Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2016: 67

Risk assessment of marking and tracing methods with regards to the welfare of farmed salmonids

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2016: 67
Risk assessment of marking and tracing methods with regards to the welfare of farmed salmonids

Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety
15.12.2016

ISBN: 978-82-8259-257-4
Norwegian Scientific Committee for Food Safety (VKM)
Po 4404 Nydalen
N – 0403 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

www.vkm.no
www.english.vkm.no

Cover photo: Espen Bierud, Havforskningsinstituttet

Suggested citation: VKM. (2016) Risk assessment of marking and tracing methods with regards to the welfare of farmed salmonids. Opinion of the Panel on Animal Health and Welfare, ISBN: 978-82-8259-257-4, Oslo, Norway.

Risk assessment of marking and tracing methods with regards to the welfare of farmed salmonids

Authors preparing the draft opinion

Stein Mortensen (chair), Tore Kristiansen, Cecilie Mejdell, Ingebrigt Uglem, Angelika Agdestein (VKM staff), Dean Basic (VKM staff)

(Authors in alphabetical order after chair of the working group)

Assessed and approved

The opinion has been assessed and approved by Panel on Animal Health and Welfare. Members of the panel are: Brit Hjeltnes (chair), Øivind Bergh, Edgar Brun, Knut Egil Bøe, Carlos Goncalo Afonso Rolhas Fernandes das Neves, Jacques Godfroid, Roar Gudding, Kristian Hoel, Cecilie Mejdell, Stein Mortensen, and Espen Rimstad

(Panel members in alphabetical order after chair of the panel)

Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority. Project leaders from the VKM secretariat has been Angelika Agdestein, Dean Basic and Tron Gifstad. The members of the working group Stein Mortensen (Panel on Animal Health and Welfare), Tore Kristiansen (Institute of Marine Research, Havforskningsinstituttet), Cecilie Mejdell (Panel on Animal Health and Welfare) and Ingebrigt Uglem (Norwegian Institute for Nature Research, NINA) are acknowledged for their valuable work on this opinion. The Panel on Animal Health and Welfare is acknowledged for comments and views on this opinion. VKM would like to thank the hearing experts Kevin Glover, Per Gunnar Fjelldal, and Vidar Wennevik (all from the Institute of Marine Research, Havforskningsinstituttet) for their contributions to sections 1.6.2, 1.5.6 and 1.4, respectively. VKM would also like to thank Lucy Robertson for proofreading the manuscript.

Competence of VKM experts

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

Table of Contents

Summary

The Norwegian Food Safety Authority (NFSA) asked the Norwegian Scientific Committee for Food Safety (VKM) for an opinion of risks of reduced welfare implications associated with the different marking and tracing methods, and combinations thereof, for farmed salmonid fish, restricted to Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*) in Norway. A working group was established comprising members from the Panel on Animal Health and Welfare and external experts from the Institute of Marine Research, the Norwegian Institute of Nature Research, and VKM staff. The Panel on Animal Health and Welfare has reviewed and revised the draft prepared by the working group and has approved the opinion.

There are environmental concerns of escaped farmed salmon interbreeding with wild fish, potentially threatening genetic integrity, and transmission of diseases. The Norwegian government intends to prevent or reduce escapes of farmed salmonids from occurring, and wishes to have those farmed fish that have escaped removed from the environment. In order to facilitate these actions, it is essential to be able to identify escaped fish. In addition, reliable methods for tracing the origin of the escaped fish are also crucial. Mandatory marking of all farmed fish in Norwegian aquaculture has therefore been suggested. On this basis, this VKM report will be used by the NFSA to evaluate which marking methods are most suitable, from both short- and long-term perspectives, in relation to the Animal Welfare Act.

In order to fulfil the requirements, the marking must enable visual identification of escaped fish and also enable an individual fish to be traced back to its origin. There is no single marking system that fulfils both these criteria.

A variety of marking and tracing methods are available for mass marking of farmed fish. These methods differ with regard to their suitability for actually distinguishing wild from farmed (escaped) fish in the field. External marks may be lost or fade over time. Morphology will often differ between wild and farmed fish. However, the differences are often small if the fish has escaped early in the production cycle. Reliable determination of origin, based on morphological variation, requires experience and handling of the fish. Adipose fin removal is the only definitely visible and 100 % permanent marking method, as the adipose fin does not regenerate.

Marking is commonly done by attaching a tag, either externally on the surface of the fish, in tissue, or internally in the body. All marking procedures involve handling fish; this is stressful to the fish, and/or may induce pain. All marking will therefore have an impact on fish welfare. With most marking methods, the risk of reduced fish welfare decreases with time.

Tracing methods differ with regard to their suitability for being used to trace the marked fish back to its origin, either on an individual level (a mark that identifies each fish) or the farm level (a mark that identifies each farm).

In order to visually identify escaped fish and enable tracing back to the farm of origin, a combination of both marking and a tracing method, with sufficient number of available codes, is necessary. Visible marking methods, such as adipose fin removal, VIA tags, VIE tags, freeze branding, or injection of pigments may be used for identifying the fish. These can be combined with tracing using natural marks or the use of CW tags or PIT tags that, with varying reliability, may enable both identification of a fish on an individual level or batch

level and from where it originated. Chemical marking along with all types of natural marks (i.e. scales, otoliths, biochemical or genetic composition in tissue) was evaluated as representing the lowest risk of reduced fish welfare. However, these marks require analysis after catch and sampling, in order to determine whether the fish is wild or farmed. All other marking methods presented in this report represent a *high risk* of reduced fish welfare during or shortly after marking. This risk is reduced to *moderate* for most of the methods on a long-term scale. Spraying of pigments and most externally attached tags remain a *high risk* of reduced welfare, on both short-term and long-term scales.

VKM concluded that there are no combinations of marking and tracing methods that are feasible without an increased risk of reduced animal welfare.

VKM also recognizes a number of uncertainties and data gaps related to how and to what extent the different marking methods affect may fish welfare. For example, the functional role of the adipose fin is still unclear, making evaluation difficult concerning how fin clipping affects fish long term. It must also be emphasized that, regardless of the method used to tag fish, there will always be higher risks of reduced fish welfare associated with large-scale marking as opposed to small-scale marking. VKM therefore highlights the need for more scientific documentation and suggests that marking methods should be tested in large-scale trials.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food Safety, marking methods, surgery, injection, natural marks, animal welfare, salmonid fish, tracing, fish farming, escapes

Sammendrag på norsk

Mattilsynet har bedt Vitenskapskomiteen for mattrygghet (VKM) om en vurdering av risiko for svekket dyrevelferd ved bruk av ulike metoder for merking og sporing, samt kombinasjoner av disse, for oppdrettsfisk. Oppdraget er begrenset til atlantisk laks (*Salmo salar*) og regnbueørret (*Oncorhynchus mykiss*) i Norge. Det ble nedsatt en arbeidsgruppe bestående av medlemmer fra VKMs faggruppe for dyrehelse og dyrevelferd, eksterne eksperter fra Havforskningsinstituttet og Norsk institutt for naturforskning samt fra VKMs sekretariat. Faggruppe for dyrehelse og dyrevelferd har gjennomgått og revidert et utkast og godkjent den endelige risikovurderingen.

Rømt oppdrettsfisk er et alvorlig miljøproblem. Oppdrettsfisk kan blande seg genetisk med villfisk og derved true det genetiske mangfoldet i ville bestander. Rømt fisk kan også spre sykdommer. Norske forvaltningsmyndigheter har som mål å forebygge rømming og fjerne rømt oppdrettsfisk fra hav og elver. For å kunne nå målene er det avgjørende å kunne skille vill og oppdrettet fisk fra hverandre, og ha metoder som gjør det mulig å spore den rømte fisken tilbake til oppdrettsanlegget den kom fra. Det har derfor vært diskutert om det skal bli obligatorisk å merke all oppdrettsfisk. VKMs rapport vil danne bakgrunn for Mattilsynet når det skal ta stilling til hvilke merke- og sporingsmetoder som er mest anvendelige – både i forhold til kort- og langtidseffekter, sett i relasjon til Dyrevelferdsloven.

For å kunne nå myndighetenes mål om merking og sporing må det både være mulig å identifisere rømt fisk visuelt, og være mulig å spore fisken tilbake til oppdrettsanlegget den kom fra. Det finnes i dag ingen merkemetode som tilfredsstiller begge kravene.

Det finnes flere metoder for merking og sporing av store grupper av oppdrettsfisk. Hvor anvendelige metodene er, varierer med hensyn på om de er egnet til å skille vill og oppdrettet fisk fra hverandre i naturen. Utvendige merker på fisken kan gå tapt eller bli usynlige over tid. Bruk av forskjeller i utseende mellom villfisk og oppdrettsfisk (f.eks. finneslitasje) for å identifisere rømt fisk vil være unøyaktig for fisk som rømte i en tidlig fase, og pålitelig identifisering på bakgrunn av morfologisk variasjon krever erfaring og innebærer at fisken må håndteres. Fettfinneklipping vurderes som den eneste utvendige merkemetoden som er helt tydelig og varig, ettersom fettfinnen ikke vokser ut igjen.

Merking kan gjøres ved at det festes et merke på fisken, eller at det injiseres et merke enten i vev eller ved implantering i bukhulen. Alle merkemetoder medfører at fisken må håndteres. Håndteringen vil i ulik grad stresse fisken og/eller forårsake smerte. Alle merkemetoder vil derved ha en effekt på fiskens velferd. Ved de fleste merkemetoder vil risiko for redusert fiskevelferd reduseres over tid.

Sporingsmetodene har ulik anvendelighet, med hensyn på i hvilken grad de kan brukes til å spore fisken tilbake til oppdrettsselskap eller anlegg, eller kunne identifisere fisken på individnivå.

For å visuelt kunne identifisere rømt fisk og spore disse tilbake til oppdrettsanlegget de kom fra, kan det være nødvendig å kombinere en merkemetode med en sporingsmetode som har det nødvendige antall tilgjengelige koder for hver gruppe eller individ. Synlige merkemetoder som fettfinnefjerning, VIA-merker (øyemerker), VIE-merker, frysemerking eller injeksjon av pigmenter kan kombineres med identifisering av naturlige merker eller bruk av CW tags (snutemerker) eller PIT-merker som med varierende nøyaktighet kan brukes til å spore fisken tilbake til anlegg eller utsettsgruppe. Kjemisk merking og ulike naturlige merker, som skjell, otolitter, biokjemiske eller genetiske markører i vev, er vurdert å gi lavest risiko for redusert fiskevelferd, men krever prøvetaking og påfølgende analyse for å kunne avgjøre om fisken er vill eller oppdrettet etter fangst. Alle andre merkemetoder som er inkludert i denne rapporten, er vurdert å medføre *høy* risiko for redusert fiskevelferd under eller like etter merkingen. Risikoen er redusert til *moderat* for de fleste metodene når effektene vurderes i et lengre tidsperspektiv. Spraying av pigment og de fleste eksterne merker gir en *høy* risiko for redusert velferd, både i et kort- og langtidsperspektiv.

VKMs konklusjon er at det ikke finnes kombinasjoner av merke- og sporingsmetoder som ikke medfører økt risiko for redusert fiskevelferd.

VKM har identifisert en rekke usikkerheter og kunnskapshull knyttet til hvordan ulike merkemetoder påvirker fiskens velferd. For eksempel er det fremdeles uklart hvilken funksjonell rolle fettfinnen spiller. Dette gjør det vanskelig å evaluere langtidseffektene av fettfinneklipping. Det må også understrekes at det - uavhengig av hvilken metode som benyttes - alltid vil være høyere risiko for redusert fiskevelferd ved storskala merking enn ved merking av mindre grupper. VKM understreker derfor at det er viktig å fremskaffe mer vitenskapelig dokumentasjon på utprøving av merkemetoder i stor skala for å kunne evaluere om og i hvilken grad ulike merkemetoder påvirker fiskevelferden.

Abbreviations and glossary

Abbreviations

BKD = Bacterial kidney disease

C&R = Catch and release

CNS = Central nervous system

CWT = Coded Wired Tag/Coded wire tagged

FA = Fatty acid

IMR = Institute of Marine Research

LA-ICP-MS = Laser ablation inductively coupled plasma mass spectrometry

NFD = The Ministry of Trade, Industry and Fisheries

NFSA = The Norwegian Food Safety Authority

NINA = Norwegian Institute of Nature Research

NMT = Northern Marine Technology

PIT = Passive integrated transponder

VIE =Visible implant elastomer

VIA = Visible Implant Alphanumeric

VKM = The Norwegian Scientific Committee for Food Safety

Glossary

Welfare = the quality of life experienced, integrated over the whole or a defined period of life.

Welfare needs = requirements as perceived by the animals, i.e. as monitored by animals' emotional/affective systems.

Welfare indicators = observable, measurable attributes of an animal and/or its environment (e.g., water quality parameters) that are correlated with the focal animal's welfare.

Animal-based welfare indicators = welfare indicators based on observations of animals themselves, ranging from observations of health and morphology, behavior, and performance, to physiological/biochemical samples. Also called outcome measures.

Resource-based welfare indicators = welfare indicators based on observations of environmental conditions and available resources (e.g., water quality, food availability, and safety). Management-based indicators (e.g., feeding system) are included or may sometimes appear as a third category. Also called input measures.

Operational welfare indicators = welfare indicators that are feasible to use to assess welfare on full-scale/commercial fish farms.

Welfare assessment protocol = a collection of parameters/measurement points, usually including both resource-based and animal-based indicators (covering health, behaviour and/or physiology), and tailored to the specific assessment task.

Overall welfare assessment = making a science-based evaluation of the welfare of a selected group of animals using a set of animal-based and resource-based welfare indicators, with the intention of covering all of the animals' welfare needs.

Background as provided by the Norwegian Food Safety Authority

Escaped farmed salmon is a serious environmental problem because it can interfere genetically with wild fish in rivers. Genetic changes in wild Atlantic salmon populations, as a result of interbreeding between wild and farmed conspecifics have been well documented. It is generally accepted that farm escapees represent a significant threat to the genetic integrity, and the long-term evolutionary capacity of recipient wild salmon populations. In order to reduce the number of escapees, there is a need for robust methods to trace escapees back to their origin. This requires both catch strategies, marking and tracing methods.

The government administration's goal is primarily to prevent escapes, but also to remove as much as possible of the escapees. In this context, it is important to find out where the fish have escaped from. Therefore, it is a political desire to consider tagging all farmed fish, cf. Stortingsmelding nr. 12 (2001-2002). Tagging of farmed salmon will make it possible to differentiate wild fish from escaped farmed fish and at the same time could trace escaped fish to the farm it came from.

The Norwegian Government wants to put into practice the principle that the fish farm that is responsible for the escape is also responsible for covering the expenses of cleanup/fishing escapees. The Ministry of Trade, Industry and Fisheries (NFD) points out that today it is not possible to implement this because farmed fish are not marked or traceable. On this basis, shall The Norwegian Food Safety Authority (NFSA) survey tagging and tracing methods for fish and assess whether they are tested and found suitable with regard to animal welfare.

Current legislation

The aquaculture industry and investigation organizations have in recent years developed new methods for marking and tracing farmed fish. The NFSA is uncertain whether these methods are tested and found suitable with regard to animal welfare, cf. § 8 of the Act of 19 June 2009 No. 97 of Animal Welfare (Animal Welfare Act). In addition, whoever is marketing or selling new methods, equipment and technical solutions for use on animals or livestock shall ensure that these are tested and found suitable with regard to animal welfare.

Paragraph 8 in the Animal Welfare Act implies that the person using methods and equipment, and who markets and sells new methods and equipment, is responsible for ensuring that they are justifiable for use. It must also be considered to what extent such testing of new methods application is required in accordance with the Regulations of 15th of January 1996 nr. 23 on experimental animals.

The Animal Welfare Act allows marking of animals so long it is performed in a justifiable way in relation to animal welfare, cf. § 9. The provision includes methods for examination and diagnosis, vaccinations, drug therapy, surgery and other therapeutic methods. Surgery is defined here as treatment that involves perforation of the skin or mucosa, as well as crushing of tissues. The term includes as well acupuncture and injections, and other perforation of skin associated with vaccination, sampling and administration of drugs and fluids. Most fish marking methods fall into this category.

When tagging animals, appropriate methods shall be used that do not cause the animal behavioral limitations or unnecessary stress and strain, cf. § 10 of the Animal Welfare Act. The preparatory works for the Animal Welfare Act (Proposition 15 (2008-2009)) states on labeling: “Labeling is defined as change of body shape or location of objects on the animal to easily identify the animal, gaining knowledge about who is its owner etc. or to register where the animal is located”. This provision must be seen in conjunction with the statutory provision regarding medical and surgical treatment.

Marking of fish is also mentioned in the Regulations on 17 June 2008 no. 822 concerning abattoirs and processing plants for aquaculture animals (Aquaculture Operation Regulations). According to § 31 Aquaculture Operation Regulations:

“Surgical intervention and removal of body parts from live fish is forbidden”.

The provision in the first paragraph is not to prevent marking which does not cause fish behavioral limitations, damage or unnecessary stress or that a veterinary surgeon is performing operations for fish health reasons.”

The term "intervention" in the first line also includes implants. Use of marking implant must therefore be in accordance with the provision requirements.

Paragraph 20 in the Aquaculture Operation Regulations provides clear documentation requirements for new methods and technical solutions. This documentation requirement is targeted towards the user. This means that the enterprise that is responsible for the fish also has a duty to ensure that the method/equipment is documented justifiable from an animal welfare point of view.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) requests the Norwegian Scientific Committee for Food Safety (VKM) to undertake a risk assessment of welfare implications of the different marking and tracing methods, and combinations thereof, for farmed fish and fish for cultivation. With farmed fish and fish for cultivation, we mean Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*).

The NFSA requests VKM to answer the following:

1. How do the described marking methods affect the welfare of farmed fish and fish for cultivation? Special attention should be given to the following risk factors:
    a. The skills of the person marking the fish.
    b. The procedure/technique used, along with post-operative complications and pain management.
    c. Factors that may affect achieving good welfare, f. ex., when shall marking methods used, environmental factors etc.
    d. The marking method, i.e.,:
        i. Suitability in relation to the fish size.
        ii. Suitability for large-scale tagging.
        iii. Pain or discomfort it causes.
        iv. Reliability and durability of the marking device.
        v. Risk of losing the marking device.
        vi. Is there a risk for at the marking device or the procedure:
            - Limits the fish's natural movements or changes fish's behavior.
            - Causes injuries or diseases.
            - Causes mortality. If possible state estimated mortality percent.
    e. Describe possible mitigation measures

2. Is there a risk of negative welfare implications when using the described tracing methods for farmed fish? If yes,
    a. Describe the risk.
    b. Describe possible mitigation measures.

3. Which combination of marking and tracing methods are feasible in practice without an increased risk of reduced animal welfare?

4. Which consequences for fish welfare have the different combinations of feasible marking and tracing methods?

Assessment

1 Introduction

1.1 Literature

Literature searches were conducted using ISI Web of Knowledge, Google Scholar, and by searching data archives at the Institute of Marine Research (IMR) and the Norwegian Institute of Nature Research (NINA). No restrictions were set on date of publication or language of articles. Individual members of the working group also provided literature that was, if relevant, included in the report, based on their expertise on the topic.

For handling-related welfare effects, Boolean searches were used containing the following keywords (or different combinations of): handling, surgery, injection, fish, effect, and stress.

Boolean searches were used for different types of marking methods containing the keywords (or different combinations of): marking, fish, effect, stress, pain, adipose fin removal, chemical marking, freeze branding, Carlin tag, Floy tag, T-bar tag, Visible Implant tag, Passive Integrated Transponder tag, Coded Wire tag, pigment injection, and spraying Pigment.

For tracing of origin based in natural markers, Boolean searches were used containing the following keywords (or different combinations of): catch, release, scale, otolith, DNA stand-by, lipids, fatty acid, and earth elements.

1.2 Fish welfare

The value of animals, and their worth in relation to human interests have been discussed throughout history. Animal welfare science explores and evaluates the welfare of animals, and the ethics perspective is necessary to decide which levels of welfare level are satisfactory. Those conditions that are regarded as acceptable for animals and those conditions that are regarded as unacceptable will depend on societal factors and thus vary among cultures and over time. The word “welfare” probably originates from the old Norse word “vel ferð”, meaning “good journey”. In the context of animal welfare, this expression implies an animal’s “good journey” through life or over a selected production period; e.g., the period in which salmon are kept in sea cages, or the time at slaughter. In order to be able to experience a “good journey” or a “bad journey”, an animal must have a sentient qualitative experience of life. The term “animal welfare” is therefore considered meaningful only to animals with a central nervous system (CNS) that enables the animal to have subjective and conscious emotional experiences. Sentience is also the key characteristic that determines whether a species or life stage is protected by the The Animal Welfare Act.

In Norway, all vertebrates, cephalopods, decapods, and honeybees have been considered to be encompassed within the moral circle and are included in The Animal Welfare Act. However, there is no scientific consensus or exact knowledge on which branches of the phylogenetic tree should be described as sentient beings. This is not surprising, given that even for humans we are still unable to explain consciousness and how it emerges from the multitude of electric and chemical signals in the brain-body axis. Even when some doubt exists, and we choose to treat an animal species (like salmon) as non-sentient, then the potential for enormous consequences in terms of suffering for an immense number of individuals should the assumption be wrong, prompts us to apply the precaution principle and act as if, despite any doubts, the species is sentient (Lund et al., 2007).

From an evolutionary point of view, sentience, emotions, and the ability to feel pain are characteristics that have evolved and provide attention, quality, and meaning to the sensory signals, and motivation to, and flexibility of the behaviour needed to fulfil an individual's needs, e.g., to seek food and protection. In an ever-changing environment, the ability to learn how to predict where to find food and how to avoid dangers is a great advantage. In order to be able to learn to predict the outcome of actions, an animal must have an evaluation system for success or failure. This is provided by the emotional "reward systems" in the brain, where successful coping is rewarded by good feelings (wellbeing, excitement, joy, comfort, good taste, etc.) and results in good welfare. In contrast, poor welfare, and learning from mistakes, arises from unsuccessful coping and ill-health, and is punished by the punishment systems (suffering, fear, pain, discomfort, etc.) (Spruijt et al., 2001; Duncan, 2005.).

Over the past few decades, animal welfare science has become a substantial part of the science related to farm animal production, including fish farming. The concept of animal welfare is referred to in thousands of papers ranging from molecular biology to animal ethics. However, the concept is rarely defined or used in a systematic or rigorous way, and different disciplines in animal welfare science use the concept in different ways, where welfare can include both the state of an animal and their living conditions. There is currently no consensus regarding the preferred or "correct" definition and conceptual clarity is lacking. Nevertheless, the relevant definitions centre around one or combinations of the following three approaches (Fraser, 2009; Duncan and Fraser, 1997): *Biological functioning* - emphasizing health, normal development and growth/production, bodily homeostasis and the ability to cope with the environment; *Affective states/feelings* - emphasizing the presence of positive feelings and lack of suffering; and *Natural living* - emphasizing that the animals can live reasonably natural lives and have the possibility of performing a repertoire of species-specific, motivated behaviours. For farmed fish, and especially when considering farm operations or procedures such as tagging, the first two approaches are more relevant. However, restricting the use of the term *welfare* to the overall emotional experience created by the CNS and conveyed through endocrine systems provides conceptual clarity and anchors the welfare concept to evolved neuro-biochemical processes occurring in individual animals, causing them to experience a certain quality of life (Torgersen et al., 2011). Factors like biological functioning and living conditions also affect an animal's welfare and are useful *welfare indicators*. This is explored further below.

In the report on the needs for animal welfare research in Norway (The Research Council of Norway 2005) the following definition of animal welfare was adopted: *Animal welfare is an individual's subjective experience of its mental and physical state, as regards its attempt to cope with its environment.* This is an expansion of Broom's definition (Broom, 1986), which emphasizes that welfare is a property of an individual, and that it addresses this individual's subjective experience of its state as the balance between positive and negative perceptions (Spruijt et al., 2001; Damsgård et al., 2006).

