Detection of Larval Remains after Consumption by Fishes

JASON D. SCHOOLEY,* ABRAHAM P. KARAM, BRIAN R. KEESNER, PAUL C. MARSH, CAROL A. PACEY, AND DARREN J. THORNBRUGH

School of Life Sciences, Arizona State University, Post Office Box 874601, Tempe, Arizona 85287-4601, USA

Abstract.—In southwestern North America, consumption of native fish larvae by nonnative predators has imperiled native populations. Field-acquired dietary analyses have provided little evidence of this cause-effect relationship. In this study, small, nonnative green sunfish Lepomis cyanellus, bluegills L. macrochirus, red shiners Cyprinella lutrensis, fathead minnow Pimephales promelas, and yellow bullheads Amieturus natalis were each fed a single larva of the native razorback sucker Xyrauchen texanus. Gut content analysis revealed that prey detection generally became increasingly difficult over a short postconsumption time period under laboratory conditions. For green sunfish, bluegills, and yellow bullheads, significant relationships between prey detection and time were revealed; the probability of prey identification was initially 50% or greater for about 30 min postconsumption, whereas few prey (3%) were identifiable at 60 min postconsumption. For red shiners and fathead minnow (poole/c for analysis), no relationship was evident; these two species completely masticated their prey, thus hindering identification. Green sunfish and bluegills swallowed prey whole, and yellow bullheads damaged larvae during consumption. Many larvae were discovered in the foregut, and 25% were regurgitated during predator fixation. Use of gut content analysis as evidence of predation on native fish larvae by small, nonnative fish is problematic and unreliable due to rapid mechanical and chemical digestion of fragile larval tissues.

Declines of native fish populations have been exacerbated in part by nonnative fish predation on early life stages (Courtenay and Stauffer 1984; Lever 1996; Fuller et al. 1999). In southwestern North America, there is increasing evidence that native fishes can be quickly extirpated when their larvae occupy the same habitat as nonnative fishes (Johnson and Hines 1999; Dudley and Matter 2000; and similarly, Lenley [1985]); there is also direct evidence of native young being consumed en masse by nonnative fishes (Marsh and Langhorst 1988). Still, few direct observations support the broad-scale recruitment losses that can occur when native fish larvae encounter nonnative fishes. Most studies have cited difficulties with identification of small larvae or eggs in predator gut contents due to rapid digestion (Hunter 1981; Folkvord 1993; Kim and DeVries 2001). Explanations for this paucity of evidence may include specimen collecting and preservation techniques (Crowder 1980), timing of collections and gut analysis (Ruppert et al. 1993), predator digestion and evacuation rates (Lohr and Fausch 1996; Brandenburg and Gido 1999), and fragility of larval tissues (Brandt et al. 1987). The intent of this study was to explore these issues with a laboratory experiment on gut analysis of nonnative predators performed within short time intervals after consumption of a native fish larva.

The razorback sucker Xyrauchen texanus is an example of a native fish with successful annual reproduction but near-total recruitment failure (Marsh et al. 2003; Minckley et al. 2003). Most field-collected evidence points to consumption of all larvae by nonnative fishes, particularly green sunfish Lepomis cyanellus, as a likely cause for population collapse (Marsh and Langhorst 1988; Johnson and Hines 1999).

Results under controlled laboratory conditions demonstrated the likely difficulties in field detection and assessment of the effect of predation by small-bodied nonnative predators.

Methods

During 23–24 February 2006, individuals of five regionally common nonnative species (Table 1)—red shiner Cyprinella lutrensis, fathead minnow Pimephales promelas, green sunfish, bluegill L. macrochirus, and yellow bullhead Amieturus natalis—were collected from the Salt River and Sycamore Creek, Maricopa County, Arizona, by use of a backpack electrofischer (Smith-Root, Inc.; Model 12-A POW [programmable output waveform]). Red shiners, green sunfish, and yellow bullheads were targeted as representative common nonnative predators of native larvae, but sampling provided less-than-adequate abundances of these species. Bluegills and fathead minnow were readily available and were therefore collected as a contingency. Fish were transported under oxygenated conditions to a laboratory at Arizona State University (ASU), where each species was assigned to one of five 37.9-L aquaria. Feed was withheld from nonnative predators for 72 h before the experiment began.

