

# Fish identification, marking and tagging methods

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# Background

In order to be able to study fish movement patterns, population dynamics, different physiological variables or to be able to track an individual over a period of time, it is often necessary to be able to identify an individual fish for a shorter or extended period of time. This can sometimes be done using the animal's natural patterns and other unique markings. In other cases, the animal must be marked or tagged in some way, which often involves a certain degree of handling and/or surgery. A number of scientific studies have been done to evaluate different marking and tagging methods. Several other documents describe and compare different methods, including a Swedish report on different marking and tagging methods for salmonids. A scientific compilation of identification methods for small fish was also recently published. But there is a lack of easy-to-follow guidance that considers the welfare of fish, economic aspects and the user's perspective. There is a demand for this type of guidance, from the regional ethical committees on animal experiments and other groups.

On behalf of Sweden's National Committee for the Protection of Animals used for Scientific Purposes we have reviewed commonly used methods of marking, tagging and identifying fish. The report contains methods that are suitable for use in lakes, watercourses and seas, but also controlled environments, such as aquariums, fish farming cages or laboratory environments. For each method reviewed here, we go over the most important factors for the user and the impact on the fish. We have listed the references we used by title and method at the end of the report. The purpose of the report is to assist the user in choosing the most suitable method and should therefore not be seen as a comprehensive handbook for marking and tagging fish. Our goal in publishing this report is to help ensure that the correct marking and tagging methods are used for the right purposes, thereby contributing to a reduction in the negative impact on the animals and reducing the number of animals used.

# Things to consider when marking and tagging

There are more than 34,000 fish species across the world, and since their morphology, physiology and behaviour vary, these factors need to be considered when choosing a marking or tagging method. It is also important that fish capture and handling is well planned and done as quickly and gently as possible. Many fish species are covered by a protective mucus layer, and it is therefore important that all handling is done in a way that has as little impact as possible on the mucus layer. For example, if netting is necessary, only knotless, silicone-coated nets should be used. Sock nets, where the fish are not exposed to air at all, are optimal. If fish are handled by hand, it is important that your hands are wet or that you use latex gloves or similar. It is also important to minimise the amount of time fish are exposed to air, especially if the species is sensitive to air exposure. In many cases, the fish must be anaesthetized before marking or tagging. Some fish, such as eels and carp, are less sensitive to air exposure, but if the handling and the marking or tagging procedure takes longer than 10 seconds, the fish should normally be ventilated by flushing oxygenated water over the gills during the procedure. During surgical procedures it is important to work with clean, sharp instruments and clean surfaces to minimise the risk of infection. The instruments, including cannulas, must be disinfected with 70% alcohol or with other disinfectants, such as Cidex.

## Education, training and ethical approval for animal research

For simpler procedures, you should have completed a fish marking and tagging course, preferably a course with the content described in SJVFS 2019:25, Chapter 5, Section 4, which in Sweden is given by the Swedish University of Agricultural Sciences. For the more advanced procedures, such as the implantation of devices in the abdominal cavity, you should also have attended an LAS (Laboratory Animal Science) programme with species-specific theoretical and practical training.

The formal training requirements differ depending on the marking or tagging method and depend on whether it is done for research or commercial purposes. If the marking or tagging is done as part of a research project, as an isolated measure or one of several measures, the person performing the marking must have completed a LAS education/training. The education is divided into two parts: theoretical and practical. The theoretical part is provided by the Nordic Consortium for Laboratory Animal Science Education and Training (NCLASET), while the practical part is held by trained staff at the workplace where the person will work after completing training. The minimum training consists of the three LAS courses covering:

- Swedish legislation, ethics, animal welfare and 3Rs
- Laboratory animal science on fish
- Practical training for the marking or tagging technique that will be used.

If fish marking or tagging will be done for research purposes, ethical approval for animal research from a regional ethical committee on animal experiments is also required. If marking is done within a fish farming operation to comply with

fisheries legislation (Ordinance 1994:1716), no ethical approval for animal research is required. However, the staff must have training that corresponds to what is stipulated in SJVFS 2019:25, Chapter 5, Section 4 (9).

First-hand experience is needed to increase speed while maintaining the level of quality in the procedures, but also to build up routines and experience working, for example using aseptic techniques. Working with an aseptic technique means adapting your work methods so that you do not contaminate either the marking or tagging device or instruments, for example by not introducing sterilised materials with contaminated hands. Studies on tagging devices that have been surgically inserted into the abdominal cavity show that the knowledge and experience of the person performing the procedure is crucial. Trained individuals cause fewer and less severe injuries. Surgical procedures require proper training to minimise the impact on the fish. Training must always be adapted according to the fish species and should, whenever possible, start by practising on dead fish or basic non-animal training models.

## **Work environment considerations**

Staff who work with marking or tagging should have workplaces adapted for this; a good work environment provides good conditions for high quality work. In most cases, staff who mark or tag fish should sit or stand at a table with a suitable working height. The premises where the procedure is performed should have strong work lighting and good ventilation. In order to prevent the fish's body temperature from rising during the procedure, the fish should be kept at the same temperature as the water it was taken from. There can be high noise levels on a fish farm and ear protections are recommended. If a large number of fish are to be marked – for example in adipose fin, pelvic fin and maxilla clipping – the work becomes monotonous and staff should take regular breaks, as long as this does not have a negative impact on the animals. Breaks should therefore be planned.

## **Anaesthesia**

Anaesthesia means “not feeling pain” and can be divided into local anaesthesia, sedation and general anaesthesia. Local anaesthesia blocks sensory (pain) impulses, sedation creates a lower level of consciousness and general anaesthesia leads to complete unconsciousness.

For procedures using sedation and general anaesthesia, there must be at least one container holding water with the selected anaesthetic and at least one container for recovery. The water in the containers must be the right temperature and be well oxygenated through aeration. For procedures that take 10 seconds or more, oxygenated water containing a sedative should generally be flushed over the gills throughout the procedure. This is due to the fact that there is a strong link between air exposure and mortality. Note, however, that sensitivity to air exposure differs significantly between different fish species. It is also worth pointing out that in certain methods, anaesthesia can be more stressful for the fish than marking or tagging without anaesthesia.

## **Known impacts of having a tag**

All forms of marking and tagging affect the fish in one way or another, but one should always strive to have the lowest impact possible on the animals. In addition to the stress that the fish experiences during the marking or tagging procedure itself, for example capture, air exposure, and anaesthesia, the presence of a tag can

also affect the fish. Being tagged can mean a reduced capacity for recovery and lower chances of reproduction and survival. Most tags are so-called external tags. These tags are often easy to attach and do not always require the animals to be anaesthetized, which can reduce recovery time in itself. But at the same time, external tags can affect the fish's behaviour and health. For example, an external tag can disturb the fish's streamlined shape so that drag increases, which can worsen the fish's swimming ability or require the fish to use more energy. Algae can also grow on an external tag over time, which can further increase drag. External tags can also get stuck in vegetation and other structures in the aquatic environment, which can lead to the loss of tags and injury to the fish. Some studies also suggest that external tags may increase the risk that a fish will be eaten by a predator or may provoke attacks from other fish.

These problems are avoided when tags are placed under the skin, in the muscles or in the abdominal cavity instead of being attached to the outside of the fish. Such tags, however, often require more complex procedures and that the fish be sedated or anaesthetized (general anaesthesia) during the procedure. Creating an opening in the abdominal cavity means that the skin's protective barrier is damaged, which increases the risk of infection. There is also a risk that the body will reject tags that are placed in the abdominal cavity, which usually occurs during the first few weeks after the procedure and is more common in warm-water fish. Both rejection and infections at the insertion site are serious animal welfare problems and are best prevented by ensuring that the procedures are only performed in an aseptic environment by well-trained staff. The size and weight ratio between the fish and the tag must also be carefully determined through measuring and weighing, as a tag that is too heavy or too large, that presses against the abdominal wall or internal organs, increases the risk of rejection and can cause inflammation.