The International Association for the Study of Pain defined human pain as 'An un-pleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' (IASP, 1979, p. 249). However, although full consensus is lacking (see Rose 2012; Key 2016 and following discussion: Animal Sentience. http://animalstudiesrepository.org/animsent/), there is growing evidence that teleost fish are sentient beings and thus able to experience states of welfare. Furthermore, fish fulfil the following criteria for pain perception (Sneddon, 2003; Chandroo et al., 2004; Braithwaite, 2010; Sneddon et al., 2014):

- Fish have nociceptors, both myelinated "fast" A delta fibers and unmyelinated C fibres, in the skin.
- There is evidence of central processing of nociception in fish involving brain areas that regulate motivated behaviour (including learning and fear), and the nociceptive processing is sensitive to endogenous modulators (opioids).
- Fish possess the functional equivalents of the limbic and dopaminergic nervous systems as in other vertebrates. These systems are linked with emotion, memory, spatial relationships, primary consciousness, reward, cost-benefit estimation and decision-making.
- Nociception in fish activates physiological responses like changes in respiration, heart rate, or hormonal (e.g., cortisol) levels.
- Fish pay selective attention to noxious stimuli, whereby the response to a noxious stimulus has high priority over other stimuli and the animal does not respond appropriately to concurrent events (e.g., presentation of predator; reduced performance in learning and memory tasks).
- Fish demonstrate avoidance learning, i.e. show alterations in behaviour over the longer term that reduce encounters with the stimulus, and show long-lasting changes in a suite of responses, especially those related to avoidance of repeat noxious stimulation.

1.2.1 Welfare indicators

As it is not possible to measure an animal's own experiences (feelings) directly, measurable, validated, and scalable indicators of the animal's welfare are required. *Welfare indicators* are often categorized as "animal-based" or "resource-based" measures, alternatively named "outcomes" and "inputs". Animal-based indicators (the outcomes) are measured on the animal itself, and may comprise measures of health/morphology, physiology, and behaviour. Examples in fish include body condition score, fin damage, skin ulcers, gill health, stress hormone levels, opercular ventilation rate, swimming behavior, and aggression. Resource-based indicators (the inputs) are measures of the resources offered to animals, and also include management (management is sometimes categorized as a third category). Examples for fish include water quality parameters (temperature, O_2, CO_2, ammonia, particles, etc.), water current, light and light programs, enrichment items (e.g., hiding places for cleaner fish), tank or cage size and design, stocking densities, feed and feeding method, number of handlings and methods used, etc.

If we consider the experience of welfare as an animal's own assessment of fulfilment of its needs, then objective assessment of the fulfilment of an animal's' needs should provide a reasonable indication of its welfare. One example is the "four welfare principles" suggested by the Welfare Quality-® project: good feeding, good housing (or living environment), good health, and appropriate behaviour, all accompanied by 2-4 specific welfare criteria. For instance, the criteria for health include: absence of injuries, absence of disease, and absence of pain induced by management procedures. In order to be able to measure welfare, welfare indicators are needed. For a measure to be called a *welfare indicator*, it should be valid, i.e., it should be correlated with the welfare status of the animal, or, more specifically, to the stated principles/criteria. Welfare indicators should be reliable in the sense that inter- and intra-observer variability, or scoring of test-retest variability, should be low. Furthermore, indicators should be feasible, i.e., practical and not too costly to use. Operational indicators are measures that may be used on farm, as checkpoints and as tools for daily management. By using a protocol comprising of welfare indicators that cover the criteria and principles, an overall welfare assessment may be performed.

The Salmon Welfare Index Model, a method for overall welfare assessment of Atlantic salmon based on the theory of semantic modelling (Bracke et al., 1999) has been developed by Stien and colleagues (2013), using both animal-based and resource-based welfare indicators. This method has been expanded to include welfare indicators to be measured by fish health services (Pettersen et al., 2013) and has been evaluated at 10 different fish farms (Folkedal et al., 2016).

The aim of the present risk assessment is to assess if and how various marking and tagging methods for fish may have any negative impacts on the welfare of farmed Atlantic salmon and rainbow trout, in both the short-term and long-term. (Short-term is defined as length of time ranging from minutes (during the handling/marking procedure) to weeks, depending on how fast the wound heals post-operation. Long-term is defined as length of time beyond this point, possibly lasting for years until death.) The tagging procedure, which generally requires the fish to be taken out of water, will impact on the animal's stress levels and their perception of fear and pain during and shortly after the process. The marking procedure will last for seconds or minutes, depending on whether this is done in conjunction with vaccination and measurements, and the post-operation period, with accompanying pain and malaise, may last for hours or days. Apart from during this relatively short aversive period, the marking procedure will have little impact on fish welfare over time. However, we are concerned about the risk of a potential long-lasting effect. A method may be associated with longer-lasting side-effects, such as an increased risk of inflammation or infections, impacts on the ability to swim/manoeuvre, competitiveness, or increased fearfulness. Depending on these factors, marking/tagging will have varying effects on fish welfare integrated over the whole production period. For this risk assessment, the short-term and long-term welfare consequences are described separately.

1.3 Production of salmonid fish in Norwegian aquaculture

Norway is the leading country globally in the production of Atlantic salmon, *Salmo salar*, and rainbow trout, *Oncorhynchus mykiss*, with a standing stock of 375 million salmon and 25 million rainbow trout in the sea in 2014. The annual production of Atlantic salmon has gradually increased over the last decades, reaching around 1.3 million tonnes in 2015. In order to achieve this production volume, an estimated 281 million smolts were released into net pens in 2014. In comparison, the registered landed catch of wild Atlantic salmon in 2015 was 56 237 individuals in the sea and 160 237 fish in the rivers (Statistisk sentralbyrå), i.e., only around 0.1 % of the salmon harvest in Norway consists of wild fish.

There is also an annual production of approximately 70 000 tonnes of rainbow trout in sea cages. A few farms produce a total of around 500 tonnes of Arctic char, *Salvelinus alpinus*. There are also numerous small hatcheries producing salmon and brown trout, *Salmo trutta*, for restocking of rivers and freshwater lakes.

1.4 Escapes

There is increasing evidence that escaped farmed salmon may threaten native and potentially locally adapted wild salmon populations through interbreeding and competition (e.g., Hindar et al., 1991; McGinnity et al. 2003; Garcia de Leaniz et al., 2007) and spreading diseases (Madhun et al., 2015). Farmed salmonids escape at all life stages. Most of the reported escape events are largely due to technical and operational failures of farming equipment during the marine farming phase (Jensen et al., 2010). Large-scale escape events are more common during the autumn months when coastal storms are most frequent and intense, resulting in structural damage to farm equipment (Jensen et al., 2010). However, farmed salmon also escape during the land-based production phase, and Jensen et al. (2010) found that 11% of reported escape events occurred from smolt production facilities.

Fish farmers have a legal obligation to report escapes to the authorities. According to the statistics made available by the Directorate of Fisheries (www.fiskeridir.no), 160 000 salmon were reported to have escaped in 2015. However, not all escape events are reported, and it is believed that smaller unnoticed or unreported escapes (so-called trickle escapes) make up a significant proportion of escapes not included in the official statistics. It has been estimated that the true number of fish escaping from net pens may be two to four times the reported number (Skilbrei et al., 2015).

Escaped farmed salmon can migrate very long distances, even over relatively short time-periods (Hansen et al., 1997; Milner and Evans, 2003; Skilbrei et al., 2010). It has been shown that a significant number of fish farm escapees return to (or never leave) coastal areas in the summer and autumn of each year, on their way to enter rivers in part of their spawning migration (Lund et al., 1991; Webb et al., 1991; Crozier, 1993; Walker et al., 2006). Escaped farmed salmon have been systematically registered in catches, both from the sea and from rivers, since 1989 (Diserud et al., 2013; Fiske et al., 2014; Anon, 2015). From 2014, a national monitoring programme has coordinated the efforts of several agencies for monitoring escaped farmed salmon in more than 140 rivers in Norway (Anon, 2015). Identification of escaped salmon is commonly based on morphological characteristics and analysis of scale-growth patterns (Lund et al., 1991; Lund et al., 1989).

The proportion of escaped salmon in recreational fisheries during the summer months has been relatively stable the over last 10 years, and has varied between 3-9 % (Anon, 2016). In 2015, the proportion was 3.4 % in 98 examined rivers, which was somewhat lower than in 2014 when 5.4 % of the recreational catches was escaped salmon (Anon, 2016). The proportion of escaped salmon has, however, been higher in out-of-season surveillance fisheries carried out during the autumn months, for which 9.1 % of the salmon captured in 2015 was farmed (Anon, 2016). This is mainly due to escaped salmon entering the rivers later in the season than wild salmon, as well as a higher probability of capturing escaped fish on sports-fishing gear. Escaped salmon found in the rivers may have originated from multiple sources (potentially farms located along the entire coastline), and this complicates the identification of the source of the escaped fish.

1.5 Methods for marking fish

Marking or tagging fish involves attachment of some kind of external or internal object/material, or removal of body parts that will not regenerate during the remaining lifetime of the fish, in order to enable subsequent identification at the individual level or as belonging to a group. The purpose of fish marking is often to recognize, trace, or track the fish in research or monitoring activities (reviewed by Håstein et al., 2001). In this section, methods for marking fish that have the potential to be used for recognizing and identifying farmed salmonids in nature are reviewed. The aim is not to give a full review of the myriad of fish tags and marking methods that have been developed during the last century, but to summarize the most relevant tags and methods that may be used for mass marking of farmed salmon and trout (Figure 1.5-1). The review serves as a background for subsequent evaluation of how these methods could affect fish welfare.

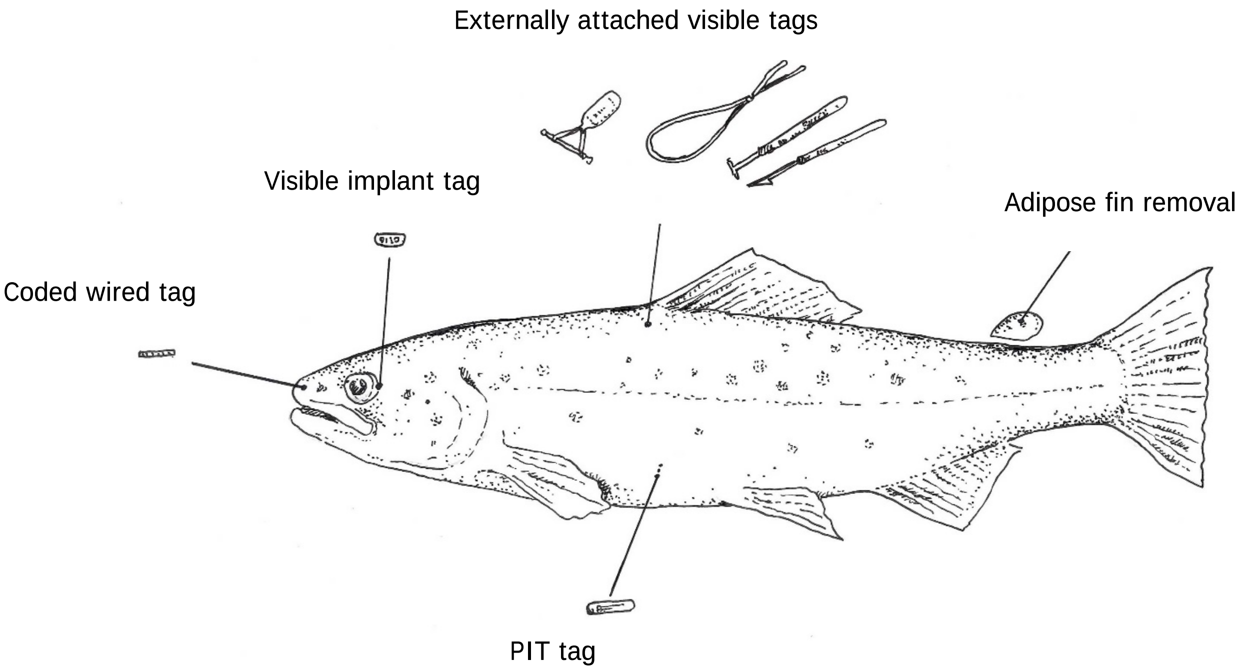

Figure 1.5-1 Illustration of various marking or tagging techniques in fish (S. Mortensen, Institute of Marine Research).

1.5.1 Adipose fin removal

The adipose fin is a small, non-rayed fin, looking like a flap of tissue between the dorsal and caudal fin, found in salmonids and in approximately 6000 ray-finned fish species. Adipose fins lack the endoskeleton, dermal skeleton, and associated musculature that characterize the other fins found in extant fishes. Instead, adipose fins tend to be passive structures, supported by rods of collagen oriented along the proximodistal axis of the fin called actinotrichia, which sandwich a sub-dermal space composed of either adipose tissue, as in some siluriformes (catfishes), or a non-adipose tissue, loosely termed ‘connective matrix’ as in salmonids (Stewart et al., 2014). Thus, despite its name, the adipose fin in salmon contains no fat. The apparently rudimentary anatomy of adipose fins inspired a long-standing hypothesis that these fins are vestigial and lack function. However, fossils do not support this hypothesis. Within ray-finned fishes, structurally elaborated, second dorsal fins (i.e., with dermal skeleton, endoskeleton and associated musculature) are found almost exclusively within the Acanthopterygii (spiny-finned fishes), which lack adipose fins (Mabee et al., 2002). Stewart and colleagues (2014) used phylogenetic and anatomical evidence to demonstrate that adipose fins originated repeatedly. This suggests that adipose fins are adaptive, although the details of their function are not yet resolved. One hypothesis suggested by Reimchen and Temple (2004) is that the adipose fin might play a role as a precaudal sensory organ. When examining the subdermal space of the adipose fin they found innervated tissue that contained many star-like astrocyte cells that are commonly found in the spinal cord and the brain (Buckland-Nicks et al., 2012), supporting the precaudal sensor hypothesis. Recently, Aiello et al. (2016) published a study on the catfish, *Corydoras amens*, in which neural activity, recorded from nerves that innervate the adipose fin, was shown to encode information on both movement and position of the fin membrane, including the magnitude of fin membrane displacement. The authors of this study concluded "that the adipose fin of *C. amens* is medianosensitive and has the capacity to function as a "precaudal flow sensor".

The function of fins is generally propulsion and precise manoeuvering in the water. In addition, various displays of the fins and body may also be used as signalling tools between individuals during sexual courtship or other social interactions, and some fins even function as taste organs. In mature salmon males, the adipose fin becomes enlarged and is believed to play role in courtship, with females selecting males with larger adipose fins to mate (Westley et al., 2008). Thus, although the adipose fin has been believed to be a vestigial (degenerated) structure with no function, and therefore loss of the adipose fin should therefore have no disadvantages for the fish and should not cause any ethical dilemma. However, the information above tends to contradict this belief, and suggests that the adipose fin is a functional organ (Buckland-Nicks et al., 2012; Stewart et al., 2014). The ethics if removing a functional organ by clipping adipose fins is therefore debatable

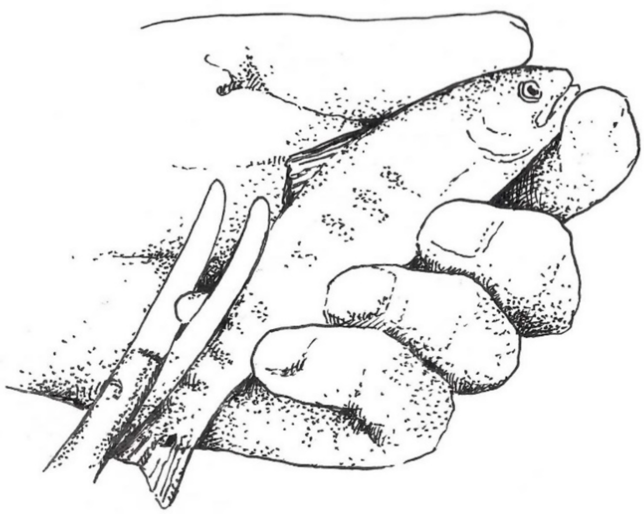

Figure 1.5.1-1. Clipping of adipose fin in Atlantic salmon parr (S. Mortensen, Institute of Marine Research).

Clipping or surgical removal of whole or parts of the fins are probably the most used methods for identifying groups of fish (Figure 1.5.1-1). These are very cheap, quick marking methods compared with most other methods (Hammer and Blankenship, 2001), and require only a small pair of surgical scissors and minimal training. Identification of a tagged fish is also quick and easy. In salmonids, clipping the adipose fin is most common, and has been used to mark billions of hatchery-reared fish that are released into the wild for stock enhancement and sea ranching in many parts of the world. In Washington State, USA, around 200 million hatchery reared chinook, *Onchorynchus tshawytscha*, and coho salmon, *O. kisutch,* are fin clipped and released annually (http://wdfw.wa.gov/hatcheries/mass_marking.html). Fin clipping is mainly done manually by cutting the fin of anaesthetized salmon parr or smolts using a small pair of curved scissors; a trained operator can mark up to 8000-10000 fish a day (Kristiansen and Skilbrei , 2012, Mortensen et al., 2013). For Pacific salmon, *Onchorhyncus sp,* an automatic adipose fin cutter has been developed that has better clip quality than hand-clipped fish (Northern Marine Technology (NMT) Inc, http://www.nmt.us/products/afs/afs.shtml) (Hand et al., 2010).

Clipping of other fins has also been used to mark groups of fish (e.g., family groups) in stock enhancement, scientific experiments or by breeding companies (e.g., Gjerde and Refstie 1988; Dietrich and Cunjak 2006; Peterson et al., 2014). Mass marking of farmed salmon by adipose fin clipping or other fin clipping, could be used to distinguish farmed from wild fish, but for further identification of escape location or owner of the fish this method would need to be combined with other marking methods with more unique codes.

Many studies have shown that adipose fin clipping is the best method for obtaining a permanent mark (Petersson et al., 2014) and has no significant effect on growth and survival (Gjerde and Refstie, 1988; Johnsen and Ugedal, 1988; Stauffer and Hansen 1969; Vincentlang, 1993; Petersson et al., 2014). Most published studies have compared return ratio and growth rates of tagged and untagged fish released for stock enhancement and sea ranching. In a study in which more than five million tagged chinook salmon were released from four hatcheries in three different years, Vander Haegen et al. (2005) compared adipose fin-clipped and coded wired tagged (CWT) fish with otolith-marked fish. They found no significant effects of CWT or adipose fin clipping. In the river Imsa in western Norway, Hansen (1988) found lower return rates for CWT and adipose fin-clipped wild Atlantic salmon smolts, but the reduced recapture rates were attributed to the anaesthesia and handling process, rather than the marking *per se*.

An important issue when using fin clipping is that some fins may regenerate, especially if only parts of the fin are clipped. The adipose fin is most suitable for long-term studies as it shows least regeneration, followed by the pelvic fins (Armstrong, 1949; Stauffer & Hansen, 1969; Weber and Wahle, 1969; Johnsen and Ugedal, 1988). Pectoral fins and the anal fin regenerate more quickly, and are probably not suitable for marking intended to last more than one year.

1.5.2 Freeze branding

Freeze branding is applied by pressing a metal bar, bearing a identifying design and cooled to sub-zero temperatures, to the body of a fish (Guy et al., 1996). Branding causes the skin to scar in the shape of the branding symbol, and pigment is either concentrated or displaced at the branding site (McFarlane et al., 1990). The fish are anaesthetised before branding. Various agents may be used to cool the metal bars, such as liquid nitrogen, compressed CO_2, dry ice and Freon. Different patterns can be used to distinguish between only a limited number of groups. The marks may be visible for years, but generally branding is a short-term mark that becomes less legible as the fish grows (Guy et al., 1996). A Norwegian commission that evaluated methods for identification escaped farmed salmon did not evaluate freeze branding as a potential methid for mass marking of farmed salmonids (Merkeutvalget, 2004). Due to the low number of unique codes and the instability of the mark, Mortensen and colleagues (2013) concluded that freeze branding was probably not a realistic solution for marking farmed salmon.

1.5.3 Externally attached visible tags

These tags comprise a range of visible markers that are attached to the fish by puncturing the skin and using one or two metal or monofilament wires, that traverse the fish body, to affix the tag (Guy et al., 1996). Alternatively, the tags can be affixed with a single wire with an intramuscular anchor, without traversing the body of the fish. Externally attached visible tags are one of the oldest and extensively used fish marking techniques, and examples of such tags are T-bar anchor tags, Dart tags, Spaghetti tags, Disc tags, Operculum tags, Streamer tags, and Carlin tags (Jacobson, 1970; MacFarlane et al., 1990, Hammer and Blankenship, 2001, www.floytag.com, www.hallprint.com). External tags are often used for marking salmonid fish in studies mapping their distribution and dispersal following some kind of experimental treatment or simulated escape from fish farm facilities. The external tags usually have a printed number code on one or several sides, and the number of unique codes typically varies from thousands to tens of thousands, although the potential number of codes could be almost infinite. The most common tags that may be used for mass marking of farmed salmon are Carlin -tags and Floy/T-bar tags. Carlin tags are small plastic plates with a printed number code, which are attached to the fish by inserting two metal wires through the body of the fish immediately below its dorsal fin (Carlin, 1955). Carlin tags are fixed by crimping the two ends of the wire together. Floy/T-bar tags are internal anchor tags, that may be inserted into the musculature or body cavity by using a semiautomatic tagging gun (Gutherz et al., 1990). In salmonids, such tags are usually implanted just below the dorsal fin and they penetrate only one side of the fish. The anchor side of the tag is T-shaped. In Norway, external tags have been used to investigate migration of wild and escaped farmed fish, fishing mortality, population sizes, and efficiency of stock enhancement (e.g. Jacobsen, 1970; Svåsand et al., 2001, 2004; Skilbrei and Jørgensen, 2010). In addition, individually numbered tags have been used in controlled experiments for individual recognition. Carlin tags have been used to study migrations of Atlantic salmon in the ocean and their return to the rivers, and many studies have used Floy anchor tags.

Merkeutvalget (2004) concluded that Carlin tags- and Floy/T-bar tags were not suitable for identification of escaped farmed salmonids due to logistic, economic, and fish welfare concerns.

1.5.4 Visible internal tags

Visible internal tags comprise several types of tags that are inserted or injected under the epidermis of the fish. They are visible for various periods following implantation, either by naked eye or by using additional light sources. Here, we consider three visible internal tag types that could be used for mass marking of salmonids; *Visible Implant Elastomer tags* (VIE), *"pigment"* tags and *visible implant alphanumeric* (VIA- or eye tags) *tags*.

Visible Implant Elastomer (VIE)

Visible Implant Elastomer (VIE) is a two-component, silicone based-material that is mixed immediately before use and that is injected as a liquid that soon cures into a pliable, biocompatible solid (http://www.nmt.us/references/vie.shtml). The tags are implanted beneath transparent or translucent tissue. VIE is available in 10 different fluorescent and non-fluorescent colours. The tags are visible under ambient light, but tag detection is enhanced if the fluorescent tags are illuminated with UV-light. Different colours and tag positions may be used to identify several group or batch codes. VIE tags have been implanted in various body locations in salmonids (Bailey et al., 1998; Olsen and Vollestad, 2001; Walsh and Winkelman, 2004), with retention and visibility being best when the elastomer is implanted in the adipose eye tissue (Close and Jones, 2002; Fitzgerald et al., 2004). Problems with reading the tags have been reported, as it is difficult to distinguish between some colours and because the tags may become less easily to identify as fish grow (Lipsky et al., 2012). As with freeze branding, VIE tags were not evaluated as a potential method for mass marking of farmed salmonids by the Norwegian commission that evaluated methods for identification of escaped farmed salmon (Merkeutvalget, 2004). Although a higher number of unique codes can be achieved by using VIE tags than with branding, the number will still be too low to allow secure tracing of an individual fish back to its farm of origin. Furthermore, the temporal instability of the VIE marks also restrict their applicability and therefore VIE tags were not recommended for mass marking of farmed salmon (Mortensen et al., 2013).

Pigment tags

"Pigments" used for marking include dyes, stains, inks, and paints, as well as microscopic plastic chips, that are applied by immersion, spraying, injection, or tattooing (McFarlane et al., 1990; Guy et al., 1996). These tags are injected under the epidermis, and therefore they may be considered as being internal tags. In the same way as for branding and VIE-tags, pigments tags may be used to identify several groups or batch codes. Pigment tags are easily visible during the initial months after tagging, but long-term detectability depends on the material and application method. To our knowledge the potential for using pigment tags for mass marking of farmed salmon has not been assessed in detail, but the applicability of the method will most likely be limited due to the same factors as for branding and VIE tags.

