Does size matter? A test of size-specific mortality in Atlantic salmon *Salmo salar* smolts tagged with acoustic transmitters

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Running headline: SURVIVAL OF SMOLTS BY ACOUSTIC TAGGING

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Abstract

Mortality rates of wild Atlantic salmon *Salmo salar* smolts implanted with acoustic transmitters were assessed to determine if mortality was size dependent. The routinely accepted, but widely debated, “2% transmitter mass: body mass” rule in biotelemetry was tested by extending the transmitter burden up to 12.7% of body mass in small (mean fork length 138.3 mm, range 115 – 168 mm) downstream migrating *S. salar* smolts. Over the short timescale of emigration (range 11.9 – 44.5 days) through the lower river and estuary, mortality was not related to *S. salar* size, no relationship was found between mortality probability and transmitter mass: body mass or transmitter length: fork length ratios. This study provides further evidence that smolt migration studies can deviate from the “2% rule” of thumb, to more appropriate study-specific measures, which enables the use of fishes representative of the body size in natural populations without undue effects.

Key Words: Biotelemetry, Migration, Transmitter effects, Tag burden

Introduction

Recent technological advances have dramatically improved our ability to track fishes in the wild (Cooke *et al*., 2013; Thorstad *et al*., 2013). Fuelled by the need to understand the movements of diadromous fishes, particularly salmon smolts, during their estuarine and early marine migration, acoustic transmitters have been miniaturised, thus opening up new and exciting aspects of fisheries research. Previously limited to larger species or older life stages, acoustic telemetry now has the potential to track small fishes through freshwater, estuarine and marine environments for considerable periods of time (Thorstad *et al*., 2013). Like all battery-powered electronic transmitters, one significant remaining constraint of this technology, for fishes, is the transmitter size relative to that of the fish, which currently precludes use of the technique on small species and very early life stages.

In fishes, the “2% rule” (Winter, 1996) has been accepted frequently as a ‘rule of thumb’ for maximum tag mass to body mass ratios (tag burden), despite criticism in recent years (Jepsen *et al*., 2005). Empirical studies have shown negative effects on fishes when tag burden is greater than this and have been used to support this position (McCleave & Stred, 1975; Ross & McCormick, 1981; Marty & Summerfelt, 1986; Adams *et al*., 1998; Lefrançois *et al*., 2001; Sutton & Benson, 2003).

More recently, the boundaries of telemetry transmitter burden impacts on small fishes have been explored, stimulated in part by the study of Brown *et al*. (1999) showing no effect on swimming performance of surgically implanted acoustic transmitters (7 x 12 mm, 0.6 g in air) up to 12% of body mass in juvenile hatchery rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (mean L_F 88.9, mean mass 7.4 g). Studies on Pacific salmon (*Oncorhynchus* spp.) from hatcheries have attempted to determine a maximum tag burden for surgically intracoelomic implanted transmitters. Species, tag size, survival rate and other measures of performance, however, have varied between studies. For example Zale *et al*. (2005) reported a small decrease in swimming performance with transmitter mass (mass 1-5 g in air, volume 0.5-1.5 cm^3) of up to 4% body mass in cutthroat trout, *Oncorhynchus clarkii lewisi* (Richardson, 1837) (mean L_T 240 mm, mean mass 132.8 g). Yearling Chinook salmon, *Oncorhynchus tshawytscha*, (Walbaum, 1792) (mean L_F 166 mm and mass 50.5 g) exhibited 80 - 100% survival rates with a combined intracoelomic implantation of an acoustic transmitter (7 x 20.5 mm, 1.8 g in air) and passive integrated transponder (PIT) tag (2.15×12.0 mm, 0.1 g in air) up to 5.6% of their body mass (Ammann *et al*., 2013). However, growth and survival impacts in *O.tshawytscha* (L_F 80 – 109 mm, mass 6.8 – 16.3 g) surgically implanted with an acoustic transmitter (mean mass 0.64 g in air; 0.28 ml volume) and a PIT tag (mass 0.10 g in air, 0.04 ml volume) were evident at transmitter burdens greater than 6.7% (Brown *et al*., 2010).

