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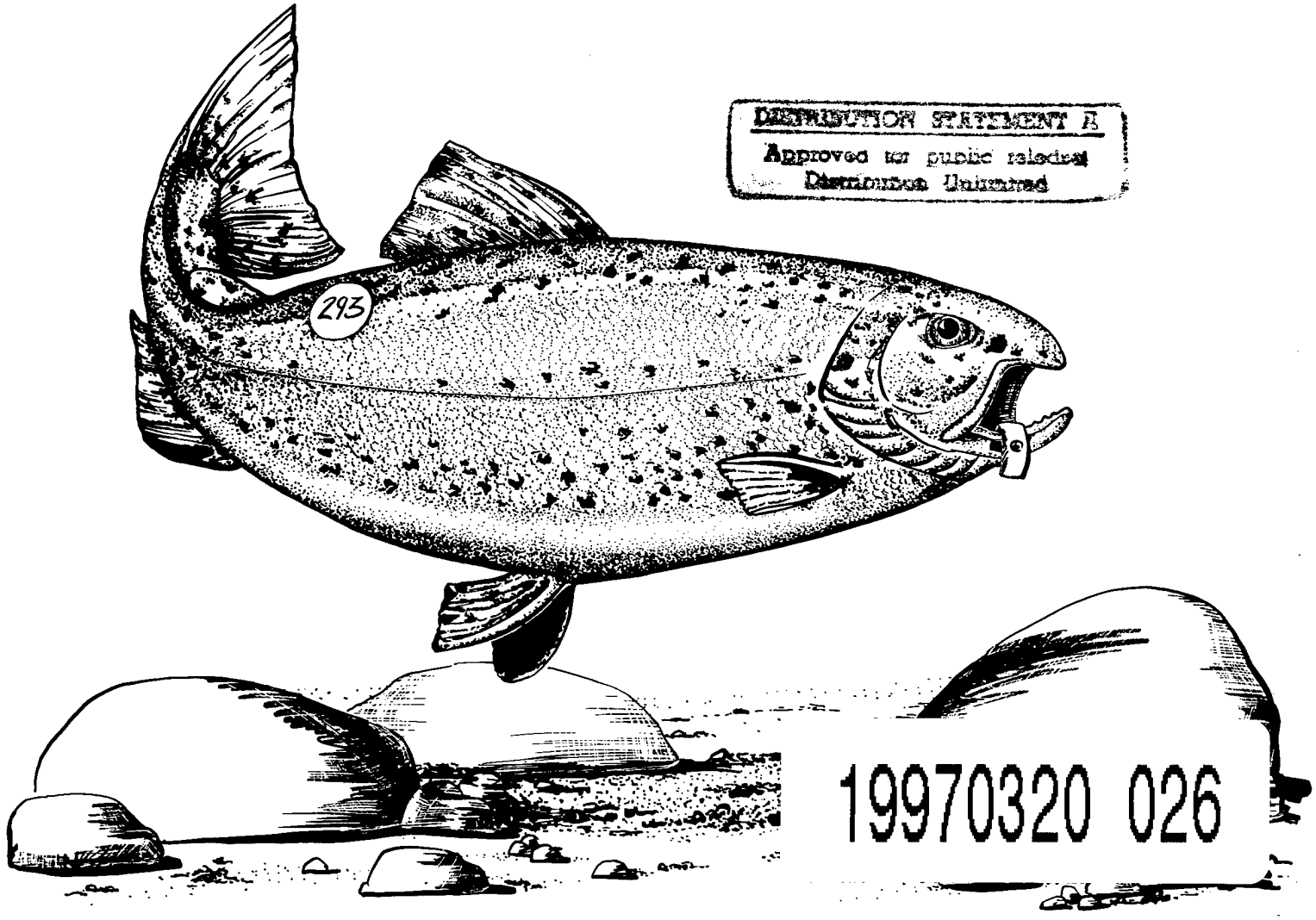
METHODS FOR LONG-TERM IDENTIFICATION OF SALMONIDS: A REVIEW



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Biological Report 88(37)
May 1988

Methods for Long-term Identification of Salmonids: A Review

by

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Introduction

Statutory mandates define the restoration responsibilities of the U.S. Fish and Wildlife Service with regard to anadromous salmonids (Anadromous Fish Conservation Act, Fish and Wildlife Act of 1956, Atlantic Salmon Conservation Act, and others). Consequently, it is important that those dealing with the population dynamics, restoration efforts, and ecology of these fish remain apprised of advances in long-term fish marking methods.

Since man began exploiting anadromous salmonids, there has been a need to identify individual fish and stocks of fish. Today the science of fish tagging and stock identification has evolved into a sophisticated art that assimilates input from varied disciplines, including electrical and mechanical engineering, genetics, mathematics, and fishery biology.

For this report, we define a long-term tagging method as one that can be detected at least 1 yr after application. Tag application, tag retention, and recovery or detection of the mark were evaluated by reviewing the literature. In addition to discussing conventional methods in which a physical marker is applied to individuals, we reviewed several newer methods of stock identification.

Computer literature searches for 1983–87 were conducted by using the BIOSIS and U.S. Fish and Wildlife Reference Service. We also solicited information from Canadian and United States researchers who are involved in fish marking or identification projects. An in depth review of nontag marking methods by Moring and Fay (1984) and an extensive bibliography of aquatic animal tagging by Emery and Wydoski (1987) provided summarized information for the years before 1984.

Our intent was not to review critically all the various methods available, but to discuss those being used or that have application to the anadromous salmonid fishery and to update the information base with recent findings. In other words, we concentrated on tags that can be applied quickly to a large number of fish and that are retained at reasonably high percentages for at least 1 yr.

Coded Wire Tags

A method of marking fish by implanting a coded wire tag (CWT) into the snout was developed by Jefferts et al (1963). Coded wire tags are bits of stainless steel wire etched with a binary code consisting of four longitudinal rows accounting for 262,144 possible numerical combinations. Standard tags are round, 1 mm long, and 0.25 mm in diameter; smaller tags (0.5 mm) are also available for small fish.

