Effect of pulsed gastric lavage on apparent survival of a juvenile fish in a natural system

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A B S T R A C T

Dietary studies are essential for studying trophic dynamics, and are often based on analysis of stomach contents. A popular method to collect stomach contents is the use of pulsed gastric lavage (PGL), wherein a stream of pressurized water forces an individual to regurgitate food items. Most past experimental studies have shown no effect of PGL on survival, but these studies are limited to laboratory or cage experiments, thereby controlling for natural effects such as predation or emigration. Using a mark–recapture/resighting approach, we determined the effect of PGL on apparent survival (φ = 1 − mortality − emigration) in a natural system. In two study sites, we marked a total of 200 juvenile common snook, Centropomus undecimalis (Bloch 1792) (mean = 251.7 mm standard length, sd = 30.7, range = 202–320 mm) with PIT tags, lavaged 89 of these snook, and resighted 90% of marked fish at least once with a telemetry array. Using the Barker survival model, we determined a significant effect of PGL on apparent survival through QAIC model selection, 95% confidence intervals of parameter estimates, and likelihood ratio testing (P = 0.017). The PGL effect reduced QAIC, model averaged maximum likelihood estimates of apparent survival by 12.0–17.4%. Since we estimated apparent as opposed to true survival, we could not fully partition lethal and sublethal (emigration) effects; however, a lower incidence of emigration in lavaged individuals suggests that emigration did not drive the declines in apparent survival. Regardless of the mechanism, we found that PGL affected individuals, which is contrary to most previous controlled studies. Future researchers using PGL must consider the influence of potential lethal/sublethal effects in natural settings.

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1. Introduction

The study of trophic dynamics is fundamental to ecology. Quantifying trophic interactions has enabled ecologists to understand energetic pathways within natural systems (Match et al., 2011; Richoux et al., 2010; Rosenblatt and Heithaus, 2011), measure effects of fragmentation and anthropogenic degradation (Layman et al., 2007), identify basal evolutionary drivers (Bolnick et al., 2003), and maintain fisheries (Wipfli and Baxter, 2010). Ideally, trophic interactions are quantified by directly observing consumption, but direct observation is often difficult or impossible. Thus, many studies quantify trophic interactions by examining recently consumed prey items in a species’ digestive tract (Dehn et al., 2007; Hyslop, 1980; Olson and Boggs, 1986; Richoux et al., 2010). Frequently, stomach content examination occurs by sacrificing and dissecting individuals to ensure no prey items are missed; however, this is infeasible with endangered species, eliminates the opportunity to resample the individual, can impact ecological studies, and can instigate conflicts with stakeholders. Thus, researchers have developed an array of minimally lethal techniques to study diet (Light et al., 1983; Miranda, 1986; Murphy and Willis, 1996).

One such technique is the use of isotopic chemical tracers, which is a non-lethal method frequently used to identify an individual’s relative trophic position as well as the relative contributions of distinct basal resource pools. However, the use of stable isotopes can be impracticable in some systems and often only provides broad descriptions of diet (Layman et al., in press; Post, 2002). Additionally, the use of stable isotopes often requires an examination of a species’ diet to guide and validate resource-use trends (Layman et al., in press), thus still requiring stomach content analysis.

Another non-lethal technique is the use of emetic chemicals, such as hydrogen peroxide, to induce regurgitation (Miranda, 1986). Emetic chemicals allow direct examination of prey items, but efficiency varies among species and the process is cumbersome (Miranda, 1986). A more convenient method of direct stomach content examination is the use of acrylic tubes to perform pulsed gastric lavage (Murphy and Willis, 1996). Pulsed gastric lavage forces the individual to regurgitate consumed food items by flushing the stomach with...
pressurized water from a tube inserted past the esophagus (Fowler and Morris, 2008; Hartleb and Moring, 1995; Light et al., 1983).

Gastric lavage is a widely used method in fisheries science and ecology, and is used on endangered species (Murphy and Willis, 1996; Shuman and Peters, 2007) due to the belief that gastric lavage is a non-lethal technique. To test the detrimental effects of gastric lavage, several studies have quantified post-lavage survival in a laboratory setting or by caging individuals in the wild (Hafs et al., 2011; Hakala and Johnson, 2004; Light et al., 1983; Shuman and Peters, 2007). However, these controlled experiments eliminate additive effects found in natural systems that may increase mortality rates (e.g. via predation) or change behavior. This is of particular importance when studying rare and endangered species, or in studies that require fish to be recaptured or detected at a later date for estimation of population size, survival, movement, or other characteristics. Also, many lavage-effect studies are completed over time scales too short (days) to observe possible longer-term (months) effects. Therefore, there is an exigent need to understand how gastric lavage affects individuals in an uncontrolled natural setting over a prolonged period.

