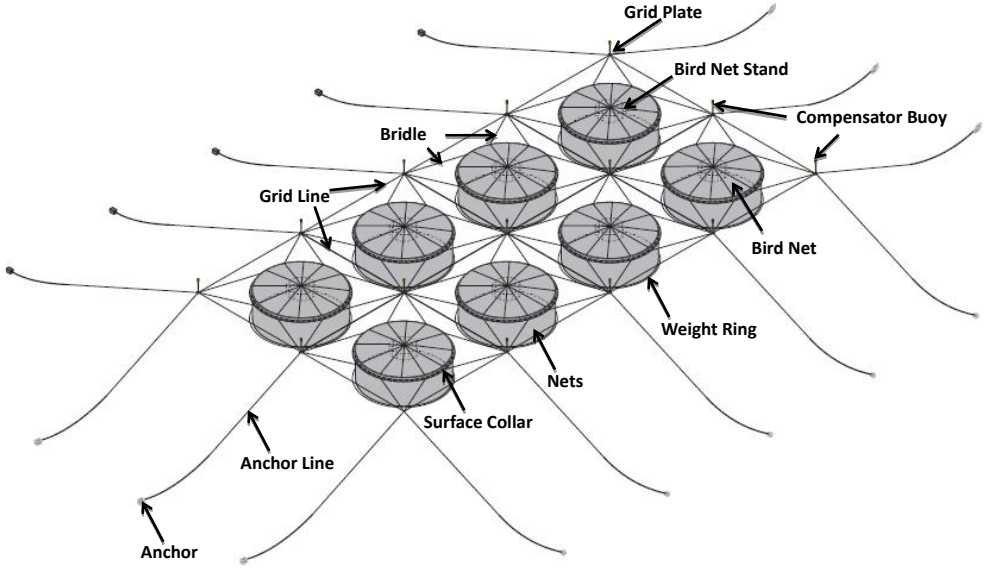
Net pens are held spatially in a leased area of ocean space using an appropriate mooring design that accounts for system restraint spatially (i.e. mooring stiffness) and an appropriate degree of movement to allow for storm surges and tidal ranges (i.e. mooring elasticity). Net pens may be moored individually or within a group, frequently referred to as a flotilla. Mooring net pens individually employs three to four mooring lines that connect the surface collar to the seabed. However, the most common mooring strategy is to use a submerged grid system, with anchor lines arranged in a catenary shape to secure a group of net pens on a site lease (Figure 3.13). The components of the anchor line (i.e. chain, rope, buoy) will be specific for the area and anticipated loads in an effort to optimise the stiffness/elasticity characteristics. The submerged mooring grid system is maintained at any depth in the water column, primarily determined by the vessel traffic that must visit the site and the oceanographic conditions present. Sites located in higher energy areas typically deploy the submerged mooring grid at a greater depth to dampen the loads experienced.

The logistics required to efficiently manage a 1 million Atlantic salmon marine grow-out site should not be underestimated. These sites tend to be remote from the nearest shore base primarily to avoid conflicts with other users of the ocean space, especially near coastal communities. Daily site visits to the farm site may be desirable but not always possible, especially in more exposed open ocean conditions due to frequent inclement weather conditions making visitation unsafe. Well-boat or road/ferry transport is most often used to deliver the smolt to the marine site to begin this stage of the grow-out cycle. Such modern well-boats can have a capacity of up to 900 m^3 and be capable of transporting up to 100 metric tonnes of live fish.

Direct handling of the culture stock is generally kept to a minimum throughout the marine grow-out period to reduce stress on the fish and resultant risks associated with poor fish

health, particularly in areas with warmer water temperatures. Certainly, the exception to this approach presently is the ongoing requirement to complete multiple transfers of Atlantic salmon to well-boats to treat for sea lice infestation. Smolt are frequently single stocked in each net pen in keeping with the desire to minimise handling, such that the harvest number and target weight provides calculated target harvest density for the specific net pen being stocked. Some fish farm operators might have little choice but to initially double or triple stock in each net pen with a plan to later size grade and split the stock into additional net pens as the fish grow. However, multiple stocking is generally not a desirable strategy given the need to time stock splitting or risk overall fish welfare issues, the inherent difficulty to equally split the stock and track numbers that are entered to each subsequent net pen, and a high risk of escape during the stock splitting procedure, especially if using an underwater swim-through approach.

Figure 3.13. **Line drawing of a group of net pens held together spatially using a submerged mooring grid**



Source: Bridger, Bridger and Jensen (2015).

Daily site visits allow the site crew to feed the fish stock, generally observe the well-being of the fish, check for the presence of predators, generally monitor the integrity of the mooring grid system and inspect collar bridle connections. Sending divers into the water at least one day each week is acceptable practice to remove dead fish and visually inspect the integrity of the containment net for holes. Technology can now replace divers to collect dead fish using air lift-up systems, but visual observations of the fish stock and nets underwater is still widely practiced. Thorough underwater inspections are also generally required in the fall to prepare for the winter storm season, in the spring as the worst storms subside, and quickly following every major storm event to observe and report on overall structural integrity of the nets and mooring system.

Rearing environment (water flow, DO, temperature lighting/photoperiod)

Early sexual maturation is one of the main problems in the production of most aquaculture species, including salmon. Sexual maturation in salmon is associated with reduced growth, loss of flesh quality and high mortality. Sexually mature salmon lose

their ability to regulate their water and salt balance in seawater, and most of them will die during the winter if they are grown in seawater. Keeping sexually mature salmon in seawater is therefore regarded as a serious welfare problem.

The occurrence of unwanted early sexual maturation (grilsing) is kept to a minimum by the use of artificial illumination. The recommended strategy for S0s is to rear them under natural light from their seawater transfer in August/October until January, after which they should be reared under continuous artificial light (Oppedal et al., 2006). This strategy reduces the incidence of sexual maturation at approximately 24 months post-fertilisation. The S1s are reared under natural light from their transfer to seawater in March-June. The artificial light is turned on in December/January. This reduces the incidence of sexual maturation at approximately 36 months post-fertilisation.

When using lights mounted above the water surface, the recommendation is to use $4W/m^2$ cage surface (metal halogen lights). Today the industry is using more and more specially developed underwater lights, which are more efficient. As a result, the unwanted illumination of the surroundings and the amount of energy needed to illuminate the cage is greatly reduced.

The fish farmer must contend with biofouling of the nets during the grow-out cycle. Net biofouling is addressed either through scheduled net changes as necessary or more frequent net cleaning while deployed in the water. Net changing requires the farm operators to carefully untie the present fouled net, place the new clean net outside of the fouled net, remove the fouled net and secure the new clean net to the collar and any weighting system at depth. Net changing is a common practice within the fish farming industry, although it does present an additional handling of fish farm infrastructure that can result in a loss of fish. The Atlantic salmon eventually grow to reach the target harvest weight. The stock is harvested, bled and returned to shore for final processing prior to being sold to the marketplace.

Fish sizes, densities and growth rates

Smolts are normally transported at densities of 30-50 kg/m^3 when the transport is done with open valves (Rosten et al., 2005). Water flow is normally not a problem as the flow in a well-boat can be three to four times the water flows used in normal smolt production. Atlantic salmon may be stocked in sea cages any time after the fish have smolted, usually when they are greater than 50 g, but typically stocking occurs at a size of greater than 70 g. It is a common practice today to stock larger smolt so the fish can better tolerate the anticipated set of sea lice that will be more of an issue for smaller fish. Production time from seawater transfer can range 7-18 months in S1s and 12-20 months in S0s. The production cycle during the on-growth period in seawater varies between companies, farms, regionally within countries and between countries. Salmon are slaughtered at a size of 2-3 kg and up to more than 10 kg. Typical target market weight is approximately 5 kg.

Feed (types, sources, composition) and feeding (rates, methods)

The same 1 million fish farm will produce upwards of 5 000 metric tonnes at a target harvest weight of 5 kg per fish. This farm will require delivery of up to 6 125 metric tonnes of feed over the course of the anticipated 18-month marine grow-out period at a food conversion ratio of 1.25. Putting this volume of feed into perspective, a standard flatbed transport truck will typically carry 22 metric tonnes as a standard load.

In a salmon farm, large quantities of feed have to be distributed every day. The feed is normally delivered to a land base or directly to the farm. The feed is transported by road or by boat in bulk or in large bags (500-800 kg). On the farm the dry feed is kept in silos and is distributed to the individual sea cages through pipes. The feed is distributed by pressurised air, or in some cases by water. The feeding systems are normally computerised and linked to a production control system. Hand feeding used to be common, but today this is only done for appetite control or as a supplement to automatic feeding during critical stages (i.e. immediately after seawater transfer). In a salmon farm there can be considerable variation in appetite between cages and from one day to another. Most farms use a system for waste feed detection, such as underwater video cameras (for inspection during feeding), dopplers that register pellets falling through the net, or lift-up systems which collect waste feed in a "funnel" hanging under the cages, and lift it to the surface with a pump.

The stock is fed multiple times each day using either feed boats that tie to individual net pens to complete the meal feeding or from a centralised feed system that manually or automatically provides calculated feed amounts to each net pen population through feed pipes that extend between the moored feeder and individual net pens. The allotted feed can be calculated from feed tables based on the biomass present, water temperature and assumed food conversion ratio. Alternatively, cameras can be used to try to monitor the feeding behaviour of the Atlantic salmon stock through detection of excess feed pellets. Successful use of cameras for this purpose can be limited in locations that have high tidal current that easily washes feed pellets away from the net pen volume, where high organic loads are present in the water column reducing underwater visibility, and in large volume net pens where the camera field of view is too small to be effective. With proper use, both feeding strategies can be used to monitor for excess feeding. In some cases a dramatic change in the feed requirement of the assumed fish population can indicate a fish health concern or loss of stock, presumably from escape, predator consumption or theft. See general nutrition references above and feed company websites for more information.

Diseases, pathogens and parasites

In salmon farming, there is a considerable problem linked to the parasitic copepod salmon louse (the main species is *Lepeophtheirus salmonis*). The salmon louse is an ectoparasite on salmon in seawater. Lice infestations can damage the skin and mucus layer and, in heavy infestation, result in osmoregulatory problems and secondary infections (Pike and Wadsworth, 1999). Injury and losses due to salmon lice are one of the main health and economic problems in salmon farming, with global estimates of cost near USD 500 million (Costello, 2009). In salmon farming regions of the world, the topic of sea lice interactions between wild and farmed fish has become quite polarised (Beamish et al., 2006, 2007; Saksida, Downey and Galloway, 2008; Marty, Saksida and Quinn, 2010; Jones and Beamish, 2011). There has been so much attention directed toward this parasite that information is now available from several salmonid host species about the immune modulation due to sea lice infection at the genomic (Braden et al., 2012) and cellular (Lewis, Barker and McKinley, 2014) level. Further research indicates the Pacific and Atlantic species of lice (*L. salmonis*) are actually quite distinct genetically and cause different pathologies, which raises the question of comparing studies from both oceans. Because of the seriousness of the situation, the Norwegian authorities have issued a regulation (FOR 2000-02-01 nr 70) as a measure for combating this problem. At sea temperatures above 4°C the fish must be inspected for sea lice at least every two weeks. The number of adult females, the number of sea lice in mobile stages (adult males and

pre-adult males and females), number of treatments, sea temperature, and the use of wrasse are to be registered and reported to the Norwegian Food Inspection Authority every month.

Between 1 December (in Troms and Finnmark, 1 November) and 1 July, fish must be treated if the average number of adult females per fish is more than 0.5, or if the sum of adult females and mobile stages is on average more than 5. Between 1 July and 1 December (1 November in Troms and Finnmark), fish must be treated if the average number of adult females per fish is greater than two, or if the sum of adult females and mobile stages is on average more than ten. Normally, all fish on the site concerned will have to be treated. An exception is made for cages with less than 0.1 mobile stages and adult females. The fish must be treated within two weeks once the threshold has been exceeded.

In British Columbia, Canada, farmed Atlantic salmon must be treated for sea lice if there are an average of three motile stages per fish, especially during the outmigration periods of juvenile wild Pacific salmon smolts (March-May).[6]

Viral diseases, in addition to sea lice, are the main problems in the marine grow-out phase of Norwegian salmonid aquaculture. Table 3.5 gives an overview of the occurrence of the most common diseases with confirmed or suspected viral aetiology. infectious salmon anemia, pancreas disease, heart and skeletal muscle inflammation, IPN and cardio myopathy syndrome (CMS) was diagnosed on 480 sites in 2011, and the vast majority of these diagnoses are from marine grow-out sites (the annual Fish Health Report from the Norwegian Veterinary Institute[7]). One of the main reasons for this situation is the lack of effective vaccines against viral diseases (Gomez-Casado, Estepa and Coll, 2011).

Table 3.5. **Number of sites diagnosed with the most common viral diseases in Norwegian salmonid aquaculture**

	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
ISA	13	14	23	21	12	8	16	11	4	7	17	10	7	1
PD	7	10	11	15	14	22	43	45	58	98	108	75	88	89
HSMI							54	83	94	162	144	139	131	162
IPN					174	178	172	208	207	165	158	223	198	154
CMS											75	76	53	74

Notes: ISA: infectious salmon anemia; PD: pancreas disease; HSMI: heart and skeletal muscle inflammation; IPN: infectious pancreatic necrosis; CMS: cardio myopathy syndrome.

The main bacterial diseases (furunculosis, vibriosis and cold-water vibriosis) are successfully controlled with the combination of vaccines and general biosecurity measures applied today in aquaculture globally. Still, cases occur sporadically but they are not considered a significant problem. *Moritella viscosa* is an important causal factor of the disease winter-ulcer, but other bacteria, mainly *Tenacibaculum maritimum*, have been increasingly linked to this condition (Olsen et al., 2011). BKD still occurs sporadically, but the number of outbreaks has been drastically reduced over the last 15 years due to good broodstock testing routines. However, the bacterium can occur in healthy carrier wild fish and the threat of horizontal transmission will always exist.

In addition to sea lice described above, a handful of other parasites occur on a regular basis. The myxozoan *Parvicapsula pseudobranchicola* is widespread in the northern parts of Norway, and infections can lead to high mortality (Karlsbakk et al., 2002). The microsporidian *Desmozoon lepeophtherii* (Freeman and Sommerville, 2011) (also known as *Paranucleospora theridion*) occurs along the entire coast, but its role as a pathogen is still unclear. Tapeworm (*Eubothrium* sp.) is relatively common but curable,

as are various surface parasites. In Chile, *Piscirickettsia salmonis*, the sea lice *Caligus rogercresseyi*, and the viral diseases of infectious salmon anemia and IPN are considered significant problems.

Pathogens that represent a concern to Atlantic salmon marine production in Canada include infectious salmon anemia virus, viral hemorrhagic septicaemia virus, infectious hematopoietic necrosis virus, IPNV, *Aeromonas salmonicida* (furunculosis), *Yersinia ruckeri* (enteric redmouth disease), *Renibacterium salmoninarum* (BKD), species of *Vibrio* and sea lice (*Caligus elongatus* and *Lepeophtheirus salmonis*).

Drug treatments, vaccines, and usage of chemicals for cleaning and disinfection

Atlantic salmon globally are typically vaccinated in fresh water before entry to sea cages so they are protected in the marine environment. There is also extensive use of different pharmaceuticals and antiseptics (e.g. hydrogen peroxide) against sea lice infestations. Antibiotics are rarely used on salmon with typically less than 1% of the production each year being treated. In addition, there is some use of pharmaceuticals against tapeworm (Praziquantel). All vaccines and pharmaceuticals must be approved by the appropriate government agencies in the jurisdiction of use.

