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REPORT OF THE WORKSHOP ON IDENTIFICATION OF FISH FARM ESCAPEES AND WILD SALMON

Trondheim, Norway, 11-13 February 1991

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1. INTRODUCTION

1.1 Terms of reference

The terms of reference were (C. Res. 1990/2:25) as follows:

"A Workshop on Identification of Fish Farm Escapees and Wild Salmon (Chairman: Dr L.P. Hansen, Norway), with members drawn from all countries participating in the Working Group of North Atlantic Salmon, will be held in Trondheim, Norway from 11-13 February 1991 at national expense to:

a) develop and report on techniques that could be used to distinguish wild salmon from fish farm escapees,

b) techniques examined should include:

i) morphology

ii) scale-pattern recognition

iii) biochemical and physiological methods

iv) genetic markers

v) large-scale group marking of salmon in farms

c) report to the Working Group on North Atlantic Salmon, the Study Group on the Genetic Risks to Atlantic Salmon stocks, and the Anadromous and Catatdromous Fish and Mariculture Committees."

The categories of fish that we would like to be able to monitor are *inter alia* escaped fish of cultured stocks, genetically modified fish (e.g. triploid or transgenic fish), and intentionally released fish (e.g. for local stock enhancement or sea ranching).

1.2 Participants

Name	Country
L.P. Hansen (Chairman)	Norway
K. Hindar	Norway
L. Karlsson	Sweden
J.H. L'Abée-Lund	Norway
R.A. Lund	Norway
H. Lura	Norway
L. Norman	Sweden
F. Økland	Norway
Ø. Skaala	Norway
J. Webb	U.K. (Scotland)
A. Youngson	U.K. (Scotland)

2. MORPHOLOGY

Several previous studies have used systematic measurements of the fin length and body size (fork length) to discriminate wild and cultured salmon (Hansen et al., 1986; Potter, 1987; Lund et al., 1987). In Norway significant differences have been found in the sizes of fins of different wild stocks of salmon. Accordingly, this may reduce the discriminatory power of these methods when sampling fish of differing origin in mixed stock fisheries; particularly when attempting to identify specimens released or lost from culture at the juvenile stage (parr and smolts).

Critical descriptions of various morphological characters of groups of adult salmon were made according to Lund et al., (1989). The groups studied included specimens of harvestable farmed salmon, escaped/released reared salmon from sea fisheries, recaptures of hatchery reared fish and of wild salmon. Seven characters were identified as being associated with the culture environment. These are - the erosion and deformation of the tail, dorsal, pectorals, the snout and gill covers together with

estimations of the level of pigment spotting on the left side of the fish between the snout and a vertical line from the front of the dorsal fin (Figure 1). Amongst these however, the first four characters are the most diagnostic and are therefore considered to be the most appropriate indicators of a specimen having spent some time in culture. Discriminatory power is usually increased by considering the greatest number of these characters in each case, together with scale characters (see Section 4 below).

The method is summarised by the conclusion that fish of farmed origin bear defects on two or more body characters in each case. Wild fish seldom bear more than one defect whereas ranched salmon had one or two defects.

Concern has been expressed as to the effects of fin regeneration on the accuracy of estimates based on the use of these criteria. Fin regeneration does occur, and is most evident amongst fish that have been at liberty for a long time. Marginal fin re-growth is most commonly found in the tail, although re-development is often to a size below that expected for a given body fork length. It is uncommon for either the dorsal or paired fins to regenerate in this way. However, where re-growth occurs, deformations of the fin rays and the associated epidermal tissue tend to remain detectable.

In Norway, data on the incidence of cultured salmon has been gathered in a number of fisheries using a score card system. It was pointed out that the discriminatory power of the methods in this case is limited by the scoring approach adopted rather than by the techniques themselves.

Field Characters

Recently, studies have been conducted in Scotland on the riverine and spawning behaviour of escaped (cage-reared) adult salmon in a small river (Webb et al., in press). Wild and farmed fish were observed from the riverbank and identified by skilled personnel under appropriate field conditions of light and water clarity

using Polariod spectacles. The origin and sex of fish at spawning could be determined by using a range of field characters;

i. Fin size, shape and form; farmed salmon bore smaller and deformed tail, dorsal and paired fins. The marginal edges of the tails of wild fish were transparent. In contrast, the same area of the tails of farmed fish were opaque.

ii. Body shape; wild salmon were more fusiform than farmed escapes.

iii. Scale loss; many of the farmed males bore extensive areas of scale loss from the middle and upper regions of their flanks; often exposing areas of flesh. In many cases, the resulting lesions did not develop secondary fungal infections (*Saprolegnia* sp.). The phenomenon was not evident amongst wild males.

iv. Behaviour; at spawning, the intrasexual behaviour of some farmed males differed from the normal behaviour previously described by Jones (1959). Intrasexual interactions between farmed males often lacked the normal aggressive threat displays.

Field conditions and the origin of escapes would effect the feasibility of these techniques being used elswhere.

In summary, despite the extensive use of morphological characters to distinguish fish of wild and farmed origin, all estimates will almost certainly be underestimations of actual values. The efficiency of methods relying on morphological characters will tend to depend on the following factors:

i. The culture environment from which fish were lost or released (hatchery or sea-cages).

ii. The developmental stage at loss or release.

iii. The intervening period between loss or release and subsequent recapture.

iv. The experience of the observer.

Within hatcheries and growing-on facilities producing table fish, the effect of regional differences in culture environments at each developmental stage is unlikely to be important. However, many hatcheries rearing fish for restocking purposes may produce juveniles bearing fewer discriminatory characters and these may be less well developed.