In this study we determined the effect of gastric lavage on the apparent survival of juvenile common snook, Centropomus undecimalis, in a natural estuarine system. It was previously established that lavage is an effective non-lethal sampling method for snook (Adams and Wolfe, 2006), but the effects were only examined under controlled conditions via caging. Here, we employed a mark–recapture/resighting approach to track lavaged and non-lavaged individuals. We calculated maximum likelihood estimates of apparent survival from the mark–recapture/resighting data for comparison between lavaged and non-lavaged populations. This approach allowed a rare, if not the first, study of post-lavage effects for a fish in a natural system, in which natural effects were not controlled.

![Figure 1](image_url)

**Fig. 1.** Study locations in southwest Florida, USA. Marking occurred in South Silcox and Culvert Creek only, but recapture/resighting effort occurred in four creeks (North Silcox, South Silcox, Yucca Pen, and Culvert Creek) to reduce potential survival bias caused by emigration.
2. Methods

2.1. Capture–recapture and lavage

This experiment was conducted from October 2010 to April 2011 on wild caught juvenile common snook, *Centropomus undecimalis*, from Charlotte Harbor, FL, USA. All capture and recapture/esigning occurred in four tidal-mangrove creeks of approximately equal length (≈1.6 km) on the eastern shoreline of Charlotte Harbor: North Silcox, South Silcox, Yucca Pen, and Culvert Creek (Fig. 1). Marking occurred in South Silcox and Culvert Creek only, but recapture/esigning effort occurred in all creeks to reduce potential survival bias caused by emigration.

For this study, we focused on juvenile snook 201–320 mm standard length (SL), which approximated age-1 individuals (Barbour and Adams, in press; McMicheal et al., 1989; Stevens et al., 2007). This experiment was conducted from October 2010 to April 2011 on wild caught juvenile common snook, *Centropomus undecimalis*, from Charlotte Harbor, FL, USA. All capture and recapture/esigning occurred in four tidal-mangrove creeks of approximately equal length (≈1.6 km) on the eastern shoreline of Charlotte Harbor: North Silcox, South Silcox, Yucca Pen, and Culvert Creek (Fig. 1). Marking occurred in South Silcox and Culvert Creek only, but recapture/esigning effort occurred in all creeks to reduce potential survival bias caused by emigration.

For this study, we focused on juvenile snook 201–320 mm standard length (SL), which approximated age-1 individuals (Barbour and Adams, in press; McMicheal et al., 1989; Stevens et al., 2007; Taylor et al., 2000). Capture–esigning of snook was conducted with a center-bag seine net (30.5 × 1.8 m, 6.3 mm mesh) and hook-and-line (Table 1). After capture, all fish were scanned with a handheld passive integrated transponder (PIT) tag reader (model no. RS601, Allflex®). All individuals were measured for SL to the nearest millimeter, and unmarked individuals were marked with uniquely coded half-duplex (HDX) PIT tags (23 mm length × 3.4 mm diameter, 0.6 g in air; Texas Instruments TIRFID S-2000).

Before surgically implanting the PIT tag, we performed pulsed gas-
tric lavage (PGL) on approximately half (44.5%) of the captured indi-
viduals. Previous and ongoing research indicate that juvenile snook
movements and apparent survival are similar throughout the season
in which the study was conducted (October–April) (Barbour and
Adams, in press; Barbour et al., 2012); therefore, we generally dedi-
cated entire sampling days to lavaging fish, or not (Table 1). On the
few days when we did not lavage all fish, we randomly selected indi-
viduals to lavage. The lavage device consisted of a 12 V battery,
22.7 L per minute bilge pump (Rule®), 2.1 m of 2.54 cm diameter
hose, and a 6.35 mm diameter nozzle (Fig. 2). We performed PGL by
forcing a stream of water past the esophagus into an individual’s
stomach. Once the stomach of the snook was satiated, we externally
massaged the stomach forcing water and recently consumed prey
items onto a mesh screen (Fig. 2). We repeated this process until
three consecutive treatments failed to produce any new items. The
treatment time for each snook ranged from 30 s to 2 min. Post-
lavage, we placed the snook in a 1 × 1 × 1 m holding pen for an ap-
proximate minimum of 30 s before marking with a PIT tag.

