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Considerations for the design and interpretation of fishing release mortality estimates

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ABSTRACT

To generate mortality estimates for fish that are captured and released in recreational and commercial fisheries, it is common to temporarily hold fish in captivity. Typically, captured fish are placed in some form of pen, cage or tank with control individuals, yet little is known about how the type of holding environment influences fish condition or mortality. Here we captured freshwater fish (bluegill; *Lepomis macrochirus*) via angling and fyke net and retained them in one of four holding environments; a round flow-through tank on shore [TANK], a knotless nylon pen with natural substrate in the lake [PEN], a knotless nylon floating cage with a rigid structure [RCAGE], and a knotless nylon floating cage that lacked rigid structure [CAGE]. Mortality was low (1%) across both capture techniques and holding environments during the 14-day retention period. All mortalities were associated with capture by fyke net. A chronic stress indicator, blood glucose, was determined for a subset of fish on day 5. Although there were significant differences in blood glucose between angled RCAGE and angled PEN (Tukey, $P = 0.047$) and angled RCAGE and fyke PEN (Tukey, $P = 0.015$), the observed levels were generally quite low (range: 1.0–3.9 mmol L^{-1}) and the differences were likely associated with differences in feeding; fish in the PEN group with access to substrate (and presumably the most food) had slightly higher glucose levels. At the conclusion of the study Fulton's condition factor was similar among all groups (ANOVA, $P > 0.05$, all terms). However, fish held in the TANK treatment had the highest levels of external protozoan parasite infection by *Ichthyopthirius* (Tukey, $P < 0.05$). This study illustrates that in situ holding environments (rather than tanks) may help reduce mortality, stress, and disease during studies that estimate post-release mortality. We encourage additional research to explore how the holding environment can influence inferences made about post-release mortality and sublethal impacts of fishing.

1. Introduction

Nearly all fisheries, whether recreational or commercial, release a component of their catch (Cooke and Cowx, 2006). Although quantifying the harvest component of fisheries mortality is relatively simple (Hilborn and Walters, 1992), generating mortality estimates for released fish is more challenging (Coggins et al., 2007). As summarized in Wilde (2003), there are several approaches to doing so. One involves the use of tagging methods where fish are released and tracked with various electronic tags to assess survival (see Donaldson et al., 2008) or marked in some manner that enables determination of survival from mark-recapture analysis (Pine et al., 2003). However, the most common approach (ICES, 2014) involves holding fish in captivity (e.g., pens, cages, tanks) to assess mortality. Wild fish do not always transition well to captivity, even if for just a period of several hours or days (Casebolt et al., 1998). Captivity for wild fish can be inherently stressful (Grutter and Pankhurst, 2000; Portz et al., 2006) and is often associated with a disinterest in

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feeding (Murchie et al., 2009) and subsequent change in fish condition (Portz et al., 2006), agonistic interactions with conspecifics (Portz et al., 2006), disease outbreaks (Robertson et al., 1987; Portz et al., 2006), extensive exploratory behavior in an attempt to escape (Donaldson et al., 2011) and abrasion from the holding vessel (Portz et al., 2006; Donaldson et al., 2011). Given these potential negative consequences of holding wild fish in captivity, it would be useful to know how holding gears (e.g., pens, cages and tanks) influence how mortality estimates or even sublethal assessments are generated.

It is increasingly recognized that control fish should be used to account for handling and holding effects (Wilde, 2003). Indeed, whenever survival is not 100%, without controls it is not possible to determine if it is the treatment (e.g., a given capture gear) or some aspect of the study method (e.g., type of holding environment) that is associated with deaths (ICES, 2014). In some cases where control fish are not used, it is possible to evaluate the relative differences between treatments (e.g., handling the fish use one technique is better than another). However, it is not possible to easily incorporate such information into fisheries management models. It is rather apparent that control/background mortality differs markedly among studies (ICES, 2014), yet it is unclear how capture method or controls for the type of holding environment used influence mortality estimates. One of the common ways in which to obtain controls is to use what are perceived to be "benign" capture methods such as use of barbless hooks or passive traps (ICES, 2014). However, are these approaches truly benign? With growing scrutiny over mortality estimates (see Wydoski, 1977; Coggins et al., 2007; ICES, 2014), there remains a need to provide direction on study design to improve future research and ensure that values used in management and conservation are reliable and accurate.

