

Russian River Coho Recovery Workgroup Monitoring and Evaluation Committee

Potential Marking and Tagging Techniques

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INTRODUCTION

The Monitoring and Evaluation (M&E) Committee of the Russian River Recovery Workgroup is tasked with recommending methods to assess the biological performance of released fish and habitat suitability of receiving streams. Development of a strategy to differentiate hatchery and naturally produced fish is fundamental to the evaluation of biological performance and is a common feature of all salmonid supplementation projects. However, the reintroduction of large numbers of selectively bred juvenile fish to six streams throughout a basin at various age classes imposes additional burdens on a marking program. Because little is known about the dynamics of the historical or extant Russian River coho population, our evaluation of program success requires accurate estimates of seasonal survival rates, growth, habitat use, abundance, distribution, and carrying capacity during freshwater residence. Our ability to manage the reintroduced population is also dependent on smolt to adult (ocean) survival rates and patterns of spawner homing and straying.

With these concepts in mind, the M&E Committee began formulating a marking/tagging strategy in February 2003. Committee members have been discussing a suite of potential techniques including visible implant elastomer (VIE) tags, passive integrated transponder (PIT) tags, coded wire tags (CWTs), visible implant alphanumeric tags, fin clips, and freeze brands. No mark or tag is perfect and the pros and cons of potential techniques must be weighed with respect to their effects on growth, behavior, survival, retention, information capacity, application requirements, recovery requirements, and cost (Guy et al. 1996). Innovations in tagging and marking techniques for juvenile salmonids have created a new volume of information in the scientific literature. We felt a review of this literature was warranted to inform the ongoing discussion of techniques and facilitate the development of a marking/tagging strategy. This document presents a brief review of methods and a proposed framework for evaluating their utility in a comprehensive monitoring and evaluation plan. We based the framework on past discussions of ecological questions, likely sampling frequencies, and sampling methods. The framework can be modified as our discussion of a comprehensive M&E plan matures but we hope this serves as a starting point.

POTENTIAL METHODS

Visible Implant Elastomer (VIE) Tags

Description: VIE tags are externally visible internal marks created by injecting a biocompatible fluorescent silicon elastomer into translucent tissue. The elastomer hardens into a pliable solid a few hours after application, fluoresces under UV light, and comes in a variety of colors. The transparent tissue posterior to the eye (adipose eyelid) is the most common marking location in salmonids (Bailey et al. 1998; Hale and Gray 1998; Close 2000; Close and Jones 2002). In age-0 brown trout (<70 mm fork length) elastomer has been injected parallel to the base of the dorsal, adipose, anal, pectoral, and pelvic fins (Olsen and Vollestad 2001). Close and Jones (2002) also marked yearling rainbow trout in the jaw.



Effects on Growth, Behavior, and Survival: No adverse effects have been reported. Bailey et al. (1998) found no differences in survival rates for adult coho that were marked as smolts. Olsen and Vollestad (2001) found no short term growth differences in age-0 brown trout.

Mark Retention: Baily et al. (1998) reported a two year retention rate of 73% for coho marked as smolts (mean FL=108 mm). Short term (24 hour) tag losses were 3-7%. Close (2000) marked fingerling rainbow trout (mean 79 mm FL) in postocular tissue and found that without UV light, marks were undetectable after 6 months. With UV light, marks were externally visible in 57% (green mark) and 87% (yellow mark) of fish. Mark detectability appeared positively correlated with size of fish at marking (larger fish retained marks at a higher rate). Dissection showed that shed rates were 12-18%. Reporting on the long term results of the same study, Close and Jones (2002) found detection rates ranging from 29-33% after fish were at large for 29-35 months. Olsen and Vollsted found 100% retention after 77 days in age-0 brown trout (mean 38 mm FL) marked along the bases of fins. Ventrally placed marks were easier to see. Post-ocular tissue marking was not recommended in small fish.

Information Capacity: VIE tags were designed for batch marking, although color and tag location combinations can be used for more detailed identification.