We inserted PIT tags into the abdominal cavity of all unmarked
fish through a 3 mm incision posterior and ventral to the pectoral
fin. For this mark, previous studies found 100% tag retention with
no mortality for *C. undecimalis* of >120 mm SL with no need for su-
tures to close the incision (Adams et al., 2006; Boucek and Adams,
2011). All fish were released within 100 m of their capture location
within the creek of capture.

To increase resighting probabilities, we employed an array of elev-
en autonomous PIT tag antennae (Adams et al., 2006; Barbour et al.,
2012). A complete description of antennae units and construction is
available elsewhere (Barbour et al., 2011; 2012). As a marked fish
passed an antenna, thereby entering its magnetic field, the PIT tag’s
unique number was read, and the antenna’s computer recorded
time and date of detection. We placed an antenna in the lower, mid-
dle, and upper strata of each creek with the exception of Yucca Pen
upper (due to financial constraints) (Fig. 2 in Barbour et al., 2012).
Each stratum was approximately 0.5 km in length. Placing antennae
outside of the primary study creeks allowed quantification of emigra-
tion, which we defined as being detected outside of an individual’s
marking creek.

2.2. Model selection and data analysis

We estimated survival with the Barker survival model (Barker,
1997, 1999) within Program MARK (White and Burnham, 1999).
This model allows for incorporation of information from physical cap-
ture–esigning during discrete primary periods, and continuous
resighting from all antennae during secondary periods (the intervals
between primary periods). We defined capture–esigning events as
the primary periods, and coded for uneven time intervals by scaling
31 days to equal an interval of length 1 (Table 1). We added two ‘dummy’ primary periods (Table 1) after the completion of physical
capture–esigning to continue monthly estimation of survival until
the end of the continuous resighting data on April 10, 2011. We
created capture histories for each individual, and assigned each indi-
vidual to one of four groups. Groups were created to classify each
individual as: (1) lavaged, marked in Culvert Creek; (2) lavaged,
marked in South Silcox; (3) not lavaged, marked in Culvert Creek;
or (4) not lavaged, marked in South Silcox.

![Fig. 2. Photograph of lavage procedure with a juvenile common snook (*Centropomus undecimalis*) regurgitating prey items onto a mesh screen. Two regurgitated prey fishes can be seen on the mesh screen.](image-url)
For the Barker model, Program MARK estimates seven parameters (Table 2) (White and Burnham, 1999). Since resightings did not occur over the entire geographic range of our study species, we hereafter refer to survival (s) as apparent survival (ϕ), which incorporates the effects of mortality and emigration (ϕ = 1 – mortality – emigration). For numerical optimization, we transformed real parameter values [0, 1] to β-values [−∞, ∞] with the logit-link function. Additionally, due to concerns over potential convergence issues with the Barker model at certain parameter values (A.B.L. manuscript in preparation), we verified model results with an alternative optimization method (simulated annealing).

The Barker model is heavily parameterized, allowing for well over 1000 base model combinations. Therefore, we made a priori assumptions to fix r, p, F, and P (Table 2) as time and group-independent (.) parameters since we had no dead recoveries and few physical recaptures, and therefore lacked sufficient data to inform time-dependent or group-wise estimates. Next, we created a series of models that allowed ϕ, R, and R’ (Table 2) to vary with time dependence (t) or independence (.), by group (G) or subgroup (L = lavaged vs. not lavaged; C = Culvert Creek vs. South Silcox Creek), and by an additive (+) or multiplicative (×) combination of time/group. For example, ϕ(C×t) estimated a unique ϕ for each of the four groups for each time period, resulting in 32 distinct ϕ estimates; ϕ(C&L) estimated a single, time-independent (.) β-value intercept for ϕ shared by all groups, and estimated a second, additive β-value to represent a difference in ϕ for lavaged individuals; ϕ(C&L) was identical, except a third β-value represented a difference in ϕ lavage effect by creek; and ϕ[C+L1] had a common ϕ β-value intercept for all four groups, a second ϕ β-value to differentiate creeks, and a third ϕ β-value to represent a shared lavage effect for the creeks.

To assess overdispersion in the model, we conducted a median ĉ goodness-of-fit test (White and Burnham, 1999) on the global model: ϕ(C×t)R[C×t]R[G×t]R[G×p]F[G×t]P[G×t]. We then applied the estimated c (variance inflation factor) to the completed model set to adjust subsequent estimates of variance and model selection criteria (Burnham and Anderson, 2002). Values outside of 1 ≤ ĉ ≤ 4 indicate a severe structural lack of fit for the given dataset and overall model (Burnham and Anderson, 2002). We ranked models using Akaike’s Information Criterion (AIC) values (Akaike, 1973). Models with lower AIC values are assumed to better explain the variation in the data with maximum parsimony. In practice, we used the small-sample, ĉ-corrected version of AIC, QAICc. Models with QAICc values differing by less than 2 were considered equivalent (Burnham and Anderson, 2004; Feare and Doherty, 2004).