# Methods



We have broken down each method point by point under the headings:


- Research question
- Brief method description
- Animal welfare
- Practical concerns


We have also categorised the methods using two symbols: a **fish symbol** that signifies animal welfare and a **tool symbol** that indicates how difficult the method is to learn from a practical standpoint and how much equipment is needed. The symbols are in green, yellow and red, where green represents the least impact on animal welfare and a method that is relatively easy to learn and does not require much equipment. At the other end of the scale, you will find the red symbols, i.e. methods with a high impact on the welfare of the fish, a high degree of difficulty and that require a lot of equipment. In the summaries, we have assumed that the marking or tagging will be performed correctly by an experienced individual.

The assessments are based on the scientific literature and the project group's collective experience with the various marking and tagging techniques. It is worth noting that the price and size of the equipment can drop as technology in the field is developing rapidly. This is especially true in genetic analyses and for various electronic tags.

## Symbols and their meanings


Symbol	The meaning of the symbol
 <p>Green fish</p>	<ul style="list-style-type: none"><li>• Little or no surgical intervention</li><li>• Little if any impact on survival and reproduction</li></ul>
 <p>Yellow fish</p>	<ul style="list-style-type: none"><li>• Some degree of mortality associated with marking</li><li>• Some impact on survival and reproduction</li></ul>

Symbol	The meaning of the symbol
 <p data-bbox="368 450 523 488">Red fish</p>	<ul data-bbox="772 338 1203 461" style="list-style-type: none"> <li>• High degree of mortality associated with marking</li> <li>• High impact on survival and reproduction</li> </ul>

Symbol	The meaning of the symbol
 <p data-bbox="288 891 507 929">Green tools</p>	<ul data-bbox="772 725 1203 792" style="list-style-type: none"> <li>• Easy to learn and perform correctly</li> <li>• Little equipment required</li> </ul>












- Relatively easy to learn and perform correctly
- May require a lot of equipment



 <p data-bbox="331 1496 523 1534">Red tools</p>	<ul data-bbox="772 1364 1230 1460" style="list-style-type: none"> <li>• Requires extensive training to perform correctly</li> <li>• Requires a lot of equipment</li> </ul>
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## Brief description of the methods









Table 1. Summary of the situations where different marking and tagging methods can be used

Method	To recognize groups	To recognize individuals	Mark lasts the entire life of the fish	Automatic reading possible	Suitable for fish $\leq 4$ cm	Symbols
Biotelemetry/biologger – internal		X		X		 Yellow fish  Yellow tools  Red tools
Biotelemetry/biologger – external		X		X		 Yellow fish  Yellow tools
Carlin tags		X	X			 Yellow fish  Red tools
Chemical branding	X					 Yellow fish  Green tools

Method	To recognize groups	To recognize individuals	Mark lasts the entire life of the fish	Automatic reading possible	Suitable for fish $\leq 4$ cm	Symbols
Clipping the adipose fin	X		X			 Yellow fish  Green tools
Clipping the maxilla	X		X			 Yellow fish  Yellow tools
Clipping the pelvic fin	X		X			 Red fish  Yellow tools
Coded-wire tags	X	X	X		X	 Green fish  Yellow tools
Dart tags		X				 Yellow fish  Yellow tools

Method	To recognize groups	To recognize individuals	Mark lasts the entire life of the fish	Automatic reading possible	Suitable for fish $\leq 4$ cm	Symbols
Freeze branding	X					 Yellow fish  Green tools
Genetic identification			X			 Green fish  Yellow fish  Green tools
Microchip (PITtag, RFID)		X	X	X		 Yellow fish  Green tools  Yellow tools
Natural patterns and unique markings		X	X	X	X	 Green fish  Green tools
Otolith marking	X		X		X	 Green fish  Green tools

Method	To recognize groups	To recognize individuals	Mark lasts the entire life of the fish	Automatic reading possible	Suitable for fish $\leq 4$ cm	Symbols
P-chip		X	X		X	 Yellow fish  Green tools
Short-term marking with plastic beads or filaments	X	X			X	 Yellow fish  Green tools
Spaghetti tags	X	X				 Red fish  Green tools
Spray marking	X					 Red fish  Green tools
Streamer tags	X	X				 Yellow fish  Green tools

Method	To recognize groups	To recognize individuals	Mark lasts the entire life of the fish	Automatic reading possible	Suitable for fish $\leq 4$ cm	Symbols
Tattoo methods	X	X				 Green fish  Green tools
T-bar anchor tags		X				 Yellow fish  Yellow tools
VI alpha tags	X	X	X			 Green fish  Green tools
VIE (Visible implant elastomer)	X	X	X		X	 Green fish  Yellow tools

# Guide for when you need to be able to distinguish between individuals

Does the mark/tag need to be readable without having to euthanise the fish?

NO



YES



Does the mark/tag need to last the entire life of the fish?

NO



YES



Method	Symbols
Carlin tags	 Yellow fish Red tools
P-chip	 Yellow fish Green tools

Method	Symbols
Coded-wire tags	 Green fish Yellow tools

Method	Symbols
Biotelemetry/biologger internal	 Yellow fish Yellow tools Red tools

Biotelemetry/biologger external	 Yellow fish Yellow tools
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Dart tags	 Yellow fish Yellow tools
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Short-term marking with plastic beads or filaments	 Yellow fish Green tools
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Microchip	 Yellow fish Green tools Yellow tools
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Natural patterns and unique markings	 Green fish Green tools
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Spaghetti tags	 Red fish Green tools
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Streamer tags	 Yellow fish Green tools
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Tattoo methods	 Green fish Green tools
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T-bar anchor tags	 Yellow fish Yellow tools
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VI alpha tags	 Green fish Green tools
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VIE	 Green fish Yellow tools
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# Guide for when you do not need to be able to distinguish between individuals

Does the mark/tag need to be readable without having to euthanise the fish? **NO** →

Method	Symbols
Otolith marking	  Green fish Green tools

**YES**  
↓

Does the mark/tag need to last the entire life of the fish? **NO** →

Method	Symbols
Chemical branding	  Yellow fish Green tools

Freeze branding	  Yellow fish Green tools
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Short-term marking with plastic beads or filaments	  Yellow fish Green tools
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Spaghetti tags	  Red fish Green tools
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Spray marking	  Red fish Green tools
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Streamer tags	  Yellow fish Green tools
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Tattoo methods	  Green fish Green tools
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VI alpha tags	  Green fish Green tools
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VIE	  Green fish Yellow tools
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**YES**  
↓

Do you need to collect information about inbreeding, effective population size and similar? **NO** →

Method	Symbols
Clipping the adipose fin	  Yellow fish Green tools

Clipping pelvic fin	  Red fish Yellow tools
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Clipping the maxilla	  Yellow fish Yellow tools
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Coded-wire tags	  Green fish Yellow tools
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**YES**  
↓

Method	Symbols
Genetic identification	   Green fish Yellow fish Green tools

## Biotelemetry/biologger – internal



### Research question

- For individual tagging
- Collect information about the fish's physiology and/or movements
- For all fish types
- Can remain in the fish for several years

### Brief method description

An electronic device is placed inside the fish (can be done two different ways that differ in the degree of difficulty).

### Animal welfare

- The fish is exposed to air
- The impact on survival and growth differs between the types of tags used
- General anaesthesia must be used
- The fish is not affected during reading

### Practical concerns

- The tags costs SEK 200 – several 100 000
- Reading possible up to a couple of kilometres away (acoustic communication and radio communication) or unlimited (satellite communication)

### Things to consider

The fish is anaesthetised to the level of general anaesthesia before inserting most types of internal biotelemetry tags, partly to reduce suffering and partly to keep the fish immobile during the procedure. Tags that are placed in the fish's stomach usually do not require surgical insertion but can be inserted into the fish's stomach using a finger or a guide. Tags that are implanted in the abdominal cavity require anaesthesia and a surgical procedure. Some tags must be anchored inside the abdomen, usually to the abdominal wall with one or more stitches. Avoid anchoring the tag in the same stitch that closes the wound in the abdominal cavity, as this can make it difficult for the fish to heal. The fish must be anaesthetized throughout the implantation procedure.