The purpose of this study was to compare mortality estimates generated for two apparently benign capture methods (i.e., rapid angling and short-set fyke nets at cool temperatures) often used as controls and to evaluate how those estimates varied relative to four replicated (3 of each) types of holding environment (a round flow-through tank on shore, a knotless nylon pen with natural substrate in the lake, a knotless nylon floating cage with a rigid structure, and a knotless nylon floating cage that lacked rigid structure). We used bluegill sunfish (*Lepomis macrochirus*) as a model given that they are an abundant freshwater fish and can be captured in large numbers to provide a reasonable sample size (e.g., Cooke et al., 2003). Bluegill sunfish are commonly targeted by recreational anglers across their range (Coble, 1988; Quinn and Paukert, 2009) and small-scale commercial fisheries exist in some regions of midwestern North America (Larocque et al., 2012). The species' general morphology allows them to forage in both open-water habitats and among substrate and vegetation in the littoral zone (Ehlinger and Wilson, 1988). We evaluated both lethal (mortality rates) and sublethal endpoints (blood glucose, external parasite burden and condition factor) to assess fish condition and health. This study was designed to identify the most appropriate methods among those commonly used to identify post-release mortality. With the most appropriate methods identified, management actions would then be based on the most reliable information which would ensure that fisheries are managed to achieve both conservation targets while maximizing fishing opportunities.

2. Methods

2.1. Study location

The experiment was conducted at Queen's University Biological Station on Opinicon Lake, Ontario (44° 34′ N, 76° 19′ W). Opinicon Lake is a shallow mesotrophic lake located along the Rideau Canal waterway. The lake contains abundant populations of warmwater fish species such as largemouth bass (*Micropterus salmoides*) and bluegill sunfish. Experimental procedures were carried out between 4 May 2014 and 18 May 2014 when water temperature averaged 14 °C (range: 12–15 °C).

2.2. Holding environments

Three replicates of four different holding environment treatments were used in this experiment: floating rigid cages [RCAGE], floating cages [CAGE], pens that reached the substrate [PEN], and tanks [TANK]. Replicates for the RCAGE treatment were assembled using a 1.22 m × 1.22 m × 1.22 m frame of 2.54 cm diameter white CPVC pipe. A knotless nylon net (material obtained from Memphis Net and Twine, Heavy Delta, 12.7 mm sq.) with one unmeshed side was placed over the structure and affixed in place with cable ties. The CAGE treatment was similar except that it had only CPVC pipe around the perimeter of the unmeshed side, i.e., the remaining mesh was free to move in the water. The open side of both the RCAGE and CAGE treatments were kept afloat by pool noodles that were fixed to this part of the structures. Alternating between RCAGE and CAGE, replicates of the pens ($n = 6$) were attached at their corners using a 61 cm length of twine. The cages were taken to approximately 2.44 m of water and anchored at either end so that the entire structure remained oriented in the same direction during the experiment, i.e., parallel to shore. To construct a PEN replicate, four 2.44 m lengths of rebar were hammered into the substrate approximately 1.22 m apart in 1.07–1.22 m of water. A knotless nylon net (same material as above) with two open sides was placed around the rebar and connected to it using cable ties. The surface side of the mesh was tied to the rebar approximately 1.83–2.44 m from the surface of the water. To reduce the chance of fish escaping near the substrate, loose mesh was covered with sand, gravel, bricks, and rocks. The TANK treatments included a row of three 1000 L outdoor circular fiberglass tanks supplied with flow-through lake water at a rate of 166 L h^{-1}, where water was exchanged 4 times per day (Fig. 1).

