



Trade-offs in experimental designs for estimating post-release mortality in containment studies

Mark W. Rogers^{a,*}, Andrew B. Barbour^b, Kyle L. Wilson^c

^a U.S. Geological Survey, Great Lakes Science Center, Lake Erie Biological Station, 6100 Columbus Avenue, Sandusky, OH 44870, United States

^b School of Forest Resources and Conservation, University of Florida, 7922 NW 71st Street, Gainesville, FL 32653, United States

^c Department of Biological Sciences, University of Calgary, 2500 University Drive N.W., Calgary, Alberta T2N 1N4, Canada

ARTICLE INFO

Article history:

Received 13 May 2013

Received in revised form 23 October 2013

Accepted 25 October 2013

Keywords:

Tagging

Containment

Post-release mortality

Experimental design

ABSTRACT

Estimates of post-release mortality (PRM) facilitate accounting for unintended deaths from fishery activities and contribute to development of fishery regulations and harvest quotas. The most popular method for estimating PRM employs containers for comparing control and treatment fish, yet guidance for experimental design of PRM studies with containers is lacking. We used simulations to evaluate trade-offs in the number of containers (replicates) employed versus the number of fish-per container when estimating tagging mortality. We also investigated effects of control fish survival and how among container variation in survival affects the ability to detect additive mortality. Simulations revealed that high experimental effort was required when: (1) additive treatment mortality was small, (2) control fish mortality was non-negligible, and (3) among container variability in control fish mortality exceeded 10% of the mean. We provided programming code to allow investigators to compare alternative designs for their individual scenarios and expose trade-offs among experimental design options. Results from our simulations and simulation code will help investigators develop efficient PRM experimental designs for precise mortality assessment.

Published by Elsevier B.V.

1. Introduction

Numerous studies have sought to estimate post-release effects of fishery or research activities on fish that are otherwise presumed to survive. Multiple examples of post-release mortality (PRM) estimation come from “catch-and-release science” (Cooke and Schramm, 2007), accounting for tagging mortality in mark-and-recapture studies (e.g., Brenden et al., 2010), and incorporating parameters for “cryptic mortality” into population models (e.g., Coggins et al., 2007). Freshwater catch-and-release (CR) mortality studies have been a large contributor to understanding handling effects. For example, Hühn and Arlinghaus (2011) identified 107 studies with 252 individual CR mortality estimates in a meta-analysis limited to genera popular in European fisheries. Efforts aimed at decreasing unintended mortality (e.g., circle hook requirements) and accounting for unintended mortality (e.g., tagging studies, fishery bycatch) necessitate robust PRM experimental designs.

Pollock and Pine (2007) provided guidance for estimating mortality and uncertainty in CR studies and discussed trade-offs among approaches (e.g., containment versus telemetry). For the most com-

mon method, containment studies (Cooke and Schramm, 2007; Raby et al., in press), Pollock and Pine (2007) recommended that: (1) control fish always be used, (2) individual fish not be treated as replicates, and (3) precision be considered before experimentation to determine if estimates would be informative. In studies with randomized designs, container-to-container effects can bias estimates of mortality for tagged compared with control containers. To account for the potential for container-specific effects, Pollock and Pine (2007) encouraged the use of stocking equal numbers of control and treatment fish in each container (hereafter called ‘paired-design’); thus any container-to-container effects will be equally distributed between control and tagged fish. Pollock and Pine (2007) focused on CR studies, yet their recommendations extend to other PRM studies with objectives of quantifying and comparing treatment effects.

Cowx et al. (2010) stated that a “plethora” of studies have aimed to measure lethal and sublethal handling effects. However, we are not aware of any in-depth exploration of Pollock and Pine’s (2007) warning for careful planning and study design. Cooke et al. (2013) provided perspectives on measuring physiological consequences of CR fishing and common limitations, yet gaps still remain for quantifying treatment effects. For fish tagging studies, in particular, guidance exists for tag selection and ensuring that the marked population is representative of the unmarked population (e.g., Pine et al., 2003) and mark-recapture literature explicitly details the

* Corresponding author. Tel.: +1 419 625 1976; fax: +1 419 625 7164.

E-mail address: mwrogers@usgs.gov (M.W. Rogers).

need and methods for accurate estimates of capture probability. Yet, methods for quantifying mortality due to handling and tagging have received less attention and could result in bias in fish tagging studies if the number-at-large is not adequately assessed. We were challenged with designing an experiment to measure PIT-tagging mortality for a yellow perch movement study at Lake Erie, and wanted to extend our result to create a broadly applicable simulation tool to inform future PRM study designs. Our objective was to use simulations to evaluate trade-offs in the ability to detect mortality in paired-design containment experiments across a range of design scenarios in which sample size (number of fish per container) and replication (number of containers) were independent variables. We also evaluated the effect of control-fish survival and among-container survival variability on the precision of mortality estimates. Lastly, we provided Program R code (R Development Core Team, 2012) that can be modified to address other scenarios.