After model selection, we conducted hypothesis testing by two methods. First, we tested for significance of the lavage effect using the QAICc selected model. We examined 95% CIs of the ϕ β-values representing the lavage effect. If the 95% CIs of these β-values did not include 0.0, a significant lavage effect was determined at the α = 0.05 level. Second, we conducted a likelihood ratio test between the QAICc selected model and a nested model with no lavage effect (α = 0.05). After the hypothesis testing, we used model averaging based on QAICc weighting to derive final values from real parameter estimates (Burnham and Anderson, 2004). These values were used to determine the magnitude of the lavage effect.

3. Results

During primary sampling periods, water temperatures ranged 15.3–27.4 °C (Table 1), well within normal physiological tolerances for juvenile snook (Shafland and Foote, 1983). In total, we marked 200 snook (mean = 251.7 mm SL, sd = 30.7, range = 202–320 mm) and lavaged 89 (Table 1). We marked 81 snook in Culvert Creek (34 lavaged) and 119 in South Silcox (55 lavaged). We recaptured 3 marked snook by seine net and resighted 180 (90%) at least once with the PIT tag antennae array. Resighting percentages by group were as follows: lavaged Culvert Creek (85.3%), not lavaged Culvert Creek (95.7%), lavaged South Silcox (78.2%), and not lavaged South Silcox (95.3%). Antennae detected a lower percentage of lavaged snook as emigrating. Of the resighted snook, emigration percentages by group were as follows: lavaged Culvert Creek (24.1%), not lavaged Culvert Creek (35.6%), lavaged South Silcox (46.5%), and not lavaged South Silcox (65.6%).

Computed model results with an alternative optimization method (simulated annealing).

Table 3

<table>
<thead>
<tr>
<th>Model</th>
<th>QAICc</th>
<th>ΔQAICc</th>
<th>QAICc, weight</th>
<th>k</th>
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<tr>
<td>ϕ[C+L1]R[G×t]R′(t)</td>
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<td>0.57</td>
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<td>0.00</td>
<td>0.06</td>
<td>28</td>
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<tr>
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<tr>
<td>ϕ[G×R[G×t]R′(t)]</td>
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<td>0.00</td>
<td>0.00</td>
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<tr>
<td>ϕ[C×R[G×t]R′(t)]</td>
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<td>26</td>
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<td>697.13</td>
<td>0.00</td>
<td>0.00</td>
<td>18</td>
</tr>
</tbody>
</table>

For model creation, we represented time-dependence (t), group effect (G), creek effect (C), and lavage effect (L) with the associated symbols. The parameter of primary interest was apparent survival (ϕ), and models that differed by ΔQAICc < 2 were considered equivalent. Number of estimated parameters (k) is listed for each model.
the creek effect (95% CI = 0.19 – 1.48). Likelihood ratio testing between the QAICc-selected lavage effect $\phi(C + L_1)R(G^t)R'(t)$ and nested, creek effect model $\phi(C)R(G^t)R'(t)$ indicated a significant improvement in model fit with the addition of the single lavage-effect $\phi$ $\beta$-value (chi-square = 5.75, df = 1, $P = 0.017$). Model averaged apparent survival estimates attributed a 17.4% reduction in the maximum likelihood estimates of $\phi$ in Culvert Creek and a 12.0% reduction of $\phi$ in South Silcox to the lavage effect (Fig. 3).

4. Discussion

Our study of juvenile C. undecimalis revealed a significant effect of pulsed gastric lavage (PGL) on apparent survival. This result conflicts with previously reported findings that PGL does not reduce survival (Hafs et al., 2011; Hakala and Johnson, 2004; Light et al., 1983; Shuman and Peters, 2007), but those studies were in controlled settings and did not allow for natural effects such as predation, competition, or emigration. Our study allowed for these additive natural effects (though this study did not address the effect mechanism) and demonstrated a significant effect of PGL on apparent survival, which suggests that field studies on other species are needed in addition to laboratory and caging studies.

The difference in apparent survival may have resulted from lethal or sublethal effects. In this study, lethal effects are those that caused direct mortality of the individual, whereas sublethal effects caused a change in some aspect of the subject that resulted in non-detection in the system.

