Accounting for Incomplete Detection when Estimating Site Occupancy of Bluenose Shiner (*Pteronotropis welaka*) in Southwest Georgia

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Abstract - *Pteronotropis welaka* (Bluenose Shiner) has a fragmented range throughout the Southeast, but its apparent rarity may reflect a low probability of detection during surveys. Our objectives were to obtain up-to-date status information for populations in southwest Georgia and to account for incomplete detection in our estimate of the proportion of sites occupied. We detected Bluenose Shiner at 5 of 39 sites (13%) sampled during 2004 and 2005 and estimated detection probability (*p*) and the proportion of sites occupied (*psi*) from seine-haul data. Models containing habitat covariates as predictors of *p* and *psi* provided a better description of the data than models without covariates for Bluenose Shiner and three other minnow species. Regardless of the model structure, the probability of detecting Bluenose Shiner during a single seine haul was substantially lower than for the other minnow species (3–8% vs. 13–33%). However, estimates of the proportion of sites occupied (corrected for incomplete detection) were similar to observed occupancy rates for all four species because of the large number of seine hauls we made at each site. The modeling approach we followed increased our confidence in survey results and provided information on where and how much to sample in future surveys. It has broad application to future surveys and monitoring programs for rare aquatic species in the southeastern United States.

Introduction

Pteronotropis welaka Evermann and Kendall (Bluenose Shiner) is distributed in Coastal Plain streams from Louisiana to Florida, but is highly fragmented throughout its range (Boschung and Mayden 2004, Gilbert 1992, Ross 2001). Warren et al. (2000) assigned this species to the vulnerable status category, indicating that it is at risk of becoming threatened or endangered. Bluenose Shiner is officially protected as a threatened species in Georgia and has special-concern status in Florida, Alabama, and Mississippi (Boschung and Mayden 2004, Gilbert 1992, Freeman 1999, Ross 2001). This species is strongly associated with deep water and aquatic vegetation, which may explain its extirpation from Mississippi headwater streams where these habitats have been altered (Ross 2001). In addition, the isolated nature of populations makes Bluenose Shiner particularly vulnerable to local extinction. Fagan et al. (2002) found that fishes comprised of geographically widespread but

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fragmented populations were much more likely to suffer global extinction than fishes with small, spatially continuous distributions. Their results have important implications for conservation because species in the former category, including Bluenose Shiner, are rarely afforded the protections (e.g., federal listing) given to species with small geographic ranges.

Bluenose Shiner also has a fragmented distribution in Georgia, where it is only known from the lower Flint River system (Apalachicola drainage; Freeman 1999). When we reviewed its status in 2003, only five sites were collectively represented in databases maintained by the Georgia Natural Heritage Program and the Georgia Museum of Natural History. These sites were spread across four different USGS 10-digit Hydrologic Unit Code (HUC) watersheds. Opportunities for dispersal between all but the two Spring Creek sites are very limited because of distance and impoundments on the mainstem Flint River (Fig. 1). Furthermore, all of the sites were known from samples that predated 1976, and the region has experienced extreme drought and intensive agricultural use since that time (Golladay et al. 2004).

Given this species' specialized habitat requirements, isolation between known populations, age-of-occurrence records, and the environmental change that has occurred in our study area, we expected that some or all of Georgia's populations of Bluenose Shiner might be extirpated. However, the apparent isolation and rarity of the species in the state could also reflect limited sampling effort or sampling methods with a low probability of detecting the target species (MacKenzie et al. 2002, O'Connell et al. 2005). We were particularly concerned about these problems because of the species association with habitats that are difficult to sample and because of the limited amount of comprehensive fish sampling that has occurred in southwest Georgia.

The objectives of our study were 1) to determine if Bluenose Shiner still persisted at historically occupied sites, 2) to identify new populations in historically occupied watersheds, and 3) to estimate detection probability for our sampling methods and account for incomplete detection when estimating the proportion of sites currently occupied.

Methods

Study area

Our study area includes several lower Flint River sub-basins within the Southeastern Plains ecoregion of southwest Georgia (Griffith et al. 2001). Most of our sample sites fell within the Dougherty Plain, a limestone karst region where groundwater discharge and agricultural water withdrawals have a large influence on stream flow patterns. Landcover in the region is dominated by irrigated row-crop agriculture (ca. 50%) and forestry lands (ca. 30%; Golladay et al. 2004.)