Visible implant alphanumeric tags (eye tags)

These tags are small (2.5/3.5mm x 1/1.5 mm) flat, rectangular tags that are implanted under transparent tissue (Bergmann et al., 1992). The tags may be numbered to facilitate individual recognition of some hundreds or thousands of fish (Haw et al., 1990). In salmonids, such tags have been implanted below the transparent tissue posterior to the eye, and have therefore been referred to as "eye-tags" Tag retention for visible implant tags is low and it is reasonable to expect tag loss of between 30 % and 50 % (Merkeutvalget, 2004 #28). Merkeutvalget (2004) also concluded that visible implant tags are not suitable for identification of escaped farmed salmonids due to logistic and economic concerns.

1.5.5 Remotely detectable internal tags

Remotely detectable internal tags are electronic or magnetic tags that are implanted into the fish, but are invisible following insertion. Since the tags are either magnetic or electronic, they can be detected with an appropriate detector. Two types of remotely detectable internal tags may be used for mass marking of salmonids; *Passive Integrated Transponder (PIT) tags* and *Coded Wire Tags (CWT)*.

Passive Integrated Transponders (PIT-tags)

A PIT-tag is a small electronic microchip encased in a biocompatible glass tube (Thorstad et al., 2013; Guy et al., 1996; Gibbons and Andrews, 2004). The tag requires an external energy source to be activated. Once activated the tag relays a unique code to a detector. PIT-tags can be injected into the body cavity or muscle of the fish with a hand-held semiautomatic tag injector. Although there is the potential for developing automatic injectors, such systems are presently not available. A range of detectors, including hand-held models, automatic tubular versions, and antennaes covering the entire width of rivers, has been developed. The length of the tags typically varies from 8 to 23 mm, while the diameter is a couple of millimetres. Due to their small size, PIT-tags can be used to tag fish down to 5 cm (Thorstad et al., 2013). The detection range increase with tag size, but is typically less than one meter. The tags can be coded with billions of unique codes. At present, several, partly incompatible, PIT-tag systems are commercially available. PIT-tags have been used in numerous studies on fish dispersal, movements and behaviour during the last decades and a large body of literature on the functionality of PIT tags exists (Cooke et al., 2013). Merkeutvalget (2004) concluded that PIT-tags (approximately 1.5 Euro per tag in 2016) would be too expensive be a suitable tag method for mass marking of farmed salmon. However, prices are expected to fall, especially for long-time contracts that concern millions of tags. Furthermore, the use of PIT-tags also represents practical challenges in large, commercial-scale farming. The transponders are embedded in a glass cylinder that may break during gutting and processing of fish at the slaughter plant, if they have not been removed beforehand.

Coded wire tags (CWT) (snout tags)

CWT are small pieces of magnetized stainless steel wires (0.25 mm in diameter, 0.5 – 2.2 mm in length) that are injected hypodermically using a range of different tag injectors, from simple, hand-held devices to automatic injectors that are custom-made for tagging large numbers of fish (Jefferts et al., 1963; see also NMT, http://www.nmt.us/products/cwt/cwt.shtml). The tags can be coded with an almost infinite number of unique codes, branded into the wire with laser technology. Due to their small size, fish down to a few centimetres may be marked with CWT. The presence of a tag can be detected using a magnetic sensor, but the tag has to be dissected out of the fish before the tag number can be read under a microscope. This means that the fish must be killed before the tag number can be read. Thus, the actual presence of the tag can be used to distinguish between tagged and untagged fish without killing the fish, but the tag number can only reveal the origin of the batch or individual after dissection. Salmonid fish are usually tagged in the snout, and CWTs are therefore often referred to as snout tags for salmonids. The tag retention is typically high in salmonids (Ísaksson, 1978; Tipping and Heinricher, 1993; Champigneulle et al., 1987). A commercial, mobile, and automatized system exists that enables tagging and vaccination of millions of salmonid parr at the same time as the adipose fins are clipped as visible markers that the fish is equipped with a CWT (www.nmt.us/products/afs/afs.shtml). This system has been developed as a “swim in” system for pacific salmonids, but has been found not to function for Atlantic salmon as they behaved differently and did not swim towards the dark area and water current into the marking unit. Merkeutvalget (2004) concluded that CWT was the most promising tag type for mass marking of farmed salmon at the time that the report was written. However, it was emphasized that the method would involve considerable logistic, economic and technological challenges before being a realistic alternative for marking all or a specific proportion of farmed salmon in Norway. These challenges include construction of automatic tagging machines that could be moved among smolt farms without risk of disease transfer, establishment of a central tag database, and development of functional logistic solutions for tagging and tracing of tagged fish in smolt farms and sea cages, as well for analysing recaptured fish. In addition, if the heads of farmed salmon are to be used for fish-meal or consumption, the small metal tag may represent a food safety issue.

1.5.6 Chemical marking

Chemical marking of fish for identification purposes has become an important tool in fisheries management and research, and recent advances in molecular science and mass spectrometry technology now allow routine application by fisheries scientists and biologists. The principle behind chemical marking is that the fish is exposed to a chemical compound (pigments, trace elements, or stable isotopes) by means of immersion, injection, or ingestion, and this results in the compound becoming physiologically incorporated within the fish on a more or less permanent basis (Guy et al., 1996). Thereafter, tissue samples may be analysed with specialized equipment for detection of the chemical compound. Here we briefly summarize two types of chemical marking: *fluorescent* and *elemental*.

Marking of otoliths with fluorescent pigments

Fluorescent pigments may be incorporated into calcified structures at early life stages by immersion in a pigment solution for a short period. The most commonly used fluorescent compounds are tetracycline, calcein, and alizarin red (Weber and Ridgeway, 1967; Szedlmayer and Howe, 1995; Beckman and Schulz, 1996; Guy et al., 1996; Mohler, 1997 ; Jones et al., 1999; Liu et al., 2009; Williamson et al., 2009; Smith et al., 2010; Wells et al, 2013). Fluorescent marking is also possible via the feed, but this method requires a longer period of treatment (Odense and Logan, 1974; Hendricks et al., 1991). If taken up by calcified structures, these compounds can be seen as fluorescent marks in scales, fin rays, vertebrae, bones, and otoliths when using ultraviolet light under a microscope. The immersion normally has minimal negative effects on fish welfare, although to improve dye uptake the calcein-tagging technique requires a high salinity dehydration step prior to the calcein bath. All the techniques may be applied on large numbers of eggs, larvae, or juvenile fish. However, the fish must be killed and the otoliths dissected out before analysis. The analyses do not require expensive equipment or a high level of expertise and may thus be applicable under field conditions. To our knowledge, automatized equipment and methodology that allow mass marking of fish with fluorescent compounds has not been developed. The number of unique codes is also limited to the number of exposures to the compound, as each exposure induces a separate ring in the otolith. Thus, it would probably be difficult to create sufficient codes to be able to use them to trace escaped salmon to a particular producer or an individual fish farm. The Norwegian commission that evaluated methods for identification of escaped farmed salmon did not evaluate the use of fluorescent compounds (Merkeutvalget, 2004).

Marking with trace elements and stable isotopes

Marking may also be performed by manipulating otolith chemistry using enriched stable isotopes (Warren-Myers, 2015). Uptake of an isotope into the calcified structures of fish, like otoliths, results in a detectable change in the relative concentration of the isotope compared with the natural composition. Thus, artificial stable isotope ‘fingerprint’ marks can be created when sufficient, but harmless, amounts of enriched isotope are introduced. The most relevant isotopes are those of Barium, Strontium and Magnesium (Warren-Myers et al., 2014). Chemical marking with enriched stable isotopes can create unique single and multiple markers with 100 % accuracy (Thorrold et al., 2006) and stable isotopes have been used successfully to mark several fish species and ontogenetic stages (Williamson et al., 2009; Thorrold et al., 2006; Munro et al., 2008; Smith and Whitledge, 2011). The isotopes can be delivered via (1) transgenerational transfer, in which enriched stable isotopes that have been injected into brood stock are passed on to the offspring (Thorrold et al. 2006; Kuroki et al., 2010); (2) immersion of larvae or juveniles in an isotope-enriched solution (Schroder et al., 1995; Smith and Whitledge, 2011; Wickström and Sjöberg, 2014); or (3) via isotope-enriched feeds (Ophel and Judd, 1968; Behrens-Yamada et al., 1979; Woodcock et al., 2013). It has also been shown that such marking may be accomplished by incorporating the marker in an injected vaccine (Warren-Myers et al., 2014). As with the use of fluorescent compounds, a suspect fish has to be killed and the otoliths dissected out before analysis. The levels of isotopes in various regions of the otoliths can be measured using high-resolution laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (Thorrold et al., 2006). A recent study, in which the potential for using the methodology in industrial mass marking was evaluated, suggested that 63 unique fingerprint marks could be made at low cost, using different Barium and Strontium isotopes, but there is also the potential for developing a higher number of marks (Warren-Myers, 2015). However, the method has to be optimized and validated for each species. Warren-Myers (2015) found no negative welfare effects on the progeny following transgenerational, immersion or injection techniques for marking the fish. Although the method has never been used as a mass marking tool, it has the potential to be used for creating an identification mark at the company level, although it would not allow tracing back to individual smolt factories or sea cage farms.

1.6 Methods for tracing fish origin based on natural marks

An alternative to conventional fish marking methodology is to utilize naturally occurring markers for tracing or determining the origin of farmed fish found in nature. One well-known example is the use of fish scales for assessing growth history of the fish, which, in turn, could be used for deciding whether a fish has been raised in a hatchery as such fish usually have a rapid, non-seasonal growth pattern in the juvenile part of their life history. It is also possible to use morphological variations to assess the origin of salmon, as overall body shape, fin condition, pigmentation, and colour often differ between farmed and wild salmon (Fiske et al., 2005; Jonsson and Jonsson, 2011). Morphological assessment of origin is however, commonly based on subjective evaluation of differences, and it is difficult to identify farmed salmon that have escaped during their early life stages. Furthermore, the reliability when using morphological variation to identify escaped salmon depends on the level of experience of the examiner and the fish has to be handled out of water. In this section, the most relevant methods for using natural markers for tracing or identifying farmed salmonids are reviewed.

Analysis of natural marks does not involve handling or manipulation of fish, and potential effects on fish welfare would be more likely to be related to sampling relevant biological material (e.g., scales or tissue) from live fish. If the material is collected from fish that are captured and killed in regular fisheries, then collection of material would not affect the fish beyond that which is commonly accepted for recreational and commercial fisheries. However, if the material is collected during extraordinary out-of-season sampling (e.g., during autumn surveillance fisheries or organized recapture of escaped fish), it could be necessary to collect material from live fish. In such fisheries, salmon that may be determined visually to be escapees are killed, whereas fish that are assumed to be wild are released following the same guidelines as in ordinary catch and release (C&R) fisheries. In order to be able to assess the proportion of escaped salmon in the sample, biological material has to be collected from both killed and released fish.

1.6.1 Tracing based on structures indicating growth pattern

A range of external and internal structures of fish can be used to estimate their age and growth patterns as they show natural growth rings (Dahl, 1910; Fiske et al., 2005). The most commonly used structures are scales, otoliths, vertebrae, fin spines, eye lenses, teeth, or bones of the jaw, the pectoral girdle, and opercular series. The variations in growth patterns can also be used for discriminating between wild and farmed fish, because the latter grow faster during their early life stages due to more favourable conditions in the hatcheries. This results in the spacing between the growth rings being larger, as well as farmed fish having weak or a lack of seasonal spacing-patterns as typically occur in wild fish (Ross and Pickard, 1990; Barlow and Gregg, 1991). It is also possible to manipulate growth ring patterns in otoliths by exposing the fish to short-term temperature fluctuations in the hatchery to induce distinctive structural marks on the otoliths. Manipulation of growth ring patterns is often referred to as “thermal marking” and is a widely used technique for identifying the origins of hatchery-produced salmonids (Volk et al., 1993). In addition, information about growth can contribute to tracing the fish after escape, as it may be possible to estimate the time in liberty due to a change in growth pattern and comparison with the growth patterns of fish from farms with those of recaptured escapees. In this section, we briefly summarize the use of scales and otoliths to identify farmed salmon, as these are the most commonly used structures for identifying escaped salmonids.

Scales

Analysis of scales provides a relatively simple and objective way of differentiating between farmed and wild salmon (Friedland et al., 1994). The scales are sampled from both dead and live fish, and are stored dry, without any need for preservation, before the analyses. The sampling is often carried out by local anglers, who may send the samples to the laboratory by mail. Sampling of scales is usually conducted by; a) using a blunt knife first to remove mucus, and thereafter to scrape off several scales or; b) using a pair of pliers to pull out single scales without prior removal of mucus. The latter method is gentler for the fish, and is the method used when sampling scales from live fish. Scales are predominantly sampled from salmon that have been captured and killed during regular recreational and commercial fisheries, both in the sea and rivers. However, scale sampling is also carried out as a part of out-of-season surveillance fisheries in rivers during the autumn and in extraordinary recapture fisheries following reported escape incidents. In the latter case, scales have to be sampled from live fish to avoid killing wild salmon, as not all escapees can be discriminated from wild fish on a morphological basis and it is necessary to assess the proportion of escapees in the rivers. In the laboratory, the scales are examined under a stereomicroscope and the origin of the fish decided, either manually or by semi-automatic image processing (Friedland et al., 1994; Thorstad et al., 2008). Usually, only a few scales are needed for differentiating between wild and farmed salmon with more than 90 % reliability (Lund and Hansen, 1991). Scales are routinely used for determination of the origin of thousands of salmon each year by several Norwegian institutes, and, besides genetic analysis, is considered the most precise way of identifying escaped farmed salmon (Fiske et al., 2005).

Otoliths

Otoliths can provide more accurate age determination than scales (Jonsson, 1976; Hindar and L'Abee-Lund, 1992), but are more difficult to obtain and to analyse. In order to retrieve the otoliths the fish has to be killed and the otoliths dissected out. Although the otoliths can be stored dry, the analysis itself may involve grinding or cutting before the otolith can be analysed under a microscope following similar principles as for scales. Because of the more laborious collection and analyses for otoliths compared with scales, Fiske and colleagues (2005) recommended that scale analyses would be more appropriate for large-scale screening aimed at determining the origin of salmonids.

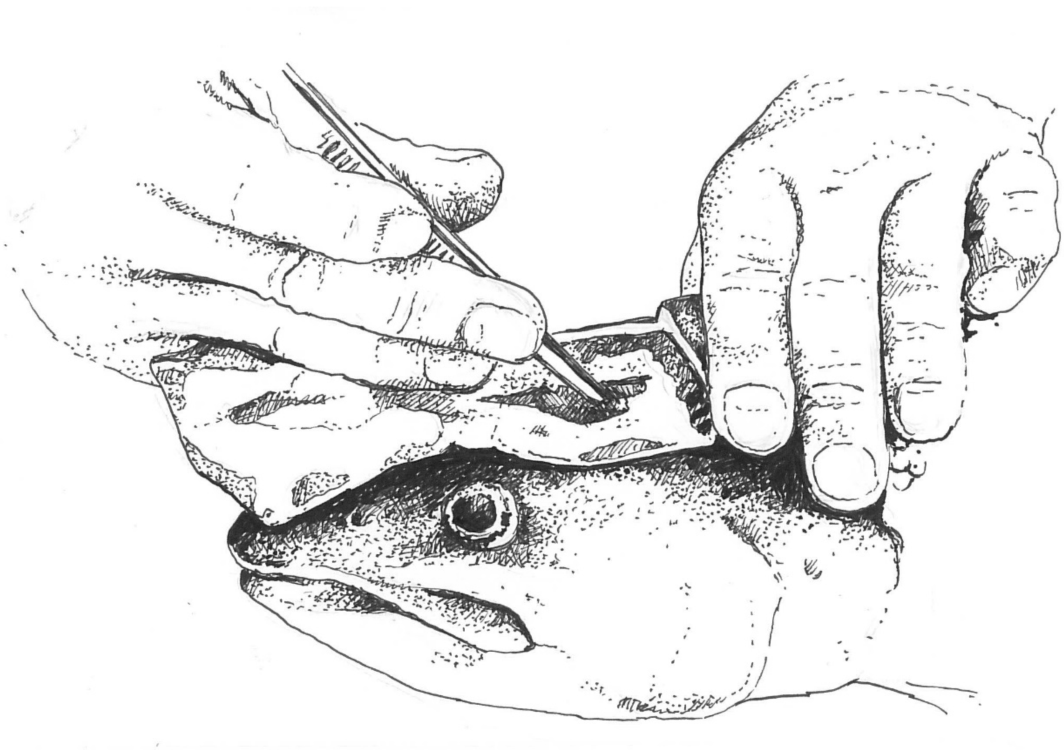

Figure 1.6.1-1. Removal of the otolith from the skull of a fish. (S. Mortensen, Institute of Marine Research).

1.6.2 Tracing based on genetic composition and variation

The DNA database method

Tracing based on a DNA database has been considered. This would represent a “top-down” approach to identification, using a database consisting of a combination of breeding information and individual DNA profiles to enable the identification of escapees through match/exclusion to family (Glover, 2010).

However, a DNA register consisting of allele frequencies for a panel of molecular genetic markers for the major strains would incur problems due to genetic drift from the breeding programmes to commercial production. The DNA register would need to be combined with rigorous documentation of fish movements across the supply chain, and perhaps would require test of genotyping proficiency. A register could provide some of the advantages that a physical tag could also provide over the DNA stand-by method, notably, diagnostic identification of escapees, even in the case of drip-leakage, and an indefinite time delay following escape.

While a DNA register approach is technically viable, and feasible within the logistical framework of individual producers who require a verification tool for monitoring and tracing own production. However, for management authorities requiring an identification tool for farmed escapees for the entire industry, this approach has major and arguably insurmountable logistical challenges. A major problem with the DNA register approach is that it cannot, alone, assign ownership of an individual fish. Maintaining a database containing this number of fish, and documenting fish-family movements between the various producers would be technically challenging. As with physical tagging, it would also incur high costs for fish that do not escape.

The DNA stand-by method

The DNA stand-by method is based on combined sampling, genotyping and statistical analyses, and enables the origin of the fish to be traced back to its cage and farm of origin.

The DNA stand-by method implements a “bottom-up” approach to identification, i.e., starting with the DNA of escapees and sampling the last plausible link within the supply chain to identify/exclude the potential source(s) of escape.

The method has a number of conditions that need to be fulfilled in order to be able to be implemented in a robust manner, and, not least, to offer the best possible chances of identification (Glover et al., 2009; 2008). Rapid recapture of a homogenous group of escapees in restricted time and space represents the most important step in identification of farmed escapees. In order to avoid having to compare the genetic profile of recaptured escapees with every single farm, which is clearly not feasible, the escapees needs to be captured close to the source of escape, before the fish disperse and mix with fish from other potential sources.

The DNA stand-by method has been implemented successfully in the identification of escapees from fish farms in Norway.

1.6.3 Tracing based on biochemical variation in tissue and internal structures

The biochemical composition of various fish tissues may be affected by the environment in several ways. Assuming that, in comparison with farmed salmon, wild fish experience different environmental conditions throughout their life history, it might be possible to use variations in biochemical composition to reveal the background of a fish and thus also its origin. Below, we have briefly summarized the use of lipids, fatty acids and earth elements for tracing the origin of Atlantic salmon.

Lipids and fatty acids

The biochemical composition of the feed used in salmon farming differs from the natural sources of feed for wild salmon. A major difference is that farm feed is composed of a high proportion of terrestrial ingredients, and, in 2013, only 30 % of the ingredients in Norwegian salmon feed were of marine origin (Ytrestøyl et al., 2015). The terrestrial ingredients generate chemical signals that can be used for assessing the origin of salmon. The potential for using variation in biochemical composition to discriminate between wild and farmed salmon has been examined using several methods including fatty acid (FA) distributions, chemical profiling with elemental analysis, 13C nuclear magnetic resonance, and the use of stable isotopes (Dempson and Power, 2004; Aursand et al., 2009; Axelson et al., 2009; Schröder and de Leaniz, 2011; Skilbrei et al., 2015). The results indicate that quantification of lipid composition and FA-profiling would, in most cases allow correct discrimination between farmed and wild salmon, and that such analyses could be a good tool for studying the history of escaped salmon. Analyses of biochemical composition would, nevertheless, probably be of limited value for tracing escapees back to smolt producers or individual salmon farms, as the feed at different farms would generally contain similar ingredients.

Natural variation in earth elements

The elemental and isotopic composition of bio-minerals in bones, teeth, scales, and otoliths may also be used to identify potential geographical differences between fish populations, because regional differences in water composition are reflected in the bio-minerals (Thresher et al., 1994; Campana et al., 1997; Severin et al., 1995). Variation in feed sources will further add to the variability, and the elemental composition in such tissues may thus be used to determine the origin of farmed salmon. For instance, Adey and colleagues (2009) found that 87 % of samples taken from six farm sites around the west coast of Scotland were correctly classified to their farm of origin on the basis of scale chemistry. Since otoliths and scales grow incrementally, by analyzing the elemental variation in specific areas in scales or otoliths they may also provide a record of the location/environment of the fish throughout its life. This may be done by using high-resolution LA-ICP-MS to ablate sections of a scale or otolith, as was done for scales by (Flem et al., 2005). Flem and colleagues (2005) found that two farmed populations differed chemically from one wild and one cultivated stock, although the two farms were only partially delineated from one another. It has also been suggested that a database of elemental fingerprints of otoliths/scales from different salmon farms could be used to identify the origin of escapees (Veinott and Porter, 2005). The Norwegian commission that evaluated methods for identification of escaped farmed salmon recommended that the use of variation in natural earth elements to trace escaped salmon should be investigated further (Merkeutvalget, 2004). During recent years, a large on-going Norwegian project has been investigating the potential for using the variation in naturally occurring elements in scales to establish a method for tracing escapees back to their source (www.fhf.no/prosjektdetaljer/?projectNumber=901016). Preliminary results indicate good discrimination in the freshwater phase of the salmon life cycle, but it is still unclear whether the method may be used for tracing fish back to individual farms with adequate precision.

2 Hazard identification and characterization

2.1 Methods for marking fish

Pre-marking procedures - anaesthesia

Successful anesthesia of salmonids is also a well-researched area and standard operational procedures exist that should ensure maximal fish welfare and minimal post-exposure effects. Marking of fish is commonly done under general anesthesia in order to immobilize the fish. As the fish are removed from the water during the marking procedure, air temperatures should not be below 0 °C. The amount of cortisol released in response to anaesthesia appears to be low, but may represent an extra load under otherwise stressful circumstances. Furthermore, anaesthetics may cause secondary adverse reactions such as acidosis and osmotic stress due to respiratory arrest and insufficient exchange of gas and ions between the blood and the water. Overall, anaesthetics may reduce stress, thereby improving welfare, but can also have unwanted side effects that reduce fish welfare and therefore should always be used with caution (Zahl et al. 2012). In the following evaluation of fish welfare effects due to marking, we assume that adequate measures will be taken to reduce negative effects during the marking procedure through appropriate administration of an efficient anesthetic

2.1.1 Adipose fin removal

Acute welfare effects during clipping

Clipping is done at the base of the adipose fin with a small pair of surgical scissors, carefully avoiding cutting into the muscle. The adipose fin will not regenerate if the whole fin is removed (Armstrong, 1947; Stauffer and Hansen, 1969; Weber and Wahle, 1969; Johnsen and Ugedal, 1988, Mortensen et al., 2013).