For many salmonids, seaward-migrating smolts are relatively small, so tag burden issues are particularly acute in these studies. In coho salmon, *Oncorhynchus kisutch*, (Walbaum, 1792) smolts L_F 95 – 130 mm, a maximum transmitter size to body size of 17% L_F and 7% by mass showed no adverse effects on survival, growth or physiology using transmitters of 6 x 19 mm, and mass of 0.9 g in air (Chittenden *et al*., 2009). Small *O. mykiss* pre-smolts (L_F 110 – 170 mm, mass 16.8 – 53.3 g) have been shown to survive intrcoelomic implantation with acoustic transmitters 8 mm diameter, 24 mm long, mass 1.4 g (with a 12 mm PIT tag embedded in the body of the tag) (Welch *et al*., 2007), however greatest survival rate in that study was with *O. mykiss* larger than 140 mm L_F.

Although there is a paucity of studies that have directly examined the effects of tag burden specifically on Atlantic salmon, *Salmo salar* L. 1758 smolts in the wild, there is good reason for concern that tag size effects may introduce unwanted biases to smolt movement and mortality studies. Many tracking studies on *S. salar* smolts have been conducted on *S. salar* which have been reared in hatcheries and are typically larger than wild *S. salar*. For study of stocked smolts, this is acceptable, but their use as a surrogate for wild *S. salar* is a poor choice. Hatchery fishes, express different physiological, behavioural and ecological traits to those of wild smolts (*e.g*. Jonsson *et al*. 1991). Physical condition along with physiological status also differs between wild and hatchery fishes due to their exposure to different selection regimes, thus migration preparedness and survival is likely to differ significantly between hatchery origin and wild smolts (McCormick *et al*., 1998). Fishes reared in hatchery conditions lack exposure to predators and this may result in increased mortality for hatchery origin individuals when released to the wild. Thorstad *et al*. (2012*a*), for example, reported low survival (12%) for hatchery reared smolts released to the wild, potentially due to reduced freshwater migratory behaviour.

Also, resulting from tag burden concerns, in most salmon smolt acoustic telemetry studies using widely available 7 x 20 mm sized transmitters, and where wild fishes are used, often only the largest individuals are selected for tagging (*e.g.* Lefèvre *et al*. 2012). Since the size of fishes is thought to play a significant role in survival, bias in initial selection may falsely represent true behaviour and/or mortality (Gingerich *et al*., 2012; Deng *et al*., 2015). There is a pressing need for smolt migration studies which focus on wild rather than hatchery reared fish and access the full size range of the natural migrating smolt populations. One route to enabling this, is to better evaluate the effects that exceeding the ‘2% rule’ may have on wild migrating smolts implanted with acoustic transmitters, particularly under natural conditions. The effect of tag burden, beyond 2% of body mass, on mortality is tested here with wild *S. salar* smolts implanted with acoustic transmitters.

Materials and Methods

The Foyle catchment (4450 km^2, 54° 736′ N; 007° 083′ W) is situated on the border between Northern Ireland (U.K.) and the Republic of Ireland (Fig. 1). Two main tributaries of the catchment are the rivers Finn and Mourne, both of which have significant migrations of *S. salar* smolts. The average size of these smolts is relatively small at around 135 mm L_F and 26 g (Loughs Agency, 2009). These two rivers form the River Foyle at their confluence, which is a transitional/estuarine water under tidal influences. Salinity levels range from 0.14 at the confluence of the rivers Mourne and Finn (River Foyle) to 22 at Culmore point (Fig. 1). This section of river (confluence to Culmore point) will be referred to as the estuarine section. At Culmore point, the Foyle discharges into a large sea lough, Lough Foyle. Lough Foyle is a shallow embayment, covering approximately 186 km^2, 20% of which is intertidal mudflats. At its mouth, the lough narrows to a 1 km wide channel before discharging into the Atlantic Ocean. Salinity in the sea lough ranges from 22 at Culmore point to 35 at its mouth and represents the early marine phase of migration for migrating smolts (Fig.1).