Salmon lice infestations have been treated with antiseptics, organic phosphates, pyrethrines and pyrethroids, avermectins and chitin synthesis inhibitors. The avermectins and chitin synthesis inhibitors are administered through the feed, while the other treatments are given as baths. Bath treatment typically involves placing a closed or semi-closed tarpaulin around the cage and adding the treatment chemical. Treatment of fish sometimes also occurs in well-boats subsequent to transfer of fish from the sea cage. Labrids (wrasse) (Bjordal, 1990) have been used as an alternative to chemical treatment for more than 15 years, primarily in Norway, with lumpfish receiving attention as a cleaner fish in more recent years. These cleaner fish remove individual sea lice from Atlantic salmon.

Disposition of waste and carcasses

Scuba divers are used to collect dead fish from cages. Dead fish are transported to land and disposed of similarly to land-based disposal. The open-system technology (e.g. net pens and cages) used for most Atlantic salmon grow-out in marine environments results in release of all inputs to the cages to the surrounding water, except what is harvested as fish. These emissions consist of nutrients and dissolved organic substances, surplus (uneaten) feeds and faeces, antifouling devices and chemicals, including medicines and disinfectants. Some of these effluents may be reduced by improvements in husbandry and technology (e.g. underwater video systems to monitor feeding and reduce the amounts of uneaten feed) while others are inevitable in aquaculture and increase with increasing production.

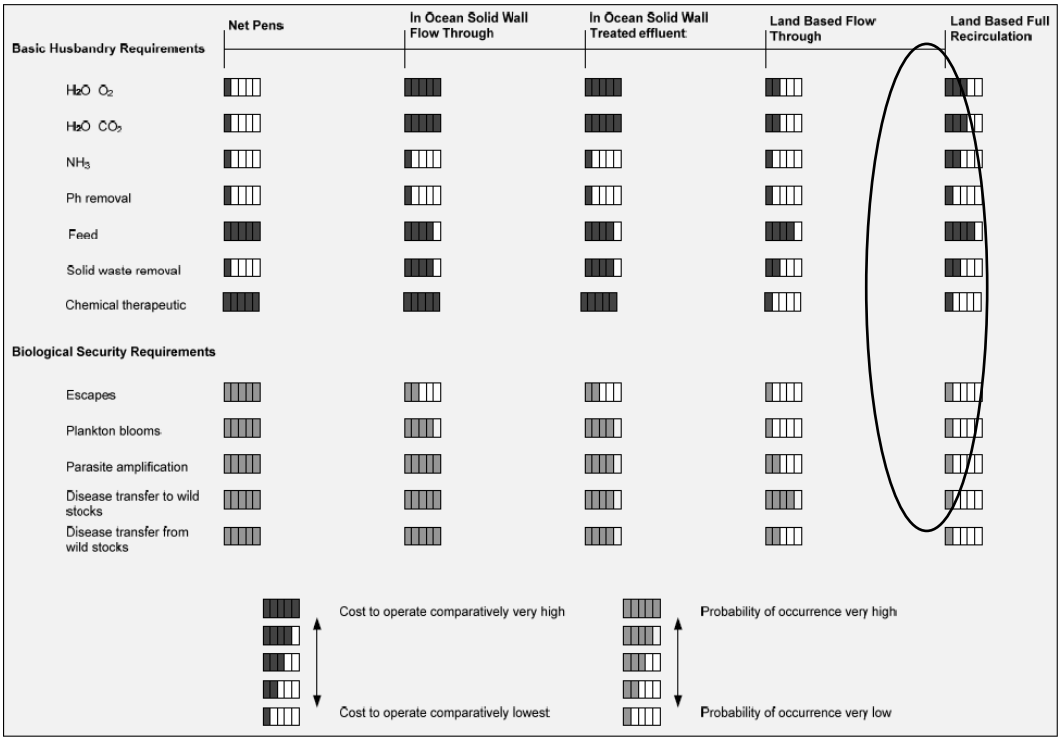
Land-based grow-out

Physical environment (tanks, nets, cages) and containment conditions

The Atlantic salmon aquaculture industry is proficient at operating land-based grow-out to produce millions of smolt in fresh water annually. Recirculation systems represent a significant portion of this smolt production globally. Continuing the grow-out so that it is entirely achieved in land-based facilities through to the target harvest weight of 4-5 kg per fish would be very similar to the smolt production systems already discussed, but on a much larger scale. From a technology perspective, raising Atlantic salmon to

market size in a closed containment system is possible as long as every component within the system is operated to reach its maximal efficiency and optimisation. However, there are many uncertainties with this approach that need to be addressed before serious investors will commit to land-based systems, including the risks associated with pathogens should they enter the facility, the variable costs to operate the facility (especially to pump water ashore), the capital costs and land footprint required to become economically feasible, and the research needed to optimise land-based grow-out operations, for example appropriate diets and genetic selection. Figure 3.14 provides one perspective on the operations of a land-based grow-out facility using full recirculation compared with other production models, including marine-based net pens.

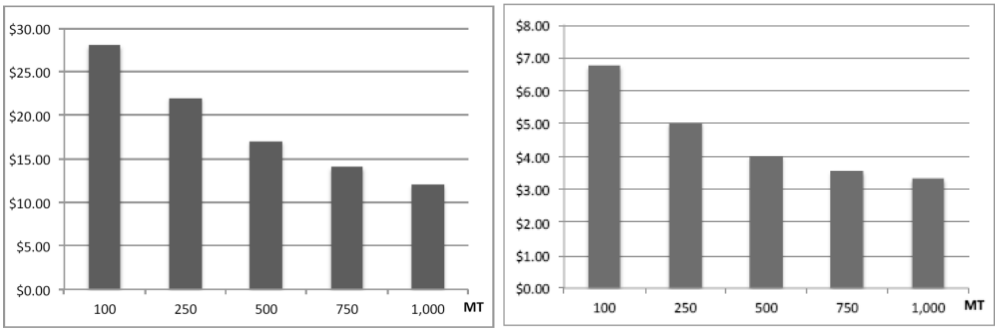
Figure 3.14. **Basic overview of requirement to raise salmon to harvest**



Source: Wright and Arianpoo (2010).

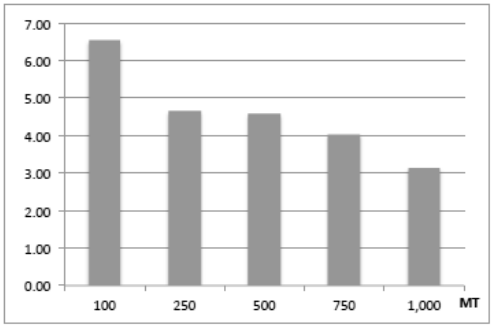
Farm site criteria will factor in transportation, distribution, land, electrical and labour costs. If fresh water alone is planned, then the facility may be located near a target destination city rather than in distant coastal communities, thereby reducing some of the operating costs associated with transportation and distribution. The scale is a significant point since it has the most influence over the future financial performance, but costs do not linearly diminish with increasing scale (Gardner Pinfold Consultants Inc., 2014; Figures 3.15 and 3.16). Once the scale is selected the components of the farm can be chosen. An example of a 100 MT module farm (20 000 individuals at 5 kg each) that grows salmon from smolt to harvest can be built in a 2.25 km² footprint (Figure 3.17). The modular system has 10 x 200 m^3 tanks that can be isolated to prevent total losses if there is a disease issue.

Figure 3.15. **Unit capital cost (left) and to-market unit operating costs (right) for 100-1 000 MT land-based farms**



Source: Gardner Pinfold Consultants Inc. (2014).

Figure 3.16. **Unit energy use for 100-1 000 MT land-based farms**



Source: Gardner Pinfold Consultants Inc. (2014).

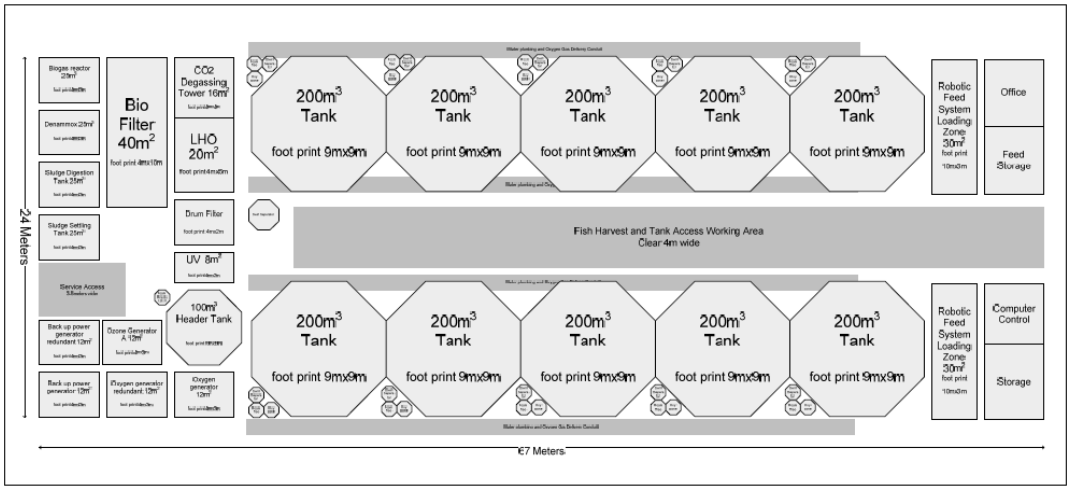
Rearing environment (water flow, DO, temperature, lighting/photoperiod)

In most cases, land-based systems will have no choice but to use intensive recirculation technology similar to the systems described for fry and smolt production, due to the large volume of the tanks and water required to raise salmon through to harvest. Knowledge of the incoming water supply is important when sizing the components needed for the farm. Ground well water is naturally filtered with the advantage of not being accessible to wildlife, which is one major disadvantage of lake and river water. Temperature fluctuations are less with well water compared with other sources. In contrast, river or lake water should be filtered and sterilised before entry into a recirculation facility given the possibility for there to be an array of pathogens present. Municipal water will enter filtered and disinfected, but also with the added costs associated with dechlorination and commercial water rates. The proposed water should be tested before use to determine whether pathogens or other water quality parameters may threaten the incoming water supply. All incoming water should be treated prior to entering the system using UV irradiation and/or ozonation regardless of the water source. UV for disinfection can be less expensive to run than ozone, but the condition of the water will dictate when either can be used. Ozone is more appropriate for water that is turbid, even for short times of the year, which would cause low UV

transmittance and therefore effectiveness (Summerfelt, 2003). Filtration of incoming water should be considered in cases where water is turbid as a result of suspended sediments.

An intensive recirculation system can reuse up to 99% of the water passing through the system and tanks. To do so, the solids must first be removed from the system as fish health and equipment can be compromised if water is not cleaned effectively. Relatively low concentrations of total suspended solids can be maintained by using a dual drain culture tank and drum filters. However, a radial-flow separator has been shown to have twice the removal efficiency compared with a swirl separator of the identical size and surface loading rate (Davidson and Summerfelt, 2004). Particulate filtered water then likely moves into a biofilter, which is the most critical component of the system as it converts toxic ammonia to less toxic nitrate. Salmon will generate 45 g of ammonia for every kilogram of feed consumed that has 45% protein (Wright and Arianpoo, 2010). Biofilters contain both nitrifying bacteria and heterotrophic micro-organisms that metabolise total ammonia (Summerfelt and Sharrer, 2004). The nitrifying bacteria require high levels of oxygen to convert total ammonia to safe nitrate ammonia. During the conversion, the bacteria produce CO_2 and this can be a high contributor to the total CO_2 for the system at up to 37% (Summerfelt and Sharrer, 2004). Outgoing water from the biofilter therefore needs to be stripped of this produced CO_2 by passing through a degasser. Reused water can be sterilised after the biofilter using an UV or ozone to eliminate any bacteria or fungus created in the system.

Figure 3.17. **Footprint of an example 100 MT land-based farm**



Source: Wright and Arianpoo (2010).

Temperature manipulation capability is one advantage of using land-based rearing, although this might come at an exorbitant cost. Land-based facilities are typically built to run at a constant temperature throughout the production cycle to allow the fastest rate of growth at optimal fish health. Oxygen concentrations should also be maintained at 8-11 mg/l, which may require oxygen to be artificially introduced into the system. Providing optimal temperature and other water quality parameters could allow faster growth of production fish, thereby offsetting some of the additional operational and capital expenditure costs.

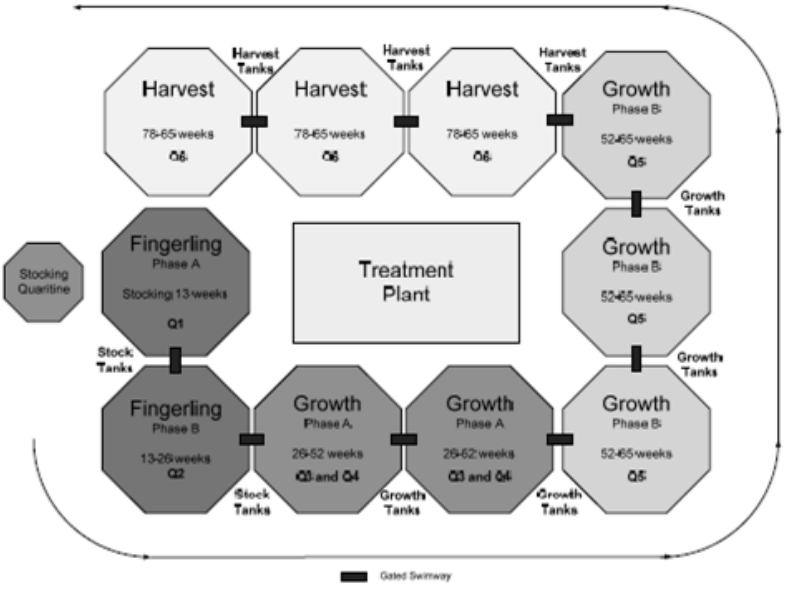
Photoperiod can also be manipulated and controlled in land-based systems. From a feeding perspective, use of a 24-hour light photoperiod would be optimal to provide additional feeding opportunities. However, when a 24-hour period was provided to

Atlantic salmon in a land-based facility, there was a 36.6% grilse rate (Summerfelt et al., 2013). Additional research is therefore needed to determine the best photoperiod to optimise growth rates while also preventing early maturation.

Fish sizes, densities, growth rates

Growth is a key aspect in land-based farming. The modular concept depends upon all stages to grow to a predicted weight at a specific time to keep stocks moving through the farm (Figure 3.18). Land-based systems allow conditions to be manipulated to decrease the time to harvest. Indeed, one grow-out trial reported that salmon reached an average weight of 4.7 kg at 372 days post-stocking in a land-based facility compared to 626 days for the same strain to achieve the target weight when reared in net pens (Summerfelt et al., 2013). Closed containment land-based operations theoretically allow a continuous sequential harvest strategy to be employed to maximise utilisation of capital assets, minimise energy costs and provide a steady production harvest (Wright and Arianpoo, 2010). A sequential harvest is required to ensure raised salmon are removed when necessary to keep stocking density under 50 kg/m^3; however, land-based systems often boast their abilities to operate around 80 kg/m^3.

Figure 3.18. **Growth phases of a modular land-based farm**



Source: Wright and Arianpoo (2010).

Feeds (types, sources, composition) and feeding (rates, methods)

Feeding can be restricted depending on water quality parameters in land-based systems. In order to keep feeding rates at a high level, the condition of the water must be excellent. Feeding can occur throughout the entire photoperiod and as the salmon grow they need to be fed larger meals less often. This requirement will allow tanks to be staggered in feeding to keep the total demand on the system low so as not to overwhelm the biofilter. Commercial Atlantic salmon diets are designed for optimal growth and food conversion ratio in open net pens. Similar extensive nutrition research is required to develop a diet specifically made to optimise fish performance while minimising the impact on the recirculation system and receiving environment from any effluent. For example,

a specific Atlantic salmon diet might need to be developed and used if the water temperature will be maintained around 15°C. Farmed Atlantic salmon raised in land-based facilities have displayed issues associated with an off flavour from the flesh that is thought to be caused by the high level of water recirculation. Depuration in flow-through water for a specific duration (up to ten days) is considered a primary means to address this issue, but perhaps a finishing diet might also be developed to assist in creating a more wholesome flesh taste for market.