Clipping is a manual procedure demanding full concentration to avoid clipping too deep and into the muscles. In order to avoid handling fish several times, fin clipping should be done during vaccination, when the fish is 25-50g (Kristiansen and Skilbrei, 2012), but it is also possible to mark fish at a smaller size (Mortensen et al., 2013). In studies of wild salmon, these are usually marked in the rivers 1-2 months prior to smolt migration, at 15-25g size (Ugedal et al. 2014). In a stressful environment, with a need to keep up with the speed of the vaccination process, errors will probably be more frequent. Furthermore, if there is a large number of fish to be clipped, and many hours per day of clipping, the risk of loss of concentration and operator fatigue, and consequent errors and injuries, may increase. Hand and colleagues (2010) compared automatic and manual fin clipping of Pacific salmon and found that only 70 % of manual clips were of good quality compared with 95 % of automatic clips. An automated machine (developed by NMT), that utilizes the natural behavior of Pacific salmon, did not function when tested on Atlantic salmon. However, should adipose fin removal be implemented in farmed Atlantic salmon, then equipment for automatic removal of adipose fins will probably be developed and included as a part of the vaccination machines (Kristiansen and Skilbrei, 2012). Over-doses or too long duration of anaesthesia may lead to mortality, but do not cause suffering in that the fish do not wake up. However, this will lead to economic losses. Vaccination and fin clipping need good logistics, and there is a risk that delays in the process lead to air exposure of fish ready for clipping being too long. The vaccination and fin clipping process have a risk of poor water quality in anaesthesia and recovery tanks. Aeration of anaesthesia tank is particularly important and requires frequent changing of the water according to tested routines. Pumping, netting, and manual handling of fish may remove mucus and scales from the fish skin, especially if the hands are dry. It is therefore important to keep the fish in water or under moist conditions and handlers should not touch the fish with dry hands.

Fish experience stress during crowding, pumping, and handling. Vaccination should proceed as quickly as possible, in order to minimize the stress associated with being exposed to air. Anaesthesia during tagging would relieve acute pain. As the adipose fin is innervated (Buckland-Nicks et al. 2012) it is possible that the fish may feel pain after recovery from anasthesia, but the extent of this is unknown.

Impacts on welfare post-clipping

A recent study showed that the small wound made by adipose fin clipping closes within a few hours and seems to cause no osmotic problems or have lasting effects on the fish, based on histological observations (Andrews et al., 2015). All fish will have a small open wound for 4-6 hours at 10-14^{o}C, and for between 6-12 hours at 4 ^{o}C (Andrews et al., 2015), which will increase the risk of infections. The wound may also influence the vaccine response, since the immune response of the fish will probably prioritize the wound (Andrews et al., 2015).

As the wound created by adipose fin removal has been reported to heal within a day after tagging (Andrews et al., 2015), it is reasonable to believe that the probability that fish experience chronic pain and stress for longer periods is low. However, neuroma formation, which may cause phantom pain, is known to occur in beak-trimmed poultry (Breward and Gentle, 1985).

Most studies report no effects from adipose fin clipping on growth and mortality (Gjerde and Refstie, 1988; Johnsen and Ugedal, 1988; Stauffer and Hansen, 1969; Vincentlang, 1993; Mortensen et al. 2014; Petersson et al., 2014). However, mass marking of all farmed salmon may increase the risk of fungal or other infections in fresh water.

Adipose fin clipping has been reported to affect swimming behaviour of small Pacific salmon (Reimchen et al. 2011). As far as we know, studies have not been done on Atlantic salmon, but since the adipose fin is believed to be a water-flow sensor in turbulent water, salmon in tanks and cages should not be seriously affected by the lack of adipose fin.

The lack of the adipose fin may be detected by eye or camera without handling the fish, but as the aim is to remove escapee fish from the rivers the fish must be caught in traps or harpooned in the rivers and killed. This is, of course stressful, but short lasting.

<u>Summary</u>

Adipose fin removal is a quick, cheap, and easy way to mark salmon, but useful only for identifying farmed salmon as a group. The wounds heal within a day and adipose fin removal has no effects on growth and survival in most studies. However, risk factors such as increases in infection pressure or injuries due to human errors, should not be disregarded if all farmed salmon are to be marked. The risk of neuroma formation is unknown. The adipose fin is a functional organ that probably acts as a flow sensor in turbulent water, and therefore probably most important for salmon living in rivers. However, whether it is acceptable to remove a functional and healthy organ is an ethical dilemma. In conclusion, adipose fin removal is a potential, but probably a minor hazard to fish welfare if it is conducted with due care and attention, and in conjunction with other procedures.

2.1.2 Freeze branding

Acute welfare effects during freeze branding

Freeze branding (or cold-branding) has been conducted with hand-held devices cooled down with either liquid nitrogen or dry ice (CO_2) (Guy et al., 1996). Following immobilization, the fish are removed from the water and the branding area blotted dry, before the brand is applied for 1-2 seconds with gentle pressure. If the branding iron sticks to the fish, it needs to be re-cooled (Guy et al., 1996). Since freeze branding involve anaesthesia and manual handling out of water, as well as manually operated tagging equipment, training and experience with the technique are important to ensure a good result and to minimize potential negative welfare impacts. To our knowledge, studies examining the relevance of training and experience to avoid welfare issues during freeze branding have not been carried out. As both the technique and the equipment are simple, the skills necessary could probably be attained relatively easily. However, it has been shown that the general trauma associated with freeze branding of rainbow trout fry may double subsequent mortality due to increased predation risk (Maynard et al., 1996). Thus, experience, training, and optimization are probably important factors for reducing the occurrence of negative effects due to freeze branding.

As salmonids in general are affected more by stress under sub-optimal conditions, it is also likely that potential negative effects from freeze branding would be related to environmental conditions. It is also possible that different ontogenetic stages and fish sizes would respond differently to tagging. To our knowledge, systematic studies in which the effects of freeze branding in salmonids have been quantified under different environmental conditions and for different stages/sizes have not been conducted. However, it has been shown that coho salmon fry (average length: 42.9 mm) suffered a mortality of 8.3 % due to the handling during freeze branding, but that the mortality rate after tagging was not significantly elevated (Peters et al., 1994). It has furthermore been shown that the position selected for branding may affect subsequent growth rate, but not survival, of roach (*Rutilus rutilus*) (Evrard, 2005). However, freeze branding has been found to have no effect on survival and growth in juvenile burbot (*Lota lota*), pinfish (*Lagodon rhomboids*), Chinook salmon and sockeye salmon (*Oncorhynchus nerka*) (Smith, 1973; Ashton et al. 2013; Matechik et al., 2013). Hence, it appears that freeze branding may have relatively minor impacts on survival and growth of juvenile salmonids, and also that such impacts cannot be completely ruled out.

We have not identified studies where stress related physiological parameters have been measured for fish, during or after freeze branding. It is therefore difficult to evaluate the extent to which the fish may feel pain during/after the tagging. However, since freeze branding results in destruction of the outer epidermis of the fish and a histopathology study reported cells that commonly are found in healing teleost wounds in the branding area (Laird et al., 1975), it seems likely that freeze branding inflicts pain. Anaesthesia during the tagging would relieve acute pain, but to what extent fish may feel pain after recovery from anesthesia is unknown.

Impacts on welfare post-branding

Freeze branding initially causes darkening of the brand area due, to destruction of melanophore control, with subsequent invasion of the area of the stratum spongiosum and hypodermis by melanin containing cells commonly found in healing teleost wounds (Laird et al. 1975). According to Laird et al. (1975), traumatic damage was completely resolved within four months after branding. This indicates that freeze branding is primarily suitable for short-term batch marking.

As the wound created by freeze branding has been reported to heal within four months after tagging, the probability that fish experience chronic pain and stress for extended periods is likely to be low (Laird et al. 1975). However, it is likely that the fish experience short-term pain and stress.

To our knowledge, published information regarding infection risks or diseases due to freeze branding is lacking. The literature suggests elevated mortality due to increased predation risk shortly after freeze branding, probably caused by the general trauma associated with marking, rather than the marking process *per se* (Maynard et al. 1996). Elevated long-term mortality has not been reported (Smith 1973; Evrard 2005; Ashton et al. 2013; Matechik et al. 2013).

The general trauma associated with tagging may affect the anti-predator behaviour of juvenile fish, and thereby increase mortality due to predation (Maynard et al., 1996). To our knowledge, no other effects of freeze branding on behaviour have been reported.

As for most external tags or marks, fish must be captured and handled during inspection of brand marks. Thus, impacts on welfare will be related to capture and handling, and not primarily to inspection of the tag. Capture and handling may be stressful for fish and thus have welfare effects. However, compared with other tagging methods, handling during inspection of brands is not particularly stressful or harmful for the fish

Summary

Freeze branding creates a short-term mark that can be used for identification of a limited number of fish batches. The mark is essentially a wound that can be identified on the fish for a limited period, until the wound is completely healed. Freeze branding may result in an increase in short-time mortality, partly from increased predation risk due to the general trauma associated with being tagged. No long-term effects on mortality and growth have been reported, although, for unknown reasons, growth rates may vary with branding locations on the fish. As freeze branding involves causing of wounds, it seems likely that it inflicts some degree of pain and stress, at least during the tagging and initial healing process. Freeze branding can therefore be considered a hazard to fish welfare.

2.1.3 Externally attached visible tags

Acute welfare effects during tagging

A variety of externally attached visible tags are available. For example, Anchor tags are injected by using a “tagging gun”. Tagging is done in a few seconds and require minimum time out of water. Carlin tags are more invasive, as two metal wires are injected by needles through the muscle and twisted on the opposite side. Since the procedure involves anaesthesia and manual handling out of water, as well as the manually operated tagging equipment, training and experience with the technique are important to ensure a good result and to minimize potential negative welfare impacts. To our knowledge, studies examining the relevance of training and experience to avoid welfare issues on external tags have not been carried out. As both the technique and the equipment are simple, the skills necessay would probably be attained relatively easily. Because of the slow speed of tagging, Carlin tags are not appropriate for mass marking. Floy tagging is fast and has been used in large tagging programmes with several 100,000 Atlantic salmon (Svåsand et al 2011).

It is reasonable to assume a negative correlation between fish size and negative effects. Salmon are also more vulnerable to scale loss near smoltification. We are not aware of any studies on optimal periods for external tagging of salmon.

As the procedure involves puncture of the skin and injection of metal or monofilament threads into the body, it seems probable that the fish will feel some pain and irritation after recovery from anaesthesia, but these are probably not severe. We did not identy studies in which stress-related physiological parameters or behaviour were measured for fish, during or after Carlin or Floy tagging. However, Hansen (1988) demonstrated that Carlin-tagged and handled Atlantic salmon smolts had poorer survival in the sea.

Impacts on welfare post-tagging

Externally attached tags on fish will create a hydrodynamic drag, which may result in movement of the tag and consequent skin irritation. For both Floy and Carlin tags, wounds around the tag are frequently observed as long as the tag remain in place (http://www.hafro.is/catag/b-fish_tags_tagging/b12-tag_mark_types/b1201-external-tags.html). A relatively large proportion of the fish have inflammations and wounds at the attachment point in the skin. As the salmon swim in very shallow waters, algae and other fouling organisms may attach to the tag and increase the drag. Both recapture rates and growth have been shown to be reduced when using external tags (ref). Open wounds around tags may act as a gateway for infection, and may explain some of the increased mortality reported in several studies (ref). Reduced growth rate, wounds in the muscles that reduce the fish quality, and increased disease risk would not be acceptable to the farming industry.

As the wound will be irritated by a moving wire in the skin, and the tag itself may grow into the muscles of larger fish, there will be long-term negative effects, most likely including pain. To our knowledge, published information regarding infection risks or diseases due to external tagging is lacking, but several studies show negative effects on survival of externally tagged fish (e.g. Strand et al., 2002, and references therein). The literature suggest mortality may be elevated due to increased predation risks shortly after marking that is caused by the general trauma associated with marking, and probably not by the marking process *per se* (Maynard et al., 1996).

To our knowledge, no other effects on behaviour due to external tagging have been reported.

As for most external tags or marks, fish must be captured and handled during inspection of the tag. Thus, impacts on welfare will be related to capture and handling, and not primarily to inspection of the tag. Capture and handling may be stressful for fish and may thus have welfare effects.

Summary

External tags can be used to identify almost unlimited numbers of individual fish. The tags are attached using metal or monofilament wires or anchors (e.g. see www.floytag.com).

The tag plates or tubes may become fouled with algae, which will increase drag. The tags have been associated with low, but significantly increased mortality, and reduced growth. A significant proportion of the fish will develop chronic wounds in the skin, that impact on both welfare and flesh quality. In general, these tags have too many negative effects on fish welfare and fish performance and quality to be suitable for mass marking programmes. Marking with externally attached tags is therefore considered a hazard to fish welfare.

2.1.4 Visible internal tags

2.1.4.1 Visible implant elastomer (VIE) tags

Acute welfare effects during tagging

VIE tags are created by injecting a liquid into the tissues of the fish (Guy et al., 1996), usually under anaesthesia. Within hours, the liquid cures into a pliable solid that makes a cohesive, well-defined mark. Coding is possible by using different colors and tag positions. As with all other tagging methods, experience during handling is probably important for ensuring fish welfare. To our knowledge, published studies, in which the effects of experience with the VIE tagging technique are assessed are lacking. As the VIE tags are injected subcutaneously, and not deep into tissue, to allow visibility, it is unlikely that the procedure itself inflicts serious harm. The immediate mortality in brown trout (*Salmo trutta*) due to tagging under field conditions has been reported to be low (0.5 %) (Olsen and Vøllestad 2001).

The potential impacts of using VIE tags under different environmental conditions have not been investigated, but, the effects of VIE tags for various size classes of fish have been examined in several species. Ward and colleagues (2015) found no effects on survival or growth for four size classes (40-49/50-59/60-69/70-79 mm total length) in humpback chub (*Gila cypha*) with VIE tags, and Wagner et al. (2013) found no effects on growth or survival for three size groups (93/138/152 mm average total length) of juvenile spotted seatrout (*Cynoscion nebulosus*) following implantation of VIE tags. Tag location, however, may affect survival and has been demonstrated for juvenile red drum (*Sciaenops ocellatus*), in which no mortality was associated with tags injected under the dorsal fin, bute 40 % mortality was observed when VIE tags were implanted above the anal fin (Bushon et al., 2007). Thus, the use of VIE implants does not generally affect mortality and growth among different sizes of juvenile fish, but survival may be affected if the tags are implanted in an unsuitable position.

Injection of VIE tags in western silvery minnows (*Hybognathus argyritis*) resulted in elevated levels of lactate in blood plasma, but not a corresponding increase in plasma cortisol levels (Neufeld et al., 2015). Neufeld and colleagues (2015) concluded that the overall stress effects were low. It has also been shown that the use of VIE tags may tempoarily affect the immune response in three-spined stickleback (*Gasterosteus aculeatus*), in particular the granulocyte:lymphocyte ratios, both for the tagged group and sham control group (Henrich et al., 2014). This indicates that puncture of the skin during injection of a tag may temporarily affect the immune system, but that the tag itself would not have any effect. Henrich and colleagues (2014) recommend a two-week recovery period following tagging with VIE implants to minimize manipulation of the immune system. Thus, application of VIE tags appears to stimulate a weak stress response and could have a negative effect on the immune system. Since use of VIE tags includes injection of a liquid and puncture of the skin it is also likely that it would involve some temporary pain.

Impacts on welfare post-tagging

We have not identified any histological studies in which the influence of injection with VIE tags on tissues are described. Weston and Johnson (2008) reported that tagging rainbow darter (*Etheostoma caerulum*) with VIE tags did not result in lesions or scars. However, Kerwath and colleagues (2006) found that VIE tags resulted in fin rot in one species of South African linefish (Fransmadam, *Boobsoidia inornata*), but not in another species (Roman, *Chrysoblephus laticeps*). The vast majority of studies in which effects of VIE tags have been examined do not report any tissue damages or inflammations, and it seems probable that the risk for such complications is low.

To our knowledge, the possibility that VIE tags may inflict chronic pain and stress on fish has not been examined. As VIE tags do not appear to cause any mortality or growth reduction in the long-term (se references below), it is unlikely that they would involve chronic pain and stress.

Apart from the observations of fin rot in a South African linefish species (Fransmadam, *Boobsoidia inornata*) (Kerwath et al., 2006), VIE tags have not, to our knowledge, been reported to increase infection risk and susceptibility to disease. However, several studies of different fish species have examined survival and growth following application of VIE tags. Almost all these studies report no significant effects on either growth or long-term survival (Olsen and Vøllestad 2001; Skinner et al. 2006; Kerwath et al., 2006; Weston and Johnson, 2008; Leblanc and Noakes, 2012; Matechik et al., 2013; Wagner et al., 2013; Ashton et al., 2013; Ward et al., 2015). One exception was 40 % mortality in juvenile red drum when the tag was injected in an inappropriate position (Bushon et al, 2007). Use of fish tags with bright colours may attract predators and thereby increase mortality due to predation (Catalano et al., 2001), but no such effect has been found for VIE tags (Haines and Modde, 1996; Malone et al., 1999; Roberts and Kilpatrick, 2004; Reeves and Buckmeyer, 2009; Bouska and Paukert, 2010).

In general, the occurrence of behavioural effects due to VIE tags has been neither investigated nor reported, apart from Suthpin et al. (2007) who found no effect on swimming performance in Sacramento splittail (*Pogonichthys macrolepitodus*) with VIE tags. With the exception of short-term behavioural effects due to the general trauma of tagging it is unlikely that VIE tags would result in long-term behavioural impairment as the tags are small implants that would not affect the body shape of the fish.

VIE tags are made for situations in which visual identification is required and sacrificing the fish is not desired (Guy et al. 1996). However, as with most fish tags, capture and handling of fish would be required during inspection; this may be stressful for fish and therefore have an effect on welfare.

Summary

In general, VIE tags have not been shown to influence either short-term or long-term survival, growth or behavior. There is some evidence that minor problems may occur. As with all taggings or marking methods, injection of VIE tags involves handling fish and also puncture of the skin. Marking fish with VIE tags thus represents a hazard to fish welfare.

2.1.4.2 Pigment tags

Tagging with pigments involves either injection of pigment under the skin or spraying pigment onto the surface of the fish. As these two approaches differ, both with respect to the technique and the subsequent consequences for the fish, they are discussed separately. We have focused on injection of either alcian blue or acrylic/latex paint and spraying with fluorescent pigment, as these two methods would be the most appropriate for mass marking of salmon.

Acute welfare effects during tagging – Injection of pigments

We found little information about how experience or functionality of equipment would affect fish welfare. As with all methods that involve handling of fish and puncture of skin it is however reasonable to believe that both varying levels of experience and functionality of the equipment would influence fish welfare. Although injection of dyes normally is a safe and reliable marking method, sudden fish death has been observed when tagging Atlantic salmon parr (Bricknell and Bruno, 1995). In this case, the marking equipment was not adjusted to the size of the fish and the dye was injected through the body wall and into the cardiac region, resulting in considerable mortality (27%) due to internal hemorrhages.

Little information exists regarding how injected pigments may affect fish welfare immediately after tagging under different environmental conditions or for varying fish sizes/stages. Malone and colleagues (1999) reported a minor depression in growth in bridled goby (*Coryhopterus glaucofraenum*) when using injected pigment tags, but it was unclear whether this was a result of handling or the tag itself. Moreover, Hayes et al., (2000) did not find any short-term mortality following injection of a photonic paint mark in adult Chinook salmon. Injections of pigments is, similar to injection of VIE tags, a minor intervention, and it is unlikely that acute welfare effects, besides those inflicted through handling, would have a significant negative effect. We have not found any studies that investigated acute pain and stress, but as injection of a dye involves puncture of the skin, short-term pain is likely to occur.

Impacts on welfare post-tagging – Injection of pigments

Thedinga et al. (1997) used histological analyses to investigate how injection of alcian blue affected the tissues of three flatfish species; yellowfin sole (*Pleuronectes asper*), rock sole (*Pleuronectes bilineatus*) and pacific halibut (*Hippoglossus stenolepis*), and found no evidence of lesions in the skin or musculature, and no alterations in either the cells or the structure of the dyed cells. In addition, they found no anomalies in hepatocytes and concluded that alcian blue tags are non-toxic and non-irritating (Thedinga et al., 1997).

As alcian blue was shown to be non-toxic and non-irritating, and did not affect cell structure in flatfish (Thedinga et al., 1997) it seems likely that the overall probability of chronic pain and stress is low. However, it is important to bear in mind that different fish species may react differently to the use of alcian blue. To our knowledge, similar tests have not been conducted for other dyes (acrylic/latex paints) that have been used for creating subcutaneous pigment tags.

Injected pigment tags do not typically affect survival and mortality after tagging (Ryan, 1975; Cane, 1981; Herbinger et al., 1990; Bridcut, 1993; Thedinga et al., 1995; Malone et al., 1999; Hayes et al., 2000; Skinner et al., 2006; Simon 2007). However, Thedinga and colleagues. (1994) found that injection of pigments might contribute to increased predation in field situations and Malone et al. (1999) found that internal pigment tags might depress growth in small fish. We have not found any information regarding infection risk and increased susceptibility to diseases.

Tagging with alcian blue using a Pan-Jet inoculator had minimal effect on behaviour of brown trout, and recapture was possible over a period of 12 months (Bridcut, 1995). We have not found any evidence of significantly negative effects on fish behaviour, due to the use of injected pigment tags.

As for most fish tags, fish must be captured and handled during inspection of injected pigment tags. Capture and handling may be stressful for fish and may therefore have an effect on welfare.

Summary – Injection of pigments

Intradermal injection of pigments is normally a safe and reliable batch-marking method, with few or no negative effects on growth, survival, and behaviour. The most common dye used (alcian blue) is non-toxic and non-irritant. However, incorrect adjustment of the tagging equipment (Panjet) has resulted in considerable rapid mortality due to injection of dye into the peritoneal cavity of Atlantic salmon parr. Injection of pigments is therefore a hazard to fish welfare.

Acute welfare effects during tagging – Spraying of fluorescent pigments

This tagging method involves a fluorescent pigment, of various particle sizes, being sprayed onto the surfaces of fish under high pressure, such that the particles penetrate into the dermal tissues. Industrial handheld abrasive blasters have been used for spraying (Schumann et al., 2013). Both user experience and design of equipment are likely to affect the outcome, as illustrated by the large variation in mortality of salmonids (0-97 %) after marking (Schumann et al., 2013 and references therein). Furthermore, Bandow (1987) showed that post-marking mortality increased with increasing pigment delivery force; from no measured mortality at 70 g to 97 % at 330 g. Correct delivery force is important, as tag retention appears to increase with increasing force. At present no consensus or best practices exist to ensure optimal spraying pressure and pigment particle size that will minimize mortality.

Mortality after spray marking has been shown to be size-dependent for several fish species. In orangethroat darter (*Etheostoma spectabile*) mortality was higher for smaller fish, whereas in plains topminnow (*Fundulus sciadicus*), larger individuals suffered higher marking mortality than smaller ones (Schumann et al., 2013). The reason for this size-dependent mortality is unclear.

To our knowledge, studies addressing stress responses during and following marking by spraying are lacking. The main reason for post-marking mortality is believed to be suffocation, as compacted pigment has been found around the gills of dead fish (Bandow, 1987; Schumann et al., 2013). It is likely that the fish that died experienced both stress and pain after marking.

Impacts on welfare post-tagging – Spraying of fluorescent pigments

We did not find any studies investigating potential tissue damage or inflammation associated with spray marking with pigments, and reports on the occurrence of chronic pain and stress due to spray marking were also lacking.

Mortality due to spray marking has been examined for a range of species. Bandow (1987) examined survival using different delivery dye forces for six fish species (Chinook salmon, lake trout, *Salvelinus namaycush*, muskellunge *Esox masquinongy*, channel catfish *Ictalurus punctatus*, largemouth bass *Micropterus salmoides* and walleye *Stizostedion vitreum*). Postmarking mortality for walleye varied from 59 to 79 %, whereas the maximum mortality for the other species was 8 %. Schumann et al. (2013) also measured post-marking mortality for six fish species (orangethroat darter, bluegill, plains topminnow, grass carp *Ctenopharyngodon idella*, black bullhead *Ameiurus melas* and channel catfish). Only black bullhead suffered no mortality, whereas between 9 and 16 % mortality was found for channel catfish, plains topminnow and orangethroat darter. All of the grass carp died, and the mortality for bluegills was 79 %. Most of the mortality due to spray marking with pigments occurs within 72 hours (Hennick & Tyler 1970; Phinney., 1974; Bandow, 1987; Schumann et al., 2013). Considerable mortality (68 %) due to spray marking has also been observed for brook stickleback (Moodie and Salfert, 1982). In addition, spray marking appears to inhibit or reduce post-marking growth, as well as increasing susceptibility to disease, in several fish species (Moodie and Salfert, 1982; Bandow, 1987; Schumann et al., 2013).

No information about the effects of spray on behaviour has been reported.

Spray marking with fluorescent pigment requires capture and handling of fish during inspection. Capture and handling may be stressful and may therefore have an effect on welfare.