2.3. Capture of fish

Bluegill were captured 4 May 2014 from Opinicon Lake, Ontario. Fykes nets ($n = 3$) each had 7 steel hoops that were 0.5 m apart and 0.9 m in diameter. Nets had two wings and a lead that was attached vertically to the mouth of each net. Wings were 4.6 m long by 0.9 m high, leads were 10.7 m long by 0.9 m high, and the mesh 2.54 cm square nylon (See Stoot et al., 2013 for more detail). Fyke nets were set in shallow weedy bays and checked twice during the day to capture bluegill ($n = 204$). Angling techniques used standard spinning gear with small hooks, bobbers, and a small piece of worm to capture bluegill ($n = 204$) from shallow weedy bays that were similar to those used during fyke netting. Angling occurred during the day when the fyke nets were fishing. To distinguish fish by a capture technique, individuals were marked by clipping either the upper or lower corner of the caudal fin. Treatment replicates were randomly populated with a similar size range of fish from each capture technique (17 fish per capture technique/replicate or 34 fish total/replicate; Table 1).

All holding environments were monitored three times a day at six-hour intervals from 4 May 2014 to 8 May 2014. After 8 May 2014, holding environments were monitored daily until 18 May 2014. Dead or moribund (e.g., loss of equilibrium, lethargy) bluegill were removed, checked for signs of trauma (e.g., fin abrasion scored as: none, moderate, heavy), external parasites (present/absent at this stage), and fin clip location. To identify whether holding vessel or capture method were potential sources of stress after four holding days, three fish/capture technique/replicate ($n = 72$) were

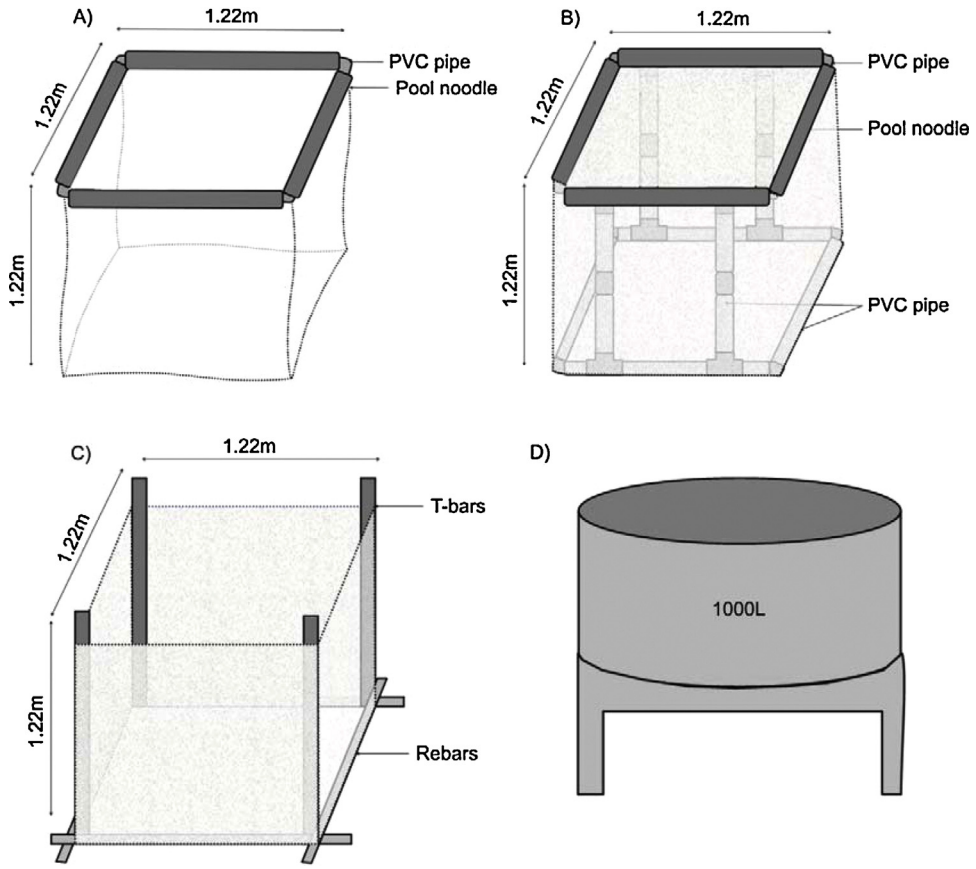

Fig. 1. Diagrams of each holding treatment. (A) Cage (B) Rigid Cage (C) Pen and (D) Tank.