Coded wire tags are implanted with an automated injection assembly that enables a biologist to tag 500 to 800 fish per hour. An adipose fin clip is often added as an external confirmation marker. The fish is then typically passed through a quality control unit (magnetic field to determine the presence of the tag in the snout) and released. Elrod and Schneider (1986) reported losses of less than 1% during their work with these tags on lake trout (*Salvelinus namaycush*). Moring and Fay (1984) summarized extensive CWT research and tag loss in Atlantic salmon (*Salmo salar*) and Pacific salmon (*Oncorhynchus* spp.).

Fish are ultimately identified as carrying a CWT by the recognition of the adipose clip or detection of the tag with magnetic field. The fish must then be sacrificed to allow for removal of the tag from the snout and decoding, which means reading the tag under a microscope. The manufacturers of CWT's estimate that 200 tags can be decoded per person per day. Moberly et al. (1977) reviewed various procedures required to insert, detect, retrieve, and process the CWT's.

In 1984, the general consensus among some CWT users was coho salmon (*Oncorhynchus kisutch*) weighing 2.27 g (200/lb) were the smallest fish that would accommodate the tags. At the same time, some users believed that fish as small as 0.38 g (1,200/lb) could be successfully implanted by an experienced tagging crew. Opdyke and Zajec (1980) tagged chum salmon (*Oncorhynchus keta*) as small as 0.8 g. Northwest Marine Technology, a commercial vendor, has advertised that fish as small as 0.25 g (1,800/lb) can be routinely tagged. The basis for this claim was a tagging study by Thrower and Smoker (1984) in which emergent Alaskan pink salmon (*Oncorhynchus gorbuscha*) were implanted with half-length CWT's. In this study the authors developed an injector head mold smaller than anything available then, which enabled them to implant these small fish at rates of 800/h with more than 90% tag retention. Of the total of 9,338 emergent pink salmon they tagged in April 1982, 17 adults were recovered in August 1983. Their estimate of return (0.4%) was similar to that for other local untagged stocks during the same period.

Advances were made in the field of binary coded wire tagging in the early 1980's. An alternative method of tag decoding was developed that did not require sacrificing the fish. Flat tags (1.5 mm long) with the binary code etched along the edge could be read by using an X-ray machine that transmitted an image of the tag (while in the fish's snout) to a television screen. This technology, which has just recently become commercially available, makes it possible to identify fish many times, or at least eliminate the time-consuming tag retrieval process (Moring and Fay 1984). The flat tags and X-ray detection equipment require a considerably greater initial investment than the round tag system; however, the system eliminates the cost of tag retrieval and is nondestructive. Based on price quotations provided by Northwest Marine Technology, individual tag cost in mid-1987 ranged from $0.03 to $0.07, depending on the quantity ordered.

A significant portion of CWT use is concentrated in Pacific coast salmon research (25 million tags per year as quoted in a Northwest Marine Technology 1987 Newsletter). Coded wire tagging is also more organized and closely monitored in this fishery than anywhere else in the world. The Pacific Marine Fisheries Commission has taken on the responsibility of coordinating the returns as they are reported. Due to the volume and variety of tags and codes being released and subsequently returned (500,000 CWT's have been recovered to date) and the number of governmental and private agencies currently involved, data processing has understandably been delayed.

The logistics of coordinating a CWT recovery program for the Atlantic salmon fishery are complicated by the comparatively wide-ranging habitat of the species and the many nations involved. Coded wire tags are currently implanted in Atlantic salmon as a means of identifying the country of origin of salmon caught on the high seas. However, the basic reason for tagging salmon has varied through the years, resulting in a reduction of the data base needed to evaluate long-term tag retention and return information (Victor Segarich, personal communication). Researchers at the Salmon Genetic Research Group reported using CWT's with various other external tagging methods, such as pheasant wing tags and a newly developed form of tattooing or panject marking (Atlantic Salmon Federation 1985–1986). The consensus of Atlantic salmon researchers is that the CWT program will expand in the future. Because fewer Atlantic salmon (in comparison with Pacific salmon) are produced and tagged annually, the initial cost of the tagging and decoding equipment has delayed the large-scale use of CWT systems by Atlantic salmon researchers.

Coded wire tags have also been used to identify stocks of cyprinids, ictalurids, and percids (Northwest Marine Technology 1987 Newsletter). Klar and Parker (1986), who compared the usefulness of CWT's and microtaggants in marking fingerling striped bass (*Morone saxatilis*), reported 100% tag retention when CWT's were implanted in the cephalic portion of the adductor mandibularis (a small muscle below the eye) and superior overall results compared to those with microtaggants.

Although the passive integrated transponder tagging system is considered state of the art, the flat CWT and X-ray technology should not be considered far behind. In fact, the CWT system will probably continue to be the most widely used technique due to its lower long-term cost per tagged fish.

Passive Integrated Transponders (PIT) Tags

Implantable transponders were first used in the early 1970's to identify livestock, specifically horses. Today, Destron Identification Devices Inc., Boulder, CO, markets the tags and decoding equipment, which have been used to mark and identify not only livestock, but artwork, machinery, and (for the last several years) fish.

Much of the information presented in the following review of this tagging system was obtained from three sources:

1. Documentation provided by Destron Identification Devices Inc.
2. Victor Segarich, U.S. Fish and Wildlife Service, Nashua National Fish Hatchery, Nashua, NH.
3. Annual reports prepared by the National Marine Fisheries Service (NMFS) for the Bonneville Power Authority, currently investigating the potential of PIT tags for use in research on anadromous salmonids.

The reader is advised to refer to these reports (Prentice and Park 1984; Prentice et al. 1985, 1986) for a detailed review of various aspects of baseline biological testing with regard to these tags.

The PIT tag is inert, consists of a microchip and antenna, and is 12 mm long × 2.1 mm wide. It was originally encapsulated in polypropylene, which unfortunately permitted moisture to enter and foul the electronic circuitry, causing an unacceptable failure rate in early tags (Prentice et al. 1986). Today the tags are encapsulated in glass, which apparently remedied the moisture problem. The tags or transponders are implanted in the body cavity with a modified hypodermic syringe and 12-gauge needle or semi-automatic tag injector. Once activated, the tag emits a low frequency radio signal (40–50 kHz), which is translated into a 10-digit alphanumeric code (there are 34 billion possible codes). The tag is decoded in vivo, which eliminates handling of both tagged and nontagged fish. Since there is no self-contained energy source, the tag's lifetime is indefinite.