2. Materials and methods

Our simulations followed a recommended experimental design with control fish (handled) and treatment fish (handled and tagged) that were then held together within containers (Pollock and Pine, 2007). We simulated survival for individuals from each treatment group as a binomial process. Each individual had a probability of surviving determined by treatment-dependent handling and tagging mortality inputs. The number of tagged and untagged fish in each enclosure were equal, thus the number of total fish per container had to be an even number. We chose to use a paired-design to ensure container-specific effects would not bias estimates of mortality between control and tagged fish. The number of containers was specified and individual containers were treated as replicates. Tag survival was estimated for each group as the difference between handling survival and tagging mortality. We calculated standard errors of the mean tagging survival for each container to determine 95% confidence intervals. To determine the average expectation for each scenario, we repeated simulations 1000 times.

Our simulation inputs were founded on a PIT-tagging mortality study for juvenile bluegill and yellow perch (Kaemingk et al., 2011). Our baseline handling survival was 95% for our simulations. We simulated a range of additive tagging mortality from 5% to 20% in increments of 5%. The number of fish in each simulated container ranged from 10 to 100 by 2, equally divided between the two treatments (i.e., 10 fish per container equated to 5 tagged and 5 control fish). We simulated a range of containers from 2 to 25. Our evaluation metrics included (1) relative bias in tagging mortality estimates relative to known input value, (2) average standard error in tagging mortality estimate, (3) percent coverage of tagging mortality estimates, and (4) the mean p -value from simulation replications for an analysis of variance (ANOVA) that tested for differences in survival rates between control and tagged fish with containers as replicates for each iteration. We calculated relative bias using the formula:

$$(\hat{M}_t - M_t) \times M_t^{-1} \quad (1)$$

where $\hat{M}_t$ is estimated tagging mortality for the i th iteration and M_t is the true mortality level. We then derived the average of the relative departures for all iterations for a given parameter scenario and reported that value as bias. We determined percent coverage as the number of iterations during which the 95% confidence interval of estimated tagging mortality included the true value for a given parameter set. We applied these outputs to select the design for our yellow perch scenario and simulated data under that design to determine if the data conformed to ANOVA assumptions. We assessed homoscedasticity assumptions with Levene's test and

normality assumptions with a Shapiro–Wilk test from 10,000 iterations (sensu Cooke et al., 2003).

We evaluated the sensitivity of simulation metrics to changes in control fish survival and among container variation in control fish survival. For sensitivity of designs to control fish survival, we compared a baseline scenario where handling survival was 95% to a scenario where handling survival was 80% with additive tagging mortality ranging from 5% to 20% in increments of 5% for both. To evaluate the effect of among container variation, we used a scenario with 10 replicates (i.e., containers), ranging from 12 to 200 fish-per-container in increments of 4, and a mean handling survival of 95%. We applied random deviates to the mean handling survival for each container. Random deviates were drawn from a normal distribution with standard deviations ranging from 0% to 25% of mean handling survival in increments of 2%. Simulation output metrics included mean relative bias, mean percent coverage, and mean ANOVA p -values as described above.

3. Results

Our simulations allowed for direct comparison of trade-offs in number of containers (i.e., replicates) versus total number of fish per container for informing PRM experimental designs. As expected, experiments with few individual fish and little replication were most biased. In some cases with few fish, the binomial process resulted in a higher number of fish dying from handling than tagging despite our known inputs with increased mortality from tagging. In experiments with low numbers of replicates, increasing the number of fish per container was marginally effective for improving bias (Fig. 1). As tagging mortality increased, bias and standard error estimates improved at lower numbers of replicates and fish per container relative to low tagging mortality (Figs. 1 and 2). For all tagging mortality values we evaluated, acceptable coverage levels (i.e., $\geq 90\%$) were only obtained with >6 containers (Fig. 3). The number of fish per container had little effect on coverage (Fig. 3) thereby requiring the use of >6 replicates when estimating tagging mortality under this survival scenario. Experiments with low tagging mortality and few replicates had a low ability to detect tagging effects with ANOVA. For our yellow perch scenario, we concluded that 8 replicates with 100 fish per container would adequately allow us to detect additive tagging mortality ≥ 0.05 (Fig. 4). This choice was informed by simulation outputs as well as considerations for the number of containers available ($N=4$) and the expected tagging period (one week). Shapiro–Wilk and Levene's tests to assess violations of normality and homoscedastic assumptions for the ANOVA determined that normality and homoscedastic violations occurred in only 13% and 6%, respectively, of 10,000 simulations in the yellow perch scenario.

Proper experimental design was dependent upon mean control fish survival and variability of mean control fish survival across containers. Lower handling survival of control fish resulted in a need for more experimental effort to maintain precision. For example, the ability to detect a significant difference between handling and tagging mortality when alpha was equal to 0.10 could be achieved when handling survival was 95% and tagging mortality was 5% by using 8 containers and 100 fish per container, but required 7 additional containers when handling survival decreased to 80% with 5% tagging mortality. When the standard deviation around the mean survival for control fish was higher than 10%, average ANOVA p -values exceeded 0.20 in simulations with 100 fish per container and 10 replicates (Fig. 5). Therefore, experimental design requires an expectation of what baseline handling mortality may be and careful consideration to minimize among container variation.

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