Any variations in the incidence of defect characters between different wild populations are unlikely to be important.

The stage at which cultured fish are liberated into the wild probably remains the most critical factor that presently effects the abilities of fishery scientists to distinguish a specimen's origin. Furthermore, the use of morphological characters in this way restricts differentiation to the level of identifying escaped or released fish with their wild counterparts rather than their progeny.

3. OTOLITHS

Evidence was presented illustrating a method by which otoliths could be used to distinguish hatchery and wild salmon parr. It was suggested that the technique could be used to distinguish farmed and wild salmon parr with a high degree of accuracy. In wild specimens, the otoliths are translucent and tend to have distinct annuli. In contrast, the otoliths of juvenile fish from hatcheries were opaque and had few or no distinct zones. It is not clear how long a juvenile fish would have to remain in either environment before its origin could be accurately determined. However, it is very probable that the discriminatory power of the technique will increase with the length of time spent either in the hatchery or with age in wild fish. Accordingly, unfed fry of

hatchery origin released into rivers and streams will probably not be detectable.

It is not known whether the differences at the parr stage are sustained in later adult life. Some otoliths taken from adult salmon that had been previously classified by other methods were examined. The results were as follows:

Origin	n	Wild#	Hatchery#
Wild	9	7	2
Hatchery	14	1	13

# denotes specimens reclassified according to Hindar and L'Abée-Lund (in prep.).

Further studies using adult specimens of known origin may illustrate whether the technique can be used to distinguish adult salmon derived from wild or hatchery populations.

4. SCALE PATTERN RECOGNITION

Salmon scales are often used in age and growth studies because they reflect growth at the different life stages of fish (e.g. Dahl, 1910; Tesch, 1968). The growth patterns of salmon differ between stocks, and detailed scale patterns have been used to distinguish between different groups of salmon, e.g. to separate salmon of European and North American origin in the high seas salmon fishery at West Greenland (Reddin, 1986). In hatcheries and salmon farms, fish are manipulated to obtain good growth which may result in growth patterns differing from those of wild fish. However, identification of escapes or releases of reared Atlantic salmon so far have been done in a qualitative manner rather than being based on quantitative definitions of the characters used. In the Baltic Sea a visual method based on different growth patterns during the freshwater stage in wild and

reared fish is in use in an international program to estimate the proportion of wild salmom in commercial catches. The scale features in this matter are (1) more distinct annuli in wild fish, and (2) more noumerous, more broken and widely spaced circuli in reared fish. Reliability tests of the method of blind samples have generally shown error rates less than 10% for Northern Baltic stocks (Antere and Ikonen, 1983; Anon., 1991). No studies so far have been able to detect one single character that separates the fish categories with high presicion. Therefore, scale reading has to be based on several characters being rare in wild fish and more common on scales of reared fish. One study submitted to the meeting presented quantitative guidelines based on this approach using material from Norwegian salmon stocks of known origin. The combined use of six scale characters, having less than 5 % occurrence in wild fish, in a score system gave good separation between farmed and wild salmon. Moreover, a large proportion of ranched fish could be identified (Table 1). The characters used were:

(1) Smolt size; when back-calculated length at smolt stage was larger than 95 % of the observations on wild smolts in a particular area.

(2) Smolt age; when smolt age was outside the range of 95 % age distribution of wild fish in the area.

(3) Transition from freshwater to saltwater; when the scales showed diffuse transition from freshwater to saltwater (Figure 2 and 3).

(4) Sea winter bands; when the sea winter bands were irregularly located, e.g. backcalculated length at first sea annulus < 35 cm or back-calculated length at the second sea annulus/back-calculated length at the first sea annulus < 1.55 (Anon., 1984).

(5) Summer checks; if there was more than one summer check within the first two sea annuli.

(6) Replacement scales; if more than 15 % of the scales in a sample had been replaced during the sea stage.

The discriminative levels proposed for the various scale

characters in this study may not be valid for any part of the Atlantic salmon's area of distribution. However, this approach may be useful in several regions with character levels based on examination of scales from local wild stocks. The list of potential characters which are rare in wild fish may be extended by including other characters , e.g. circuli scores and circuli spacing within the freshwater and the sea zones or within annual zones. L'Abée-Lund and Sægrov (in press) have reported significantly higher circuli counts in the first and the second year in reared brown trout than in the corresponding wild stock. Circuli countings within the freshwater zone have also proved to have fairly high classification success in discriminant function analysis using material from wild and reared Baltic salmon (Anon., 1991).

Generally discriminant function analysis based on multiple scale characters may provide further precision in classifying samples.

Manual scale reading is to some extent a matter of subjective desicions. However, image processing of scales reduces reader subjectivity and allows further scale parameters to be tested which it may not be possible to include in manual scale reading. Pilot studies have suggested that both capacity and precision of scale reading can be improved by image processing.

5. BIOCHEMICAL AND PHYSIOLOGICAL MARKERS

Biochemical techniques of proven usefulness in distinguishing farmed and wild salmon do exist. Other possibilities have been identified but are largely untried. In general the means of discrimination rely on dietary modification in reared fish. Some are inadvertent but others are intentional and in two cases, related to flesh pigmentation. The pigments canthaxanthin and astaxanthin are fed to reared fish, but only in the sea phase of life.

5.1 Canthaxanthin

The red-orange pigment canthaxanthin (Carophyll Red) has been used widely in the past as an additive to feed supplied to salmon grown in sea-cages. Canthaxanthin is not a natural pigment in salmonids. Wild adults feeding in the sea accumulate the pigment astaxanthin instead. In the past canthaxanthin has been fed alone, or in combination with astaxanthin, but its use is now being phased out in the fish-farming industry.