Passive Integrated Transponders are currently being tested in juvenile and adult salmonids in the Pacific Northwest (Prentice and Park 1984; Prentice et al. 1985, 1986) and on a smaller scale in adult Atlantic salmon at the Nashua National Fish Hatchery.

From 1983 to 1985, NMFS researchers in Seattle, WA, experimented with various anatomical locations for PIT tag implantation. Dummy (nonfunctional) PIT tags were injected into the body cavity, opercular region, and the dorsal and caudal musculature of juvenile salmonids. Adult salmonids were also tested for injection in the nose. The body cavity was chosen as the best anatomical site for implantation for all life stages. Passive Integrated Transponders implanted in the body cavity of adult Atlantic salmon were not as easily decoded as those implanted in the nose; however, glass-encapsulated tags seemingly can be read easily while in the body cavity (Earl Prentice, personal communication). Further testing with the dummy tags and subsequent work with functional tags (Prentice et al. 1985, 1986) yielded the following guidelines for body cavity implantations:

1. As judged by survival data, tag retention, and tissue response, the PIT tag could be injected and retained in the body cavity of juvenile salmonids weighing as little as 1.3 g.
2. Implantation of the tags at a pelvic or pectoral insertion was satisfactory in juveniles, but pectoral insertion is recommended due to the eventual position of the tag in the body cavity.
3. The tag did not appreciably affect survival in any of the test groups.
4. Growth was not significantly affected in any of the tagged test groups.
5. Tag retention was not markedly different between pectoral and pelvic treatments; however, the pectoral site tended to be slightly better.
6. Tag wounds appeared to close sufficiently within 8 to 12 d after implantation to prevent tag loss or wound infection.
7. If the gut was perforated with the tagging needle, there was an immediate change in fish color or behavior, usually followed by death within 5 d.
8. If a tag was lost (not retained in the fish), it was usually lost within 3 d after implantation.
9. There was no long-term behavior difference between tagged and untagged fish; however, this observation should be tested further (Prentice et al. 1986).

In 1984, NMFS, which began studying the efficiency of juvenile PIT tag monitors under simulated field conditions, found the equipment to be more than 90% efficient. In 1985, NMFS conducted field tests on a PIT tag monitor system for juvenile fish at the McNary Dam on the Columbia River (see Figures 10 and 11 *in* Prentice et al. 1986). Results of a series of tests with juvenile spring and fall chinook salmon (*Oncorhynchus tshawytscha*) indicated that the monitor functioned at 95% efficiency and 99% accuracy.

In 1986 and 1987, NMFS continued research with the newly developed glass-encapsulated PIT tags (Earl Prentice, personal communication). The glass tags are about 1 to 2 mm longer than the polypropylene model (a size increase that was necessary to accommodate a more economically efficient, automated connection between the antennas and the microchip), but have proven superior to their predecessors in three ways:
1. Excellent retention; no sign of mesentery tissue attachment, as was occasionally noticed with the polypropylene tags.
2. Improved tag longevity due to a more reliable moisture seal.
3. Greater range of transmittance; tags have been decoded at a distance of 10 cm from the fish's body (an improvement over the polypropylene tags).

The PIT system was recently compared with the more traditional tagging methodology of freeze-branding. Prentice et al. (1986) indicated that survival and behavior of juvenile chinook salmon implanted with PIT tags or freeze-branded were not significantly different. In addition, the number of test fish could be reduced by 90%–95% in a PIT tagging study to attain the same results and statistical significance. Fish tagged with PIT tags are handled once during a complete study, whereas other tagging methods require handling of both tagged and untagged fish during each detection effort.

Inasmuch as most PIT-tagged smolts released to the sea have been tagged with the polypropylene tags, the detection rate of the returning adults is not expected to be high. The first adults with the glass tags are expected to return in 1988; however, there was only one detection station (McNary Dam on the Columbia River near Umatilla, OR) by late 1987. The emphasis of the study to this point has been directed at the basic feasibility of the tags and detection system, the effect of this type of tagging on fish physiology and behavior, and preliminary research with the PIT tag as a tool for studying outmigrations of juvenile anadromous salmonids.

Passive Integrated Transponder tag detection devices have been incorporated into existing CWT trapping facilities, and adult salmonids can be automatically checked as they pass through detection systems in Denil fish ladders (Prentice et al. 1985). Passive Integrated Transponder reading efficiency can be expected to exceed 95%; it has been as high as 100% in migrant adult steelhead, *Salmo gairdneri* (Prentice et al. 1986).

With current equipment, an experienced PIT-tagging crew can tag fish at a rate of more than 100 juvenile salmonids (1.3–3.5 g) per hour. Personnel at NMFS have recently designed a gravity-fed tag-to-syringe loading device that may increase this rate to 400 per hour.

As would be expected with a system that incorporates state-of-the-art technology and body cavity implantation (a PIT jaw tag has also been used to monitor adult salmonid movements), its high cost and slow tagging rate may limit its utility. Cost per tag decreased after recent design modifications, and depends partly on the number ordered. The NMFS in 1987 procured 100,000 tags at a cost of $3.50/tag – a cost that may decline further as technology advances. The initial cost of detection systems varies with the complexity of the operation.

The usefulness of the PIT tag is being investigated in Norway, Sweden, and Australia, and is being considered in New Zealand. Most research has been done by NMFS in the Pacific Northwest. There has been more preliminary research into the effects of this tag on the behavior and biology of the target animal than for perhaps any other tagging system (Earl Prentice, personal communication).

The PIT tagging system will not replace traditional tagging methods (branding, attached tags, coded wire tags, etc.), but should provide the researcher with a new, versatile tool to complement these methods. Inter- and

intra-agency organization, similar to that for the coded-wire program in the Pacific Northwest, will be necessary to aid effort and reduce cost of future PIT tagging projects.

Branding

Several types of brands have been used over the years to mark fish. Freeze-branding may be the most popular technique (Everest and Edmundson 1967; Fujihara and Nakatani 1967; Mighell 1969; Smith 1973; Laird et al. 1975; Nahhas and Jones 1980; Gunnes and Refstie 1980; Fay and Pardue 1985; Burgeois et al. 1987), followed by thermal-branding (Groves and Jones 1969; Niggol 1969), or branding with electric current (Johnson and Fields 1959; Owens and Gebhardt 1968; Jenkins and Klain 1969). Less successful or less popular techniques include caustic (silver nitrate) brands (Thomas 1975; Harshbarger 1979) and laser branding (Brock and Farrel 1977).