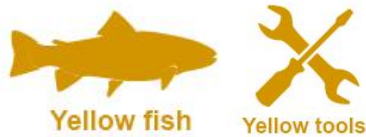
This method is used to collect information about the fish's movement patterns, behaviour and physiology. Data is transmitted wirelessly, usually through the use of radio waves (in fresh water and air), acoustic signals (in water; the receiver must also be in the water) or via satellite communication (in air; the tag sends data after it releases from the fish and floats up to the surface). Many devices also act as biologgers and store information locally on the device. Reading is done using the implant's associated equipment, often through a computer connection. The method is suitable for all types of fish, but the tag must be adapted according to the size of



the fish, which in practice means that tagging fish <100 g is seldom possible with the current technology.

The cost of a tag depends on the size and sensors used for measurement. The cost of the software and hardware needed for programming and reading the tags, tagging equipment and anaesthetics also needs to be considered. The method requires training in surgical procedures. A poorly performed tagging procedure can result in the rejection of the implant. Studies show good survival after the tagging process, but it can take several weeks for a fish to fully recover after a major surgical procedure.

## Biotelemetry/biologger – external



### Research question

- For individual tagging
- Collect information on fish movement patterns and the physical environment
- For all fish types
- Can remain on the fish for several years

### Brief method description

An electronic device is attached to the fish

### Animal welfare

- The fish is exposed to air
- The impact on survival and growth depends on the size, placement and design of the implant
- The fish usually need to be sedated
- The fish is not affected during reading

### Practical concerns

- The tags costs SEK 200 – several 100 000
- Reading possible up to a couple of kilometres (acoustic communication and radio communication) or unlimited (satellite communication)

### Things to consider

The fish is sedated before tagging for most types of external biotelemetry tags, partly to reduce suffering and partly to keep the fish immobile during the procedure. One or more sutures (synthetic or stainless steel) are typically used to attach the tag to the fish's skin using a needle and needle holder. For larger wild fish, tags are sometimes attached using a harpoon. For this method, the fish usually needs to be completely or partially exposed to the air.

This method is used to collect information about the fish's movement patterns, behaviour and physiology. Data is transmitted wirelessly, usually through the use of radio waves (in fresh water and air), acoustic signals (in water; the receiver must also be in the water) or via satellite communication (in air; the tag sends data after it releases from the fish and floats up to the surface). Many types of telemetry devices also act as bio-loggers and store information locally on the device. Reading is done using the tag's associated equipment, often through a computer connection. The method is suitable for all types of fish, but the tag must be adapted to the size of the fish, which in practice means that tagging fish <100 g is seldom possible.

The cost of a tag depends on the size and the type of sensors used for measurement. The cost of the software and hardware needed for programming and reading the

tags, tagging equipment and anaesthetics also needs to be considered. A poorly performed tagging procedure can result in the rejection of the implant or that it falls off within a few weeks. Some devices are designed so that they float and can thus be recovered. Some techniques are easy to learn, but practice is required to acquire reasonable speed and maintain quality.

## Carlin tags



### Research question

- For individual tagging
- For fish  $\geq 15$  cm in open environments
- If performed correctly, remains attached for the entire life of the fish

### Brief method description

Two stainless steel wires are pulled through two inserted cannulas placed under the fish's dorsal fin. The cannulas are then pulled out of the fish. The steel wires are twisted tightly against the fish's body, holding a metal plate with a code.

### Animal welfare

- The fish is exposed to air
- The method may lead to a somewhat higher mortality
- General anaesthesia must be used

### Practical concerns

- Each tag costs approximately SEK 40
- Visual reading

### Things to consider

The fish is anaesthetized to the level of general anaesthesia before tagging, partly to reduce suffering and partly to keep the fish immobile during the procedure. Without anaesthesia, there is a high risk that the fish will be injured. The double cannulas (outer diameter 0.9 mm, length 38 mm) are attached to a cannula holder and are inserted through the fish at the height of the centre of the pterygiophores (the bones that supports the dorsal fin). The exit holes must be at the same height as the entrance holes, and the steel wires that are pulled through the cannulas must sit directly against the body of the fish, otherwise a wound can easily develop in the fish's body because the tag itself and the thread through the fish are movable. A small metal plate is attached to the steel wire with an alphanumeric code. Tagging also requires needle holders with narrow jaws (1–1.5 mm), cutting pliers for stainless steel wire and a fish cradle made of stainless steel or plastic where the fish is placed during the tagging procedure.

This method is mostly used for salmonids (order Salmoniformes), but can principally be used for all fish larger than 15 cm. The technique is difficult to learn and requires a lot of practice. Costs to consider include the tags (approximately SEK 40 each), anaesthesia and tools. Environmental rulings that apply for certain watercourses contain requirements that a certain proportion of the fish that are reared must be tagged with Carlin tags. Through a ruling of the Land and Environment Courts, this requirement has been waived in at least one case (2018). A certain degree of reduced survival has been seen in some studies: untagged fish

have about 5% higher probability of survival than fish tagged with Carlin tags. Preliminary studies suggest that one-year-old smolts experience more adverse effects from the tag than two-year-old smolts (even if the one-year olds meet the size requirements for tagging). A certain degree of tag loss has been noted (<1%). The method should not be used on species that live in habitats with rocks, branches and vegetation, as the tag can get stuck, and in the worst case scenario, tear off.

## Chemical branding



### Research question

- For group marking (primarily)
- Suitable for fish that do not have overly dark scales, salmonids  $\geq 7.5$  cm
- Visible 2–3 weeks

### Brief method description

In this method, different chemicals are used to create a brand on the fish's skin.

### Animal welfare

- The fish is exposed to air
- Increased risk of infection
- The fish must receive local anaesthetic or be sedated

### Practical concerns

- Visual reading
- Can be seen from a distance of a couple of metres in clear water with good light

### Things to consider

The fish need to receive local anaesthetic or be sedated before it can be chemically branded, partly to reduce suffering and partly to keep the fish immobile during the procedure so that the branding result is better. In this method, different chemicals are used to create a brand on the fish's skin. Commonly used chemicals are potassium permanganate or silver nitrate, mixed with water. For the best results, the mucus layer must be scraped off the fish where the brand will be placed.

This is primarily a group marking method, but it can also be used to differentiate individuals in shorter studies through combinations of brands on the fish's body. This marking method is suitable for many groups of fish but has mostly been used for salmonids (order Salmoniformes). The few studies that have been done show that brands created using potassium permanganate and silver nitrate usually disappear after two to three weeks. For the first few days after branding, the dark spots can be seen from a couple of metres away in clear water with good light.

Branding requires chemicals and something to distribute the chemical mixture with, such as a brush, narrow tube or a syringe with a narrow opening. The chemical mixtures used can also be irritating to human skin, eyes and the respiratory system. Preparation of stock solutions and ready-to-use solutions must always be done under a fume hood. Gloves, safety goggles and good lighting are important. Costs include chemicals, anaesthetics and tools.

Few studies have been done using this type of marking, and it is rarely used in modern fish research. The fact that the brand disappears after a short period of time reduces the usefulness of the method and the necessity to remove the fish's mucus layer for the best results increases the risk of infection in the fish.

## Clipping the adipose fin



### Research question

- For group marking
- For fish with adipose fins, for example salmonids
- Visible the entire life of the fish

### Brief method description

The adipose fin is removed using scissors or a razor blade

### Animal welfare

- The fish is exposed to air
- Little or no effect on survival and growth
- The fish must be sedated

### Practical concerns

- Visual reading

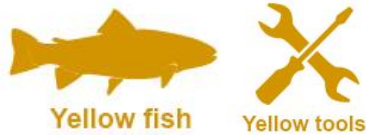
### Things to consider

The fish must be sedated before the adipose fin is removed, partly to reduce suffering and partly to keep the fish immobile during the procedure. The fish is placed with its back upwards and the fin is cut away with a razor blade in a special razor blade holder or cut off using scissors with curved shanks. A poorly performed marking procedure, where the fin is not entirely removed, can result in a certain degree of fin regeneration, which can make it difficult to see whether the fish has been marked or not. The technique is easy to learn, but practice is required to acquire reasonable speed. Costs to consider include anaesthesia and tools.

