

CONSERVATION GENETIC MANAGEMENT OF BROWN TROUT (*Salmo trutta*) IN EUROPE

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1. INTRODUCTION

This report has been produced within the framework of the EU Fisheries and Agriculture Research (FAIR) project "Concerted action on identification, management and exploitation of genetic resources in the brown trout (*Salmo trutta*)" (abbreviated as "TROUTCONCERT"; FAIR CT97 3882) coordinated by Dr Michael M. Hansen at the Danish Institute for Fisheries Research.

The purpose of the report is to describe the current knowledge of the conservation genetic status of the brown trout in Europe, and to review the main threats towards the biological diversity at the gene level of brown trout populations. Further, the report summarizes currently available conservation genetic guidelines that are applicable to brown trout, and provides some general recommendations for the genetic management of brown trout populations in Europe.

It should be stressed that the goal of this presentation is not to provide a detailed strategy or action plan for conservation and sustainable management of brown trout biodiversity. However, the summary and general overview presented here represent a necessary basis for further activities aimed at promoting conservation genetic management of the species. The views expressed are those of the authors and do not reflect official standpoints of the EU.

1.1 THE STRUCTURE OF THIS REPORT

This report is directed towards everyone with a professional or private interest in conservation genetic management of biological resources in fishes, and to those specialised in brown trout management. Target groups include both fishery and wildlife managers as well as representatives for conservation oriented authorities. Several sections providing basic background information (e.g., 1.2, 1.4, 1.5 and 1.7) have been included, and more informed readers may skip these sections.

The Introductory section (1) provides basic information on the brown trout (1.2), and on the particular conservation challenges of this species (1.3). For the reader who is not at all familiar with population genetics, section 1.4 presents a very basic background including elementary terminology, and section 1.5 includes a short introduction to laboratory techniques for studying genetic variability in brown trout. Section 1.6 describes the present level of knowledge regarding local adaptation in brown trout, and 1.7 provides briefly the general goals of genetic conservation. The second section (2) describes what is currently known regarding the population genetic structure of brown trout in the different European regions, and also comments on the available information regarding temporal genetic variation (2.6).

Section 3 presents the major categories of threats to brown trout biodiversity and describes the three general processes through which genetic diversity is reduced and lost. A particular focus is devoted to the genetic consequences of stocking (3.1 and 3.2) because this measure is frequently regarded as beneficial to the natural population, but may, in fact, constitute a serious threat to brown trout biodiversity.

Section 4 exemplifies international agreements that are applicable to brown trout conservation, and in 4.1 the official threat status of the species over the European range is

reviewed. This overview is based on national Red Lists and similar documents.

The overview of the conservation genetic situation of brown trout in individual European countries is continued in chapter 5. The discussion in this chapter is based on the answers to a questionnaire to TROUTCONCERT participants, and exemplifies aspects of both the legal system, the major threats, and the general attitude towards brown trout conservation in some countries.

Currently available guidelines for genetic conservation applicable to brown trout are summarized in section 6. These guidelines include both general conservation recommendations (6.1; Table 6) and more specific advice associated with particular activities (6.2; Table 9). The general guidelines section (6.1) include discussions on how to define conservation units (6.1.2), and how to construct protocols for prioritizing brown trout populations for conservation (6.1.3). A summary of the report is presented in section 7, which also contains specific recommendations extracted from the various parts of the report (7.1).

1.2 THE BROWN TROUT - BRIEF BACKGROUND

The natural distribution of the brown trout is essentially restricted to Europe, and the species is widely found within this range. In the east, the species range also extends into Asia, and the southern limits are in the Atlas mountains of North Africa (Elliott 1994). Brown trout have been introduced to at least 24 countries outside Europe, including the USA, Canada, and Australia, as well as several countries in South America, Africa, and Asia. Most of these introductions were carried out in the late 1800s and first half of the 1900s, before the biological risks with introduction of exotic species were generally recognized.

The brown trout is ecologically very diverse. For instance, both resident and migratory life-history types exist within the species. The migratory forms consist of both anadromous and lake-dwelling trout. Anadromous trout (often called "sea trout") migrate from the rivers or streams in which they are born to the sea where they forage until reaching sexual maturity. They return to their native rivers to spawn, and many also return for overwintering. The lake-dwelling forms migrate from their natal rivers to lakes, and also return to the rivers to spawn. Resident trout spend their entire life in a river or a small stream, and often spawn in smaller tributaries of the area (Elliott 1994). The strong instinct of the brown trout to return to the natal river and stream to spawn is known as "homing". Migratory and resident trout may co-exist in rivers, and some studies (e.g., Hindar et al. 1991a) suggest that the two forms interbreed. However, the precise mechanisms and possible genetic factors underlying this variation in life-histories still remain largely unresolved. The diet of the brown trout may vary with age, geographic area, and environment and may consist of benthos, zoo-plankton, insects, and fishes. In many lakes in northern Europe the brown trout is the only naturally occurring fish, and thus the only top predator. It interacts ecologically with other species and healthy populations of brown trout are, for instance, necessary for the reproduction of the freshwater pearl mussel (*Margaritifera margaritifera*). The larvae of the mussel live on the gills of the trout (Young & Williams 1984a,b; Grundelius 1987). The freshwater pearl mussel populations are decreasing and threatened all over its range. The species is classified as "Endangered" in many European countries, including Denmark, Finland, France, Germany, The Republic of Ireland, Sweden and the United Kingdom (IUCN 1996).

Together with the North American cutthroat trout (*Oncorhynchus clarkii*), the brown trout is one of the genetically most substructured vertebrate species currently known to exist (Allendorf & Leary 1988). A large proportion of the intraspecific biological diversity of the brown trout is represented by genetic differences between populations, and this genetic divergence is often coupled with pronounced phenotypic variation. For instance, in northern Europe approximately 40% of the genetic variation is due to differences between populations (Ryman 1983). This figure increases to about 65% when also considering trout populations in Central Europe and the British Isles (Ferguson 1989 and references therein), and is expected to increase even further if genetic data from the southern and eastern ranges are included. For a species with such a distinct genetic population structure, it is particularly important that conservation measures and strategies are focused on the population level.

In some countries brown trout is important for commercial fisheries and the species is a very popular target for angling. In several European countries (for instance Sweden, Denmark, Germany, Switzerland, Portugal, The Republic of Ireland, and large parts of France, the UK, and Spain) it is one of the most important species for sport fisheries, and as such supports a large tourist industry. The brown trout is particularly important to tourism in regions where industry is less developed and, consequently, opportunities for employment of local inhabitants are limited (e.g., Western Ireland and Galicia in Spain). The sea-run brown trout is especially valued by sport fishermen. In many countries of the EU "sea trout" are caught in rivers and in some countries, particularly Sweden, Norway and Denmark, angling for "sea trout" takes place directly on the coast as well. If this sort of angling could be developed in more countries, the opportunities for trout angling will be ample due to the many thousand kilometres of available coastline.

1.3 WHAT IS SPECIAL ABOUT BROWN TROUT CONSERVATION?

Large parts of the intraspecific variability of the brown trout have been lost due to environmental degradation, harvest and stocking. The remaining parts are threatened. Therefore, addressing the conservation genetic status of the brown trout in Europe is important. In addition to the ecological significance of the species, its high value for sport and commercial fisheries gives it considerable socioeconomic status. At the same time, the biological characteristics of the brown trout and the many serious threats towards it make conservation efforts particularly challenging. There are several reasons why brown trout conservation is special:

1. Extensive genetic studies have revealed that considerable genetic heterogeneity exists within the taxon referred to as "brown trout". There are several major evolutionary lineages, and within those lineages there is further heterogeneity represented by pronounced differentiation among local populations. In the brown trout an unusually large part of the genetic variation is represented by differences between populations. It is essential for conservation that the management of this species is focused on local populations.
2. Environmental degradation has exterminated or reduced many local populations all over the species range. Because of the species' genetic structure this has resulted in an unusually large loss of intraspecific biodiversity as compared to species with a less pronounced substructuring.

3. Because of the species' economic value, remaining populations are further threatened by activities such as stocking and harvest. Ironically, these activities are frequently considered harmless or even beneficial, but may, in fact, be devastating as shown by recent population genetic research.

4. Additional attributes connected to the brown trout and its conservation situation which make this species particularly interesting as a model organism include:

- No comprehensive delineation has, as far as we know, been made regarding the threat and conservation situation for the species over its European range.
- The brown trout intraspecific structure varies over the European range which, to some degree, necessitates different conservation and management strategies in different parts of Europe. The wide distribution of the species also makes it a primary target species for genetic monitoring over a wide geographic range.
- Habitat protection is complex, particularly for the migratory forms of brown trout. These forms move over large ranges and their conservation may be affected by the environmental status of different kinds of habitats, the policies of multiple countries, and several forms of exploitation.
- In many countries, the fact that the brown trout is a species of economical interest to fisheries impedes efficient conservation as a result of a complex legislative situation. As for other aquatic species subject to harvest, the management of brown trout is regulated by multiple legal acts.
- The brown trout is subject to a large number of management and conservation activities, including stocking, hatchery breeding, and translocations. At the same time, there are indications that many of these programs do not work well from a conservation genetic standpoint.
- There is a general need for an increased conservation focus on the aquatic environment. To date, much more effort has been concentrated towards various conservation activities in the terrestrial environment. Conservation of aquatic biological diversity still lags behind terrestrial conservation despite several cautionary reports on this issue.

One major reason why it is possible to identify these special features characterising the conservation and management situation of the brown trout is the extensive research effort that has been directed towards this species. It is interesting to note that these efforts have predominantly been conducted at the initiative of the scientific community, and represent the result of basic research that was in many cases not specifically aimed at providing information regarding management and conservation. It is possible that future studies will reveal similar situations for other species. Presently, however, it is imperative that appropriate action is taken for the brown trout on the basis of available information. Such action will be important not only for conserving the biodiversity of brown trout, but also for providing guidelines that can be applied to other species.

1.4 BIOLOGICAL DIVERSITY AT THE GENE LEVEL - A BASIC BACKGROUND

The genetic information of an individual is stored in the *DNA molecule* in the *nucleus* of every cell. The DNA molecule is made up of four separate building blocks called *nucleotides*. The genetic information consists of numerous combinations of the order of the four nucleotides into the so called *DNA sequence*. A specific part of the DNA molecule which includes the sequence information for a particular product is called a *gene*, and the physical location of a gene on the DNA strand is denoted *locus*.

The genes are transferred from one generation to the next, and every individual has two copies of each gene - one copy inherited from its mother and the other from its father. The DNA sequence of a specific gene may not always be exactly the same. There may be some differences in the sequence, resulting in different variants of the same gene. Such variants of a specific gene are called *alleles*. The existence of different alleles implies that *genetic variation exists*. The different alleles of a specific locus often occur in different frequencies in different populations (*allele- or gene frequencies*). The genetic variation of a species is therefore distributed both *within populations*, expressed as different allele combinations between individuals (so called *genotypes*), and *between populations* (in the form of differences in occurrence and frequency of alleles between populations). The diversity at the gene level is often referred to as *intraspecific variability* as it represents *biological diversity within a single species*.

Individuals of a species are often divided into more or less separate groups, *populations*, distributed over the species range. For a species such as the brown trout it is obvious that such groups are restricted to the specific water system in which they exist/spawn. Without human intervention, fish cannot migrate between such water systems if there is no connecting water between them. Also, even when migration between spawning grounds is possible, the strong homing instinct (i.e., sexually mature individuals return to spawn in the stream in which they were born) of the brown trout results in the formation of more or less reproductively isolated populations. Complete lack, or restricted exchange, of individuals between populations results in individuals from the same population being more genetically alike than individuals from different populations.

A population acquires new alleles primarily through the immigration of individuals from surrounding populations (*gene flow or genetically effective migration*), and through the process of random change of the DNA sequence (*mutation*). Apart from these two processes the number of alleles in a population is determined by the size of the population (see below) and the selective forces that particular alleles may be subjected to. Mutations occur very seldom, and this process for recreating genetic variability that has been lost, can usually be ignored within the time frames typically surveyed by human activities (tens or hundreds of years). From an evolutionary perspective (tens of thousands of years), however, mutations are very important. The process of mutation is the only way in which genetic variability is created, and without mutations there would be no biological diversity.

Different populations diverge genetically through the processes of *natural selection* and *genetic drift*. The DNA sequence difference between alleles of an individual gene may result in the function of the products of the alleles being somewhat diverse. Alleles may, for instance, be more or less "good" for the individual in a given environment. Through the process of *natural selection* individuals which carry particular alleles in specific environments

are favoured; they have enhanced survival and/or reproduction, and their alleles are spread to a larger extent than those of individuals who do not carry such selectively favoured alleles.

In all populations of a restricted size the frequency of particular alleles change randomly from one generation to the next. This process, called *genetic drift*, may also result in loss of genetic variation. By pure chance some of the alleles that exist in the parent generation may not be passed on to their offspring. The smaller the population, the more dramatic the fluctuation of allele frequencies, and the faster the loss of genetic variation.

Another consequence of small population size is *inbreeding*, i.e., the production of offspring from matings between close relatives. If a population is small and isolated, inbreeding is inevitable. In many species inbreeding is coupled with reduced viability and reproduction, reduced mean values of meristic traits, as well as increased occurrences of diseases and defects, so called *inbreeding depression* (Frankel & Soulé 1981). In salmonid fishes, inbreeding depression has, for instance, been documented in Atlantic salmon (*Salmo salar*; Ryman 1970), rainbow trout (*Onchorhynchus mykiss*; Aulstad & Kittelsen 1971; Kincaid 1976; Gjerde et al. 1983), brook trout (*Salvelinus fontinalis*; Cooper 1961) and is indicated also in studies of brown trout (Ståhl & Ryman 1987; Ståhl 1987).

The rate of genetic drift and inbreeding is not determined by the actual, census, population size but by a parameter denoted *effective population size* or N_e (Crow & Kimura 1970; Ryman et al. 1995a). *Effective population size* is perhaps one of the most important concepts in population genetics with respect to conservation biology, and it refers to the size of an "ideal" population that would have the same rate of drift as the observed, actual population. The effective population size of a natural population is typically much less than the census size and depends on such factors as sex ratio, variance in family size (i.e., variability in numbers of offspring per individual), temporal fluctuations in numbers of breeding individuals, overlapping generations, etc. (Crow & Kimura 1970). Minimum effective population sizes of 50 to 5000 per generation have been suggested by various workers as being necessary to avoid significant losses of genetic variability over various periods of time (Franklin 1980; Allendorf & Ryman 1987, in press; Lande & Barrowclough 1987; Franklin & Frankham 1998, Lande 1995, Lynch & Lande 1998).

In addition to the DNA of the nucleus of every cell (*nuclear DNA*) a small amount of DNA also exists in small organelles in the cytoplasm outside the nucleus called *mitochondria*. The DNA of the mitochondria (*mitochondrial DNA* or *mtDNA*) has several characteristics that are different from those of the nuclear DNA. For instance, mtDNA consists of a single, circular DNA-molecule. This means that the mitochondrial genome is *haploid*, i.e., there is only one copy of each gene. All the genes in the mtDNA circle are inherited together. Differences in DNA sequence in the mitochondrial genome result in the occurrence of different *haplotypes* (cf. alleles of the nuclear genome).

Since the mitochondria are located in the cytoplasm outside the cell nucleus, mtDNA is maternally inherited; only the eggs contain cytoplasm, not the sperm. The maternal inheritance implies that the process of, for example, genetic drift occurs at different rates for mtDNA compared to nuclear DNA; a reproducing male and female have the potential of transferring four nuclear alleles to the offspring, but only one mtDNA haplotype. Because of the maternal inheritance, the observed genetic structuring using mtDNA markers reflects the female segment of the population(s).

Biological diversity is frequently considered on the three levels of ecosystems, species, and genes (e.g., McNeely et al., 1990). Although there is a practical dimension of such compartmentalization, all levels of biotic diversity -- past, present, and future -- in reality represent nothing but a continuum of genetic differences (cf. Antonovics, 1990). Genetic variability is the basis of the biological evolution of the planet (Frankel 1970, 1974). The presence of genetic variation within species (between populations as well as between individuals within populations) is essential for their potential for survival, and for successfully evolving in response to both short-term and long-term environmental changes (e.g., Soulé and Wilcox, 1980). Natural selection favours alleles that are superior in a particular environment: without something to "choose from" better adapted individuals can not be created.

The intensifying rate of environmental change caused by various forms of human activities increases the need for genetic variability in natural animal and plant populations to respond to these changes. At the same time, human manipulations are reducing the genetic variability of these populations at a continuously increasing rate. Loss of alleles and allelic combinations can occur extremely rapidly, i.e., within a single generation. The different rates at which genetic variation can be generated and lost is a basic concern for conservation of genetic variability within species.

Despite the obvious importance of within species genetic variability, most contemporary concerns about losses of genetic variation appear to be directed at the species level and above. For instance, few attempts have been made to involve local populations in the collection of threatened biological resources or in the management of protected areas (NINA 1990). This is particularly unfortunate for a species like the brown trout where distinct genetic differences are common between populations (e.g., Ryman 1981).

1.5 TECHNIQUES FOR ASSESSING GENETIC VARIATION

During the past few decades the development in molecular genetics has been rapid, and several laboratory techniques are now available for the accumulation of population genetic data on brown trout and other species. So far, the most widely used method has been electrophoresis of allelic variants at protein coding loci, so called allozymes (e.g. Aebersold et al. 1987; Utter et al. 1987; Morizot and Schmidt 1990; May 1992). Although many new molecular genetic techniques have been developed more recently, protein electrophoresis must still be considered a very valuable tool: it is comparatively cheap and easy to use, and it detects considerable levels of genetic variation in the brown trout (e.g. Ferguson 1989). An additional advantage is that extensive reference data sets are available for allozymes, allowing comparisons between samples collected from populations separated in space and/or time.

The relatively recent development of new and highly variable genetic markers, such as maternally inherited mitochondrial DNA (e.g., Avise 1994), and hypervariable mini- and microsatellite DNA (e.g., Estoup & Angers 1998; Goldstein & Schlötterer 1998) has led to new research opportunities that were not possible using allozymes only. Mitochondrial DNA has proven useful for identifying major evolutionary lineages in brown trout (Bernatchez et al. 1992). In addition, this type of marker can be used to track demographic features exclusively for the female proportion of a population (Laikre et al. 1998).

Microsatellites have enabled the assessment of genetic population structure of many species, including brown trout, at much smaller geographic scales than has traditionally been possible (Estoup et al. 1998). Also, mini- and microsatellites are very useful for assignment of parentage (Marshall et al. 1998) and could be used, for instance, to estimate variance in reproductive success among individuals. This could allow estimation of the relative fitness of individuals. Mini- and microsatellites are also useful for estimation of relatedness among individuals (Queller et al. 1993) and have been used for identification of sibling groups among individuals in salmonid fish populations (Hansen et al. 1997a; Fontaine & Dodson 1999). Statistical tests that are particularly suitable for mini- and microsatellites have been developed for detecting recent population bottlenecks (Cornuet & Luikart 1996). Finally, it is possible to analyze DNA (in particular microsatellites) from old archival material, such as old scale samples, and thereby obtain data on the genetic composition of populations over very long time-spans (Nielsen et al. 1997, 1999a,b; Miller & Kapuscinski 1997; Tessier & Bernatchez 1999).

The applications of the techniques mentioned above are not restricted to purely scientific problems, but are also useful tools for conservation and management of brown trout and other salmonid fishes. For instance, knowledge of the amount and distribution of genetic variation is important for any genetically sound management or conservation program. Further, the delineation of small scale structures within populations is likely to be useful for the design of measures for the conservation of genetic biodiversity (Avice 1994), especially when migrating populations conform to a metapopulation structure, as is likely to be the case for sea-run brown trout (Ferguson et al. 1995). Assignment tests can be used for tracking the genetic impact of stocking non-native fish into wild populations and for assessing whether or not present populations are indigenous or have been founded by straying or stocked fish (Nielsen et al. 1997; Hansen et al. 2000a, b). Testing for population bottlenecks is likely to be a useful method for monitoring reduced effective population sizes both in wild populations and domesticated strains.

1.6 LOCAL ADAPTATION

The large diversity in morphology, behaviour and life-history patterns among local brown trout populations has for long been recognized as evidence of adaptations to local environmental conditions. Population genetics theory predicts the conditions under which local adaptation is likely to evolve, and there are strong reasons to believe that these are fulfilled in the brown trout. First, the species is naturally distributed across a variety of environmental conditions (from Iceland to Turkey, from high altitude to low altitude streams, etc.) and it exhibits a number of life history types (e.g. stream resident vs. anadromous) which suggests that local populations are subject to different selection regimes. Second, molecular studies have revealed a relatively high degree of genetic differentiation among geographically related populations, indicating limited exchange of genetic material (gene flow) so that the necessary condition of reproductive isolation is met.

Although much of the observed phenotypic variation among conspecific salmonid populations is generally thought to represent adaptations to local environmental conditions, this presumption is typically based on little direct evidence (Hindar et al. 1991b; Taylor 1991). Proof of the existence of local adaptation requires (1) that it can be demonstrated that variation in the trait under consideration has a genetic basis, and (2) that it enhances survival

and/or reproduction (i.e. fitness) in the particular environments of the local populations. For practical reasons these facts are indeed very hard to establish experimentally for wild salmonid populations.

Even though relatively few direct examples, fulfilling the criteria mentioned above, can be found, there are several examples that indirectly suggest the presence of local adaptation in salmonids (see e.g. Taylor 1991; Hindar et al. 1991b; Adkison 1995; Hindar & Jonsson 1995, and references therein). For example, a large number of studies have shown that there is a genetic basis for differences among local populations for phenotypic characters such as migratory and homing behaviour (e.g. Bams 1976; Kelso et al. 1981), body morphology (e.g. Taylor & McPhail 1985), age and size at maturity (e.g. Beacham & Murray 1987; Palm & Ryman 1999), as well as many other traits. Further, a clear negative correlation has been shown between return rate (and indirectly survival rate) and geographical distance of the source populations in transplantation experiments (Altukhov & Salmenkova 1987; Reisenbichler 1988).