* Corresponding author: jschooley@gmail.com
† Present address: Division of Biology, Kansas State University, 232 Ackert Hall, Manhattan, Kansas 66506, USA.

Received August 3, 2007; accepted December 22, 2007 Published online June 19, 2008
Three-hundred larval razorback suckers (subsample \( n = 26 \); mean total length [TL] = 11.4 mm; range = 10.3–12.8 mm) were collected from Lake Mohave, Arizona–Nevada, after dark on 25 February 2006 with hand-held aquarium dip nets and submersible 12-V lights (after Mueller 1995), transported under oxygenation to the ASU laboratory, and placed in two 37.9-L aquaria. To avoid starvation mortality in razorback sucker larvae, the fish were fed on 27 February 2006 with high-boiled chicken-egg yolk suspended in water. All aquariums were maintained at ambient room temperature (20°C) in aerated, dechlorinated water treated with Stress Coat (Aquarium Pharmaceuticals, Inc., Chalfont, Pennsylvania) and buffered formalin (25 mg/L of water) to prevent infection caused by *Ichthyophthirius multifiliis* (Hoffman 1999). The experimental temperature of 20°C was within the range documented during razorback sucker spawning in the lower Colorado River (10.5–21°C; hatch occurs in 7–21 d; Minclelcy and Deacon 1991).

On 1–2 March 2006, randomly assigned individual predators were placed in 1-L jars containing 500 mL of fresh, oxygenated, 20°C water. Larger specimens were placed in 1.5-L jars (0.75 L of water) or 2.0-L jars (1.0 L of water). Jars were housed in individual cardboard isolation chambers to reduce visual stimulation and stress. Predators were acclimated in jars for 30 min before introduction of a single razorback sucker larva per predator. Jars were visually monitored and the exact time of prey consumption was noted. In the event of unsuccessful feeding attempts (including premature regurgitation) or prey injury or mortality, larvae were replaced with fresh specimens. Each predator (within species) was randomly assigned a digestion period of 15, 30, 45, or 60 min, after which the predator was manually extracted from the jar, euthanized and fixed in 10% buffered formalin, and transferred to 70% ethanol. In some cases, prey was regurgitated during euthanasia and this material was retained for examination. Predators were measured for TL (mm), digestive tracts were excised, and gut contents were examined under a dissecting microscope (7–42× magnification), categorized, and photographed.

A categorical grading system was employed to rank the state of digestion of the larvae retrieved from individual digestive tracts: (1) fully intact (easily recognized as a fish larva); (2) partially digested or damaged (mechanically or chemically) but with identifying characters intact (e.g., lenses still attached to head, melanophores visible, tissues with some identifiable shape or consistency but possibly separated into multiple pieces); (3) extensively digested and exhibiting no intact identifying characters (e.g., lenses detached from head, some sort of matter available but without larval form); and (4) no prey material present (fully digested or not visible).

For two-dimensional visual and analytical interpretation of data, the digestive state was secondarily categorized as a binary index of larval identification: a value of 1 indicated identifiable remains (pooling of digestive states 1 and 2), and a value of 0 indicated an absence of identifiable remains (pooling of digestive states 3 and 4). Regurgitated larvae were subject to the same categorization systems; the only difference was that the predator was not dissected.

Logistic regression was used to determine the relation between digestive state and elapsed time after consumption (Menard 1995). The regression modeled the probability that larval tissues were identifiable (i.e., probability that the binary index was equal to 1; \( P(Y = 1) \)) at a given time postconsumption through maximum likelihood estimation using the LOGISTIC procedure in the Statistical Analysis System (SAS) version 9.1 for UNIX. The logistic probability curves used the following formula:

\[
P(Y = 1) = \frac{e^{(\alpha + \beta X)}}{1 + e^{(\alpha + \beta X)}},
\]

where \( \alpha \) (intercept) and \( \beta \) (slope) are parameters from logistic regression, \( X \) is time (min) since consumption, and \( e \) is the base of natural logarithms. Here, \( P(Y = 1) \) is a decreasing function of time. Model significance was demonstrated by the statistic \( G_M \) (or model chi-square \( \chi^2 \)).