Diseases, pathogens and parasites

The advantage of the closed system is the ability to filter and sterilise incoming and recirculated water to assist in the removal of pathogens. This advantage was evident from a trial run in an intensive recirculation system in West Virginia, where there were no drugs or chemicals used to treat the salmon. Additionally, the salmon had not been vaccinated. Salt was used to treat any low level fungus outbreaks and weak fish were removed from the system to keep the population strong (Summerfelt et al., 2013). This same advantage becomes a significant disadvantage should a pathogen ever get introduced into the system. Evidence suggests that fish present may be treated to alleviate fish health concerns from many diseases; however, the prospect of fully removing many pathogens following introduction into a system is very low.

Drug treatments, vaccines, and usage of chemicals for cleaning and disinfection

Land-based closed-containment systems must be treated with vigilance for biosecurity. If a problem occurs then the tanks should be able to be isolated or operated as a quarantined area. If a fish health problem occurs with a facility using a high level of recirculation, then all salmon could be rapidly exposed. It is important that all technicians are trained to notice any abnormal behaviour or conditions within the fish stock and are able to deal with these issues quickly. All equipment and tanks should have cleaning and regular maintenance schedules to reduce organic loading.

Disposition of waste and carcasses

Phosphorus removal is one of the largest environmental concerns for a land-based aquaculture facility. Aquaponics can be integrated to help remove high nitrite levels from the system as the phosphorus will be absorbed as nutrients by the plants. If this cannot be used then the phosphorus will need to be removed using different effective methods. Each kilogram of feed averages 0.25 kg of solids in the water, and recirculation systems provide an opportunity to remove much of the phosphorus levels through solid waste removal using mechanical filtration. A combination of equipment types and methods can be used, including drum filters, swirl separators, foam fractionators and settling ponds.

Transportation for grow-out, harvest and processing

Transportation typically occurs in well-boats.

Harvest and processing

At least two to three days of starvation is recommended (depending on temperature) to eliminate the contents of the gut and to calm the fish down before transport and harvest.

Some companies grade their fish before slaughter because populations may have considerable individual variation. It is more common especially within large operations to use well-boats with integrated grading equipment to pump the fish on board. Fish smaller

than a predetermined size fall through the grids and are returned to an empty cage for continued grow-out.

There is some variability in harvesting techniques. One methodology is to stun the salmon using an automated stunner or a blow to the head. Gill arches are then cut to allow the salmon to bleed before immersing them into ice water. At this point, the salmon are often transferred to the processing plant for gutting and additional processing depending on the market – head on gutted/dressed, fillets, portions, etc. A preprocessing step might require that the fish be gutted prior to transfer to the processing plant if the processing plant is not located near the sea cage sites.

Biocontainment

Chromosome set manipulation (triploidy)

Triploidisation is considered the most effective method for producing sterile fish for aquaculture (Benfey, 1999, 2015; Tave, 1993). The methodology to produce triploid fish routinely results in populations that are >98% triploids (Benfey, 2015). The methods for producing sterile fish are simple, easily applied on a commercial scale and the required investments quite low (Benfey, 2015). Triploid salmon are sterile because they cannot produce a balanced set of chromosomes when the three homologous chromosomes are to be distributed among the developing gametes. There are three ways to induce triploidy: 1) duplication of the paternal genome; 2) duplication of the maternal genome; or 3) crossing tetraploids with diploids (Benfey, 2009). Induction of triploidy by duplication of the paternal genome has been achieved in rainbow trout, but not in Atlantic salmon (Benfey, 2015). Triploidy can be easily induced through duplication of the maternal genome by preventing the second polar body from leaving the egg shortly after fertilisation. The method to induce triploidy in salmon involves the use of heat or hydrostatic pressure (Benfey and Sutterlin, 1984; Johnstone, 1985; Quillet and Gaignon, 1990; Johnstone, McLay and Walsingham, 1991). Use of hydrostatic pressure is the preferred method for inducing triploidy as it is easier to ensure that all eggs are exposed to identical treatment in a sealed pressure vessel and the optimum pressure treatment is independent of temperature (Benfey, 2009). A female triploid salmon is for all practical purposes sterile and does not produce functional gametes. A male triploid salmon is also sterile as it does not produce functional sperm. Males still go through sexual maturation and can produce sperm capable of fertilising eggs (Fjelldal et al., 2014); however, the embryos produced from a triploid male are aneuploid and die early in development (Benfey, 2015). Farm operations that raise triploid salmon are therefore normally based on monosex female populations to avoid losses in growth experienced by males that still undergo sexual maturation, and the potential loss of wild breeding potential should a male triploid escape and mate with wild diploid females.

Production of triploid salmon has been tested in Canada (e.g. Friars and Benfey, 1991; O'Flynn et al., 1997; Pepper, Nicholls and Collier, 2004), France (Quillet and Gaignon, 1990), Ireland (Cotter et al., 2000), Norway (Oppedal, Taranger and Hansen, 2003), Scotland (Johnstone, McLay and Walsingham, 1991; Johnstone, 1993; McCarthy et al., 1996), Tasmania (Jungalwalla, 1991) and the United States (Galbreath et al., 1994; Galbreath and Thorgaard, 1995). All-female triploid Atlantic salmon are presently commercially raised in the Tasmanian aquaculture industry (Benfey, 2015). Use of triploids in production may have some risks. Triploids have experienced higher mortalities in comparison to diploids (Hansen et al., 2007) throughout the production cycle and have a lower tolerance for

suboptimal environmental conditions (Altimiras et al., 2002; Ojolick et al., 1995; Pepper, Nicholls and Collier, 2004; Hansen et al., 2015). The frequency of lower jaw, gill and vertebral deformities may also be higher in triploid populations (Sutterlin, Holder and Benfey, 1987; Jungalwalla, 1991; Sadler, Pankhurst and King, 2001; Pepper, Nicholls and Collier, 2004; Lijalad and Powell, 2009; Powell, Jones and Lijalad, 2009; Fjelldal and Hansen, 2010; Leclercq et al., 2011; Fraser et al., 2013, 2014; Taylor et al., 2013, 2014; Tibbetts et al., 2013). Post-smolt triploid Atlantic salmon are more prone to cataracts (Wall and Richards, 1992; Leclercq et al., 2011; Taylor et al., 2014, 2015). However, this occurrence can potentially be reduced by an elevated level of dietary histidine, which might also improve feed conversion efficiency (Taylor et al., 2014). Triploids have a lower relative abundance of B-cell lymphocytes (Fraser et al., 2012), may have more antibiotic-resistant intestinal bacteria (Cantas et al., 2011), reduced innate immune response to bacterial pathogens (Langston, Johnstone and Ellis, 2001) and potential issues with adhesions and pigmentation after vaccination (Fraser et al., 2014; Larsen et al., 2014). However, triploid Atlantic salmon are also less likely than escaped diploids to outcompete or displace native salmon for these reasons (Benfey, 2015). Research on triploids is ongoing, with recent projects adding to the body of knowledge on triploid Atlantic salmon, while taking advantage of advances in salmon husbandry and genetic improvements to potentially remove many of the current drawbacks to the use of triploids in a commercial setting (Benfey, 2015).

Sex control technologies

There are two methods to create all-female Atlantic salmon. The first method produces all female diploid Atlantic salmon by irradiating sperm before fertilisation, followed by administration of heat or pressure shocking so only the two maternal chromosome copies will be functional, producing gynogenetic diploid (all-female) offspring (Kirpichnikov, 1981; Quillet and Gaigon, 1990). The second method produces female fish that function as males and can later be used as broodstock to produce all female triploids. All female triploids are desirable because, as mentioned previously, they are sterile and reproductive organs do not develop. Genetic female Atlantic salmon (XX), may also be treated with androgens or aromatase inhibitors that allow them to develop as functional males. These "neomales" yield all-female offspring when crossed with normal females (Benfey, 2009). The androgens used are 17α-methyltestosterone (MT) and 17α-methyldihydrotestosterone (MDHT) administered through the diet or bath treatments. At first feeding, fry are fed a diet containing a target concentration of MT or MDHT, resulting in females having functional sperm-producing testes. The immersion treatments have been shown to be simpler and effective, suitable for commercial scale use in hatcheries, and offer other advantages compared with dietary manipulation treatments (Lee, King and Pankhurst, 2004). Administering MT or MDHT requires an identification/confirmation of the neomales (having only functional XX chromosomes) compared with normal males (XY chromosomes).

Neomales can be distinguished from normal males within a population of Atlantic salmon by using a sex-specific genetic marker that was initially developed for rainbow trout, but has since been adapted for Atlantic salmon. Previously, this identification was difficult and required examination of dissected testes for abnormalities (constrictions, diminished or absent sperm ducts, presence of some ovarian tissue with visible oocytes) (Benfey, 2015). Eggs are fertilised with the sperm of confirmed neomales and then the newly fertilised eggs undergo pressure treatment (as described above) to produce all-female triploid progeny.

Interactions with the external environment

Escapees

Atlantic salmon escape from hatchery and marine grow-out sites despite the general economic incentive of the farm operator to retain all fish for eventual harvest. Escapes may be classified as either chronic or acute losses as follows:

- Chronic losses are represented by the potential leakage of stocked fish to the outside environment occurring anytime during the grow-out cycle. Chronic losses may occur without the knowledge of the farm operator, are sometimes difficult to detect and can potentially occur over an extended period of time, thus making it very difficult to ascertain the actual number of losses. Examples include escapes through typical handling and site operations occurring outside of the confines of the containment netting, such as during size grading.
- Acute losses tend to occur from a single severe event, sometimes without notice, which may lead to the escape of a significant number of fish. Acute losses may follow severe weather, devastating predator breach of the containment netting, catastrophic failure of equipment or unexpected vandalism of the containment net.

Farm escapes may pose a risk to wild populations and ecosystems based on: 1) the likelihood (probability) for escape at a specific time; 2) the magnitude (numbers) of escapees involved; and 3) the impact on wild populations or ecosystem (Naylor et al., 2005). Financial losses to the operator and risks to wild populations are only eliminated if the farm successfully contains all Atlantic salmon stock through to harvest.

Recapture of escapes directly by the fish farm staff, a third party contracted by the industry or regulatory agency is often cited as a potential means to eliminate the impact from escapes. The implementation timeline, effort duration and spatial boundary for the recapture will all limit the effectiveness to recapture Atlantic salmon based on the reported escaped fish behaviour. For instance, Solem et al. (2012) reported that half of the tracked Atlantic salmon 12 hours following release covered an area of 17.17 km^2, while all of the tracked escapes encompassed 226.29 km^2. The required recapture effort will also need to be significantly more than seven days and beyond the site boundary. Skilbrei and Jørgensen (2010) reported that an effort over 4 weeks and 40 kilometres from the release site was required to recapture 37.8% and 44.6% of the Atlantic salmon that were 5.5 kg and 1.5 kg, respectively, following release in September.

Numbers and proportion (compilation by country)

Escape of Atlantic salmon from aquaculture facilities occurs in all jurisdictions allowing commercial aquaculture operations. Naylor et al. (2005) summarised regulations associated with aquaculture containment and escape reporting and monitoring by region up to 2003. Thorstad et al. (2008) provided a review of documented incidences of Atlantic salmon escapes from fish farming activities located in numerous jurisdictions globally. Acquiring a complete picture of the global numbers and incidences of Atlantic salmon escapes is not practical primarily due to the general lack of official data available from the majority of Atlantic salmon farming jurisdictions.

The lack of reliable escape data is further exasperated by the difficulty to enumerate escapes from single chronic or acute events and escape reporting is expected to underestimate the actual number of escapes per incident. Chronic leakage or incidents resulting in a small number of escapes are generally unreported or not reported if considered below

a government-specified threshold in some jurisdictions. Further, escape numbers are self-reported by the farm operator and tends to be optimistically underestimated following severe acute incidents. In reality, the only quasi-accurate inventory number of Atlantic salmon raised within each net pen, site and region is acquired after the Atlantic salmon have been harvested and enumerated while being packed for sale. Even then the discrepancy between stocked versus counted mortality and harvested fish can be great and there may be substantial numbers of unaccounted for or unexplained escapes. Regardless, the level of underestimation of farm escapes is considered to be quite high by some observers, with Sægrov and Urdal (2006) estimating that only 12-29% of the actual number of escapes may be reported. A more recent estimate based on a number of experimental releases suggests that the actual number of escaped farmed salmon is two to four times higher than the reported number (Skilbrei and Jørgensen, 2010).

Norway and Scotland both require mandatory reporting of escapes and maintain publicly available databases associated with these numbers and incidents.[8] Jensen et al. (2010) analysed fish escape statistics from the Norwegian Directorate of Fisheries as reported from farm operators. The paper described several broad categories of potential escape events from September 2006 to December 2009. The analysis indicated that the most prevalent causes of Atlantic salmon escape were the result of equipment structural failures (68% of all reported escapes), land-based related incidents (11%), farm operational failures (8%), external factors (8%) and unknown reasons (5%). Reported structural failures occurred as a result of large storm events that may combine with farm component fatigue coupled with human error when initially installing the site or subsequently operating/maintaining its components. During the 2009-12 timeframe, there were a total of 506 000 saltwater and 59 492 freshwater Atlantic salmon escapes reported to the authorities by fish farm operators in Scotland. The primary causes for these escapes as a percentage of total escapes for the consolidated period are provided in Table 3.6.

Table 3.6. **Causes and numbers of Atlantic salmon escapes in the Scotland Atlantic salmon aquaculture industry as reported by fish farm operators, 2009-12**

Reported cause of escape	Reported number escaped	% of total number in period
Fresh water		
Hole in net (unknown)	0	0
Hole in net (predator)	43 927	73.83
Human error	12 385	20.82
Equipment failure	0	0
Weather	3 180	5.35
Total	59 492	100.00
Salt water		
Hole in net (unknown)	83 332	16.47
Hole in net (predator)	29 740	5.88
Human error	13 262	2.62
Equipment failure	1 092	0.21
Weather	378 574	74.82
Total	506 000	100.00

Survival and migration

Survival of escaped Atlantic salmon is affected by many factors. Hansen, Døving and Jonsson (1987) found that farmed salmon tagged and released during summer in Norway were apparently homeless and some of the immature fish were captured north of the Faroe Islands. Hansen and Jonsson (1989; 1991) studied tagged post-smolts held in salt

water and sequentially released for one year finding interannual variation in migration pattern and survival with poor survival in salmon that were released during later summer and autumn, and poor homing precision of fish released during winter. Hansen (2006) found large salmon released ("escaping") during the winter at Bersagel in Norway did not home back to the area where they escaped, but were recaptured along the coast in marine fisheries and rivers to the north and south-east of the release site. All recaptures of salmon released at Meløy were north of the release site. Salmon travelled a bit differently here, but there was no consistent evidence that they were homing to rivers close to the release site. The geography of the two release sites was quite variable (Hansen, 2006).

When escaping from marine net pens, the survival and dispersal of farm salmon depend on the time of year they escape. Winter escapes of farm salmon are associated with high mortality and wide dispersal (hundreds of kilometres); post-smolts escaping during spring and summer seem to survive better and disperse to nearby rivers of the marine location (Hansen, Døving and Jonsson, 1987; Hansen and Jonsson, 1989, 1991; Skilbrei and Jørgensen, 2010). Farmed adult salmon escaping from sea cages in the spring and summer, a few months before sexual maturity, have a relatively high survival (Hansen, 2006; Chittenden et al., 2011).