Future studies will most probably result in increasing evidence of local adaptation in brown trout and other salmonids. For instance, reciprocal transplantation experiments or transplantation of different populations to the same foreign environment could be very useful for the confirmation of an adaptive basis for phenotypic differences among populations. Furthermore, many characters believed to be responsible for adaptation represent so called quantitative traits, i.e., traits that are controlled by several gene loci and that are often influenced by environmental factors to a large extent. Thus, an increased understanding of quantitative genetics, possibly linked to future advances in molecular genetics (Ferguson & Danzmann 1998), will also be valuable in this respect.

1.7 GENERAL GOALS FOR GENETIC CONSERVATION

The general goal of conserving biological diversity at all levels from genes to ecosystems has been established internationally, and, for instance, apply to all nations that have ratified the Convention on Biological Diversity (Rio de Janeiro 1992). Most European countries, including the EC, have ratified this convention (Table 2). The convention stresses that "States are responsible for conserving their biological diversity and for using their biological resources in a sustainable manner".

Ethical as well as practical reasons justify the conservation of genetic variation in natural animal and plant populations, including fishes (e.g., FAO/UNEP 1981, Ryman 1991). From the perspective of fishery and conservation management, the goal should be to maintain genetic resources without avoidable and irreversible losses of genetic diversity resulting from management interventions or actions (Riggs 1990; Ryman 1991). It is important to emphasize in this context that genetic diversity refers to hereditary differences between as well as within populations (section 1.4). In particular, maintenance of genetic variation between populations is critical for the concept of local adaptation (Hindar et al. 1991a; Ryman 1991).

We know virtually nothing about the economical, ecological, or evolutionary value of most genes or populations. Therefore the main focus of genetic conservation must be non-specific, i.e., striving towards maintaining as much genetic diversity within and between populations as possible (Frankel 1970, 1974; Ryman & Ståhl 1980; Utter 1981; Meffe 1986; Ryman 1991).

On the basis of this perspective it is the responsibility of managers to maintain genetic integrity and health of the populations within a given jurisdiction. Management must recognize the biological reality of existing genetic diversity and devise appropriate management strategies on the basis of this reality to conserve genetic variability effectively (Ryman 1991).

2. BROWN TROUT POPULATION GENETIC STRUCTURE

The genetic diversity of the brown trout is distributed at several hierarchical levels. A schematic illustration of this hierarchy is shown in Figure 2.1. It should be stressed that at present, the existence of an hierarchical population genetic structure has been confirmed, but the number and properties of the "levels" within the hierarchy, the number and properties of groupings within different levels, as well as the amount of interaction between groupings and "lineages" largely remains unresolved.

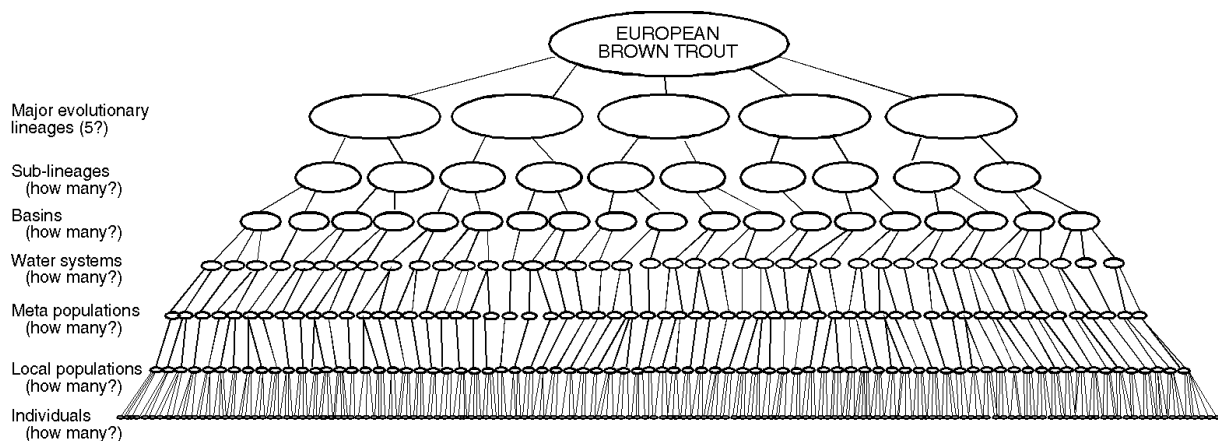


Figure 2.1. Very schematic illustration of the hierarchical population genetic structuring within the brown trout species. It is presently clear that an hierarchical structure exists and that it probably looks something like this, but it is unclear (1) how many "levels" there are, (2) how many groupings within levels there are, (3) the degree of interaction between grouping within and between levels.

At a large geographical scale, a number of highly divergent evolutionary lineages have been proposed for the southern and eastern range of the species based on analyses of mitochondrial and nuclear DNA (e.g., Bernatchez et al 1992; Giuffra et al. 1994, 1996; Bernatchez and Osinov 1995; Garcia-Marin and Pla 1996; Largiader and Scholl 1996; Antunes et al. 1999). In the northern range, that has been strongly affected by Pleistocene glaciations, one evolutionary "lineage", often referred to as the "Atlantic race", appears to be predominant (Bernatchez et al. 1992). However, several studies indicate that even here the situation is more complex, as postglacial recolonizations from more than one glacial refuge may have taken place, although it is currently unclear how many recolonization "lineages" can be identified (Ferguson & Fleming 1983; Hamilton et al. 1989; Hynes et al. 1996; García-Marín et al. 1999). The current pattern regarding the distribution of five major evolutionary lineages over the Eurasian range was proposed by Bernatchez (1995) on the basis of mitochondrial DNA analyses, and is illustrated in Figure 2.

At a more local scale, medium to strong genetic differentiation has been observed between brown trout populations in many European countries representing different basins, drainages, river systems and spawning sites (e.g., Ryman et al. 1979; Ryman 1983; Crozier & Ferguson 1986; Ferguson & Taggart 1991; Apostolidis et al. 1997; Hansen & Mensberg 1998; Estoup et al. 1998). This differentiation is due partly to the strong homing instinct of brown trout, resulting in limited gene flow among populations. Gene flow among populations may also be restricted due to impassable geographic barriers that promote genetic differentiation. In addition, different "evolutionary lineages" can be found in a mosaic pattern of distribution at a very fine geographic scale presumably due to complex colonisation histories.

In general, little or no correlation has been observed between genetic and geographical distances between landlocked populations. However, this is not necessarily the case for sea-run brown trout where populations may be connected by gene flow (Moran et al. 1995; Hansen & Mensberg 1998; Bouza et al. 1999). It must be stressed that very little is still known about the genetic population structure of "sea trout" and of the relationship between anadromous and resident populations, though most published data suggest that coexisting resident and anadromous trout interbreed and are part of the same population (Campbell 1977; Jonsson 1985; Hindar et al. 1991a; Cross et al. 1992).

2.1 TAXONOMIC CONFUSION MUST NOT IMPEDE CONSERVATION

There is considerable confusion regarding the taxonomy of the brown trout (cf. Elliott 1994; Kottelat 1997). This confusion stems from an ongoing discussion on how to classify the various morphologically and/or genetically distinct groupings that have been identified. Some suggest that these groupings should receive species status, and up to 57 species names for various forms of brown trout have been proposed since the beginning of the modern nomenclature system in the middle of the 18th century. Some of these classifications have been based on minor morphological and/or life history forms, probably reflecting mainly environmental and phenotypic plasticity (Bernatchez et al. 1992). In brown trout, morphology or life history appears not to be sufficient for delineation of taxonomic units with evolutionary significance and can be misleading. For example, existing data indicate that populations classified as subspecies in terms of life history forms, i.e., *S. t. trutta* (anadromous form), *S. t. lacustris* (lake dwelling form), and *S. t. fario* (stream resident form), do not necessarily represent monophyletic groups (Ryman 1983; Hindar et al. 1991a; Cross et al. 1992).

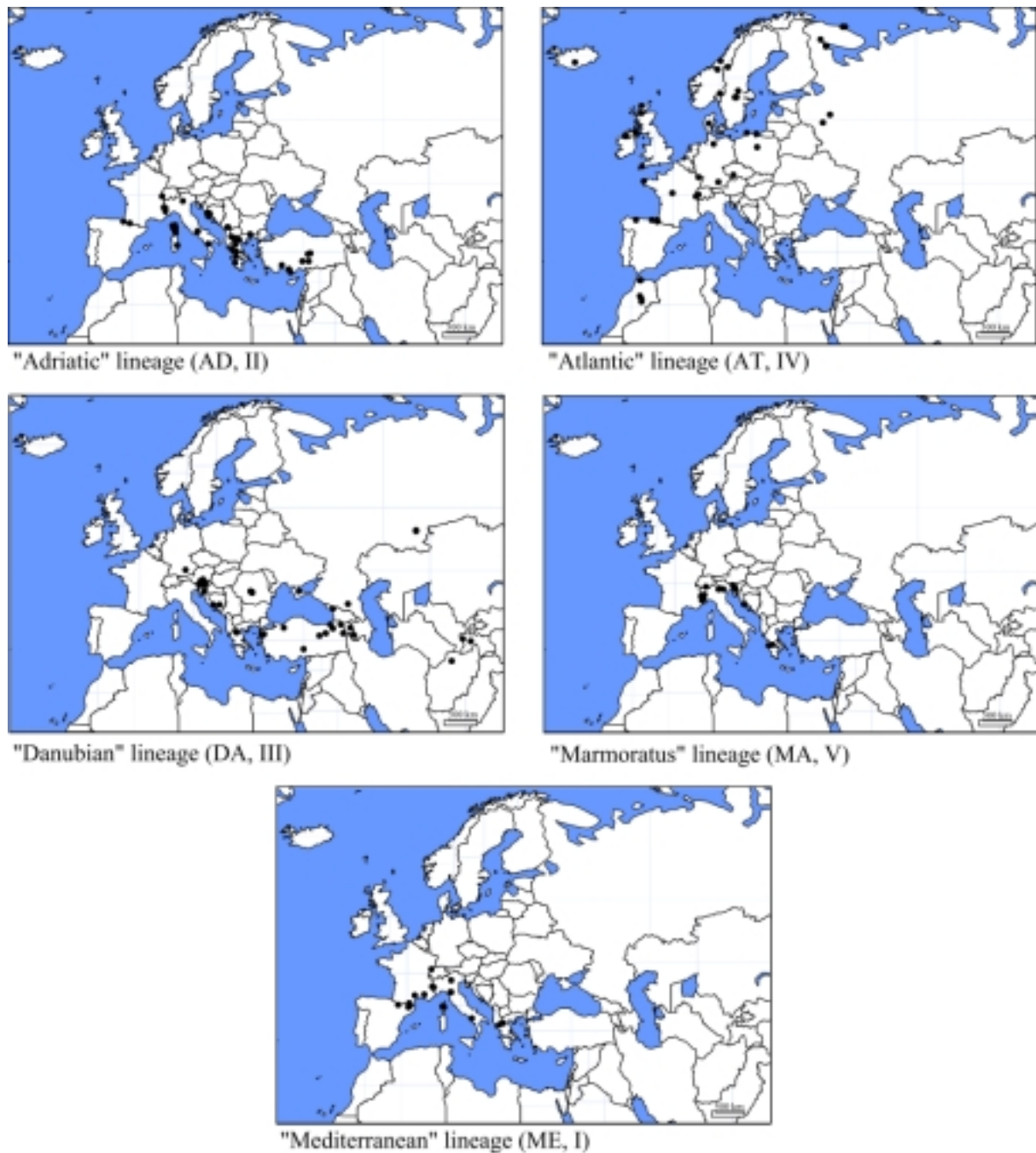


Figure 2. Geographical distribution of the five major evolutionary lineages of brown trout as proposed by Bernatchez (1995). Please note that the figure is an updated version of the corresponding figure in Bernatchez (1995) and it contains unpublished data. The figure is printed with kind permission of Dr. Bernatchez (Department of Biology, University of Laval, Sainte-Foy, Quebec, Canada) and will appear in one of his coming publications, **IT IS NOT TO BE COPIED/REPRODUCED!**

Clearly, classification of population genetic groupings should be based on genetic information. However, even with data indicating a genetically distinct unit, it is unclear what this unit should be called; a species, a subspecies, or a genetically distinct population within a species. In the sections describing the genetic diversity in the three major European regions (2.2-2.4) the classification used is that preferred by the various TROUTCONCERT research members of that particular region. Thus, the use of, for instance, "*Salmo marmoratus*" does not imply that all of the authors of this report prefer that designation before "*Salmo trutta marmoratus*".

In the section on the current threat status of the brown trout (4.1) we use the nomenclature of the publications cited. The apparent taxonomic discrepancies between countries reflect the fact that there is considerable taxonomic disagreement (e.g., what species concept should be applied) in contemporary literature (Berg 1948; Lelek 1987; Kottelat 1997). As an illustration of these opposing views, Lelek (1987) considers brown trout a single species and also lists a few subspecies names, whereas the most recent checklist by Kottelat (1997) that also takes genetic data into account, proposes more than 20 different species and indicates that this listing is probably incomplete.

The unclear taxonomy described above is not unique to the brown trout; similar discussions exist concerning other species as well (e.g., cutthroat trout; Allendorf & Leary 1988; whitefish; Bernatchez 1995). *It is important to stress, however, that the taxonomic discussions do not affect measures necessary for conservation and management. Effective conservation of the brown trout must be based on the genetic differences between populations regardless of whether we call these populations species, subspecies or local populations.*

2.2 DIVERSITY OF BROWN TROUT IN THE MEDITERRANEAN-ADRIATIC REGION

The Mediterranean-Adriatic Province is the region in Europe where the *Salmo trutta*-complex exhibits the highest phenotypic diversity (Behnke 1968). Several morphs of brown trout with variable taxonomic status depending on the authors, have been recognized in this area, most of them in the Balkanic regions and Turkey (*S. trutta macrostigma*, *S. trutta dentex*, *S. trutta peristericus*, *S. marmoratus*, *S. carpio*, *S. obtusirostris*, etc.). Lists of these putative species or subspecies can be found in Behnke (1965, 1968), Banarescu et al. (1971), Economidis & Banarescu (1991), Kottelat (1997) and Dorofeeva (1998).

Studies based on variation of nuclear and mitochondrial DNA have confirmed high genetic diversity among brown trout populations in the Mediterranean-Adriatic region. However, only two different entities have presently been clearly distinguished based on genetic data: *S. marmoratus* and the Mediterranean populations of *S. trutta*. Most of the earlier taxonomic classifications that were based on phenotypic variation and which resulted in complex biogeographic structuring of, for instance, the populations of the Balkan Peninsula, have not been confirmed by the data generated using recent molecular techniques (Karakousis & Triantaphyllidis 1990, Apostolidis et al. 1997).

***S. marmoratus*:** The marble trout shows strong morphological and ecological characteristics which easily distinguishes it from other Mediterranean brown trout populations (Sommani 1961; Behnke 1968). The marble trout also exhibits substantial genetic divergence from other

identified Mediterranean populations as well as from the "lineages" of the Danubian and Atlantic regions (Bernatchez et al 1992, Giuffra et al. 1994, 1996). Genetic distance estimates based on allozyme variation and nucleotide divergence of mtDNA suggest that *S. marmoratus* differentiated from the other "lineages" 1-3 million years ago. The distribution of the marble trout is restricted to basins of the upper Adriatic sea, and there are no indications of pronounced population substructuring within that "lineage".

In some tributaries of the Pô river, the marble trout is found in zones of narrow overlap (i.e., parapatry) with native populations of *S. trutta* (Giuffra et al. 1996). Some authors suggest that this situation prevailed before the introduction of exotic *S. trutta* which resulted in hybridization between the two forms. If the marble trout really did coexist with the native *S. trutta*, this would support the contention that it is a "true species" (Sommani 1961; Behnke 1968), but no clear documentation of this situation is available. Presently, this taxon is threatened by industrial and agricultural pollution, and it is very difficult to find populations which are not heavily introgressed by Atlantic stocks. The risk of extinction and complete introgression is high. Owing to its restricted distribution and the probability that only a few small uncontaminated populations exist, a conservation program is urgently needed for this unique morph.

Mediterranean *S. trutta*: Among the remaining populations of *Salmo trutta* distributed around the Mediterranean area no apparent large scale population genetic structure can be detected using mtDNA and protein electrophoresis. These populations harbour two substantially differentiated mtDNA haplotypes (Bernatchez et al. 1992; Bernatchez 1995). The occurrence of these two differentiated haplotypes suggests that a minimum of two distinct groups existed in the past. The two mtDNA lineages are now scattered across the region in a rather unpredictable pattern and can be found within the same population (Giuffra et al 1994). However, nuclear markers indicate a highly complex population structuring. In a small geographic area populations exhibiting fixed differences at one or more loci were found, in some cases these genetic dissimilarities were associated with ecological and phenotypic differences (Krieg & Guyomard 1985; Apostolidis et al. 1996a; Giuffra et al. 1996).

A particularly interesting morph is the one endemic to the Garda Lake in the Pô basin: *S. carpio*. It has been identified as a true species because it exhibits very specific ecological and reproductive traits (exclusively lake dwelling, two reproductive periods per year, and deep-water spawning) which differentiate it from a sympatric form of brown trout, identified as a population of *S. trutta lacustris* (Behnke 1972). Recent molecular studies (Giuffra et al 1994) suggest that *S. carpio* originates from a rather recent hybridization event between *S. trutta* and *S. marmoratus*, followed by intensive genetic drift. However, its species status remains unclear since the sympatric populations of *S. trutta lacustris* form is now extinct. *S. carpio* does not show any evidence of genetic introgression with introduced Atlantic stocks, but is threatened by pollution and overfishing. The risk of extinction is very high. Although its species status is not established and its origin could be quite recent, this form presents pronounced genetic and phenotypic distinctiveness within the microgeographic context of the Pô valley, and thus deserves urgent conservation actions.

The taxonomic status of many populations in the Balkan countries and Turkey remains to be confirmed using genetic techniques. For instance, it is presently unclear whether some of the morphs identified in those areas (i.e., *S. trutta macrostigma*, *S. obtusirostris*) are really

genetically distinct. The degree of introgression of alleles from Atlantic populations currently used in enhancement programs in the Balkan countries and Turkey is also unknown for most of the populations.

The Mediterranean region is probably the region where additional macrogeographic studies of genetic and phenotypic variability patterns are most urgently needed. It is also important to clarify the current status of the original diversification of brown trout in this region. Ongoing stocking activities with releases of large quantities of exotic brown trout is thought to constitute a severe threat to the local populations, but data is largely lacking.

2.3 DIVERSITY OF BROWN TROUT IN THE BLACK, CASPIAN AND ARAL SEA BASINS

Compared to other parts of the native range of brown trout, very few populations in the eastern range (which represents over 50% of the total) have been genetically studied (Bernatchez et al. 1992, Bernatchez & Osinov 1995, Riffel *et al.* 1995, Largiadèr & Scholl 1995, Osinov & Bernatchez 1996). The area within the eastern range from which samples have been analysed encompasses the Black, Caspian and Aral Sea basins. The scarcity of available genetic data from these three basins is well illustrated by the fact that the most conclusive results summarised here are based only on ten populations from within the limits of the former Soviet Union (Bernatchez & Osinov 1995, Osinov & Bernatchez 1996). Within this region, two of the five major phylogeographic groupings, as defined by mitochondrial DNA genotypes, the "Danubian" grouping and the "Atlantic" grouping were detected (Bernatchez et al. 1992, Bernatchez & Osinov 1995, Osinov & Bernatchez 1996). Most populations studied possessed exclusively "Danubian" haplotypes, whereas Atlantic haplotypes were only found in a few individuals from the headwaters of the Danubian drainages (Bernatchez et al. 1992) and in one population from a tributary of the Upper Volga (Caspian Sea basin; Osinov & Bernatchez 1996). In both cases, it was not possible to infer whether these occurrences of the Atlantic haplotypes have resulted from natural colonisations or of artificial introductions. The same applies for the two occurrences of "Danubian" haplotypes that so far have been detected outside of the Black, Caspian and Aral Sea basins in two populations from Mediterranean drainages, i.e., in a Greek population (Apostolidis et al. 1997), and in a population in former Yugoslavia (Bernatchez et al. 1992).

Congruence between mitochondrial and allozyme variation was observed when comparing populations of the "Atlantic" and "Danubian" groupings (Bernatchez & Osinov 1995, Osinov & Bernatchez 1996). In these studies, two allozyme loci *LDH-C1** (=LDH-5) and *MEP-1** were found to be the most discriminatory nuclear markers with respect to the two phylogeographic groupings. The geographic variation at these two loci also suggests that secondary contact (natural and artificial) between the "Atlantic" and "Danubian" groupings has been common in the headwaters of the Danubian and northern Caspian drainages (Bernatchez & Osinov 1995, Riffel et al. 1995, Largiadèr & Scholl 1995, Osinov & Bernatchez 1996). The allozyme data further indicate that these contacts were in some cases followed by introgressive hybridisation. However, the level of mixing between the two groupings appears to be very limited, when considering the total range of the Danubian grouping. Further studies, using both nuclear and mitochondrial markers, are urgently needed for a better assessment of the natural or artificial origin of presumed "Atlantic" genes in these particular areas. Such studies are a prerequisite for a sustainable management of the natural

genetic resources of brown trout of several European countries (e.g. Austria, Germany, and Switzerland).

The genetic differentiation at the nuclear level between "Danubian" trout and the major groupings occurring in the Mediterranean-Adriatic region has not yet been clearly demonstrated. This is due to a lack of studies that assess genetic differentiation at nuclear loci between Mediterranean populations and populations from the "Danubian" grouping using the same techniques. As is the case for allozymes, for example, electrophoretic techniques and studied loci differ considerably between laboratories, and hence comparisons between results of different studies can be misleading. In this context, it is noteworthy that one study from Turkey found fixed differences at three allozyme loci between two populations of the Black and Mediterranean Sea basins, respectively (Togan et al. 1995). However, so far no mtDNA data has been published for these two populations and it would be too speculative to assume that these differences are representative for the nuclear differentiation between brown trout of the "Danubian" and "Mediterranean" groupings.