We sampled 39 sites between July 2004 and September 2005 (Fig. 1). We sampled all five historical sites and three randomly selected sites within historically occupied USGS 12-digit HUC watersheds (hereafter, small watersheds). We randomly selected two additional small watersheds within each

historically occupied 10-digit watershed (hereafter, large watershed) and sampled up to three randomly selected sites within each. A recent occurrence of Bluenose Shiner in a new large watershed was reported to us during 2004, and we treated this site as if it were a historical site in terms of sample-site selection. Thus, our original design was to sample nine randomly selected sites and one historical site within each of the historically occupied large watersheds. However, access problems prevented us from sampling all 10 sites in each watershed. Compared to a completely randomized design, our design ensured

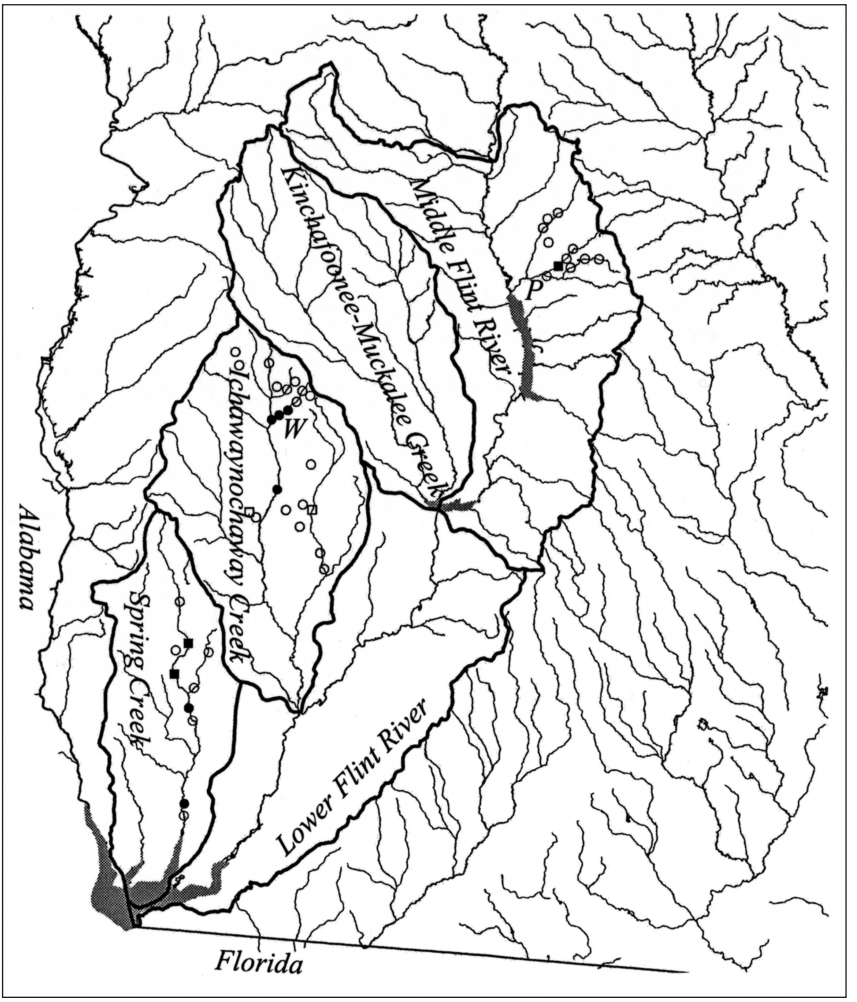

Figure 1. Location of survey sites in the Flint River system of southwest Georgia. Filled squares and filled circles indicate historical and new sites, respectively, where *Pteronotropis welaka* (Bluenose Shiner) was detected during this survey. Empty squares and empty circles indicate historical and new sites, respectively, where Bluenose Shiner was not detected during this survey. P and W mark the locations of Pennahatchee and Wolf creeks, respectively.

that we would sample a broad array of habitats and stream sizes while minimizing travel between sampling sites. Compared to a design that emphasized historical sites and sites near historical sites, our emphasis on random sample sites allowed us to obtain a relatively unbiased estimate of the proportion of sites occupied in historical watersheds.