Summary – Spraying of fluorescent pigments

Due to the inconsistency in post-marking survival and growth within and among species, fluorescent spray procedures need further investigation and refinement to ensure that this method would not have a negative impact on fish welfare. In some cases, the immediate mortality after marking is unacceptably high. As the reason for mortality appears to be suffocation, the welfare of the marked fish must be assumed to be impaired. The overall impact of spray marking on fish welfare thus seems to vary, depending on species and size. Spraying of pigments is thus a hazard to fish welfare.

2.1.4.3 Visible implant alphanumerical (VIA) tags

Acute welfare effects during tagging - VIA tags

Visible implant alphanumerical (VIA) tags are small pieces of plastic, with alphanumeric characters on them, which are inserted under transparent tissues on fish, e.g. in the eye region (Guy et al., 1996). We have not been able to find any information regarding how experience on inserting VIA tags and functionality of the equipment used might affect fish welfare during and immediately after tagging, although experience appears to be important to ensure optimal tag retention (Wenburg and George, 2011).

Information regarding effects of implantation of VIA tags in relation to environmental variation and fish/size stage is sparse. However, one study indicated that use of VIA tags in two species of rockpool fish (*Girella elevata* and *Bathygobius cocosensis*) *may* result in increased mortality (20 % vs 7 % in the control group) (Griffiths, 2002).

Information on pain or stress associated with implantation of VIA tags is also sparse, but Neufeld et al. (2015) found a minor increase in lactate in blood in western silvery minnow

(*Hybognathus argyritis*), which was interpreted as indicating a low level of stress. As the tagging method involves handling and puncture of the skin, it is likely that some temporary pain/stress may occur.

Impacts on welfare post-tagging – VIA tags

The tissues appear to heal rapidly after insertion of VIA tags (Wenburg and George, 2011), and occurrence of inflammation has not been reported. Mourning et al. (1994) reported that 1 % of juvenile rainbow trout was classified as "seriously injured" following insertion of VIA tags. Published information regarding the potential for chronic pain and stress due to insertion of VIA tags is lacking.

Mortality and growth have been examined for a range of fish species following implantation of VIA tags. Generally, VIA tags are not reported to have any effects on mortality or growth ,(Mourning et al., 1994 – rainbow trout *O. mykiss*; Halls and Azim, 1998 – spot barb *Puntius sophore,* Indian major carp *Catla catla*, snakehead *Channa striatus*, tank goby *Glossogobius giuris*, climbing perch *Anabast testudineus*; Malone et al., 1999 – *Coryphopterus glaucofraenum*; Rikardsen et al., 2001 Arctic charr *Salvelinus alpinus*; Woods, 2005 – pot-bellied seahorse *Hippocampus abdominalis*; Wagner et al., 2013 – spotted seatrout *Cynoscion nebulosus*; Davis et al., 2014 – Rainbow trout *O. mykiss*; Turek et al., 2014 – tiger muskellunge *Esox masquinongy*, cutthroat trout *O. clarki*, rainbow trout *O. mykiss*; Barriga et al., 2015 - Patagonian catfish *Hatcheria macraei*). One exception, however, are small rockpool fish (see above), for which a somewhat increased mortality was found following tagging. We did not find any information on increased infection risk and diseases due to application of VIA tags.

To our knowledge, behavioural effects due to use of VIA tags have not been investigated. Inspection of VIA tags requires capture and handling of fish, which may be stressful for the fish and therefore have an effect on welfare.

Summary

Although VIA tags seem to have few or minor impacts on survival and growth, studies on other potential effects on fish welfare are lacking. Marking fish with VIA tags is nevertheless a potential hazard to fish welfare.

2.1.5 Remotely detectable internal tags - CWT and PIT tags

2.1.5.1 CWT

Acute welfare effects during tagging - CWT

Coded Wire Tags (CWTs) are usually inserted into the muscle, adipose tissue or fibrous tissue in the snout of salmonids. CWT is a well-tested method and automatic equipment has been developed for Pacific salmon, but is still not functional for Atlantic salmon. More than a billion Pacific salmon have been tagged with CWT (Johnson, 1990). Tagging is done by pressing the snout of the fish towards a head mould, whereupon the tag is automatically injected into the snout tissue. This procedure, including vaccination and adipose fin clipping, is completed within a few seconds. The technique and the equipment are simple to use, and the skills needed for manual tagging could be attained relatively easily. If this method is intended for mass marking of farmed Atlantic salmon, a new automatic method for tagging should be developed that is acceptable to fish welfare and practically feasible for Atlantic salmon juveniles.

As CWTs are very small (length: 0.5-2.0 mm, diameter: 0.25 mm) they can be used on salmon sizes down to 1 g. In very small fish (less than 3 cm in length) care must be taken to avoid injury to olfactory nerves and organs (Habicht et al., 1998). Morrison and Zajac (1987) found that 18 of 44 salmon fry tagged with 0.5 mm CWTs had substantial damage to one of the main-stem olfactory nerves. Mass marking of salmon is most appropriate during vaccination, when the fish are 20-40 g and will tolerate the tagging. It is important to adjust the head mould to the size of the fish to ensure correct placement of the tag.

Morrison and Zajac (1987) found inflammation in the tagging area lasting for about 10 days after tagging. Studies in which stress-related physiological or behaviour parameters have been measured for CWT-marked fish are apparently lacking. However, CWT-tagged and adipose fin-clipped fish have been shown to experience stressed during the handling and tagging procedure, but recover within few hours (Sharpe et al., 1998).

Impacts on welfare post-tagging – CWT

We found no reports of long-term injuries caused by CWTs, and CWT will probably not cause chronic pain and stress. Vander Haegen and colleagues (2005) report indications of smaller size of CWT-tagged Pacific salmon at return. However, in Icelandic sea ranching experiments, returning CWT-tagged salmon were larger than Carlin-tagged salmon.

To our knowledge, information on infection risks or diseases due to CWT tagging in Atlantic salmon is lacking. However, Zajac and colleagues (1988) report bacterial kidney disease (BKD) infection related to CWT tagging, so care should be taken should disease, such as BKD, be present in the hatcheries.

Effects on behaviour associated with CWT has not been reported, but whether the magnetized tag may affect homing ability in migrating fish has been discussed (Morrison and Zajac, 1987). However, no indications of increased mis-migration failures have been reported (Vander Haegen et al., 2005).

In order to retrieve CWT-data from individual fish, the CWT must be surgically removed, which, in practice, means killing the fish. For escaped farmed fish that would be killed anyway, this does not constitute an additional welfare problem. Benign recovery of CWTs without killing the fish is possible by placing the tag in shallow tissue (e.g., post-ocular tissue or between fin rays). Oven and Blankenship (1993) described one such approach; they used a magnetized scalpel to recover tags from between fin rays, and a modified syringe to extract tags from the adipose fin and from post-ocular tissue. They also found that a 2 mm biopsy punch was an effective way of removing tags from the adipose fin. An important feature of this method is that the tag should be visible once it has been magnetically detected; “blind” recovery may be more difficult or may involve more trauma to the fish. The rainbow trout used by Oven and Blankenship (1993) more than doubled in size during the experiment and all tags remained visible. However, the efficacy of this approach for recovery of tags from adult salmon that were tagged as parr or smolts, for example, would need careful evaluation.

2.1.5.2 PIT tags

Acute welfare effects during tagging – PIT tags

In small fish, PIT tags are surgically inserted into the body cavity under anaesthesia. The tag is inserted with a hypodermic needle or by making a small cut in the body cavity wall and pushing the tag through. The cut punctures the skin, body wall muscles, and peritoneum, and there is a risk of injuring internal organs if the cut is too deep. Therefore, the tagging operator must be suitably trained, have the necessary surgical skills, and follow the tagging manual (use of preloaded, single-use injectors is recommended by one tag producer (Biomark.com)). Routines must be followed to avoid injuries of internal organs when the tags are placed in the body cavity. Puncturing the body cavity by a needle or scalpel may cause some initial pain and stress, but the fish will soon recover.

Impacts on welfare post-tagging – PIT tags

Surgical implantation of 23 mm PIT tags under anaesthesia without suture closure of the tagging incision was found to be a feasible method for individual marking of Atlantic salmon 100 to 135 mm fork length. (Larsen et al., 2013). Reseachers are cautioned about the use of sutures to close the surgical incisions, due to high rates of fungal infection and tissue inflammation around the incision sites.

PIT tags can be read remotely using PIT antennae on live fish, indicating the possibility of collecting individual data during the production phase.

Summary – CWT and PIT tags

Both CWT and PIT tags are well-tested tags that have been used on millions of fish with few obvious implications for welfare. CWTs have low impact on welfare, performance, and survival of farmed Atlantic salmon, especially if anaesthesia is used. However, any tagging procedure involving handling and anaesthesia involves some risk of errors during the process that could result in some mortality and welfare hazards (Zahl et al., 2010).

PIT tags have minimal impact on performance or welfare if used on sufficiently large fish and provided that the personnel inserting the tags have the necessary surgical skills and follow recommended procedures (Prentice et al., 1990) However, the tagging procedures involve some welfare hazards.

2.1.6 Chemical marking

Acute welfare effects during chemical marking

The potential effects on fish welfare due to chemical marking of otoliths or other calcified structures are evaluated. As the potential welfare effects are likely to be similar for the different substances used for chemical marking, we have not differentiated between the use of pigments or isotopes/trace elements.

To the best of our knowledge, welfare effects associated with varying levels of experience or functionality of equipment have not been investigated for chemical marking of fish. However, chemical marking involves handling, as the fish are exposed to the marker either by immersion, injection, or ingestion. As with all other handling of fish, experience is important to reduce the risks to welfare. Furthermore, experience of using and functionality of equipment is also of importance for markers that are injected, as this involves puncturing the skin with some kind of marking device. In mass marking of farmed salmon, injection of chemical markers would probably be implemented during vaccination (e.g., Warren-Myers et al., 2014). It is highly unlikely that chemical marking during vaccination will affect fish welfare beyond that which is normally experienced with vaccination.

Chemical marking by immersion is usually carried out on fish eggs and larvae. Immersion can take place in two ways; either direct immersion in water with ambient salinity or by momentary immersion in a hypersaline bath (osmotic induction) immediately prior to immersion in water with the relevant chemical (de Braux et al., 2014). Compared with conventional immersion, osmotic induction reduces the time for marker immersion time by increasing the rate of marker uptake. Both methods have been used for marking salmon with isotopes, and no mortality for either treatment following immersion has been reported (Braux et. al. 2014). In general, unacceptable short-term effects on mortality or growth have not been observed following chemical marking through immersion (Bashey, 2004; Taylor et al., 2005; Lu et al. ,2014;2015; Hansen et al., 2015), although immersion in very high concentrations over prolonged periods may result in excessive mortalities during or after immersion (Beckman and Schulz, 1996). Information on immediate mortality due to chemical marking through ingestion or injection that can be ascribed to the marking procedure was not identified.

Injection of a chemical marker will, as for all marking techniques that involve puncture of the skin, involve short-term pain. However, it is difficult to assess the degree of pain experienced, but it is likely to be comparable to the pain experienced with vaccination by injection, which is carried out routinely on all farmed salmon. Chemical marking through ingestion is unlikely to inflict pain or stress. Osmotic induction of the chemical marker may, in theory, be stressful for the fish, but we have not identified any studies in which this effect is assessed in terms of pain or stress. Nevertheless, it seems that any pain or stress experienced following osmotic induction would be considerably less than that associated with delousing in hyposaline water, which is frequently used by the fish farming industry.

Impacts on welfare post-marking

As far as we know, tissue damage, inflammation, chronic pain, and stress have not been reported following chemical marking.

Most studies that have examined the effects of chemical marking in fish have concluded that this type of marking has no persistent effect on the marked organism (e.g. Blom et al., 1994; Tsukamoto, 1988, Tsukamoto et al., 1989, Baumann et al., 2005; Liu et al., 2009; Hansen et al., 2015). However, Meyer and colleagues (2012) found sub-lethal effects due to marking Atlantic cod eggs and larvae with alizarin, in terms of reduced hatching success and reduced growth rates and first feeding success, possibly because the larvae had ingested the compound. In contrast, Blom et al. (1994) and Svåsand (1995) did not find comparable effects when marking similar stages of Atlantic cod.

As far as we know, the long-term effects of chemical marking on fish behavior have been neither studied nor reported.

In most cases in which chemical markers are used, the fish must be killed to allow inspection of the marker. Provided that fish are killed according to established and commonly accepted methods for euthanizing fish, it seems likely that chemical marking would not affect fish welfare beyond that which is accepted in this context

Summary

The majority of studies that have examined potential welfare effects of chemical marking in fish have concluded that this type of marking has no major short-term or persistent effects. However, some concerns have been raised regarding the possibility of reduced viability of larval fish, especially if fish are exposed to unnecessarily high concentrations of markers for prolonged periods. Chemical marking is therefore a potential hazard to fish welfare.

2.2 Methods for tracing fish origin based on natural marks

Use of natural marks for determining the origin of fish is based on analyses of biological samples collected from either live or dead fish. The purpose of collecting samples from live fish is to avoid killing fish with uncertain identity. As no live-capture methods are able to select escapees only, collection of live fish for subsequent tracing of origin means that both escaped farm fish and wild fish are caught. Under such circumstances, visual determination of origin is difficult, as the fish must be handled carefully and rapidly. In order to avoid killing fish that appear to be wild, these are released after sampling. The main purpose of collecting such samples is to determine the actual proportion of escapees in salmon populations as a tool for stock management.

Our evaluation of the potential implications for fish welfare is framed on the following basis:

- If samples are collected from dead fish, then the method of killing should follow procedures that are representative for normal recreational or commercial fishing. As evaluation of methods for killing fish during recreational or commercial fishing are beyond the scope of this report, we have evaluated sampling from live fish only.
- We have not evaluated welfare implications for capture of salmon with conventional gear used in recreational or commercial fishing *per se*, as the objective of this report is not to evaluate welfare during fishing, but welfare issues that result primarily from tagging and tracing of farm-produced salmonids. Thus, we have restricted our assessment to effects from releasing live fish caught by various methods and collection of samples from live fish.
- We have not differentiated between unidentified escaped fish and wild fish when assessing potential effects on fish welfare, as we currently have no reason to assume that the welfare effects will differ between escapees and wild fish.
- As collection of samples from live fish is conducted in the same way for the different methods for tracing origin on the basis of natural marks, we have not distinguished between the different analytical approaches used subsequent to sampling.
- Salmon are captured by using either passive or active equipment (reviewed in Næsje et al., 2013). Passive gear includes: bag nets, fyke nets, permanent fish traps, or gill nets. Active collection methods include use of sports fishing gear, driftnets, shore seines, electrofishing, harpooning, and use of light in combination with hand nets during the night (light fishing). The choice of collection method depends on the situation. In the marine environment, bag nets, fyke nets, gill nets, and drift nets are the most commonly used and suitable collection methods. In rivers, sports fishing gear, shore seines, electrofishing, harpooning, and light fishing are the most appropriate methods. Although electrofishing is an effective and accepted method for live-capture of small fish, it is generally not used for live-capture of large/adult fish in rivers, and therefore is not evaluated here. Harpooning implicitly involves the fish being killed and is not evaluated. Nevertheless, it is important to bear in mind that harpooning could involve fish welfare issues as an unknown proportion of fish are wounded before they are killed. The most common methods for collection of samples from live salmon for tracing their origin are the use of sports fishing gear, bag nets, fish traps, and light fishing. We have therefore focused mainly on the potential welfare effects due to release of fish caught by these methods (hereafter referred to as capture and release or C&R).
- Advances in genetically based analytical methods means it is now possible to use a single fish scale to genotype an individual fish, and thus sampling of any other tissue than scales is not required. Analysis of otoliths involves the fish being killed in order to collect samples

and is not relevant here. Thus, the potential effects on fish welfare due to sampling of scales from live fish are the focus of assessment in this report.

2.2.1 Release of salmonids captured alive for tracing based on natural marks

Acute welfare effects

Little has been reported on the importance of experience and functionality of equipment for C&R of salmon using bag nets, fish traps or light fishing, and the associated effects on fish welfare. This is partly because, as fish with gear-inflicted injuries are typically killed and not released, studies investigating the fate of injured fish have not been carried out. Like all other methods that involve handling of fish it is, however, likely that experience and optimization of equipment are important. It has, for instance, been shown that some of the salmon caught in bag nets are injured in the net to such an extent that their survival if released is uncertain (Rikstad, 2004; Hvidsten and Fiske, 2012). Fiske and Wennevik (2011) reported that approximately 20 % of fish caught in a bag net was seriously injured. Occurrence of injuries can be reduced by using nets with a smaller mesh size than used by commercial fishermen (Rikstad, 2004), but this will increase the by-catch of smaller fish (Strand and Heggberget, 1996). It is also essential to develop an adequate screening protocol to be able to select fish with critical damage, such that these could be killed humanely and not released. Telemetry studies in several rivers have indicated that salmon caught in bag nets, and subsequently tagged with radio transmitters before release, show high survival and most likely also normal progression during up-migration in rivers, provided that only healthy and undamaged fish are tagged (Thorstad et al., 2011). C&R of salmon using sports fishing gear and its influence on fish welfare have been evaluated by VKM in 2009 (VKM, 2009). One of the main conclusions reached in this assessment was that "welfare issues including survival can be improved by selection of correct fishing tackle, handling procedures, and training of anglers and guides". Several guidelines for optimal C&R of salmon caught with sports fishing gear exist.

Water temperature is assumed to be important for survival of salmon after C&R using sports fishing gear. In general, mortality appears to increase when the water temperature exceeds 18 °C (Havn et al., 2015). The size of the salmon may also be related to mortality after C&R angling. As larger fish are stronger, they are often more difficult for anglers to land before they are exhausted, and due to their longer play times they suffer greater physiological disturbance than smaller fish (Dempson et al., 2002; Thorstad et al., 2003). However, Booth et al., (1995) found that post-angling physiological impacts were greater for small salmon (grilse) than for much larger multi-sea-winter fish. Proximity to spawning is also thought to influence survival and behaviour following release of salmon caught with sports fishing gear. It is assumed that salmon become more tolerant of disturbances closer to the spawning season and it has been shown that mortality due to C&R is lower during the autumn than earlier in the season (Booth et al., 1995). In contrast, C&R of spawning fish may affect reproductive behaviour more than if fish were released well before the spawning season.

Mortality due to C&R is mainly acute, as it predominantly occurs during the first 24 hours after release (Muoneke and Childress, 1994; Havn et al.. 2015). Typically, the acute mortality due to C&R varies from zero to 11-12 % at water temperatures below 18 °C (Havn et al., 2015 and references therein; Lennox et al. 2015). In a recent Norwegian study on effects of C&R on salmon, the average mortality in several rivers was found to be 7 % (Uglem pers med.). Most of the fish that died in this study had injuries, had been handled in a sub-optimal way, or had been caught and released at high water temperatures. C&R with sports fishing gear is also often accompanied by the released fish moving a certain distance downstream immediately after release, and a delay in subsequent upstream migration (e.g. Thorstad et al., 2003; 2007; Lennox et al., 2015),. However, provided that the fish are released during the normal fishing season, the majority will be able to continue and finish their spawning migration before the spawning season. How behaviour is affected by C&R in rivers close to the spawning season is not known, and little is known about effects of C&R when using equipment other than sports fishing gear, but it is assumed that smaller fish will suffer more than larger fish when some kind of net is used, as smaller fish are more easily trapped in the net (Næsje et al., 2013)

On the basis of a substantial literature review, VKM (2009) concluded that C&R using sports fishing gear may harm the fish, as well as inflicting pain, but also that the welfare implications were probably temporary. Hence, it is likely that stress and pain in salmon due to C&R using sports fishing gear, with the purpose of collecting samples for tracing the origin of the fish, are only short-term. The potential for fish that are captured with other relevant gear for collection of samples for tracing their origin to experience stress and pain following release has not been reviewed nor, to our knowledge, addressed in any relevant studies. Should fish be seriously injured and then released, or suffer mortality during capture, then it is likely that they experience pain and stress. However, the extent to which fish experience pain and stress is however difficult to assess.

Long-term welfare effects

Capture of salmonids for collection of samples for tracing their origin may typically cause injuries and tissue damages in two ways; either by causing scale loss, skin abrasions, and fin damage due to poor handling, or by injuries in the mouth regions due to catching the fish on a hook (VKM, 2009). Skin damage may occur when using gear that involves physical handling of the fish and could lead to chronic stress and, in turn, increase susceptibility to infections and pathogens. Potential pathogens include fungi (*Saprolegnia* spp.) and opportunistic bacterial infections (e.g. *Aeromonas* spp., *Pseudomonas* spp.) (Ferguson, 2006). Hook damage may occur when using sports fishing gear to capture fish. For instance, Meka (2004) found that 30 % of rainbow trout in a C&R fishery in Alaska had at least one scar consistent with hooking. The extent to which injuries or damage due to different types of gear used for capturing salmon for tracing their origin affects survival or welfare after release is not known, but it is reasonable to assume that some negative welfare effects will occur.

Occurrence of chronic pain or stress after release of fish caught for collection of samples for tracing of the origin of salmonids has, to our knowledge not been investigated for fish in the wild. As most of the mortality, following C&R of fish caught, for example, with sports fishing gear, seems to occur within a few days after release, the probability of long-term effects, in terms of pain and chronic stress, may be relatively limited.

Most of the mortality of fish captured and released with sports fishing gear occurs during the first days after release and the long-term mortality appears to be low (Muoneke & Childress, 1994; Havn et al., 2015). To our knowledge, long-term mortality following release has not been investigated or determined for other relevant fishing gear, but several experiments on radio-tagged salmon caught in bag nets indicate that long-term survival is high (Thorstad et al., 2011 and references therein). Fish caught and released are subject to wounds during hooking, and the skin may be damaged due to handling prior to release (VKM, 2009).

However, it is has not been demonstrated whether fish caught with sports fishing gear and thereafter released are more prone to secondary infections through wounds, but this cannot be excluded. Injuries (skin damage, gill lesions etc.) may be invaded by opportunistic aquatic pathogens (particularly *Saprolegnia* spp.) that may cause severe lesions and increased mortality (Ferguson, 2006; VKM, 2009).

C&R using sports fishing gear may result in a delay in migration to the spawning areas for salmon (Thorstad et al., 2003; 2007; Lennox et al., 2015). In some cases, this delay may be several weeks. Despite a significant delay in migration, reproduction would probably not be seriously affected as the majority of the salmon that experience delayed migration are observed at known spawning areas during the spawning season. Behavioural effects on fish captured and released with other relevant gear, or close to the spawning seasons, have not been investigated or reported.

Summary - release of salmonids captured alive for tracing of origin

Apart from C&R of salmon with sports fishing gear, little is known about the welfare effects due to release of fish captured for collecting samples for tracing their origin. However, it is well documented that C&R of fish caught with sports fishing gear has a negative effect on welfare for a proportion of the fish, and it seems likely that the same would also be the case for fish captured with other relevant fishing gear. In addition to optimization of handling procedures, it is important to establish adequate routines for selecting and euthanizing fish that, due to gear-inflicted injuries, are likely to experience reduced welfare after release. If seriously injured fish are euthanized, the welfare effect on released fish is assumed to be low.

2.2.2 Collection of scales for tracing origin based on natural markers

Scales are collected from live fish with a pair of pliers or tweezers. Between four and eight scales are collected from each fish (Anon, 2016). Anaesthesia is not used. Studies on the effects of scale sampling from live fish, which are subsequently released, are lacking. Thus, our assessment is based on own knowledge and personal communications.

Acute welfare effects - Scales

Scale sampling from salmon for tracing their origin involves handling relatively large and strong live fish. Hence, experience with fish handling is likely to be important for reducing potential negative impacts. The equipment used is very simple, but as scales may be difficult to remove, especially close to the spawning season, experience will be important to ensure that scales are sampled rapidly and efficiently (Fiske, P. pers. comm.). It seems likely that potential welfare effects will be reduced with experience with the method.

It is common that fish react by “jumping” or “wincing” when scales are removed (Thorstad, E. pers comm.). This is seldom observed when items such as external radio transmitters are attached under the dorsal fin of fish, and may be because scales are collected from an area close to the lateral line, which is a particularly sensitive area. The extent to which this reaction reflects pain is not known. As with all methods that involve handling of live fish, it is reasonable to assume that scale sampling will be stressful for fish to some extent.