On the basis of morphological and ecological variation, populations of the Black, Caspian and Aral Sea have been classified into distinct taxa (Berg 1948), i.e. populations from the Black Sea basin are recognised as *S. t. labrax*, those from the Caspian Sea basin as *S. t. caspius*, and those from the Aral Sea basin as *S. t. oxianus*. In addition, distinct ecophenotypic forms from Lake Sevan (Caspian Sea basin) are recognised as a separate species (*S. ischchan*). In the studies of Bernatchez & Osinov (1995) and Osinov & Bernatchez (1996), the combined results of allozyme and mitochondrial markers provided weak support for this taxonomic distinction (cf. Fig.2 of Bernatchez & Osinov 1995). For example, it could be clearly demonstrated that *S. ischchan* represents a morphologically and ecologically unique trout form that evolved recently and belongs to the same evolutionary lineage as all other trout from the Caspian, Black and Aral Sea basins. This trout form is therefore not a species derived from a primitive ancestor of all brown trout populations as has been hypothesised by Behnke (1986).

In the context of conservation and management, the most relevant finding of the aforementioned studies is the high level of population genetic differentiation that was found within the "Danubian" grouping. *All populations from the Black, Caspian and Aral Sea possessed private alleles or mtDNA genotypes and were genetically distinct.* Therefore, as was stated by the authors, *they represent unique gene pools that warrant individual recognition for conservation and management* (Bernatchez & Osinov 1995, Osinov & Bernatchez 1996). Finally, if taking into account that the findings are based on a very small number of populations of the "Danubian lineage" relative to its enormous geographic range, it appears likely that a substantial part of the existing genetic variability within this major evolutionary lineage still remains undetected. Thus, *additional genetic studies for a firmer assessment of genetic population structure of brown trout within the eastern basins are urgently needed.*

2.4 DIVERSITY OF BROWN TROUT IN THE ATLANTIC REGION

The Atlantic phylogeographic group, as defined by mitochondrial DNA genotypes (Bernatchez et al. 1992), is found throughout the Atlantic river systems from Iceland and Norway in the north to Iberia and the Atlas mountains of Morocco and also in Baltic and

White Sea drainages. However, significant differences in nuclear and mitochondrial DNA markers distinguish Atlantic Iberian populations from the more northern Atlantic populations (Moran et al. 1995; Antunes et al. 1999; Bouza et al. 1999; García-Marín et al. 1999; Weiss et al. 2000). The northern part of the Atlantic region was ice covered during the last glaciation and thus many populations have existed only since postglacial times, i.e. during the past 10,000 to 18,000 years. During the last glaciation the main distribution of the Atlantic lineage would have been in France, Iberia and North Africa. However, in addition to the brown trout populations being present in these unglaciated parts of the Atlantic region, one or more glacial refugia probably existed at the margins of the ice sheets. The ice cover was not continuous for the glacial period but there were major advances and retreats of glaciers during this time and associated with these were changes in sea level. These could have allowed periods of allopatry followed by secondary contact.

Although the extent of overall genetic differentiation resulting from a few thousand years of separation would be small, rapid evolution of ecological and other life history specializations could have occurred (Behnke 1972). The natal homing behaviour of brown trout means that differences in life history characteristics such as time and place of spawning, even though these may only involve a few genes, can produce reproductive isolation. Genetic differentiation is reinforced and extended by natural selection when the populations come into secondary contact. Current brown trout populations in Northwestern Europe have thus arisen by postglacial colonization from one or more "Atlantic" glacial refugia. It is also possible that colonization occurred from the Southeastern part of the range through late glacial river exchange between the Volga and the Baltic rivers. Thus, in parts of the range there has possibly been some secondary contact and introgression with the Danubian lineage (Osinov & Bernatchez 1996).

On the basis of discontinuities in *LDH-C1**(=*LDH-5**)*100* and **90* allele frequencies, Ferguson and Fleming (1983) proposed that Britain and Ireland had been colonised in the postglacial period by two "races" of brown trout. The first colonist was referred to as the "ancestral race" as it is characterised by the *LDH-C1*100* allele that, on the basis of comparison with other salmonid species, is the ancestral allele at this locus. The second race was called the "modern race" and was characterised by the more recent *LDH-C1*90* allele that is unique to brown trout. This dual colonization hypothesis was extended by Hamilton et al. (1989) to the rest of NW Europe on the basis of additional analyses. The phylogeny of mtDNA haplotypes and the pattern of haplotype distribution suggested that the post-glacial colonization of NW Europe was more complex than the dual colonization previously proposed (Hynes et al. 1996).

On the basis of genetic variation at *LDH-C1** and *CK-A1**, García-Marín et al. (1999) proposed that northwestern Europe was colonised by three lineages characterised as follows:

Lineage I: *LDH-C1* 90 CK-A1*100*;
Lineage II: *LDH-C1* 100 CK-A1*115*;
Lineage III: *LDH-C1* 100 CK-A1*100*.

García-Marín et al. (1999) proposed that colonization occurred from (i) a northern and eastern radiation from a refuge centred near the English Channel, (ii) a northern expansion from a refuge in Atlantic drainages of Iberia and southern France, and (iii) a north-western migration from an eastern Mediterranean-Caspian refuge. They suggest that most current populations in

the formerly glaciated area are introgressed combinations of these lineages. In a recent study, however, this hypothesis is re-evaluated, and it's suggested that gene flow from refugia in central (e.g., France and Germany) and eastern continental Europe alone can explain the current geographic distribution of mtDNA haplotypes and putative diagnostic allozyme alleles throughout previously glaciated regions of northern Europe (Weiss et al. 2000).

Irrespective of the number and exact pattern of postglacial colonization it is clear that more than one postglacial colonization took place. Current genetic diversity in northwestern Europe is thus the result of independent postglacial colonization by genetically distinct brown trout "lineages" together with genetic differentiation that has occurred in isolation during postglacial times. In practice, however, it is difficult with currently available phylogeographic information to differentiate between these two aspects. Introgression between "forms" has probably occurred (Osinov & Bernatchez 1996; Garcia-Marin et al. 1999a) and possibly also sympatric/micro-allopatric speciation.

Many populations of brown trout are unique in one or more aspects. Often populations are genetically unique as shown by protein or DNA studies. This is particularly so in the unglaciated southwestern part of the Atlantic range where populations have persisted for much longer than in the northwestern part. Specific alleles often occur in only one or a few populations with up to 66% of the total genetic variation in northwestern Europe being distributed among populations (Ferguson 1989).

2.5 GENETICALLY DISTINCT SYMPATRIC POPULATIONS

An interesting and important phenomenon that has been observed is the existence of reproductively isolated and genetically differentiated populations that occur sympatrically on a small geographic scale. The occurrence of such sympatric populations was first reported in Lake Bunnarsjöarna in Sweden (Allendorf et al. 1976, Ryman et al. 1979). Following a routine genetic investigation, it was discovered that this very small mountain lake was actually inhabited by two co-existing, genetically distinct, brown trout populations. The reproductive isolation appeared complete due to the apparent fixation for different alleles at a locus coding for lactate dehydrogenase. Further, because of this alternate fixation it could be easily determined which population each fish belonged to, and it could thus be shown that the populations were characterized by significantly different growth rates (Ryman et al. 1979).

Subsequently, a similar situation of sympatry has been reported to occur in Lough Melvin in Ireland (Ferguson & Mason 1981; Ferguson & Taggart 1991). The brown trout of Lough Melvin have been subjected to very detailed studies. L. Melvin is a lake of some 21 km² situated in northwestern Ireland. Three types of brown trout, known locally as gillaroo, sonaghen and ferox, have been described from the lake. They are distinct morphologically (Cawdery & Ferguson 1988) and have different feeding preferences (Ferguson 1986). Allozyme studies (Ferguson & Mason 1981; Ferguson & Taggart 1991) showed major differences in the occurrence and frequency of alleles indicating a high degree of reproductive isolation between the three types. Examination of mitochondrial DNA restriction fragment length polymorphisms (McVeigh et al. 1995; Hynes et al. 1996), multi-locus DNA fingerprints (Prodöhl et al. 1992), single-locus minisatellite variation and microsatellite variation (A. Duguid & P. Prodöhl, pers. comm.) further confirm the genetic discreteness of the three types. This reproductive integrity of the Melvin trout types is maintained by

geographical separation of the spawning sites through natal homing (Ferguson & Taggart 1991). The gillaroo spawn in the only outflowing river of the lake, whereas sonaghen spawn in the inflowing rivers. The ferox spawn in the lower deeper section of one of the inflowing rivers, which is also used further upstream by sonaghen. Another example of genetically and morphologically distinct sympatric populations in Ireland involves brown trout known locally as dollaghan and salmon trout in the L. Neagh system (Crozier & Ferguson 1986).

In a number of lakes in Britain and Ireland, as well as "normal" brown trout, long-lived piscivorous trout, often referred to as ferox, are found and these in some cases can exceed 10kg in mass. In at least two lakes in Britain and Ireland ferox appear to be reproductively isolated from sympatric trout. In a lake in the Hardangervidda area of Norway trout with a variant allele, which result in a fine-spotted pattern, coexist with other brown trout (Skaala & Jorstad 1987). This spotting pattern is controlled by a single locus with two codominant alleles (Skaala & Jorstad 1987). A morph with a marmorated coloration exists alongside trout of "normal" coloration in the River Otra in Norway (Skaala & Solberg 1997).

Finally, it should be stressed that it is still unclear to what extent sympatric populations represents a common phenomenon in the brown trout. The presence of different morphotypes within a single lake does not in itself provide enough evidence of multiple populations until this has been confirmed by genetic data. Further, for statistical reasons, it may be difficult to detect the existence of multiple populations when they are not distinguished by fixed (or very large) allele frequency differences, or when different morphotypes (potentially representing different populations) are absent which could serve as a natural starting point for making allele frequency comparisons. For example, Jorde & Ryman (1996) describe a situation where it took several years of sampling (i.e. several hundreds of fish) until it became evident that two of their lakes actually harboured multiple genetically distinct and co-existing populations. Since most population genetic surveys are typically based on relatively limited sample sizes (say, 50-100 individuals per population), the existence of sympatric brown trout populations on a small geographic scale may be more common than is presently recognized.

2.6 TEMPORAL HETEROGENEITY

A large amount of data has been generated on the frequency and geographical distribution of different alleles at genetic marker loci in natural brown trout populations in Europe. However, information regarding the temporal dynamics of those marker alleles is still relatively sparse despite the fact that such information is essential for conservation and sustainable management. Typically, population genetic investigations include sampling at one particular occasion only, i.e., they lack temporal replication. This situation is by no means unique to brown trout, but reflects a general phenomenon.

Several workers have compared gene frequencies from natural populations of several species sampled at 2-3 occasions (e.g., Krimbas & Tsakas 1971; Begon et al. 1980; Ryman 1983; Burns & Zink 1990; Waples & Teel 1990; Hedgecock et al. 1992; Ruzzante et al. 1997), and a few such studies have also been conducted on brown trout (Ryman & Ståhl 1980; Hansen & Loeschke 1996). For most species, however, few studies exist that systematically follow genetic changes within a population over extended periods of time (e.g., DeSalle et al. 1987; Turner et al. 1999). This fairly limited knowledge of the extent of temporal variation of

genetic markers influences the interpretation of observed spatial patterns; it is largely unclear if they are stable over time (Ryman 1983; Burns & Zink 1990).

In a couple of recent studies the genetic composition of populations over several years has been reported for a few natural brown trout populations in Sweden (Jorde & Ryman 1995, 1996; Laikre et al. 1998). In those studies material was collected over more than ten years, and the main results may be summarized as follows: 1. The existence of genetic change over time has been established. 2. These changes may be relatively large even between consecutive cohorts. 3. The demography of species with overlapping generations (which applies to the brown trout) affects the genetic dynamics of the population - evaluation of genetic data based on discrete generation theory may therefore obscure the interpretations. 4. The amount of allele frequency change is larger in populations with over-lapping generations as compared to populations with discrete generations. 5. Cohorts (age classes) born approximately 1 generation apart are genetically more similar than cohorts born fewer or more years apart. 6. Temporal genetic data combined with demographic data may be used to estimate the genetically effective population size (N_e ; section 1.3). 7. Estimates of N_e indicate that the effective size of populations in lakes of similar size may vary considerably and may be relatively small. The female effective size in one population was estimated to approximately half that of the total effective size (Jorde & Ryman 1996; Laikre et al. 1998; Palm et al., in prep).

Some other recent studies on Atlantic salmon (*Salmo salar*) compared microsatellite markers from old scale samples with contemporary data from the same populations. Nielsen et al. (1997, 1999a,b) studied Atlantic salmon populations from Denmark over a period from 1913 to the present and found that the genetic structure of populations was remarkably stable over time. A qualitatively similar result was obtained by Tessier & Bernatchez (1999) in a study of landlocked salmon covering a time-span of app. 30 years.

The limited data that do exist on temporal genetic variability for salmonid populations indicate that observed patterns of spatial differentiation are relatively stable over time (Ryman 1997a). For instance, in the study mentioned above on natural brown trout populations in Sweden the spatial component of genetic diversity (i.e. between lakes) was estimated to 4.5% whereas the temporal component (i.e. between years within lakes) was estimated to 0.5% (Jorde & Ryman 1996; Ryman 1997a). Therefore, there is no reason to “panic” and automatically conclude that the information on spatial genetic population structure of brown trout (or other salmonids) generated over the years is “useless”. However, it is obvious that extended studies of temporal genetic variability in brown trout populations (as well as populations of other species) are needed to increase the understanding of the genetic dynamics of natural populations. A better understanding for these processes is necessary for adequate management and conservation measures. It is extremely difficult to monitor and evaluate the effects of various activities on the biodiversity at the gene level if the magnitude of the "normal" variation in genetic composition over time is unknown. Studies needed include not only empirical observations, but also theoretical developments. For instance, research focus on genetic models for age-structured populations with overlapping generations is needed to permit interpretation of empirical data, and to better understand the impact on natural populations of various activities (Ryman 1997b).

3. THREATS TO BROWN TROUT POPULATIONS

In this section the threats to brown trout populations are reviewed. Many brown trout populations are threatened by various types of human activities, and these activities can be divided into three general categories: *environmental degradation*, *harvest*, and *enhancement* (Laikre & Ryman 1996).



Illustration: Nils Peterson

Environmental degradation involves both direct, physical destruction of the natural habitat of the brown trout through, for example, construction of power plants which make migration to the spawning locations impossible, and indirect degradation of e.g., the chemical properties of the water through various forms of pollution. It is important to stress that pollution may have both local and long distance effects. Just like airborne

pollutants (such as acid rain), contaminants that are transferred by water may have negative environmental affects far from the site of discharge. Environmental degradation also includes the alteration of the species composition by, e.g., introduction of exotic species or extermination of existing species with which the brown trout naturally coexists.

Harvest includes commercial as well as sport fisheries. Harvesting obviously implies removal of individuals from the population and thus it tends to reduce the population size, this in turn may result in reduced population viability and loss of intrapopulation genetic diversity.

Enhancement of populations through the release into the wild of fishes bred in hatcheries or transfered from other locations, is an increasingly common management practice. This is often demanded as a consequence of reduced population sizes and increased demand for harvest. Stocking represents a particularly serious threat since it is generally regarded as beneficial, and a means to "help" the natural population, but it may in reality result in the extinction of local, wild gene pools (Ryman & Utter 1987; Allendorf & Leary 1988; Ferguson 1989; Hindar et al. 1991b; Waples 1991a; Taylor 1991; Leary et al. 1993; Hansen & Loeschke 1994; Ryman et al. 1995a; Allendorf & Waples 1996; see also sections 3.1 and 3.2).

Similarly, there are three major processes through which intraspecific diversity in general is reduced and lost; *extinction*, *hybridization*, and *loss of genetic variability within populations* (Ryman et al. 1995b; Laikre & Ryman 1996). These processes relate to each of the different hierarchical levels of intraspecific genetic diversity; i.e., between alleles within individuals, among individuals within populations, between populations within geographical regions, and so on. Genetic diversity between populations (or at other levels in the hierarchy) is reduced whenever a genetically distinct population ceases to exist (extinction) or when its integrity is compromised through hybridization (Ryman et al. 1995b). Activities within the three general

threat categories environmental degradation, harvest, and enhancement may trigger the three major processes for reducing genetic variability as illustrated in Figure 3.1.

Activity	Extinction	Hybridization	Loss of variation within populations
Harvest	X		X
Stocking	X	X	X
Environmental degradation	X	(X)	X

Figure 3.1. Schematic illustration of the three general categories of activities that constitute threats to the intraspecific variability of the brown trout, and how they relate to the three major processes through which genetic diversity is lost. (Modified from Ryman 1993).

Extinction of local populations may result in the complete loss of alleles or allele combinations. Environmental degradation is the major cause of extinction of local brown trout populations, but over-harvest and enhancement may also result in extinction.

Enhancement causes extinction if such masses of fish are released that the natural population is "swamped" or "outnumbered", or if the stocking program is carried out for such a long time that the natural population gradually is replaced by the released stock. In either case the result is that the gene pools of indigenous and possibly locally adapted wild populations are replaced by the gene pools of non-native domesticated fish. Moreover, whereas wild brown trout populations are in general highly variable and differ genetically from each other (Ryman 1983; Ferguson 1989) loss of genetic variability has often taken place in hatchery strains and they are generally weakly genetically differentiated from each other due to exchange of fish among hatcheries (Ryman & Ståhl 1980; Garcia-Marin et al. 1991; Hansen et al. 1997b). In addition to loss of variability due to a small number of parent fish, hatchery strains are frequently subjected to unnatural selective pressures. Such selection may be unintended but may nevertheless result in genetic changes, which may in turn cause e.g., behavioural, physiological and morphological changes, i.e., domestication (Hale 1969; Ruzzante 1994). "Swamping" may therefore lead to the loss of several genetically different wild gene pools at the expense of one or a few gene pools of domesticated trout. Enhancement may also cause extinction if the released individuals carry parasites and/or diseases against which the natural fish lack, or have insufficient, resistance (Figure 3.2).

No parallels exist for terrestrial vertebrates to the enormous numbers of fishes that are released into nature (Allendorf et al. 1987; Ryman et al. 1995b). For the brown trout these releases are

intentional and are carried out to compensate for lost or reduced natural reproduction due to habitat alterations, or to generally increase the amount of fish for fisheries.

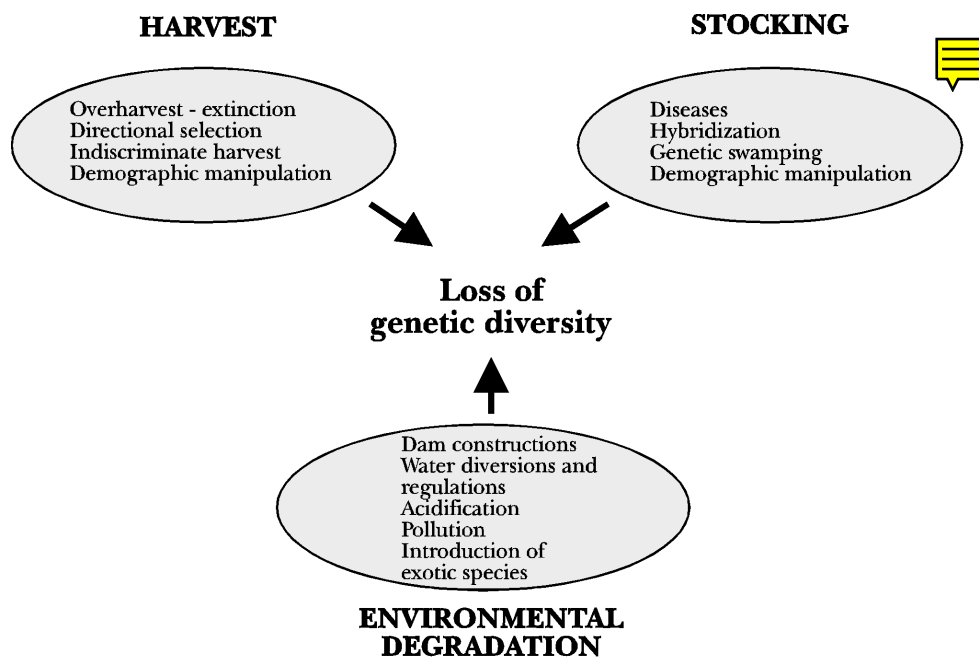


Figure 3.2. Examples of potentially negative effects on genetic diversity related to environmental degradation, and to harvest and stocking of natural brown trout populations. (Modified from Laikre & Ryman 1996.)

Hybridization may occur both between and within species. The process does not necessarily imply the loss of individual alleles, but it results in the rearrangement of previously existing gene combinations that most likely can never be recreated and that may result in the breakdown of adaptations to particular local environments (cf. Hindar et al. 1991b; Carvalho 1993). At the interspecific level, the brown trout may hybridize with, for instance, the Atlantic salmon (*Salmo salar*). An alarming increase of such hybridizations has been observed, for example, in some Swedish water systems (The Swedish National Board of Fisheries 1995a), and is believed to be associated with the large numbers of captively bred salmon that are released into these areas (Laikre & Ryman 1997).

Hybridization at the intraspecific level represents an increasing threat to the genetic integrity of local populations of several species, including the brown trout. For the brown trout this threat is associated with stocking (Figure 3.2). The release of cultured fish causes a genetic problem when the released fish interact and reproduce with the wild fish (e.g., Ryman et al. 1995b). Cultured fish may differ genetically from natural populations by origin or through selection or inbreeding. At the same time, it is known that many traits, including traits involved in local adaptations, are the result of the interaction between many genes that make up so-called "co-adapted gene complexes". If trout with different "co-adapted gene complexes" interbreed these gene complexes may be broken down resulting in loss of adaptations, so-called outbreeding depression (Templeton & Read 1984; Templeton 1986; Gharrett & Smoker 1991; Ballou 1995).

Loss of genetic variability within populations occurs through selection, intentional or unintentional, and genetic drift and inbreeding due to restricted population size. Selection occurs when particular genotypes contribute proportionally more offspring than others in response to environmental factors (see section 1.4). Such differential reproduction rates result in increased frequencies of the alleles and genotypes being favoured. The corresponding decrease of those alleles that are unfavoured may ultimately result in loss of genetic diversity (Ryman et al. 1995b).