Application Requirements: Tags are injected with either a hand held syringe or pneumatic marking gun. Tagging rates with the hand held syringes average 200-300 fish per hour (Bailey et al. 1998; Close 2000). Northwest Marine Technology (NMT) claims 600 fish/hr can be marked with the gun.

Recovery Requirements: Tags are visible under natural light or supplemental UV light (wavelength 250-360 nm). Bailey et al. (1998) found tags readily visible in adult coho under ambient and UV light. NMT supplies a filtered halogen flashlight (480 nm) and amber glasses that supposedly enhance tag visibility. The halogen flashlight does fluoresce tags but most researchers reported little success with the amber glasses (Close and Jones 2002). Viewing fish with a UV light under darkened conditions can reveal marks that are otherwise externally undetectable – fluorescing dissected tissue under UV light can reveal deeply imbedded tags.

Cost: NMT is the sole source provider. Hammer and Blankenship (2001) described total application costs (tags, tagging equipment, tagging trailers, anesthetic, and salaries) for a wide variety of marks and tags used in salmonid research. They listed VIE application costs of \$0.11 per fish and coded wire tag (CWT) + adipose fin clip + VIE tag combination costs of \$0.22 per fish. Decoding costs were negligible for VIE tags and ranged from \$3-5/fish for the CWT + Clip + VIE combo. Current pricing from the NMT website is similar – the pneumatic marking gun (recommended for large batches of fish) can be rented for \$550 per month or purchased for \$6,600.

Discussion: The utility of VIE tags for short (<6 month) and long (>24 month) duration studies is dependent on fish pigmentation and size at marking. Suitable marking locations are limited in fish less than 100 mm FL. The only long term study in coho evaluated fish that were >100 mm at tagging. The long term retention of marks applied to the base of fins in fish <100 mm has not been reported. Although the tags are cheap they may not be easy to apply – taggers should be trained extensively and mark retention tests should be conducted.

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Olsen, E. M., and L. A. Vollestad. 2001. An evaluation of visible implant elastomer for marking age-0 brown trout. *North American Journal of Fisheries Management* 21: 967-970.

Passive Integrated Transponder (PIT) Tags

Description: PIT tags are small (12 mm long x 2.1 mm diameter) glass encapsulated electronic devices that carry a unique code. The tag is inserted into the body cavity with a hypodermic needle or surgical techniques (Guy et al. 1996; Gries and Letcher 2002). When energized from an external power source (tag reader/interrogator) the tag transmits its code. Hand held and powerful stationary tag readers are used to recover codes without injuring fish.



Effects on Growth, Behavior, and Survival: No adverse effects have been reported in 70-100 mm Chinook, sockeye, and steelhead (Prentice et al 1990). Dare (2003) evaluated survival and retention in juvenile Chinook and found mortality over a 28 day period was less than 1% (325 total mortalities out of 144,450 tagged fish). Peterson et al. (1994) found no overwinter (7 month) growth or survival differences between coded wire and PIT tagged juvenile coho – even among the smallest size class of fish (53-70 mm FL). They concluded that either tagging method was effective in 65 mm coho.

Mark Retention: PIT tag retention rates typically approach 100% (Prentice et al. 1990). Dare (2003) reported a 99.996% retention rate in juvenile Chinook over 28 days. Peterson et al. (1994) found no tag loss in juvenile coho during a 72 hour trial. Using surgical techniques to implant tags in age-0 Atlantic salmon, Gries and Letcher (2002) reported 99.8% retention after 9 months.

Information Capacity: The electronic code allows unique individual identification but the tags cannot be programmed.

Application Requirements: Tags are injected with either a syringe or multi-use pistol grip style injector. Salmonids greater than 60 mm FL can be tagged (Peterson et al. 1994; Gries and Letcher 2002). Dare (2003) reported mean tagging rates of 230-279 fish/hour.