The function of the adipose fin has not been researched extensively, but there is evidence to suggest that it primarily functions as a flow meter in turbulent water. This means that salmonids most likely need the adipose fin when in flowing water and less so when they are in the open sea. The method does involve an amputation but is likely the mildest amputation a fish can be subjected to. The method is mandatory for all farmed salmon and trout released into the sea, Lake Vänern, Lake Vättern, Lake Mälaren, Lake Hjälmaren and Lake Storsjön.



## Clipping the maxilla



### Research question

- For group marking
- Suitable for many fish species, salmonids  $\geq 10$  cm
- Visible the entire life of the fish

### Brief method description

The flap in the corner of the mouth is clipped away (the maxilla – sometimes also the supramaxilla).

### Animal welfare

- The fish is exposed to air
- Can impact survival and growth
- The fish must be sedated

### Practical concerns

- Visual reading

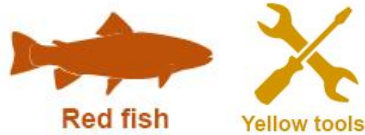
### Things to consider

The fish must be sedated before the maxilla is removed, partly to reduce suffering and partly to keep the fish immobile during the procedure so the quality of the mark is ensured. The anaesthetized fish is held on its side with its head angled towards the person performing the marking procedure. Straight shank surgical scissors must be used. One shank of the scissors is gently inserted under the flap (the outermost part of the maxilla) which is then clipped off. It is very important to avoid injuring the eye of the fish with the shank of the scissors and to avoid pushing the scissors too far in under the flap. You should also be sure not to clip twice because it can easily cause unwanted injury to the fish.

The marking method is suitable for many groups of fish but is primarily used for salmonids (order Salmoniformes), and the fish should be at least 10 cm. Fish anatomy differs between different fish species, and for many species this type of marking is not a suitable option. Costs to consider include anaesthesia and tools.

Few studies have been done on this type of marking, and it is rarely used in fish research. A study on farmed rainbow trout showed no negative effects on growth, survival or sexual maturity. Another study on Coho salmon (*Oncorhynchus kisutch*) showed lower survival and less growth in individuals with a clipped maxilla compared to unclipped controls. As this marking method involves an amputation and there is a lack of recent studies, this method should only be used if there are very good reasons to do so. Other, more animal-friendly methods, are often suitable to use.

## Clipping the pelvic fin



### Research question

- For group marking
- Suitable for all fish, salmonids  $\geq 7$  cm
- Visible the whole life of the fish

### Brief method description

One of the pelvic fins is removed using scissors.

### Animal welfare

- The fish is exposed to air
- Effects on survival and growth depend on the species
- The fish must be sedated

### Practical concerns

- Visual reading

### Things to consider

The fish must be sedated before the pelvic fin is removed, partly to reduce suffering and partly to keep the fish immobile during the procedure so the quality of the mark is ensured. During the marking procedure, the anaesthetized fish is kept with its head away from the person performing the procedure and on its back. Surgical scissors with straight shanks must be used, with one shank of the scissors inserted under the selected fin, the rays of the fin are then clipped as close to the base of the fin as possible. The technique is fairly difficult to learn; practice is needed to develop speed while ensuring the clipping procedure is done correctly. A poorly performed marking procedure, where the fin is not entirely removed, can result in a certain degree of fin regeneration, which can make it difficult to see whether the fish has been marked or not.

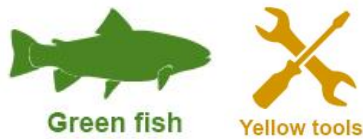
The degree of impact on the fish largely depends on the species, since the pelvic fin serves a slightly different purpose in different species. Some studies in salmonids have shown reduced growth or survival after a pelvic fin was removed. Other studies show no or only a small effect on the survival and growth of the fish when a pelvic fin is removed. A Swedish study on brown trout showed a 30% reduction in survival if both pelvic fins were removed.

The marking method is suitable for all fish, but is primarily used for salmonids (order Salmoniformes), and the fish should be at least 10 cm. By removing the right or left pelvic fin, you can create two different groups of fish. Costs to consider include anaesthesia and tools.

Considering that this marking method involves an amputation, which in all likelihood has a significantly greater negative impact on the fish than, for example

adipose fin clipping, one should avoid using this method unless there are very good reasons to use it. Other, more animal-friendly methods are suitable in most cases. If this marking method is used, both pelvic fins must never be removed on an individual.

## Coded-wire tags



### Research question

- For group tagging (primarily)
- Suitable for fish  $\geq 2.5$  cm
- Often lasts the entire life of the fish

### Brief method description

A small metal pin is inserted into the fish, usually into the cartilage of the snout.

### Animal welfare

- The fish is exposed to air
- No known impact on survival
- Sedation is recommended

### Practical concerns

- Each tag costs approximately SEK 1
- The tagging machine costs  $>$  SEK 300,000, but it is possible to rent a machine
- Fish must be euthanised to read the code

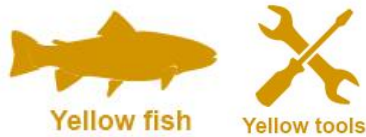
### Things to consider

The fish should be sedated before tagging so that they can be handled quickly and smoothly and to avoid unnecessary stress to the fish. The snout tag is a small metal pin that is inserted into the fish's tissue, usually into the snout cartilage that ensures that the tag will not travel around the fish's body and the risk of rejection is lower. Special equipment is needed to mark the fish. The tags are delivered on a spool and the tagging machine magnetises, cuts and inserts the tag into the fish. This marking method is mostly appropriate as a group marking method and works best for very large groups (hundreds of individuals). This method can also be used for individual marking, but then it would be the equivalent of three tags per individual because the tagging machine cuts slightly wider than the number marking on each tag. This means that tags before and after the tag used need to be sacrificed in order to ensure that a full tag number is applied. A detector can be used to determine if a fish is marked or not. In order to read the engraved code, the fish must be euthanised before the tag is removed and the code is read under a microscope.

Fish 2.5 cm and up can be marked with the method and the tag is suitable for all types of fish. The method usually uses tags that are 1.1 mm long and 0.25 mm in diameter, but for fish that are less than 4 cm, tags that are 0.5 mm in length should be used. For larger fish, there are also tags that are 1.6 and 2.2 mm in length. The tags costs between SEK 0.90–1.50 each, depending on how many are ordered. A basic detector costs approximately SEK 2,000. The tagging machine costs approximately SEK 325,000, but can also be rented for about SEK 25,000/month. Add to this costs for anaesthesia.

Tag loss is normally a few per cent (study of American paddlefish in ponds 0–3% and brook trout 1.5–2.3%). Most fish that lose the tag do so within the first two weeks after tagging. In a large study of Chinook salmon (*Oncorhynchus tshawytscha*), it was found that the snout tag did not affect survival from the juvenile stage to adulthood when fish returned to spawn. Less than one per cent of tagged fish get an infection at the tag site. Of all the marking methods, this method probably affects the fish the least. For a small fish, snout tagging can even have a lower impact than swabbing the mucus layer for genetic identification.

## Dart tags



### Research question

- For individual tagging
- For fish  $\geq 20$  cm in open environments
- If performed correctly, the tag remains attached for at least a year

### Brief method description

In this method, a barbed plastic tag is inserted under the dorsal fin of the fish using a cannula.

### Animal welfare

- The fish is exposed to air
- General anaesthesia must be used

### Practical concerns

- Each tag costs approximately SEK 20
- Visual reading

### Things to consider

The fish is anaesthetized to the level of general anaesthesia before tagging is performed, partly to reduce suffering and partly to keep the fish immobile during the procedure. Without anaesthesia, there is a high risk that the fish will be injured. A special tag applicator is used when tagging. The tip is sharpened so the tag can easily penetrate the skin and muscles. With the cannula attached to the tag applicator, a scale is removed just below the base of the dorsal fin and the tag is then attached. The barb on the tag must hook securely into the pterygiophores (the bones that supports the dorsal fin), otherwise the tag will come loose. Most of the tag then hangs outside the fish's body like a stiff, narrow tube. The tag is colour-coded, pre-printed with relevant text and numbered by the manufacturer according to the requests of the end user.