"Unnatural" selective pressures that result in changes in the genetic composition of the local populations may be caused by selective harvest where individuals of a particular genotype are caught preferentially. If particular genotypes frequently are coupled with, for instance, large body size or fast growth then those genotypes will be removed to a larger extent if this type of individuals are preferentially caught. Pollution and other forms of change of environmental properties may also result in an altered direction of selection. Under captive conditions (such as a hatchery) selective forces may be different from those in nature, which may result in "domestication" of the hatchery stock (Petersson & Järvi 1993, 1995; Johnsson et al. 1996). It is generally very difficult to quantify or foresee which selective pressures are acting on a population and how this will affect the genetic composition of a population (Ruzzante & Doyle 1991; Ruzzante 1994).

Loss of genetic variation through genetic drift and inbreeding always occurs in populations of a restricted size. The smaller the population the more dramatic these losses may be, and the rate of genetic drift and inbreeding is determined by the *effective population size* (N_e ; section 1.3). Because the effective size is determined by several demographic parameters, manipulation of such parameters may decrease the effective size. For instance, selectively removing a particular sex or age class from the population may reduce N_e and therefore result in loss of genetic variability. Similarly, increasing the reproductive rate of some individuals in the population, but not all, may reduce the effective size (i.e., supportive breeding; section 3.2). Obviously, any activity resulting in long or short-term depletion of the effective population size below a critical level threatens the maintenance of genetic variability within a population. Such activities include all those causing habitat degradation, overharvest, and direct or indirect manipulation of sex ratios and reproductive rates in connection with aquaculture and other categories of artificial propagation.

As exemplified in Table 1 (see also Table 3) activities within at least two of the three major threat categories have caused reduction of intraspecific variability in brown trout in various parts of Europe; some very serious losses have occurred due to environmental degradation and enhancement operations. Documentation of reduced variability due to over-harvest or selective harvest appears to be scarce, however, occurrence of negative effects can be inferred from indirect observation. For instance, in eight populations of anadromous brown trout in western Norway, Sættem (1995) found a significant negative correlation between the density of adult fish (> 3/4 kg) and the proportion caught by anglers. In three of the rivers, fewer than 60 adult fish remained in the river for spawning, after having been subjected to catch rates near 50% during the previous angling season. In such situations, harvesting by anglers reduces the size of the population to the extent that an increased loss of genetic variability may become evident. A negative correlation between population density and catchability has also been demonstrated for gillnetting of resident brown trout in mountain lakes (Borgström 1992). This suggests that harvesting may have genetic consequences both in (sparsely populated) lakes and rivers, although we are unaware of any quantitative assessment of this effect.

Table 1. Examples of human activities reducing the genetic diversity in brown trout in Europe (modified and extended from tables in Ryman & Utter 1987, Ryman et al. 1995b, Laikre & Ryman 1997).

General threat category	Activity	Result (threat)	Reference
Environmental degradation	Dam constructions and water diversions	Two thirds of the large-sized populations of brown trout in the Lake Vänern area (Sweden) extinct in less than 100 years due to migratory obstructions.	Ros 1981
		About 46 out of approximately 52 natural populations of sea migrating brown trout ("sea trout") in Finland extinct due to habitat destruction or stocking.	Koljonen & Kallio-Nyberg 1991
	Pollution	Acid rain increasingly threatens freshwater environments of the northern temperate zone. Acid water destroy eggs and fry. Massive kills of trout observed in Norway after heavy acid rains. More than half of the lakes of southern Norway are presently barren of fish (e.g. brown trout), and the number has doubled in the past 15-20 years. Over 8000 brown trout populations have been exterminated in Norway, and approximately 4000 populations are negatively affected by acid rain.	Henriksen et al. 1989; Hesthagen 1997; Hesthagen et al. 1999
		Almost 40% (52/133) of a set of natural brown trout populations investigated where threatened by pollution (Finland).	Koljonen & Kallio-Nyberg 1991
	Introduction of exotic species	Decline in brown trout recruitment following introduction of minnow (<i>Phoxinus phoxinus</i>) in a Norwegian subalpine lake. The minnow has been introduced to many mountainous lakes supporting brown trout populations.	Borgstrøm et al. 1996, Tysse 1995
Stocking	Release of non indigenous/genetically different populations	Introgression rates of up to 80% between released hatchery fish and local populations in France.	Guyomard 1989
		Introgression rates of 19-91% between released hatchery fish and local populations in Lower Lough Erne in Northern Ireland.	Taggart & Ferguson 1986
		Introgression rates of approx. 75% between transplanted trout and local populations in rivers from Greece.	Apostolidis et al. 1996, 1997
		Complete replacement of natural populations with hatchery fish in the Cares and Cubia rivers, and progression towards elimination of natural populations in Llobregat River (Spain).	Garcia-Marin et al. 1991
		Breakdown of apparent natural population structure in River Skellefteälven (Sweden) due to release of hatchery fish produced through matings of fish from different geographic regions.	Ryman 1981
	Replacement of several natural populations with hatchery stocks in lagoons and reservoirs in Galicia (northwestern Spain).	Martínez et al. 1993, Arias et al. 1995	

General threat category	Activity	Result (threat)	Reference
Stocking (cont.)	Cultivation and captive breeding	Dramatic genetic differences between hatchery populations of brown trout and the natural populations they were presumed to represent; the proportion of variable loci reduced by 50% in hatchery stocks (Sweden). Differences between hatchery strains supposed to represent the same natural population.	Ryman & Ståhl 1980, 1981, Ryman 1981
		Hatchery strain supposed to represent a natural population of the Lake Saimaa (Finland) genetically different from the source population. Average heterozygosity in hatchery strain reduced by 33%	Vuorinen 1984
		Observations in the context of conservation breeding programs of large-sized populations from the Lake Vänern area (Sweden): Dramatic loss of genetic variability in hatchery populations. Several occurrences of hybridization between Atlantic salmon and brown trout in hatcheries detected through genetic analysis. Unusually large proportions of defect fry probably due to inbreeding.	Ståhl & Ryman 1987 Ståhl 1987
		Strikingly different genetic characteristics of hatchery populations used for stocking and natural populations (Spain).	Garcia-Marin et al. 1991
		Loss of variability in domesticated trout used for stocking, very little genetic differentiation among the quantitatively most important strains due to exchange of fish among fish farms (Denmark).	Hansen et al. 1997b
Harvest	Overharvest	Over 45% (61/133) of a set of natural brown trout populations investigated where threatened by overharvest (Finland).	Koljonen & Kallio-Nyberg 1991



Illustration: Nils Petersen

Because the activities causing the documented losses presented in Table 1 are widespread, it is likely that similar reductions of intraspecific biodiversity have occurred on a large scale in the European countries. However, no serious attempts appear to have been made to review what actually has been lost, and what is at risk of being lost, in terms of local brown trout populations and the genetic resources they represent. **It is recommended that such overviews are carried out both on a national and international level.**

3.1 STOCKING AS A TOOL IN MANAGEMENT AND CONSERVATION OF BROWN TROUT

As stated in the previous section stocking is generally regarded as a main tool in conservation and enhancement of brown trout populations. Stocking may indeed be considered an important measure in supplying "first-aid" to populations that are in immediate danger of going extinct due to demographic factors. However, in other cases inappropriate stocking practices are themselves the major threat to the genetic integrity of populations. In this section we highlight the different types of motivation for stocking trout. We provide some examples of stocking activities and we stress the need for well-considered decision-making procedures before stocking is initiated and for monitoring stocking programmes in order to assess both their efficiency and possible negative consequences. Several of the points have been raised previously in a publication by Cowx (1994), and we strongly recommend managers to consult this paper.

Many stocking programmes lack clear definitions of success criteria, alternative methods of enhancement such as habitat improvement are not considered and, finally, no monitoring is undertaken to assess the efficiency of the programmes (Cowx 1994). To ensure proper decision-making many factors have to be considered and it is therefore fruitful to adopt a formalised procedure as suggested by Waples (1991a) and Cowx (1994). Before going into a detailed description of decision-making procedures it is useful to list the three major types of stocking activities that involve brown trout.

1. Conservation releases: This type of stocking serves the purpose of saving populations from extinction, i.e if stocking is not undertaken it is likely that the population will perish due to demographic factors. Accordingly, conservation releases involve the use of local broodstock for production of stocking material (supportive breeding). Conservation releases should only be considered a temporary solution. In the long run there is little sense in continuing releases unless the factors responsible for the population decline are identified and alleviated.

Example: Ruhlé (1996) provides an example from Lake Constance (Germany/Austria/Switzerland). A dam was established in 1962 at Reichenau that impaired migration of trout from the lake to the main spawning grounds in the Alpine Rhine. This resulted in a dramatic decline of the spawning population size. A supportive breeding programme was undertaken, based on adult spawners caught below the dam. At the same time other measures were taken to improve environmental conditions and in particular to facilitate passage of the dam. The establishment of a supportive breeding programme has secured the survival of the population until migration to the area above the dam has been reestablished, which is currently under way.

2. Releases for reestablishing populations: Many populations of brown trout have been eradicated due to unfavourable environmental changes such as building of dams and destruction of proper spawning grounds. In cases where conditions have improved to an extent that renders the habitat suited for a self-sustained population it may be necessary to reintroduce fish from other populations if natural recolonisation is impossible. As in all other cases of stocking activity, stocking material should not derive from hatchery strains, as these may have undergone some genetic changes such as loss of variability and domestication due to small effective population sizes and altered selection regimes in hatcheries. Instead wild fish from neighbouring populations inhabiting similar environments should be preferred.

Example: We are aware of some examples of this kind of stocking activity (for instance from Sweden, Scotland and Denmark) and have chosen an example from Denmark (Hansen et al.

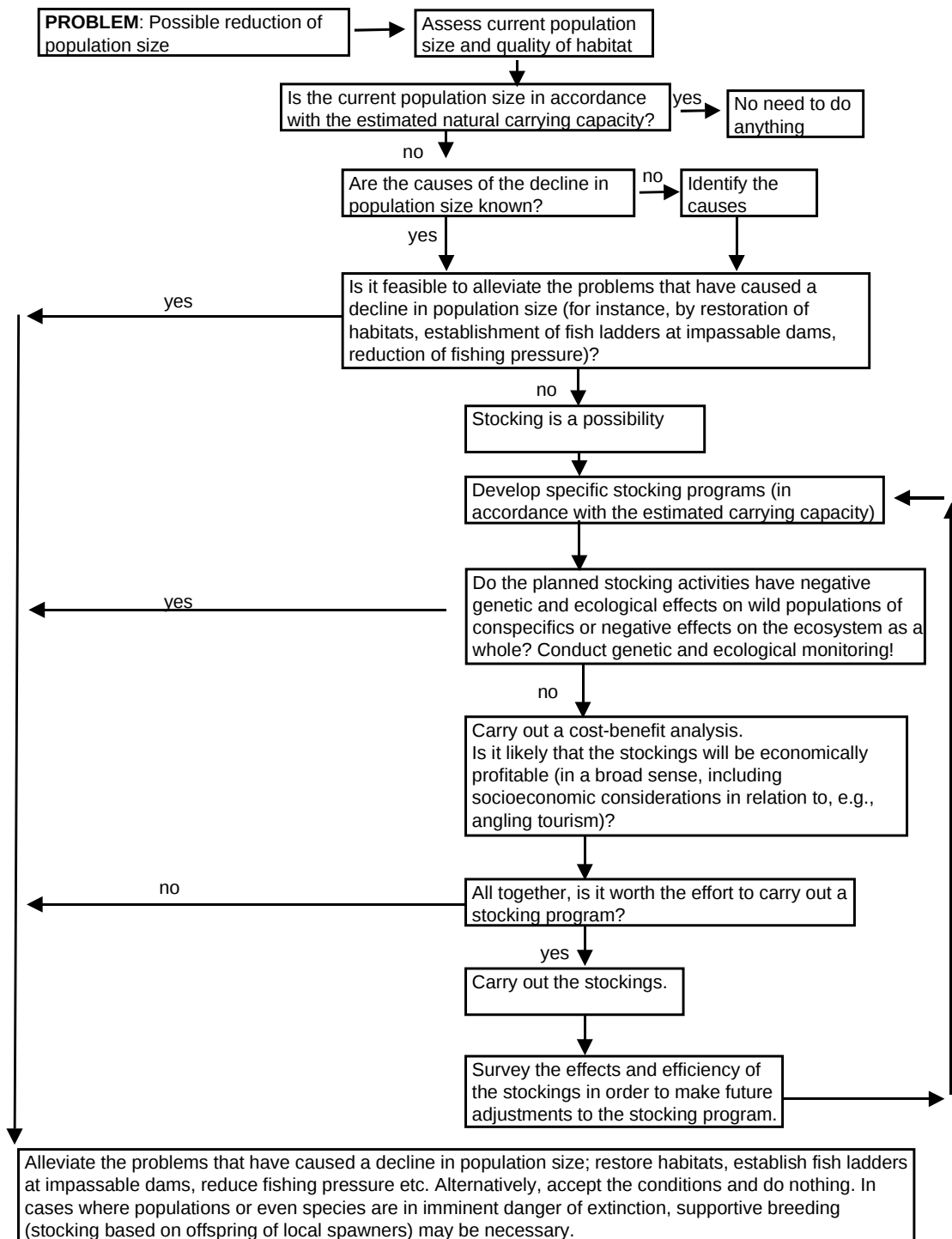
2000a). On the Danish Island of Zealand nearly all indigenous trout populations have been extirpated during the 1960's and 70's. However, during the 1990's habitat restoration in several rivers has resulted in conditions suitable for brown trout. At the same time (using microsatellite DNA markers) two surviving trout populations on the island have been identified and they have been found to be essentially unaffected by released hatchery trout. A stocking program for introducing trout to restored rivers has now been initiated based on offspring of wild spawners from the two indigenous populations.

3. Releases for improving fisheries: Most brown trout stocking programmes belong to this category. The purpose of the releases is to increase population sizes to the benefit of fisheries (mainly angling). The stockings are based on the assumptions that original population sizes have decreased due to environmental degradation and increased fishing pressure. In many cases, for instance when a river is dammed, the production capacity decreases for obvious reasons. However, in several other cases an actual decrease in population size has in fact not been documented. Stocking programmes are in these cases initiated solely as a response to public demands for more fish. It is particularly problematic if the addition of stocked fish to the wild population leads to an increase of the total population size above the natural carrying capacity, as this may induce intense competition between wild and stocked fish.

Example: There are numerous examples of this kind of stocking activity. A typical example has been described by Taggart & Ferguson (1986), where a large number of hatchery trout were stocked into tributaries of Lough Erne, Northern Ireland. Their study of the genetic impact of stocking activity on native Lough Erne trout is summarised later.

Decision-making procedures: From the description of different types of stocking activities it must be stressed that only type 1 (conservation releases) and 2 (releases for reestablishing populations) could help to provide long-term solutions. However, it must be stressed that they must be accompanied by proper measures to reduce the underlying factors that have caused population declines. Releases for improving fisheries (3) do not solve the real problems. In order to provide a solution to these problems, it is necessary to identify the exact causes leading to declines of population sizes, for instance the presence of impassable barriers to migrating fish, destruction of suitable spawning/nursery areas and over-fishing. The solutions then include, for instance, construction of fish ladders to facilitate passage of barriers, restoration of spawning grounds by putting out new gravel, and regulation of fisheries. Finally, when a decision has been made to initiate a rehabilitation programme it is important to monitor the actual impact. This requires collection of data (in particular information on population sizes) both before and after implementation of the rehabilitation measures. Formalised descriptions of decision-making procedures have been developed by several authors. In Figure 3.3 a flow diagram (modified from Cowx 1994) is presented that shows the basic steps in the procedure.

Fig. 3.3. Flow diagram describing the decision-making procedures before stocking is initiated. Modified from Cowx (1994).



3.1.1 Efficiency and impact of stocking programmes

It is relatively uncomplicated to assess whether conservation releases and reintroduction releases are in fact successful. If the populations targeted for conservation releases nevertheless fail to survive or if reintroduction efforts fail to lead to the establishment of self-

sustaining populations, the whole program must be considered unsuccessful. In contrast, the vaguely defined goals of many releases for improving fisheries renders it difficult to evaluate their efficiency. In cases where the efficiency of stocking programmes have in fact been monitored, by using either external tags or genetic markers, a number of different outcomes have been observed in terms of survival and long-term genetic contribution by the stocked fish. In the following we will briefly mention some examples of stocking activities that have clearly resulted in survival and reproduction of stocked trout and other stockings that have not resulted in a detectable genetic contribution of the stocked fish. It is not the intention to summarise all studies that have focused on these issues but rather to mention some representative examples.

A. Stocking programmes where survival and introgression of genes from stocked trout has been demonstrated. Taggart & Ferguson (1986) report an example where hatchery trout were stocked into tributaries of Lough Erne, Northern Ireland. Hatchery trout and native trout were fixed for different alleles at the allozyme locus *LDH-C**. Using this locus as a genetic marker it was demonstrated that the stocked trout reproduced in the wild and interbred with native trout. Though not specifically monitored, it must be assumed that the stocked trout made a contribution to the angling in the lake.

Largiader & Scholl (1996) studied a situation where hatchery trout belonging to the Atlantic phylogeographical group were stocked into the Doubs River (Switzerland and France) which is inhabited by a phenotypically divergent form of trout belonging to the Mediterranean phylogeographical group. Their data, based on several diagnostic allozyme loci, indicate that the hatchery trout reproduced in the river and interbred with native trout. However, mating between native and stocked trout was apparently not random; there were indications of positive assortative mating (i.e., stocked and native trout, respectively, preferred to mate with individuals from their own group). Though not specifically monitored, it must be assumed that the stocked trout made a contribution to the angling.

Poteaux et al. (1998) report on hatchery trout belonging to the Atlantic phylogeographical group that were stocked into French rivers inhabited by trout belonging to the Mediterranean phylogeographical group. The impact of stocking activity was monitored using allozyme loci and mitochondrial DNA markers. Reproduction by hatchery trout and interbreeding with native trout was demonstrated. However, the genetic contribution by hatchery trout appeared to diminish over time, indicating selection against hatchery trout and their offspring.

A study by Apostolidis et al (1996b, 1997) concerns stocking of trout in Greece, where trout from one geographical locality within the country (Acheloos River, draining into the Ionian Sea) were stocked into the Nestos River in East Macedonia. The actual contribution to the fisheries was considered to be small. However, using mtDNA markers it was estimated that approximately 75% of the indigenous population had been replaced by stocked trout.

B. Stocking programmes with little or no detectable genetic contribution by stocked trout. Brown trout stocking in Spain is mainly based on hatchery trout introduced from northern Europe. Hatchery and wild trout are fixed for different alleles at the *LDH-C** allozyme locus. This locus has been used for assessing stocking impact and efficiency in several parts of Spain. In nearly all cases stocking programmes appear to have been inefficient. Genetic contribution of stocked trout has only been demonstrated in a few cases, mainly in slowly running waters (Moran et al. 1991; Martinez et al. 1993; Garcia-Marin et al. 1999b).

Hansen et al. (1995, 2000a) have studied the effects of stocking hatchery trout into a wild trout population in the Karup River, Denmark. The river is inhabited by anadromous and resident trout. Using mitochondrial DNA and microsatellite markers it was found that the stocked hatchery trout had hardly made a genetic contribution to the present population. However, a later study showed that the situation might be more complex (Hansen et al. 2000b) as a large proportion of hatchery x wild trout “hybrids” were observed among resident trout but not among sea trout. This suggests that the potential for interbreeding between hatchery and wild trout is higher for stocked hatchery trout that become resident compared to hatchery trout that become anadromous. Unpublished tagging data from the 1980's showed that some stocked hatchery trout were recaptured in the river as adult sea-run brown trout, but in much smaller numbers than expected.

As stated previously, these examples are not provided in order to give an overview of all studies aimed at estimating the impact of brown trout stocking activity. Similarly, it is not the intention to try to explain the many different outcomes of stocking activities that have been observed; this must be considered a highly complex, and as yet unresolved, problem involving both genetic (e.g. origin of stocking material vs. indigenous populations, extent of local adaptations, degree of domestication of hatchery strains) and non-genetic (e.g. life stage of stocked fish and number of stocked fish in relation to carrying capacity) factors (Garcia-Marin et al. 1999b).

Finally, it must be considered a problem that there are very few examples of studies where really intensive studies of the contribution of stocked trout to the fisheries (for instance based on physical tags) have been combined with the use of genetic markers to study reproduction and introgression by stocked trout. Lack of genetic contribution by stocked trout does not provide a definitive proof that the stocked trout have not contributed to the fisheries; in the most extreme case where all stocked trout have been caught (= strong contribution to the fisheries) it would of course not be expected to observe a genetic contribution by the stocked fish. Nevertheless, even if these reservations are considered the results still raise concerns about the efficiency of stocking programmes; many stocking programmes are likely to have little or no positive effects to the fisheries. At the same time it is well established that stocking of non-native fish into wild populations may lead to negative genetic effects (e.g. Hindar et al. 1991b). The case from Greece described above (Apostolidis et al. 1996b, 1997) shows that it is even possible that stocking of brown trout provides little contribution to fisheries but at the same time has a negative effect on indigenous populations. This as well as the other examples mentioned stresses the need for 1) *only initiating a stocking program when it is in fact a viable management option* and 2) *monitoring the efficiency and possible negative consequences of stocking programs once they have been initiated*.

3.2 SUPPORTIVE BREEDING - A CAUTIONARY NOTE

A particular form of stocking refers to "supportive breeding", a type of breeding-release program where the released fish descend directly from the receiving population (Ryman & Laikre 1991). A fraction of the wild parental fish is brought into a hatchery for artificial reproduction, and the offspring are released into the natural habitat where they mix with the wild fish. The logic of this procedure is generally to increase survival through breeding in a protected, captive environment.

Supportive breeding has previously been regarded as entirely beneficial, in contrast to situations where the released fish represent genetically different populations (see above). In the case of supportive breeding no exogenous genes are introduced to the wild population. Although it is generally preferable to use indigenous populations if stocking is to be carried out (see 4.1), supportive breeding may also have strongly negative genetic effects. For instance, supportive breeding may cause the rate of inbreeding to increase dramatically as compared to the situation without supportive breeding. The loss of overall genetic variability may also increase through this form of practice. This loss of biodiversity at the gene level may occur even though the absolute number of fish actually increases from the release.