Data collection and analyses

Sites were generally sampled with 0.48-cm mesh seines, but we also carried out supplemental dipnet sampling in areas that were too difficult to seine because of depth or dense aquatic vegetation. A 2.4-m x 1.8-m seine was used at most sites, but a 1.8-m x 1.8-m seine was used when obstructions prevented the efficient use of the larger seine. We made up to 30 seine hauls at each site and attempted to standardize the area sampled during each seine haul. To minimize disturbance to target species, we sampled different areas as we moved through the site in an upstream direction. Furthermore, hauls were typically separated by an obstruction (e.g., a log), a change in habitat, or at least 1 linear meter of stream channel. Data on the occurrence of Bluenose Shiner and other focal species (see below) were recorded separately for each seine haul (hereafter quadrat), which resulted in a vector of ones (present) and zeros (absent) for each site. When necessary, quadrat-specific voucher specimens were retained for laboratory identification. We also measured maximum depth to the nearest cm and visually assessed current velocity (0 = sluggish or no perceivable current, 1 = moderate to swift current) and aquatic vegetation coverage (0 = 0–25% coverage, 1 = greater than 25% coverage) within each quadrat. Conductivity and turbidity were measured at one location within each site using an YSI Model 85 and a LaMottee 2020 Turbidimeter, respectively. Site location within the watershed was measured as the distance of the site from the largest stream in the large watershed (hereafter distance to mainstem); distances were measured in ArcView 3.3 (Environmental Systems Research Institute, Inc.) by tracing over a 1:24,000 digital stream layer using the measure tool.

Surveys that do not account for incomplete detection of the target species may underestimate the true proportion of sites occupied, which can lead to biased assessments and monitoring programs for rare species (MacKenzie et al. 2004). Detection probability and site occupancy were estimated from the quadrat data using the approach described by MacKenzie et al. (2002). For our application, detection probability (p) is the probability of detecting a focal species within a single quadrat when the species is present within the site. Site occupancy (*psi*) is the proportion of sites occupied within the overall study area. The approach is a modification of closed-population mark-recapture models and assumes that sites are closed to changes in occupancy for the duration of the survey period; the short duration (i.e., within a single day) of sampling at each site ensured that this assumption was met for our study. The method also assumes that target species are not falsely detected, which emphasizes the importance of confirming identifications in the laboratory. Finally, the model assumes that detecting a species at one

site is independent of detecting it at other sites. This assumption would be violated if greater effort were allocated to sites near historically or currently occupied sites or if sampling sites were purposefully selected upstream and downstream of occupied sites. Neither of these conditions characterizes our sampling design.

One of the key strengths of the MacKenzie et al. (2002) approach is that it allows estimates of *psi* and *p* to be conditioned on both quadrat- and site-specific covariates. Including covariates may allow for more accurate estimates of *psi* and *p* and may also help identify habitats to sample in future surveys. Based on the known microhabitat affinities of Bluenose Shiner, we predicted that *p* would be positively associated with maximum depth and the presence of aquatic vegetation and negatively associated with current velocity within quadrats. Conductivity—an index of Floridian aquifer input—and distance to mainstem varied considerably across our study sites and were modeled as covariates of *psi*. We chose the distance to mainstem variable after inspecting the spatial distribution of occupied sites in the watershed. This was appropriate because our emphasis was on getting the best estimate of occupancy rather than evaluating specific hypotheses about factors influencing occupancy.

Models were built using the occupancy-estimation procedure in Program MARK (White and Burnham 1999). First, we built a simple model with no covariates. Next, we built a global model for *psi*, which included both predictor variables, and then identified the best fitting detection model by adding detection covariates to the global model one variable at a time. Small sample size prevented us from building larger models or models with interactions. Relative model fit was assessed using Akaike's Information Criterion (AIC) as corrected for small sample size (AIC_c; Burnham and Anderson 1998). Because covariate data can be expensive to collect, we compared model fit and parameter estimates between models with and without covariates. We also compared model-estimated occupancy rates to our naïve estimate of occupancy from the survey data (i.e., percent of sites occupied, uncorrected for detection). One detection of Bluenose Shiner occurred during dipnetting, but not seining. Because our dipnetting protocol did not permit the estimation of detection probability, this site was included in the overall analysis, but was not used to estimate detection probability.

To provide a basis for comparison to models generated for Bluenose Shiner, we also built models for three additional cyprinid species that were collected in the study: *Notemigonus crysoleucas* (Mitchell) (Golden Shiner), *Notropis harperi* Fowler (Redeye Chub), and *Pteronotropis grandipinnis* (Jordan) (Apalachee Shiner). To evaluate the effectiveness of our survey methods for all focal species, we calculated cumulative detection probabilities for the average number of seine hauls (N) made at survey sites using the following equation: $(1 - p)^N$. We used estimates of *p* from models without covariates to determine cumulative detection because these estimates reflect average detection over the wide range of habitats we encountered.