Impacts on welfare post-removal of scales

As scales protect the skin against the external environment, it is possible that scale removal may result in tissue damage and inflammation in the same way as for injuries caused by different capture methods. However, fish are able to regrow scales that, over time, will cover the exposed area. Since only a few scales are removed, the probability of major damage is relatively low. Nevertheless, the actual effects of scale removal of this magnitude have not been investigated.

If a fish develops secondary infections due to sampling of scales, it is also possible that it will experience chronic pain and stress. The probability of developing infection and disease due to scale sampling from live fish for tracing their origin is not known. As for skin damage and lesions caused by C&R there is, in general, a potential for development of disease and infection, with subsequent mortality (VKM, 2009). Effects on behaviour due to scale sampling from live fish have, to our knowledge, not been investigated or reported.

Summary – Collection of scales for tracing of origin based on natural markers

Investigations of whether sampling of scales from live salmon affects their welfare following release have not been conducted. As salmon show an immediate behavioural response during scale removal, it is possible that they experience short-term pain, but the extent of this is difficult to assess. Furthermore, because removal of scales may be associated with development of secondary infections, there is also the potential for chronic effects to occur. Scale sampling is, nevertheless, a relatively minor intervention compared with many tagging methods, and is unlikely to pose a considerable hazard to fish welfare.

3 Risk characterization

The risk of reduced fish welfare is defined as the probability that a hazard will cause welfare reduction, multiplied by the degree of consequence of this welfare reduction.

Parameters such as pain, discomfort, infections, mortality and stress responsiveness were taken into account with equal measures when addressing reductions in fish welfare.

In this assessment, the project group has chosen a three-grade scale concerning probabilities and consequences. The term «fish» refers to the relevant part of the fish population, i.e. fish subjected to the given method of marking. Numbers are estimated.

- Low probability: <1 % fish will experience reduced welfare
- Moderate probability: 1 – 10 % fish will experience reduced welfare
- High probability: >10 % of fish will experience reduced welfare

In this context, consequence relates to the severity of the effects of the different marking methods on fish welfare, graded as:

- Limited consequence: No or limited consequence, e.g. low grade or only transient pain, fear, or other negative affective states, for fish welfare
- Moderate consequence: Moderate consequence, e.g. more prominent or lasting pain, fear or other affective states, for fish welfare
- Serious consequence: Serious consequence, e.g. severe or long-lasting pain, fear, or other negative affective states (including increased mortality and reduced fitness), for fish welfare

The effects related to marking may be acute (during the process of marking or shortly after) or appear as a consequence of the marking, causing chronical or long term effects. The risk characterization is therefore divided and presented as two different tables; one presenting acute effects, the other presenting long-term effects.

In the following risk charts of this chapter, different levels of risk are represented as follows:

- Low risk: Green
- Moderate risk: Yellow
- High risk: Red

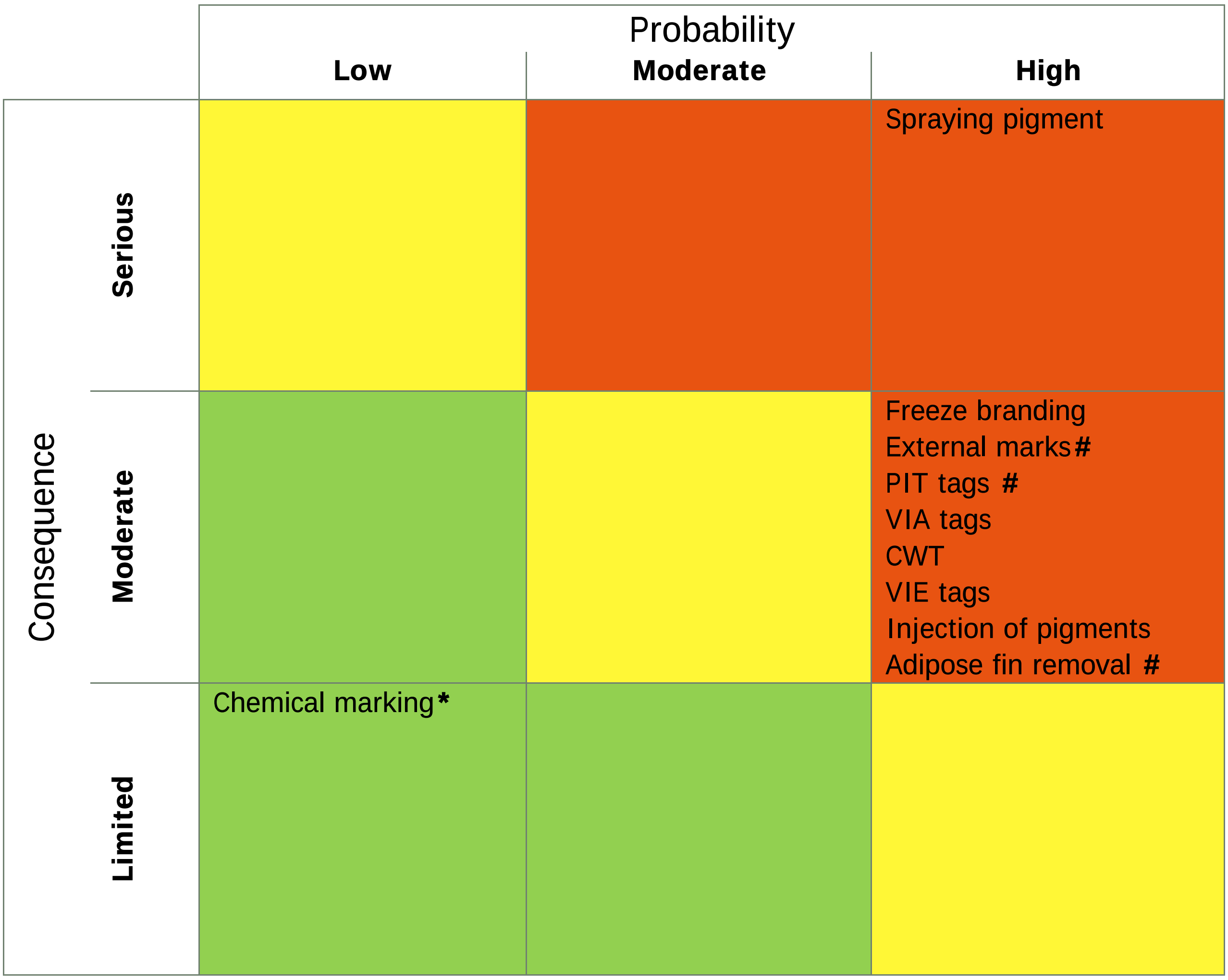

Figure 3-1. Estimates of the risk of acute or short-term reductions in fish welfare associated with marking methods. * indicates that the fish can be tagged in combination with vaccination, # indicates knowledge gaps associated with ranking of risk estimation.

Consequence		Probability: Low	Probability: Moderate	Probability: High
	Serious			Spraying pigments
	Moderate	PIT tags**#**	Adipose fin clipping**#**	External marks**#**
	Limited	Chemical marks*****		Freeze branding**#** VIE tags Injection of pigments VIA tags CWT

Figure 3-2. Estimates of the risk of reductions in fish welfare associated with persistant, long-term or chronic effects of marking methods. ***** denotes that the fish can be tagged in combination with vaccination, **#** indicates knowledge gaps associated with ranking of risk estimation.

4 Uncertainties and data gaps

In this risk assessment, some uncertainties and data gaps remain in relation to how marking methods affect fish welfare. These include:

- Removal of body parts, such as the adipose fin. Since the adipose fin is innervated, it is possible that the fish will feel pain following removal. Information on the functional role of the adipose fin is scarce.
- Information on how some of the marking methods (for example freeze branding, externally visible tagging methods or visible internal tags) affects fish under different environmental conditions or different life stages is limited or non-existing.
- Overall, few studies on fish welfare, concerning pain or behavior, are available within this context. Most of these have rather focused on fish health parameters, such as growth and survival.
- Most of the available information, regarding effects of marking methods on fish welfare, is based on small-scale trials.
- It is difficult to assess to what extent fish experience aversive stress responses and fear, in relation to C&R for subsequent sampling and analysis

.

5 Answers to the terms of reference

1. **How do the described marking methods affect the welfare of farmed fish and fish for cultivation?**

In this section, the effects on fish welfare for each of the described marking methods are addressed. As mentioned in the beginning of chapter 2, we assume that sufficient measures have been taken to reduce the negative effects on welfare during the marking procedure through appropriate administration of an efficient anesthetic. Details underlying the classification of risks are provided in chapters 2 and 3.

Adipose fin removal

Removal of the adipose fin may involve a risk of affecting fish welfare, both on a short-term and long-term time-scale. In terms of acute effects, surgical removal of a fully functional body part from the fish will result in a ***high*** risk of reduced welfare. Although the clipping would be performed in connection with other procedures (e.g., vaccination) when the fish is anaesthetized and will feel no pain during the surgery, there will probably be some pain during recovery, before the wound is healed. Although the technique is easy to learn, the skills of the person clipping the fin is crucial and operator fatigue may occur. There is also a risk of clipping too deeply when this is done manually, and this may also cause pain. The risk of permanently reduced fish welfare is, however, considered ***moderate***, with some uncertainties related to the lack of knowledge. For most individuals, the wound heals within a short period after clipping. Clipping too deep may not only increase wound healing time, but may also increase the risk of a long-lasting wound infection. The functions of the adipose fin are not fully understood, and lack of the fin may affect swimming behaviour and possibly social behaviour on the spawning grounds. These negative effects are probably low in caged salmonids.

Clipping is usually done on juveniles, during vaccination or handling (transfer to a new environment). Although adipose fin clipping requires extra handling, it may be carried out on a large scale. Clipping may also be automatized. The adipose fin does not regenerate, and thus the marking is permanent. As fin clipping allows differentiation into one of two categories, (i.e., marked or unmarked), the method is only adequate as a visual mark, that indicates whether a fish is farmed (clipped) or wild (unclipped).

Freeze branding

Freeze branding affects the welfare of fish, both on a short-term and long-term timescale. The risk of reduced fish welfare in the short-term is considered ***high***. The fish may experience stress linked to handling, anaesthesia, and marking. The mark is essentially a wound that heals, and it is assumed that marking results in temporary pain or stress. The short-term effects may also include increased mortality due to predation of the marked fish, probably because of general stress inflicted during tagging. On a more long-term time-scale, freeze branding is considered a ***moderate*** risk for reduced fish welfare.

The freeze branding method is simple and easy to learn, but skills in fish handling fish are necessary. Freeze branding has been used for marking fish of salmon parr/smolt size, but is probably not suitable for smaller fry. No automated methods for large-scale freeze branding exist. The mark will fade as the skin heals, and is therefore a short-term mark. As the number of codes that can be constructed is relatively low, freeze branding is suitable for batch marking, and not for individual identification of a large number of fish.

Externally attached visible tags

A variety of external tags are available. Marking with external tags affects the welfare of fish, both short-term- and long-term. To varying extents, these marks irritate the skin and increase the risks of wounds and infections. From a short-term perspective, the risk of reduced fish welfare is considered ***high***. The smaller types of external tags can be used on small fish, but are generally not recommended as the negative effects appear to be negatively correlated with fish size. Long-term effects include elevated mortality and increased predation risk; thus external tags remain a ***high*** risk of reduced fish welfare.

External tagging involves minor surgical interventions and a certain level of skill is necessary in order to place the tag correctly and reduce the risk of infections. Although general skills in fish handling is necessary, the method is usually easy to learn. Small external tags can be used for salmon parr/smolt size. Automated methods for mass marking do not exist at present.

The tag retention varies depending on type of external tags, but tag loss always occurs to some extent. Therefore, the use of external tags does not represent a reliable permanent marker, particularly as the degree of tag loss is usually difficult to estimate in a natural situation. The number of possible codes is very high and therefore external tags could be suitable for individual identification of a high number of fish, although tag loss will result in some uncertainty.

Visible internal tags

Visible internal tags include pigments that are injected into the tissues of the fish, either as a liquid that solidifies into a coloured mark (VIE mark), or as a liquid that forms a coloured or fluorescent mark in the skin.

The risk of reduced fish welfare in the short-term is considered ***high***, mainly due to puncturing of the skin and injection of a liquid substance. A certain level of skill is required, although the method is usually easy to learn. General skills in fish handling are also necessary. Visible internal tags can be used on small fish and therefore this is an adequate method for tagging salmon parr/smolt-sized fish. If tagging is done correctly, the risk of reduced fish welfare on a longer timescale is assumed to be ***moderate***

Fully automated methods for mass marking do not currently exist. Tag retention varies depending on types of visible internal tags, and, in some cases, the visibility of the tags decreases as the fish grows because, over time, the tags become positioned to deeply in the fish tissues to allow visual detection. Considerable tag loss has been reported, and therefore, visible internal tags do not represent an adequate permanent marker. The number of codes is limited and visible internal tags are applicable only for batch marking.

Injection of pigments

Intradermal injection of pigments is normally a safe marking method, with few or no negative effects on growth, survival, and behaviour. However, erroneous adjustment of the tagging equipment may result in considerable mortality. Although the technique is easy to learn, a certain level of skill with the marking equipment is necessary, as well as experience in fish handling. The marking method can be used on small fish and is thus suitable for marking of salmon parr/smolt-sized fish. Semi-automated equipment for injection of pigments exists, and there is the potential for development of fully automated equipment that could be used for marking fish during vaccination.

Tag tag retention is high for some pigments, but it is uncertain whether the retention will be adequate for tracing the origin of escaped salmonids, especially if fish are tagged as parr or smolts. The number of codes that can be constructed is limited and injection of pigments is most suitable for batch marking. In terms of acute effects, tagging fish with injection of pigments poses a ***high*** risk for reduced fish welfare. This risk is reduced to ***moderate*** from a long-term perspective.

Spraying of pigments

Pigments may be sprayed onto the skin of fish under high pressure, penetrating into the tissues. There are several reports of significantly increased mortalities after marking, most likely due to suffocation. The mortalities vary among species and according to methodological approaches. A standardized methodology that ensures minimal welfare effects has not been developed. The risk of reduced fish welfare is considered ***high***, both short-term and long-term.

The tagging method is, in itself, simple and the necessary skills would be easily attained. General skills in fish handling are also required. In theory, the method can be used on all sizes of the fish, but the negative effects appear to be more pronounced with smaller fish. The mark fades over time and, thus, this method is not suitable for long-term marking. Automated methods for large-scale marking do not exist. The number of codes is limited and spray marking is thus a batch-marking method.

Visible implant alphanumerical tags (VIA or eye tags)

Visible implant alphanumerical (VIA) tags are small pieces of plastic that can be implanted under transparent tissues, e.g., in the eye of fish. The short-term risk of reduced fish welfare is considered ***high***, which is reduced to ***moderate*** in longer timescale. Use of VIA tags involves minor surgical interventions and a certain level of skills is required, although the method is usually easy to learn. General skills in fish handling are also necessary. VIA tags can be used for salmon parr/smolt-sized fish. Automated methods for mass marking do not exist. A high degree of tag loss has been reported, and thus, this method is not reliable for long-term marking. The number of codes can be very high and therefore, if tag loss could be reduced, VIA tags could be used for individual identification of a large number of fish.

Coded wire tags (CWT or snout tags)

Coded wire tags (CWT) are very small (0.5-2.2mm long, 0.25 mm diameter), coded, magnetized stainless steel rods that can be inserted into the tissues. In salmon, the most common tagging area is the snout of the fish, usually in combination with adipose fin clipping. Except for the general risk of stress from handling the fish, there are few impacts on welfare from the tagging. In very small fish, there is a risk of injuring olfactory nerves. Relatively little training is required to become proficient in CWT tagging, and, by following well-tested procedures, the operator can insert CWT with little risk of harming the fish or causing later mortality. CWT have minimal effects of growth, mortality, or behaviour.

As for all methods, fish should be healthy and tolerant of handling. Fish should be at least 50 mm at tagging, and the method is thus suitable for salmon parr/smolt. The risk of tag loss is low, provided that the tagging is perfomred properly, and the high number of possible codes makes it possible to identify a virtually infinite number of fish. CWT can be remotely detected, but, to allow identification of individual fish, must be surgically removed from the fish *post mortem* and read under a binocular microscope. In terms of acute effects, CWT-marking poses a ***high*** risk of reduced fish welfare.

The tagging procedure is relatively fast and the tags are cheap and suitable for mass marking of fish. However, reading the tags is slow and expensive, and industrial-scale use would involve considerable logistical issues. For tagged fish to be detected without using an electromagnetic reading device, the method should be combined with adipose fin removal. For the long-term, CW tags represent a **moderate** risk of affecting fish welfare. It should be noted that CWT that are not removed may represent a problem for consumers if fish heads are used as food. However, since the CW tag is biologically inert, it represents no hazard to human health (http://www.nmt.us/support/appnotes/apc03.pdf)

Passive Integrated Transponders (PIT-tags)

PIT-tags are surgically implanted into fish. Relatively little training is required to perform this technique, and, by following well-tested procedures, operators can perform the tagging with little risk of harming the fish or causing later mortality. Tags should be located in the ventral area of the abdominal cavity, somewhere between the pyloric ceca and the pelvic girdle, generally in the fatty tissue just posterior to the pyloric ceca. Tagging is done under anaesthetics, either using a tag injection needle or by making a small cut with a scalpel and inserting the tag by hand. Subsequent pain management is common, and post-operative complications are unusual.

As with all methods, fish should be healthy and tolerant of handling; their length should be at least 65 mm, but preferably above 80 mm. The risk of tag loss is low, if the tagging is performed properly, and the high numbers of possible codes means it should be possible to identify an infinite number of fish. The tags can be read remotely (distance from tag < 1 m). Tagging usually has no effects on growth and behaviour. However, as the tag is made of glass and metal it may represent a food safety issue. The tagging procedure is relatively slow, and the current price of tags probably means that this method is too expensive for mass marking of fish.

PIT tags represent a ***high*** risk of having a negative short-term effect on fish welfare, but this risk is reduced to ***low*** over time.

Chemical marking

Chemical marking involves marking otoliths or other calcified structures with a suitable chemical by exposing the fish by immersion, injection, or ingestion. Most of the studies that have investigated the potential welfare effects of chemical marking in fish, have concluded that this type of marking has no major short-term or long-term effects, provided that immersion in very high concentrations over prolonged periods is avoided. If the chemical is injected, puncture of the skin may inflict temporary pain. The risk of reduced fish welfare due to chemical marking is considered ***low***, on both the short-term and long-term scale.

As chemical marking may involve exposure through immersion, as well as puncture of the skin, good fish handling skills are required. The marking methods are not difficult to learn, but, as with all marking of fish that involves handling and skin puncture, experience is important for achieving optimal results. Chemical marking can be performed for all life stages of fish, but there are some concerns regarding the possibility of reduced viability of larval stages, especially if fish are exposed to unnecessarily high concentrations of chemicals over longer periods. Automated methods for mass marking do currently not exist, but there is the potential for developing automated vaccination equipment that simultaneously injects a chemical marker. The chemical markers are integrated into calcified structures and the marking is permanent. The number of possible codes, however, is relatively low and the method is most suitable for batch marking; that is, it may be possible to compose individual codes that identify the origin the fish at the company or fish farm level.

2. **Is there a risk of negative welfare implications when using the described tracing methods for farmed fish? If yes,**
    a. **Describe the risk.**
    b. **Describe possible mitigation measures.**

Tracing the origin of salmonids on the basis of natural marks, e.g,. genetic variation or composition of earth elements, has been regarded as a method with few or no negative effects on fish welfare, as the tracing is not based on tagging *per se*, but merely uses a natural intrinsic signature. Thus, in general, tracing on the basis of natural marks will not be associated with reduced fish welfare due to the tracing method *per se*. provided that the necessary biological samples are collected from fish using accepted methods in recreational or commercial fisheries, or similar.

However, if the samples are collected under extraordinary fishery efforts, with the intention of collecting samples for the sole purpose of tracing escaped salmonids, fish welfare may be compromised. If the extraordinary sampling is conducted following the accepted principles for capture in commercial or recreational fisheries, no additional negative welfare effects should occur. However, if fish are released after collection of biological samples (scales) fish welfare may be compromised as a result of: a) negative impacts due to C&R or b) due to scale sampling from live fish that are subsequently returned to the river.

C&R practice was evaluated by VKM in 2009 and it was concluded that it has the potential to harm fish, and thus, implicitly, may also reduce fish welfare. Little is known about how scale sampling from live fish affects its welfare, but observations indicate that fish show a “startle reaction” when scales are removed, and there is also the potential for secondary infections to occur due to removal of scales. More knowledge is needed regarding effects of C&R in monitoring/surveillance fisheries during autumn and close to the spawning period, as well as the potential for infections due to scale removal. Mitigation measures should be based upon the recommendations made by VKM in 2009 for C&R, and only the minimum number of scales sampled.

However, as the negative welfare effects of C&R are normally to be considered minor, as far as concerning long-term perspective, and scales only a few are sampled from each fish, it seems likely the overall fish welfare impact associated with tracing on the basis of natural marks would be minor.

The potential for negative welfare effects associated with tracing of origin by marking of fish, i.e., by using artificial marks instead of natural marks, is addressed in the previous section.

3. **Which combinations of marking and tracing methods are feasible in practice without increasing the risk of reduced animal welfare?**

In this context, it is assumed that combinations of marking/tracing methods should include a marking method that primarily provides a visible indication of whether a fish has escaped from a farm, and a tracing or marking method that enables identification of the origin of escaped fish. The rationale behind this approach is that a visible mark would provide a means for identifying escaped salmon and removing them from the river without the risk of killing wild fish, and subsequent analysis of a tag or utilization of biological samples from these fish could then be used for tracing fish back to the appropriate level. We have evaluated possible combinations on the basis of their effects on fish welfare, and not on the basis on their reliability or economic feasibility, as this is not within the remit of the assessment.

There are no combinations of marking and tracing methods that are feasible without an increased risk of reduced animal welfare.

Chemical marking and PIT tags were evaluated as the single visible marking methods that have the least effects on fish welfare, as far as concerning long-term scale. The methods for subsequent tracing that were assessed as having the least effect on fish welfare were all types of natural marks, followed by removal of adipose fin, the use of CW tags, VIA tags, VIE tags, freeze branding, and injection of pigments. These visible marking methods can be combined with the tracing of natural marks with a *low* to *moderate* probability of increased risk of reduced animal welfare.

4. **Which consequences for fish welfare has the different combinations of feasible marking and tracing methods?**

As there are many combinations that individually represent a low or moderate risk of reduced welfare, there are also a multitude of combined consequences. The potential consequences from each method are summarized in section 2. Empirical knowledge regarding the consequences from combinations of marking and tracing methods is lacking.

6 Conclusions

A variety of marking and tracing methods are available. The marking methods differ regarding their suitability for differentiating between wild and farmed (escaped) fish in the field.

Adipose fin removal is a permanent marking method, as the adipose fin does not regenerate as long as the entire fin is removed. Other external marks may be lost or fade over time. All externally applied marks have an impact on fish welfare. With most marking methods, the risk of reduced fish welfare reduces over time. Natural or chemical marks have the lowest risk of reduced fish welfare, but require analysis after catching and sampling.

Tracing methods differ regarding whether they can be used to trace fish back to their origin, at the individual level or the farm level; this is mainly due to the potential number of codes that can be derived with the different methods.

In order to identify escaped fish and trace them back to their farm of origin, a combination of a marking approach and a tracing method, with sufficient codes, is necessary. Visible tracing methods, like adipose fin removal, VIA tags, VIE tags, freeze branding, or injection of pigments may be combined with tracing of natural marks or use of internal CWT- or PIT tags that can enable identification of fish on an individual level.

Most marking methods represent a ***high*** risk of reduced fish welfare during or shortly after marking, as a very high number of fish would be involved, but ***low*** to ***moderate*** increased risk of reduced fish welfare on a long-term scale. Spraying of pigments and most externally attached tags represent the highest risk of reduced welfare from a long-term perspective.

There are no combinations of marking and tracing methods that are feasible without an increased risk of reduced animal welfare. These conclusions are summarized in Table 3.