Technically it is relatively easy to distinguish canthaxanthin and astaxanthin qualitatively using thin-layer chromatography (TLC) (Craik and Harvey, 1986). Samples can be screened rapidly and large scale sampling programmes can be executed. The same techniques can be used to (1) identify farmed fish of both sexes when these have been fed canthaxanthin in culture and when they still retain the pigment in their tissues, (2) identify the ova of females which have carried canthaxanthin by examining whole ova or the yolk-sac of developing embryos, and (3) identify the progeny of such females until the first few weeks of free-feeding by extracting pigment from the yolk-sac remnant, the alimentary tract, or later, from the integument.

In escapes, the detection of canthaxanthin must often be made when astaxanthin is also present, fed as a partial colourant in culture or accumulated in marine feeding. The TLC technique is qualitative but can probably detect canthaxanthin at 5 - 10% of the total pigment load. The half-time of canthaxanthin in the body is not known but canthaxanthin was still present in detectable quantities in escapes from sea-cages returning to freshwater 18 months after their escape.

The identification of canthaxanthin has proved of great value in past studies as a means of distinguishing wild and farmed fish and their progeny (Webb et al., in press). It seems likely that this will cease to be the case in future because of consumer pressure to withdraw canthaxanthin from use in aquaculture.

5.2 Astaxanthin

Astaxanthin is the main natural red colour pigment of salmonids. In recent years synthetic astaxanthin (Carophyll Pink) has been added to salmon feed to provide red flesh colouration. The pigment is fed throughout the entire seawater phase. Astaxanthin exists as three different optical isomers and the ratio of these isomers differs greatly in synthetic astaxanthin and the astaxanthin accumulated by wild salmon. Since more than 95% of the carotenoid in use, at least in Norway, is of synthetic origin, isomeric ratios in tissue are a potential means of identifying farmed salmon lost from sea-cages. The different isomers are separated by means of High Pressure Liquid Chromatography (HPLC) via camphanic acid esters (Vecchi and Müller, 1979), a method which has been widely used in studies of natural astaxanthin isomer occurrence in many marine organisms and of carotenoid metabolism in salmonids.

The isomeric content of fish flesh reflects the diet of the animal and the isomers appear to be transferred to gonadal tissues in the same relative proportions (Lura and Sægrov, in press a). Astaxanthin is thus a marker, transferred maternally to ova in the same way as canthaxanthin. Individual eggs contain enough carotenoid to perform a reliable analysis. A drawback of the method, compared to detection of canthaxanthin, is that it requires access to an HPLC system which is a rather expensive equipment and is relatively time consuming. A trained person can perform 15-30 samples a day.

Since 1987 astaxanthin has been the only carotenoid in use in the Norwegian industry, except for a period during summer 1990 when 30% of feed contained some canthaxanthin because of a shortage of astaxanthin. Astaxanthin has also been fed widely in Scottish aquaculture but usually in combination with canthaxanthin. However it is the express intent of both the Norwegian and Scottish industries to use only astaxanthin in future. It is possible that in future the industrial synthesis

of astaxanthin will exploit the synthetic capacity of genetically selected yeasts. It seems likely that the isomeric ratios produced by these methods will differ from those which result from current industrial methods. However, it is also likely that the isomeric ratios will also differ markedly from those present naturally in salmon tissues.

The use of astaxanthin isomeric ratios to identify escaped farmed fish and the females' offspring is a rather recent development. The dilutionary effect of natural astaxanthin consumption after escape has not been closely examined so far but wild salmon have very narrow ranges of isomeric ratios, suggesting that the method will prove informative. Indeed, farmed and wild fish can be distinguished readily from a knowledge of the ratios of the three isomers of astaxanthin and the ova of escaped farmed salmon differ from the ova of wild fish in the same way. This has been used to assess the spawning succes of farmed escapes in rivers in Norway (Lura and Sægrov, in press b).

5.3 Antibiotics

Antibiotic residues can be detected and will be specific to farmed fish but their usage is irregular, several compounds are used and the formulations and dosages are intended to ensure that residues should not persist. However, residues of one of the commonly used treatments, oxytetracycline, fluoresce in hard tissues, under UV light. The other antibiotics do not. It is not envisaged that examining antibiotic residues will be a useful addition to the range of techniques which will be used to distinguish wild salmon and farmed escapes.

5.4 Fatty acids

The primary production of fatty acids (FAs) differs in the freshwater and marine environments. The lipids of freshwater and marine fish reflect differences in their dietary FA profiles because fishes tend to absorb and assimilate FAs unchanged.

Indeed it has been shown experimentally that eels for instance, will assume the FA profiles characteristic of herring, if herring are their diet (Lovern, 1964). The FA profile of salmon varies with diet in the same way (Viga and Grahl-Nielsen, 1990). Since marine and freshwater FA profiles differ and since fish accumulate FAs unchanged, it would be expected that cultured parr in freshwater, fed a diet compounded of marine species, would differ from wild parr in a predictable manner. The differences might be maintained for some time after escape to freshwater or transfer to the sea. However, the rapid turn-over of lipids and growth itself would be expected to erode differences quickly, especially if the initial differences were small. Moreover, even in wild salmon FA profiles are inconstant because of differential mobilisation or utilisation of FAs in times of negative energy balance. The same effects must be considered in relation to the FA profile of the "prey" species from which commercial diets are formulated. The use of FA profiles to distinguish farmed and wild fish is untested but unlikely to prove useful.