We will not go into the theoretical details of supportive breeding (for more expansive discussions the reader is referred to Ryman & Laikre 1991; Ryman 1994; Ryman et al. 1995a; Waples & Do 1994; Hedrick et al. 1995; Nomura 1999; Ryman et al. 1999). It should simply be recognized that the reason for the possible increase of inbreeding and genetic drift (section 1.4) is that the manipulations of the reproductive rates of some of

the parents (the fish brought into captivity have more offspring than the wild fish if the program is successful) affects the overall effective population size (N_e ; section 1.4).

Supportive breeding activities should always be preceded by an analysis of the inferred genetic consequences and, if necessary, modified to eliminate or minimize the negative genetic effects (Ryman et al. 1995b; Table 9). A computer based program that will aid the manager in conducting such evaluations is currently under construction (N. Ryman, unpublished).

4. INTERNATIONAL AGREEMENTS AND OFFICIAL THREAT STATUS

At the political level, several measures have been taken in response to the increased awareness of the general need for the conservation of natural resources. In Table 2 some of these measures that are of relevance to brown trout conservation are summarized. All countries that are members of European Union (EU) have, by approving several international conventions and agreements, agreed to conserve viable populations of naturally occurring species in the respective countries, and to conserve and sustainably use biological diversity at all levels, from genes to ecosystems. For instance, these countries are legally committed to the Habitat Directive (European Commission 1992), and have also ratified the Bern convention and the Biodiversity Convention.

Table 2. Examples of international, political measures of relevance to brown trout conservation.

Type of measure	Example	No. of European countries* involved	Comments
EU Council Directive	The Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora (European Commission 1992) - "The Habitat Directive"	15 (EU** members)	Includes a list in Appendix II of animal and plant species of community interest whose conservation require the designation of special areas of conservation. The brown trout is not included in this list.
International conventions	The Convention on the Conservation of European Wildlife and Natural Habitat (Bern 1976) - "The Bern Convention"	40 (incl. EU**)	Includes an appendix (II) of strictly protected fauna species. No fishes are listed.
	The United Nations Convention on Biological Diversity (Rio de Janeiro, 1992) - "The Biodiversity Convention"	22 (incl. EU**)	The intention of the convention is to conserve and sustainable use biological diversity at the ecosystem, species, and gene level.
	The Convention on Fishing and Conservation of the Living Resources in the Baltic sea and the Belts (Gdansk 1973) - "The Gdansk Convention"	EU** + 5 non-EU** countries	Aims at conserving the living resources of the Baltic and the Belts. The International Baltic Sea Fishery Commission (IBSFC) constructs the rights and obligations of member countries. The Commission deals primarily with issues concerning fisheries (quotas, methods, etc.).
	The Convention on Straddling and Migratory Fish Stocks (UN 1995)	UN-member (all European countries)	Aims at regulating the exploitation of migratory fish populations outside the exclusive economic zones of individual countries.
	The United Nations Convention on the Law of the Sea (UNCLOS) 1994.	UN-members (all European countries)	Aims at regulating the exploitation of marine biodiversity, including fishes, outside the exclusive economic zones of individual countries.
International guidelines	International Council for the Exploration of the Sea Code of Practice on the Introductions and Transfers of Marine Organisms (ICES 1994).	17	Guidelines for reducing the risks for harmful effects of deliberate introductions and translocations.
	Agreement for the Implementation of the Provisions of the United Nations Convention on the Law of the Sea of 10 December 1982 relating to the Conservation and Management of Straddling Fish Stocks and Highly Migratory Fish Stocks (e.g., FAO 1998)	All	Agreement aiming at ensuring the long-term conservation and sustainable use of straddling fish stocks and highly migratory fish stocks.

Type of measure	Example	No. of European countries* involved	Comments
International Guidelines (cont.)	Code for Conduct of Responsible Fisheries (FAO 1995)	All	Sets out principles and international standards of behaviour for responsible practices with a view to ensuring the effective conservation, management and development of living aquatic resources. Stresses that each country is obliged to conserve native populations and their genetic characteristics.
	The Precautionary Approach (e.g., FAO 1995, 1998; Rehbinder 1994; NASCO 1999; The Biodiversity Convention, see above)	All	Implies that if there is a risk that a particular measure may have harmful effects on e.g., biodiversity, actions should be taken to remove or reduce the risk, even if scientific data confirming the threat is not yet available.
Identification of species of special concern	The IUCN Red List of Threatened Animals (IUCN 1996)***	41 European countries surveyed	Provides a basis for international conservation prioritizing. Six brown trout taxonomic units listed (see section 4.1)

* Europe includes around 41 countries.

** Formally, all environmental law issues are managed by the EC. All EU members are also members of the EC.

*** The IUCN list of threatened animals is maintained in a searchable database by the World Conservation Monitoring Centre (WCMC).

The so-called "Red Lists" of threatened animals and plants classifies species into "threat categories", i.e., Critically Endangered, Endangered, Vulnerable, etc. (IUCN 1996). At the international level these classifications are produced by the World Conservation Union (IUCN). There are also national Red Lists (cf. Table 2), which are produced within each country. Not all European countries have Red Lists, and the system for evaluating threat status may vary. Also, even when there are Red Lists, not all of the vertebrate classes may have been assessed. For instance, not all European countries have evaluated their fish fauna for Red Listing. At a workshop (Faunistic surveys and vulnerability assessments) held at the Symposium on the Conservation of Endangered Freshwater Fish in Europe (Berne, July 1994) it was concluded that the present level of information is inadequate regarding vulnerability assessment of European Freshwater fish species for Europe as a whole (Maitland 1995). For example, of the 15 countries represented at the Workshop, only seven had detailed country-wide maps of freshwater fish distribution.

4.1 OFFICIAL THREAT STATUS OF BROWN TROUT IN EUROPE

Species prioritized for conservation are frequently those occurring on various lists of threatened animals, i.e., the appendices of the Bern convention and the Habitat Directive, and the IUCN Red List for Threatened Animals (see section above). Although the listings of

threatened animals constitute valuable conservation tools, they have several shortcomings (e.g., Ryman et al. 1995b; Leidy & Moyle 1998). *Two of the major limitations are that fish species in general have not been evaluated for conservation status and that the threat classification system does not take into account the intraspecific population genetic structure of the species. The latter point is particularly devastating for a species like the brown trout that is characterized by high degree of genetic divergence among local populations.*

The brown trout is not included on the list of the Bern convention. In the EU Habitat Directive Appendix 2 which lists "species of common interest that need particular conservation areas" the brown trout forms *Salmo marmoratus* and *Salmo macrostigma* are listed (Table 3). According to the latest version of the IUCN list of threatened animals (IUCN 1996) the brown trout is not threatened at the international level, although the three forms *Salmo carpio*, *Salmo letnica*, and *Salmo platycephalus* are listed as threatened, and three other forms (*Salmo marmoratus*, *S. dentex*, and *S. montenegrinus*) are listed in the category of species for which data is deficient (Table 3; IUCN 1996). As discussed in section 2.1, confusion still exists regarding the taxonomy of the brown trout complex, and some would argue that the forms listed by the EU Habitat Directive and IUCN are not to be regarded as separate species (e.g., Elliott 1994).

Table 3 exemplifies how the brown trout is ranked in some national red lists for threatened animals. Obviously, the classification varies considerably between countries. To a large extent, however, this is probably more a reflection of the level of awareness among those involved in the classification procedure than of the actual threat situation. For instance, some countries do not recognize intraspecific units, which does not allow the genetic diversity among and within populations in highly structured species such as the brown trout to be adequately accounted for. Other countries have not assessed their fish fauna at all. Further, as pointed out by Allendorf (1988), the Red List of threatened fishes to a large extent reflects the geographic distribution of fish biologists rather than that of threatened fishes.

Table 3. Examples of threat classification of brown trout taxa in national Red lists. When the threat category classification follows the one outlined by IUCN (1996) the classification abbreviation is given in parenthesis.

Country	"Taxon"	Threat category	Reference
World wide	<i>Salmo carpio</i> <i>Salmo letnica</i> <i>Salmo platycephalus</i>	Vulnerable (VU) Vulnerable (VU) Critically Endangered (CE)	IUCN 1996
	<i>Salmo dentex</i> <i>Salmo marmoratus</i> <i>Salmo montenegrinus</i>	Data deficient (DD) Data deficient (DD) Data deficient (DD)	
European Union	<i>Salmo marmoratus</i> <i>Salmo macrostigma</i>	Need conservation areas Need conservation areas	European Commission 1992
Czech Republic	<i>Salmo t. trutta</i>	Extinct in the wild	Lusk 1996
Denmark	<i>Salmo trutta f. trutta</i> <i>Salmo trutta f. lacustris</i> <i>Salmo trutta f. fario</i>	Rare Rare Rare	Danish Red List 1997
Country	"Taxon"	Threat category	Reference

Estonia	<i>Salmo trutta trutta</i> (sea migrating) <i>Salmo trutta trutta morpha fario</i> (resident)	Vulnerable Care demanding	Estonian Red Data Book
Finland	<i>Salmo trutta m. trutta</i> <i>Salmo trutta m. lacustris</i> <i>Salmo trutta m. fario</i>	Endangered (EN) Endangered (EN) ¹ In need of monitoring ²	Finnish Ministry of the Environment 1991
France	<i>Salmo t. trutta</i> L. ("sea trout")	Vulnerable	Keith & Allardi 1996
- Corsica	<i>Salmo t. macrostigma</i> ("large spotted trout")	Rare	Keith & Allardi 1996
Germany	not listed	-	
Greece	<i>Salmo trutta</i>	Endangered	Economidis 1991
Hungary	not listed	-	Keresztessy 1996
The Republic of Ireland	not listed	-	Quigley & Flannery 1996
Latvia	not listed	-	
Lithuania	not listed	-	
Norway	not listed	-	
Portugal	<i>Salmo trutta</i> L. anadromous form	Vulnerable	Portuguese Red Data Book (SNPRCN 1991)
Russia (incl. some former USSR states)	<i>Salmo t. labrax</i> (anadromous form, the Black Sea)	Endangered (EN)	Red Data Book of the Russian Federation (Flint et al., in press)
	<i>Salmo t. aralensis</i> (anadromous form, the Aral Sea)	Extinct in the wild (EW)	Red Data Book of the USSR (Borodin et al. 1984)
	<i>Salmo t. caspius</i> (anadromous form, the Caspian Sea)	Endangered (EN)	Red Data Book of Kazakskaya SSR 1978; Red Data Book of the Russian Federation (Flint et al. in press)
	<i>Salmo ischchan</i> (Lake Sevan, Armenia and the Caspian Sea)	Vulnerable (but one out of four forms is already lost)	Red Data Book of USSR (Borodin et al. 1984)
	<i>Salmo t. trutta</i> (the Baltic Sea)	Vulnerable (VU)	Red Data Book of the Russian Federation (Flint et al. in press)
	<i>Salmo t. exenami</i> (Lake Exenami, Caspian Sea)	Vulnerable (VU)	Red Data Book of the Russian Federation (Flint et al. in press)
	<i>Salmo t. m. fario</i> (the Volga and Ural river basins, Caspian Sea)	Data deficient (DD)	Red Data Book of the Russian Federation (Flint et al. in press)

Country	"Taxon"	Threat category	Reference
Slovakia	<i>Salmo t. m. lacustris</i> L.	Indeterminate	Holcík 1996
	<i>Salmo t. m. trutta</i> L.	Extinct in the wild	Holcík 1996
Slovenia - Danubian basin	<i>Salmo t. m. fario</i> L.	Vulnerable	Povz 1996
	<i>Salmo t. lacustris</i> L.	Endangered	
- Adriatic basin	<i>Salmo marmoratus</i> L.	Endangered	
Spain	<i>Salmo trutta</i> L.	Vulnerable	Blanco & González 1992, Elvira 1996
Sweden	<i>Salmo trutta</i> L.	Lower risk (LR) ³	Ahlén & Tjernberg 1996
Switzerland - Northern part (North Sea, Black Sea and Mediterranean basin)	<i>Salmo t. trutta</i>	Extinct in the wild (EW) Vulnerable (VU)	Swiss Red List 1994
	<i>Salmo t. lacustris</i> <i>Salmo t. fario</i>	Rare	
- Southern part (Adriatic basin)	<i>Salmo t. lacustris</i> <i>Salmo t. fario</i> <i>Salmo t. marmoratus</i>	Vulnerable (VU) Rare Endangered (EN)	Swiss Red List 1994
United Kingdom	not listed	-	Maitland & Lyle 1996, Sweetman et al. 1996

¹ A new version of the Finnish Red List is under way and the classification will probably change to "Vulnerable (VU)".

² A new version of the Finnish Red List is under way and the classification will probably change to "Near threatened (LR, nt)".

³ In the new version of the Swedish Red List (will apply from year 2000) the classification will be "Near threatened (LR, nt)".

The scientific experts of the TROUTCONCERT network are in agreement that the threats towards the brown trout are currently not taken seriously enough. It is not acceptable that conservation efforts are focused only on the species level, ignoring the biological diversity within species. The valuable biological resources that local brown trout populations represent are being lost at an alarming rate. It is vital that the current threat situation is recognized and that measures are initiated to halt and reverse the negative trend.

5. THE CONSERVATION SITUATION IN INDIVIDUAL COUNTRIES

In some respects the conservation genetic situation of the brown trout varies considerably between the European countries. For instance, evolutionary factors have resulted in differences in the amount and structure of biological diversity of the species in the individual countries. In some countries, such as Switzerland, Spain, Italy, Greece, and France, molecular genetic studies indicate that two to five of the major "evolutionary lineages" exist, whereas only one of these lineages appear to occur in e.g., Norway, Sweden, Denmark, and the U.K (Bernatchez 1995; Figure 2). Similarly, the remaining number of natural, undisturbed, brown trout populations varies between countries, indicating that some areas may retain a greater proportion of the remaining gene diversity of the species although the exact situation in this respect remains unclear. Further, the "biological significance" of the brown trout may vary

between countries. In Sweden for instance, it represents the only fish species in many lakes and streams, whereas in Spain, outside the most head streams the brown trout is commonly found together with minnows (*Phoxinus phoxinus*) or barbels, such as *Barbus graellssii*, *B. haasi* and *B. meridionalis*.

To review the conservation situation for the brown trout in individual European countries a questionnaire was sent out to all TROUTCONCERT participants. The information obtained, representing 14 European countries, is summarized below.

Although the type of activities resulting in the main threats to brown trout biodiversity described in section 3 occur all over Europe, there are differences between countries with respect to the degree to which different activities are carried out, and to the magnitude of the impact these operations have had. These differences between countries are reflected both in the national red listings (Table 3) and in Table 4 where the current major threats of some individual countries are shown. As an example, poaching appears to be a considerable danger to the brown trout in Estonia and in Greece (in the latter country even dynamite is occasionally used by poachers), whereas illegal fishing is not regarded as a critical threat in the other European countries.

Table 4. Examples of current major threats to brown in some European countries.

Country	Habitat degradation				Harvest		Stocking
	Dams	River regulation	Pollution	Spread of diseases and parasites	Overfishing	Poaching	Release of domesticated and/or non-local stocks
Denmark	X	X			X		X
Estonia	X	X	X		X	X	
Finland		X	X		X		
France		X	X				X
Germany	X						X
Greece	X	X	X		X	X	X
The Republic of Ireland		X	X	X	X		X
Norway		X	X	X			X
Portugal	X	X	X		X		X
Russia		X	X		X		
Spain	X	X			X		X
Sweden	X		X				X
Switzerland	X	X	X				X
United Kingdom		X	X	X	X		X

Table 5. Examples of the legislative situation in some European countries with respect to brown trout management and conservation, based on information from TROUTCONCERT participants.

Country	Legislation regulating management and conservation	Management and law enforcement	Legislation aims explicitly at sustainable management	Management and conservation part of the same law	Conservation/ management of fish regulated by the same law as other animals
Denmark	Protection of species and habitats regulated by the Nature Protection Act, Environmental Protection Act and Watercourses Act. Management and stocking regulated by the Fisheries Act.	Law enforcement and monitoring of population sizes and habitats by the Danish Institute for Fisheries Research. Practical management on a local scale (counties, anglers' clubs, etc.)	Yes	Partly	Partly
Estonia	The Law of Exploitation and Protection of the Fauna, The Fisheries Law, The Water Law, The Law of Sustainable development, etc.	Various departments of the Ministry of Environment, as well as regional county departments of environment.	Yes, in the sense of exploitation purposes.	Partly	Partly
Finland	The Fisheries Act, The Nature Conservation Act	The Ministry of Agriculture and Forestry, Water owner associations, fisheries communities	No	No	No
France	Freshwater Fisheries Law (1984), Law on Water (1992), Nature Protection Law (1976)	National Fishing Council (Conseil Supérieur de la Pêche) under control of the Ministry of Environment. Management by 4200 approved anglers' associations grouped in 93 departmental federations	Partly, stocking is considered the only sustainable management option	Freshwater Fisheries Law for management, Nature Protection Law for conservation	Conservation measures can be used for fish, but this rarely takes place in practice
Germany	?	Local Agriculture and Fisheries Ministries, except when concerning national parks	Yes	Yes	No
Greece	Several laws. Management and stocking is regulated by the Law of Fisheries, and there is other legislation regulating management and conservation	The Department of Fisheries and Forestry, controlled by the Ministry of Agriculture, as well as local authorities.	Yes	Partly	Partly

Country	Legislation regulating management and conservation	Management and law enforcement	Legislation aims explicitly at sustainable management	Management and conservation part of the same law	Conservation/ management of fish regulated by the same law as other animals
The Republic of Ireland	?	Department of the Marine. Management undertaken by the Central Fisheries Board and seven Regional Fisheries Boards, local owners and clubs.	Not in the biological sense. Current legislation aims to maintain populations for angling purposes.	Partly, but not in practice.	Potentially yes, but not in practice
Norway	Several laws including: Salmonid Fish and Freshwater Fish Act	Directorate for Nature Management	Yes	Yes	No
Portugal	Freshwater Fisheries Law	The Ministry of Agriculture and Forestry, Ministry of Environment when concerning national parks	Yes (?)	No (?)	No (?)
Russia	The Federal Law of Animals World, The Federal Law of Environmental Protection, The Fisheries Rules	The State Committee of Fisheries and its Regional (individual basins) Departments of Protection and Management of Fish Resources. The State Committee of Ecology.	Partly	Yes	Yes
Spain	The Freshwater Fisheries Legislation	Autonomous governments (e.g., in Galicia, Catalonia, etc.)	Not really, this perspective is introduced in recent legislation, but very timidly	Partly, varies between regions	Partly
Sweden	Fisheries Act, The Environmental Code	National Board of Fisheries, County administrations, Coastal guards, Fisheries owners	Yes	Yes	No
Switzerland	Federal and Cantonal Fisheries laws	Cantonal Fisheries department	Yes	Partly	No
United Kingdom	The Fisheries Law, The Countryside and Wildlife Law	England & Wales: the Environment Agency, management by fishery owners and angling clubs Scotland: the Scottish Office: Agriculture, Environment and Fisheries Department. Overall control by Environment and Fisheries Department. Sea-run brown trout management by District Salmon Fisheries Boards (mainly fishery owners), local owners. Northern Ireland: The overall control is with the Department of Agriculture (except the Rover Foyle system) and they undertake reseach. Most management is by individual angling clubs.	Not in the biological sense. Current legislation aims to maintain populations for angling purposes.	Partly, but not in practice.	No, potentially conservation could be carried out under the Wildlife Legislation or Biodiversity Action Plans, but it has not been done.

Perhaps most importantly, however, the conservation situation varies because of differences in attitude towards conservation issues and progressiveness in implementing management strategies for conservation. To some degree this is reflected in the national laws and regulations affecting brown trout conservation and management. In Table 5 some basic information regarding the national legal situation in some European countries are exemplified. In less than half of the countries the legislation aims explicitly at biological sustainable management. This is despite the fact that all of the countries of Table 5 have ratified the Convention on Biological Diversity (section 4). In some of the countries (e.g., the Republic Ireland, the U.K. and some regions of Spain) the foremost objective of the legislation is to maintain fish stocks for exploitation.

In most of the exemplified countries the conservation and management of brown trout fish are regulated under both fisheries and general wildlife legislation. In contrast to this, wild terrestrial vertebrates and economically unimportant fish species (those which are not harvested for human consumption) are managed under general wildlife and habitat legislation only. This situation appears to have resulted in the prioritisation of exploitation interests over the general interests of conservation of biological diversity. It is imperative that this predicament is altered in all European countries.

5.1 COUNTRY BY COUNTRY OVERVIEW

Here we comment briefly on a few aspects of the conservation situation for the brown trout in some individual European countries.

British Isles (Great Britain and the Republic of Ireland): Many different activities constitute threats to brown trout biodiversity on the British Isles. Major perils involve stocking, habitat loss, overexploitation, introduction of diseases and parasites, water abstraction, pollution (including heavy metals, sheep dip, salt run off from road gritting, and acid rain), salmonid farming, hydroelectric schemes, eutrophication and competition with introduced species (e.g., pike and rainbow trout).

The brown trout is not regarded as threatened on the British Isles (Table 3) and is not included in any wildlife legislation. The species is not listed in Red Data Books or in the Biodiversity Action Plans. These lists are based on species or subspecies designations in the Republic of Ireland and Britain, and as there are no trout populations currently designated as such, they fail to be protected by these measures.

Legally, management of brown trout is controlled under the Fisheries legislation, which mainly aims at sustaining populations for angling purposes. Theoretically the Countryside and Wildlife Legislation also applies to brown trout, but does not appear to have been practically applied to conserve brown trout populations. In practice, local Fisheries Boards carry out most management activities.

In Scotland, stocking of sea migrating trout populations requires approval of the District Salmon Fisheries Board, but there is no control on stocking of non-migratory brown trout. In Northern Ireland and the Republic of Ireland, permission from the Department of Agriculture

is needed before stocking is allowed, but this is only from a disease and parasite point of view, not genetics. In England and Wales, all stocking is controlled by the Environment Agency. Salmon and Freshwater Fisheries Act 1975 (England & Wales) could be used to prevent stocking on a precautionary basis but only where previous stocking has not taken place. Throughout the United Kingdom, "nature research"- or "site of special scientific interest"-designation could be applied to protect brown trout habitats but this measure has not even been invoked. In a few areas angling clubs and management bodies are voluntarily beginning to use native broodstock only for stocking purposes.