Farm salmon tagged in the feeding areas off the Faroe Islands have poorer survival than wild fish tagged in the same area (Hansen and Jacobsen, 2003). Farm escapes seem to approach the coast and enter rivers later in the season than wild fish, many of them after the angling season (Fiske et al., 2001). There is a significant correlation between the intensity of fish farming in an area (estimated as density of farms, or total numbers of smolts put into net pens) and the occurrence of escaped farm fish in the rivers (Fiske et al., 2006).

Juvenile stages of farm fish escaping into fresh water locations have a migratory behaviour that is more similar to wild fish. Generally, the homing precision of adults released as freshwater juveniles or as smolts in rivers is much higher than that for fish escaping from or being released at marine sites, without any connection with a river (Hansen and Jonsson, 1994; Hansen and Quinn, 1998). However, even when migrating in the same river, the homing precision of farm fish is lower than that of wild fish. Moreover, farm salmon from the commercial strains home less well to a river than farm fish developed from the local population (Jonsson, Jonsson and Hansen, 2003).

Reproduction

Escaped Atlantic salmon have been shown to spawn in fresh water (e.g. Gausen and Moen, 1991; Crozier, 1993; Butler, Cunningham and Starr, 2005) at which time they interbreed with other cultured salmon (if present) and wild salmon. Their reproductive success is less than that of wild salmon (e.g. Fleming et al., 1996, 2000; Fleming, Lamberg and Jonsson, 1997). This is partially a result of how long the salmon have been escapees and how successful the escaped salmon have been at foraging in the wild. Commercial pelleted feed is made for a growing salmon and varies from a broodstock diet (see previous sections). When escaped salmon introgress with wild salmon, they may be reducing the fitness of a population as the wild salmon have genetically successfully adapted to a specific area, whereas the cultured salmon have been selectively bred for traits important to the industry. The only way to prevent escaped salmon from interbreeding with wild salmon is to make the cultured salmon functionally sterile (see the section on "Biocontainment" above).

Experiments in stream tanks designed to simulate natural breeding conditions suggest that escaped farmed salmon typically have lower spawning success than wild salmon (Fleming et al., 1996, 2000; Fleming, Lamberg and Jonsson, 1997; Weir et al., 2004).

When farmed salmon are kept in a fish farm until just before spawning, their spawning success is very much reduced relative to wild salmon. Farmed males attain only a few per cent of the spawning success of wild males, whereas farmed females may have about a third the success of wild females (Fleming et al., 1996).

Even when the fish have been in culture for only half a generation, as in sea/ocean ranching (i.e. from fertilisation until the smolt stage), the spawning success of males may be halved relative to that of wild fish. Sea-ranched females, on the other hand, seem not to experience reduced spawning success (Fleming and Einum, 1997).

Successful spawning of farmed Atlantic salmon escaping to Norwegian and Scottish rivers has been documented on the basis of observations of distinct pigmentation differences between the eggs of wild and farmed fish (Lura and Sægrov 1991a, 1991b; Webb et al., 1991). Such analyses suggested a mean farmed female spawning success of 82% relative to wild females in six Norwegian rivers (Lura, 1995). An experimental release of farmed salmon in the River Imsa indicated that they had 19% reproductive success (i.e. breeding and early survival) of native fish (Fleming et al., 2000). In extreme situations, like in the River Vosso, at a time when few wild females were present, nearly all eggs may have been spawned by escaped farm females (Sægrov et al., 1997).

Males maturing sexually at the parr stage are known to fertilise a variable proportion of eggs during the spawning of anadromous individuals (Jones and Hutchings, 2002). Experiments by Garant et al. (2003) and Weir et al. (2005) suggest that mature male parr resulting from crosses between escaped farmed salmon, or between farmed and wild fish, may attain an individual spawning success up to four times higher than that of wild offspring. The two experiments were, however, quite similar with respect to the total proportion of offspring fathered by parr (24% and 23%, respectively).

Ability to establish population

Outside the natural range of the species, deliberate and accidental releases of Atlantic salmon have failed to establish self-reproducing populations, with very few exceptions (MacCrimmon and Gots, 1979). Freshwater resident populations appear to have established in Argentina and New Zealand (Lever, 1996). Naturally produced offspring of Atlantic salmon have recently been found in rivers in British Columbia (Volpe et al., 1999), likely as a result of successful spawning of escaped farmed salmon. Whether or not this will lead to self-sustaining populations remains to be seen.

The failure of introductions of Atlantic salmon to establish sustained populations, in spite of hundreds of release attempts on several continents, is in stark contrast to brown trout introductions which have led to self-sustaining populations in North America, South America, Africa and Oceania (MacCrimmon and Marshall, 1968). The reasons why Atlantic salmon have failed where brown trout have succeeded are not known, although several hypotheses can be entertained (Gross, 1998; Waknitz et al., 2002).

Within its natural range, Atlantic salmon readily establishes self-sustaining populations following human intervention to open new river stretches by building fish ladders (Jones, 1959) or by improving water quality (Hesthagen and Larsen, 2003). Releases appear to speed up the recolonisation process in comparison with natural recolonisation.

Ecological (non-genetic) effects

Escaped farmed salmon are likely to survive at least temporarily in the wild, because the environment has the ability to support their needs for food and shelter. It is possible and likely that they are in competition with other Atlantic salmon (wild or escaped) and

potentially other species. There are many areas where overfishing, overexploitation, industry, pollution, etc. have created habitat that is open and underutilised as well. However, generations of selective breeding and the structured aquaculture environment (e.g. regular feeding to satiation decreasing competition) put escaped farmed Atlantic salmon at a disadvantage to wild Atlantic salmon or other wild species with regards to altered spawning behaviour, subsequent survival of eggs, altered predator avoidance and competitive ability (see Bridger and Garber [2002]; Thorstad et al. [2008]). Escaped farmed salmon appear to consume similar food resources as wild salmon on feeding grounds in the Atlantic Ocean, but it is unlikely that Atlantic salmon production is limited by the availability of food and food competition from escaped farmed salmon (Thorstad et al., 2008). Offspring of escaped farmed Atlantic salmon show high growth rate in the wild, especially if feeding conditions are favourable, and have in artificial streams been shown to reduce early survival of wild juveniles (Sundt-Hansen et al., 2015).

Pathogen transfer

The flow of pathogens can occur readily between wild and domesticated stocks of finfish due to the connectivity of the aquatic environment in which they live. As aquaculture continues to expand, and given the dynamic nature of intensive aquaculture, there are multiple pathways of transmission between wild and farmed fish, and in the case of viral pathogens, some unique drivers of viral adaptation (Kurath and Winton, 2011). Theory predicts that common aquaculture practices may favour evolution toward higher pathogen virulence. However, theory also predicts that viruses, in particular, move from wild fish reservoirs to infect domestic fish in aquaculture more readily than viruses from domesticated fish move across the interface to infect wild stocks. This is because, among other things, the selective pressures that favour higher virulence pathogens in aquaculture are not present in wild stocks (Kennedy et al., 2015).

Drugs and chemicals

Drugs and chemicals that are used on salmon farms will typically be released directly into the surrounding marine environment, usually without any prior treatment or removal. For this reason most countries have strict requirements on how and when the chemicals and drugs are used, and they undergo an extensive evaluation prior to approval to ensure that their use will not result in significant environmental impacts outside of the immediate farm area.

Feed and faeces

Waste feed and faeces pass through cages and into the benthic environment. Countries with salmon aquaculture have benthic monitoring programmes that track changes to the benthic environment to prevent negative effects. Atlantic salmon cages are often attractants to benthic or other organisms for these reasons (presence of feed and faeces).

Genetics of Atlantic salmon

Genetic information

The Atlantic salmon has long been recognised as a phenotypically variable species. For example, the variation in its life history, migrations, growth rate and body size at maturity is matched by few vertebrates (Allendorf, Ryman and Utter, 1987; Hutchings and Jones, 1998). The large phenotypic variation is not, however, necessarily associated with greater genetic variability. A higher susceptibility to environmental factors such as temperature, food and density is part of the explanation why salmon and many other fish species are more phenotypically variable than other vertebrates.

In order to obtain knowledge about the level and distribution of genetic variation in Atlantic salmon, one needs to study variation directly at the level of genes, their building blocks (nucleic acids), their direct products (proteins) and/or their large-scale organisation (chromosomes). Genetic variation can also be inferred from controlled experiments where the phenotypes of inter-related crosses are compared under "common-garden" standard conditions, as in quantitative genetics, or by studying how gene variants or phenotypic characters vary across environments, as in ecological genetics. Recently, major international research initiatives – merging molecular and quantitative genetic approaches – have been initiated to study the entire genome of Atlantic salmon with an aim to map the genes that are important for performance traits. This chapter makes use of all of these pieces of information to describe the genetics of Atlantic salmon.

Cytogenetics

The ancestor of all extant salmonids is believed to have undergone genome duplication some 25-100 million years ago (Allendorf and Thorgaard, 1984). The duplication of a diploid genome (tetraploidisation) is still detectable in the form of duplicate loci for many genes in Atlantic salmon and other salmonids, and thus, these species may be considered pseudo-tetraploid. The whole genome sequence of Atlantic salmon, including duplicated regions was recently published (Lien et al., 2016).

The Atlantic salmon has a variable number of chromosomes (2n = 54-60), while the number of chromosome arms is more stable (NF = 72-74) (Kirpichnikov, 1981). Chromosomal differences exist between widely separated populations of Atlantic salmon. For example, the standard European karyotype is 2n = 58, NF = 74, whereas Canadian fish may have 2n = 54, NF = 72 (Hartley, 1988; Phillips and Hartley, 1988). However, chromosome polymorphisms are also found within populations, and may even occur among offspring of the same female. Brown trout, a congeneric species, has 2n = 78-82 and NF = 98-100, whereas species within the genus *Oncorhynchus* have 2n ranging between 52-74 and NF = 102-108, and species within the genus *Salvelinus* have 2n = 76-84 and NF = 96-100 (Kirpichnikov, 1981).

Molecular population genetics of Atlantic salmon

The Atlantic salmon is strongly genetically structured compared to most fish species, particularly those living in the marine environment (Ward, Woodwark and Skibinski, 1994). Enzyme electrophoresis of protein variants (so-called allozymes) shows that approximately one-third of the total genetic diversity (or heterozygosity) of Atlantic salmon results from genetic differences between populations. In a study of 53 natural and hatchery populations from all of the distribution area of Atlantic salmon, analysing 19 enzymes encoded by 38 loci (genes), Ståhl (1987) estimated an F_{ST} of 0.36. F_{ST} is the relative difference between the genetic diversity in the total population, H_T, and the average genetic diversity in the sub-populations, H_S, or $F_{ST} = (H_T - H_S)/H_T$. It varies from 0.0 when all populations have the same allele frequency to 1.0 when different populations are fixed for alternate alleles (Wright, 1969).

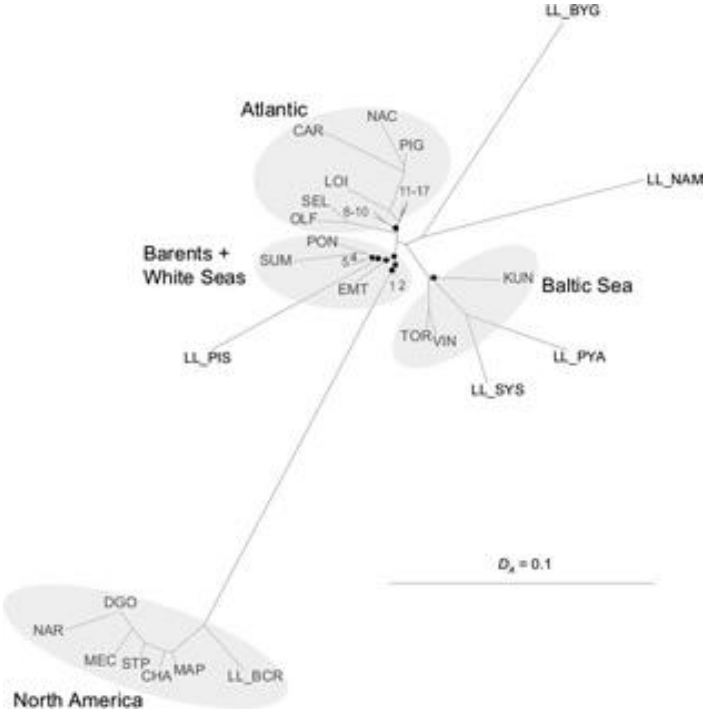
The genetic differentiation between Atlantic salmon populations worldwide arises first from a major genetic dichotomy between populations from either side of the North Atlantic Ocean, and second from genetic differences between European populations in Baltic and Atlantic drainages (Ståhl, 1987). There is also some evidence for further regional sub-structuring, both in Europe and North America (Verspoor, 2005), as well as genetic differentiation of local populations between rivers (Bourke et al., 1997; Skaala et al.,

1998; Koljonen et al., 1999) and within rivers (Møller, 1970; Heggberget et al., 1986; Ståhl and Hindar, 1988). Landlocked or resident populations do not form a single genetic grouping but belong to their respective geographic regions.

In absolute terms, Atlantic salmon is not highly genetically variable as a species; the total allozyme heterozygosity in Ståhl's (1987) study ($H_T = 0.04$) is in the low range of what is found in fish species (*cf.* Ward, Woodwark and Skibinski, 1994). Baltic populations of Atlantic salmon are commonly less variable than populations along the Atlantic coast.

These early findings from studies of enzyme electrophoretic variation have been supported by later electrophoretic studies (Bourke et al., 1997; Verspoor et al., 2005) that employed a larger number of genetically variable loci, on a smaller number of populations, than used by Ståhl (1987). Bourke et al. (1997) found some support for the divergent Baltic group of populations in Europe, and also indicated that this group is related to northern coastal Atlantic populations. This relationship was also noted by Koljonen et al. (1999) in a detailed study of Baltic populations, where populations in the western Baltic area showed some resemblance to a Norwegian population. Populations in the north-east (north-western Russian Federation and north-eastern Norway/northern Finland) form a separate phylogeographic unit in Europe, in line with suggestions by Kazakov and Titov (1991) and Skaala et al. (1998). A dendrogram based on allozymes (focusing on variation in Europe) is shown in Figure 3.19, and compared with a recently published dendrogram (Bourret et al., 2013a) based on a large number of single nucleotide polymorphisms (SNPs) using a 7k SNP-chip.

Figure 3.19. **SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*)**



Source: Bourret et al. (2013).

The lessons from studies at the protein level are supported by several, more recent studies of DNA. A genetic dichotomy between North American and European Atlantic salmon is evident in both mitochondrial DNA (mtDNA) and nuclear DNA (Bermingham et al., 1991; McConnell et al., 1995; Taggart et al., 1995; Verspoor et al., 1999; King et al., 2001; Nilsson et al., 2001; Bourret et al., 2013). Moreover, local populations of Atlantic salmon are genetically distinct (Galvin et al., 1995; Nielsen, Hansen and Loeschcke, 1996; Sánchez et al., 1996; McConnell et al., 1997; Norris, Bradley and Cunningham, 1999) and a significant relationship exists between geographic and genetic distance ("isolation by distance") on both small and large geographical scales (King et al., 2001; Primmer et al., 2006). Analyses of DNA microsatellites extracted from archived scales suggest that the local genetic structure of Atlantic salmon may be temporally stable, even over several decades (Nielsen, Hansen and Loeschcke, 1999, 1997; Tessier and Bernatchez, 1999; Vähä et al., 2008; Glover et al., 2012). Comparative genetic analyses at several institutions in Europe have recently been carried out in order to create a large database of microsatellite genotype data in Europe and North America (Ellis et al., 2011).