5.5 Minerals

Mineralisation of structural tissues may differ in wild and cultured fish. This may reflect differences in the relative contents of minerals in fresh and seawater (Mg particularly) or because of the proximity of metal structures (e.g. galvanised metal, copper and brass fittings, anti-foulant paints) in aquaculture. The use of relative mineral content as a possible discriminant of wild and farmed fish will be dependent on the retention times of specific minerals in tissues. Possible means of discriminating wild fish and escapes from farms are untested and the techniques found necessary will almost certainly be expensive and time-consuming.

6. **GENETIC MARKERS**

6.1 Rationale

We define a genetic marker as an inherited character with a simple mode of inheritance, with little or no effect on reproduction and survival of its carrier, and which can be identified in parents and offspring by available methods.

The value of genetic markers as opposed to other marks or tags is that they are transmitted to subsequent generations, all size groups from eggs to adults can be marked, there is no loss of marks, and there is no differential mortality resulting from the mark itself or from handling of the fish. Although they may be used to identify individual fish in special cases, their primary use is not identification, but rather the estimation of gene flow from the marked group (e.g. farmed escapes) to wild populations. Here we consider four types of genetic markers (1) morphological-genetic markers, (2) biochemical-genetic markers or isozymes, (3) DNA-fragments, and (4) cytological or chromosomal markers. In addition, immunogenetic methods which were abandoned in the early 1970s as other techniques became available, might prove useful in species where immunogenetic variation exists in spite of low levels of genetic variation detected by enzyme electrophoresis (Utter and Seeb, 1990). We are however not aware of any immunogenetic variation that can presently be used for genetic marking of salmonids.

The different types of genetic markers have somewhat different areas of application. Morphological-genetic markers and biochemical-genetic markers are generally used at the population level, while DNA fragments give a higher resolution and may prove efficient for identification of families and individuals. Cytological markers are normally used at the sub-species level and above.

6.2 Methods

1. Morphological-genetic markers are those alleles that have a major morphological effect on a trait which is not affected by the environment (Kirpichnikov, 1981). An example of a

morphological-genetic marker is the fine-spottedness of brown trout (Skaala et. al, 1990).

2. Biochemical-genetic markers are those detected by enzyme electrophoresis, which is a technique to separate and stain for proteins encoded by specific genes (Hedgecock et al., 1976; Allendorf and Utter, 1979). By electrophoresis, information can be drawn from many genes (some of which are polymorphic) in large samples of fish with relative ease (see Utter et al., 1987). Many species of fish have been well characterized by this technique, and baseline data on Atlantic salmon are now rapidly accumulating from throughout the species' range.

3. Nuclear DNA variation represents the ultimate genetic information. However, the techniques for obtaining this information are laborious and expensive, and very little is known about nuclear DNA variation in salmonids.

Extranuclear DNA refers to the genetic information in ribosomes (rDNA) and mitochondria (mtDNA). Especially the latter are useful for genetic analyses, because the DNA content of the mitochondria is limited (and therefore more easily analysed than nuclear DNA) and mtDNA is strictly maternally inherited (Ferris and Berg, 1987).

4. Cytological genetic methods are routinously used for ascertaining the number of chromosomes in a particular species (Hartley, 1987). It is also possible with special staining techniques to produce special banding patterns of the chromosomes. The usefulness of these techniques is at present somewhat limited by the difficulties of identification of all the chromosomes, and by the observation that the chromosome number may show intra-individual variation.

6.3 Genetic variation in Atlantic salmon

The morphological variation of Atlantic salmon is considerable

both between and within drainages, but all the morphological characters that we are aware of are influenced by the environment to the extent that they are not useful as genetic markers. The same conclusion holds true for population-specific differences in ecological traits and traits related to disease resistance. Enzyme electrophoresis has provided the most extensive data set for evaluating genetic variation in Atlantic salmon. Ståhl (1987) analysed 29 samples of natural populations and 24 of hatchery stocks from most of the species' range. The level of genetic variation is in the lower half of that found in fish species, with an average expected heterozygosity of about 4%. Considerable genetic differences exist between regions (i.e. eastern North America, European drainages to the Atlantic, and the Baltic), and significant genetic differences exist between Atlantic salmon from different rivers within each region, as well as between samples from various parts of the same river (Ståhl, 1987).

The differences detected by electrophoresis refer to differences in frequencies of particular alleles, and not to presence (other than at very low frequencies) or absence of those alleles. Therefore, there is no possibility of identifying an individual to its source population, but a potential exists for using the allele frequency variation in order to produce cultured stocks that are genetically divergent from the wild ones.

Some data are available for comparisons of the genetic variation in farmed and wild Atlantic salmon in Norway (Ståhl, 1987, Ståhl and Hindar, 1988) and Scotland (Youngson et al., 1989 and in press). These data are summarized in Table 2, and clearly show the large overlap in allele frequencies of the respective groups.

Nuclear DNA variation has been detected in a limited number of Atlantic salmon by the application of the so-called "DNA fingerprinting" technique, both by hybridization to multiple-locus and single-locus probes (Fields et al., 1989; Taggart and Ferguson, 1990). These techniques will most probably prove very useful in future experimental studies of reproductive success.

Mitochondrial DNA variation has been detected within and between populations of Atlantic salmon, but the number of fish studied so far is somewhat limited (Gyllensten and Wilson, 1987; Hovey et al., 1989; and references therein). The available evidence suggests that considerable variation in frequencies of mtDNA fragment patterns exists between North American and European populations of Atlantic salmon, and between salmon from Baltic and Atlantic drainages in Europe. However, as with enzyme electrophoresis, it is not possible to assign individuals to populations by this technique, and the use of mtDNA variation may prove most successful in experimental situations.