Table 3. A summary of the methods of marking and tracing of fish evaluated in this risk assessment. The table distinguishes between methods that identify fish visually (usually after catching the fish), with a sensor, or after killing the fish and subsequent analysis. The risk of reduced fish welfare at a long-term scale relates to Figure 3-2.

Method	Remotely detectable (after catching)	Risk of long-term effects of reduced welfare	Identification at individual (I)- or farm (F) level	Comment
Adipose fin removal			F	
Freeze branding			F	Mark fades over time
Externally attached visible tags			I	High loss of markers, risk of infections / complications
Injected pigments			F	
Sprayed pigments			F	
VIE tags			I	
VIA tags			I	
PIT tags	**Detectable with sensor**		I	Marker embedded in glass
CWT	**Detectable and readable with sensor *after* killing fish**		I	May be detected with sensor at very close range
Chemical marking			F(?)	Limited number of codes
Natural marks			F(?)	Limitation on number of codes
DNA			I	Register required

7 References

Adey E.A., Black K.D., Sawyer T., Shimmield T.M., Trueman C.N. (2009) Scale microchemistry as a tool to investigate the origin of wild and farmed *Salmo salar*. Mar Ecol Prog Ser. 390:225-235.

Aiello B.R., Stewart T.A., Hale M.E. (2016) Mechanosensation in an adipose fin. Proceedings of the Royal Society B-Biological Sciences 283. DOI: 10.1098/rspb.2015.2794.

Andrews M., Stormoen M., Schmidt-Posthaus H., Wahli T., Midtlyng P.J. (2015) Rapid temperature-dependent wound closure following adipose fin clipping of Atlantic salmon *Salmo salar* L. J. Fish Dis. 38:523-531.

Anon. (2016) Status for norske laksebestander i 2016. Rapport fra Vitenskapelig råd for lakseforvaltning nr 9, 190 s.

Anon. (2015) Status for norske laksebestander i 2015. Rapport fra Vitenskapelig råd for lakseforvaltning nr. 8, 300 pp, + Vedleggsrapport nr. 8b, 785 pp (in Norwegian).

Armstrong G.C. (1949) Mortality, Rate of Growth, and Fin Regeneration of Marked and Unmarked Lake Trout Fingerlings at the Provincial Fish Hatchery, Port Arthur, Ontario. Transactions of the American Fisheries Society 77:129-131. DOI: 10.1577/1548-8659(1947)77[129:MROGAF]2.0.CO;2.

Ashton N.K., Ireland S.C., Cain K.D. (2013) Artificial Marker Selection and Subsequent Tagging Evaluations with Juvenile Burbot. Transactions of the American Fisheries Society 142: 1688-1698.

Aursand M., Standal I.B., Prael A., McEvoy L., Irvine J., Axelson D.E. (2009) 13C NMR pattern recognition techniques for the classification of Atlantic salmon (*Salmo salar* L.) according to their wild, farmed, and geographical origin. J Agric Food Chem 57:3444-3451.

Axelson D.E., Standal I.B., Martinez I., Aursand M. (2009) Classification of wild and farmed salmon using Bayesian belief networks and gas chromatography-derived fatty acid distributions. J Agric Food Chem 57.

Bailey R.E., Irvine J.R., Dalziel F.C., Nelson T.C. (1998) Evaluations of visible implant fluorescent tags for marking coho salmon smolts. North American Journal of Fisheries Management 18:191-196.

Bandow F. (1987) Fluorescent pigment marking of seven Minnesota fish species. Investigational Report No. 393. Minneapolis: Minnesota Department of Fisheries 1987.

Barlow C.G., Gregg B.A. (1991) Use of circuli spacing on scales to discriminate hatchery and wild barramundi, *Lates calcarifer* (Bloch). Aquaculture and Fisheries Management 22:491-498.

Barriga J.P, Chiarello-Sosa J.M., Juncos R., Battini M.A. (2015) Photo-identification and the effects of tagging on the Patagonian catfish *Hatcheria macraei* Environ Biol Fish 98: 1163-1171.

Bashey F. (2004) A comparison of the suitability of alizarin red S and calcein for inducing a nonlethally detectable mark in juvenile guppies. Trans Am Fish Soc. 133: 1516–1523.

Baumann H, Peck MA, Herrmann JP (2005) Short-term decoupling of otolith and somatic growth induced by food level changes in postlarval Baltic sprat, Sprattus sprattus. Marine and Freshwater Research 56: 539–547.

Beckman D.W., Schulz R.G. (1996) A simple method for marking fish otoliths with alizarin compounds. Trans Am Fish Soc 125:146-149.

Behrens-Yamada S., Mulligan T.J., Fairchild S.J. (1979) Strontium marking of hatchery-reared coho salmon (*Oncorhynchus kisutch*, Walbaum) J Fish Biol 14:267-275.

Bergmann P.K., Haw F., Blankenship H.L., Buckley R.M. (1992) Perspectives on design, use, and misuse of fish tags. Fisheries 17:20-25. 17:20-25.

Blankenship H. L. (1990) Effects of time and fish size on coded wire tag loss from chinook and coho salmon. Fish-Marking Techniques 7():237-243.

Blom G, Nordeide JT, Svåsand T, Borge A (1994) Application of two fluorescent chemicals, alizarin complexone and alizarine red S, to mark otoliths of Atlantic cod, Gadus morhua L. Aquaculture and Fisheries Management 25, 229–243.

Booth R.K., Kieffer J.D., Davison K., Bielak A.T., Tufts B.L. (1995) Effects of late-season catch and release angling on anaerobic metabolism, acid–base status, survival, and gamete viability in wild Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 52: 283–290.

Bouska W.W., Paukert C.P. (2010) Effects of visible implant elastomer mark colour on the predation of red shiners by largemouth bass. Fisheries Manag Ecol 7: 294–296.

Bracke M.B.M., Metz J.H.M., Spruijt B.M. (1999a) Overall animal welfare reviewed. Part 2: Assessment tables and schemes. Netherlands Journal of Agricultural Science 47:293-305.

Bracke M.B.M., Spruijt B.M., Metz J.H.M. (1999b) Overall animal welfare assessment reviewed. Part 1: Is it possible? Netherlands Journal of Agricultural Science 47:279-291.

Bracke M.B.M., Spruijt B.M., Metz J.H.M. (1999c) Overall animal welfare reviewed. Part 3: Welfare assessment based on needs and supported by expert opinion. Netherlands Journal of Agricultural Science 47:307-322.

Braithwaite V.A. (2010) *Do fish feel pain?* Oxford: Oxford University Press.

Breward J., Gentle M.J. (1985) Neuroma formation and abnormal afferent nerve discharges after partial beak amputation (beak trimming) in poultry. Experientia 41:1132-4.

Bricknell I.R., Bruno D.W. (1995) Sudden fish death associated with alcian blue marking. Bull. Eur. Ass. Fish. Pathol 15: 91-92.

Bridcut E.E. (1995) A Coded Alcian Blue Marking Technique for the Identification of Individual Brown Trout, *Salmo trutta* L.: An Evaluation of Its Use in Fish Biology. Biology and Environment: Proceedings of the Royal Irish Academy 93B: 107-110.

Broom D.M. (1986) Indicators of poor welfare. British Veterinary Journal 142(6):524-526.

Buckland-Nicks J.A., Gillis M., Reimchen T.E. (2012) Neural network detected in a presumed vestigial trait: ultrastructure of the salmonid adipose fin. Proceedings of the Royal Society B-Biological Sciences 279:553-563. DOI: 10.1098/rspb.2011.1009.

Bushon A.M., Stunz G.W., Reese M.M. (2007) Evaluation of visible implant elastomer for marking juvenile red drum. North American Journal of Fisheries Management 27:460-464. DOI: 10.1577/m06-157.1.

Campana S.E., Jones C.M., Gunther D., Tubrett M., Longerich H., Jackson S., Halden N.M., Kalish J.M., Piccoli P., de Pontual H., Troadec H., Panfili J., Secor D.H., Severin K.P., Sie S.H., Thresher R., Teesdale J., Campbell J.L. (1997) Comparison of accuracy, precision, and sensitivity in elemental assays of fish otoliths using the electron microprobe, proton-induced X-ray emission, and laser ablation inductively coupled plasma mass spectrometry. Can J Fish Aquat Sci 54:2068-2079.

Cane A. (1981) Tests of some batch-marking techniques for rainbow trout (*Salma gairdneri* Richardson). Fisheries Management 12: 1-8.

Carlin B. (1955) Tagging of salmon smolts in the River Lagan. Rep Inst Freshwat Res Drott 36:57-74.

Catalano M.J., Chipps S.R., Bouchard M.A., Wahl D.H. (2001) Evaluation of injectible fluorescent tags for marking centrarchid fish: retention rate and effects on vulnerability to predation. North American Journal of Fisheries Management 21: 911–917.

Champigneulle A., Escomel J., Laurent P. (1987) Marquage d'ombles chevalier (*Salvelinus alpinus*) de petite taille par injection de miromarques magnétisées (in French). Bull. Fr. Pêche. Piscic. 304:22-31.

Chandroo K.P., Duncan I.J.H., Moccia R.D. (2004) Can fish suffer?: perspectives on sentience, pain, fear and stress. Applied Animal Behaviour Science 86:225-250. DOI: 10.1016/j.applanim.2004.02.004.

Close T.L., Jones T.S. (2002) Detection of visible implant elastomer in fingerling and yearling Rainbow Trout. North American Journal of Fisheries Management 22:961-964.

Cooke S.J., Midwood J.D., Thiem J.D., Kimley P., Lucas M.C., Thorstad E.B., Eiler J., Holbrook C., Ebner B.C. (2013) Tracking animals in freshwater with electronic tags: past, present and future. Animal Biotelemetry Cooke, S.J., Midwood, J.D., Thiem, J.D., Klimley, P., Lucas, M.C., Thorstad, E.B., Eiler, J., Holbrook, C. and Ebner, B.C. 2013. Tracking animals in freshwater with electronic tags: past, present and future. Animal Biotelemetry, 1:5. DOI: 10.1186/2050-3385-1-5.

Crozier W.W. (1993) Evidence of genetic interaction between escaped farmed salmon and wild Atlantic salmon (*Salmo salar* L) in a Northern Irish river. Aquaculture 113:19-29. DOI: Doi 10.1016/0044-8486(93)90337-X.

Dahl K. (1910) Alder og vekst hos laks og ørret belyst ved studier av deres skjæl Centraltrykkeriet, Kristiania (Oslo).

Damsgård B., Juell J.E., Braastad B.O. (2006) Welfare in farmed fish. Tromsø; Fiskeriforskning 5:1-111.

Davis J.L., Barnes M.E., Kientz J.L., Galinat A.G. (2014) Effects of Fish Length and Anatomical Placement on Retention of Visible Implant Alpha Tags in Hatchery-Reared Rainbow Trout. North American Journal of Fisheries Management 34: 932-937.

de Braux E, Warren-Myers F, Dempster T, Fjelldal PG, Hansen T, Swearer SE (2014) Osmotic induction improves batch marking of larval fish otoliths with enriched stable isotopes. Ices journal of marine science 71:2530-2538. doi:10.1093/icesjms/fsu091

Dempson J.B., M. P. (2004) Use of stable isotopes to distinguish farmed from wild Atlantic salmon, *Salmo salar*. Ecol Freshw Fish 13:176-184.

Dempson B., Furey G., Bloom M. (2002). Effects of catch and release angling on Atlantic salmon, Salmo salar L., of the Conne River, Newfoundland. Fisheries Management and Ecology 9, 139–147.

Dietrich J.P., Cunjak R.A. (2006) Evaluation of the impacts of Carlin tags, fin clips, and Panjet tattoos on juvenile Atlantic salmon. North American Journal of Fisheries Management 26:163-169. DOI: 10.1577/m05-032.1.

Diserud O.H., Fiske P., Hindar K. (2013) Forslag til kategorisering av laksebestander som er påvirket av rømt oppdrettslaks - oppdatering for perioden 1989-2012. NINA Rapport 976, 22 pp (in Norwegian).

Duncan I.J.H. (2005) Science-based assessment of animal welfare: farm animals. Revue Scientifique Et Technique-Office International Des Epizooties 24:483-492.

Duncan I.J.H., Fraser D. (1997) Understanding animal welfare. Edited by: Appleby, M. C.; Hughes, B. O. Animal welfare. 19-31.

Evrard G. (2005) Use and effect of freeze branding on roach (*Rutilus rutilus* L.). Bulletin Francais de la Peche et de la Pisciculture 374: 35–42.

Ferguson, H.W. 2006. Systemic Pathology of Fish. Scotian Press, London.

Fiske P., Aronsen T., Hindar K. (2014) Overvåking av rømt oppdrettslaks i elver om høsten 2013. NINA Rapport 1063, 44 pp (in Norwegian).

Fiske P., Wennevik V. (2011) Overvåking- og utfisking av rømt oppdrettslaks i Namsen og Namsenfjorden 2007 – 2009. Oppdragsrapport Kunnskapssenteret for Laks og Vannmiljø, 11: 1-18.

Fiske P., Lund R.A., Hansen L.P. (2005) Identifying fish farm escapees, in: C. S.X., et al. (Eds.), Stock Identification Methods; Applications in Fishery Science, Elsevier Academic Press, Amsterdam. pp. 659-680.

Fitzgerald J.I., Sheenan T.F., Kocik J.F. (2004) Visibility of visual implant elastomer tags in Atlantic salmon reared for two years in marine net-pens. North American Journal of Fisheries Management 24.

Flem B., Moen V., Grimstvedt A. (2005) Trace element analysis of scales from four populations of Norwegian Atlantic salmon (*Salmo salar* L.) for stock identification using laser ablation inductively coupled plasma mass spectrometry. Appl Spectrosc 59:245-251.

Folkedal O., Pettersen J.M., Bracke M.B.M., Stien L.H., Nilsson J., Martins C., Breck O., Midtlyng P.J., Kristiansen T. (2016) On-farm evaluation of the Salmon Welfare Index Model (SWIM 1.0): theoretical and practical considerations. Animal Welfare 25:135-149. DOI: 10.7120/09627286.25.1.135.

Fraser D. (2009) Assessing animal welfare: different philosophies, different scientific approaches. Zoo Biol 28:507-18. DOI: 10.1002/zoo.20253.

Friedland K.D., Esteves C., Hansen L.P., Lund R.A. (1994) Discrimination of Norwegian farm, ranch, and wild origin Atlantic salmon by image processing. Fish. Management. Ecol. 1.

Garcia de Leaniz C., Fleming I.A., Einum S., Verspoor E., Jordan W.C., Consuegra S., Aubin-Horth N., Lajus D., Letcher B.H., Youngson A.F., Webb J.H., Vollestad L.A., Villanueva B., Ferguson A., Quinn T.P. (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews 82:173-211. DOI: 10.1111/j.1469-185X.2006.00004.x.

Gibbons J.W., Andrews K.M. (2004) PIT tagging: Simple technology at its best. Bioscience 54:447-454. DOI: Doi 10.1641/0006-3568(2004)054[0447:Ptstai]2.0.Co;2.

Gjerde B., Refstie T. (1988) The effect of fin-clipping on growth-rate, survival and sexual maturity of Rainbow trout. Aquaculture 73:383-389. DOI: 10.1016/0044-8486(88)90071-3.

Glover K.A. (2010) Forensic identification of fish farm escapees: the Norwegian experience. Aquac Environ Interact 1:1-10. DOI: 10.3354/aei00002.

Glover K.A., Ottera H., Olsen R.E., Slinde E., Taranger G.L., Skaala O. (2009) A comparison of farmed, wild and hybrid Atlantic salmon (*Salmo salar* L.) reared under farming conditions. Aquaculture 286:203-210. DOI: 10.1016/j.aquaculture.2008.09.023.

Glover K.A., Skilbrei O.T., Skaala O. (2008) Genetic assignment identifies farm of origin for Atlantic salmon *Salmo salar* escapees in a Norwegian fjord. ICES J. Mar. Sci. 65:912-920. DOI: 10.1093/icesjms/fsn056.

Griffiths S.P. (2002) Retention of visible implant tags in small rockpool fishes. Marine Ecology Progress Series 236: 307–309.

Gutherz E.J., Rohr B.A., Minton R.V. (1990) Use of hydroscopic molded nylon dart and internal anchor tags on red drum American Fisheries Society Symposium 7:152-160.

Guy C.S., Blankenship H.L., Nielsen L.A. (1996) Tagging and marking, in: B. R. Murphy and D. W. Willis (Eds.), Fisheries techniques, 2nd edition, American Fisheries Society, Bethesda, Maryland. pp. 353-383.

Habicht C., Sharr S., Evans D., Seeb J.E. (1998) Coded wire tag placement affects homing ability of pink salmon. Transactions of the American Fisheries Society 127:652-657. DOI: 10.1577/1548-8659(1998)127<0652:cwtpah>2.0.co;2.

Haines G.B., Modde T. (1996) Evaluation of marking techniques to estimate population size and first-year survival of Colorado squawfish. North American Journal of Fisheries Management 16: 905–912.
Hansen T., Fjelldal P.G., Warren-Myers F., Swearer S., Dempster T. (2015) Detecting and tracing farmed salmon with natural geo-element otolith 'fingerprint' tags: developing and validating tag delivery techniques. Institute of Marine Research Report 11/2015 90pp.
Halls AS, Azim ME (1998) The utility of visible implant (VI) tags for marking tropical river fish. Fisheries Management and Ecology 5: 71–80.
Hammer S.A., Blankenship H.L. (2001) Cost comparison of marks, tags, and mark-with-tag combinations used in salmonid research. North American Journal of Aquaculture 63:171-178. DOI: 10.1577/1548-8454(2001)063<0171:ccomta>2.0.co;2.
Hand D.M., Brignon W.R., Olson D.E., Rivera J. (2010) Comparing Two Methods Used to Mark Juvenile Chinook Salmon: Automated and Manual Marking. North American Journal of Aquaculture 72:10-17. DOI: 10.1577/a08-065.1.
Hansen L.P. (1988) Effcts of Carlin tagging and fin clipping on survival of Atlantic salmon (Salma salar) released as smolts. Aquaculture 70:391-394. DOI: 10.1016/0044-8486(88)90122-6.
Hansen L.P., Reddin D.G., Lund R.A. (1997) The incidence of reared Atlantic salmon (*Salmo salar* L) of fish farm origin at West Greenland. ICES J. Mar. Sci. 54:152-155. DOI: DOI 10.1006/jmsc.1996.0178.
Havn T.B., Uglem I., Solem Ø., Cooke S.J., Whoriskey F.G., Thorstad E.B. (2015) The effect of catch-and-release angling at high water temperatures on behaviour and survival of Atlantic salmon Salmo salar during spawning migration. 87: 342-359.
Haw F., Bergman P.K., Fralick R.D., Buckley R.M., Blankenship H.L. (1990) Visible implanted fish tag. American Fisheries Society Symposium 7:311-315.
Hayes M.C., Focher S.M., Conto C.R. (2000) High-pressure injection of photonic paint to mark adult chinook salmon. North American Journal of Aquaculture 62:319-322.
Hendricks M.L., Bender T.R., Mudrak V.A. (1991) Multiple marking of American shad otoliths with tetracycline antibiotics. . N Am J Fish Manag 11:212-219.
Hennick D.P., Tyler R.W. (1970) Experimental marking of emergent pink salmon (*Oncorhynchus gorbuscha*) fry with sprayed fluorescent pigment. Trans Am Fish Soc 99: 397-400.
Henrich T., Hafer N., Mobley K.B. (2014) Effects of VIE tagging and partial tissue sampling on the immune response of three-spined stickleback Gasterosteus aculeatus J Fish Biol 85: 965-971.
Herbinger C.M., Newkirk G.F., Lanes S.T. (1990). Individual marking of Atlantic salmon: evaluation of cold branding and jet injection of alcian blue in several fin locations. Journal of Fish Biology 36: 99–101.
Hindar K., L'Abee-Lund J.H. (1992) Identification of hatchery-reared and wild Atlantic salmon juveniles based on examination of otoliths. Aquaculture and Fisheries Management 23:235-241.
Hindar K., Ryman N., Utter F. (1991) Genetic effects of cultured fish on natural fish populations. Canadian Journal of Fisheries and Aquatic Sciences 48:945-957. DOI: 10.1139/f91-111.
Hvidsten N.A., Fiske P. (2012) Innsig av villaks til Trondheimsfjorden og andel rømt oppdrettslaks ved Ytre Agdenes Merkestasjon i 2011. NINA Minirapport 388. 14 s
Håstein T., Hill B.J., Berthe F., Lightner D.V. (2001) Traceability of aquatic animals. Revue Scientifique Et Technique De L Office International Des Epizooties 20:564-583.
Ísaksson Á., Bergman P. (1978) An evaluation of two tagging methods and survival rates of different age and treatment groups of hatchery-reared Atlantic salmon smolts. J. Agr. Res. Icel. 10:74-99.

Jakobsson J. (1970) On fish tags and tagging. Oceanography and Marine Biology: an Annual Review, 8: 457-499.

Jefferts K.B., Bergman P.K., Fiscus H.F. (1963) A coded-wire identification system for macro-organisms. Nature (London) 198:460-462.

Jensen Ø., Dempster T., Thorstad E.B., Uglem I., Fredheim A. (2010) Escapes of fishes from Norwegian sea-cage aquaculture: causes, consequences and prevention. Aquaculture Environment Interactions 1:71-83.

Johnsen B.O., Ugedal O. (1988) Effects of different kinds of fin-clipping on over-winter survival and growth of fingerling brown trout, Salmo trutta L., stocked in small streams in Norway. Aquaculture Research 19:305-311. DOI: 10.1111/j.1365-2109.1988.tb00434.x.

Jones G.P., Milicich M.J., Emsile M.J., Lunow C. (1999) Selfrecruitment in a coral reef fish population. Nature 402:802-804.

Jonsson B., Jonsson N (2011) Ecology of Atlantic salmon and Brown trout: habitat as a template for life histories. Fish and Fisheries Series 33 Springer Netherlands. 708 p. DOI 10.1007/978-94-007-1189-1.

Jonsson B. (1976) Comparison of scales and otoliths for age determination in brown trout, *Salmo trutta* L. Norwegian Journal of Zoology 24: 295-301:295-301.

Kerwath S.E., Götz A., Wilke C., Attwood C.g., Sauer V.H.H. (2006) A comparative evaluation of three methods used to tag South African linefish. African Journal of Marine Science 28: 637-643.

Kristiansen S. T., Skilbrei O. (2012). Evaluering av fettfinneklipping av all oppdrettslaks for lettere identifikasjon ved utfisking av rømt laks i lakseelvene. Havforskningsinstituttet, Rapport Fra Havforskningen Nr 20. 2012 https://www.imr.no/filarkiv/2012/12/hi-rapp_20-2012_til_web.pdf/nb-no

Kuroki M., Buckley R.M., LeClair L., Hauser L. (2010) Validation and efficacy of transgenerational mass marking of otoliths in viviparous fish larvae. J Fish Biol 77:292-298.

Laird L.M., Roberts R.J., Shearer W.M., McArdle J.F. (1975) Freeze branding of juvenile salmon. J. Fish Biol 7: 167–171.

Larsen M.H., Thorn A.N., Skov C., Aarestrup K. (2013) Effects of passive integrated transponder tags on survival and growth of juvenile Atlantic salmon *Salmo salar*. Animal Biotelemetry 1:19 doi: 10.1186/2050-3385-1-19.

Le Blanc A., Noakes D.L. (2012) Visible Implant Elastomer (VIE) Tags for Marking Small Rainbow Trout. North American Journal of Fisheries Management 32: 716-719.

Lennox R.J., Uglem I., Cooke S.J., Tor F.. Næsje T.F., Whoriskey F.G., Havn T.B., Ulvan E.M., Solem Ø, Thorstad E.B. (2015) Does Catch-and-Release Angling Alter the Behavior and Fate of Adult Atlantic Salmon During Upriver Migration? Transactions of the American Fisheries Society 144: 400-409.

Lipsky C., Hastings E., Brown R., Dill R. (2012) Correcting visual implant elastomer tag identification to better evaluate adult Atlantic salmon returns from multiple stocking sites in the Penobscot River, Maine, USA., in: B. Parsons, et al. (Eds.), Advances in Fish Tagging and Marking Technology, American Fisheries Society Symposium. pp. 63-75.