In general, a change in stocking practices and legislation, habitat improvements, and more comprehensive management strategies are highly warranted conservation requirements in the British Isles. In addition, conservation measures specific to individual populations are imperative.

Denmark: Habitat destruction, particularly dams, weirs and non-functional fish ladders in connection with rainbow trout hatcheries, constitutes a predominant peril to natural Danish brown trout populations. In addition, a general problem is the lack of suitable spawning substrate, partly due to the burial of spawning redds in the sand caused by river regulations. Overfishing, which often includes catch of returning anadromous trout spawners in nets close to the river outlets (resulting in large proportions of the potential parental fish being removed) and high angling pressure on resident trout, also threatens wild populations.

Release of fish to support wild populations is not allowed in Denmark unless it takes place according to specific plans approved by the Ministry of Food, Agriculture, and Fisheries. However, even though current guidelines advise against using domesticated stocks, this nevertheless still takes place and release of large numbers of such fish into areas of natural populations is a major concern. Stocking is often done by anglers' clubs that frequently have their own hatcheries. Restoration of habitats is sometimes also done by anglers' clubs, but is generally handled by local communities and counties. The management is controlled by the Danish Institute for Fisheries Research, and the over all responsibility for management rests with the Ministry of Food, Agriculture and Fisheries.

Environmental management is regulated in the Nature Protection Act, Environmental Protection Act and Watercourses Act, whereas management of fish species subject to exploitation is regulated by the Fisheries Act. All of these laws clearly aim at biologically sustainable management. Both management and some conservation regulations are included in the Fisheries Act, but specific conservation regulations, such as Red Lists, etc. are covered by separate acts and a separate ministry (Ministry of Environment and Energy). Therefore conservation and management of fishes are partly regulated by a separate law as compared to other animal species.

A positive trend in Denmark is that supportive breeding, often combined with habitat restoration, is increasingly replacing stocking with non-native domesticated trout. Local anglers' clubs finance these activities by a rod license that all anglers need to pay annually. However, some anglers' clubs have technical difficulties in relation to rearing of trout and some of them are not entirely aware of how conservation genetics guidelines should be implemented in practice. Consequently, a consultant in management and conservation (a biologist) has been employed, funded by rod licenses. This consultant is responsible for arranging courses for anglers and others involved in management of trout (and other fish

subject to angling) and, in general, the consultant is available full-time for giving advice on these matters. This arrangement seems to work very well and no doubt has significantly improved management and conservation of brown trout and other fish species in Denmark.

The most urgently needed conservation measures include making weirs and dams passable for trout, not only by construction of fish ladders and similar devices, but also by assuring that these constructions actually work. In many rivers the need for restoration of spawning grounds is also critical.

Estonia: The brown trout is wide spread in Estonia and occurs in many inland streams and in many of the rivers flowing into the Baltic Sea. Overfishing is considered to be a major threat to brown trout populations in Estonia. This also involves illegal fishing, which is widespread and includes electrofishing. In addition, habitat destruction through dams, channelization, and water regulations, as well as pollution by drainage of industrial and public waste into rivers pose threats to Estonian brown trout populations.

Enhancement operations are currently relatively limited in Estonia (as well as in *Latvia* and *Lithuania*), and concentrated to the coastal areas. However, 20-30 years ago it was a common practice to stock sea migrating brown trout fry into inland streams, thus mixing fish from different watersheds. No guidelines for stocking are available. A licence from the county administration is necessary to carry out stocking, and to obtain this licence a veterinary certificate confirming that the stocking material originate from a disease free source is necessary.

The legal system is under continuous reconstruction in Estonia due to the process of harmonization of Estonian laws with EU legislation, but at present the responsibility for conservation and management rests with the regional county departments of environment and, on a national basis, with the Ministry of Environment. Several laws apply to brown trout conservation and management (Table 5), including the Fisheries Law and the Nature Conservation Law. Some paragraphs of these laws overlap and may be interpreted quite differently.

The most urgently needed conservation actions include control of exploitation and elimination of the poaching problem. A reduction of pollution and habitat restoration is also urgently needed.

Finland: Overharvest is one of the main threats to brown trout populations in Finland. This includes gill-net fishing in lakes and both trap- and gill-net fishing in the coastal sea areas. Overfishing with respect to both recruitment and size occur widely. Undersized brown trout often occur as bycatch in whitefish fisheries. Habitat alterations such as dredging and ditching for drainage in reproductive areas, and acidification also constitute serious threats. According to Koljonen & Kallio-Nyberg (1991) the major threats to 33 reviewed populations of sea migrating trout are (listed in order of importance): pollution (19 cases), construction of dams (14 cases), land use including forestry and farming (12 cases), fishing (10 cases). For 71 reviewed populations of freshwater, migratory trout the most important threat is harvest (35 cases), and for resident brown trout land use, dredging, and ditching constitute major threats.

Originally, all the larger river basins in Finland contained migratory brown trout, but today only about six out of 52 natural sea migrating populations have avoided extinction or mixing with hatchery trout (Ikonen 1984; Koljonen & Kallio-Nyberg 1991).

The conservation of most wild animals and plants species in Finland is regulated under the Nature Conservation Act and administrated by the Ministry of Environment. However, the exploitation of game animals is regulated under the Hunting Act and that of commercially valuable fish species (29 species including brown trout) is regulated under the Fisheries Act administrated by the Ministry of Agriculture and Forestry. Fish species that do not have a commercial value are managed under the Nature Conservation Act.

Stocking programs are considered to be at least part of the reason for decline of wild populations. The southern and central parts of Finland are those most heavily stocked, and coincide with those regions where the wild populations are most threatened. No official guidelines for how to conduct a biologically sound stocking program exists, but the general principles are relatively well known and discussed among managers. Stocking is generally allowed, although some transfer of fishes is restricted due to the disease problem. For instance, transfer of sea migrating trout to inland waters are subject to a licence.

Important conservation measures needed in Finland include regulation of fishing, including increase of allowable mesh size, habitat restoration in rivers, and improvement of water quality.

France: Stocking activities are intense in France, and are a serious peril to wild populations. The same domesticated strain is used for enhancing native populations all over the country. No legislation on enhancement practices exist, in fact, the brown trout can be freely translocated all over the country. The importation and release of brown trout from other countries is also allowed. Similar to other European countries systematic information on the extent of stocking activity and its effects on wild populations is lacking. In addition to enhancement, habitat destruction such as pollution, river regulations and water diversions for small hydropower plants cause decline of the biodiversity of brown trout in France. Reproduction is destroyed in some areas due to soil erosions, which cause sediments to aggregate on redds.

Management is the responsibility of fisheries rights owners, and in practice is conducted by local anglers' associations. The aim of these associations is primarily to sustain populations for angling purposes, using their own fish farms and hatcheries to produce fish for stocking.

Germany: Stocking is regarded as the most serious threat to German brown trout populations, although habitat destruction, particularly damming, also causes reasons for concern. Altering the present legislation on stocking is a primary conservation need, as well as a systematic identification of remaining populations which are uninfluenced by stocking activities. At present, the regulations concerning various enhancement practices differ among autonomous governmental regions, and guidelines, if at all available, are highly dependent on the opinion of individual local fisheries officials. Recently, several local German states have implemented a statement in their fisheries and stocking guidelines saying that fishes used for stocking should derive from the same drainage as the receiving natural populations. However, the implementation of this statement is rarely followed up, and almost impossible to control.

Legally, management and conservation of freshwater fish are the responsibilities of the Agriculture and Fisheries ministries of the different local states, whereas conservation and management of other species are handled by Environmental ministries.

Greece: Overfishing is considered to be one of the most serious threats to brown trout populations in Greece. This also includes illegal fishing, involving the use of dynamite and trap fishing. Habitat destruction is another important reason for threat, and include dam constructions, canalizations and water regulations, as well as pollution from agricultural and domestic wastes. Like in many other countries systematic information on extent of stocking activities and its effect on wild populations are lacking. However, there are indications of stocking activities having been carried out in at least some rivers. The management and conservation of freshwater fish in Greece are the responsibilities of the Department of Forestry at the Ministry of Agriculture.

Norway: The major current threats to the brown trout in Norway are acid rain, hydropower regulation, agricultural and domestic pollution, indiscriminate stocking (including other species), and spread of salmon lice through aquacultural activities.

Despite actual documentation of serious losses of brown trout biodiversity (Hesthagen 1997, Hesthagen et al. 1999; Table 1) the species is not officially regarded as threatened. There is, however, some concern for particular morphotypes such as the fine spotted trout (Skaala & Jorstad 1987) and "large sized" trout.

It is not allowed to stock in Norway, but it is possible to apply for permission for stocking according to two schemes. For anadromous populations only local populations can be used, whereas for landlocked populations any broodstock can be used as long as the species is present in the area where the fish will be released. The latter scheme, however, is now changing to be more similar to the scheme for anadromous populations (in countywise stocking plans). Genetic guidelines for stocking are found in some reports from management authorities but they do not appear to be well spread.

Perhaps the most urgent conservation initiative in Norway is to identify those populations which are in greatest danger of disappearing.

Portugal: Dam constructions, hydroelectric power schemes, water diversions, pollution and overfishing are the major threats to natural brown trout populations in Portugal (SNPRCN 1991). Stocking is often restricted to fisheries reserves. Despite the indication of no displacement and minimal introgression observed until now between hatchery and wild populations (Antunes et al. 1999), stocking activities deserve more detailed examination as they may contribute to the reduction and loss of intraspecific diversity.

Although the resident form of brown trout is not considered endangered in Portugal, the anadromous form, that has its southernmost occurrence in northern Portuguese rivers, is listed as vulnerable (SNPRCN 1991).

Important conservation measures include establishing a management strategy aimed at maintaining sustainable natural brown trout populations. This strategy should include plans for the reduction of pollution, and general environmental protection. Special focus should be put on maintaining spawning areas, and constructing suitable fish ladders or similar devices. Stocking

activities should be avoided altogether until further information regarding the influence on wild populations of enhancement operations is available and an extensive genetic characterisation of Portuguese brown trout populations has been carried out.

Russia: The main threats to the brown trout in Russia include river regulations, pollution and overfishing. As a result of considerable environmental alterations in southern sea basins the anadromous form of the "Danubian lineage" has been lost in the Aral Sea and is very rare in the Caspian and Black Sea basins. Many populations of the resident form of the "Danubian lineage" are small and may be lost in the near future. For some parts of the species range in Russia the situation is more or less unknown. It is necessary to have a license from the Basin Department of Protection and Management of Fish Resource for angling any brown trout population, but illegal fishing is widespread. Stocking is a limited practice in Russia, and it is currently not a threat to wild brown trout populations in this country.

Spain: In Spain the general attitude towards the brown trout is that it is a resource for sport and entertainment, and this reflects the conservation situation. The species is not regarded as endangered in any way, although population geneticists of the country consider the situation alarming. The threats to the brown trout differ between geographical regions. For instance, in the upper watercourses overfishing is predominant, while in middle and low watercourses contamination and diseases, together with overfishing are the major problems. Stocking is also a real threat, perhaps not too much in the region of Galicia, where stocking incidence is low, but in the rest of Spain, where many introgressed populations exist, specially in the medium and low watercourses. Currently, a common policy in, for instance, the region of Galicia is to construct hybrid stocks (German stock x local populations), which are likely to be more "successful" than "pure" exotic stocks in surviving and thus reproducing and spreading exogenous genes to native populations. There is an obvious risk that this initiative will increase introgression rates, and thus genetic contamination of natural populations. Further, the monitoring of these operations will be much more difficult than in the case where "pure" hatchery trout are stocked, as the released fish will not be "genetically tagged".

Regulations that control stocking vary among the different autonomous governments, and enhancement practices depend on the initiative of local fishermen. The only existing restriction concerning release of exogenous fish refer to introduction of exotic species, which is regulated.

Obviously, the overall attitude towards conservation of biodiversity and fishes needs to change in Spain, as well as in many other European countries. The current neglect of the destruction of the biological resources that brown trout populations represent is clearly not in line with international agreements that Spain has accepted.

Sweden: The need for conservation management measures that recognize and preserve the intraspecific variability in association with the exploitation of Swedish brown trout and other fish populations were first pointed out in the 1960s and 1970s (Svärdson 1966; Ryman et al. 1977; Ryman et al. 1979; Ståhl & Ryman 1979), and the argumentation has been repeated and extended ever since (e.g., Ryman & Ståhl 1980, 1981; Ryman 1981; Hynes et al. 1981; The Swedish National Board of Fisheries 1984; Ryman & Utter 1987; Ståhl & Ryman 1987; Grundelius et al 1991; Järvi et al. 1998). Following many years of ignorance of conservation genetic aspects from fisheries managers and authorities, the attitude is now changing fairly rapidly (The Swedish National Board of Fisheries 1995b). Today, the threat situation of the

brown trout is widely recognized among authorities, and the species is classified as being at "Lower risk - near threatened" (Table 3).

A number of human activities, including dam constructions, acid rain, agriculture and forestry, are known to have caused the extinction or severe reduction of many Swedish brown trout populations (Torbjörn Järvi, National Board of Fisheries, pers. comm.; Ros 1981; Table 1). However, no systematic study of exactly how much has been lost has been conducted. Similarly, stocking activities have been extensive, and to a large extent still occur indiscriminately. No systematic analyses of the effects of stocking on the genetic diversity of wild populations have been conducted. To be able to stock, permission from the regional authorities is necessary. A new and more restrictive policy program for stocking fish and crayfish will be approved by the National Board of Fisheries shortly.

Similar to the situation in Norway, many managers focus management and conservation concern pronouncedly on large sized, "ferox" morphotypes. Populations for stocking and captive breeding are frequently selected exclusively on the basis of the average or maximum size, and the underlying rationale generally rests on the presumption that body size is to a large extent genetically determined in fishes. However, empirical evidence supporting this assumption is generally weak or nonexistent, and a recently published study on transplanted stocks of brown trout indicates that bodysize may be largely environmentally influenced (in contrast to other characters such as age at maturity and migratory behaviour; Palm & Ryman 1999). Also, from a conservation genetic perspective, management should aim at conserving the overall biodiversity. Concentrating conservation efforts on particular morphological traits with unclear genetic background will not achieve this goal, but may result in serious losses of the species evolutionary potential represented by the populations that are not, for instance, "large sized" and therefore receive less protection.

Critical conservation measures include systematic documentation of the threat situation and the development of a serious conservation strategy for the brown trout.

Switzerland: The new Federal Laws of Water Protection (1992) and the new Federal Legislation on Fisheries, in force since January 1st 1994, have considerably improved the prospects of protecting fish and their environment in Switzerland. This legislation is focused on the protection of endangered species and intraspecific units and the protection and restoration of habitats. The management of fish populations and detailed regulations about fishery apparatus and methods are the responsibility of the cantons. Nevertheless, the cantons are obliged to manage the fishery activities in such a way that fishery remains sustainable. The new Federal law on Fishery also contains restrictive regulations concerning the import and introduction of live fish intended for stocking. For each introduction of both alien species and genetically poorly adapted races or strains in natural systems, an authorization is required, so a restrictive practice can be enforced. Current practice aims at stopping introductions of non-indigenous fish in all open natural waters altogether.

6. GUIDELINES FOR CONSERVATION GENETIC MANAGEMENT

The continuing erosion of the biological resources of brown trout populations by human activities calls for strategies to reverse the current trend. It is necessary to develop protocols for conservation and sustainable use of these resources. This section is aimed at presenting

guidelines for conservation genetic management relevant to the management of brown trout populations in Europe. Numerous reports have been written on the subject of conservation genetics, and here we summarize some of those that are relevant to the brown trout. We have chosen to divide this section into two major parts where the first section (6.1) presents general conservation directives (summarized in Table 6) and the second section (6.2) deals with recommendations that have been presented in association with particular activities and threats (summarized in Table 9).

6.1 GENERAL CONSERVATION GENETIC GUIDELINES

Two of the most fundamental general conservation genetic recommendations are to maintain as much as possible of the natural ecosystems and to assess the genetic population structure (Table 6). By maintaining intact ecosystems large populations may be conserved in a natural setting to which adaptation may have occurred, and in which the evolutionary forces may continue to act. An extensive proportion of the natural habitat of the brown trout has been affected by various activities that have altered the natural state of the ecosystem. Therefore, it is particularly important that future manipulations of remaining unexploited areas are avoided as much as possible, and preceded by extensive cost/benefit analyses that include biological considerations.

To enable adequate management and conservation of natural brown trout populations it is vital to gather information on the population genetic structure (e.g., Ryman 1981; Allendorf et al. 1987). This information is essential for illuminating basic issues such as "what are we managing?" and "what do we want to conserve?". For instance, it is obviously necessary to know if a particular water system contains one or more populations, and if there is gene flow between them or not. Identifying populations and population interactions may seem like a trivial task, but in the case of the brown trout (and many other species) genetic studies have been shown to be required for such identification (Ryman et al. 1979; Ryman & Ståhl 1981; Ryman 1983; Crozier & Ferguson 1986; Ferguson & Taggart 1991; Apostolidis et al. 1997; Hansen & Mensberg 1998; Estoup et al. 1998). Several studies have, for example, revealed that genetically separated brown trout populations may coexist sympatrically within the same lake (Ryman et al. 1979; Ferguson & Taggart 1991). The genetic barrier between such populations may be due to differences in site or time of spawning (Ryman et al. 1979; Fahy 1989; Ferguson & Taggart 1991), and the existence such barriers may be impossible to detect without population genetic studies.

Knowledge of the population genetic structure is also a necessary prerequisite for evaluations of the genetic effects of different manipulations, and for monitoring biological diversity. For instance, in order to determine the genetic effects of a stocking program information on the genetic composition of the population(s) both prior and after the stocking program is required. There are a number of laboratory techniques that can be used for accumulating the adequate data in this respect (see section 1.5). It should be stressed that the statistical evaluation of the data is just as important as the generation of the raw data in the lab.

Local brown trout populations are often connected to each other in a metapopulation type of structure (Jorde & Ryman 1996; Ferguson et al. 1995; Hansen & Mensberg 1998). It is important that such structures are taken into account when planning conservation and/or exploitation activities. For instance, gene flow between subpopulations may be necessary to

maintain the viability of the individual populations, and activities directed toward one or a few local populations may affect others within the metapopulation.

Table 6. General conservation genetic guidelines applicable to the management of natural brown trout populations.

Conservation objective	Guideline/Recommendation	References
General conservation, management and exploitation	Assess the genetic population structure. Knowledge of the amount and distribution of genetic resources is essential for any form of management.	Ryman 1981, 1991; Frankel & Soulé 1981; Ryman & Ståhl 1981; Schonewald-Cox et al. 1983; Meffe 1986; Hindar et al. 1991b; FAO 1993, Ferguson et al. 1995; Ryman et al. 1995a; Allendorf & Waples 1996; Largiadèr et al. 1996; Harvey et al. 1998
	Maintain natural ecosystems in as natural a state as possible and over large enough areas to avoid causing reductions in population numbers and local extinctions.	
	Maintain as much genetic variability within and between populations as possible.	
	Focus conservation efforts at metapopulations, i.e., systems of interconnected local population.	Jorde & Ryman 1996; Spruell et al. 1999
	Address the issue of local adaptation. The high degree of genetic differentiation between local populations may be coupled with adaptations to particular environmental conditions.	Hindar et al. 1991b; Waples 1995a; Allendorf et al. 1997; Palm & Ryman 1999
Biodiversity monitoring	Monitor genetic variability of natural brown trout populations by i) continuously collecting data on survival/extinction/ demographic status of local populations ii) gather data on genetic population structure, iii) collect data on change of genetic composition over time to identify man mediated loss and change of genetic diversity, iv) collect data on previous release activities in areas supporting natural populations, v) any future release activity should be preceded by a biological consequence analysis.	FAO/UNEP 1981; McNeely et al. 1995; Laikre & Ryman 1997, in prep.
Identification of conservation units	Identify appropriate units for conservation (ESUs) by estimating evolutionary relationships among populations. Decide the appropriate hierarchical level on which to focus conservation efforts (OCUs) by considering biological factors as well as social, economical, ethical and legal ones.	Ryder 1986; Waples 1991b, 1995b; Moritz 1994, 1995; Moritz et al. 1995; Dodson et al. 1998
Prioritizing populations for conservation	Base conservation priorities on evaluations of a combination of extinction risk and biological consequences of extinction using available questionnaires and guidelines.	Allendorf et al. 1997

6.1.1 Monitoring genetic diversity

The aim of the nations that have ratified the Convention on Biological Diversity (1992, cf. Table 1) is to conserve and sustainably use biological resources at all levels (from genes to ecosystems). To accomplish these goals, methods for monitoring biodiversity must be available. If the amount and distribution of biological diversity is not systematically studied over time it will not be possible to detect negative changes and reductions of this diversity. Programs for monitoring biodiversity at the ecosystem and species levels are available in at least some countries (Paulsen 1997; Rasmussen & Geertz-Hansen 1998; Norwegian Agency for Nature

Conservation 1998; Estonian Ministry of Environment 1997; The Swedish National Environmental Protection Agency 1999). However, protocols for genetic diversity monitoring seem to be completely absent even though the need for developing such programs has been stressed (e.g. UNEP 1995). In a review of the available information on biological diversity at the gene level and the need for monitoring genetic diversity in Sweden, suggestions for initial genetic monitoring programs and model organisms were presented. The brown trout is one of the species that is particularly recommended as a model organism for monitoring programs (Laikre & Ryman 1997, in prep.).

Because of the generally strong genetic differentiation between individual brown trout populations, monitoring the number, distribution and status of existing populations provide at least a basic idea of changes in intraspecific variability. Such monitoring of population existence and status does not require advanced laboratory techniques, but can be handled by local managers. The knowledge that such monitoring would provide is currently largely lacking in European countries. For instance, it is apparent that many brown trout populations have been reduced in size or lost completely (cf. Tables 1 and 3) and that the activities causing loss of brown trout intraspecific variability are widespread and are likely to have caused many undocumented losses. However, none of the European countries appear to have systematic information on how many populations have actually been lost, or on the rate at which the loss of populations proceeds.