Recovery Requirements: Fish generally need to be in hand but remote detection systems are available. Tag readers come in a variety of styles from hand held devices with no memory to stationary antennas with datalogging receivers (www.biomark.com). Tag detection distances vary between the devices – less expensive models have detection distance of 2.5-7.5’.

Cost: Biomark, Inc. is the primary supplier for fisheries applications. Tags are \$3.90 each for quantities >1000. Tag readers range from \$395 to \$9,500 – the unit in use at Warm Springs costs about \$3,000. Hammer and Blankenship (2001) list application costs (based on purchase of >10,000 tags) that ranged from \$2.50-5.50/fish for PIT tags and \$3.60-6.50/fish for a PIT+CWT+adipose fin clip combination.

Discussion: The small size, large information capacity (unique codes for each tag), high retention rate, and negligible effect on behavior and survival make PIT tags a nearly ideal choice for our coho releases. They are, however, the most expensive tags (aside from radio transmitters) and cost may be prohibitive for large numbers of fish. PIT tagging smolts captured in outmigrant traps or smaller groups of parr could improve growth, juvenile survival, and smolt to adult survival rate estimates.

References:

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Coded Wire Tags (CWTs)

Description: Coded wire tags are small (0.5-2.2 mm long x 0.25 mm wide) pieces of stainless steel wire imprinted with laser etched numeric codes. The laser etched coded tags or Decimal Coded Wire Tags (DCWT) replace an older system that relied on a series of notches in the wire to produce a binary code. The codes can be used to identify groups of fish (batch codes) or individuals (sequential codes). Developed in the 1960s and used primarily to identify hatchery of origin in Pacific salmonids, CWTs are the most widely applied tags in the world (Guy et al. 1996). Nearly 40 million juvenile salmonids receive CWTs annually (Johnson 1990). In juvenile salmonids, tags are typically implanted in the snout using syringes or automated injectors. Additional tagging locations include the cheek muscles, nape, and base of the dorsal, adipose, caudal, and anal fins (Guy et al. 1996; Hale and Gray 1998; Munro et al. 2003). Because CWTs are internal, the adipose fin is typically excised to signal the presence of a tag. Because recent ESA listings have prompted the widespread use of adipose clips to differentiate wild and hatchery fish, many coho salmon currently receive an adipose clip but are not implanted with a CWT. This mass marking of hatchery coho necessitates the use of electronic detection in all ad clipped coho (Vander Haegen et al. 2002; Blankenship and Thompson 2003). Tags can only be recovered from most body locations by post mortem dissection. Codes must be read under magnification.



Effects on Growth, Behavior, and Survival: Effects of snout tagging are generally minimal (Guy et al. 1996). Blankenship and Thompson (2003) evaluated 1.6 and 2.2 mm long CWTs and found no effects on growth, survival, or homing in coho that were 96-115 mm at tagging. Peterson et al. (1994) found no overwinter (7 month) growth or survival differences between CWT (1.1 mm tags) and PIT tagged juvenile coho as small as 53 mm FL. Barnes (1994) reported that CWT and adipose fin clipping of rainbow trout had no effect on feed conversion, growth, or condition factor. Munro et al. (2003) found no difference in mortality between tagged and untagged age-0 rainbow trout. A study of snout tagged spring Chinook found that CWT implantation promoted the transmission of the pathogen *Renibacterium salmoninarum* (Elliot and Pascho 2001). Habicht et al. (1998) suggested that tags implanted in pink salmon fry could damage sensitive olfactory organs and negatively affect adult homing.

Mark Retention: Munro et al. (2003) evaluated retention in age-0 rainbow trout (<80 mm) tagged in five body locations: the snout, nape, and musculature at the base of the dorsal, anal, and caudal fin. Retention rate was 95-100% after 8 weeks and 90% after 5 months to 3 years. They successfully tagged fish as small as 40 mm and found no differences in retention among 10 mm size classes. Hale and Gray (1998) tagged larger trout (80-314 mm TL) in multiple body locations and found 92-100% retention after 30 days. Most tag loss occurred within 3 weeks.