Liu Q., Zhang X.M., Zhang P.D., Nwafili S.A. (2009) The use of alizarin red S and alizarin complexone for immersion marking Japanese flounder *Paralichthys olivaceus* (T.). Fish Res 98:67-74.

Lü H.J., Zhang X.M., Xi D., Gao T.X. (2014) Use of calcein and alizarin red S for immersion marking of black rockfish Sebastes schlegelii juveniles. Chin J Oceanol Limnol 32:88–98.

Lü H., Chen H., Fu M., Peng X., Xi D., Zhang Z. (2015) Experimental evaluation of calcein and alizarin red S for immersion marking grass carp Ctenopharyngodon idellus. Fish Sci. 81:653–662

Lund R.A., Hansen L.P. (1991) Identification of wild and reared Atlantic salmon, *Salmo salar* L, using scale characters. Aquaculture and Fisheries Management 22:499-508.

Lund R.A., Hansen L.P., Järvi T. (1989) Identification of reared and wild salmon by external morphology, size of fins and scale characteristics. NINA Forskningsrapport, 1: 1–54 (in Norwegian with English summary).

Lund R.A., Okland F., Hansen L.P. (1991) Farmed Atlantic salmon (*Salmo salar*) in fisheries and rivers in Norway. Aquaculture 98:143-150. DOI: Doi 10.1016/0044-8486(91)90379-L.

Lund V., Mejdell C.M., Rocklinsberg H., Anthony R., Hastein T. (2007) Expanding the moral circle: farmed fish as objects of moral concern. Diseases of Aquatic Organisms 75:109-118. DOI: 10.3354/dao075109.

Mabee P., Balhoff J.P., Dahdul W.M., Lapp H., Midford P.E., Vision T.J., Westerfield M. (2012) 500,000 fish phenotypes: The new informatics landscape for evolutionary and developmental biology of the vertebrate skeleton. Journal of Applied Ichthyology 28:300-305. DOI: 10.1111/j.1439-0426.2012.01985.x.

Madhun A.S., Karlsbakk E., Isachsen C.H., Omdal L.M., Sorvik A.G.E., Skaala O., Barlaup B.T., Glover K.A. (2015) Potential disease interaction reinforced: double-virus-infected escaped farmed Atlantic salmon, Salmo salar L., recaptured in a nearby river. Journal of Fish Diseases 38:209-219. DOI: 10.1111/jfd.12228.

Malone J.C., Forrester G.E., Steele M.A. (1999). Effects of subcutaneous microtags on the growth, survival, and vulnerability to predation of small reef fishes. Journal of Experimental Marine Biology and Ecology 237: 243–253.

Matechik C.V., Mickle A., Stallings C.D. (2013) Experimental test of two marking methods on survival, growth, mark retention and readability on young-of-year pinfish (*Lagodon rhomboides*). Journal of Experimental Marine Biology and Ecology 440: 49–53.

Maynard D.J., Frost D.A., Waknitz F.W., Prentice E.F. (1996) Vulnerability of marked age-0 steelhead to a visual predator. Transactions of the American Fisheries Society 125:330-333. DOI: 10.1577/1548-8659(1996)125<0330:vomast>2.3.co;2.

McFarlane G.A., Wydoski R.S., Price E.D. (1990) Historical review of the development of external tags and marks in: N. C. Parker, et al. (Eds.), Fish-marking techniques, American Fisheries Society Symposium 7, Bethesda. pp. 9-19.

McGinnity P., Prodohl P., Ferguson K., Hynes R., O'Maoileidigh N., Baker N., Cotter D., O'Hea B., Cooke D., Rogan G., Taggart J., Cross T. (2003) Fitness reduction and potential extinction of wild populations of Atlantic salmon, Salmo salar, as a result of interactions with escaped farm salmon. Proceedings of the Royal Society B-Biological Sciences 270:2443-2450. DOI: 10.1098/rspb.2003.2520.

Meka J.M. (2004) The influence of hook type, angler experience, and fish size on injury rates and the duration of capture in an Alaskan catch-and-release rainbow trout fishery. N. Am. J. Fish. Manage. 24, 1309–1321.

Meyer S., Sørensen S.R., Peck M.A., Støttrup J.G. (2012) Sublethal effects of alizarin complexone marking on Baltic cod (Gadus morhua) eggs and larvae. 324–325: 158–164.

Merkeutvalget. (2004) Identifisering av rømt oppdrettslaks. Fiskeridirektoratet (in Norwegian).

Milner N.J., Evans R. (2003) The incidence of escaped Irish farmed salmon in English and Welsh rivers. Fish. Manag. Ecol. 10:403-406. DOI: DOI 10.1111/j.1365-2400.2003.00348.x.

Mohler J.W. (1997) Immersion of larval Atlantic salmon in calcein solutions to induce a non-lethally detectable mark. N Am J Fish Manag 17:751-756 N Am J Fish Manag 17:751-756.

Moodie G.E.E., Salfert I.G. (1982) Evaluation of fluorescent pigment for marking a scaleless fish, the brook stickleback. Progressive Fish-Culturist 44: 192-5.

Morrison J., Zajac D. (1987) Histologic effect of coded wire tagging in Chum Salmon. North American Journal of Fisheries Management 7(3):439-441.

Mortensen A., Puvanendran V., Hansen Ø.J. (2013) Fysisk merking av oppdrettslaks. Norima rapport 1/2013 (in Norwegian).

Mourning T.E., Fausch K.D., Gowan C. (1994). Comparison of visible implant tags and floy anchor tags on hatchery rainbow trout. North American Journal of Fisheries Management 14: 636–642.

Munro A.R., Gillanders B.M., Elsdon T.S., Crook D.A., Sanger A.C. (2008) Enriched stable isotope marking of juvenile golden perch (*Macquaria ambigua*) otoliths. Can J Fish Aquat Sci 65:276-285.

Muoneke M.I, Childress W.M. (1994) Hooking mortality: a review for recreational fisheries. Reviews in Fisheries Science 2, 123–156.

Neufeld K., Blair S., Poesch M. (2015) Retention and Stress Effects of Visible Implant Tags When Marking Western Silvery Minnow and Its Application to Other Cyprinids (Family Cyprinidae). North American Journal of Fisheries Management 35: 070-1076.

Næsje T.F., Barlaup B.T., Berg, M., Diserud O.H., Fiske P., Karlsson S., Lehmann G.B., Museth J., Robertsen G., Solem Ø., Staldvik F. (2013) Muligheter og teknologiske løsninger for å fjerne rømt oppdrettsfisk fra lakseførende vassdrag. NINA Rapport 972. 84 s.

Odense P.H., Logan V.H. (1974) Marking Atlantic salmon (*Salmo salar*) with oxytetracycline. J Fish Res Bd Can 31:348-350.

Olsen E.M., Vollestad L.A. (2001) An evaluation of visible implant elastomer for marking age-0 Brown Trout. N Am J Fish Manag 21:967-970.

Ophel I.L., Judd J.M. (1968) Marking fish with stable strontium. J Fish Res Bd Can 25:1333-1337.

Oven J., Blankenship H.L. (1993) Benign recovery of coded wire tags from rainbow trout. North American Journal of Fisheries Management 13: 852-855.

Peters R.J., Knudsen E., Pauly G.B. (1994) Effects of freeze branding on growth and survival of choho salmon fry. US Fish and wildlife service report. 6 pp.

Petersson E., Rask J., Ragnarsson B., Karlsson L., Persson J. (2014) Effects of fin-clipping regarding adult return rates in hatchery-reared brown trout. Aquaculture 422:249-252. DOI: 10.1016/j.aquaculture.2013.12.020.

Pettersen J.M., Bracke M.B.M., Midtlyng P.J., Folkedal O., Stien L.H., Steffenak H., Kristiansen T.S. (2014) Salmon welfare index model 2.0: an extended model for overall welfare assessment of caged Atlantic salmon, based on a review of selected welfare indicators and intended for fish health professionals. Reviews in Aquaculture 6:162-179. DOI: 10.1111/raq.12039.

Phinney D.E. (1974) Growth and survival of fluorescent pigment marked and finclipped salmon. Journal of Wildlife Management 38: 132-137.

Prentice E.F., Flagg T.A., McCutcheon C.S., Brastow D.F., Cross D.C. (1990) Equipment, methods, and an Automated Data-Entry Station for PIT Tagging. American Fisheries Society Symposium 7:335-340.

Reeves K.S., Buckmeier D.L. (2009) Mortality, Predation, and Tag Visibility of Fish Marked with Visible Implant Elastomer Tags. North American Journal of Fisheries Management 29: 323-329.

Reimchen T.E., Temple N.F. (2004) Hydrodynamis and phylogenetic aspects of the adipose fin in fishes. Can. J. Zool. 82:910-916. DOI: 10.1139/z04-069.

Rikardsen A.H., Woodgate M., Thompson D.A. (2002) A comparison of Floy and soft VI-alpha tags on hatchery Arctic charr, with emphasis on tag retention, growth and survival. Environ Biol Fish 64: 269–273.

Rikstad A. (2004) Sorteringsfiske etter laks med kilenot I salvassdraget, Fosnes commune, NT. Fylkesmannen i Nord-Trøndelag, Miljøvernavdelingen Rapport Nr. 5- 2004 1-11.

Roberts J.H., Kilpatrick J.M. (2004) Predator feeding preferences for a benthic stream fish: effects of visible injected marks. Journal of Freshwater Ecology 19: 531–538.

Ross W.R., Pickard A. (1990) Use of scale patterns and shape as discriminators between wild and hatchery striped bass stocks in California, in: N. C. Parker, et al. (Eds.), Fish-Marking Techniques, American Fisheries Society Symposium 7. American Fisheries Society, Bethesda, Maryland. pp. 71-77.

Ryan PA (1975) Fish tagging with injected dyes. Mauri Ora 3: 55-61.

Schroder S.L., Knudsen C.M., Volk E.C. (1995) Marking salmon fry with strontium chloride solutions. Can J Fish Aqua Sci 52:1141-1149.

Schumann D.A., Koupal K.D., Hoback W.W., Schoenebeck C.W. (2013) Evaluation of Sprayed Fluorescent Pigment as a Method to Mass-Mark Fish Species. The Open Fish Science Journal 6: 41-47.

Schröder V., de Leaniz C.G. (2011) Discrimination between farmed and free-living invasive salmonids in Chilean Patagonia using stable isotope analysis. Biol Invasions 13:203-213.

Severin K.P., Carroll J., Norcross B.L. (1995) Electron microprobe analysis of juvenile walleye pollock, *Theragra chalcogramma*, otoliths from Alask: a pilot stock separation study. Environ Biol Fishes 43:269-283.

Sharpe C.S., Thompson D.A., Blankenship H.L., Schreck C.B. (1998) Effects of routine handling and tagging procedures on physiological stress responses in juvenile Chinook salmon. Progressive Fish-Culturist 60:81-87. DOI: 10.1577/1548-8640(1998)060<0081:eorhat>2.0.co;2.

Simon J. (2007) Evaluation of marking European silver eels with visible implant elastomer tags and alcian blue. J Fish Biol. 70: 303–309.

Skilbrei O.T., Heino M., Svasand T. (2015a) Using simulated escape events to assess the annual numbers and destinies of escaped farmed Atlantic salmon of different life stages from farm sites in Norway. ICES J. Mar. Sci. 72:670-685. DOI: 10.1093/icesjms/fsu133.

Skilbrei O.T., Holst J.C., Asplin L., Mortensen S. (2010) Horizontal movements of simulated escaped farmed Atlantic salmon (*Salmo salar*) in a western Norwegian fjord. ICES J. Mar. Sci. 67:1206-1215. DOI: 10.1093/icesjms/fsq027.

Skilbrei O.T., Jorgensen T. (2010) Recapture of cultured salmon following a large-scale escape experiment. Aquaculture Environment Interactions 1:107-115. DOI: 10.3354/aei00011.

Skilbrei O.T., Normann E., Meier S., Olsen R.E. (2015b) Use of fatty acid profiles to monitor the escape history of farmed Atlantic salmon. Aquac Environ Interact 7:1-13. DOI: 10.3354/aei00132.

Skinner ,A., Courtenay S.c., Parker W.R., Curry R.A. (2006) Evaluation of Techniques for the Marking of Mummichogs with Emphasis on Visible Implant Elastomer North American Journal of Fisheries Management 26: 1003-1010.

Smith J.E., Macreadie P.I., Swearer S.E. (2010) An osmotic induction method for externally marking saltwater fishes, *Stigmatopora argus* and *Stigmatopora nigra*, with calcein. J Fish Biol 76:1055-1060.

Smith K.T., Whitledge G.W. (2011) Evaluation of a stable isotope labelling technique for mass marking fin rays of age-0 lake sturgeon. Fish Manag Ecol 18:168-175.

Smith J.R. (1973) Branding Chinook, Coho, and Sockeye salmon fry with hot and cold metal tools. Progressive Fish-Culturist 35: 94–96.

Sneddon L.U. (2003) The evidence for pain in fish: the use of morphine as an analgesic. Applied Animal Behaviour Science 83:153-162. DOI: 10.1016/s0168-1591(03)00113-8.
Sneddon L.U., Elwood R.W., Adamo S.A., Leach M.C. (2014) Defining and assessing animal pain. Animal Behaviour 97:201-212. DOI: 10.1016/j.anbehav.2014.09.007.
Spruijt B.M., van den Bos R., Pijlman F.T.A. (2001) A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. Applied Animal Behaviour Science 72:145-171. DOI: 10.1016/s0168-1591(00)00204-5.
Stauffer T.M., Hansen M.J. (1969) Mark Retention, Survival, and Growth of Jaw-Tagged and Fin-Clipped Rainbow Trout. Transactions of the American Fisheries Society 98:225-229. DOI: 10.1577/1548-8659(1969)98[225:MRSAGO]2.0.CO;2.
Stewart T.A., Smith W.L., Coates M.I. (2014) The origins of adipose fins: an analysis of homoplasy and the serial homology of vertebrate appendages. Proceedings of the Royal Society B-Biological Sciences 281. DOI: 10.1098/rspb.2013.3120.
Stien L.H., Bracke M.B.M., Folkedal O., Nilsson J., Oppedal F., Torgersen T., Kittilsen S., Midtlyng P.J., Vindas M.A., Overli O., Kristiansen T.S. (2013) Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. Reviews in Aquaculture 5:33-57. DOI: 10.1111/j.1753-5131.2012.01083.x.
Strand R., Finstad B., Lamberg A., Heggberget T.G. (2002) The effect of Carlin tags on survival and growth of anadromous Arctic charr, Salvelinus alpinus. Environmental Biology of Fishes 64:275-280. DOI: 10.1023/a:1016091619937.
Strand R., Heggberget T. (1996) Kilenotfisk; maskeviddeens betydning for fangst-selektivitet og størrelsesseleksjon. NINA Oppdragsmelding 440: 1-13.
Szedlmayer S.-T., Howe J.C. (1995) An evaluation of six marking methods for age-O red drum, *Sciaenops ocellatus*. Fishery Bulletin 93:191-195.
Sutphin Z.A., Myrick C.A., Brandt M.M. (2007) Swimming Performance of Sacramento Splittail Injected with Subcutaneous Marking Agents, North American Journal of Fisheries Management 27: 1378-1382.
Svåsand T., Agnalt A.-T., Skilbrei O.T., Borthen J., Heggberget T. (2004) An integrated development programme for marine stocking: the Norwegian example. In: D.M. Bartley & K.M. Leber (eds), Marine Ranching. FAO Fisheries Technical Paper 429, 19–72.
Svåsand T. (1995) Massemerking av tidlig livestadier av torsk med alizarin. TemaNord. Nordisk Ministerråd, København. 30 pp.
Taylor M.D., Fielder D.S., Suthers I.M. (2005) Batch marking of otoliths and fin spines to assess the stock enhancement of Argyrosomus japonicus. J Fish Biol 66:1149–1162.
Thedinga J.F., Moles A., Fujioka J.T. (1997) Mark retention and growth of jet-injected juvenile marine fish. Fishery Bulletin 95: 629-633.
Thedinga J.F, Johnson S.W. (1995) Retention of jet-injected marks on juvenile coho and sockeye salmon. Trans. Am. Fish. Soc. 124:782-785.
Thorrold S.R., Jones G.P., Planes S., Hare J.A. (2006) Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. Canadian Journal of Fish Biology 49:668-677.
Thorstad E.B., Fleming I.A., McGinnity P., Soto D., Wennevik V., Whoriskey F. (2008) Incidence and impacts of escaped farmed Atlantic salmon *Salmo salar* in nature. NINA Special Report 36, 110 pp.
Thorstad E.B., Næsje T.F., Fiske P., Finstad B. (2003) Effects of hook and release on Atlantic salmon in the River Alta, northern Norway. Fisheries Research 60: 293–307.

Thorstad E.B., Næsje T.F., Leinan I. (2007). Long-term effects of catch-and-release angling on Atlantic salmon during different stages of return migration. Fisheries Research 85, 330–334.
Thorstad E.B., Fiske P., Staldvik F., Økland F. (2011) Beskatning og bestandsstørrelse av laks i Namsenvassdraget. NINA Rapport 747: 1-32.
Thorstad E.B., Rikardsen A.H., Alp A., Økland F. (2013) The use of electronic tags in fish research - an overview of fish telemetry methods. Turk J Fish Aquat Sci 13:881-896. DOI: 10.4194/1303-2712-v13_5_13.
Thresher R.E., Proctor C.H., Gunn J.S., Harrowfield I.R. (1994) An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae). Fish Bull 92:817-840.
Tipping J.M., Heinricher J.R. (1993) Use of magnetic wire tag locations to mark tiger muskellunge. N Am J Fish Manag 13:190-193.
Torgersen T., Bracke M.B.M., Kristiansen T.S. (2011) Reply to Diggles et al. (2011): Ecology and welfare of aquatic animals in wild capture fisheries. Reviews in Fish Biology and Fisheries 21:767-769. DOI: 10.1007/s11160-011-9221-y.
Tsukamoto K. (1988) Otolith tagging of ayu embryo with fluorescent substances. Nippon Suisan Gakkaishi 54: 1289–1295.
Tsukamoto K., Seki Y., Oba T., Oya M., Iwahashi M. (1989) Application of otolith to migration study of salmonids. Physiology and Ecology, Japan 1: 119–140.
Turek K.C., Pegg M.A, Pope K.L. (2014) Short-term evaluation of visible implant alpha tags in juveniles of three fish species under laboratory conditions. Journal of Fish Biology 84: 971–981.
Ugedal O., Berg M., Bongard T., Bremset G., Kvingedal E., Diserud O.H., Jensås J.G., Johnsen B.O., Hvidsten N.A., Østborg G.M. (2014) Ferskvannsbiologiske undersøkelser i Surna. Slutrapport for perioden 2009 - 2013. NINA Rapport 1051. 129 s + vedlegg.
Vander Haegen G.E., Blankenship H.L., Hoffmann A., Thompson D.A. (2005) The effects of adipose fin clipping and coded wire tagging on the survival and growth of spring Chinook salmon. North American Journal of Fisheries Management 25:1161-1170. DOI: 10.1577/m04-011.1.
Veinott G., Porter R. (2005) Using otolith microchemistry to distinguish Atlantic salmon (*Salmo salar*) parr from different natal streams. Fish Res 71:349-355.
Vincentlang D. (1993) Relative survival of umarked and fin-clipped coho salmon from Bear Lake, Alaska. Progressive Fish-Culturist 55:141-148. DOI: 10.1577/1548-8640(1993)055<0141:rsouaf>2.3.co;2.
VKM (2009) Risk Assessment of Catch and Release: Opinion of the Panel on Animal Health and Welfare of the Norwegian Scientific Committee for Food Safety. ISBN: 978-82-8082-376-2. 79 pp.
Wagner J.P., Blaylock H.B., Peterson M.S. (2013) Evaluation of Internal Tag Performance in Hatchery-Reared Juvenile Spotted Seatrout. North American Journal of Fisheries Management 33: 783-789.
Walker A.M., Beveridge M.C.M., Crozier W., O Maoileidigh N., Milner N. (2006) Monitoring the incidence of escaped farmed Atlantic salmon, *Salmo salar* L., in rivers and fisheries of the United Kingdom and Ireland: current progress and recommendations for future programmes. ICES J. Mar. Sci. 63:1201-1210. DOI: 10.1016/j.icesjms.2006.04.018.
Walsh M.G., Winkelman D.L. (2004) Anchor and visible implant elastomer tag retention by hatchery rainbow trout stocked into an Ozark stream. N Am J Fish Manag 24:1435-1439.

Ward D.L., Persons W.R., Young K.L., Stone D.M., Vanhaverbeke D.R., Knight W.K. (2015) A Laboratory Evaluation of Tagging-Related Mortality and Tag Loss in Juvenile Humpback Chub. North American Journal of Fisheries Management 35: 135-140.

Warren-Myers F.W. (2015) Enriched stable isotope mass marking techniques for aquaculture and fisheries. PhD-thesis, School of BioSciences, The University of Melbourne, August 2015.

Warren-Myers F.W., Dempster T., Fjelldal P.G., Hansen T., Jensen A.J., Swearer E. (2014) Stable isotope marking of otholiths during vaccination: a novel method for mass-marking fish. Aquaculture Environment Interactions 5:143-154.

Webb J.H., Hay D.W., Cunningham P.D., Youngson A.F. (1991) The spawning behavior of escaped farmed and wild adult Atlantic salmon (*Salmo salar* L) in a Northern Scottish river. Aquaculture 98:97-110. DOI: Doi 10.1016/0044-8486(91)90375-H.

Weber D., Ridgway G.J. (1967) Marking Pacific salmon with tetracycline antibiotics. J Fish Bd Can Res 24.

Weber D., Wahle R.J. (1969) Effect of Finclipping on Survival of Sockeye Salmon (Oncorhynchus nerka). Journal of the Fisheries Research Board of Canada 26:1263-1271. DOI: 10.1139/f69-112.

Wells R.J.D., Smith S.E., Kohin S., Freund E., Spear N., Ramon D.A. (2013) Age validation of juvenile shortfin mako (*Isurus oxyrinchus*) tagged and marked with oxytetracycline off southern California. Fish Bull 111.

Wenburg J.K., George G.W. (2011) Placement of Visible Implant Tags in the Anal Fin of Wild Coastal Cutthroat Trout. North American Journal of Fisheries Management 15: 874-877.

Westley P.A.H., Carlson S.M., Quinn T.P. (2008) Among-population variation in adipose fin size parallels the expression of other secondary sexual characteristics in sockeye salmon (*Oncorhynchus nerka*). Environ Biol Fishes 81:439-446.

Weston M.R., Johnson R.L. (2008) Visible Implant Elastomer as a Tool for Marking Etheostomine Darters (Actinopterygii: Percidae). Southeastern Naturalist 7: 159-164.

Wickström H., Sjöberg N.B. (2014) Traceability of stocked eels – the Swedish approach. Ecol Freshw Fish 23:33-39.

Williamson D.H., Jones G.P., Thorrold S.R. (2009) An experimental evaluation of transgenerational isotope labelling in a coral reef grouper. Mar Biol 156:2517-2525.

Woodcock S.H., Grieshaber C.A., Walther B.D. (2013) Dietary transfer of enriched stable isotopes to mark otoliths, fin rays and scales. Can J Fish Aquat Sci 70:1-4.

Woods C.M.C. (2005) Evaluation of VI-alpha and PIT-tagging of the seahorse Hippocampus abdominalis. Aquac Int 13: 175–186.

Ytrestoyl T., Aas T.S., Asgard T. (2015) Utilisation of feed resources in production of Atlantic salmon (Salmo salar) in Norway. Aquaculture 448:365-374. DOI: 10.1016/j.aquaculture.2015.06.023.

Zahl I.H., Kiessling A., Samuelsen O.B., Olsen R.E. (2010) Anesthesia induces stress in Atlantic salmon (Salmo salar), Atlantic cod (Gadus morhua) and Atlantic halibut (Hippoglossus hippoglossus). Fish Physiology and Biochemistry 36:719-730. DOI: 10.1007/s10695-009-9346-2.

Zajac D., Brunson R., Comstock R., Gilliam K. (1988) Relationship between coded wire tagging and bacterial kidney-disease in coho salmon. Progressive Fish-Culturist 50:187-188. DOI: 10.1577/1548-8640(1988)050<0188:rbcwta>2.3.co;2.