Due to an apparent similarity in results between green sunfish and bluegills and between fathead minnow and red shiners, a stepwise contingency table analysis was conducted to determine whether pooling the two sunfishes or the two minnow species would significantly affect results (CATMOD procedure in SAS; SAS Institute 2004). Species, time (early: 15 and 30 min pooled; late: 45 and 60 min pooled), and prey

<table>
<thead>
<tr>
<th>Predator species</th>
<th>Number collected</th>
<th>Mean TL (mm)</th>
<th>TL range (mm)</th>
<th>Number of larvae consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluegill</td>
<td>21</td>
<td>67</td>
<td>55–88</td>
<td>21</td>
</tr>
<tr>
<td>Fathead minnow</td>
<td>27</td>
<td>59</td>
<td>52–66</td>
<td>8</td>
</tr>
<tr>
<td>Green sunfish</td>
<td>24</td>
<td>92</td>
<td>58–116</td>
<td>16</td>
</tr>
<tr>
<td>Red shiner</td>
<td>5</td>
<td>56</td>
<td>50–64</td>
<td>3</td>
</tr>
<tr>
<td>Yellow bullhead</td>
<td>33</td>
<td>76</td>
<td>58–113</td>
<td>24</td>
</tr>
<tr>
<td>Totals</td>
<td>110</td>
<td></td>
<td></td>
<td>72</td>
</tr>
</tbody>
</table>

TABLE 1.—Number of collected predators of each species (obtained from Salt River and Sycamore Creek, Arizona), mean and range of predator total lengths (TLs), and number that successfully consumed the single razorback sucker larva that was presented to each predator under experimental conditions in 2006.
Table 2.—Parameters (mean ± SE; α = intercept, β = slope) of a logistic regression model used to determine the relationship between the probability that consumed razorback sucker larvae (1 larva/predator) were identifiable and the elapsed time postconsumption during a laboratory experiment in 2006. Due to similarity in results, sunfish predator species (green sunfish and bluegill) were pooled for analysis.

<table>
<thead>
<tr>
<th>Group or species</th>
<th>α</th>
<th>β</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunfishes</td>
<td>4.7528 ± 1.5495</td>
<td>-0.1364 ± 0.0422</td>
<td>1</td>
</tr>
<tr>
<td>Yellow bullhead</td>
<td>1.8288 ± 1.0673</td>
<td>-0.0480 ± 0.0281</td>
<td>1</td>
</tr>
</tbody>
</table>

Detection (yes or no) were used in a three-dimensional analysis of count data.

Results

Sixty-five percent of experimental predators consumed a razorback sucker larva (Table 1). Of the larvae consumed, 54 of 72 (75%) were retrieved upon examination of the predator or preservation jar. Of the fish sacrificed, 18 of 72 (25%) were observed regurgitating the larva into the preservation jar during the brief period before death. In the majority of cases where the larva was not regurgitated, the larva or larval remains were found in the predator’s gill basket or foragit. Differential feeding mechanisms across taxa (families) probably had an effect on results.

Log-linear results supported the pooling of sunfishes and the pooling of minnow species. Species had a nonsignificant effect in each model (sunfishes: $\chi^2 = 4.22, P = 0.3764$; minnows: $\chi^2 = 1.25, P = 0.5358$). Fortunately, pooling increased the sample sizes for logistic regression.

Pooled sunfishes had a significant relationship between identification index and time (logistic regression: $G_M = 20.96, P < 0.001$; Table 2). All eight larvae were identifiable at 15 min postconsumption, whereas none of the eight larvae was identifiable at 60 min (Figure 1). The sunfish logistic model predicts a 50% or better probability of prey identification within 35 min postconsumption. Sunfishes predominantly swallowed prey whole, and mastication was not observed. Similar results were obtained for green sunfish that were allowed to feed freely for 24 h on larval plains killifish Fundulus zebrenus and other food items (Lohr and Fausch 1996).