Since the tag applicator needs to be able to hold the entire tag, it is important to use tags with as small a barbed tip as possible. Applicators that are too large create unnecessarily large wounds, which can allow the tag to move and cause unnecessary pain and discomfort for the fish. Since a dull tag applicator creates unnecessarily large wounds, the cannula of the tag applicator must be sharpened or replaced regularly.

This marking method is suitable for all groups of fish but should be avoided for species that move frequently between rocky habitats and in vegetation, as the tag can then be scraped or torn off. Dart tags are available in a range of sizes and designs that differ in the amount of space provided for text. The tag you choose to use can therefore vary significantly, depending on the size of the fish and other factors. The tag is suitable for fish that are 20 cm or larger. One study showed that

tag loss with this method is about 2% per year, but other studies show larger and smaller degrees of tag loss. The tag is usually used for studies that are shorter in duration (up to 12 months). Costs to consider include the tags (approximately SEK 20 each), anaesthesia and tools. Practice is needed to become skilled at attaching the tag using this method.

## Freeze branding



### Research question

- For group marking (primarily)
- Suitable for fish with scales that are smooth and not overly dark, salmonids  $\geq$  7.5 cm
- Clearly visible for several months and can be felt for up to a year

### Brief method description

A metal object that has been cooled to a very low temperature (usually with liquid nitrogen) is pressed against the fish's skin for 3 seconds.

### Animal welfare

- The fish is exposed to air
- No impact on survival and growth
- The fish must receive local anaesthetic or be sedated

### Practical concerns

- Visual and tactile reading
- Liquid nitrogen is used

### Things to consider

The fish must receive local anaesthetic or be sedated before freeze branding, partly to reduce suffering and partly to keep the fish immobile during the procedure so the quality of the brand is ensured. In addition to liquid nitrogen, metal rods are needed, e.g. small Phillips head screwdrivers or special holders for liquid nitrogen to which a metal rod is attached so that the metal can be cooled by the nitrogen. If metal rods are used, they need to be placed in liquid nitrogen for a few minutes before branding. The metal rod is then pressed lightly against the fish for three seconds; pressing any longer can create a wound, which, for example increases the risk of infection.

Two things happen during freeze branding. First, a dark spot is temporarily created, and second, the fish's scales are deformed. The dark spot disappears after about a month, while the deformation of the scales can be seen at certain light angles and felt with the fingers for up to one year, sometimes longer. This deformation can be seen and felt best on fish with smooth scales (cycloids). This is primarily a group marking method, but it can also be used to differentiate individuals in shorter studies through combinations of freeze brands. The method has mostly been used for salmonids (order Salmoniformes).

Costs to consider are liquid nitrogen, anaesthesia and tools. Caution must be exercised when working with liquid nitrogen; gloves and safety goggles are required. Good ventilation and lighting are also important.



Few studies have been done using freeze branding, and the method is seldom used in fish research. If performed correctly, this marking method has no impact on the fish (survival and growth), though it is not clear whether the method causes pain to the fish. In the few studies where freeze branding has been used, fish have not been observed to try to rub off the brand.

## Genetic identification



### Research question

- Information on effective population size, kin recognition, etcetera
- For all fish (that have identified biomarkers)

### Brief method description

DNA is collected by swabbing the mucus layer of the fish or from tissue samples from the fish's fins or scales (if necessary, blood). Different sample collection methods entail different levels of pain and discomfort for the fish.

### Animal welfare

- Fish are exposed to air
- The level of anaesthesia needed depends on the species and how the DNA is collected

### Practical concerns

- Sampling costs from SEK 200 per animal
- Genetic markers for the species is required

### Things to consider

There are four main methods for collecting tissue samples. Generally fin sample and swabbing are preferred.

The **fin sample** is the most common method used to date and is easy to learn. A small piece of fin (usually 1-2 mm<sup>2</sup>) is clipped off at the tip, which causes relatively little injury to the fish, and the tissue is placed in ethanol. When collecting a fin sample, the fish should be sedated so that it can be handled without being injured. This is especially true for small fish. With proper training, a sample can be collected from larger fish ( $\geq 1$  kg) without anaesthesia, which often leads to lower stress for the fish than sedation, sampling and recovery.

**Swabbing** the mucus layer of the fish has recently been shown to provide the same quality results as fin tests in 95–100% of cases and is an easy technique to learn. Swabbing should primarily be considered for fish  $\geq 5$  cm. In many cases, swabbing can be done without anaesthesia, but this will depend on how active the species is. The use of this technique does have a negative effect on the mucous layer, which can lead to infection. This mainly applies to smaller fish, since a larger proportion of the mucus layer is affected. Swabbing should therefore be avoided for wild fish smaller than 5 cm. this guideline differs for captive fish, as they can be monitored and treated in case of infection.

**Scale samples** should only be taken for age determination, as this method causes damage to the fish's mucous layer, which increases the risk of infection. If you are performing age determination, a portion of the scales can also be used for genetic

analysis. The same guidelines that apply for anaesthesia in fin sampling also apply for scale sampling. The level of difficulty involved in collecting scale samples depends on the species and age of the fish.

**Blood sampling** is used rarely because it is very difficult to take samples from a fish, and unless blood samples are being collected for other reasons, we do not recommend using this technique.

Genetic identification can provide better answers to some questions that are also answered through typical fish marking and tagging, such as effective population size, whether populations differ and the degree of inbreeding. However, genetic identification cannot answer questions about, for example migration patterns, movement in the water and growth.

## Microchip (PITtag, RFID)



### Research question

- For individual tagging
- For all fish types
- Usually stays in place for several years

### Brief method description

A microchip is placed in the abdominal cavity or in the muscular tissue. The chip can be placed in a number of ways, each with different degrees of difficulty.

### Animal welfare

- The fish is exposed to air
- Low impact on survival and growth
- Sedation may be needed depending on the technique selected and fish size

### Practical concerns

- Each tag costs from SEK 15
- Reading can be done automatically or manually

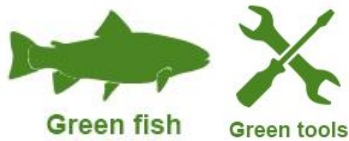
### Things to consider

If the tag is to be injected into muscular tissue, no preparation is usually needed, unless sedation makes it easier to handle the fish. The process of sedation, tagging and recovery is usually more stressful for the fish than simply tagging the fish. This is especially true in field research where large fish will be tagged, but some fish species (for example rainbow trout) may need to be sedated to be handled quickly and gently. General anaesthesia must be used if the tag is to be injected into the abdominal cavity, which is standard for fish under 1 kg. Injection into the muscle tissue is relatively easy to learn, while injecting into the abdominal cavity is more difficult. Another technique is to inject the tag into the abdominal cavity with a cannula. This method is somewhat easier to learn but creates a larger wound in the fish. Yet another technique is to pierce a hole in the abdominal wall with a pointed blade scalpel and push the mark in with your fingers (using gloves and as aseptic as possible). This method is more difficult to learn and requires dexterity and a sharp scalpel, but it creates a smaller wound in the fish that heals much better and more quickly. There is a risk of infection when the tag is injected either into the muscle tissue or into the abdominal cavity. Experience from the Fisheries Research Station in Älvkarleby shows that 0.1% of fish are infected when this method is used.

The method is often called the PIT tag (Passive Integrated Transponder tag) method or RFID (Radio Frequency IDentification). The tag is activated by a signal emitted from a reader. The tag then emits a unique code which is shown on the display of the reader or is stored and then transferred automatically to a computer. The most commonly used tag can be read at a distance of 30 cm, but larger tags

have a longer range. There are automatic readers that can detect fish as they swim past; this is usually done by building an obstacle so that fish must pass close to the reader. If manual reading is done, the fish must be taken up in the air to be scanned with the scanner. The cost of a tag is about SEK 15 and up, depending on the size. In addition, there is the cost of the tagging equipment, anaesthesia and ethanol for cleaning. The tag can be adapted to the size of the fish. The microchips used are usually in round glass ampoules, usually 12 mm (diameter 2 mm) or 23 mm (diameter 3.7 mm) in length. Tag rejection occurs 0–80% of the time. Initial rejection can be especially high for small fish (less than 50 mm), in which abdominal implants are always used. Once the wound has healed after the injection, the tag has very little effect on the fish (if the size of the tag is properly adapted to the size of the fish). In one study that looked at tagged brown trout and untagged brown trout, the tagged fish had 95–99% survival and the same growth rate. The tag itself usually lasts for 20–30 years.