Mitochondrial DNA can be a particularly useful marker to reveal large-scale geographic groupings. It is maternally inherited, has a relatively high mutation rate and lacks recombination, hence, current distribution patterns of maternal genetic lineages can be identified. Studies have shown good separation of mtDNA types between Atlantic salmon from North America and those from Europe (Bermingham et al., 1991). There is also a distinction between European coastal (Atlantic) populations and the Baltic populations (Verspoor et al., 1999; Nilsson et al., 2001). Baltic populations show less variation than Atlantic populations. As for allozymes, there is only weak evidence for clear geographic groupings within the Atlantic area. Populations in the north-east (Russian Arctic coast) share mtDNA (as well as allozyme) variants with North American populations, suggesting that early northern colonisers included fish of North American origin (Makhrov et al., 2005).

Molecular markers at the DNA level continue to provide more detailed knowledge of the genetic population structure of Atlantic salmon from local to global scales. Several glacial refuges seem to be involved in the colonisation of the European coasts, as well as the Baltic Sea and White Sea drainages (Consuegra et al., 2002; Asplund et al., 2004; Säisä et al., 2005; Tonteri et al., 2005; Verspoor et al., 2012). Some of the more divergent groupings of populations, e.g. the south-eastern Baltic group and the White Sea group, may have been colonised from ice-dammed lakes in the north-western part of the Russian Federation (Bourret et al., 2013), whereas an Iberian refuge seems a more likely origin of European populations along the Atlantic coast. In the far north, several refugia may have contributed to the current population structure. Recently, a study of 1SW and MSW Atlantic salmon populations from the three phylogeographic lineages of Atlantic salmon in Europe, based on using a 220 000 SNP-chip, has identified a gene that strongly affects sea age at maturity in salmon (Barson et al., 2015), and also revealed a mechanism for maintaining genetic variation by sex-dependent dominance in the heterozogytes. The same gene, vestigial-like family member 3 gene, was at the same time identified as strongly affecting male maturation in wild and farmed populations (Ayllon et al., 2015).

Estimates of gene flow from molecular markers

If we assume that salmon populations are in approximate equilibrium for molecular markers (meaning that random genetic drift within populations are balanced by gene flow between them), then Wright's (1969) island model

$$F_{ST} = 1/(4\ N_e m + 1)$$

can be applied to provide a rough estimate of gene flow or "the number of genetically effective migrants" (N_em) that are exchanged between wild populations in each generation. For simplicity, N_em can be thought of as the number of immigrant individuals successfully reproducing in a population. Between continents, estimates of F_{ST} suggest $N_em<<1$ per generation, and salmon populations on the North American and Eurasian continent seem to evolve independently. Caution needs to be exercised when estimating equilibrium number of migrants from genetic data. Interestingly, tagging studies also suggest little exchange between continents, as thousands of tagged salmon have been recaptured but less than a handful of individuals have been found crossing the North Atlantic to reproduce.

Between natural Atlantic salmon populations occurring in different rivers on the same continent, estimates of N_em usually vary from 2 to 12 genetically effective migrants between rivers each generation (Ståhl, 1987; Bourke et al., 1997), suggesting low to modest amounts of gene flow. Tributaries to smaller rivers may show higher levels of gene flow, e.g. in River Conne, Newfoundland, an F_{ST} estimate suggests $N_em>20$ on a ~10 km geographical scale (Beacham and Dempson, 1998). Tributaries to some of the larger rivers may show levels of differentiation similar to that between rivers, e.g. in the River Tana/Teno on the Norwegian/Finnish border, $F_{ST} = 0.047$ and N_em ~4 on a ~100 km scale (Ståhl and Hindar, 1988; Elo, Vuorinen and Niemelä, 1994). Recent, more detailed studies of microsatellites of Atlantic salmon within the Tana/Teno watercourse have revealed considerable differences between mainstem and headwater streams on the one hand, and tributary populations on the other, with respect to effective population size and gene flow (Vähä et al., 2008). Also, the study estimated at what geographical scale local adaptations could develop in this large river system.

Co-existent freshwater resident and anadromous salmon in Little Gull Lake, Newfoundland, showed a very low estimate of gene flow at $N_em = 0.1$ (Verspoor and Cole, 1989), suggesting that these sympatric forms are completely genetically isolated. In other cases, the evidence suggests no genetic differentiation between co-existing forms, which should be considered tactics within a single population.

Cultured stocks

In hatchery stocks used for ranching and supplementation in Baltic rivers, Ståhl (1987) and Koljonen (1989) found 10-25% less within-population genetic variability (heterozygosity) compared to wild stocks. The relative genetic divergence between populations was higher for hatchery stocks than for wild stocks, consistent with the operation of founder effects and genetic drift in cultured stocks.

In farmed salmon, Mjølnerød et al. (1997) found that a principal farm strain in Norway, founded from a number of different rivers (Gjedrem, Gjøen and Gjerde, 1991), had higher levels of allozyme heterozygosity than two wild populations, but lower levels of allelic richness. Other protein studies (Verspoor, 1988b; Cross and NiChallanain, 1991; Youngson et al., 1991) have shown genetic differentiation of farm strains from their wild origin. These studies also noted reductions of genetic variability in farm strains both in terms of number of alleles and mean heterozygosity. Skaala, Taggart and Gunnes (2005) compared the broodstocks of the five major Norwegian farm strains with four wild populations in Norway at eight polymorphic enzyme coding loci. The genetic distance between one farm strain and its source populations was about ten times higher than that observed between three wild populations. Mean F_{ST} was 0.161 among the farm strains, compared to 0.021 among the four wild populations studied. The mean number of alleles was about 12% lower in farm strains than in wild stocks, percentage polymorphic loci

was 14% lower in farm strains and mean heterozygosity was about 17% lower in farm strains than in wild stocks.

DNA studies using mini- and microsatellites have demonstrated that farm salmon have even greater reductions in genetic variability than shown by protein studies. Clifford, McGinnity and Ferguson (1998a; 1998b) found that an Irish farm strain of Norwegian origin had 56% of the number of alleles and 53% of the mean heterozygosity over three minisatellite loci compared with local wild populations. Norris, Bradley and Cunningham (1999), examining the same strain with 15 microsatellites, found between 52% and 80% of the alleles present in wild salmon. Skaala et al. (2004), using 12 microsatellite loci, found strong reductions in the number of alleles at all loci in the farm strains. A direct comparison of allelic variability between a farm strain and its wild source showed that 50% of the alleles in the wild population were retained in the farm strain. The genetic differentiation observed between this farm strain and its wild founder populations was two to six times higher than the genetic differentiation observed among wild populations. Karlsson, Moen and Hindar (2010) found significantly lower microsatellite genetic diversity in farm strains than in wild salmon from Norway, although the difference was small and largely related to loss of rare alleles. On the other hand, mtDNA diversity was higher in some farm strains than in wild populations, suggesting that when farm strains are made of crosses of genetically divergent populations, they can attain a high mtDNA diversity and keep it for many generations of selective breeding.

A 7k SNP-chip was recently employed to find loci that differentiate generically between wild and farm salmon in Norway (Karlsson et al., 2011). A major finding was that when employing the top-ranked 60 SNPs with respect to F_{ST} between a pool of wild population and a pool of farm strains, it was possible to allocate individuals to farm or wild, irrespective of population of origin (Karlsson et al., 2011). The SNPs discriminating farm from wild fish were located on all but two chromosomes and suggest that molecular changes have occurred throughout the salmon genome during the domestication process.

Quantitative genetics

Knowledge about the genetic basis of biological characteristics of Atlantic salmon is derived from aquaculture-related, quantitative genetic research, carried out for selective breeding programmes (Gjerde, 1993). Table 3.7 lists several biological characteristics of Atlantic salmon that have been evaluated for heritability. Heritability is generally regarded as the ratio of additive genetic variance to total phenotypic variance and simply reflects how much of the observed variation in a trait can be attributed to purely genetic effects. Hence:

$$V_P = V_E + V_G + V_I$$

where V_P = total phenotypic variance, V_E = the environmental variance, V_G = the genetic variance and V_I = genetic/environmental interactions. The genetic variance can be divided into additive V_A and dominance V_D genetic variance, where the former goes into the expression for heritability $h^2 = V_A/V_P$. Statistical techniques, based on resemblance between relatives in controlled breeding experiments, are used to disentangle genetic and environmental sources of variation.

Many morphological, life-history and behavioural traits show significant heritable variation both within and among populations of Atlantic salmon (García de Leániz et al., 2007a; Carlson and Seamons, 2008). Ultimately, these traits may be critical at a particular life stage(s) in influencing survival and reproduction, and thus overall net fitness. In addition, some fitness-related traits may affect growth or development rates, which can indirectly affect survival and/or reproduction. The timing of key life-history events,

such as the onset of migration and spawning, also influences fitness components and, although primarily triggered by environmental cues, has a heritable component.

In general, heritability may range from near 0% to over 50%, depending on the trait (Table 3.7). Heritability less than 15% would be regarded as low; 16-25% as low to intermediate; 26-40% as medium to high; and above that as high to very high (e.g. Rye and Refstie, 1995). Traits having high to very high heritability (typically morphometric and meristic traits; not shown here) would display a generally high degree of constancy of that character, even under conditions of environmental change. Conversely, traits having low levels of heritability (often fitness-related traits) could differ among stocks and within stocks between years largely as a result of differing environmental conditions experienced.

Body size and growth rate have high and intermediate heritabilities, respectively (Table 3.7), and have been successfully targeted in selection programmes (Gjøen and Bentsen, 1997; Thodesen et al., 1999). Moreover, many characteristics that correlate with body size and growth rate show similarly intermediate to high heritabilities. For example, age at smoltification has intermediate heritability, as does sea age at maturity (Table 3.7), which is considered a major stock characteristic (Schaffer and Elson, 1975). As noted above, knowledge about the genetic basis for sea age at maturity has taken a leap forward as a result of the publications of Ayllon et al. (2015) and Barson et al. (2015). Stocks with a given sea age at maturity are likely to continue to express this to some extent even if environmental conditions change. For example, Norwegian stocks used for salmon farming in Ireland show significant retention of their sea age and growth rate characteristics under culture in Ireland, even under rearing and release from a local river (McGinnity et al., 2003). Avoidance of early sexual maturity (i.e. at weights below market size) has been an important target for breeding programmes (Gjøen and Bentsen, 1997). Timing of the spawning run seems to have a strong heritable component (Hansen and Jonsson, 1991), even among tributaries within the same river system (Stewart, Smith and Youngson, 2002). It is also likely that the timing of spawning has a genetic component, as spawning time seems to be adjusted to ensure appropriate timing of emergence of juveniles in different rivers (Heggberget, 1988; Jensen, Johnson and Heggberget, 1991).

Survival rates in both fresh water and sea water usually have low heritabilities (Table 3.7). Significant differences in marine survival rates have been reported for different strains of Atlantic salmon reared and ranched at the same site, suggesting retention of some degree of genetic based differences among stocks (Jonasson, 1996; Crozier, Moffett and Kennedy, 1997). The heritability of marine survival has been calculated under cage culture for Atlantic salmon (Standal and Gjerde, 1987); however, these studies do not replicate true marine survival conditions. The best information on heritability of marine survival in the wild environment for Atlantic salmon comes from a study of heritability of return rates in salmon ranched from Iceland (Jonasson, Gjerde and Gjedrem, 1997). Heritability in return rate was generally low, ranging from 7% to 24% in 1SW-fish and 1-7% in fish maturing after 2SW. As return rate in ranched salmon is to a large extent a fitness trait, comprising many life-history traits such as migration behavior, disease resistance, predator avoidance, etc. (Jonasson, Gjerde and Gjedrem, 1997), it is not surprising that heritability for this characteristic is quite low. Furthermore, marine survival is known to vary in response to changing conditions at sea (Friedland, Hansen and Dunkley, 1998), indicating that any genetic basis is heavily modified by the environment. An advantage of the native stock in comparison with non-native stocks was found by Ritter (1975) when comparing return rates of hatchery stocks in rivers other than their native ones.

Table 3.7. **Summary of heritability estimates (h^2s) for various traits in Atlantic salmon computed from the sire component of variance or mixed model analysis**

Trait	Heritability (h^2_s) estimate	
	Range	Mean
Growth and body composition		
Body length (cm)	0.08-0.57	0.28
Body weight (g or kg)	0.00-0.44	0.23
Condition factor	0.05-0.37	0.23
Specific growth rate (% Body weight/day)	0.04-0.26	0.14
Swimming stamina	0.24	--
Filet colouration/carotenoid concentration	0.01-0.60	0.31
Fat content	0.09-0.35	0.25
Slaughter yield (%)	0.03-0.20	0.12
Belly flap thickness	0.16	--
Daily feed intake (% Body weight)	+	--
Thermal growth coefficient	+	--
Feed efficiency ratio	+	--
Amino acid absorption	+	--
Mineral absorption	+	--
Life-history and survival		
Age at smelting	+	--
Age at maturity (grilse)	0.04-0.65	0.18
Age at maturity (MSW)	0.08-0.17	0.13
Survival (alevin/fry)	0.09-0.29	0.13
Survival (eyed ova)	0.29	--
Return rate (grilse)	0.12	--
Return rate (MSW)	0.08	--
Health condition and disease resistance		
Total haemolytic activity (% standard)	0.04-0.35	0.20
Resistance furunculosis (a. titre or % survival)	0.00-0.53	0.20
Non-specific haemolytic activity (% standard)	0.02-0.32	0.19
Resistance vibriosis/hitra (a. titre or % survival)	0.01-0.69	0.18
Lysozyme activity (% standard)	0.08-0.19	0.14
Total immunoglobulins (IgM, g/l)	0.00-0.12	0.06
Post-stress cortisol level (ng/ml)	0.05-0.07	0.06
Red blood cells (RBC) membrane fragility	0.60	--
Specific haemolytic activity (% standard)	0.29	--
Spinal deformities (%)	0.25	--
Resistance to bacterial kidney disease (% survival)	0.23	--
Resistance to infectious salmon anaemia (% survival)	0.19	--
Resistance salmon lice (No. sea lice)	0.19	--
α_2-antiplasmin level (% human reference)	0.19	--
α_2-macroglobulin level (% human reference)	0.12	--
Fibrinogen level (% human reference)	0.11	--
α_1-antiproteinase level (% human reference)	0.10	--
Resistance diphteria toxoid (a. titre)	0.09	--
Post-stress glucose level (mg/ml)	0.03	--
Antithrombin level (% human reference)	0.03	--
Serum iron concentration (μg/ml)	+	--

Notes: +: significant variation between full- and/or half-sib groups. MSW: multiple sea winter

Source: García de Leániz et al. (2007a).

Quantitative trait differences between cultured and wild fish

Farm salmon differ genetically from wild salmon in morphological, behavioural and ecological traits that are affected by domestication. Fleming and Einum (1997) compared a seventh-generation strain of farm salmon in Norway with its principal founder population from the wild: the River Namsen population. The fish were reared in a common environment and compared for several fitness-related traits. Farm salmon showed more robust bodies and smaller fins. Farm juveniles were more aggressive in a tank environment, but wild juveniles dominated in a stream-like environment. Farm juveniles were also more risk-prone, reappearing from cover soon after a simulated predator attack (see also Johnsson, Höjesjö and Fleming [2001]). Growth performance in farm juveniles was higher than in wild juveniles (see also Thodesen et al. [1999]). Similar results were obtained in comparisons between another strain of farm salmon and two wild populations (Einum and Fleming, 1997). These results suggest that farming generates rapid genetic change due to genetic drift and intentional and unintentional selection in culture, and that some changes involve important fitness-related traits. Quantitative genetic components of fitness have recently been studied in cultured, hybrid and wild Atlantic salmon in Norway (Besnier et al., 2015) and Ireland (Reed et al., 2015). The former study identified a quantitative trait locus (QTL) with a strong effect on survival.

The higher growth rate of farm salmon also carries over in the wild where farm and farm × wild offspring have shown higher growth rates than offspring resulting from wild × wild crosses (Einum and Fleming 1997; McGinnity et al., 1997, 2003; Fleming et al., 2000; Sundt-Hansen et al., 2015).