Information Capacity: Tags can be ordered in groups to serve as batch codes or sequential codes for individual identification. Implanting tags in different body locations greatly expands the ability to identify subgroups from batch codes.

Application Requirements: Tags are implanted with hand held single, multi-shot, or stationary automatic injectors. Tagging devices typically have a sleeve that regulates the depth of needle insertion. Molds that match the size and shape of a fish's head are used to implant snout tags. Proper insertion is verified with a magnetic tag detector (quality control device) immediately after tagging. Tagging rates range from a few hundred to 1,000 tags/hour for the multi-shot and automated injectors respectively (www.nmt-inc.com).

Recovery Requirements: Externally marked (typically adipose fin clipped) fish are passed through electronic magnetic detectors to verify the presence of tags and pinpoint where they are imbedded. Detectors are available in a variety of configurations including hand held wands and tunnels. Blankenship and Thompson (2003) reported samplers using wands and tunnel detectors had detection efficiencies of 99-100%. Hand held wand detection rates may decrease with increasing fish size and larger CWTs (1.6 or 2.2 mm long) can be detected in deeper tissue (Vander-Haegen et al. 2002; Blankenship and Thompson 2003). Fish must be dead to remove snout implanted CWTs but tags have been successfully removed from live fish tagged in postocular tissue, dorsal, and adipose fins (Heinricher-Oven and Blankenship 1993). CWTs placed in distinct body locations can also be detected using hand held wands without sacrificing fish (Munro et al. 2003). Munro found 100% correct identification of tags at five body locations in 178-450 mm FL rainbow trout that were <80 mm at tagging. Hale and Gray (1998) correctly identified 91-98% of tags in multiple body locations using wand detectors in live trout >80 mm. Because the wand detector had a 2 cm range, certain body locations were too close to one another to be used in multiple mark combinations.

Cost: Northwest Marine Technology (NMT) is the sole source provider and quotes a tag price of \$0.072-0.076 for quantities > 10,000 (www.nmt-inc.com). Tag injectors range in price from \$6,800-29,400, detectors are \$6,000-20,200, and quality control devices are \$11,650. However, CWT equipment is used at Warm Springs Hatchery and throughout California – using existing equipment would lower initial costs substantially – all equipment can also be rented from NMT. Hammer and Blankenship (2001) list an application cost of \$0.108-0.136 per fish for a CWT + adipose fin clip combination. Decoding costs are \$3-5 per fish.

Discussion: The small size, high information capacity, high retention rate, negligible affect on biological performance, and low cost of CWTs make them ideal candidates for use in the coho recovery program. Lack of external visibility is a disadvantage. However, implanting tags in different body locations (either separately or in combinations) could greatly enhance recognition of seasonal release groups in live returning adults. Tagging body locations with CWTs in conjunction with a short term external mark to indicate age at release may allow accurate external identification of fish during juvenile and adult sampling.

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Fin Clips

Description: The punching, notching, or removal of fins is the easiest and oldest method for marking salmonids. When punches and notches heal they form detectible irregularities in fin rays. The marks in regenerated rays can be subtle, however, and punches and notches are best used as short-term marks (Guy et al. 1996). The complete removal of pelvic fins has been used extensively to mark salmonids but the practice can result in high mortality and is generally not recommended (Guy et al. 1990; Sweeting et al. 2003). Excision of the adipose fin is widely used to identify hatchery fish and signal the presence of a coded wire tag.

Effects on Growth, Behavior, and Survival: No adverse effects have been reported from adipose fin removal. Sweeting et al. (2003) cite several studies that reported 6-50% mortality from pelvic fin removal in juvenile coho. Hammer and Blankenship (2003) reviewed five studies that reported variable survival rates for pectoral, dorsal, and anal fin removal. While survival was affected growth was not.