## Natural patterns and unique markings



### Research question

- For identification of individuals (primarily)
- For fish with an appearance that varies between individuals

### Brief method description

With the use of still photos or video, images of individuals are taken which are then analysed.

### Animal welfare

- No effect on the fish

### Practical concerns

- Water quality determines reading distance
- Reading can be done automatically or manually

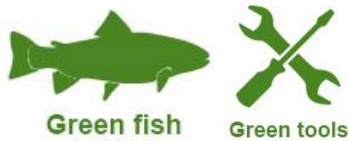
### Things to consider

The method is based on the use of still images or videos taken of individual fish, which are then analysed manually or using digital image processing. Using this method, fish are not marked in any way. Photo identification instead relies on the animals having stable and individual differences in appearance, such as colour, patterns and scars. For shorter studies, the method is suitable if differences persist during the project period.

There is no direct cost per animal; costs are instead associated with investment in the equipment needed to take high-resolution photos and any software needed if automatic identification is desired. Expect higher payroll costs if the images or videos are to be read manually. The method requires a still image or video using a camera with high enough resolution. Lighting may be needed. If there is a risk that normal lighting will disturb the fish, infrared lighting can be used, as most fish species are unable to see light in the infrared range. In this case, cameras should be infrared sensitive and equipped with a daylight filter. However, if infrared light is used, information on fish colour is lost, which means that if colour is an important part of identification, infrared light cannot be used.

The length of time it takes to learn the technique depends on previous knowledge of photography. Staff must be able to take photos with a relatively high and consistent quality. Ergonomic concerns mainly apply to manual photography, not when cameras are set up to capture images of the fish. Cameras can be set up either to capture images at regular intervals or when a fish moves into the camera's field of vision. In order to capture quality images/video, it may be necessary to build obstacles that lead the fish past the camera within a suitable distance.

## Otolith marking



### Research question

- For group marking
- For bony fish
- Lasts the entire life of the fish

### Brief method description

The appearance of the otoliths (located in the inner ear of the fish) are changed.

### Animal welfare

- Little if any impact on survival and growth

### Practical concerns

- Easy technique to learn
- The fish must be euthanised for reading
- $\geq$  SEK 0.1 per fish (temperature marking)

### Things to consider

The appearance of otoliths, which are bones found in the inner ears of bony fish, changes as the fish grow and form zones like growth rings in a tree. There are a number of ways to influence this pattern in order to create a unique mark.

Temperature marking is a safe, cheap and simple technique. Fish are exposed to variations in water temperature of at least 3 °C a number of times, which creates darker and lighter bands in the otoliths. The technique is reliable and does not seem to have a negative impact on the animals; the cost has been estimated at around SEK 100–200 per thousand individuals. An equivalent mark can also be achieved by manipulating the length of the day. Another technique is to bathe the fish or eggs in an aqueous solution with a fluorescent dye (for example calcein or alizarin) which is absorbed into the otoliths. It is also possible to create clear “banding” in the otoliths by bathing the fish in a strontium chloride solution or feeding the fish food that contains the metallic substance strontium.

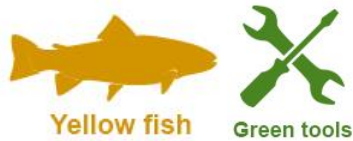
Two techniques that we do not recommend are dry marking eggs and the use of Tetracycline. Dry marking eggs can result in a reduced hatching rate, and Tetracycline is an antibiotic that should only be used for disease control.

Otolith marking should work on most species of bony fish, but the literature mainly reports marking of different salmon species. Otoliths must be removed and sanded or sectioned to view the growth rings. Light/dark and temperature markings are viewed with an ordinary light microscope, fluorescent substances require UV light to view, and if strontium is used, reading is done with an electron microscope with special detectors.

The cost depends on the technique used, but is the lowest per individual when marking large groups. The easiest and cheapest techniques are temperature marking and light/dark marking, which do not require any harmful chemicals and which are simple and safe for staff to perform. If marking is done with fluorescent dyes or strontium, knowledge of how these substances should be handled is needed along with knowledge of the health risks associated with using the substances. Fish only need to be handled if they will be moved to a special marking tank. The marking process has very little, if any, impact on the fish.



## P-chip



### Research question

- For individual tagging
- For all types of fish, especially small fish
- Usually remains in place for the entire life of the fish.

### Brief method description

A very small microchip is superficially inserted into the dorsal muscle tissue.

### Animal welfare

- The fish is exposed to air
- Low impact on survival and growth
- Sedation may be needed depending on fish size

### Practical concerns

- Each tag costs approximately SEK 50
- Reading is done manually after sedation

### Things to consider

P-chips are very small (0.5x0.5x0.1 mm), and it is therefore possible to tag small fish, such as zebrafish that are larger than 2 cm. When tagging small fish (5 cm or less), sedation is required, and the process of sedation, tagging and recovery is stressful for the fish.

Sedation is also needed when reading the chip, as the fish must be immobile and the reader held close to the fish's skin. Reading cannot be done in the water; therefore, fish must be lifted up and exposed to air. Despite these disadvantages, the small size of p-chips makes them preferable to PIT tags when marking, for example, zebrafish.

A P-chip is a small electronic device with a unique code. The chip is activated by laser light from the reader and then emits its unique code which is shown on a display and transferred to a computer. The cost of a tag is about SEK 50. Add to this cost for marking equipment and anaesthesia. The chips are supplied in sterile syringes. Chip rejection occurs in about 10% of all fish marked. To minimise rejection, it is important to "massage" the injection-site wound so that it closes. Once the injection-site wound has healed, the chip has very little impact on the fish and is not expected to have any effect on behaviour, growth or survival.

## Short-term marking with plastic beads or filaments



### Research question

- For group tagging (primarily)
- For fish in a laboratory setting
- Only for short studies (1-2 weeks)

### Brief method description

A cannula, needle or special tagging gun is used to thread a steel wire, nylon filament or similar through the fish. Either the colour of the threads is varied or plastic beads or similar are placed on the threads/filaments before attaching them to the fish.

### Animal welfare

- The fish is exposed to air
- The fish must be sedated

### Practical concerns

- Each tag costs from SEK 1
- Visual reading  $\leq 2$  m

### Things to consider

Sedation is required to reduce suffering and to immobilise the fish during the procedure. A cannula or needle is used to thread a steel wire, nylon filament or similar through the fish under the dorsal fin and between the pterygiophores (the bones that supports the dorsal fin). You either vary the colour of the wire/filament or attach plastic beads or similar to the ends. Another variation of the method is to thread a plastic filament that is burned at both ends with a small flame or heated rod and the bumps that form are coloured with for example nail polish. The tag must be adapted to the size of the fish so that it does not stick out so much on the sides that it can get stuck on different structures in the aquarium. In order to minimise the risk of injury, it is important that the tag sits tight against the fish's body. An advantage of this method is that it can be adapted for fish that are smaller than 4 cm, for example zebrafish.

This method is suitable for all types of fish in shorter laboratory studies where the animals are observed daily. The method is not suitable for use on wild fish as the tag may cause damage long term. At the end of the study, the tag must either be removed, or the fish must be euthanized. If the tag stays in the fish too long, it can have a negative impact on the animal, the tag can, for example, start to rotate and cause a wound. The method is quite easy to learn, quick and easy to perform, and does not have requirements for long durability like other marking and tagging methods.

## Spaghetti tags



### Research question

- For group tagging (primarily)
- For fish  $\geq 40$  cm in open environments
- Stays attached for up to one year.