Wild *S. salar* smolts were tagged over a 2 year period (2013 to 2014). Individuals were captured by electrofishing (backpack) in the upper tributaries of the Mourne and Finn in 2013 and by rod and line only in the Mourne in 2014. *S. salar* were implanted with acoustic transmitters and released close to their capture site (Fig. 1) following a short period of recovery (approximately 30 minutes) post capture. *S. salar* were anaesthetised with clove oil (0.5 mg l^{-1}); their mass (g) and fork length (L_F, mm) were recorded prior to being placed on a v-shaped surgical sponge saturated with river water. The gills were aspirated with 100% river water throughout the procedure. An incision (11-13 mm) was made along the abdominal wall, anterior to the pelvic girdle. A coded acoustic transmitter (either, Model LP-7.3, 7.3mm diameter, 18mm length, 1.9g mass in air, Thelma Biotel AS, www.thelmabiotel.com or Model V7-2x, 7 mm diameter, 18 mm length, 1.9 g mass in air, Vemco Ltd, , www.vemco.com) was inserted into the intracoelomic cavity. The incision was closed with two independent sterile sutures (6-0 ETHILON, Ethicon Ltd, http://www.ethicon.com/) with a surgeons knot. On completion of the procedure, *S. salar* were placed into a keep-box which was positioned in an area of gentle flow in the river overnight; *S. salar* were released in their tagging groups the following day. No mortality occurred before release. Work was undertaken in accordance with UK Home Office licencing.

An acoustic receiver array was established to monitor tagged *S. salar* smolts. In this study, specific automatic listening stations [ALS (Vemco VR2W)] from within a larger array were utilised to determine the survival of migrating *S. salar*. Receivers were deployed in March and recovered in the July of each year. Transmitter life was expected to extend into mid-July, receivers were recovered after this point, thus it is assumed all migrating *S. salar* would have been detected within the deployment period of receivers. Fish were deemed to have initiated migration upon detection at ALS M1 or F1 (Fig. 1). Detection on ALS L1 indicated successful freshwater and estuarine migration by tagged *S. salar* and are referred to as successful migrants. Detection at ALS, L2 and/or L3 identified *S. salar* migrating through the sea lough into the Atlantic Ocean. It is assumed any *S. salar* not detected at the consecutive ALS (L1 or L2 and L3) was a mortality (unsuccessful migrant) within that specific stage (Fig. 1). De-smoltification has not been previously reported for this population.

Extensive range tests were undertaken throughout the array, and specifically at ALS L2 and L3 (Fig.1) to ensure detection coverage at this location was adequate to determine escapement success. To test for acoustic breaches at L2 and L3, an acoustic transmitter (Model LP-7.3, 139 dB re 1 μPa power, Thelma Biotel AS, Trondheim, Norway 2013) was suspended at 3 m depth and trolled (~1500 m x 4; ebbing and flooding tide) by a drifting boat (engine off). Tests identified an acoustic range of 450 m ensuring an overlap in detection ranges of ALS L2 and L3. Transmitter failure rate reported by manufacturers is low (<2%); for Thelma transmitters of the same model used here, Gauld *et al*. (2013) reported control transmitter failure rates of 0% within field test environments. Thus relevant precautionary steps were taken to maximise detection efficiency within the study and enable the determination of transmitter fate.

The hypothesis that tag burden affects survival in *S. salar* smolts was tested by examining the influence of four characteristics (F_L, *S. salar* mass, transmitter length to F_L ratio and transmitter mass to body mass ratio) on mortality. Tests were conducted on all tagged (AT) *S. salar* to investigate outright mortality, along with a subset of these which initiated migration (ST) to investigate the effect of tag burden during migration. ST *S. salar* were analysed separately as a subset of AT as they were deemed to have initiated migration and thus may be exposed to delayed mortality post tag implantation. *S. salar* were grouped depending on their survival outcome, Welch's two sample *t*-tests were used to compare between each group (survive vs. mortality) for each variable. All analysis was conducted using R statistical computing package (R Development Core Team, 2014).