Genotype-x-environment (GxE) interactions

Phenotypic variation may result from three basic sources: 1) from purely genetic effects; 2) from purely environmental effects; and 3) from the interaction between genes and the environment (García de Leániz et al., 2007a). Genotype-by-environment (G×E) interactions will produce different phenotypes when animals with the same genetic background are exposed to different environmental conditions. For example, both Atlantic salmon and coho salmon that are raised in culture show altered growth, morphology, colouration, egg size, fecundity and spawning ability compared to wild fish with similar genetics (Fleming et al., 1996; Fleming, Lamberg and Jonsson, 1997; Bessey et al., 2004). Similar findings have been made for transgenic coho salmon (Devlin et al., 2004; Devlin, Sundström and Muir, 2006; Sundström et al., 2007) and Atlantic salmon (Moreau, Conway and Fleming, 2011; Moreau and Fleming, 2012a; reviewed in Moreau and Fleming [2012b]). These findings point to the difficulty of trying to study fitness-related traits in the laboratory where results may not mimic those for fish in the wild. The inability to predict the outcome of G×E interactions in nature without the use of large-scale mesocosms presents a major obstacle for modeling and understanding the ecology of this species.

Complicating matters further, many of the phenotypic traits that are affected by environmental conditions also have a genetic component. Evidence for genetic variation in several fitness-related traits has been demonstrated both among and within populations of Atlantic salmon, and many of these traits show G×E interactions (Table 3.8). Other traits for which G×E interactions have been shown include age at sexual maturity, male parr maturity, timing of hatching, aggression levels and body size (García de Leániz et al., 2007a).

Table 3.8. **Fitness-related traits with evidence of genetic variation among and within populations of Atlantic salmon**

Among populations		Within populations	
Trait	Environment	Trait	Environment
Body size*	Wild release and lab	Body size	Wild release and lab
Digestive rate	Lab	Feeding rate	Lab
Growth efficiency*	Lab	Growth efficiency	Lab
Growth rate*	Wild release and lab	Growth rate	Lab
Survival*	Wild release and lab	Survival*	Lab
		Timing of maturity	Lab
		Stress	Lab
		Sea louse infection	Lab

* Indicates traits for which there is evidence of a genotype-by-environment (GxE) interaction.

Source: Modified from Table 7.1 in García de Leániz et al. (2007b); see original article for references supporting each trait.

The available evidence suggests there are G×E interactions in all populations of Atlantic salmon that have resulted in the emergence of locally adapted ecotypes with variations in life-history. Some traits and life-history variations may have strong genetic determination (and therefore potentially could be altered rapidly by selective breeding efforts), whereas others are more responsive to environmental determinants and show greater plasticity. Phenotypic expression of a genetic trait can also vary as a function of the genetic background in which it is found, e.g. due to pleiotropy (i.e. where a gene influences multiple phenotypic traits).

Phenotypic plasticity in wild Atlantic salmon populations is a broad reflection of the wide heterogeneity in the wild Atlantic salmon genome. Many different alleles have been documented in the global populations for a given gene locus. In addition, the chromosome number varies in wild populations from 2n = 54-60 as a result of a partial genome duplication during the evolution of these fish, which can be considered to be pseudo-tetraploid, in that for some genes, at least, there are duplicate diploid loci. This introduces the complications of gene/allele dosing, in addition to G×E epigenetic variability, as potential mechanisms for adaptation to environmental conditions. Given the complexity of this system, prediction of phenotypes or phenotype responses to any given environmental condition or stress is very difficult, if not impossible, at this time.

Ecological genetics

Until the publication of Barson et al. (2015), the most well-defined and studied example of ecological genetic variation due to a single locus in Atlantic salmon is the malic enzyme locus (MEP-2*). Verspoor and Jordan (1989) found that a significant latitudinal variation in malic enzyme MEP-2* variation among rivers in both North America and Europe was strongly correlated to summer temperatures (see also Jordan et al., 2005). Populations inhabiting warm rivers tend to show higher frequencies of the MEP-2*100 allele than populations living in cold rivers, which tend to show higher frequencies of the alternative (*125) allele. Furthermore, just as the frequency of the *100 variant increased with increasing temperature among rivers, the same correlations have been observed within one Irish and three Scottish rivers (Verspoor and Jordan, 1989; Verspoor, Fraser and Youngson, 1991). MEP-2* variation has also been reported to be associated with phenotypic traits such as mean size at age, specific growth rate and sea age (Verspoor et al., 2005). Experimental studies by McGinnity (1997) have found associations of MEP-2* genotype with survival and growth in early life-stages in addition to those with smolt age and male parr

maturation. Recent studies have also shown that salmon population components differing in run timing have genetic differences at the allozyme and DNA levels (Consuegra et al., 2005).

Individual allozyme heterozygosity has been suggested to be positively associated with fitness. For example, heterozygous individuals may show increased developmental stability compared to more homozygous individuals. Blanco et al. (1990) found lower levels of asymmetry in bilateral traits (e.g. paired fins) to be associated with increased heterozygosity in Atlantic salmon, although this does not seem to be a general result in salmon (Vøllestad and Hindar, 1997).

Spatial variation in life-history traits of Atlantic salmon throughout its geographic range provides indications about genetic differences between populations (Verspoor, 1997). Hutchings and Jones (1998) reviewed the variation in 13 life-history variables for 275 populations, grouped into 12 regions (8 in Europe; 4 in North America). Population averages within arbitrarily chosen regions differed significantly for traits such as parr length, mean smolt age, smolt length, grilse length, mean sea age, per cent grilse, per cent female grilse and total age at maturity. However, egg-to-smolt survival, smolt-to-grilse survival and per cent mature male 1+ parr did not differ significantly among regions.

Large regional differences were evident in age-specific parr length, with mainland European populations being larger than Canadian and Norwegian populations (Hutchings and Jones, 1998). Smolt age differences were also large, ranging from 1.04 years (France) to 5.85 years (Quebec), with mean length at smoltification also varying greatly, especially within European regions. Grilse growth rate at sea differed by 20% amongst the regions, with the lowest in the western Atlantic and the highest for British and mainland European populations. Mean sea age at maturity differed among regions as well. Norwegian and mainland European fish spend on average 60-70% more time at sea than those from Newfoundland and Ireland. Incidence of grilse in populations ranged from 5% for American populations to 86-91% for Newfoundland and Irish populations. Total age at maturity differed significantly within European regions, with southern European stocks on average maturing younger than Northern European stocks. The Northern European stocks more closely matched North American stocks.

Data on temporal variation in some of these traits were available for several rivers with multi-year data. These data indicated that the percentage of grilse varied little in some populations (5%), whereas it varied by up to 30% in others. Inter-annual fluctuations in grilse sex ratio ranged from 5% up to 50%, with 10-20% being typical. Smolt age fluctuated relatively little, varying by less than 0.5 year within populations. Annual changes in grilse length typically varied by around 2-3 cm, while annual changes in smolt length were typically less than 1 cm.

Whereas part of this variation may reflect genetic adaptation to different local environments (Schaffer and Elson, 1975; Taylor, 1991; Stewart, Smith and Youngson, 2002), care is needed to separate the influences of environment from underlying traits that have a genetic basis. One example is mean smolt age. Metcalfe and Thorpe (1990) testing geographical determinants of smolt age in salmon from 182 rivers across the North Atlantic range noted that although smolt age was positively correlated with latitude within three large regional groupings (Atlantic Canada, western Europe and eastern Europe), a large amount of this variation (82%) was explained by annual changes in both temperature and day length. Hence, mean smolt age from similar latitudes in Canada and Europe would differ as a result of the differing temperature/day light regime in these locations. Environmental factors may also strongly influence other traits such as egg-to-smolt survival and percentage precocious male parr (Hutchings and Jones, 1998).

Genomics: Linking molecular and quantitative genetics

Major research initiatives are underway in Canada (cGRASP[9]) and Norway (SGP), among other countries, to identify large numbers of genes and proteins related to disease resistance, reproduction, growth, environmental tolerance, product quality and nutrition. These genomics-oriented projects focus on gene function and genome organisation through the development of genetic and physical maps and gene sequences, and data interpretation using bioinformatic approaches.[10] It is anticipated that information generated by these projects will increase our understanding of salmonid evolution, improve selection programmes, and accelerate knowledge and investment in fish health and vaccine development.

New research tools are being developed, such as linkage maps for Atlantic salmon (Moen et al., 2004a; Gilbey et al., 2004; Danzmann et al., 2008; Lien et al., 2011), large numbers of DNA sequences and microarrays (Rise et al., 2004), and a large-insert genomic library (Thorsen et al., 2005). Application of the linkage map to challenge tests with a viral disease, infectious salmon anaemia, has indicated the location of gene(s) with an effect on this quantitative trait, a QTL for disease resistance (Moen et al., 2004b). One study with microarrays (3 600 genes arrayed on glass plates) suggests different gene expression profiles of farmed and wild salmon, with indications of parallel changes taking place in Canadian and Norwegian farmed strains (Roberge et al., 2006; 2008).

Linkage mapping, combined with physical mapping and karyotyping, has led to identification of the sex-determining locus of Atlantic salmon on chromosome 2 (Artieri et al., 2006). This may facilitate production of all-female lines for farming of Atlantic salmon. Work is underway to characterise a large number of SNP markers in Atlantic salmon (Hayes et al., 2007; www.cigene.no; Barson et al., 2015; Aquagene, n.d.). A detailed map of the salmon genome has been recently published, and will improve the precision of QTL mapping and marker-assisted selection.

Inbreeding and outbreeding depression

Inbreeding depression

Inbreeding can be defined as the mating between individuals that are more closely related than individuals drawn by chance from the population. Increased rates of inbreeding in outbreeding species often show a decline in fitness, referred to as inbreeding depression (Frankel and Soulé, 1981). Fitness-related traits such as individual growth rate, survival and fecundity may be negatively affected at 5-10% inbreeding in laboratory populations. Moreover, most inbred lines of laboratory animals go extinct (Frankham, 1995; 1998).

A recent review of inbreeding in salmonids suggests that a 10% increase in inbreeding results in a reduction in fitness from about 3-15% under rapid inbreeding to 1-5% under slow inbreeding (Wang, Hard and Utter, 2002). It has proven difficult to study the consequences of inbreeding and loss of genetic variation in the wild. For example, Wang, Hard and Utter (2002) found only one study in salmonids that was carried out in a near-natural situation. In this study, Ryman (1970) showed inbred Atlantic salmon were recaptured at a lower rate than outbred individuals after release into Swedish streams.

Outbreeding depression

When interbreeding between genetically different populations results in a reduction in fitness relative to both parental genotypes, it is often referred to as "outbreeding depression". The mechanisms responsible for outbreeding depression fall into two different categories: 1) local adaptation, where the hybrid population lacks adaptations to its

environment; and 2) coadaptation, where the hybrid population contains combinations of alleles at different loci that are not adapted to each other (Templeton, 1986). Outbreeding depression may occur in the first hybrid generation, or among their offspring (Lynch, 1991). The degree of fitness loss seems to depend on how distant a cross is (i.e. the extent of genetic differentiation between the parents), but quantitative data are largely lacking on the frequency and severity of outbreeding depression in animals (Frankham, 1995).

Releases of artificially propagated salmonids provide some evidence for reduced fitness and lack of local adaptation of hybrids between native and non-native populations (Hindar, Ryman and Utter, 1991). In an Irish experiment with first- and second-generation offspring of farm and wild salmon (McGinnity et al., 2003), the highest egg mortality occurred in the F_2 hybrid group (median 68%), which was significantly higher than all other groups (e.g. wild 3%). Since the first-generation backcrosses, which used aliquots of the same eggs as F_2 hybrids, showed significantly lower mortality (8%) this high F_2 hybrid mortality is not due to maternal or egg quality effects and most likely reflects outbreeding depression (McGinnity et al., 2003). Another case of outbreeding depression is provided by the crossing of anadromous and landlocked Atlantic salmon (Sutterlin, Holder and Benfey, 1987), where lower early survival rates and morphological abnormalities were found in hybrid (landlocked x anadromous) offspring.

Effective population size

Principles for the conservation of genetic variation in natural populations have been related to the population's effective size, which is defined as the size of an ideal population that is losing genetic variation at the same rate as the actual population (Wright, 1969). The effective population size is inversely proportional to the rate of inbreeding of the population. The effective population size also affects the rate of loss of heterozygosity and of genetic variance in quantitative traits, such as body size, fecundity, survival, and ultimately, fitness. Empirical evidence from laboratory and domestic animals suggests that increased inbreeding and loss of genetic variation can have negative consequences for a number of fitness-related traits. Moreover, loss of genetic variation can reduce the possibility for a population to adapt to changing environments (Lande and Shannon, 1996). For short-term conservation, it has been suggested to maintain effective population sizes above 50 per generation to keep the rate of inbreeding low to avoid inbreeding depression (Frankel and Soulé, 1981). For long-term conservation, it has been suggested to maintain effective population sizes above 500 to 5 000 in order to preserve typical levels of genetic variability in quantitative characters (Lynch and Lande, 1998).

Effective population size has been used as one criterion for determining the extinction risk and setting conservation limits (CLs) of single populations (and/or species), e.g. in international (IUCN) guidelines for categorising threatened species (Mace and Lande, 1991). Such criteria are not well-developed, however, for anadromous Atlantic salmon populations that are interconnected by gene flow and living in different environments (Hindar et al., 2004). Such a group of populations is what population geneticists refer to as a "subdivided population" (Wright, 1969), and what many ecologists have termed a "metapopulation" (Pulliam, 1988).

A theoretical model with constant local population sizes and a fixed but arbitrary pattern of migration suggests that the total effective (meta)population size can be computed using numerical methods (Tufto and Hindar, 2003). The effective population size in a set of interconnected subpopulations depends on both the rate and pattern of gene flow. Low, symmetric migration rates between subpopulations increase the total effective size (relative to the subpopulation sizes). Asymmetric migration, on the other hand, decreases

the total effective size. In the extreme case, that is, one-way migration, the total effective size eventually becomes equal to the effective size of the subpopulation emitting migrants (Tufto and Hindar, 2003).

Precise estimates of the effective population sizes of Atlantic salmon populations have rarely been made. Some methods exist to find rough estimates of the effective size or of the ratio of effective to census size. A review of estimates from many species suggests that the effective size is often as low as 10-20% of the census population size (Frankham, 1995); some experiments with salmonids suggest that the figure may be close to 20%. With a generation time of approximately five years, a rough estimate suggests that the effective population size per generation may be close to the census size per spawning season (Hindar et al., 2004). In that case, the effective population size in most Atlantic salmon populations may be quite small, as the census size of the majority of Atlantic salmon spawning populations may be in the order of hundreds (Hindar and Jonsson, 1995).

Recent microsatellite studies of the River Tana/Teno in Norway/Finland suggest variable local effective population sizes in this river system from N_e = 35-70 in some tributaries to *ca*. 500 in mainstem Tana/Teno to more than 1 200 in Iesjohka, a major headwater stream. Similar studies in four small rivers of northern Spain suggested local effective population sizes on the order of 30, or 80, depending on whether an open or closed migration system was assumed (Kuparinen et al., 2010).

Empirical evidence of interspecific hybridisation (evidence from nature)

Natural hybrids between Atlantic salmon and its congener, brown trout (*S. trutta*), have been detected at low frequencies in many studies, beginning with Payne, Child and Forrest's (1972) estimate in samples of adult salmon (mean, 0.4%). More recent studies have found higher hybridisation rates with mean values ranging between 0.9% and 13.2% (reviewed by Jordan and Verspoor [1993]).

High hybridisation rates are often found where one species is introduced (as brown trout in Newfoundland). Fish culture may also contribute to increasing rates of hybridisation. In Scotland (Youngson et al., 1993) and Norway (Hindar and Balstad, 1994), elevated hybridisation rates show associations with the spawning of escaped farm salmon. Other causes for high hybridisation rates may be reduced population size of one of the species to such low levels that it is difficult to find conspecific spawners. Other types of disruption of the breeding system (e.g. habitat alteration) may also contribute to high hybridisation rates.