A nonsignificant relationship between identification index and time was observed for pooled minnows ($G_M = 0.2105, P = 0.646$). Only 1 of 11 larvae examined was recognizable regardless of the time interval, and none of the larvae examined was readily identifiable at 15 min postconsumption (Figure 1). Mastication of larvae in the pharyngeal mill of red shiners and fathead minnow rendered most larval remains unrecognizable (Figure 2), and subsequent digestion of prey was rapid (<30 min). Ruppert et al. (1993) found that fish larvae, including native bluehead suckers Catostomus discobolus, were only identifiable in the foragets of red shiners. Identification of larval remains from these and other minnow species may depend on the remains being located in the foraget or gill basket, where digestion and mixing with other food items are minimal.

Yellow bullhead stomach contents indicated an inverse relationship between time postconsumption and larval prey identification (logistic regression: $G_M = 3.31, P = 0.069$; Table 2). Attainment of a 50% or greater probability of larval identification required yellow bullhead gut contents to be analyzed within approximately 38 min of prey consumption. Larval prey condition was variable, as some fish were observed to consume, regurgitate, and reconsume their prey, resulting in damaged remains. Mastication of larvae was variable for yellow bullhead, but digestion occurred rapidly (<1 h postconsumption) and prey quickly became unrecognizable (Figure 2).
Discussion

The probability of detecting larval remains in the guts of predators from field collections appears remote. In a laboratory setting, where the gut contents were not complicated by variable quantities and types of prey items and where the exact feeding time and prey species were known, it was difficult to identify razorback sucker larvae after 30 min postconsumption. These intentionally favorable conditions are unlikely for field-acquired material, and the results outlined here may represent a best-case scenario for identifying larval remains.

Other studies have shown that digestion hastens with increased temperature (Peters et al. 1973; Eccles 1986). In this study, the ambient laboratory temperature was within the observed occurrence range for razorback suckers in the lower Colorado River basin, although it was near the warmer extreme. Digestion probably would occur more slowly at lower temperatures.

The fixation-preservation process can also affect results. In this study, direct transfer to formalin resulted in regurgitation by a number of fish. Gastric regurgitation by fish simply held in a collection bucket also is common. Ruppert et al. (1993) carefully euthanized field-collected red shiners in tricaine methanesulfonate (MS-222) to reduce the potential of regurgitation and found that larvae were present in the for guts of 15% of the red shiners. Other larval predation studies that directly preserved fish in formalin found few to no larvae in fish guts (Jennings and Saiki 1990; Brandenburg and Gido 1999). When multiple specimens are preserved in lots, these fragile stomach contents can be regurgitated, lost among the additional materials at the bottom of a collecting jar, and discarded. If the goal is to quantify or verify larval predation, it is critical to plan accordingly, because casual collection and gut analysis is not likely to yield adequate information (e.g., Jennings and Saiki 1990; Muth and Snyder 1995).

In cases with strong indirect evidence for predation
on larvae such as those of razorback suckers, the inability of routine gut content analysis to detect larval remains should not lead investigators to dismiss larval predation as a major factor in population decline. As this and other studies show, identification of larval prey requires (1) consumption to occur almost immediately before the predator is captured, (2) use of an active capture method (e.g., electrofishing, seining, or angling) rather than a passive method (i.e., with a time lag between capture and retrieval; gill nets, trammel nets, or traps), and (3) selection of preservation techniques that limit regurgitation. Even under these circumstances, gut content analyses are probably biased. Incorporating an alternative approach, such as larval tagging (Mohler 2003), bioenergetics modeling (Cartwright et al. 1998), immunoassay (Theilacker et al. 1986), or other modern options, is recommended for verification of results.

Acknowledgments

James C. Lee assisted with field collections, Michael R. Schwenm assisted with experimental procedures and manuscript review, and anonymous reviewers provided valuable comments and suggestions. Work was performed under U.S. Fish and Wildlife permit number TE039716-0 and Arizona State University Institutional Animal Care and Use Committee protocol number 05-767R.

References