### Brief method description

Spaghetti tagging is an imprecise term. In this context it is the use of a metal cannula to push a wire or a thin plastic tube through and under the back section of the dorsal fin (or just behind) and then attach the ends together.

### Animal welfare

- The fish is exposed to air
- Uncertain if growth and survival are impacted
- We recommend the fish to be sedated

### Practical concerns

- Each tag costs approximately SEK 10
- Visual reading
- Can be seen in clear water  $\leq 2$  m

### Things to consider

Although the procedure is relatively quick, we recommend sedation to reduce suffering, the risk of infection, and to keep the fish immobile during the procedure. The fish's mucus layer can be damaged by measures used to keep it immobile when working without anaesthesia. Spaghetti tags are threads or thin tubes made of vinyl or other flexible plastic which are inserted under the back section of the dorsal fin of the fish (or just behind) using a metal cannula and then pulled through. The ends of the thread/hose should be equal in length on both sides of the fish. A knot is then tied on the thread or the ends are attached together with a plastic or metal clip. The most serious mistake that can be made is attaching the tag under the dorsal fin in such a way that the tag damages or hinders the function of the dorsal fin. The colour of the threads is usually varied if the tagging method is used as a group tagging method. It is also possible to fit the threads with numeric and/or letter codes so that the method can be used to distinguish individuals. However, this is seldom done.

This tagging method is suitable for all groups of fish but should be avoided for species that move frequently between rocks and in vegetation, as the tag can then be scraped or torn off. Spaghetti tags are best suited for fish that are at least 40 cm. The tag is usually used for shorter studies (up to 12 months); only in exceptional cases does the tag last for several years. One study showed that tag loss was 50% after 190 days (two species of buffalofish) and 250 days (brown bullhead),

respectively. Costs to consider include anaesthesia and tools. Few studies have been done to determine how the tag affects the fish; this tagging method is not so used very frequently due to the high rate of tag loss. As this tagging method has its shortcomings, both in terms of reliability and animal welfare, the method should only be used if there are very good reasons to do so. Other, more animal-friendly methods, are suitable in most cases.

## Spray marking



### Research question

- For group marking
- Requires the fish to have a thin epidermis (outer skin) and fixed scales
- Visible 1–5 months depending on the species

### Brief method description

In this method, dye particles are applied under such high pressure that they penetrate the fish's epidermis, the outermost part of the skin.

### Animal welfare

- The fish is exposed to air
- Usually the fish does not need to be sedated
- Can lead to high mortality

### Practical concerns

- Visual reading, possibly using ultraviolet light

### Things to consider

The fish is usually not sedated before marking. In this method, dye particles are applied under high pressure using a spray gun or similar equipment that is held about 30 cm from the fish. The method requires the fish to be completely exposed to the air or in a very small amount of water such that one side of the fish is completely exposed to the air. Markings using non-fluorescent dye particles can be difficult to read after a few months. Fluorescent dye particles are usually used instead because they are more reliable, although ultraviolet light is required for reading. Older literature indicates that the marking can last for several years, but recent studies indicate that the marking disappears after 1–5 months, depending on the fish species. This method is not used very often and is not suitable for all types of fish. This is partly because the fish's epidermis must be thin and permeable enough for the dye particles to penetrate, and partly because the scales must not sit so loosely that they get blown off by the high pressure. The method is not suitable, for example for spawning salmon (the epidermis is too thick) or salmonids near the smolt migration (the scales sit too loose).

The cost per fish can be low, between SEK 1–3, and there is also the cost for spray gun. Staff performing the marking procedure must wear appropriate personal protective equipment, such as full body protective clothing, safety goggles and a mask.

It is a quick marking method for large groups of fish, but due to several factors, we do not recommend using this method. During the standard procedure, water is first drained from a trough until the fish is lying on the bottom floundering. Since the fish is not sedated, this means that the fish are exposed to intense stress. Secondly,

recent studies have shown that marking mortality for some species can be close to 100%. If this method is used, there must be very good reasons to do so. Other methods may not be as quick and easy, but they are much better for the fish and likely generate more reliable results.

## Streamer tags



### Research question

- For group tagging (primarily)
- For fish (1–5 cm over the back) in open environments
- Depending on the species, the tag remains attached for a few weeks or months

### Brief method description

Streamer tagging is an imprecise term. In this context it is the use of a plastic strip with a needle at one end and a notch in the middle, which is pushed through the fish just below the dorsal fin.

### Animal welfare

- The fish is exposed to air
- Uncertain if growth and survival are impacted
- We recommend the fish to be sedated

### Practical concerns

- Each tag costs approximately SEK 10
- Visual reading

### Things to consider

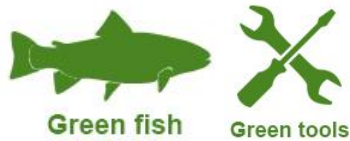
Although the procedure is relatively quick, we recommend sedation to reduce suffering, risk of infection, and to keep the fish immobile during the procedure. The fish's mucus layer can be damaged by measures used to keep it immobile when working without anaesthesia. It may be possible to tag large fish such as spawning salmon and brown trout without sedation, but the fish's head should then be covered with a wet towel to calm the fish. The needle at one end of the streamer tag is pushed through the fish just below the dorsal fin and the tag is then pulled through. The narrow part of the tag (the notch) must sit inside the fish, and the wider parts of the tag should hang freely on each side of the fish. It is not the length of the fish that matters the most, but the width just below the dorsal fin; the smallest tags have a notch at 1 cm, the largest 5 cm. Once the tag is in place, the needle is cut away or broken off. Studies have been done where divers tagged fish without anaesthesia.

For group tagging, the colour of the tags is varied, but if the streamers are fitted with number and/or letter codes, the method can be used to distinguish between individuals. This tagging method is suitable for all groups of fish but should be avoided for species that move frequently between rocky habitats and in vegetation, as the tag can then be scraped or torn off. Costs to consider include the tags (approximately SEK 10 each), anaesthesia and tools. It is possible to see the tag from a distance in clear water. Few studies have been done to investigate how the tag affects the fish. In a study performed on northern pike, no difference was found

in the survival of streamer-tagged fish and control fish. This tagging method can be good for shorter studies where tag loss does not have time to become a significant factor. In a study on flatfish, all streamer tags had come loose after four months, while in a study on northern pike, 80% of the tags had come loose after 325 days. Of all the external fish tagging methods, this is probably the one that is easiest to learn to do correctly. For shorter studies, streamer tags are a better choice than a T-anchor tags, spaghetti tags or dart tags, especially if the staff who label the fish have limited experience in fish tagging.



## Tattoo methods



### Research question

- For group marking (primarily)
- Suitable for many types of fish  $\geq 20$  g
- Usually visible for one year on salmon and brown trout

### Brief method description

The method entails injecting an ink solution into the skin.

### Animal welfare

- The fish is exposed to air
- The fish usually does not need to be sedated

### Practical concerns

- Visual reading

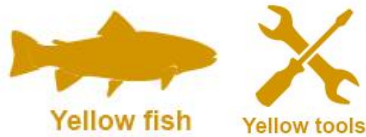
### Things to consider

The procedure is quick and easy, and the fish usually does not need to be sedated. If the fish is especially active, it should be sedated, primarily to keep the fish immobile during the procedure. The method uses a modified microdispenser to inject an ink solution into the skin. The method usually uses Alcian blue dissolved in distilled water, but there are a few other substances described in the literature. For smaller groups of fish, injection can be done using a syringe with a cannula about 0.8 mm in diameter. The needle holder is modified so that the protruding length of the cannula is limited to 3 mm. The method is generally used on larger fish ( $\geq 500$  g) but can also be used on smaller fish ( $\geq 20$  g). When marking smaller fish, the protruding length of the cannula should be shorter. For marking larger fish, the paint can also be applied with a microjet injector (a needle-free pressure injector).