Hybrids survive well but rarely reproduce (Anon, 1997), and thus may lower the productivity of local populations and in rare cases lead to introgression of genetic material from one species into the other.

Interbreeding between Atlantic salmon and species from the phylogenetically closest genera (Crespi and Fulton, 2004), Pacific salmon (genus *Oncorhynchus*) and charrs (genus *Salvelinus*), is not known to occur in the wild. Laboratory experiments suggest that some intergeneric crosses may lead to viable offspring but are unlikely to be produced in nature (Chevassus, 1979).

History of artificial reproduction in salmon

Artificial reproduction of salmonids (brown trout) was mastered by Stephan Ludwig Jacoby in Germany in the middle of the 18th century. From the 1850s onwards, this technique was used to supplement populations of several salmonid species, including the Atlantic salmon, all over the northern hemisphere (stock enhancement, Egglishaw et al., 1984).

Following developments of salmon-rearing technologies, releases of juvenile Atlantic salmon at the smolt stage (after one or more winters in a hatchery) became widespread during the 1950s, whereas the first successful attempts to raise Atlantic salmon to market size in marine enclosures were carried out in Norway from the late 1960s (Heen, Monahan and Utter, 1993).

Current level or status of intraspecific crosses

No absolute barrier to crossing exists between Atlantic salmon from different regions of the distribution area, between landlocked and anadromous populations, or between cultured strains and wild salmon. The genetic differentiation observed between populations is therefore related to the fragmented nature of the spawning habitat, the homing behaviour of migrating salmon and the typically reduced fitness of interpopulation crosses (Hindar, Ryman and Utter, 1991).

Deliberate releases are used for various reasons: conservation of endangered populations, augmentation of non-endangered populations (i.e. enhancement; Ritter, 1997), compensation for habitat lost by human activities, re-establishment of extinct populations, and for increasing catch in put-and-take fisheries and sea/ocean ranching (Isaksson et al., 1997). Accidental releases occur when Atlantic salmon escape from hatcheries or fish farms. Large escapes are known to occur, particularly from net cages in the marine environment. One example is the escape of 490 000 salmon from one Norwegian fish farm in 2005, representing a total weight (1 300 tonnes) which exceeded the total weight of wild Atlantic salmon caught in sea and river fisheries in Norway that year (Statistics Norway, 2006).

Currently, the number of artificially reproduced Atlantic salmon amounts to more than 300 million annually. The large majority of these are released into net cages for farming (Statistics Norway, 2010), whereas releases for sea/ocean ranching amount to approximately 8 million smolt per year (Isaksson, 1988; Isaksson et al., 1997).

Sea-ranched salmon in the Baltic Sea, and escaped farmed salmon in Norway, represent cases where artificially propagated salmon may make up large proportions of wild populations. More than 45% of the salmon caught at sea in the Bothnian Bay during 2000 were hatchery-produced (Koljonen et al., 2005), whereas escaped farmed salmon made up on average 11-35% of Atlantic salmon spawning populations in Norway over the period 1989-2000 (Fiske et al., 2001).

Interspecific and intergeneric crosses

Viable Atlantic salmon x brown trout hybrids are readily produced in the laboratory (Refstie and Gjedrem, 1975; Chevassus, 1979). First-generation hybrids seem to be intermediate between the two species in morphological, ecological and behavioural traits (Anon, 1997; Hindar, 1998). Some male hybrids are fertile and have been back-crossed with female Atlantic salmon (Wilkins, Courtney and Curatolo, 1993; Anon, 1997). Back-crosses are largely either non-viable or triploid (Galbreath and Thorgaard, 1995), and thus, genetic introgression between the two species must be considered a rare event (Garcia-Vazquez et al., 2003).

Hybrids between Atlantic salmon and Pacific salmon or charrs may be produced in the laboratory, although with typically very low early survival. In crosses between Atlantic salmon, brown trout, rainbow trout (*O. mykiss*), pink salmon (*O. gorbuscha*) and Arctic charr (*Salvelinus alpinus*), all combinations produced some offspring except crosses involving pink salmon females or rainbow trout males (Refstie and Gjedrem, 1975; Gjedrem 1979).

Crosses between Atlantic salmon and Arctic charr were found to show a high growth rate in fresh water, but lower growth rate than Atlantic salmon in sea water (Gjedrem, 1979).

In reciprocal crosses between Atlantic salmon and seven species of Pacific salmon, Devlin (cited in Waknitz et al. [2002]) found only one cross (female steelhead [anadromous rainbow] trout x male Atlantic salmon) to produce more than 1% survival to hatch. This cross was made using cryopreserved sperm because the natural spawning time of the two species differs by several months. Another partly successful cross was made from female Atlantic salmon x male pink salmon, with 0.36% survival to hatch. Rearing of survivors for four years did not result in signs of sexual maturation.

Production of triploid hybrids between salmonid species results in sterile fish that sometimes survive better than diploid interspecific hybrids. Gray, Evans and Thorgaard (1993) did not find viable triploid hybrids between Atlantic salmon and Pacific salmon.

Genetic and ecological information on deliberate and accidental releases

Experiences from releases of Atlantic salmon within its natural range are reviewed within this section, as these are more relevant for discussing the genetic impacts of releases. Information is obtained from deliberate and accidental releases of salmon at various stages of domestication, concentrating on recent experiments where the performance of farm and wild Atlantic salmon has been compared in whole-river environments (McGinnity et al., 1997, 2003; Fleming et al., 2000; Skaala et al., 2012).

Cultured fish may be distinguished from their wild counterparts by differences in external morphology (Lund, Hansen and Järvi, 1989; Fleming et al., 2002), growth patterns in scales and otoliths (Lund and Hansen, 1991; Hindar and L'Abée-Lund, 1992), pigmentation (Lura and Sægrov, 1991a; 1991b) (for a comprehensive review, see Fiske, Lund and Hansen [2005]), molecular genetic markers (Karlsson et al., 2011), and growth rate (of offspring) in a hatchery (Solberg et al., 2013). However, the longer the fish have been in the wild, the more difficult it is to use such characters to distinguish them from wild fish. In some instances, fin-clipping and external or internal tags has been used to identify fish of cultured origin. Genetic differences between cultured and wild fish may also be used as a basis for separation of the two groups and their offspring (Skaala et al., 2004).

Fate of released fish

Releases of cultured, first-generation offspring of native salmon, particularly when carried out at an early life stage, seem to produce fish that perform similarly to naturally reproduced salmon. Larger differences from native salmon are found when a non-native stock is used, when the fish have spent part of their life in hatcheries before release (as in stock enhancement based on summer-old parr, or sea ranching based on smolts) and when the donor stock has been subject to selective breeding for one or more generations (as in accidental releases from fish farms). The relative importance of non-native origin, hatchery experience and level of domestication is only partly known; differences between native and released fish are evident even when a neighbouring wild population is compared with native fish (McGinnity et al., 2004).

Genetic consequences – intraspecific

Genetic data

Genetic risks from introductions include homogenisation of the genetic structure of the species through swamping a region with a common gene pool, loss of entire populations caused by disease or ecological interactions, loss of local adaptations through interbreeding, and failure of populations to readapt to local conditions if the introductions continue (Ryman, Utter and Hindar, 1995). The amount of genetic change caused by interbreeding is a function of the genetic difference between introduced and local populations, and the rate of gene flow between the two. Longer term genetic impacts depend on the extent to which evolutionary processes in the local population counteract the genetic change generated by the introductions.

Interbreeding between farmed and wild fish has been demonstrated experimentally in spawning arenas (Fleming et al., 1996), in a river following release of genetically marked fish (Fleming et al., 2000) and by studying genetic markers in the wild (Crozier, 1993, 2000; Clifford, McGinnity and Ferguson, 1998a; 1998b). Clifford, McGinnity and Ferguson (1998a) used mtDNA variants to demonstrate that escaped farm females left offspring in two Irish rivers. They showed that farm female spawning was highly heterogeneous within each river, with up to 70% at some sites and complete absence in others. In addition, these authors used a bi-parentally inherited minisatellite locus to demonstrate the presence of pure farm offspring in the rivers. The authors also noted the breeding of farm males with wild females in a different part of the river from the area in which farm female spawning took place. Clifford, McGinnity and Ferguson (1998b), using the same two markers, showed that farm fish escaping into a river at the juvenile stage completed the life cycle in the wild to return to that river to breed and interbreed with wild fish.

Other examples where molecular genetic information has demonstrated farm salmon contributions to wild populations come from the observation that farm and wild adults entering the same stream differ in allozyme allele frequencies, and that the offspring generation change occurred in the direction of the farm fish. If the alleles recorded are found in both parental groups, factors other than the successful spawning of farm fish can explain this observation. However, when the farm escapes have alleles that are not found in wild fish, these have been used to demonstrate farm contribution of alleles to the wild population (Crozier, 1993; 2000).

Recently, Glover et al. (2012) analysed 21 rivers in Norway over a period of up to 30 years using 22 microsatellites. They found temporal genetic changes in six populations (significantly so in four) and a reduction in the genetic diversity among these populations over time. They found that these genetic changes most likely were caused by escaped farm salmon spawning in these rivers. Another study by Glover et al. (2013) using the SNPs that can distinguish between farmed and wild salmon, showed significant introgression of farmed to wild populations in several rivers. Also, a technique has been developed to estimate farmed to wild genetic introgression without a historical reference (Karlsson et al., 2014). This technique has now been used to demonstrate significant genetic introgression of farmed to wild salmon in more than 50 wild Atlantic salmon populations in Norway (Diserud et al., 2016, in Vitenskapelig råd for lakseforvaltning [2016]).

Phenotypic and behavioural data

A review of the literature on the genetic effects following releases of non-native salmonid populations suggested two broad conclusions (Hindar, Ryman and Utter, 1991):

- The genetic effects of (intentionally or accidentally) released salmonids on natural populations are typically unpredictable; they vary from no detectable effect to complete introgression or displacement.
- Where genetic effects on performance traits have been detected following releases of salmonids, they appear to be negative in comparison with the unaffected native populations. For example, reduced total population size and reduced performance traits have been observed following introductions of non-native salmonid populations.

Salmonid populations are believed to be adapted to their local environments (Schaffer and Elson, 1975; Taylor, 1991; Hendry and Stearns, 2004; Myers et al., 2004; Bourret et al., 2011; Barson et al., 2015), and thus, introduced populations or crosses involving introduced populations would be expected to perform worse/less than the native ones.

Lifetime fitness and productivity

Fitness and productivity in whole-river experiments

The life-cycle experiments carried out in the Burrishoole system, western Ireland, studied first- and second-generation hybrids between wild and farmed salmon in the freshwater and marine life-history phases. Three cohorts (hatched 1993, 1994 and 1998) of Atlantic salmon were released above a fish trap in the Burrishoole system in western Ireland. Multiple families of the following groups were studied, having equal representation at release: native wild (all cohorts); Norwegian farmed (all cohorts); F_1 hybrid wild x farm (male and female reciprocal groups, 1993-94 cohorts); BC_1 backcrosses to wild (1998 cohort); BC_1 backcross to farm (1998 cohort); and F_2 hybrid wild x farm (1998 cohort). As the aim of the experiment was to look at genetic differences, without the confusion of behavioural differences, eggs and milt were stripped from mature adults and artificially fertilised, and group identification determined by DNA profiling (see McGinnity et al. [2003] for experimental details).

In the Burrishoole, farm salmon showed significantly lower representation than wild salmon in the samples of 0+ parr of all three cohorts from the experimental river at the end of the first summer. "Hybrids" (i.e. first-generation hybrids [F_1Hy], second-generation hybrids [F_2Hy] and first-generation backcrosses to wild salmon [BC_1W] and farm salmon [BC_1F], respectively) were intermediate or not significantly different from wild fish (Table 3.9). During the period from May 0+ to September 1+ (i.e. second year), the highest proportion of emigrant parr, taken in the experimental trap, was from the wild group and the lowest was from the farm group, with "hybrids" intermediate in representation. In the river 0+ parr, it was found that farm parr were the largest in size, wild parr the smallest, and "hybrids" intermediate, as expected from the selection of farm strains for increased growth rate. Thus, downstream migration was inversely proportional to parr size, and proportional to cohort density over the three cohorts, indicating competitive displacement of wild parr by the larger farm and "hybrid" fish. Although displaced wild parr were found to survive downstream under the experimental conditions used, such survival would not occur if a suitable unoccupied habitat is not available. This could occur when a river is at parr-carrying capacity or where the spawning area enters directly to sea (as may be typical for escaped farm salmon spawning in some circumstances) (Table 3.9).

Adult salmon returned from sea after 1SW or 2SW. In the 1SW returns, all groups except the backcross to wild showed a significantly lower return relative to wild. In the 2SW returns, all groups except farm of the 1998 cohort showed a proportionately greater return. However, the Burrishoole population is primarily a 1SW stock and the wild 2SW

return was only 2.5% of the total return. Overall the farm group showed a 0.3% return compared with 8% for wild smolts. Taking account of the differential egg production of 1SW and 2SW females, total egg deposition of returning fish was significantly higher for wild salmon than for all groups except BC_1 backcross to wild (Table 3.9).

Another life-cycle experiment was undertaken in the River Imsa, south-western Norway, to quantify the lifetime success (adult to adult) and behaviour-ecological interactions resulting from farmed salmon invading a native population (Fleming et al., 2000). The fish were sexually mature and had been selected such that native wild and farmed salmon were homozygous for different gene variants (alleles). Releases were made in autumn 1993 above a two-way fish trap where the population could be counted at the smolt and returning adult stages (see Fleming et al. [2000] for details). In parallel with the release experiment, farmed and wild salmon were introduced into a semi-natural spawning arena where their breeding performance could be closely monitored by direct observation and video 24 hours a day (*cf.* Fleming et al., 1996).

In the River Imsa, farm and wild adults had similar migration patterns and nesting locations; however, farm females spawned before wild females. Courting of females by both farm and wild males began shortly after release of the fish into the river. Wild males, however, courted females more often and retained less of their initial testes unspawned than did farm males. Offspring (age-0 parr) from the spawnings were sampled by electrofishing the River Imsa in autumn 1994. The proportion of wild to farm genotypes among the offspring (age-0 parr) from spawnings in the river had shifted significantly from the proportion of wild to farm spawners (Figure 3.20). Most of the fish were now of wild origin (65%); farm genetic representation occurred mainly through hybridisation with wild fish. Mitochondrial DNA analysis suggested that most, if not all hybrids had farm mothers.

Table 3.9. **Lifetime successes of the wild, farm and "hybrid" groups in the Burrishoole experiments**

Group	Fertilisation to eyed egg	Eyed egg to smolt[1]	Eyed egg to smolt[2]	Smolt to adult	Lifetime success[1]	Lifetime success[2]
Wild	1.0	1.0	1.0	1.0	1.0	1.0
BC_1W	1.0	0.89	1.0	1.0	0.89	1.0
F_1HyW	1.0	0.73	1.0	0.58	0.42	0.58
F_1HyF	0.87	0.50	0.63	0.61	0.27	0.33
F_2Hy	0.34	1.0	1.84	n.a.	(0.34)	(0.63)
BC_1F	1.0	0.79	1.59	0.39	0.31	0.62
Farm	0.79	0.41	0.76	0.07	0.02	0.04

Notes: Survival of the wild group is taken as 1.0. Where another group is not significantly different from the wild group, it is also given a value of 1.0. When significantly different, then the actual survival relative to the wild group is used. Data for marine survival of F_2 hybrids are not available and were set at 1.0 (from McGinnity et al. [2003]). Results averaged over several cohorts where available.