Freeze-branding tools, constructed of either silver or stainless steel, are dipped in a coolant, typically liquid nitrogen. Time of exposure to the brand is from 1 to 2 s (Park and Ebel 1974; Raymond 1974; Fay and Pardue 1985; Burgeois et al. 1987). Long-term mark retention (up to 2 yr) has been reported for anadromous salmonids (Park and Ebel 1974; Refstie and Aulstad 1975; Gunnes and Refstie 1980); however, two recent studies reported unsatisfactory results when cold brands were used as long-term markers for rainbow trout (Fay and Pardue 1985) and Atlantic salmon (Burgeois et al. 1987). Another freeze-branding technique that may hold promise involves the use of a cold jet of liquid nitrogen or other coolant. The marking instrument is commonly used by dermatologists to remove skin lesion or warts. It is readily portable (hand-held) and resembles a small blowtorch but has the ability to focus a jet of coolant directly on a small spot, making it ideal for use on small fish. We know of no experimental trial of this equipment but suggest it here as a method having potential.

Thermal-branding requires heating the branding tool in boiling water, and electric branding involves a wire loop connected and heated with a microscope illuminator transformer or similar device. Biologists at the North American Salmon Research Center reported 90% readability of electrically applied brands on Atlantic salmon marked at a weight of 60–80 g (Moring and Fay 1984). Researchers at the Center have found "hot brands" on Atlantic salmon parr to be readable in fish at the stage at which they return from the sea, and consequently use the technique as a primary means of identifying fish (Gary Friars, Salmon Genetic Research Group, personal communication).

Another thermal-branding method that remains (to our knowledge) untested, but that may prove suitable for marking small fish, involves the use of electronic epilators (instruments used to permanently remove human hair). These instruments use a hair-thin needle through which an electrical current of known intensity and duration can be applied. In marking fish, the needle could be inserted subcutaneously and activated to disrupt melanophore development and consequently produce a brand. High intensity pulses could possibly produce a much higher temperature in a more confined area than more common heat branding devices. Again, investigative research would be required to determine the potential of such a system.

As mentioned by Moring and Fay (1984), freeze-branding remains the most popular of the two branding techniques, even though electric branding is comparatively faster. They listed the following advantages and disadvantages.

Advantages

1. Equipment and supplies are inexpensive; overall cost per fish is low compared to tagging (Laird et al. 1975; Dumas 1977; Nahhas and Jones 1980; Fay and Pardue 1985).
2. There is flexibility in marking permutations if different symbols are used.
3. Branding does not affect behavior (Groves and Novotny 1965; Mighell 1969; Dumas 1977; Fay and Pardue 1984) or long-term survival and growth (Stolte 1973; Champion and Hill 1974; Dumas 1977; Fay and Pardue 1985).
4. Marking mortality is low.
5. Marking rate is fast (Piggins 1972; Refstie and Alstad 1975; Turner et al. 1974; Fay and Pardue 1984).
6. Field identification of brands can be made without specialized equipment on live or dead specimens (Moring and Fay 1984).
7. Excellent short-term retention and fair to good long-term retention (Moring and Fay 1984).

Disadvantages

1. Symbol clarity may be obscure with various letters.
2. Effective recognition of the brand, particularly after 1 yr, depends on the experience of trained observers (Moring and Fay 1984).
3. Symbol recognition becomes difficult over long periods and rapid growth—perhaps a disadvantage in Atlantic salmon (Fujihara and Nakatini 1967; Raleigh et al. 1973; Raymond 1974).
4. Variation in methodology can greatly affect the mark (Moring and Fay 1984).
5. Smoltification tends to obscure brands (Dumas 1977).

Tetracycline

Tetracycline (TC), a broad spectrum antibiotic, was apparently first discussed as a marker for fish by Weber and Ridgeway (1962). It is bound in the growing fish at calcifying regions such as vertebrae, ribs, fin rays,

opercula, and mandibles soon after administration. The mark forms a band in the bony material, invisible in normal light but which fluoresces yellow under ultraviolet light. Three forms of TC have been used for marking fish, including the parent form, oxytetracycline (OTC) and chlortetracycline. Oxytetracycline is commonly used in the hatchery system because it does not affect palatability of the diet, and mark retention is high (Weber and Ridgeway 1962, 1967; Weber and Wahle 1969; Odense and Logan 1974; Koenings et al. 1968).

Tetracycline is typically administered in one of three ways: ingestion of TC-dosed diet, intraperitoneal injection, or immersion in a TC solution. In the dietary method, the most widely used (Moring and Fay 1984), efficiency increases as the calcium content of the feed is reduced. Glucosamine, dimethyl sulfoxide, and terephthalic acid (referred to as potentiators) can assist in the uptake of TC from the diet and incorporation into calcified structures (Weber and Ridgeway 1967; Schidmore and Olsen 1969).

Contrary to the findings of Weber and Ridgeway (1962, 1967), Koenings et al. (1986) determined that the length of the feeding period was secondary in importance to marking fish at a size above which a large percentage of the OTC assimilated was not incorporated. Koenings et al. (1986) suggested that juvenile sockeye salmon (*Oncorhynchus nerka*) must be larger than 40 mm (0.6 g) before orally administered OTC produces a functional mark. They also reported a new fluorometric technique that detects OTC in the skeletal structure before a visible ring is formed. This new technique offers the additional advantage of being able to distinguish the different forms of TC, increasing its usefulness as a tagging agent.

The immersion process has not been as useful as orally administered TC for tagging fish (Choate 1964; Schidmore and Olson 1969; Hettler 1984); however, encouraging results were reported recently when eggs and larvae of ayu (*Plecoglossus altivalis*) were immersed in TC solutions (200–300 mg/L for 24–48 h for eggs and 200–300 mg/L for 3–24 h for larvae). Mark retention was 100% at 100 d post-immersion, with no special preparations for detection (Tsukamoto 1985).

Injections of tetracycline have been used successfully in marking killifishes (Bevelander and Goss 1962) and flounders and cods (Jensen and Cummings 1967); however, mark incorporation may be slow with this method (Moring and Fay 1984).

Moring and Fay (1984) listed the following advantages and disadvantages.