Mark Retention: The retention of adipose fin clips is dependent on the amount of tissue removed. Thompson and Blankenship (1993) evaluated adipose fin regeneration in adult coho that were completely or partially clipped as smolts (120 mm FL). After 21 months at large, no regeneration was observed in fish that had all adipose tissue removed during marking. In fish that had two-thirds of their fin removed at marking, 23% completely regenerated the fin and an additional 35-63% had partially regenerated fins.

Information Capacity: Fin clips are used only as batch marks.

Application Requirements: Minimal – scissors or other cutting device. An automated adipose fin clipping device is available from Northwest Marine Technology for mass marking in conjunction with CWT insertion.

Recovery Requirements: Negligible.

Cost: Hammer and Blankenship (2001) list an application cost of \$0.015-0.020 per fish.

Discussion: Complete adipose fin removal is an indelible mark with no adverse affects on fish performance and will be used to identify coded wire tagged fish. All other fin excisions are not recommended for survival, retention, and aesthetic reasons. Caudal fin notches may be used as a short-term mark to aid population estimation – particularly estimates derived from downstream migrant trap efficiency testing.

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Freeze Brands

Description: Freeze brands are marks (scars) created by holding a cooled piece of metal (branding iron) against the body of a fish. Cooled with liquid nitrogen or pressurized carbon dioxide, the branding iron can be configured into a variety symbols to form batch marks. The marks are temporary and fade as fish grow. Freeze brands have been widely applied to juvenile salmonids (Guy et al. 1996; Hammer and Blankenship 2001). Bryant and Walkotten (1980) branded 100 mm TL coho and rainbow trout and Bryant et al. (1990) marked Dolly Varden down to 40 mm. LaJeone and Bergerhouse (1991) marked fingerling walleye as small as 50 mm TL.

Effects on Growth, Behavior, and Survival: No adverse effects have been reported and marking mortality is low (Fay and Pardue 1985; Guy et al. 1996).

Mark Retention: Freeze brands are generally used as short-term marks. Fay and Pardue (1985) reported retention rates of 96% at 13.5 weeks and 92% at 22 weeks in 178-320 mm TL rainbow trout. Bryant (1990) found marks visible after 6 months in age-0 Dolly Varden.

Information Capacity: Brands are used only as batch marks.

Application Requirements: Branding equipment must be constructed. Tagging rates of 300-2,000 fish per hour have been reported in salmonids (Bryant 1990).

Recovery Requirements: Negligible.

Cost: Hammer and Blankenship (2001) list costs of \$0.026-0.05 per fish.

Discussion: Freeze brands are cheap and have no adverse effects on fish performance. However, retention and information capacity limit them only to use as temporary batch marks. Combinations of marks and tags with higher retention rates diminish their utility.

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Visible Implant Alphanumeric (VI Alpha) Tags

Description: VI Alpha tags are small (2.5 mm long x 0.08 mm thick) pieces of plastic film imprinted with letters and numbers that form a unique code. The tags are inserted with a modified 30 gauge hypodermic needle into transparent tissue. The tags have been applied widely in salmonids but retention is variable and can be poor in fish less than 200 mm FL (Bryan and Ney 1994; Hughes et al. 2000; McMahan et al. 1996; Shepard et al. 1996). VI Alpha tags are typically placed in postocular tissue (similar to VIE tags), but the small target area in fish <125 mm FL precludes tagging. Because we are interested in tagging juvenile coho less than this size, we did not give this method further consideration.

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DEVELOPMENT OF A MARKING STRATEGY

Monitoring Variables, Sampling Methods, and Frequency

We are interested in six main variables for the biological performance component of the coho broodstock monitoring program: 1) population abundance, 2) survival rates, 3) growth rates, 4) distribution, 5) habitat use, and 6) interspecific competition. Our consideration of sampling methods and frequency is brief and deserves more detailed consideration. We assume, however, that outmigrants and adults will be trapped during spring and early winter, respectively, and that juveniles will be sampled during summer and fall by snorkeling, seining, and electrofishing. Monitoring actions are shown in Figure 1a. Arrows indicate the general time period over which monitoring would take place, in relation to release times, and release age of fish.