Results

Sixty eight wild *S.salar* smolts were implanted with acoustic transmitters (39 in 2013 and 29 in 2014) over a 2 year period. *S. salar* fork length (L_F) ranged from 115 to 168 mm and mass from 15 to 44 g (Table I). A lower proportion of *S. salar* (41%) was detected within the array in 2014 compared to 85% in 2013. There was no difference in L_F or transmitter mass to body mass ratio between fish tagged in the Mourne 2014 detected within the array and those not detected ($L_{F,}$ *t*-test, t = -0.8, df = 23.3, P = >0.05. transmitter mass: body mass, *t*-test, t = 1.3, df = 27.0, P = >0.05). Similarly there was no difference between *S. salar* detected in the array and those not in 2013 in the Mourne ($L_{F,}$ *t*-test, t = -1.4, df = 2.9, P = >0.05. transmitter mass: body mass, *t*-test, t = 1.2, df = 2.6, P = >0.05) or between all *S. salar* in the study ($L_{F,}$ *t*-test, t = -0.9, df = 35.7, p = >0.05. transmitter mass: body mass, *t*-test, t = 0.9, df = 36.6, P = >0.05). All *S. salar* were detected in the array from the river Finn in 2013. The exact fate of undetected *S. salar* cannot be directly determined.

Across the size range of *S. salar* tagged in this study (L_F 115 – 168 mm, mass 15 – 44 g), (Table I) there was no evidence to support the hypothesis that tag burden had any effect on survival. *t*-tests between all measured parameters of *S. salar* size and transmitter size to *S. salar* size ratios showed no significant difference between successful [*S. salar* detected at L1

(Fig. 1)] and unsuccessful migrants (Table I). This holds true for all tagged *S. salar* (AT, n = 68) as well as a subset of these *S. salar* (ST, n = 41) which were deemed to have initiated migration.

Indeed, the smallest tagged *S. salar* within the study (L_F = 115 mm, mass = 15 g) successfully migrated through fresh water and the estuary. Of the 10 smallest fish within the study (mean L_F = 120.1 ± 3 mm, mean mass = 18.5 ± 3 g) six were successful migrants, entering the sea lough. Similarly, of the 10 largest fish within the study (mean L_F = 160.5 ± 5.8 mm, mean mass = 38.0 ± 5.0 g) six were also successful migrants reaching the sea lough. The two fish with highest transmitter mass to body mass ratios (both 12.7%) also survived. Mean time ± S.D. from release to escapement into Atlantic Ocean (last detection within the array for successful migrants) was 24.9 ± 8.8 days (range 11.9 – 44.5 days).

Mortality within the sea lough was high, only seven individuals were detected at L2 and L3 of the initial 41 detected entering the Lough. A two sample *t*-test between *S. salar* which were successful in migrating to L2/3 and those successful in reaching L1 but not L2/3 (Fig.1) showed no difference in transmitter mass to body mass ratio (*t*-test, t = 0.1, df = 10, P = 0.9).

Discussion

The range of sizes (Table I) of *S. salar* used in this study include some of the smallest *S. salar* smolts used in electronic tagging studies, providing a unique opportunity to determine the effect of tagging on short term (up to 44 days) survival rates and migration patterns of these fish. Mortality of small, wild *S. salar* smolts implanted with acoustic transmitters, was not associated with tag burden, for transmitters 7 x 20 mm in size and 1.9 g mass in air. Survival of the smallest *S. salar* in the study to the sea lough, with a transmitter mass to body mass ratio of 12.7% and 115 mm L_F along with another *S. salar* of the same tag burden, 12.7% (L_F 123 mm), demonstrate the ability of small *S. salar* to successfully cope with relatively large acoustic transmitters. This is supported by the high survival rate (60%) to the sea lough of the 10 smallest *S. salar* within the study, equivalent to that of the largest 10 (60%). Despite only small numbers of *S. salar* being detected exiting the sea lough, no size difference in mortalities was present. No tagged *S. salar* were recorded on an ALS which had not been recorded previously at an upstream ALS. Combined with no acoustic breaching during range tests and high transmitter reliability, it is assumed the telemetry array design was adequate to determine migration success. High mortality within the lough (83%) was probably due to predation, although mortality by other means (*e.g.* osmoregulatory incompetence) cannot be ruled out. High estuarine predation is commonly reported in smolt migration studies (Hvidsten & Møkkelgjerd, 1987; Serrano *et al*., 2009; Hedger *et al*., 2011; Thorstad *et al*., 2012*b*). Reduced numbers of *S. salar* were detected within the array in 2014 despite this not being related to size. No mortalities occurred during the tagging process. This difference might be due, in part, to the change in capture method between the 2 years but the exact fate of these individuals could not be determined. Indeed the need for further investigation on the effects of capture and handling in fishes telemetry studies has recently been highlighted (Jepsen *et al*., 2015).