quickly removed from each replicate (via angling) for phlebotomy to measure blood glucose. High levels of glucose could indicate an energetic response to a stressor (Barton et al., 2002). Once removed, fish were immediately placed supine in a water filled trough. Blood was taken from the caudal vessel using a 25 gauge needle and heparinized syringe (1 ml). Glucose concentrations were determined immediately with a whole-blood field glucose meter (Accu-Check Compact Plus, Roche, Basal, Switzerland) that was calibrated and used for bluegill (Fobert et al., 2009). Bluegill sampled for glucose were released immediately. At the end of the study and prior to release, all surviving fish were assessed for fin abrasion and external parasites (more were present than at earlier evaluation so actual numbers were recorded). Fish were supplemented with diced dew worms twice a week until the conclusion of the experiment. Fish were collected and handled in accordance with the Carleton University and Queen's University Animal care Committees and with scientific collection permits provided by the Ontario ministry of Natural Resources.

Table 1
Bluegill sunfish mean total length (mm) ± 1 SD and mean weight (g) ± 1 SD by treatment replicate and capture method.

Treatment replicate	Capture method	Mean TL (mm) ± SD	Mean mass (g) ± SD
CAGE1	ANGLED	178 ± 20	92 ± 30
CAGE2	ANGLED	163 ± 26	76 ± 37
CAGE3	ANGLED	158 ± 18	65 ± 26
PEN1	ANGLED	177 ± 23	92 ± 34
PEN2	ANGLED	165 ± 15	71 ± 22
PEN3	ANGLED	172 ± 33	81 ± 47
RCAGE1	ANGLED	165 ± 29	74 ± 37
RCAGE2	ANGLED	164 ± 28	79 ± 41
RCAGE3	ANGLED	170 ± 20	80 ± 30
TANK1	ANGLED	166 ± 15	75 ± 25
TANK2	ANGLED	168 ± 19	76 ± 30
TANK3	ANGLED	168 ± 25	84 ± 38
CAGE1	FYKE	171 ± 16	78 ± 20
CAGE2	FYKE	177 ± 12	93 ± 22
CAGE3	FYKE	181 ± 15	102 ± 26
PEN1	FYKE	184 ± 17	101 ± 30
PEN2	FYKE	180 ± 14	92 ± 19
PEN3	FYKE	193 ± 6	114 ± 21
RCAGE1	FYKE	176 ± 12	90 ± 20
RCAGE2	FYKE	182 ± 12	100 ± 22
RCAGE3	FYKE	180 ± 15	99 ± 23
TANK1	FYKE	177 ± 21	92 ± 32
TANK2	FYKE	171 ± 21	84 ± 26
TANK3	FYKE	182 ± 17	91 ± 27

2.4. Analyses

Data were first explored using Cleveland dotplots, boxplots, and conditional scatterplots to identify influential observations and relationships among the predictor variables: (holding vessel [categorical factor], capture method [categorical factor], and body size [continuous covariate]) and the dependent variables: (glucose [mmol L^{-1}], body condition [Fulton's K (Nash et al., 2006) = scaling factor × (W/$\hat{L3}$)], and parasite burden [yes/no]). Collinearity was not identified among the predictor variables. For all analyses, estimates were generated by pooling across replicates ($n = 72$). Initial validation procedures for a generalized linear model (GLM) of glucose indicated heterogeneity in the residuals for holding vessel and capture method. Rather than transform the data, we used generalized least squares (GLS) and a variance-covariance structure to account for the non-constant variance in the residuals (Pinheiro and Bates, 2000). A two-way ANOVA with an interaction term was used to find any significant difference in body condition across holding vessels and capture methods. For the analysis of parasite burden,