Population Abundance: Abundance would be assessed twice in the spring and once in fall. During spring, a dive/electrofishing survey would precede the release of fingerlings and outmigrating fish would be enumerated at the downstream migrant trap. The spring (or early summer) dive/electrofishing survey would allow growth and survival analysis for wild steelhead and initial abundance of naturally produced age-0 coho after year four. During fall, dive/electrofishing surveys would be used to count fish. Electrofishing during both the spring and fall surveys would be used in only riffles and a small subsample of units to validate snorkel counts. From Year 4 onward, results from fall electrofishing would also be used to distinguish program coho from naturally produced coho - by identification of fish with and without adipose fin clips.

Survival rates: Summer survival rate would be estimated from spring and fall dive/electrofishing abundance surveys. Overwinter survival would be estimated from fall dive/electrofishing surveys and spring outmigrant trapping.

Growth rates: Growth could be estimated over the same time intervals as survival rates – using results from the same surveys. A more detailed evaluation of seasonal growth could be generated by seining. Sub-sections of the areas sampled for abundance could be seined at any interval (spring-summer or winter-spring) assuming that a sub-sample would be adequate to estimate size and growth.

Distribution of young-of-the-year coho: Distribution is of interest since YOY coho may outmigrate shortly after release, instead of over-summering and over-wintering. Early outmigration may be related to over-stocking or low food supply. Early outmigration would be detected by operating the outmigrant trap through early summer. Visual observation of coho distribution in reaches that correspond to hydrologic sampling may also reveal gross trends in survival or movement (i.e., presence of absence of fish in previously stocked areas).

Habitat Use: Habitat use may be of particular interest in the winter, when low flow refugia may be critical. Coho use of flow refugia could be observed by snorkeling and/or seining during low winter base flow.

Interspecific Competition: Competition between coho and steelhead may affect coho growth and survival, and existing steelhead populations. Relative density and size of coho and steelhead will be estimated from spring to fall through snorkel surveys, visual estimates, and seining. Note: visual estimates for survival, and seining for growth will already be taking place during this time period.

M&E Framework for Tagging / Marking

Tagging options necessary to accomplish the proposed monitoring strategy are shown in the bottom half of Figure 1a. Arrows indicate the time that marking would take place and duration of tag function.

A Proposed Marking Strategy

We assume that in each stream fish will be released in two groups, spring (fingerling) and fall (advanced fingerling). All fish will be **adipose fin clipped** to distinguish between program fish and wild fish. Spring release fish will be **coded wire tagged** in the snout. Fall release fish will be implanted with a **CWT** in the snout and caudal region. It should be possible to distinguish the location of the CWT and determine which release group a fish is from by “wanding” fish caught in the adult weir/trap (fish are released unharmed). There will be a **unique CWT code for each stream and release year**; using CWT data from carcasses we can get estimates of stray rates, spawner age, and survival. Fall release fish will also be given a visual implant elastomer (**VIE**) tag. VIE tagging will allow the distinction between spring and fall (fingerling/advanced fingerling) release fish during fall abundance and spring outmigrant sampling. We assume that when fish are small it will not be possible to distinguish the location of the CWT, so the VIE tag is necessary at this stage.

It would also be possible to **PIT tag** advanced fingerlings and smolts caught in the downstream trap. Returning fish would be scanned with a PIT detector, and their stream, age, and release group could be traced. If fall release fish were PIT tagged prior to release from the hatchery, they may not require a CWT. PIT tagging is more costly than coded wire tagging. However, PIT tagging survival rates are very high, and more information would be gathered from returning fish. Note that by using CWTs, age and stream information would only be gathered from carcasses, and carcass recovery rates may be very low.

Figure 1b shows the same information as Figure 1a, but includes blank spaces for additional input. Figure 1c contains sampling variables and tag options but is otherwise completely blank.

Figure 1a. Monitoring variables and tagging options for Russian River coho broodstock releases.