More advanced monitoring programs require direct studies of the gene diversity and include *i*) gathering data on genetic population structure, *ii*) collecting data on change of genetic composition over time to identify man mediated loss and change of genetic diversity, *iii*) collecting data on stocking activities, including genetic data on stocking material, in geographical areas where natural populations are stocked, *iv*) preceding any release activities with a biological consequence analyses (Laikre & Ryman 1997, in prep.). The county administrative in the province of Värmland, Sweden, has initialized a preliminary program for this type of monitoring of natural brown trout populations within the province (Laikre et al., in prep.).

6.1.2 Defining conservation units

Deciding what to conserve is the basic and crucial issue in any conservation activity. Following the recognition of the biological diversity within species, the species focus in conservation has become outdated. Conserving species is not enough; their evolutionary potential, represented by the genetic variability within and between populations must also be conserved (e.g., Frankel & Soulé 1981). Several workers have discussed the topic of what should be considered the appropriate units for conservation. A consensus of these studies is that conservation efforts should focus on evolutionary lineages within the species (Ryder 1986; Waples 1991b, 1995b; Moritz 1994, 1995, 1999). Such evolutionary groupings within species are called "Evolutionary Significant Units" or ESUs. (Box 1). The ESU concept has been applied primarily to the Pacific salmon (Waples 1991b), but as originally suggested (Ryder 1986), it is applicable to any species.

Box 1. ESU - Evolutionary Significant Unit

Definition (Waples 1991b):

"A population, or group of populations, that (1) is substantially reproductively isolated from other conspecific population units, and (2) represents an important component in the evolutionary legacy of the species."

The "evolutionary legacy" of a species is the genetic variability that is a product of past evolutionary events and which represents the source on which future evolution depends (Waples 1991b).

Methods for identifying ESUs include collection of genetic and ecological data, and analysis of these data by phenetic and cladistic techniques to determine hierarchical genetic/ecological relationships among the populations (see Waples 1991b, 1995b; Nielsen 1995). When these relationships among populations have been determined a decision must be made regarding the appropriate level on which to focus conservation efforts (Dodson et al. 1998). This decision implies deciding what should be regarded as being an important component in the evolutionary legacy of the species (Box 1). No standard amount of significant differentiation can be specified in this respect (Nielsen 1995), but decisions on what could be regarded as biologically/evolutionary significant must be determined on a case by case basis by professional population geneticists and evolutionary biologists (Waples 1995b). Consequently, there is currently no straightforward answer to the question on how brown trout ESUs within Europe should be defined. However, addressing this question scientifically on both international and national levels is urgent.

Box 2. OCU - Operational Conservation Unit

Definition (Dodson et al. 1998):

"The unit of conservation that results from the interplay between biological requirements and socio-economic issues. The biological requirements are largely found within the ESU. The OCU therefore reflects the ESU and its interaction with socio-economic issues. In some cases, sufficient economic resources and desire may exist within society to preserve all ESUs and thus the ESU's become the OCU's. In most cases, however, the OCUs may be larger than individual ESUs, encompassing several ESUs into a single OCU."

Similarly, the lack of adequate biological data for delineating the exact evolutionary relationships between populations may not be available when action is required, and in the absence of all necessary data, temporary operational conservation units should be defined.

In most practical situations decisions on conservation efforts will not be based on biological aspects only. Economic, social, and legal aspects are important factors in the process.

Similarly, biological information necessary for addressing the question of ESU-definition may be incomplete. The concept of "Operational Conservation Units" (OCUs) has been introduced to describe the unit of conservation that results from the interaction between biological and socio-economic factors (Box 2; Dodson et al. 1998).

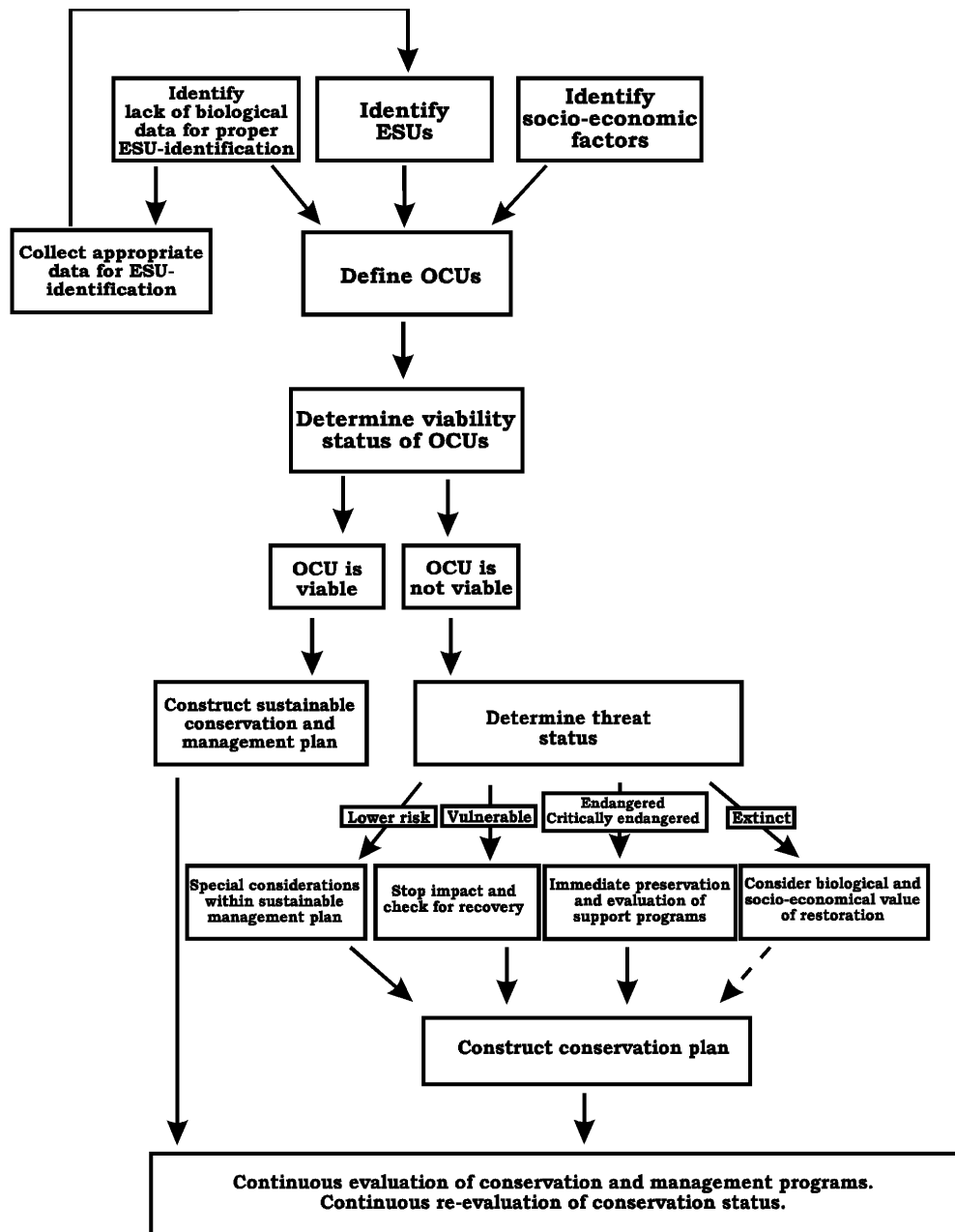


Figure 6.1. Suggested steps involved in developing a conservation and management plan for brown trout based on the ESU/OCU-approach. Modified from Dodson et al. 1998. (ESU = Evolutionary Significant Unit, OCU = Operational Conservation Unit, see text for details and references).

A schematic illustration of the steps involved in determining a conservation plan based on an OCU-approach is shown in Figure 6.1. Clearly, the current status of brown trout conservation can be dramatically improved by identifying ESUs/OCUs and focus conservation efforts and red list evaluations on these groupings instead of the species as a whole.

6.1.3 Prioritizing populations for conservation

Given the large number of natural brown trout populations currently threatened by various human activities (sections 3-5), and the practical impossibility of "conserving everything", it may become necessary in some situations to prioritize populations for conservation. There are several reasons why a method for prioritizing brown trout populations may be of value, not only from the perspective of producing a "conservation ranking list". First, a priority ranking will aid in allocating the limited resources typically available for conservation. Second, prioritizing may assist in providing a clearer focus of conservation efforts, which, in turn, may lead to more efficient programs. Third, a priority method will have to take various information regarding the populations into account, and the gathering of relevant facts may aid in identifying data that need to be accumulated for the populations under investigation. Fourth, the system used for prioritizing could also provide a useful tool for an effective monitoring of biodiversity. For example, if the process is carried out on a regular basis it will be possible to follow the changes in threat status and conservation concern of the populations under consideration.

Several different approaches for how to set conservation priorities have been proposed (e.g., Given & Norton 1993; Linder 1995; Petit et al. 1998). Recently, Allendorf et al. (1997) proposed a biologically based process for prioritizing Pacific salmon (*Oncorhynchus spp.*) populations for conservation. They suggested an approach that *i*) tries to identify the relative risk of extinction faced by different populations, and *ii*) evaluates the biological significance of the different populations. We believe that such a two-pronged approach could be useful for the brown trout as well, and below we give a brief presentation of the system proposed by Allendorf et al. for how to rank threats and priorities. When major modifications have been made to the original protocol, these are justified in the text. Otherwise, the reader should consult the original paper (Allendorf et al. 1997) for details and arguments behind the various criteria used.

The first step in the process consists of assessing the relative risk of extinction (Table 7). This is preferably done by a so-called population viability analysis (PVA) in which demographic, environmental and genetic information on the populations is used in order to estimate their probability of extinction (Gilpin & Soulé 1986; Lacy 1993). Practical help with conducting PVA analyses through workshop arrangements is provided by the Conservation Breeding Specialist Group of the IUCN (www.cbsg.org). When a PVA cannot be performed, a number of qualitative criteria may be used instead as surrogates (Table 7). If adequate data for making a direct or indirect risk assessment is missing, a population should be classified as *data deficient*, which implies that more information is required (cf. IUCN 1996).

Table 7. Criteria for assessing the level of threat for brown trout populations: modification of the model proposed by Allendorf et al. (1997) and the IUCN Red List (1996).

Criteria	Level of threat			
	Critically Endangered	Endangered	Vulnerable	Data deficient
Probability of extinction (using PVA)	50% within 5 years	20% within 20 years	5% within 100 years	Get: demographic, genetic and environmental data
	or any TWO of the following criteria:	or ONE critically endangered criterion or TWO of the following:	or ONE endangered criterion or TWO of the following:	
Effective population size per generation (N_e)	$N_e \leq 50$ or	$N_e \leq 500$ or	$N_e \leq 1000$ or	Get: genetic and/or demographic data
Total population size per generation (N)	$N \leq 250$	$N \leq 2500$	$N \leq 5000$	Get: census data
Population decline	Precipitous decline (number of spawners < 500; progeny:parent < 1)	Chronic decline or depression	Declining \geq 10% per year over the last 2-4 generations	Get: census data over time
Recent catastrophe	>90% reduction in population size within one generation	50-90% reduction in population size within one generation	10-50% reduction in population size within one generation	Get: historical or genetic data (to estimate bottleneck effects)

In the second step, the conservation importance of the individual populations is determined. By answering a set of questions (Table 8), each population is given "points" that should reflect its significance for conservation; the higher number of points a population receives, the higher priority it should have. Here we have chosen to also include some questions regarding the socio-economic and scientific value of the populations (questions no. 12 and 13; Table 8), in addition to the questions regarding the genetic/evolutionary and ecological legacy that were given by Allendorf et al. (1997). Further, since introduction of non-native fish constitutes a major threat to many brown trout populations (section 3), we suggest that populations known to be introgressed by "non-native" genes should receive less points than others. This may be achieved by removing points in relation to the estimated introgression percentage (question no. 6; Table 8).

Finally, when the level of threat and priority for conservation has been determined, the obtained information can be combined into a two-dimensional "profile" where the compared populations are arranged along the two dimensions of threat and priority (Allendorf et al., 1997). Using this profile it is then possible to identify populations with a high risk of extinction and where extinction would have pronounced negative consequences.

Applying this model for prioritizing populations may appear straightforward. However, this is not necessarily the case. There are several issues that have to be considered before applying it to real populations. For example, as commented by Wainwright and Waples (1998), available PVA models have to be modified in order to be fully appropriate for salmonids. Increased scientific knowledge will most certainly also lead to future modifications with respect to the various criteria used when determining levels of threat and priority (Tables 7 and 8). Nevertheless, natural populations are threatened now, and we agree with Currens et al. (1998) that the present system should still be workable and that there is a value with a system that "does not require waiting for perfect data or a full evaluation". Finally, it should be pointed out that the prioritisation procedures were developed specifically for Pacific salmon. Brown trout exhibit different life history variability. In particular, brown trout may be migratory or resident/landlocked. In landlocked populations genetic drift is bound to make populations less polymorphic, but at the same time more divergent, compared to migratory populations that receive gene flow from other populations. As a result, landlocked populations by default tend to score more points in question 1 (Table 8) than migratory populations. We therefore suggest keeping landlocked and migratory populations separate in the prioritisation process. In order to illustrate how the system could be used, we have applied it to four natural brown trout populations from Scotland (see the Appendix).

It is important to stress that populations which do not rank highly should not necessarily be ignored. Many of the recommendations in this report involve changes in management and conservation practices which should be applied throughout the species range where possible (section 6). It is conservation efforts such as these that are likely to prove most effective in conserving the genetic resources of the species as a whole. Nevertheless, where populations face specific, recognized threats, prioritisation of resource allocation may become necessary, and use of this scheme will be helpful. Further, in many cases, brown trout populations that score highly on the "priority axis" will not necessarily be recognized as threatened of extinction. In such cases the present prioritisation model should still be of use in assigning levels of protection to populations. In this way, populations that score highly on the biological significance scale, and thus are particularly important to the species as a whole, can be given particular protection from future stocking and other threats.

Table 8. Worksheet for determining the conservation importance of a population (modified from Allendorf et al. 1997). For each population of concern, answer the following questions.

Legacy	Question	Answer		
		Yes (1 point)	No (0 points)	No information (0 points; more data needed)
Genetic and evolutionary	1. Does this population have high genetic divergence from other populations of the same species as measured by accepted genetic techniques?			
	2. Does this population exist in a unusual habitat relative to other populations of the same species?			
	3. Does this population possess life-history traits (with a genetic basis) that are unusual for the species?			
	4. Does this population possess morphological traits (with a genetic basis) that are unusual for the species?			
	5. Has this population been long isolated geographically from other populations with little or no opportunity for migration?			
	6. Has this population remained free from introduction of non-native fish that could potentially interbreed with it?		If No go to question 6a	
	6a. Is there evidence that <u>no</u> genetic introgression has taken place?		If No go to question 6b	-----
	6b. What is the estimated introgression percentage?	1-33% (-2 points)	34-66% (-4 points)	67-100% (-6 points)
	7. Has this population avoided any severe bottlenecks in the past?			
	8. Does this population occur at, or near, the extreme of the distributional range of the species, or in marginal habitat?			
Ecological	9. Is this population a member of a native species assemblage that is unusual or rare for the species?			
	10. Are adjacent and nearby populations of the same species extinct, declining or relictual?			
	11. Would protecting the population play an umbrella role, encouraging recovery of other imperiled populations in the river basin for which limited data are available?			
Socio-economic and scientific	12. Is this population considered to be of particular cultural, economical or recreational importance?			
	13. Has this population been subject for extensive genetic/ecological studies?			

Score:

The present scheme has been designed to be flexible, and it is anticipated that it can be used at different geographical scales. The scale chosen will depend on the nature of the resources being allocated. For example, it could be used to design conservation plans at the regional level, such as within a national park. Alternatively, this scheme could be used at a national level to apportion larger scale conservation efforts. However, we do not feel that it is appropriate at this time to prioritize brown trout populations for conservation effort at scales larger than the national level. At such a large scale, the levels and types of threat and the biological significance of each population become too varied to allow meaningful comparisons to be made.

6.2 SPECIFIC CONSERVATION GENETIC GUIDELINES

Table 9 provides a summary of conservation genetic guidelines and recommendations that have been suggested in relation to particular threats or activities. Some of these recommendations may appear trivial. For instance, advice like "avoid dam constructions", "avoid pollution", and "avoid overexploitation" could seem over-simplistic. However, in the perspective of the growing human population and the increased pressure on the environment it is necessary to repeatedly point out that there is a cost coupled with our harsh exploitation of nature. Ways of life that have been taken for granted in the past, i.e., to be able to fish when and wherever you want, are no longer acceptable if biological resources are to be maintained for future generations. Any exploitative activity should be accompanied by cost/benefit-analysis that includes genetic resources.

Many of the negative effects of environmental exploitations, like dam constructions, can be reduced by proper planning. For instance, the effects of a dam may be dramatically dependent on the exact location within a water system. A dam in the upper part of the system will cause fewer number of spawning grounds to be destroyed. In some regions, such as Spain, dams will cause less destruction if they are located in the intermediate parts of the river system. Similarly, construction of functional fish ladders helps reduce the negative effects on the brown trout populations. This type of compensatory effort is used in several parts of Europe. However, the use needs to be extended. In Spain, for instance, hardly any of the migratory routes have been reconstructed through fish ladders or corresponding devices. Also, the introduction of exotic species is well known as a major agent in causing extinctions of native populations and species, but is still carried out in many countries.

The potentially negative effects of release of conspecifics, i.e., re-introductions or stockings, appear to be less well recognized among managers despite an extensive focus on these aspects in the scientific literature (see section 3 and Table 9). Re-introductions of brown trout to areas in which the natural population have been exterminated should only be considered if the causes of the extinction have been eliminated (IUCN 1987). The re-introduced fish should be as genetically similar to the original population as possible (IUCN 1987). It is important that the genetic material of the introduced fish is not too narrow; the number of unrelated individuals founding the new population should be as large as possible and comprise at least 50 genetically effective founders (Allendorf & Ryman 1987). A risk analysis considering the potential effects on the re-introduction on native populations that may exist in locations nearby the release site should always precede any re-introduction (and any other

translocation), and the re-introduction program should be monitored and continuously evaluated (Waples 1991a).

Stocking should generally be avoided, and weak populations should preferentially be supported by habitat restorations (section 3; Waples 1991a). If stocking nevertheless is carried out, the native population should be used for production of fish for release (supportive breeding; section 3.2). Exogenous genes should not be spread. A risk analysis should always precede any stocking activity, and the longterm effects of the releases followed systematically in a monitoring program. The risk evaluation should include an analysis of the potential effects on the total effective population size (Ryman et al. 1995a; section 3.2) and the supportive breeding operation adjusted accordingly.

Aquaculture activities frequently result in unintentional release and escapes of fish, and thus constitute a threat to native fish populations in the adjacent areas. Facilities used for aquaculture should thus be constructed so as to minimize the risk of escape, and be located at sites where minimal impact on wild populations will follow escapes. The risk of transmission of parasites and diseases from the cultivated fish to the wild populations should be considered in this respect. Cultured populations subject to potential escape should always be genetically tagged (Hindar et al. 1991b, Ryman 1997 a,b).

Exploitation of brown trout populations through commercial and sport fishing must be regulated to avoid overexploitation. This is an obvious guideline, which is nevertheless currently ignored in several European countries (section 5). Problems with overharvest can frequently be reduced by appropriate fishing practices with respect to e.g., the smallest allowable size of fishes caught, for instance by increasing the mesh size of gill nets. At the same time it should be recognized that a heavy fishing pressure on a few age classes may affect the demographic parameters of the population in a fashion that increases loss of genetic diversity through reducing the genetically effective population size (e.g., Laikre & Ryman 1996; Ryman 1997a,b).

Harvest of mixed populations is a problem in many fisheries as fishes are caught in areas where individuals from several populations mix. If calculation of fishing pressure is based only on the stronger populations there is a risk of overexploiting weak populations. Mixed fishery analysis using genetic stock identification techniques provide a means for assessing the genetic composition of fishery landings, which in turn allows managing the fishery to avoid overexploitation of weak populations (e.g., Pella & Milner 1987; Utter & Ryman 1993).

Table 9. Specific conservation genetic guidelines relating to particular threat categories.

General threat category	Activity/Process	Recommendation	References
Environmental degradation	Dam constructions and water diversions	Generally put in effort to avoid dam constructions in rivers and streams inhabited by brown trout. Concentrate dams to particular regions to minimize the number of affected populations. Locate dams to the upper, or in some regions the intermediate parts of the water system to reduce the number of spawning sites destroyed. Reduce the amount of destruction by providing measures such as fish ladders and similar devices that help circumvent the migration obstacles. Make sure that these devices really work. Maximize the minimum water flow, and change the current in a slow, gradual fashion. Conserve large areas of natural habitat.	Näslund 1993; NASCO 1999; cf. Table 4
	Acidification	Reduce the rate of acidification. Restore acidified water systems through, e.g., liming. Restore lost/weakened populations through genetically sound re-introduction or stocking (see below).	Näslund 1993
	Introduction of exogenous species and populations	Avoid introductions.	Allendorf 1991; FAO/UNEP 1981; Ryman 1981; IUCN 1987
Stocking	Re-introduction	Re-introductions should only take place where the original causes of extinction have been removed.	IUCN 1987
		<i>Origin of released fish:</i> The re-introduced fish should be of as similar a genetic background to the original population as possible.	IUCN 1987
		<i>Amount of released fish:</i> The released individuals should represent a minimum of at least 50 genetically effective founders.	Allendorf & Ryman 1987
		<i>Risk analysis:</i> The potential effects on still existing native populations should be evaluated.	
		<i>Monitoring:</i> The effects of the re-introduction should be monitored.	

