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Research Paper

Enriched stable isotope marking of hatchery trout via immersion: A method to monitor restocking success

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ABSTRACT

Suitable habitat for many temperate freshwater species of salmonids is predicted to dramatically decline, yet many hatcheries still release millions of juvenile salmonids into rivers and lakes annually with little or no post release monitoring. This is, in part, because marking of hatchery reared fish is often not compulsory and currently available marking methods are either costly, cause high mortalities, are inconvenient to apply, or have poor long-term retention rates. To help overcome these limitations, we tested two recently validated stable isotope mass marking methods for Atlantic salmon (larval and egg immersion), to determine if a suitable low cost, easy to apply, fish friendly marking method could be achieved for two of the most widely stocked freshwater salmonids worldwide: brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*. Egg immersion using 1000 μg L^{-1} of ^{137}Ba and ^{136}Ba over a 2 h period did not create detectable marks in the otoliths of brown or rainbow trout. In contrast, larval immersion using 100 μg L^{-1} of ^{137}Ba and ^{136}Ba over a 24 h period returned a 100% mark-success rate in the otoliths of brown and rainbow trout at an estimated marking cost of $US 0.004 per fish. Larval immersion marks were clearly definable in the otoliths, with isotope ratios in marked fish 11 times greater than ratios measured in control fish. Furthermore, the process of marking was easy to apply, with < 0.5% mortality during marking. We conclude that larval immersion marking is a suitable method for long-term monitoring of restocking success of hatchery-reared trout. If adopted, the method would enable hatcheries to cost effectively and accurately assess the real contribution of restocked fish to wild populations.

1. Introduction

Suitable habitat for a range of salmonid species is estimated to decline by 35–80% within the next 50 years (Kennedy et al., 2009; Wenger et al., 2011, 2013) which is of great concern, particularly for the survival and abundance of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta*. Wild-caught and hatchery-reared forms of rainbow and brown trout have been transplanted or introduced for food or sport in at least 45 countries around the globe (MacCrimmon and Marshall, 1968; Crawford and Muir, 2008; GISD, 2017a,b) and are two of the most popular recreationally fished salmonids worldwide. However, habitat range for rainbow and brown trout is predicted to constrict, as both species have a low upper thermal tolerance limit (Jonsson and Jonsson, 2009). Furthermore, altered habitat quality and availability may lead to an increased threat of parasites and disease, higher mortality rates during dry seasons, delayed (or altered) spawning, and decreased post-juvenile growth, survival, and maturity (Jonsson and Jonsson, 2009).

The continual restocking of wild fish populations with hatchery reared fish plays a major role in conserving recreational fisheries for trout (e.g. Halverson, 2008). However, the extent to which restocking enhances fisheries that include both wild and hatchery produced fish is often poorly understood, due to difficulties in accurately identifying the origin of fish caught during recapture surveys. Monitoring restocking is a crucial part of fisheries management, and it may be necessary in the future to mark or tag all hatchery produced fish pre-release for the purpose of ensuring best-practice fisheries management.

There are numerous mass marking methods that have been used for marking hatchery reared salmonids for monitoring purposes (Hammer and Blankenship, 2001), for example adipose fin clipping (Johnsen and Ugedal, 1988; Vander Haegen et al., 2005), coded wire tags (Johnson 1990; Courtney et al., 2000), otolith thermal marking (Volk et al., 1999; Morita et al., 2013), calcein marking (Negus and Tureson, 2004; Stubbing and Moss, 2007) and to a lesser extent, alizarin complexone (Van der Walt and Faragher, 2003) and otolith dry marking (Rogatnykh et al., 2001). Of these, adipose fin clipping by itself or combined with

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another tag is the most widely used method to help assess trout stocking (e.g., Armstrong, 1949; Hayes and Livingstone, 1955; Baxter et al., 1988; Vander Haegen et al., 2005). However, if no automated system is available, adipose fin clipping is done by hand, meaning the process is time consuming. In addition, recent studies suggest there are possible side effects of adipose fin clipping on swimming ability (Reimchen and Temple, 2004; Buckland-Nicks et al., 2011), which may have a fitness cost for released fish.

Marking salmonids with coded wire tags can produce accurate sequential marks if detected, but the cost to mark (> $US 0.06 per fish, Hammer and Blankenship, 2001) means it is often financially unfeasible. Conversely, otolith thermal marking can be cost effective to mark, but there are problems with correctly identifying marks (Hagen et al., 1995). Calcein marking via immersion is a viable alternative marking method that is more efficient to apply than adipose fin clipping. Calcein produces internal and external green marks for salmonids (Mohler, 1997; Mohler, 2003) and has been trialled on rainbow trout in North America (Negus and Tureson, 2004; Elle et al., 2010). If applied at the appropriate concentration and exposure time, marking can be 100% effective, with little effect on mortality or growth (e.g. Mohler, 1997; Crook et al., 2009). Internal marks in the otolith are thought to be permanent, but there are ongoing issues with long term correct identification of external marks (Elle et al., 2010; Ingram et al., 2015) and the cost to mark, which is approximately $US 0.05 per fish (estimated from Crook et al., 2009 and www.chemical-reagent.com). This leaves many hatcheries without an easy to apply, low cost, accurate, welfare friendly, marking method for salmonids grown for restocking purposes.