Body size is a limiting factor in acoustic tagging studies, and although the effects of tagging on Pacific salmonids (*Oncorhynchus* spp*)* are relatively well studied (Jepsen *et al*., 2005), extrapolation of data across even closely related species should be done with caution (Ebner *et al.*, 2009). The findings of the study presented here do not define tag size or a limit to tag mass ratios, however they do specifically demonstrate the potential to successfully implant small wild *S. salar* smolts with acoustic transmitters at a size much smaller than previously reported. Lacroix *et al*. (2004) recommend a transmitter mass of 8% body mass and a transmitter length of 16% or less of L_F for juvenile *S. salar* following a laboratory experiment. Several studies utilising *S. salar* smolts for tagging have not identified any abnormal mortality rates despite using transmitter mass: body mass ratios above 2%. Urke *et al*. (2013) although not specifically reporting on the effect of tag size, indicate high survival rates to sea for wild smolts (775 survival, mean L_F 127 mm, mean mass 16.5 g) implanted with acoustic transmitters (7.3 mm diamter, 1.2 g in water) and hatchery *S. salar* (85% survival, mean L_F 157 mm, mean mass 40.8 g) with transmitter mass to body mass ratios equating to approximately 7%. In addition Thorstad *et al*. (2007) indicated no effect of transmitter to body mass ratio (mean = 6%) on survival of wild *S. salar* post smolts (mean L_T 152 mm, mean mass 25 g) implanted with acoustic transmitters (7 x 19 mm 1.9 g in air). Lefèvre *et al*. (2012) utilised transmitter mass (9 x 20 mm, 2.9 g in air) to body mass ratios of up to 14% (mean 12%) with wild *S. salar* smolts and post smolts (>131 mm L_F and >20 g) with no reported effect on mortality.

This study adds to the growing evidence challenging rigid application of the ‘2% rule’ in biotelemetry (Brown *et al*., 1999; Jepsen *et al*., 2005). Brown *et al*. (1999) for example suggest moving away from the 2% rule towards a new standard with a more scientific basis which takes into account the relative buoyancy of a tag and physical dimensions. They argue that there may be a requirement of a fish to compensate for tag buoyancy by transferring gas into their swim bladder. Hence a more buoyant tag may have less impact upon a fish compared with a denser tag of similar dimensions. Jepsen *et al.* (2005) similarly argue that any tag/fish size relationship should be driven by the study objectives and empirical evidence. In some cases, large tags may be utilised without significant effects on behaviour and physiology, whilst in other circumstances, effects such as reduced growth and swimming ability may result from the use of smaller tags (Jepsen *et al*., 2005; Thorstad *et al*., 2013). Nevertheless, several longer-term studies have shown growth impacts on fishes with higher tag burdens (Larsen *et al*., 2013) and concerns over subtle impacts on behaviour and the need to minimize impacts in handling and tagging continue to drive forward tag miniaturisation processes (McMichael *et al*., 2010; Deng *et al*., 2015).

Telemetry has helped unlock an understanding of fish migration ecology providing essential knowledge to manage and conserve declining anadromous fish populations. The ability to identify migration routes, bottlenecks, sources of mortality and species interactions will enable development of more effective conservation strategies. The study presented here has shown that the 2% tag mass to body mass ratio is not an immutable threshold for tagging studies. If *S. salar* smolt migration studies are to adequately represent wild salmon behaviour there is a requirement to move away from the 2% tag mass to body mass rule of thumb adhered to in the past, and towards tested criteria which are species-specific and suitable to address study outcomes, without compromising the natural behaviour of the individual.

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Table I: Tests of the differences in a range of *Salmo salar* and tag parameters in smolts that were successful [detected at ALS L1 (Fig. 1) and unsuccessful in migrating to the sea lough [not detected at ALS L1 (Fig. 1)], and descriptive statistics for each variable. Tag mass: body mass (mass %) and tag length: fork length (Length %) ratios are expressed as a percentage. *Salmo salar* are grouped as all tagged *S. salar* (AT) and a subset of these S. salar which were detected within the acoustic array and deemed to initiate migration (ST)