Cytological variation exists in Atlantic salmon both in the number of chromosomes (2n=54-60) and in the number of chromosome arms (NF=72-74) (Hartley, 1987; Garcia-Vazquez et al., 1988). The available data are limited but suggest that variation exists between North American and European populations in the number of chromosome arms, and possibly (but with considerable overlap) between northern and southern European populations in the number of chromosomes. On a more limited geographical scale, Hartley (1988) concluded that Scottish populations of Atlantic salmon are cytogenetically homogeneous.

6.4 Application of genetic markers

6.4.1 Identification of individuals and groups

On the above background, it appears that classification of individual Atlantic salmon to its natural population, or to a farmed or wild background, is not possible. It is possible to identify individual Atlantic salmon to the correct continent with very high precision using isozymes and other markers (Verspoor and Reddin, 1989), and consequently also possible to identify any farmed fish that were transplanted between continents. It may also be possible to find Scottish or Norwegian salmon released in Spain (Garcia de Leaniz et al., 1989) or Baltic salmon released to Northern Norway (Ståhl and Hindar,

1988), but with a more limited precision.

In the future, we may expect to be able to identify transgenic fish, where non-salmon specific gene sequences have been inserted into the genome. Also, triploid fish can be identified with flow cytometry by their higher DNA content in the cells (Allen, 1983).

By and large, the best we can achieve at present without producing genetically marked groups of Atlantic salmon, is to identify groups of salmon having different allele frequencies in one or more genes. It should be noted that these genetic differences must be quite large in order to detect hybridisation between the different groups in subsequent generations (Allendorf and Ryman, 1987).

6.4.2 Estimation of reproductive success

One of the reasons for concern about escaped farmed fish, is the potential negative genetic effects they may have on wild populations. Therefore, ideally all farmed fish (and other farmed organisms) should carry genetic marks, making it possible to study the possible long term effects on the genetic variability of wild fish. For practical reasons it is not likely, however, that genetic marks will be used on such a large scale. Being aware of the fact that the use of genetic marks is the only way to achieve information about the reproductive success of farmed individuals, and introgression, we therefore regard it as extremely important to incorporate genetic marks in studies of interactions between farmed and wild populations on a more local scale. The empirical information about the genetic impact from farmed fish on wild populations that can be gained in a set of local experiments can be extrapolated to other cases. Further, as the problem of interaction between farmed and wild populations is not limited to Atlantic salmon, but may take place in all cases where farmed organisms escape or are released, there will also be a lot of information relevant to Atlantic salmon from similar studies on related fish species and other organisms.

In the Fisheries Laboratory at Lowestoft, U.K., interactions between cultured and wild salmon are studied using tagged and genetically typed fish. The DAFS Marine Laboratory in Aberdeen will assess the genetic consequences of farmed salmon for the native Polla stock over the next years if they succeed in finding a suitable regionally based genetic marker. The intention is further to compare performance of genetically tagged wild and farmed juvenile salmon in a natural stream. A mtDNA marker has been identified which appears to be useful in studying genetic input from Norwegian farmed salmon into Scottish populations.

In the Fisheries Research Laboratory, Department of Agriculture for Northern Ireland, detailed inheritance studies have now been conducted on a number of allozymes. Some of these, for instance the variants described in AAT-3, IDH-3, SDH-2, and probably PGI-3 and MDH-3,4, confirmed a Mendelian inheritance pattern, and have a potential as genetic markers (Crozier and Moffett, 1990). Mendelian inheritance was not verified in PGM-2, and the commonly observed variants at the ME-2 locus may not be useful as genetic markers as their variation may be directly or indirectly associated with fitness (Jordan et al., 1990).

6.5 Relevant experience from other species

In other fish species than Atlantic salmon genetic markers have been used in studies on interaction between wild and introduced stocks. The results from these studies show a varying degree of reproductive success in the introduced stocks and in genetic impact on wild stocks from the introduced stocks (Kreuger and Mentzel, 1979; Murphy and Nielsen, 1983; Campton and Johnston, 1985; Carmichael et al., 1986; Chilcote et al., 1986; Seeb et al., 1986; Taggart and Ferguson, 1986; Altukhov and Salmenkova, 1987; Leider et al., 1990). The information from such studies must be extracted, so that the factors of importance for the varying degree of genetic impact from introduced or farmed stocks can be identified.

7. CYTOLOGICAL MARKERS

The triploidisation of salmonids in aquaculture is widespread. triploid females do not become sexually mature. Males do become mature however, (although they are sterile) and methods have been developed to render stock for aquaculture all-female before making them triploid by exposing eggs to heat or pressure shock. The use of triploid female stock has some advantages for salmon farmers and the technique is already in use. In time, its use may become more widespread. Natural triploids are rare.

This document addresses methods for identifying farmed escapes among wild salmon with a view to answering specific questions which have bee raised. The general use of female triploid salmon would, in itself, alleviate many of the potential problems. But for the purpose of fisheries evaluation at least, triploid salmon can be identified reliably and with ease, using a blood sample, because of their greater cell nuclear size. The mark is life-long.