Table 2
The list of candidate models for blood glucose (GL) concentration (mmol L^{-1}) and the probability of parasite burden (PROB). TREAT is treatment, CM is capture method, and TL is total length. K is the number of estimated parameters for each model, AICc is the second-order AIC, ModelLik is the relative likelihood of the model given the data, AICcWt is the weight of evidence in favor of a given model among the candidate set, *LL* is the log likelihood of each model, and Cumul.Wt is the cumulative Akaike weights. Extra parameters (*K*) in the glucose models are a result of variance structures.

Model #	Model name	*K*	AICc	ΔAICc	ModelLik	AICcWt	LL	Cumul.Wt
1	GL = TREAT	9	120.882	0.000	1.000	0.435	−49.989	0.435
2	GL = TREAT + CM + TREAT:CM	13	122.639	1.757	0.415	0.181	−45.182	0.616
3	GL = TREAT + CM	10	122.832	1.950	0.377	0.164	−49.613	0.780
4	GL = TREAT + CM + TL + TREAT:CM	14	124.211	3.329	0.189	0.082	−44.421	0.862
5	GL = TREAT + CM + TL + CM:TL	12	124.576	3.694	0.158	0.069	−47.644	0.930
6	GL = CM	7	125.595	4.713	0.095	0.041	−54.923	0.972
7	GL = TREAT + CM + TL + TREAT:CM + CM:TL	15	126.682	5.800	0.055	0.024	−44.055	0.996
8	GL = TREAT + CM + TL + TREAT:TL	14	132.136	11.254	0.004	0.002	−48.384	0.997
9	GL = TREAT + CM + TL + TREAT:CM + TREAT:TL	17	132.146	11.264	0.004	0.002	−43.407	0.999
10	GL = TREAT + CM + TL + TREAT:TL + CM:TL	15	132.588	11.706	0.003	0.001	−47.008	1.000
11	GL = TREAT:CM:TL	21	140.262	19.380	0.000	0.000	−39.891	1.000
12	PROB = TREAT	4	329.656	0.000	1.000	0.436	−160.762	0.436
13	PROB = TREAT + CM	5	330.638	0.982	0.612	0.267	−160.219	0.702
14	PROB = TREAT + CM + TREAT:CM	8	333.141	3.485	0.175	0.076	−158.328	0.778
15	PROB = TREAT + CM + TL + CM:TL	7	333.157	3.501	0.174	0.076	−159.391	0.854
16	PROB = TREAT + CM + TL + TREAT:CM	9	333.913	4.257	0.119	0.052	−157.653	0.906
17	PROB = TREAT + CM + TL + TREAT:TL	9	334.510	4.854	0.088	0.038	−157.951	0.944
18	PROB = TREAT + CM + TL + TREAT:CM + CM:TL	10	335.057	5.401	0.067	0.029	−157.156	0.974
19	PROB = TREAT + CM + TL + TREAT:TL + CM:TL	10	336.155	6.499	0.039	0.017	−157.705	0.990
20	PROB = TREAT + CM + TL + TREAT:CM + TREAT:TL	12	337.710	8.053	0.018	0.008	−156.322	0.998
21	PROB = TREAT:CM:TL	16	340.695	11.039	0.004	0.002	−153.407	1.000
22	PROB = CM	2	385.996	56.340	0.000	0.000	−190.978	1.000

data were modeled using a GLM where parasite burden was the response (i.e., present/absent, binomial distribution) with treatment, capture and body size as explanatory variables. Since the analyses of glucose and parasite burden contained multiple combinations of three explanatory variables, we used AIC to determine the most parsimonious model from a candidate set (Akaike, 1974). The final GLS model was fitted using restricted maximum likelihood and validated by plotting the normalized residuals against each explanatory variable, including those not in the final model (Zuur et al., 2009). The model for body condition was validated using a similar procedure to that of the GLS model. The final model for parasite burden was validated following the procedures outlined by Zuur et al. (2009). Tukey post hoc pairwise comparisons were used to determine significant differences between groups. All analyses were performed in the R statistical environment using the package "nlme" (Pinheiro et al., 2014; R Core Team, 2014). Graphics were produced in R using the package "ggplot2" (Wickham, 2009).