Group	Test variable	Successful (*n*) Mean ± SD	Unsuccessful (*n*) Mean ± SD	Range	DF	*t*-value	*P*-value
AT	Length (mm)	(41) 138.8 ± 12.7	(27) 138.3 ± 13.8	115-168	56.8	-0.2	0.8
AT	Length %	(41) 14.5 ± 1.3	(27) 14.6 ± 1.4	11.9-17.4	57.0	0.3	0.8
AT	Mass (g)	(41) 28.6 ± 6.5	(27) 28.1 ± 7.1	15-44	58.4	-0.2	0.8
AT	Mass %	(41) 7.2 ± 1.9	(27) 7.2 ± 1.9	4.3-12.7	62.3	0.2	0.9
ST	Length (mm)	(33) 139.1 ± 12.2	(8) 143.0 ± 13.5	115-168	9.5	0.8	0.5
ST	Length %	(33) 14.5 ± 1.3	(8) 14.1 ± 1.3	11.9-17.4	10.0	-0.8	0.4
ST	Mass (g)	(33) 28.6 ± 6.6	(8) 30.65 ± 7.3	15-44	9.5	0.8	0.5
ST	Mass %	(33) 7.1 ± 2.0	(8) 6.5 ± 1.3	4.3-12.7	14.3	-1.1	0.3

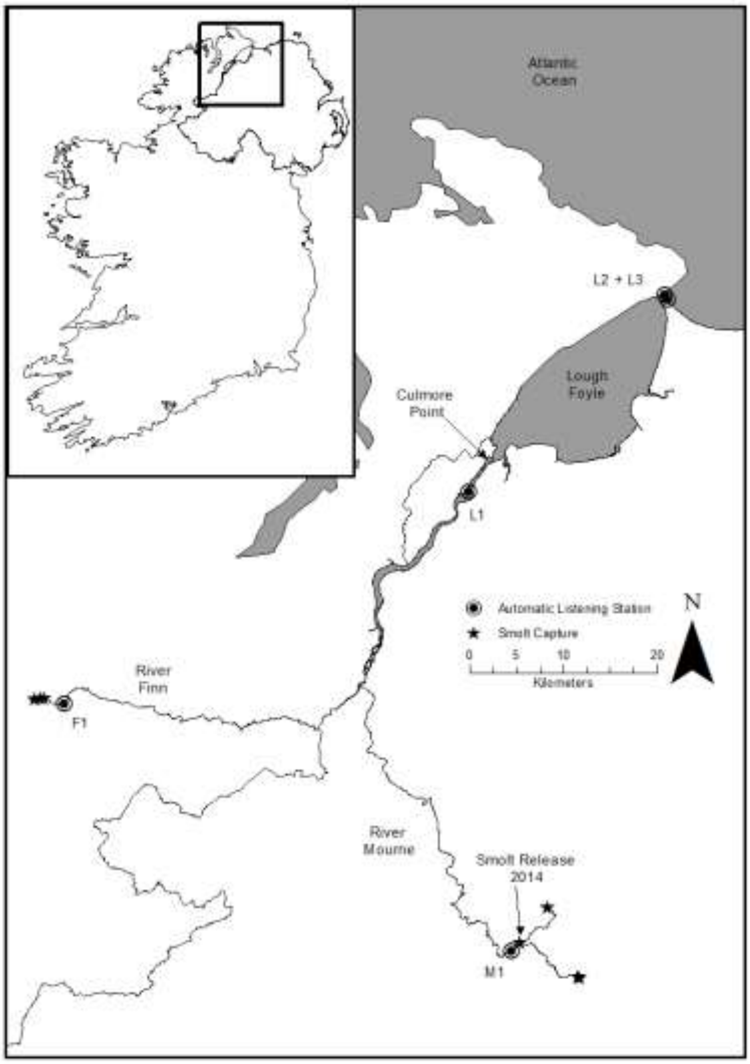

FIGURE 1: The Foyle catchment showing location on the border between Northern Ireland and the Republic of Ireland within the small inset, and the study site location. The large map outlines the study site, Automatic Listening Station (ALS) locations along with smolt capture and release points in 2013 and a single point in 2014. The river section between the confluence of the Mourne and Finn and Culmore point is estuarine.

Comment [M1]: I believe the capture points only represent those sites in 2013 and this should be stated in the legend and/or on the figure, that they are capture AND release sites 2013.