Advantages

1. Marks are essentially permanent (Jensen and Cumming 1967; Weber and Ridgeway 1967; Weber and Wahle 1969; Trojnar 1973; Odense and Logan 1974).
2. Effect on survival and growth or behavior is limited (Arnold 1966; Weber and Ridgeway 1967; Weber and Wahle 1969; Schidmore and Olson 1969).
3. No handling or anesthetic is required, unless fish are injected (Weber and Ridgeway 1967; Trojnar 1973; Odense and Logan 1974).
4. Large quantities of fish can be tagged with relatively little effort.
5. Coding and data processing are simple.
6. Life stages and sizes from egg to smolt can be marked (Trojnar 1973; Tsukamoto 1985).
7. Long-term storage of samples is possible (Trojnar 1973).

Disadvantages

1. Limited permutations for marked groups (Weber and Ridgeway 1967; Thomas 1975; Raymond 1974).
2. Wild fish are difficult to mark.
3. Lack of an external identifier, although external bony parts (fins and opercles) fluoresce for a short period (Choate 1964).
4. Fish must usually be sacrificed to enable detection.
5. Extraction and mark identification are tedious.
6. Sunlight deactivates TC fluorescence until melanophores are developed (Choate 1964; Trojnar 1973; Odense and Logan 1974).
7. TC marks are more readily detected in the fin rays of small fish and the bones of larger fish (Trojnar 1973).
8. Hatchery stocks treated with TC for disease are inadvertently tagged—which can be a source of confusion in marking studies.

Fluorescent Pigments

When fish are marked with fluorescent pigments, the objective is to spray pigment granules onto or beneath the skin (beneath the scales) and later detect their presence under ultraviolet light. This method was first described in 1959, when it was used to mark landlocked Atlantic salmon (Jackson 1959). Thereafter the technique was used on a variety of species (Andrews 1979).

In the marking of smolt-sized salmon, pigment granules are sprayed through a sand-blasting apparatus at pressures of 7.0 to 8.4 kg/cm^2, at a distance of 20 to 46 cm from the fish (Pribble 1976; Everhart and Youngs 1981; Evenson and Ewing 1985). Fish are typically dip-netted onto an inclined, plastic-lined trough (Moring and Fay 1984; Evenson and Ewing 1985) or conveyor belt (Pribble 1976) on which they pass under the pressurized spray. Marking rates are high and have been reported to be 32,000 sockeye salmon smolts per hour by a three-person crew (Everhart and Youngs 1981); or 25,000 juvenile chinook or 40,000 juvenile rainbow trout (*Salmo gairdneri*) on a conveyor system (Pribble 1976). And recently, 35,000 spring chinook salmon and summer steelhead were tagged per hour by a four-person crew at the Cole River Hatchery on the Rogue River,

OR (Evenson and Ewing 1985). Retention of the mark varies greatly in small fish, 50 mm long (Phinney 1966; Phinney et al. 1967; Hennick and Tyler 1970; White 1976; Strange and Kennedy 1982; Bax 1983).

Evenson and Ewing (1985) reported that the transparent tissue around (but not in) the eye was one of the most common and visible areas of pigment retention—an observation that corroborates the findings of Andrews (1972) in his work with fathead minnows (*Pimephales promelas*). The caudal peduncle is also a likely location for pigment retention, as evidenced in tagging work in largemouth bass, *Micropterus salmoides* (Englehardt 1977) and in chinook salmon and steelhead (Evenson and Ewing 1985).

Moring and Fay (1984) summarized the efficiency of this tagging method for various species and life stages.

Initial mortality was reportedly low, accounting for losses of only 0.4% (Strange and Kennedy 1982) and 3.8% (Phinney 1974) in two studies.

There appears to be a sexual difference in mark retention in chinook salmon and steelhead, the males of both species retaining the pigment for the shorter periods (Evenson and Ewing 1985). These researchers reviewed possible explanations for this anomaly and warned prospective users of this tagging method of the potential shortcoming. Advantages and disadvantages listed by Moring and Fay (1984) follow.

Advantages

1. Efficient mass marking (Phinney et al. 1967; Mattson and Bailey 1969; Andrews 1972; Phinney and Mathews 1973).
2. No anesthesia required and low cost (Phinney et al. 1967; Pribble 1967).
3. Low mark-related mortality (Phinney et al. 1967; Andrews 1972; Phinney 1974; Strange and Kennedy 1982).
4. Ease of use of marking equipment, limited training required, and simple detection with portable equipment (Reinjtes 1963; Pribble 1976; McAfee 1980; Bax 1983).

Disadvantages

1. Lack of permutations, which must be considered the main deterrent associated with this marking method; lack of an external identifier, and need for special (although relatively inexpensive) detection equipment.
2. Less efficient with smaller fish (Mattson and Bailey 1969; White 1976; Strange and Kennedy 1982; Bax 1983).

Dyes and Microtaggants

Subcutaneous injections of dyes and liquid latex were first tested as a tagging method by Wigley (1952) and Davis (1955). Microtaggants (color-coded plastic particles) were originally manufactured by the 3M Company to identify explosives, tools, and other equipment. Johns (unpublished manuscript) suggested using the microtaggants to mark wild animals. In 1985, Microtrace Incorporated, Minneapolis, MN, began manufacturing Microtaggants brand particles (Klar and Parker 1986), which are small laminated colored plastic chips up to seven layers thick. The tags are now also available with fluorescent or magnetic layers to aid detection. Smith Root Inc. also manufactures color-coded wire tags that are made with a stainless steel alloy (magnetically detectable). These tags are cylindrical (0.25 mm in diameter and 1 mm long) and were advertised as being compatible with all tag injection systems.

A variety of chemical compounds and commercial dyes have been injected as fish identifiers (see Moring and Fay 1984 for details). Short-term retention of some of these compounds has been excellent (Chapman 1957; Kelly and Loeb 1964; Lotrich and Meredith 1974; Fay and Pardue 1985). Moser et al. (1986) also

Species	Age or length (years) (cm)	Retention (%)	Term (months)	Reference
Coho salmon	0	97–99	12–24	Phinney and Mathews 1973
Coho salmon	0	97	12	Phinney 1974
Brown trout	0	100	20	Strange and Kennedy 1982
Atlantic salmon	0	100	7	
Coho salmon	1	100	29	Duncan and Donaldson 1968
Chinook salmon	16.6–18.7	95–60	12–54	Evenson and Ewing 1985
Steelhead	21.7	82–80	24–46	

experimented with various vital stains in bait as a means to mark fish in situ. Their results indicated that only one dye, reactive red 8, was detectable in the stomach mucosa after 11 weeks.