8. LARGE-SCALE GROUP MARKING OF SALMON IN FARMS

The large-scale group marking of farmed and ranched salmon is particularly desirable in order to identify and assess the number of escaped fish and their interaction with natural populations. The ideal situation is that all farmed salmon should be marked so that they could be recognized in fisheries and spawning populations and separated from the wild brood fish in stock enhancement programmes. However, several problems arise when marking large numbers of salmon. These are of practical, economical and biological nature. It is of great importance that the marks easily can be dectected during the entire life span of the fish.

8.1 External marks

Several techniques are in current use in fisheries science. The

method probably the most applicable to farmed fish is the removal of the adipose fin. This method is labour consuming and poses stress to the fish. Furthermore, it will also interact with coded wire tagging programmes where the adipose fin clip is reserved for tagged individuals as recommended by ICES. Mechanical tags and tattooing techniques are thought to be of limited interest due to amount of labour required and the difficulties in detection respectively.

8.2 Internal marks

The coded wire tag (CWT) and the passive integrated transponder (PIT tag) are the main internal tags used. Both methods would also require an external mark, e.g. an adipose fin clip. PIT tags are very expensive and of limited value for large-scale group marking. The CWT tag is much cheaper, but the fish has to be sacrificed. Furthermore, reading CWT tags is very labour consuming.

8.3. Biological marks

The rearing procedure in hatcheries and fish farms results in several differences between reared and wild fish. The morphology and scale pattern of the groups differ, and this method is now in current use. However, these methods underestimate the proportion of reared salmon, especially as a relatively large part of salmon escaping at an early stage can not be identified. Data presented to the workshop suggested that there were differences between wild and reared salmon in the transparency of the otoliths, and that this may provide a useful tool in discriminating between the groups. However, further testing is required. If the use of triploid female salmon in culture increases, their identification will be informative in the context of stock assessment. The mark is life-long.

8.4 Chemical marks

Commercial salmon feed contains artificial colouration such as canthaxanthin and astaxanthin. These pigments remain in salmon for a long time, and are detected by chemical analysis. They can also be detected in the eggs and alevins. These artificial pigments may be useful in mass-marking salmon in farms.

8.5 Genetic marks

In some cases the natural differences in allele frequencies are large enough to function as genetic markers, but in most cases a genetically marked broodstock has to be developed through breeding of rare heterozygotes. The resulting homozygotes will then be used as parents for the production of genetically marked homozygous individuals. This protocol can be followed without altering the performance of the cultured stock (Parker et al., in press), provided that the appropriate recommendations (e.g. for minimizing inbreeding) are followed. This method will have special relevance for farmed fish in certain defined geographical areas where it is important to monitor the genetic characteristics of valuable wild populations more closely.

9. CONCLUSIONS AND RECOMMENDATIONS

Genetic marks or tissue pigments are the only way to achieve information about the reproductive success of farmed individuals. Investigating subsequent genetic introgression is totally dependent on genetic techniques. We therefore regard it as extremely important to use genetic marks particularly, in experimental studies of interactions between farmed and wild populations. Empirical information about the genetic impact from farmed fish on wild populations can be gained in a set of local experiments, and this information can be extrapolated to other cases. The problem of interaction between farmed and wild populations is not limited to Atlantic salmon, but may occur in all cases where farmed organisms escape or are released.

Accordingly there is some information relevant to Atlantic salmon from similar studies on other fish species. The application of genetic marks in studies on interactions between farmed and wild stocks of salmon as well as other species is strongly encouraged.

1. Having considered all the methods currently available to distinguish wild salmon from farmed escapes, the Workshop considers that morphological characters, scale characters and otolith structure, used alone or in combination, are the least expensive and most discriminatory methods available. The Workshop notes that these methods will almost certainly underestimate the true members of escaped farmed fish in mixed groups of farmed and wild salmon. It is recommended that these methods continue to be used as widely as possible and that the methods continue to be developed. In particular the Workshop recommends that automated, passive methods for scale analysis be developed to reduce subjectivity of interpretation and to speed analysis. Particular attention should be given to achieving discrimination between wild fish and escapes or releases made early in freshwater life. The Workshop also recognises a requirement for the development and use of regional and local keys to scale characters since these vary markedly in relation to environment and stock.

2. In some circumstances, tissue pigment analysis is diagnostic for farmed escapes. The technique can be used to support non-chemical methods. It is of particular use in some situations for distinguishing the progeny of escaped farmed females (but not males) from those of wild females, although only through the egg stage and for the first few weeks of free swimming life. The Workshop recommends that these methods continue to be developed because of their special usefulness and power. In particular HPLC methods for detecting the isomers of astaxanthin should be further developed. Using available methods the kinetics of the isomers, in relation to time, growth, assimilation and transfer between tissues should be fully explored. The intended withdrawal of canthaxanthin as a feed pigment makes development of methods for examining isomeric ratios of astaxanthin of special

importance.

3. In some circumstances, fortuitously or by experimental design, genetic methods may be used to distinguish wild salmon from farmed escapes. In exceptional circumstances only, existing genetic methods may be sensitive enough to follow the progeny of wild and farmed fish by examining the introgression of genes (rather than fish) from farmed escapes of both sexes into natural populations. In the same way, the subsequent spread of the same genes within and between populations and their propagation through later generations might be assessed. The Workshop recommends that genetic methods continue to be developed with a particular view to following gene flow in natural populations exposed to farmed escapes because of the potential, biological importance of gene flow for natural populations. The Workshop also recommends further development of genetic tags, because of their special power, for use locally in the field or in experimental situations.