1. This assumes that displaced parr have the same survival as parr of the same group remaining in the experiment river, i.e. that the river is not at its parr-carrying capacity and spare habitat is available for displaced parr.

2. This assumes that displaced emigrating from the experimental river do not survive, i.e. that the river is at its parr-carrying capacity.

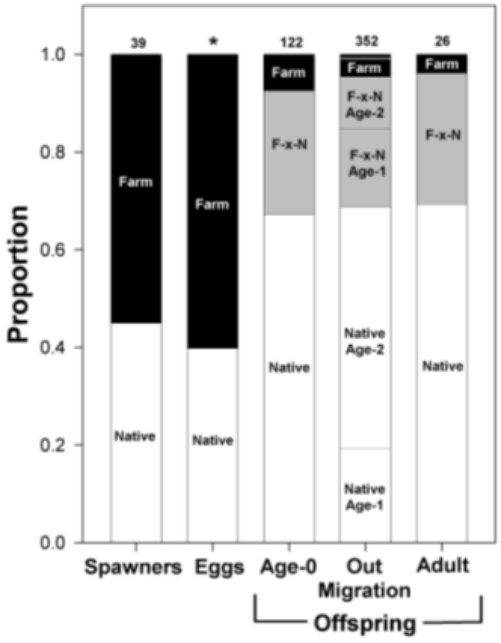
Production of smolts relative to the estimated total potential egg deposition was compared to the population's stock-recruitment relationship (Jonsson, Jonsson and Hansen, 1998). The total production of smolts (i.e. migrants to the ocean) from the spawnings was 28% below that expected based on the estimated potential egg deposition and the stock-recruitment

relationship for River Imsa (Jonsson, Jonsson and Hansen, 1998). Moreover, smolt production by wild females was 31-32% below that expected from their estimated potential egg deposition in the absence of farm females.

There were distinct behavioural and life-history differences among the smolts. Smolts produced by farm fish (farm smolts) descended earlier and at a younger age than did wild smolts, and hybrids descended at a time that was intermediate to that of the farm smolts and wild smolts. Hybrid smolts were also longer and heavier than native smolts, whereas farm smolts weighed less for a given length than did their wild counterparts.

There was no significant difference among the offspring types in survival from seaward migration to return as sexually mature individuals. The lifetime reproductive success, adult-to-adult, of the farm salmon was 16% that of the wild salmon. All adult recaptures occurred in either the coastal fishery or the River Imsa; no fish were reported as straying into other rivers. The mean age-at-maturity of hybrid fish (3.4 years) was less than that of native fish (4.2 years).

Figure 3.20. **Changes in the proportional constitution of the Atlantic salmon population in the River Imsa following the release of native wild and farm spawners**



Notes: * Potential egg deposition was 19 443 for native females and 29 388 for farm females.

The number above each bar represents either the total population size (spawners and adult offspring) or the sample size examined at each life stage (age 0 and out-migration). Two age groups of out-migrants existed, age 1 and age 2, and are stacked on top of each other for each offspring type. Solid bars represent farm offspring; open bars represent native offspring; hatched bars represent hybrid offspring (from Fleming et al. [2000], Figure 1).

Source: Fleming et al. (2000).

A recent experiment in Canada designed to study potential local adaptation to acidified rivers in Atlantic salmon populations and whether or not repeated interbreeding with farm salmon influenced this adaptation, found mixed evidence for reduced local adaptations by interbreeding (Fraser et al., 2010). Wild juveniles had higher survival in acidic water

than farm salmon or wild-by-farm hybrids. In contrast, the backcrosses and second-generation wild-by-farm hybrids performed equally well if not better than wild salmon in acidic water for the life stages studied. Follow-up studies on farm-wild hybridisation across divergent wild populations and multiple traits found evidence that hybrid fitness decreased with increasing divergence between the hybridising populations, but limitations to what extent changes in specific traits could be predicted (Fraser et al., 2010). Skaala et al. (2012) planted farmed, hybrid (farmed x wild), and wild Atlantic salmon eggs in the River Guddalselva, western Norway, much in the same manner as the Burrishoole experiment reported above but detailed to performance at the family level. They found initially a high growth rate and high survival to smolts in farmed families, whereas later releases at higher standing density showed a reduced growth and lower survival of farmed than hybrid families, as well as lower in comparable hybrid than wild families.

It has been observed that farmed females may destroy the redds of wild salmon in nature (Lura and Sægrov, 1991b). Thus, even when escaped farmed salmon have low spawning success, they can reduce the success of local wild fish.

Effects on effective population size

Supportive breeding

As the survival of early life stages in hatcheries can be substantially higher than for comparable life stages in the wild, release programmes have the capacity to overwhelm anatural population with fish from a limited number of breeders. This may create a situation where the total population size increases while the total effective population size decreases, in particular if a proportionally large input of released fish is produced from a proportionally small broodstock population (Ryman and Laikre, 1991). However, if supportive breeding results in a substantial and continuous increase of the census size over multiple generations, it is possible to increase also the effective population size of the supported population.

Escaped farm salmon

It has been estimated that the major strains of farm Atlantic salmon in Norway (which are also used in a number of other countries) have an average effective population size of about 80 individuals (Mork et al., 1999). If we ignore genetic differences between each of the four major strains, the total effective size of the major strains of farm salmon is roughly 320 individuals (even though their descendants count millions of individuals). The total effective size of the wild Atlantic salmon is not known, but it is probably on the order of 10^4 or 10^5 per generation if we assume that it is near the per-spawning census size (Tufto and Hindar, 2003).

A large number of fish escape from farms annually and make up a significant proportion of the spawners in wild salmon populations. Even though their reproductive success is less than that of wild fish, it has been estimated that the average one-way gene flow from farm into wild salmon in Norway is around 7-8% (Mork et al., 1999; Fleming et al., 2000), a more recent estimate from a demographic model being 4.5% (Hindar et al., 2006). Recent studies suggest that current introgression levels of farmed to wild salmon in Norway varies among populations from 0% to more than 40% (Glover et al., 2013; Diserud et al., 2016).

Under a scenario of one-way migration, the total effective size of the farm plus wild salmon is simply $N_e = 320$ individuals. Tufto and Hindar (2003) have estimated that the time needed for this asymptotic effective size to be attained (for one-way gene flow

at 7.5%), is of the order of 13.7 generations. With two-way gene flow, allowing 5% migration from the wild population back into the selected strains, the total effective size would increase to N_e = 880 individuals and the time to reach it decrease to 8 generations (Tufto and Hindar, 2003). However, more details are needed in the model to realistically predict the long-term outcome of interactions between several farm strains and numerous wild populations.

Genetic consequences: Interspecific

Interbreeding between Atlantic salmon and brown trout is the most likely cause of interspecific genetic consequences. The rate of hybridisation between the two species appears to be increasing (Youngson et al., 1993; Hindar and Balstad, 1994), and this increase may be partly related to salmon culture.

Interbreeding (i.e. a direct genetic effect) can be neglected when Atlantic salmon interact with other species than brown trout, for example when they are farmed in the Pacific. Indirect genetic effects, such as loss of genetic variability (reduced effective population size) or genetic change in response to new selective regimes (*cf.* Waples, 1991), must be considered if cultured Atlantic salmon establish feral populations outside the species' range, and/or if they otherwise cause population declines of native species.

Indirect genetic effects on other species can occur even if feral populations are not established. For example, if high numbers of fish escape at both fresh water and salt water life stages of Atlantic salmon, the released or escaped fish may be a key ecological factor (Soto, Jara and Moreno, 2001). The likelihood of population establishment increases with the number of introductions, and with the time over which introductions take place.

Dramatic effects of fish introductions are often associated with the concomitant introduction of a disease organism. The lack of testing of these organisms during fish introductions has made historical studies difficult to interpret, but this situation may change following developments in molecular epidemiology (Naylor et al., 2005). Deliberate releases of fish, and fish farming not operating in fully enclosed systems, will always be associated with the possibility that disease organisms are transferred from farmed to wild fish (or from wild to farmed and back to wild at considerably higher densities). Historical studies suggest that transport of fish and/or fish eggs is an important vector for disease organisms, and that these organisms may have dramatic effects on the population size of native species (Johnsen and Jensen, 1991, 1994; Bakke and Harris, 1998; Naylor, Williams and Strong, 2001).

Notes

1. www.miljødirektoratet.no/Villaksportalen.
2. www.nasco.int/implementation_plans_cycle2.html and www.nasco.int/pdf/agreements/williamsburg.pdf.
3. www.dirnat.no/content/2475/Handlingsplan-forslag-mot-lakseparasitten-Gyrodactylus-salaris.

4. https://www.skretting.com.
5. www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20080617-0822.html.
6. See: www.pac.dfo-mpo.gc.ca/aquaculture/reporting-rapports/mar-rep-rap/2011-2014/sec_1-eng.html.
7. http://wwweng.vetinst.no/eng/Publications/Fish-Health-Report.html
8. See: http://www.fiskeridir.no/ and www.scotland.gov.uk/Topics/marine/Fish-Shellfish/18364/18692/escapeStatistics.
9. http://web.uvic.ca/cbr/grasp.
10. See www.ncbi.nlm.nih.gov/About/primer for an explanation of terms.

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Annex 3.A1.
Selected research on genetically engineered Atlantic salmon

Several research groups have conducted, or are currently conducting, research on Atlantic salmon that have been genetically engineered or genetically modified via the use of recombinant DNA technologies. These fish are sometimes referred to as "transgenic" if the DNA used to make the modification came from another fish or animal species.

Most of the research to date has been conducted on an Atlantic salmon that has been genetically engineered for enhanced growth through the addition of a growth hormone gene from a chinook salmon. A partial listing of research published on this growth-enhanced transgenic salmon is presented below.

Recent developments in targeted mutagenesis by the so-called CRISPR/Cas9 system should be added to this list (e.g. Edvardsen et al. [2014] for Atlantic salmon).

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List of OECD consensus documents on environmental safety assessment, 1996-2017

	Consensus document	Lead country(ies)	Year of issue	Volume
Facilitating harmonisation	Designation of a Unique Identifier for Transgenic Plants (revised version) (guidance document)	Working Group	2006	Vol. 3
	Introduction to the OECD Biosafety Consensus Documents	Working Group	2005	Vol. 1, 3, 4, 5, 6, 7
	Low-Level Presence of Transgenic Plants in Seed and Grain Commodities: Environmental Risk/Safety Assessment, and Availability and Use of Information	Working Group	2013	Vol. 6
	Molecular Characterisation of Plants Derived from Modern Biotechnology	Canada	2010	Vol. 3
	Points to Consider for Consensus Documents on Biology of Cultivated Plants	Working Group	2006	Vol. 3
Traits	Crop Plants Made Virus Resistant through Coat Protein Gene-Mediated Protection	Task Group	1996	Vol. 1
	Genes and their Enzymes that Confer Tolerance to Glyphosate Herbicide	United States, Germany and Netherlands	1999	Vol. 1
	Genes and their Enzymes that Confer Tolerance to Phosphinothricin Herbicide	United States, Germany and Netherlands	1999	Vol. 1
	Herbicide Metabolism and the Residues in Glufosinate-Ammonium (Phosphinothricin)-Tolerant Transgenic Plants	Germany	2002	Vol. 1
	Transgenic Plants Expressing *Bacillus thuringiensis*-Derived Insect Control Protein	United States	2007	Vol. 3
Micro-organisms	***Information Used in the Assessment of Environmental Applications of Micro-organisms***			
	Acidithiobacillus	Canada	2006	Vol. 2
	Acinetobacter	Canada	2008	Vol. 4
	Baculovirus	Germany	2002	Vol. 2
	Pseudomonas	United Kingdom	1997	Vol. 2
	Guidance Documents on Biosafety Aspects of Bacteria			
	Horizontal Gene Transfer Between Bacteria	Germany	2010	Vol. 4
	Methods for Detection of Micro-organisms Introduced into the Environment: Bacteria	Netherlands	2004	Vol. 4
	Use of Information on Pathogenicity Factors: Bacteria	Netherlands and Canada	2011	Vol. 5
	Use of Taxonomy in Risk Assessment of Micro-organisms: Bacteria	Canada and United States	2003	Vol. 4

	Consensus document	Lead country(ies)	Year of issue	Volume
Biology of crops	Bananas and plantains (*Musa* spp.)	Spain	2009	Vol. 4
	Brassica crops (*Brassica* spp.)	Canada	2012	Vol. 5
	Cassava (*Manihot esculenta*)	Brazil, NEPAD-ABNE and ILSI-CERA	2014	Vol. 6
	Chili, hot and sweet peppers (*Capsicum annuum*)	Korea, Mexico and United States	2006	Vol. 1
	Common bean (Phaseolus vulgaris)	Brazil and ILSI-CERA	2015	Vol. 6
	Cotton (*Gossypium* spp.)	Spain	2008	Vol. 4
	Cowpea (*Vigna unguiculata*)	Australia	2015	Vol. 6
	Maize (*Zea mays* subs. *mays*)	Mexico	2003	Vol. 1
	Squashes, pumpkins, zucchinis and gourds (*Cucurbita*)	Mexico and United States	2012	Vol. 5
	Oyster mushroom (*Pleurotus* spp.)	Korea	2005	Vol. 1
	Papaya (*Carica papaya*)	United States	2005	Vol. 1
	Potato (*Solanum tuberosum* subsp. *tuberosum*)	Netherlands and United Kingdom	1997	Vol. 1
	Rice (*Oryza sativa*)	Japan	1999	Vol. 1
	Oilseed rape (*Brassica napus*): ***replaced with* Brassica *Crops (2012) in Vol. 5***	Canada	1997	Vol. 1
	Sugar beet (*Beta vulgaris*)	Switzerland	2001	Vol. 1
	Sugarcane (*Saccharum* spp.)	Australia	2013	Vol. 6
	Sunflower (*Helianthus annus*)	France	2004	Vol. 1
	Sorghum (*Sorghum bicolor*)	South Africa and United States	2016	Vol. 7
	Soybean (*Glycine max*)	Canada	2000	Vol. 1
	Tomato (*Solanum lycopersicum*)	Spain and Mexico	2016	Vol. 7
	Wheat (*Triticum aestivum*)	Germany	1999	Vol. 1
Biology of trees	***Timber trees***			
	Birch: European white birch (*Betula pendula*)	Finland	2003	Vol. 2
	Douglas fir (*Pseudotsuga menziesii*)	Canada	2008	Vol. 3
	Eucalyptus (*Eucalyptus* spp.)	Australia	2014	Vol. 6
	Larches: North American larches (*Larix lyalli, Larix occidentalis, Larix laricina*)	Canada	2007	Vol. 3

	Consensus document	Lead country(ies)	Year of issue	Volume
Biology of trees (*continued*)	Pines: Eastern white pine (*Pinus strobus*)	Canada	2002	Vol. 2
	Pines: Jack pine (*Pinus banksiana*)	Canada	2006	Vol. 3
	Pines: Lodgepole pine (*Pinus contorta*)	Canada	2008	Vol. 3
	Pines: White pine (*Pinus monticola*)	Canada	2008	Vol. 3
	Poplars (*Populus* spp.)	Canada	2000	Vol. 2
	Spruces: Black spruce (*Picea mariana*)	Canada	2010	Vol. 3
	Spruces: Norway spruce (*Picea abies*)	Norway	1999	Vol. 2
	Spruces: Sitka spruce (*Picea sitchensis*)	Canada	2002	Vol. 2
	Spruces: White spruce (*Picea glauca*)	Canada	1999	Vol. 2
	Fruit trees			
	Bananas and plantains (*Musa* spp.) [listed above in "Crops"]	Spain	2009	Vol. 4
	Papaya (*Carica papaya*) [listed above in "Crops"]	United States	2005	Vol. 1
	Stone fruits (*Prunus* spp.)	Austria	2002	Vol. 2
Biology of animals	Atlantic salmon (*Salmo salar*)	Finland, Norway and United States	2017	Vol. 7

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