Unfortunately, tissue growth obscures injected dyes or latex, precluding long-term visual detection. If expected growth exceeds 1000% of initial weight, it is likely the mark will no longer be visible (Kelly 1967b; Loeb 1968). Therefore, injectable dyes or liquid latex provide the experimenter with greater utility when older fish are used.

Ronald Williams (Oregon Department of Fish and Wildlife, personal communication) reported using an air-powered, needleless injector (Panjet, manufactured in the United Kingdom) to mark salmonids with various dyes. He found, however, that only two materials (India Ink, and Alcian blue – also known as National Fast Blue) were retained for acceptable lengths of time (up to 1 yr for Alcian blue) in salmonids longer than 90 mm.

The use of microtaggants is relatively new, which explains the paucity of information on their efficiency. As in the application of dyes and liquid latex, the use of a jet inoculator greatly increases tagging rate. Two recent studies dealing with the Microtaggant system employed a needleless hypodermic injector manufactured by the Vernitron Medical Products Inc., Carlstadt, NJ. In the first of these studies (Klar and Parker 1986), the microtaggant system was compared and contrasted to the coded wire tagging system in fingerling striped bass and blue tilapia (*Tilapia aurea*). Fish were injected with microtaggants behind the pectoral fin or below the dorsal. Tag retention was 98% at 270 d; however, the microtaggant system was completely unsatisfactory in comparison to the coded wire tags for periods longer than 1 year.

In another study, Thompson et al. (1986) compared microtaggants with fluorescent pigment injections in Yazoo darters (*Etheostoma* sp.) and fingerling striped bass. Initial mortality was low for both techniques. Microtaggants were detectable in 96% of the surviving darters after 6 mo, in 88% after 12 mo, and in 75% after 24 mo; results for striped bass were similar. The only advantage of using the microtaggants in this study seemed to be their greater number of permutations. Further research is needed if the full potential of this tagging method is to be realized. Moring and Fay (1984) listed the advantages and disadvantages of dyes and liquid latex, and of microtaggants as follows.

Advantages of dyes and liquid latex:

1. Excellent short-term retention (less than 1 yr).
2. With appropriate substances and techniques there is potential for longer term retention.
3. No apparent effect on long-term survival, growth, and behavior (Chapman 1957; Kelly and Loeb 1964; Kelly 1967a, b).
4. Permutations are possible if several colors and locations are used.

Disadvantages of dyes and liquid latex:

1. Potential diffusion of the mark.
2. Necessity for trained observers to identify marks (Gerking 1963; Arnold 1966; Kelly 1967).
3. Training experience necessary to apply the marks (Kelly 1967b; Loeb and Kelly 1969).
4. Anesthetic required (Smith 1970).

Advantages and disadvantages of microtaggants:

1. Advantages of using microtaggants over other injected compounds: permutations are greater; magnetically detectable; mark does not diffuse; consistent from tag to tag.
2. Disadvantages: more costly; not as readily available; requires an external marker (could confuse CWT tagging effort in the area).

Meristics and Morphometrics

Meristic characters in fish can be influenced by environmental variables (Gabriel 1944; Lindsey 1954; Barlow 1961) and genetic variation among stocks (Vernon 1957; Barlow 1961; McPhail 1984). This difference in meristic characters has been used for stock identification (Dempson and Misra 1984; Meng and Stocker 1984). Meristic and morphometric counts are made from actual specimens or radiographs. The results are typically tested for groupings or similarities by using such statistical techniques as cluster, discriminant, or multivariate analyses.

Seven river stocks of juvenile Baltic–Atlantic salmon were subjected to biometric analysis by MacCrimmon and Clayton (1985); before their study, geographical variation in Baltic salmon morphology had been based on egg size (Larsson and Pickova 1978). MacCrimmon and Clayton (1985) were able to separate these seven stocks on the basis of morphometric characters, but meristics did not prove as useful. In the same study, a dichotomy in morphometric characters (different from those used to delineate the adult stocks) was also found between immature and mature parr.

Sockeye salmon have been identified to their country of origin by biometrical analysis (Fukhara et al. 1962; Landrum and Dark 1968). Concern over stock identification in high seas fisheries prompted the initiation of a long-term survey of meristic characters of stocks on the spawning grounds. Between 1956 and 1984, more than 23,000 sockeye salmon from 17 geographical locations ranging from the Columbia River to the Nome River in northwestern Alaska were sampled (Beacham 1985). This enormous sample was analyzed for number of gill rakers and vertebrae and various measurements. Beacham reported that sockeye salmon could be traced to their broad respective

geographical region by the use of morphometrics, but that differences in meristic characteristics were not great enough to identify stocks.

Although meristic characteristics are generally believed to be genetically determined, environmental factors such as temperature, light, and salinity have been shown to influence meristic phenotype (Gabriel 1944; Taning 1952; Barlow 1961; Kwain 1975). For this reason, meristic characters appear to be useful for stock identification when the annual variability of these characters is examined to determine if differences in the meristic counts among the stocks remain consistent from year to year (Dempson and Misra 1984; Beacham 1985).

Electrophoresis and Serological Techniques

In the mid-1950's, George Ridgeway (NMFS) proposed that Pacific salmon (*Oncorhynchus* spp.) could be identified to their country of origin by serological differences that presumably reflected genetic differences. In these studies, serum protein variants were detected in sockeye salmon through techniques of immunodiffusion, immunoelectrophoresis, and the use of antisera developed in rabbits. These serological techniques differentiated populations of sockeye salmon from Bristol Bay, AK, and the Columbia River, WA (Wydoski and Emery 1983). However, difficulties in producing adequate quantities of potent antisera, coupled with the indication that some of the variations detected may have been artifacts of the preservation process rather than valid genetic difference, led to the discontinuation of this type of study (Utter et al. 1974). Serological techniques were eventually replaced by electrophoresis as a means of identifying inter- and intra-specific variation of fish species. This new type of biochemical genetic study had the advantage of providing valid genetic interpretation directly from raw data. The basic genetic principles, procedures, and interpretation of electrophoresis were outlined by Utter et al. (1987).