4. The Workshop understands that canthaxanthin and astaxanthin are used widely as colourants in the food industry. In the past, both colourants have been widely used in aquaculture. The Workshop is aware of consumer pressure on aquaculture to cease usage of canthaxanthin and of the intention of the industry to comply. The Workshop is not aware of the scientific basis on which synthetic astaxanthin is preferred to canthaxanthin but expects the use of synthetic astaxanthin alone, to become general in fish-farming. However the Workshop is aware of the advantages to fisheries management which would follow from the continued inclusion of canthaxanthin in fish-feeds by all feed compounders, at low levels and in combination with synthetic astaxanthin which will continue to be used. The Workshop recommends that the advantages for fishery managers of the continued use of canthaxanthin should be noted and reserved.

10. WORKING DOCUMENTS

1. Crozier, W.W. The identification of fish farm escapees in N. Ireland fisheries: Methods currently in use and future development of techniques.

2. Hindar, K. and J.H. L'Abée-Lund. Examination of otoliths can distinguish between hatchery-produced and wild Atlantic salmon parr.

3. Lund, R.A. and L.P. Hansen. Identification of wild and reared Atlantic salmon (Salmo salar) using scale characters.

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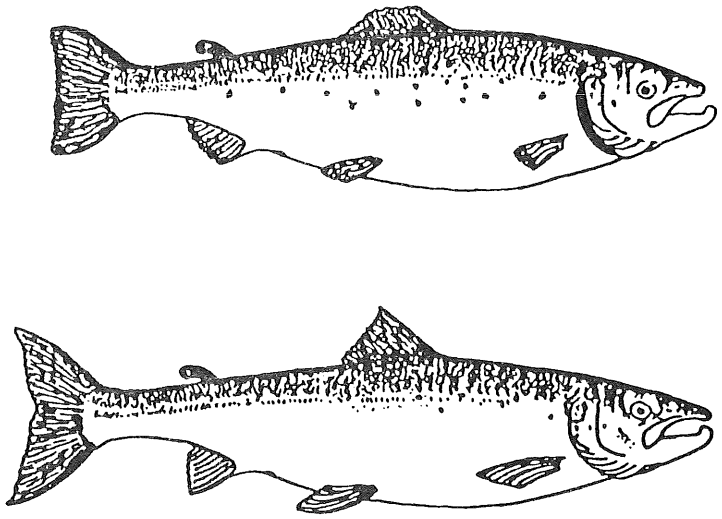

Fig. 1. Diagammatic drawing of an adult farmed salmon bearing morphological characters described in the text (A). A wild adult salmon is also shown (B).