Tattooing is a simple, fast and inexpensive subcutaneous colour marking method for groups of fish in the field, fish farms and laboratory environments. The easiest way to apply the mark is a square dot system with up to 3x3 dots. If a mark is applied to additional places on the fish, the number of possible combinations is increased. The placement of the tattoo should be determined according to the best readability, which depends on the species and sex, but unlike VIE (Visible Implant Elastomer), the method does not require transparent skin to be visible. The marking method has mostly been used on salmonids (order Salmoniformes) – and in salmon and brown trout; the tattoo should be placed in the areas in front of the pelvic fins, under the pelvic fins, under the pectoral fins and/or behind the anal fins to enable reading during spawning. Otherwise, the fish's natural pigmentation covers up the tattoos.

Costs to consider include chemicals, anaesthetics and tools. Preparation of the ready-to-use alcian blue solution, which is a strong dye, must always be done in a fume hood with gloves, protective clothing and safety goggles. This marking method has very little effect on the fish; it feels one or a few small pricks. The amount of alcian blue injected is 0.02-0.04 ml per prick. The effect on the mucus layer is also small, and in fish farming, tattoo marking has not been linked to fungal infections or other types of infection. Human consumption of fish marked with tattoos does not present any known risks.

## T-bar anchor tags



### Research question

- For tagging individuals
- For fish  $\geq 15$  cm in open environments
- Stays attached for up to one year.

### Brief method description

The method involves inserting a T-shaped plastic tag under a dorsal fin using a cannula, where the T-shape then forms an anchor for the tag.

### Animal welfare

- The fish is exposed to air
- Can result in reduced growth, mainly for small fish
- General anaesthesia must be used

### Practical concerns

- Each tag costs approximately SEK 15
- Visual reading

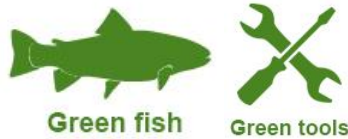
### Things to consider

The fish is anaesthetized to the level of general anaesthesia before tagging, partly to reduce suffering and partly to keep the fish immobile during the procedure. Without anaesthesia, there is a significant risk that the fish will be injured. When using the tag applicator with a cannula, a scale is removed just below the base of the dorsal fin, the cannula is then inserted, and the tag is attached. You then pull gently on the tag to feel that it is securely attached. The majority of the tag hangs outside the fish. The T on the tag must be hooked securely into the pterygiophores (the bones that supports the dorsal fin), otherwise it will come loose. The tag is colour-coded, pre-printed with relevant text and numbered by the manufacturer according to the requests of the end user. It is the same type of tag that is used to mark clothing with price tags, and the most serious error that can be made is pushing the cannula straight through the fish – which results in the T sitting outside the fish's body on one side and the rest of the tag sitting on the other side, where the two parts are connected only by the thin plastic thread. The tag will then rub and rotate back and forth, which can create a hole straight through the body of the fish.

This tagging method is suitable for all groups of fish but should be avoided for species that move frequently between rocks and in vegetation, as the tag can then be scraped or torn off. T-bar anchor tags, which are marketed by Floy Tag, are available in several different sizes and designs with varying amounts of space for text, which allows for customisation according to the size of the fish and other factors. The tag can be used on fish that are 10 cm or larger, although 15 cm is the

recommended lower limit. A study on an African catfish showed that tag loss is about 20% after one year, while other studies (on other catfish, rainbow trout, tilapia and muskellunge (North American relative of pike) showed both larger and smaller rates of tag loss. The tag is usually used for studies that are shorter in duration (up to 12 months). If applied correctly, the tag has relatively little effect on the fish, but some studies suggest that fish tagged with T-bar anchor tags show reduced growth and that this affects small fish in particular; the difference is not significant in larger fish. Costs to consider include the tags (approximately SEK 15 each), anaesthesia and tools. Practice is needed to become skilled at using this method.

## VI alpha tags



### Research question

- For individual tagging (primarily)
- Primarily for fish  $\geq 15$  cm
- Requires skin area with very little pigmentation
- Usually remains in place for the entire life of the fish

### Brief method description

A small fluorescent tag with an alphanumeric code is inserted under the skin

### Animal welfare

- The fish is exposed to air
- Probably little effect on survival and growth
- The fish should be sedated

### Practical concerns

- Visual reading
- Each tag costs approximately SEK 8

### Things to consider

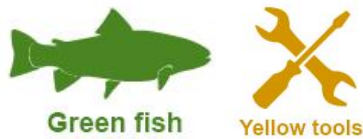
In order to ensure the optimum placement of the VI Alpha tag, the fish should be sedated. VI Alpha stands for “Visible Implant Alphanumeric”. The tag is made of with a bio-compatible, fluorescent, elastic material (elastomer) and contains an alphanumeric code. The tag is inserted under the skin using special injector in a location where the level of pigmentation is low (for example around the eye or in the fin membrane) so that the tag can be read through the skin. The standard tag is 1.2x2.7 mm and has a very lightweight, which means that they can be used on most fish. However, the tags are available in larger sizes of 2x5 mm. The company that manufactures the tags recommends the fish to be over 15 cm in length, but considering the small size and low weight, the tags should also be suitable for smaller fish. In that case, a veterinarian or a person designated as an expert according to the Swedish Board of Agriculture’s regulations and general advice on laboratory animals L150 should be consulted.

Reading is done visually, with or without the use of a UV light from a hand-held torch. Lighting increases contrast and visibility and is important, especially if the skin is pigmented where the tag has been inserted. The tags are available in several different colours, and if it is enough to be able to identify the colour of the tag, reading can be done at a relatively long distance and in the water. If the alphanumeric code also needs to be read, this is done from a few decimetres away and the fish must be taken out of the water. There is no information to indicate how long the tags remain under the skin, but if properly applied, they should last for the entire life of the fish.

A starter package costs around SEK 3,000 and contains 100 tags, equipment for inserting the tags and a light to increase readability. The injector needle needs to be replaced after a number of injections as it becomes dull. Replacement needles cost around SEK 200 each, and VI Alpha tags cost between SEK 7 and 9 each. The tags are easy to inject when suitable areas on the fish have been identified, but it is important that staff have knowledge of how the injector works and how deep into the skin the tag needs to sit so that it remains in place, but is still readable. It is also important to learn how to maintain and clean the injector, especially when it is used in a saltwater environment, and to replace the needle when necessary.

If performed correctly, neither the tagging process nor the tag itself should have a significant effect on the fish.

## VIE (Visible Implant Elastomer)



### Research question

- For group or individual marking
- Suitable for many types of fish
- Correctly placed implants are visible for the entire life of the fish.

### Brief method description

A coloured two-component plastic is injected into the fish's epidermis or fins.

### Animal welfare

- The fish is exposed to air
- The fish should be sedated

### Practical concerns

- Visual reading

### Things to consider

In order for the fish to be handled, it should be sedated or anaesthetized depending on the species, mainly to keep the fish immobile during the procedure. A coloured two-component plastic colouring is injected into the fish's epidermis or fins with an injection syringe. The use of different colour and fin combinations on different individuals make it possible to distinguish between large numbers of fish at the individual level.

VIE is a simple, fast and inexpensive subcutaneous colour marking method for groups of fish in the field, fish farms and laboratory environments. In lab environments, the implant works well as an individual mark during the course of an experiment. The implantation process is easy to perform, and by using several different colours and implant sites on the fish, the number of possible combinations increases. Bright colours, such as neon colours, are the most commonly used, fluorescent colours are also used so that the tags can be easily seen using a UV light.

This marking method is suitable for many groups of fish but has mostly been used for smaller fish in laboratory experiments (down to zebrafish size). In large fish and fish with dark colouration, it may be more difficult to see the implant than in small fish and fish with light colouration. The implant can also be difficult to see if the growth of the fish is significant and pigmented tissue grows over the implant site. VIE implants does not disappear, but placement should be decided based on the best readability, which depends on the species and gender. The best placement for the implant is in light areas and fin rays, but even semi-transparent and transparent tissue may be suitable for the implants. Clear tissue is the optimal place for an implant. Clear tissue is for example found behind the eye of salmonids, as well as behind and over the eye in many other families of fish. In addition to the

low cost for two-component plastic colouration, the cost of anaesthesia and tools should be added.

This marking method has a very low impact on the fish. The amount of elastomer injected is 0.02-0.04 ml per marking site. The impact on the mucus layer is also minimal.



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