4.1. Lethal effects

Lethal effects may have been caused by an increase in predation susceptibility following lavage or by damage from the lavage procedure itself. Increased mortality from predation could have occurred due to risky foraging behavior following loss of the lavaged meal, or through a loss of equilibrium, which would alter the body systems that normally function to avoid life-threatening situations (Beitinger et al., 2000; Danylchuk et al., 2007). In this study, a loss of equilibrium was unlikely, as we held fish in a recovery pen pre-release for several minutes – a reasonable time for recovery (Munday and Wilson, 1997) – and then released fish by hand without noticing any apparent effects. Additionally, we rarely encountered predators, such as larger fish and piscivorous wading birds (Miranda and Collazo, 1997), capable of preying upon the size-class of the snook we studied. Moreover, previous research on cannibalism by adult snook, the most common large predatory fish in these systems, showed that although small juvenile snook were cannibalized, juveniles in the 201–320 mm SL were not (Adams and Wolfe, 2006). Thus, it is unlikely that predation was a major driver of mortality.

Fig. 3. Barker QAICc model averaged apparent survival estimates for juvenile common snook (Centropomus undecimalis) marked and lavaged vs. marked and not lavaged in two coastal mangrove creeks plotted with 95% confidence intervals. In Culvert Creek, 81 fish were marked and 34 lavaged. In South Silcox, 119 fish were marked and 55 lavaged. Model results were adjusted with a variance inflation factor ($\hat{c}$) of 1.74. Substantial overlap of 95% CIs exists due to model averaging, the $\hat{c}$ adjustment, the use of a single $\beta$-value to represent the lavage effect, and transformation of optimized $\beta$-values from the logit-link function.
Alternatively, damage to internal organs during lavage may have caused death or inability to feed effectively. Internal organ damage could be caused by: (1) the use of highly pressurized water inducing hemorrhaging of the stomach lining, (2) the tail-first removal of prey fishes allowing dorsal or anal spines to puncture the stomach or esophagus of the study subject, or (3) stomach distention due to a rapid increase of stomach volume during high-pressure flushing. Such internal injuries are rarely reported in other species following lavage (Brosse et al., 2002; Hakala and Johnson, 2004; Wanner, 2006), but may have occurred in this study due to a combination of PIT tagging, the lavage procedure, and small body size. Since a previous caging study (Adams et al., 2006) demonstrated 100% survival for the study species after PIT tagging of juvenile snook of >120 mm SL, it is unlikely that this procedure has caused mortality or other negative effects associated with intracoelomic tagging (Cooke et al., 2011). Although it is possible that the combined effect of tagging and lavage caused internal damage, based on extensive tagging and lavage experience with juvenile and adult snook (Adams et al., 2006, 2012; Barbour and Adams, in press; Boucek and Adams, 2011), we feel that this is unlikely, and that a lavage-specific effect caused the decline in apparent survival.

Although internal injuries are rarely documented after lavage, internal hemorrhaging of the stomach lining was reported in large-mouth bass (Micropterus salmoides) of similar body size to the juvenile fish in our study (Hakala and Johnson, 2004). This suggests an increased susceptibility to internal injury from lavage at low body size, which was supported by increased susceptibility to internal injury from lavage at low body pressure flushing. Such injuries are rarely reported in other species following lavage (Brosse et al., 2002; Hakala and Johnson, 2004; Wanner, 2006), but may have occurred in this study due to a combination of PIT tagging and lavage procedures. However, the dataset would likely be overdispersed if lavaged and non-lavaged individuals were treated as a single group, as the two groups would have heterogeneous probabilities of movement (Schmidt et al., 2002). Furthermore, migration is an inherently high-risk behavior (Adams et al., 1994), meaning an increase in emigration would increase predation risk and potentially increase mortality. However, a lower incidence of emigration in lavaged individuals in this study suggests emigration did not drive the declines in apparent survival.

5. Conclusions

Although an assumption exists that PGL is an effective means of non-destructive sampling to study diet, this may not always be the case. Most studies on lavage effects have been conducted in controlled situations, which do not expose the organisms to conditions they face when lavaged and released in the field. Thus, these studies may underestimate the effects of lavage. Our field study demonstrated that lavage had a negative effect on juvenile snook, and provided direction to further examine the implications of PGL. For example, does lavage impact all life stages of snook, or just juveniles? More work is needed to determine to what extent our findings were due to lethal or sublethal effects since each has different implications for the population and for parameter estimates resulting from mark–recapture/resighting data. Finally, our findings should be a caution to others to use lavage as a non-destructive sampling tool only after fully testing for effects in both controlled and field experiments, since lavage effects may be species or life-stage specific. Clearly, lavage is preferred compared to destructive sampling for diet, but effects do need to be recognized and accounted for.

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