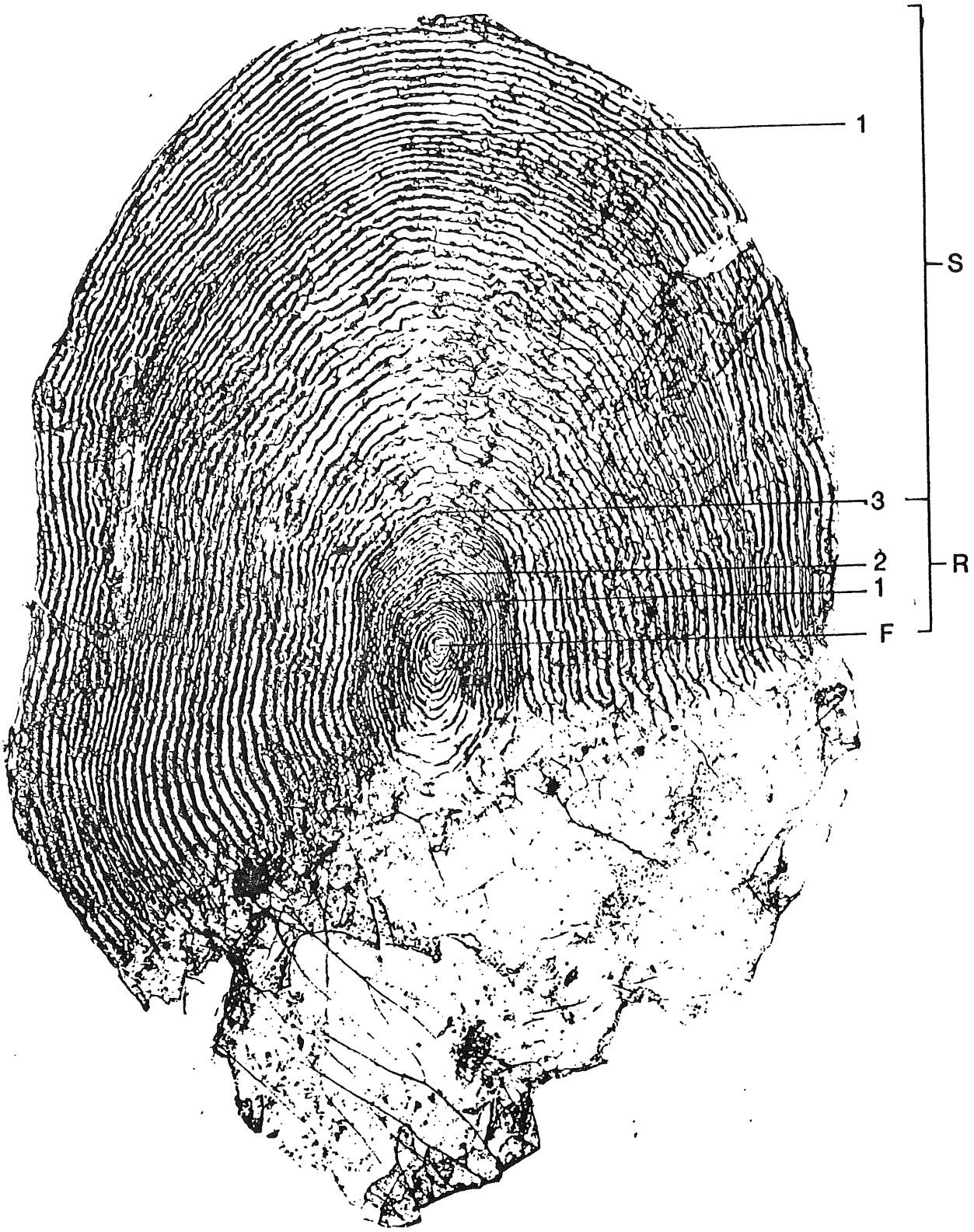

Fig. 2. A scale from a wild salmon caught in a marine fishery in Mid-Norway in July 1979. The scale is aged 3.1+ and has a clear transition between the freshwater and saltwater zones and distinct annuli in both zones. F = scale focus; R = freshwater zones; S = sea zone.

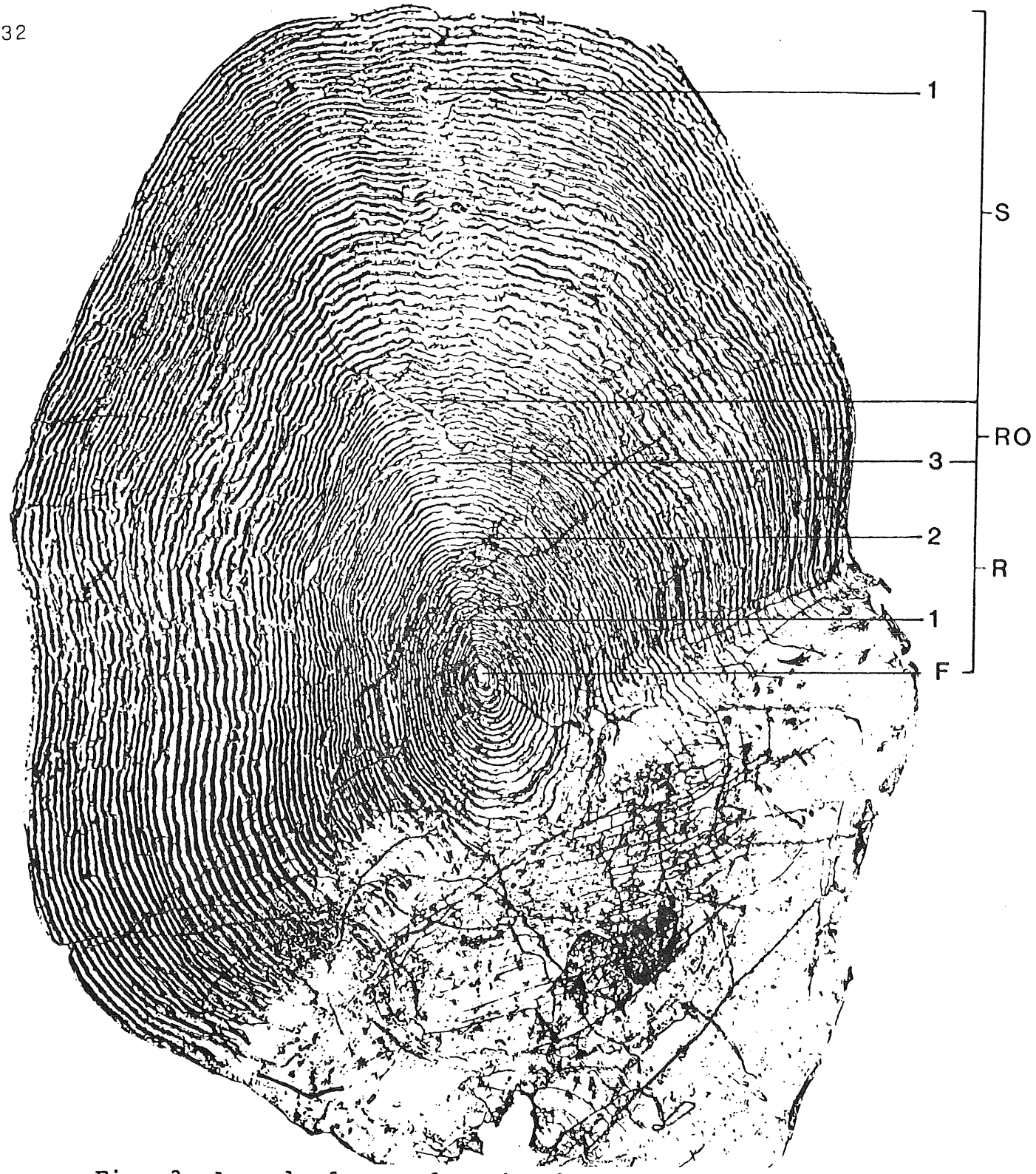

Fig. 3. A scale from a farmed salmon with the known age 1.1+. The scale has a diffuse transition between the freshwater and the seawater zones, and the age of the fish is read to 3.1+. F = scale focus; R = freshwater zones; S = sea zones; RO = runout.

Table 1. Proportion of salmon with presence of 0-6 characters on their sacales. N = number of salmon examined.

Fish category	N	0	1	2	3	4	5	6
Farmed	172	0.0	2.9	30.8	41.3	22.1	2.9	0.0
Ranched	92	14.1	30.4	41.3	10.9	3.3	0.0	0.0
Wild (mixed stocks)	244	75.0	23.4	1.2	0.4	0.0	0.0	0.0