General threat category	Activity/Process	Recommendation	References
Stocking (cont.)	Population Enhancement	Avoid stocking generally, improve natural habitats by, e.g., restoring/creating spawning grounds.	Ryman 1981, Hansen & Loeschcke 1994
		<i>Origin of released fish:</i> Use the original population to produce fish for release. Do not spread exogenous genes.	Ryman 1981, Berg & Hansen 1996, FAO 1993
		<i>Amount of released fish/gene flow:</i> Should be based on data on the genetic differentiation between the donor and recipient populations.	Allendorf & Ryman 1987, FAO/UNEP 1981
		<i>Conservation breeding:</i> The effective population size in hatcheries should be at least 50, and preferably higher for long term breeding programs.	Ryman et al. 1995a,b
		<i>Selection of individuals for release:</i> Should be based on pedigree-data regarding genetic relationships, etc. Should not be based on phenotypic characteristics.	
		<i>Risk analysis:</i> The potential biological effects of the stocking program should be evaluated. This includes <i>i</i>) genetic characterization of the donor and recipient populations, <i>ii</i>) investigation of the population genetic structure of the brown trout in the area of release, <i>iii</i>) determination of potential risks of the release, based on answers from point <i>i</i> and <i>ii</i> and previous experiences.	
		<i>Monitoring:</i> Monitor the effects of the release on the genetic diversity.	
		<i>Supportive breeding:</i> The effect of supportive breeding on the rate of inbreeding and genetic drift in the total population (wild + captive) should always be evaluated before carrying out any supportive breeding activity. In general, supportive breeding should not be carried out if it results in an increased amount of drift as compared to the situation without supportive breeding.	Ryman 1981, Laikre & Ryman 1997 Ryman & Laikre 1991; Ryman et al. 1995a, 1999
	Induced gene flow	Maximum acceptable levels of introgression should be based on estimated genetic differentiation between populations (F_{ST} or similar appropriate measure).	Ryman 1991; Ryman et al. 1995b

General threat category	Activity/Process	Recommendation	References
Stocking (cont.)	Aquaculture	<i>Unintentional release/escapes</i> : Aquacultural activities should be conducted so as to minimize potential gene flow and spread of diseases and parasites to natural populations. Aquacultural facilities should be located and designed to minimize risk of escaping. Locations should be selected on the basis of having minimal impact upon natural populations. Cultured populations subject to potential escape should be genetically tagged.	Hindar et al. 1991b; Ryman 1997; NASCO 1999
Harvest	Commercial and sport fishing	Regulate commercial and sport fishing to avoid overexploitation.	FAO 1993
		Help avoid overexploitation by limiting the size of fish allowed to catch, and by increasing the allowable size of gill net meshes. Adequate limiting sizes in this respect varies between populations, and must be based on growth data from the exploited population.	Ryman 1981
		Apply different fishing regimes management of harvest for strict anadromous trout populations vs. resident populations.	
		Avoid selective harvest that may have a negative effect on genetic variability within and between populations.	
	Harvest of mixed populations	Use mixed fishery analysis for instance by means of genetic stock identification (GSI) algorithms, or similar methods, to monitor harvest of the different populations, particularly making sure that weak populations are not overharvested.	Pella & Milner 1987, Utter & Ryman 1993, Smouse et al. 1990

7. SUMMARY AND CONCLUSIONS

This report has been produced within the framework of the EU Fisheries and Agriculture Research (FAIR) project "Concerted action on identification, management and exploitation of genetic resources in the brown trout (*Salmo trutta*)" (abbreviated as "TROUTCONCERT"; FAIR CT97 3882; Dr Michael M. Hansen, coordinator). The report describes the current knowledge of the conservation genetic status of brown trout in Europe, reviews the main threats towards the biological diversity at the gene level of brown trout populations and summarizes available conservation genetic guidelines that are applicable to the species.

The brown trout is an ecologically, economically, aesthetically and scientifically highly valuable fish species whose poor conservation status in the European countries calls for further attention and action. One of the main reasons for the inadequate protection and conservation of brown trout in European countries appears to be a general ignorance of the biological diversity that exists as genetic variation within the species. It is not enough to simply conserve species, but their evolutionary potential, represented by differences at the gene level within and between local populations of the species, must also be conserved. The general goal of conserving biological diversity at all levels from genes to ecosystems is established internationally, and applies to all nations that have ratified the Convention on Biological Diversity (i.e., most European countries, including the those of the EU). However, these intentions are generally not yet being transformed into adequate actions. Recognition of the importance of conserving intraspecific biodiversity is particularly critical for a species like the brown trout, which is characterized by very large genetic differences between local populations. Loss of local brown trout populations may result in the extermination of comparatively large parts of the species gene pool.

Numerous guidelines and recommendations for conservation genetic management that are applicable to the brown trout have been available for many years (some of them are summarized in Tables 6 and 9 of the present report). Today, the problem in many cases is not lack of knowledge but rather lack of implementation of what is already known. Of course, there will always be a need for better information and more research, but requests for more facts cannot be tolerated as excuses for inadequate action. We know enough to halt the ongoing erosion of brown trout biodiversity, and to reverse the current negative trends.

There are three major types of human activities that constitute potential threats to wild animal and plant populations: habitat alterations, harvest, and release of exotic species and translocated conspecifics. The brown trout is subject to all these types of activities. In some regions they are carried out on very large scales, and they are known to have caused the extinction or reduction of brown trout populations all over the species range. No serious attempts, however, appear to have been made to review what actually has been lost, and what is at risk of being lost, in terms of local brown trout populations and the genetic resources they represent. It is strongly recommended that such overviews are carried out both on a national and international level.

One particular activity that results in serious threats to brown trout populations in many European countries refers to "stocking". Wild populations are "enhanced" by the release of large numbers of translocated fish, often derived from hatcheries. Stocking represents an especially severe threat because it is generally regarded as beneficial and a means to "help" the natural population, but it may in reality result in the extinction or severe reduction of local wild gene pools. The fish used for stocking are frequently genetically distinct from the wild populations. Often the released fish represent more or less "domesticated" hatchery strains. Further, even if the fish released are from the same population (i.e., supportive breeding) there is a risk for negative genetic effects.

The brown trout has been subjected to far more population genetic studies than most other wild animal and plant species. Compared to other species, much is known about the genetic structure of brown trout populations. The genetic diversity of the brown trout is distributed at several hierarchical levels. At a large geographical scale, a number of highly divergent evolutionary lineages have been observed in the Southern and Eastern range of the species. In the Northern range, that has been strongly affected by Pleistocene glaciations, one evolutionary lineage, often referred to as the "Atlantic race", is predominant. However, several studies indicate that even here the situation is more complex, as postglacial recolonizations from more than one glacial refuge may have taken place, although it is currently unclear how many recolonization lineages can be identified.

There is considerable confusion regarding the taxonomy of the brown trout. This confusion stems from an ongoing discussion on how to classify the various morphologically and/or genetically distinct groupings that have been identified. Some suggest these groupings should be classified as individual species, whereas others propose that the differences reflect within species diversity or, perhaps, subspecies. It is important to stress, however, that the taxonomic discussions do not affect measures necessary for conservation and management. Effective conservation of the brown trout must be based on the genetic differences between populations regardless of whether we call these populations species, subspecies or local populations.

In Europe, species prioritized for conservation are frequently those occurring on the national and international Red Lists for Threatened Animals. Two major shortcomings of these lists are that fish species in general have not been systematically evaluated for conservation status and that the threat classification system often does not yet take into account the intraspecific population genetic structure of the species. An overview of some national and international Red Lists indicates that the brown trout is generally not regarded as threatened. The species *Salmo trutta* has received some threat classification status in only three countries (Greece - Endangered, Spain - Vulnerable, Sweden - Lower risk, near threatened). In many other countries, however, various "forms" or "subspecies" are classified as threatened. For instance, in Finland three recognized forms, *Salmo trutta m. trutta*, *S. t. m. lacustris* and *S. t. m. fario*, are classified as Endangered, Vulnerable and In need of monitoring respectively. In 11 of the 17 countries surveyed 1-4 brown trout "forms" are listed as threatened.

The scientific experts of the TROUTCONCERT network are in agreement that the current Red Lists in most cases do not adequately recognize the threat situation of the brown trout; the perils facing this species are currently not taken seriously enough. The valuable biological resources that local brown trout populations represent are being lost at an alarming rate. It is vital that the current threat situation is recognized and that measures are initiated to halt and reverse the negative trend.

A survey among TROUTCONCERT participants revealed that dams and river regulations severely damage brown trout populations in many European countries - all 14 of the surveyed countries state these types of habitat alterations as a major concern. Various sorts of pollution (including acid rain, and agricultural wastes) are a large problem in 11 of the countries. Overfishing constitutes a major peril in 8 countries, and this includes illegal fishing, which is regarded as extensive in Estonia and Greece. Stocking is hardly practiced in Estonia, Russia and Greece but

is an important concern in most of the other countries. In France and Spain, for example, stocking is carried out more or less indiscriminately at a very large scale. In France a particular domestic strain is used to stock populations all over the country, and in Spain foreign stocks representing a different evolutionary lineage is commonly used. No practically effective restrictions concerning stocking appear to exist in any of the surveyed countries, although some progress in this respect have been made in, for example, Denmark, Norway and Sweden.

The attitude towards conservation issues and progressiveness in implementing management strategies for conservation varies between the 14 surveyed countries. For instance, in only two thirds of the countries the legislation aims explicitly at biological sustainable management. In some of the countries (e.g., the Republic of Ireland, Great Britain and some regions of Spain) the foremost objective is to maintain fish stocks for exploitation. This represents an outdated attitude, which is not in line with international agreements and conventions. In the majority of the surveyed countries the conservation and management of fishes are at least partly regulated under a different legal device as compared to other species. This is primarily due to the fact that many fish species (including brown trout) are exploited for human consumption to a considerably larger degree than, for example, terrestrial vertebrates. Therefore particular fisheries legislation often regulates management and conservation of fish whereas that of terrestrial vertebrates is frequently regulated by general habitat and wildlife legislation. This situation tends to prioritise exploitation interests compared to general conservation interests. It is important that this predicament is altered in all the European countries so that fishes receive the same conservation attention as other vertebrates.

7.1 RECOMMENDATIONS

1. Documentation of what has been lost and what is at risk of being lost in each of European countries inhabited by natural brown trout populations is highly warranted. Such documentation includes mapping of localities previously inhabited by brown trout where the species has disappeared, causes of disappearance or reduction, and identification of populations at high risk of being lost. Surveys of this sort are essential for adequate management and conservation action, and a necessary prerequisite for monitoring of future changes.
2. The intraspecific structure of the brown trout must be recognized, and the aim of management and conservation must focus on maintaining this structure. For instance, it is essential that the five major "evolutionary lineages" that have currently been identified using molecular genetic techniques are maintained. Countries where two or more of these lineages occur (e.g., Switzerland, Spain, Greece, or Italy) should, at the very least, concentrate conservation efforts at maintaining viable metapopulation systems of all lineages. Artificially induced interbreeding of individuals from different lineages, i.e., through stocking activities, is unacceptable. In case of occurrence of such activities they should be stopped immediately. Countries where only one of the major evolutionary lineages occurs (e.g., Great Britain, Norway, Denmark, Sweden and Finland) should focus first on identifying and maintaining major groupings and metapopulation systems within the lineage.

3. Available general and specific guidelines for conservation genetic management of the brown trout should be incorporated into conservation and management routines. Guidelines for general conservation and management issues including identification of conservation units, prioritisation of populations for conservation and biodiversity monitoring are available (Chapter 6; Table 6). Similarly, guidelines applying to specific activities, such as introductions, re-introductions, habitat alterations, stocking, hatchery breeding, induced gene flow, aquaculture and fishing have also been presented (Chapter 6; Table 9). We urge policy makers, managers and everyone else involved in brown trout management to recognize and incorporate these guidelines into directives, regulations and practical management programs on international, national, as well as regional levels.
4. Stocking (supplementing wild populations through the release of translocated fish) should generally be avoided. If a population is declining, the cause of this decline should be identified and, if possible, removed. For instance, if habitat alterations are causing the decline the aim should be to restore the habitat so that the population may recover on its own. Similarly, if excessive fishing pressure is causing the decline, this pressure must be reduced. Exploiting any form of biodiversity beyond what is biologically sustainable is unacceptable and must be stopped, even at the cost of, for example, reduced fishing opportunities. Stocking should only be used if it represents a biologically viable management option and the fish used for stocking originate from the natural population stocked. An extensive risk analysis should always precede a stocking program and the effects of the program should be monitored using population genetic knowledge and techniques to enable the detection of possible negative effects on native populations.

In conclusion, by combining the different recommendations of this report we propose an overall strategy for conservation of genetic diversity of brown trout in Europe.

- First of all, management of brown trout must be in accordance with the conventions for protection of biological diversity that have been signed by the European countries (Chapter 4). Some of these, like the "Biodiversity Convention", are aimed at general principles for conservation, whereas others, like the IUCN Red List of Threatened Animals, specifically mention individual species, including brown trout.
- Second, it is imperative to recognise that it is insufficient and inappropriate to regard the species "brown trout" as the basic conservation and management unit. Effective management and conservation must focus on the genetic diversity that is present at the intraspecific level (Chapters 2 and 6). Brown trout consists of several major evolutionary groupings, and it is important to conserve all these. The concept of Operational Conservation Units (OCUs) provides a tool for delineating and focusing on these major lineages (Chapter 6). However, it must also be stressed that OCUs are not necessarily the basic units for management and conservation. The basic units for management and conservation are LOCAL POPULATIONS. By conserving populations within OCUs genetic diversity within OCUs is also conserved, and by conserving OCUs genetic diversity within brown trout as a whole will also be conserved.
- In some cases there may only be resources for conserving some populations within a geographical region. In those cases it will be necessary to prioritise populations for conservation. A suggestion for a protocol for this is described in section 6.1.3.

- Finally, there are many guidelines available for conservation of local populations (Chapter 6). Some of these describe general principles of conservation biology, whereas others aim specifically at conservation of populations of salmonid fishes, including brown trout. It is imperative that these guidelines and principles are integrated in the management of brown trout populations in the different European countries, and that all countries develop strategies for conservation of brown trout.

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10. APPENDIX:

PRIORITIZING BROWN TROUT POPULATIONS FOR CONSERVATION: A CASE STUDY

This section is intended as an example, to show how the prioritisation tables (Tables 7 and 8) can be used. Four populations, representing a variety of situations have been chosen. However, it is not the intent of this section to set conservation priorities for actual, named populations at this time. It was therefore decided to withhold the names of the populations used. Nevertheless, the populations used in this section are all based on actual Scottish populations. Existing data (both published and unpublished) are used. It should be noted that in this example we have concentrated exclusively on landlocked populations of brown trout, and there is no sea trout component in any of these populations. As pointed out and described in section 6.3.1 such a distinction needs to be made as isolated, landlocked populations otherwise tend to score more “prioritisation points” than populations accessible to gene flow.

A detailed description of Population A is provided here. This information is then used to give a complete example of the working involved in using this prioritisation scheme (Tables 10 & 11). A brief background is also given on the three other populations (B-D), which were also subject to the same working (not shown here). The results of the questionnaire for all four populations are given in Table 12. This information is then discussed, to show how the results of the prioritisation process could be interpreted.

Description of Population A

This population is found in a relatively small upland lake. The lake is situated above impassable falls, meaning that the population is isolated from both the sea, and other trout populations in the area. The population consists exclusively of resident brown trout which are known to spawn in the lake, rather than surrounding streams. Individuals from this population are notable for their increased longevity, large size and unusual golden colouration, as compared to other brown trout populations in Scotland. Because of this, they are prized by anglers. Nevertheless, the population is not heavily exploited, largely because of its remote location. The population has been the study of genetic and ecological studies for the past 20 years. During this time it has become apparent from catch data that the population is extremely small, and appears to be declining. Current estimates suggest that the total population size per generation is less than 500 individuals.

The scoring process for the assessment of extinction risk of population A is given in Table 10 and a breakdown of the responses to the “conservation importance” questions in Table 11.

Table 10. Extinction scores for population A

Criteria	Response to Criteria
Probability of extinction	Insufficient data to conduct PVA (Population Viability Analysis). Deferred to alternate criteria.
Effective population size per generation	Insufficient data.
OR	
Total population size per generation	Total population size per generation believed to be less than 500 from catch data. Level of Threat = Endangered
Population decline	Data suggest that population has suffered serious decline in recent years. However, the exact level of decline cannot be accurately calculated. Nevertheless, the spawning population <500, so even if the population is stable, it can be classified as chronically depressed. Level of Threat=Endangered
Recent Catastrophe	Insufficient data.

There were two responses in the endangered category. Therefore, overall, the level of threat to population A is classified as **Endangered**.

Table 11. Conservation importance of population A

Question	Response
1. Does this population have high genetic divergence from other stocks of the same species, as measured by accepted genetic techniques?	Yes. Several different techniques indicate that this stock is genetically highly divergent from other populations of brown trout in Scotland.
2. Does this population exist in an unusual habitat relative to other populations of the same species?	Yes. Due to the high altitude, and geographical location, the local habitat is extremely harsh, and the lake freezes over completely in the winter months. It is unusual for trout in Scotland to exist in this kind of environment.
3. Does this population possess life-history traits (with a genetic basis) that are unusual for the species?	Yes. The population consists of lake spawning individuals, which are unusual for brown trout in Scotland. They are also late maturing and long-lived trout. These traits have been shown to have a high heritability in other salmonids.

Question	Response
4. Does this population possess unusual morphological traits (with a genetic basis) that are unusual for the species?	Yes. Individuals exhibit an unusual golden colouration and spotting pattern. These traits have also been shown to have a genetic component in other salmonid species.
5. Has this population been long isolated geographically from other populations with little or no opportunity for migration?	Yes. It is landlocked above impassable falls, which must have existed for several thousand years.
6. Has this population remained free from any introduction of non-native fish that could potentially interbreed with it?	Yes. This is supported by genetic data, which suggest that this population is divergent from any possible hatchery source.
7. Has this population avoided any severe bottlenecks in the past?	No. Standard genetic techniques have revealed extremely low genetic variation in the population. This suggests that the population has undergone repeated bottleneck effects in the past.
8. Does this population occur at, or near, the extreme of the distributional range of the species, or in a marginal habitat?	Yes. Scotland is not particularly near the edge of the distributional range of the species. However, the extreme conditions in the lake, means that it can be described as marginal habitat for trout.
9. Is this population a member of a native assemblage that is unusual or rare for the species?	No. The minnow (<i>Phoxinus phoxinus</i>) is the only other fish species present in the lake. Brown trout and minnows are commonly found together throughout Northern Europe.
10. Are adjacent or nearby populations of the same species extinct, declining or relictual?	Yes. A genetically similar population in an adjacent loch appears to be declining at a similar rate to population A.
11. Would protecting the population play an umbrella role, encouraging recovery of other imperiled populations or species in the river basin for which limited information is available?	Unknown. Little is known about the plants and invertebrates in the local area. It is possible that rare and endangered species or populations are present.
12. Is this population considered to be of particular cultural, economical or recreational importance?	Yes. The large size attained by individuals from this population, together with the golden colouration mean that they have historically been of recreational importance.
13. Has this population been subject to extensive genetic/ecological studies?	Yes. In addition, the extremely low amounts of genetic variation in the population, mean that it is of particular scientific interest.

Overall Score: 10

Unknown: 1

All populations

Population B

This large population consists entirely of resident brown trout. It is found in a relatively large upland lake that is situated at the top of a large catchment, above impassable falls. Genetic studies have indicated that it is completely isolated from neighbouring populations.

Population C

This resident trout population is found in a small upland lake at the top of a large catchment. It is inaccessible to other trout populations. It is not heavily exploited by anglers, due to its isolation, and the relatively small average size of individuals in the population. It has not been stocked with hatchery trout in the past.

Population D

Again, this population is found in a small upland lake, above impassable falls. The population appears to be large, and trout densities are high. There is a lot of angling in this area. The population has been extensively stocked in the past, and genetic studies have indicated that introgression rates are extremely high.

Comparison of All Populations

The questionnaire was then repeated for the above populations. The results were then collated and presented in a table, to allow the relative level of threat and conservation importance of each population to be compared (Table 12).

Table 12. The outcome of the prioritisation questionnaire for all six populations.

Level of threat	Conservation importance												
	-2	-1	0	1	2	3	4	5	6	7	8	9	10
Critically Endangered													
Endangered													A
Vulnerable													
Not Classified	D				C					B			

Analysis of Outcome

It is clear from these results that population A is of high conservation importance, and also faces a high level of threat. A valuable biological and socio-economic resource is in immediate danger of extinction. This population clearly ranks above the others in terms of priority. It would be recommended that conservation effort is directed towards identifying and removing the causes behind the decline of this population. Where legislation can be used to protect this population against specific threats, such as over-exploitation, or stocking with exogenous material, this should also be done. In this example, Scottish fisheries legislation is only likely to be able to afford incomplete protection, as it is aimed more at maintaining the number of trout for angling purposes, rather than conserving existing biodiversity. Wildlife legislation could perhaps be used more effectively in this case. This would involve the formation of a Site of Special Scientific Interest (SSSI), specifically centered on the brown trout population. This could specifically protect against the introduction of exogenous brown trout. It should also be noted that there was insufficient data to calculate most of the extinction risk criteria. It should therefore also be a priority to gather further data, to enable this to be done. If PVA could be calculated, then the risks facing this population could be more accurately assessed

The other three populations do not appear to be facing such a high level of threat as population A, and are as such are unclassified by this part of the prioritisation scheme. In this type of situation, the prioritisation scheme can be used to identify which populations should be afforded some form of protection. Of the three populations, B obviously represents the most important resource. While not immediately threatened, it is recommended that this kind of population is monitored over time to ensure the position does not change. Despite the perceived low threat of extinction, it is important to remember that all brown trout populations are potentially threatened by stocking with exogenous brown trout, which can permanently destroy the genetic makeup of native populations in extremely short periods of time. Populations with a high conservation importance such as B in this example should be legislatively protected against such events.

Populations C and D are of less conservation importance. Nevertheless, they are still intrinsically valuable in both biological and recreational terms. It should also be pointed out that as this prioritisation scheme is necessarily simplistic, some important aspects of a population might be overlooked. Furthermore, the importance of a particular population both to the general ecology of the surrounding habitat, and genetic resources of the brown trout species are often not fully understood. With this in mind it is important to stress that a conservative approach should be applied. Even populations that have low prioritisation scores should, as far as possible, be managed in accordance with the general guidelines and principles set out in this report.