We investigated two alternative mass marking methods for hatcheries that will enable marking of all hatchery-reared rainbow and brown trout that are restocked for enhancing recreational fisheries. The two methods, larval immersion (de Braux et al., 2014) and egg immersion (Warren-Myers et al., 2015a), mark the otoliths of fish with enriched stable isotopes and were recently modified for marking Atlantic salmon *Salmo salar,* but have not been tested on rainbow and brown trout. Stable isotope marking has been successful with a range of barium isotopes, which means it is capable of marking each year's production from a hatchery with a different unique mark with 100% mark success (Woodcock et al., 2011b; Warren-Myers et al., 2015a). This would enable long term, accurate assessments of the contribution hatchery produced rainbow and brown trout make to wild fisheries. The minimum isotope concentration required and immersion time to achieve 100% mark success using immersion marking varies among fish species (e.g. 30 μg L^{-1} for golden perch *Macquaria ambigua* immersed for 1 day, Woodcock et al., 2011a; 100 μg L^{-1} for murray cod *Maccullochella peelii* immersed for 6 days, Woodcock et al., 2011b; 30 μg L^{-1} for Atlantic salmon immersed for 1 h, de Braux et al., 2014), which means validation and optimisation of the marking methods is necessary for rainbow and brown trout eggs and larvae before hatchery-scale use. Once optimised, both mark delivery methods can be cost effective to mark ($US 0.01–0.002 per fish, de Braux et al., 2014; Warren-Myers et al., 2015a), easy to apply and are more welfare friendly compared to fin clipping. We assessed mark success and mark strength in the otoliths of rainbow and brown trout for both delivery techniques, and outline the associated practicality of marking with stable isotopes in hatcheries.

2. Method

2.1. Marking process

Eggs and larvae of rainbow and brown trout broodfish from the Snobs Creek Hatchery, Eildon, Victoria, were used to test the two enriched stable isotope mass marking methods, namely egg immersion (Warren-Myers et al., 2015a) and larval immersion (de Braux et al., 2014). Enriched forms of the rare isotopes ^{136}Ba (7.8% prevalent) and ^{137}Ba (11.2% prevalent) were selected for marking and are compared to the more abundant form ^{138}Ba which is 71.7% prevalent. Marking via egg immersion was tested in May 2016 using a single isotope at one (^{136}Ba) or two (^{137}Ba) concentrations (500 or 1000 μg L^{-1}; Table 1), and followed the protocols of Warren-Myers et al. (2015a). Briefly, this involved mixing eggs stripped from brood females (n = 22) and fertilising with sperm from five males in a 10 l bucket. Two minutes after fertilisation, three batches of eggs per treatment (~250 ml per batch) were then placed into individual 5 l buckets containing an isotope-enriched solution. Eggs were left to swell for 2 h at 8 °C after which the isotope solution was drained off, eggs rinsed once with fresh hatchery water and then placed into hatchery rearing trays.

Table 1
Experimental treatments for marking via egg immersion.

Species	Isotope	Conc. (μg L^{-1})	Egg Vol. (L)	Vol. (L)	Time. (h)	Replicates
S. trutta	^{137}Ba	500	0.25	0.50	2	3
S. trutta	^{137}Ba	1000	0.25	0.50	2	3
S. trutta	^{136}Ba	1000	0.25	0.50	2	3
O.mykiss	^{137}Ba	500	0.25	0.50	2	3
O.mykiss	^{137}Ba	1000	0.25	0.50	2	3
O.mykiss	^{136}Ba	1000	0.25	0.50	2	3

Marking via larval immersion was tested in July 2016 using a combination of the isotopes ^{137}Ba and ^{136}Ba at two different concentrations (100 or 200 μg L^{-1}; Table 2), and followed the protocols of de Braux et al. (2014). Briefly, this involved taking batches of yolk sac larvae (n = 45 larvae per batch) within one week of hatching and immersing them in an enriched stable isotope solution for 24 h. Following immersion, batches of larvae were then placed in hatchery rearing trays.

2.2. Grow out phase and sampling

Replicate batches from the egg immersion experiment were grown in separate incubating containers at ~8 °C. Immediately prior to reaching first feeding, a sub-sample of 20 larvae per batch was collected and stored in plastic containers containing 70% ethanol. Similarly, for the larval immersion experiment, each batch of larvae was reared in separate incubating containers and a subsample of 20 larvae taken immediately prior to first feeding (4 weeks post immersion). These larval sub-samples were frozen before being transferred to plastic containers containing 70% ethanol.

2.3. Otolith sampling and preparation

Three larvae taken from each subsample (larval immersion 3 × 12 = 36; egg immersion 3 × 18 = 54 per species) and from each of two unmarked batches of hatchery control larvae (3 × 2 = 6 per species) had their sagittal otoliths dissected and removed, cleaned of any adhering tissue, air dried, and stored individually in plastic tubes for otolith analysis. Sagittal otoliths were cleaned as per Warren-Myers et al. (2014). Any remaining organic tissue was removed by immersing

Table 2
Experimental treatments for marking via larval immersion.

Species	Isotope	Conc. (μg L^{-1})	# Larvae	Vol. (L)	Time. (h)	Replicates
S. trutta	^{137}Ba + ^{136}Ba	100	44	0.400	24	3
S. trutta	^{137}Ba + ^{136}Ba	200	44	0.400	24	3
O. mykiss	^{137}Ba + ^{136}Ba	100	44	0.400	24	3
O. mykiss	^{137}Ba + ^{136}Ba	200	44	0.400	24	3

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