3. Results

Four mortalities were observed during the 14-day post-capture period, and all were captured by fyke net. Two mortalities were found in the PEN treatment, one in the TANK treatment, and one mortality in the CAGE treatment. Model selection indicated that glucose concentration was best explained by holding environment (Table 2). Significant differences were identified between bluegill glucose in the PEN and RCAGE treatments (Tukey: $P = 0.028$, Fig. 2). While blood glucose was the highest in the PEN treatment, overall the levels were low (e.g., mean_{PEN}: 2.65 mmol $L^{-1} \pm 0.12$ SE) and only marginally higher than in other treatments (Fig. 2). Condition factor did not differ among treatments ($F_{3,298} = 1.45$, $P = 0.23$),

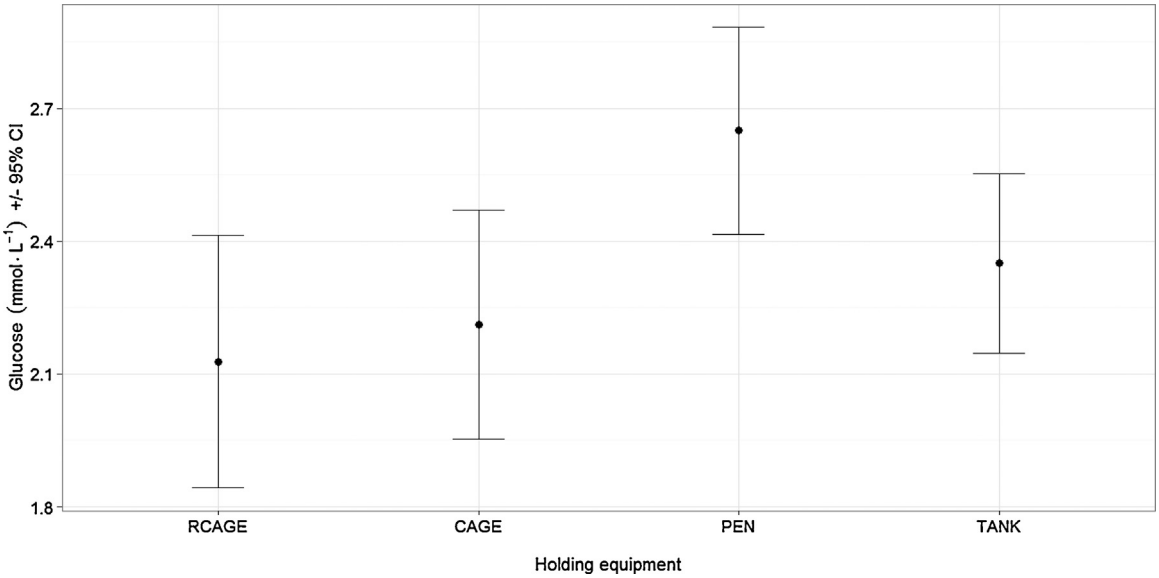

Fig. 2. Predicted glucose concentration (mmol $L^{-1} \pm 95\%$ Confidence limits) in bluegill captured and held in one of four holding gears including a rigid cage (RCAGE, $n = 18$), cage ($n = 18$), pen ($n = 18$), and tank ($n = 18$).