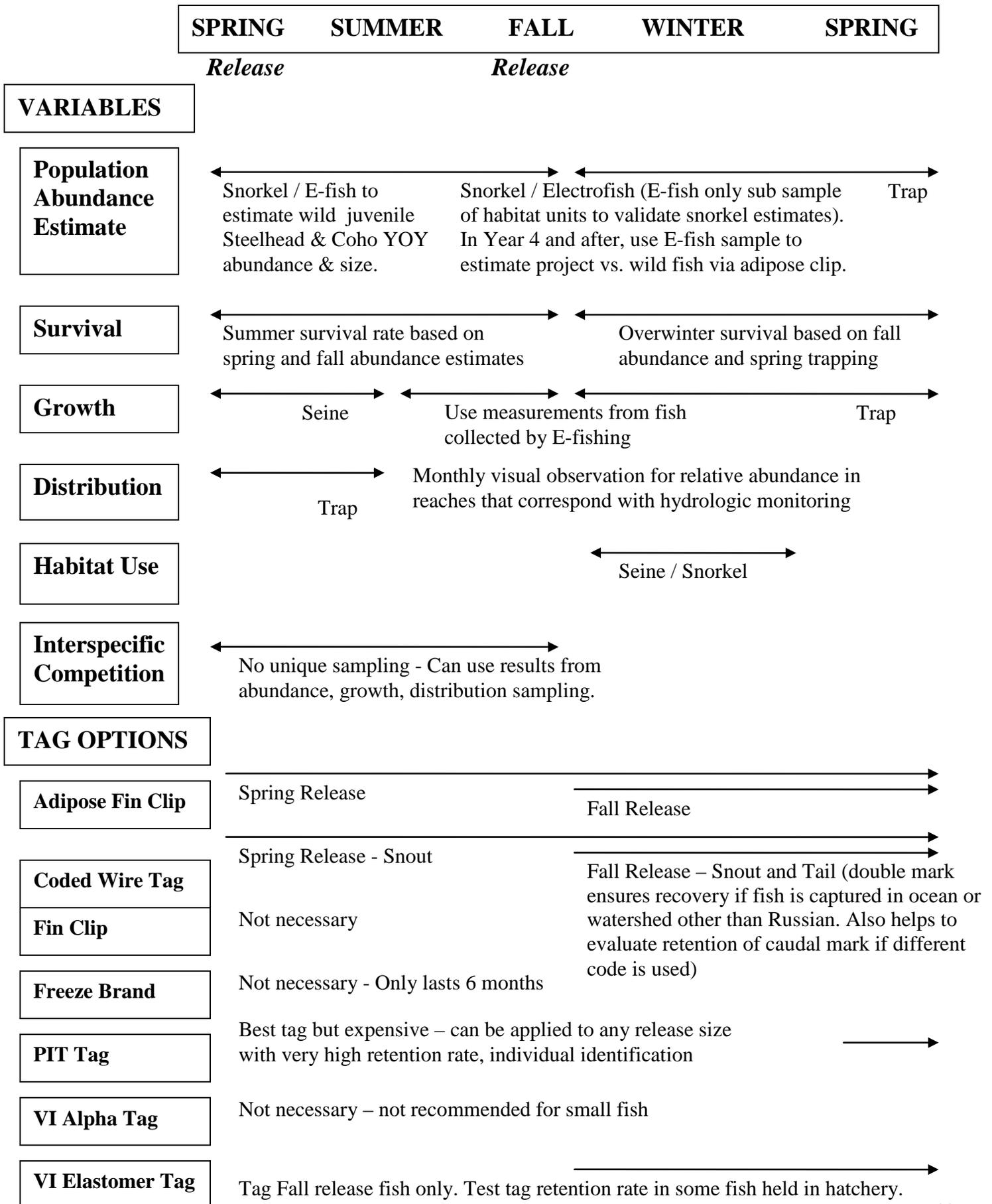


Figure 1 b. Monitoring variables and tagging options for Russian River coho broodstock release, with blanks for additional input.