In its most basic sense, researchers identify allelic variation at polymorphic loci by using starch gel electrophoresis, enabling them to differentiate discrete fish populations or stocks by examining individual loci of genes (Allendorf et al. 1975).

Isozyme electrophoresis was used to determine whether landlocked striped bass in Kerr Reservoir in Virginia–North Carolina belonged to distinct subpopulations (Rogier et al. 1985). In this study only 3 of 56 loci that could be scored were polymorphic. Because of low degree of genetic variability resolved by using isozyme electrophoresis, the population could not be subdivided into distinct stocks. Previous electrophoretic analyses have also demonstrated a low genetic variability in anadromous striped bass compared with that in other fish species (Otto 1975; Grove et al. 1976; Sidell et al. 1980).

Similar findings were reported on electrophoretic analysis of supposedly separate stocks of Atlantic cod, *Gadus morhua* (Odense et al. 1969; Lush 1970; Mork et al. 1980; Mork and Sundnes 1983). On the other hand, investigators studying serology (immunochemical characteristics within blood groups) suggested discrete subpopulations throughout the range of Atlantic cod (Frydenberg et al. 1965; Sick 1965a, 1965b; Moller 1967, 1968, 1969; Jamieson 1975; Cross and Payne 1978). Mork et al. (1985) discovered a significant correlation between genetic variation and geographic distance among Atlantic cod sampled at nine locations throughout their range. Their ultimate conclusion was that the absolute amount of genetic variation was low, and that this lack of differentiation could be attributed to the interstock exchange that has been revealed by tagging experiments (Hansen 1949; Jensen 1953; Joensen 1953; Tiews and Lamp 1974; Templeman 1974, 1976, 1979; Godo 1983).

Biochemical studies have proven useful in separating and distinguishing stocks of salmonids (Hodgins et al. 1969; Utter et al. 1974; Allendorf and Utter 1979). Beacham et al. (1985), who examined genetic variation in even and odd year brood line stocks of pink salmon in southern British Columbia and Puget Sound, found them to be reasonably distinct through cluster analysis by allelic frequency. Alaskan sockeye salmon of the Russian River (Grant et al. 1980) and the Karluk River systems (Wilmot and Buger 1985) showed significant differences in allelic frequency, as did fish of their respective early and late runs. Additional efforts in recent years have contributed to the knowledge of the genetic makeup and variability of Pacific salmon (Aspinwall 1974a, 1974b; Johnson 1979; Okazaki 1981; McGregor 1982, 1983; Fournier et al. 1984).

The Atlantic salmon has been severely depleted in both European and North American drainages. To supplement natural runs, fish culturists have operated hatcheries on both continents for more than a century (MacCrimmon and Gots 1979). The need to identify the genetic structure of Atlantic salmon populations became apparent as these hatchery stocks increased and as the high seas fishery intensified (Stahl 1987). In the West Greenland fishery, the Atlantic salmon harvested come from both North America and Europe (Saunders 1966, 1981). Present data suggest at least four loci that provide the combined capability of identifying fish to region of origin with high precision (Stahl 1987).

Although the European populations are genetically more varied than present-day North American Atlantic salmon (Henry Booke, personal communication), artificial propagation imposes the risk of reducing the total genetic diversity, as has already occurred in North America (Ryman and Stahl 1980, 1981; Ryman 1981;

Allendorf and Phelps 1980, 1981a; Cross and King 1983; Stahl 1983). Recent studies have shown that reproductive units of Atlantic salmon with morphologic and genetic differences still exist in Scandinavia and other parts of the world (Ryman and Stahl 1981; Thorpe and Mitchell 1981). In the River Alta, north Norway, three apparently genetically undisturbed (Heggberget et al. 1986) populations of Atlantic salmon were detected on the basis of difference in growth patterns, corroborated by differences in allelic frequencies.

Electrophoresis generates large volumes of data on genotypic and allelic frequency; however, much of the genetic variation in fish remains undetected. Current technology is expanding to reveal previously undetected alleles through techniques such as the modification of buffer and gel concentrations and testing of different thermal stabilities of proteins (Singh et al. 1976; Coyne 1982) and enzyme analysis of mitochondrial DNA (Utter et al. 1987). Polyacrylamide and starch gel electrophoresis and isoelectric focusing provide the researcher with useful tools to identify stocks of fish under many circumstances; however, the techniques have limitations, as do all other methods of fish identification.

Genetic Tagging

The intentional manipulation of naturally occurring allelic variation through artificial propagation programs has been explored as an alternative to physical tagging as a means of identifying stocks of fish (Schweigert et al. 1977; Grant et al. 1980; Murphy et al. 1983; Beacham et al. 1985). Differences in protein structure that are caused by genetic variation and that have been identified electrophoretically are generally inherited according to simple Mendelian principles (Seeb et al. 1986). Allelic frequencies often differ in reproductively isolated salmonid populations, and thus provide an opportunity for genetic marking (Allendorf and Utter 1979).

Genetic marking has been applied to freshwater species other than salmonids, including walleye, *Stizostedion vitreum vitreum* (Clayton et al. 1974; Murphy et al. 1983); common carp, *Cyprinus carpio* (Moav et al. 1976), and largemouth bass (Carmichael et al. 1986; Williamson et al. 1986).

In a large-scale, production-oriented study by Seeb et al. (1986), in which chum salmon of Kennedy Creek, WA, were genetically marked, electrophoretic techniques described by May et al. (1979) were used to detect polymorphisms at gene loci in extracted eye and muscle tissue. Two loci from the muscle tissue that expressed relatively low frequency alleles were chosen for the genetic markers. In a genetically structured breeding program started in 1976 and continued thereafter, 20% of the total 1980 chum salmon run in Kennedy Creek was genetically marked and 29% in 1981. Knowing the number of marked juveniles released enabled researchers to estimate the number of juvenile chum salmon produced in the area.

Taggart and Ferguson (1984) identified an allele of limited distribution among native brown trout (*Salmo trutta*) in Great Britain and Ireland that was also present in low frequencies in hatchery stocks. They suggested breeding homozygosity for this hatchery-specific allele, which would provide a useful mark in identifying the hatchery stock.