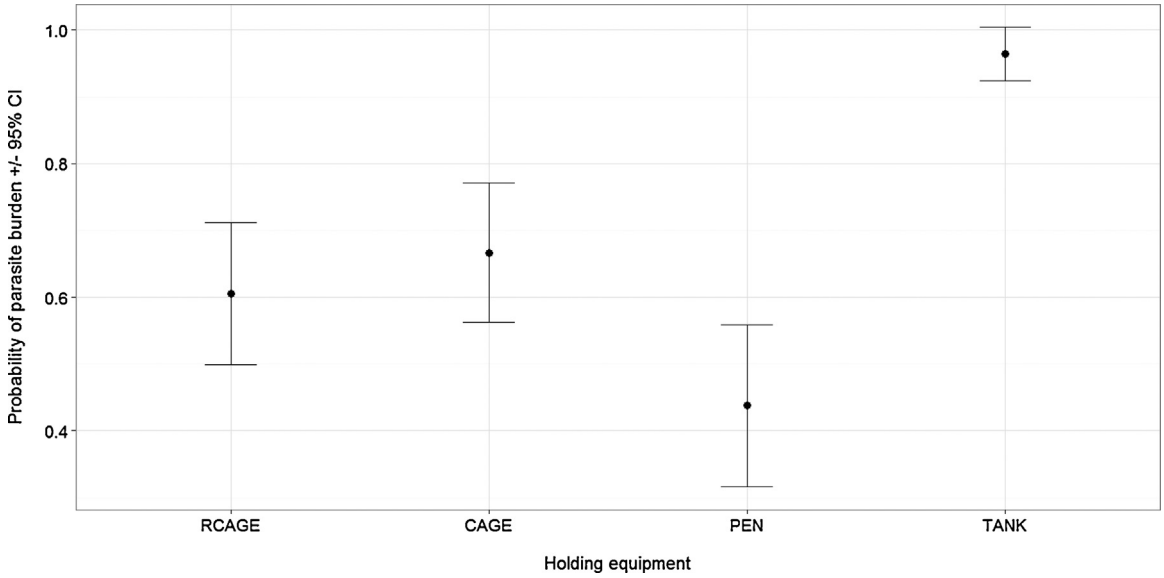

Fig. 3. Predicted probability of parasite burden (±95% Confidence limits) in blue gill held in one of four holding gears including a rigid cage (RCAGE, $n = 81$), cage ($n = 78$), pen ($n = 64$), and tank ($n = 83$).

between capture methods ($F_{1,298} = 1.73$, $P = 0.19$) or by a capture method * holding environment interaction ($F_{3,298} = 1.21$, $P = 0.30$). Model selection indicated that parasite burden was best explained by a model that contained only holding environment (Table 2). Multiple comparisons showed that after being held for 14 days, bluegill in the TANK treatment were significantly more likely to carry parasites than other treatments ($P < 0.001$, all cases). Fish held in the PEN treatment were the least likely to carry parasites and significantly less likely to carry parasites than those in the CAGE treatment (Tukey: $P = 0.031$; Fig. 3).

4. Discussion

We set out to evaluate how capture method and holding environment affected post-release mortality and a variety of sub-lethal endpoints. Statistical analysis of mortality data was not feasible since only four fish died after being held for two weeks. All of the fish that died had been captured by fyke net with the mortality occurring within several days of beginning the experiment and spread across three of the four holding environments. Fish captured by fyke net are confined during capture and can be subject to stress and injury (especially epithelial disturbance; Colotelo et al., 2013a,b) that can promote opportunistic infections by *Saprolegnian* spores (Van West, 2006). Previous research on the capture of bluegill by fyke net showed that immediate mortality was 0.23% for 2-day net sets and 2.71% for 6-day net sets (Larocque et al., 2012). After six days of holding in the fyke net, some of the fish that Larocque et al. (2012) found in the net were deceased and exhibited *Saprolegnian* infections, which is not unlike what we observed. The fish captured by rod and reel would have received comparatively less dermal abrasion and epithelial damage than fish captured by fyke net, as landing nets were not used and anglers had wet hands (Colotelo and Cooke, 2011). Moreover, barbless hooks were used which enabled rapid hook removal with minimal tissue damage and resulted in only short-term air exposure (Cooke et al., 2001).

It is not possible to discuss patterns in mortality among holding environments given that few mortalities were observed. However, some of the sublethal endpoints evaluated were informative. After five days of captivity we compared blood glucose and found a significant effect of holding environment (Fig. 2). Blood glucose is often used as an indicator of chronic stress as it becomes elevated in response to stimulation of the HPI axis (Barton et al., 2002). However, blood glucose can also become elevated in response to food intake (Cousineau et al., 2014). We found the highest glucose levels among fish held in the pen which had access to natural substrate. Although fish were not autopsied to determine gut fullness, it is conceivable that bluegill in this treatment had access to more food resources given that food could drift/swim in (through the sides – similar to the two cage treatments), fall in (on top – similar to all other treatments), and be accessed on the substrate (the only treatment with access to natural substrate). The actual difference in glucose among treatments was quite small (e.g., relative to an acute stressor; see Cousineau et al., 2014) and may not be biologically meaningful with respect to stress. Indeed, condition factor, an indicator of overall fish health and condition (Barton et al., 2002) was similar among treatments.

Perhaps the most biologically meaningful difference observed in this study related to the presence of ectoparasites after holding fish for 14 days. Parasites are often used for environmental monitoring given that they indicate stress at the ecosystem level and at the level of individual fish (Landsberg et al., 1998). We noted a major outbreak of *Ichthyophthirius multifiliis* among fish being held in all three of the tanks. *I. multifiliis* (also known as white spot disease) is a common and persistent freshwater fish protozoan ectoparasite that leads to rapid loss in fish condition and eventually death (Dickerson and Dawe, 1995; Matthews, 2005). The tanks were on flow through and independently plumbed so that the water in each turned over ~4 times per day. The in-lake holding environments were comprised of knotless mesh where water was constantly renewed. While bluegill in the lake and the tank would have all been exposed to water-borne pathogens, those in tanks could have been abraded by the hardened walls of the tank. Previous work in the same tanks at Queen's Biology Station that involved long-term holding of bluegill also noted infection by *I. multifiliis* after several weeks of holding, particularly among fish that were intentionally starved (McConnachie et al., 2012). We cannot identify the specific mechanism responsible for the parasite infection in the tanks but it was unlikely related to food availability given that condition factor was similar across groups; fish had access to natural food as well as supplemental feeding with dew worms. Although handling

stress is known to promote development of *I. multifiliis* (e.g., Davis et al., 2002) the handling in the tanks was no different than that in the other treatments again pointing to an issue with stress from captivity. Had the experiment continued, fish in the tank treatment would have died from *I. multifiliis* given its pathogenicity (Dickerson and Dawe, 1995) or would have been euthanized as a humane endpoint. *I. multifiliis* can be controlled with a variety of treatments (Dickerson and Dawe, 1995; Noga, 2010) but doing so certainly complicates the assessment of fisheries mortality studies.

The findings from this study suggest that pens and cages appear preferable to holding tanks for post-release fishing mortality studies that last several weeks. Stress and epithelial damage that influence fish condition and promote disease appear to be important considerations in selecting a holding environment for post-release fishing mortality studies that last days to weeks. For studies that are of shorter duration (e.g., hours), holding environment may not be as important and disease would unlikely be a factor unless fish entering the experiment are already in a compromised state (Miller et al., 2014). Greater effort should be given to the use of holding environments that expose fish to ambient conditions and do not require supplemental feeding. Although mortality estimates would ideally be generated from free-swimming animals in nature, there are limitations with doing so (Donaldson et al., 2008). As such, we suggest that additional research is needed to compare the effects of holding environments on post-release fishing mortality estimates. In this study there was negligible mortality so our ability to provide specific advice is somewhat restricted. We hope that other researchers will continue with this line of enquiry and incorporate different holding environments into future studies of post-release fishing mortality. Doing so will enable us to identify optimal holding environments that will generate credible and realistic estimates of capture-and-release mortality (ICES, 2014).

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