Salmonid species are particularly suited for biochemical stock identification because they show a relatively high degree of protein polymorphism and substantial heterogeneity among populations (Utter et al. 1980; Ryman 1983). Electrophoretic identification of component stocks from ocean commercial catches has been reported for a number of anadromous salmonid species (Nyman and Pippy 1972; Allendorf and Utter 1979; Grant et al. 1980; Payne 1980; Milner et al. 1983).

Genetic marking has advantages and disadvantages (summary follows), compared with standard tagging procedures (Taggart and Ferguson 1984; Seeb et al. 1986).

Advantages

Genetic tagging lacks the following limitations of standard tagging procedures.

1. The inability to mark tiny larvae (Jamieson 1974; Hedgecock et al. 1976).
2. The potential loss of the mark through fin regeneration, brand illegibility, or tag loss (Stuart 1958; Foerster 1968; Ricker 1975).
3. Differential mortality as a result of the tag or tagging procedure (Foerster 1936; Ricker 1975).
4. Differential mark recovery resulting from altered behavior in the tagged group (Ricker 1975).

Disadvantages

1. When the genotype is altered, stocks of fish – not individuals – are "tagged," and recoveries are assigned to that stock on the basis of probabilities.
2. When genetically tagging a population of fish, the researcher must consider to what degree that population might be inbreeding. Ryman and Stahl (1980) suggested using no fewer than 30 fish of the least numerous sex in any one generation.
3. A final consideration in genetic marking was raised by Allendorf and Utter (1979) when they proposed that a potential genetic pitfall may lie in breeding a strain of fish homozygous to a rare allele if that allele (or one linked to it) causes some survival disadvantage to its carriers. In other words, this allele may have been rare for this very reason. Kimura (1983), however, contended that rare alleles are typically at structural loci and are considered structurally neutral.

Genetic tagging provides a method whereby allelic frequency may be stabilized in the hatchery system, enabling an estimate of the intentional or unintentional

contribution of the hatchery stock (Stahl 1987). This general review has dealt with the use of genetic marks as a means of delineating a population structure. Genetic marking is also used to examine population mixing when the structure is already known. (For a review of this aspect of genetic marking see Pellan and Milner 1987.)

Parasites

The presence of parasites is sometimes used for identifying various groups or stocks of fish and determining fish movements and migration patterns (Sindermann 1961). Toward this end, parasites have been investigated in the Atlantic salmon (Pippy 1969a, 1969b; Nyman and Pippy 1972; Hare and Burt 1976) and in Pacific salmon (Margolis 1956; Bailey and Margolis 1987).

Geography, like the trophic status of the lake and other biotic variables, apparently influences the characteristics of the parasite fauna. In a recent study of the parasites of sockeye salmon from 15 Fraser River lakes, statistical clusters of parasites were found between lakes within biogeoclimatic zones and of similar trophic status; however, overall there appeared to be much overlap (Bailey and Margolis 1987).

No parasite is 100% incident or exclusive within a stock or depends entirely on host availability and movement patterns. In addition, the parasite must be present continually throughout the year and capable of surviving fluctuating environmental conditions (Moring and Fay 1984). This technique of stock identification probably serves best as a secondary or backup method to more reliable techniques. Moring and Fay (1984) and Wydoski and Emery (1983) offered the following advantages and disadvantages.

Advantages

1. Natural.
2. Applies to a large number of fish.
3. No time or cost to apply the tag.

Disadvantages

1. Requires much preliminary parasite survey data to determine if identification of stocks is possible with the parasite fauna present.
2. Requires the availability of technicians with a knowledge of parasitology.

Conclusions

Moring and Fay (1984) reported that they believed the CWT and fluorescent pigments were probably the most promising techniques as judged by several attributes (one of which was cost). Injecting fluorescent pigments remains one of the least expensive methods to mark fish; however, the problems of few permutations and the relatively short retention times remain. From the recent literature it would seem that the fluorescent pigment tagging method has not greatly increased in popularity over the last few years. However, as with all tagging methods, there remain situations for which fluorescent pigment tagging is tailored, as well as researchers who prefer this method.

The CWT program has certainly not declined during the past few years, as evidenced by Northwest Marine Technology's recent claim of 200 million tags implanted. It is currently the marking method of choice in any high-volume operation. With the advent of the flat tag and the recently available X-ray detection equipment, the CWT soon may be the standard marking method for ocean-going salmonids worldwide.

The PIT tag has emerged on the tagging scene in the past few years, and is still in the experimental stage. The recently improved encapsulation material and process, in addition to extensive baseline biological investigations conducted by NMFS for the Bonneville Power Administration, account for the increasing attention this system has received. The costliness of this system and the current size of the transponder may prevent the PIT system from equaling the widespread use of the CWT; however, the PIT system is sure to become a common tool in the marking of anadromous salmonids in the future.

The various methods of stock identification reviewed here have proven useful in recognizing discrete populations of fish in several river systems throughout the world. The degree of resolution associated with these methods varies with species and locale; however, electrophoresis seems capable of routinely distinguishing European and American stocks of Atlantic salmon, thus providing the biologist with another useful technique. As biochemical stock identification advances (as in the variation of mitochondrial DNA analysis), so should its application to research on anadromous salmonids.

With recent advances in super-conductor technology, new materials may soon be developed that will benefit fish marking. Perhaps powerful new magnets could be built that would be capable of extracting very small metal tags (CWT's?) directly from the fish without serious injury or death. Perhaps smaller, more powerful PIT tags will be developed. Satellite tracking of fish stocks around the world may someday become commonplace and the possibilities of applying genetic engineering to fish stock marking seem endless.

Considering the tremendous worldwide investment and importance of fishery resources and the potential benefits from development of an effective, economical marking technique, it would seem that an all-out effort to develop the "perfect" tag would not be inappropriate. Unfortunately, as in most research, advances will come in small increments, probably by individuals or small groups of workers – and perhaps totally outside fishery biology. Consequently it behooves all researchers to remain aware of new developments in other disciplines and to pursue any promising leads that develop.

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The current status of techniques and developments relating to the long-term identification of salmonid fish is reviewed and described. The literature that may relate to future techniques is discussed. Techniques include coded wire tags, passive integrated responders, branding, tetracycline, fluorescent pigments, dyes and microtaggants, meristics and morphometrics, electrophoresis and serological techniques, genetic tagging, and parasites.

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