Results

Bluenose Shiner was detected at five of our 39 sites (13%): one historical site in the Pennahatchee Creek system, two historical sites in the Spring Creek system, and one new site each in the Wolf Creek and Spring Creek systems (Fig. 1). Because of the clustering of sites along the lower reaches of tributaries and in larger mainstem creeks, we conducted additional non-random sampling along Spring Creek, Wolf Creek, and Pennahatchee Creek during 2005. This sampling resulted in the detection of one new occurrence in both Wolf and Spring Creeks. Finally, after examining a specimen provided to us by the Georgia Department of Natural Resources Stream Survey Team, we confirmed an additional new occurrence on Ichawaynochaway Creek. All totaled, Bluenose Shiner is currently known from nine sites in Georgia. While the among-site connectivity of the populations represented at these sites is unknown, the spatial clustering of sites suggests that fewer than nine populations exist. Habitat characteristics of occupied and unoccupied sites are given in Table 1.

We sampled a total of 864 quadrats within our 39 initial sites (mean = 22/site). Bluenose Shiner was only detected within 11 of these quadrats. Capture histories at occupied sites were characterized by a single or small number of detections (max = 4 quadrats) and many non-detections. The small number of occupied quadrats and sites makes it difficult to make definitive statements about habitat use. However, Bluenose Shiner was detected in a higher proportion of quadrats with >25% vegetative coverage (3.2%) compared to quadrats with <25% vegetation coverage (0.74%) and was never collected within a quadrat with moderate to swift current velocity (Table 2).

Based on AIC_c values, the model containing distance to mainstem and conductivity as predictors of *psi* and current velocity as a predictor of *p* provided the best description of the data (Table 3). Estimates (standard errors) from this model for *psi* and *p* were 0.11 (0.07) and 0.03 (0.04), respectively. The best-fitting model was 14.8 times (0.872/0.059) more likely than the model containing only covariates for *psi*, suggesting a strong effect of current velocity on model fit (Table 3). Coefficients for covariates suggest that occupancy is negatively associated with distance to mainstem and positively associated with conductivity and that detection is negatively associated with current velocity. In contrast to our expectations, models containing vegetation and depth as covariates of *p* did not fit the data better than the global model or the model without covariates.

As in those for the Bluenose Shiner, models for the other focal species containing covariates had lower AIC_c values than models without covari-

Table 1. Mean (SD) conductivity, turbidity, and distance to the largest stream in the watershed (i.e., mainstem; DM) for sites where *Pteronotropis welaka* (Bluenose Shiner) was and was not detected. Data for the additional non-random sites sampled in 2005 are not included.

Detected	Conductivity (mhmos)	Turbidity (NTU)	DM (km)
Yes	178.8 (86.0)	9.9 (7.8)	5.3 (5.9)
No	114.6 (63.0)	16.5 (13.0)	16.1 (8.3)

ates. Model weights for the best-fitting covariate models ranged from 0.73 to 0.99, but were never larger than 0.001 for models without covariates. Coefficients for covariates were in agreement with the known habitat-use patterns of each focal species. For example, the best-fitting models indicated that detection was negatively associated with current velocity for Golden Shiner, positively associated with vegetation for Redeye Chub, and positively associated with current velocity for Apalachee Shiner.

Quadrat detection probabilities varied widely across species and were substantially lower for Bluenose Shiner than the other species (Fig. 2). Estimates were lower from the best-fitting models for all species except Redeye Chub, but standard errors varied little between models or among species. Cumulative detection probabilities climbed much more slowly for Bluenose Shiner, but were high (>80%) for all species for the average number of seine hauls (n = 22) we made at each site (Fig. 3). Consistent with high cumulative detection probabilities, estimated and observed occupancy rates were similar for all four species (Fig. 4). Estimates and standard errors differed little between models with and without habitat covariates.

Discussion

Despite low rates of detection and occupancy, our study was successful at documenting the current status of Bluenose Shiner in Georgia. Extensive sampling throughout historically occupied watersheds resulted in detection at only 13% of our sites. Collection sites are clustered within or near three mainstem creeks: Spring Creek, Ichawaynochaway Creek, and Pennahatchee Creek. The former two systems appear to be especially

Table 2. Mean maximum depth (SD) within sample quadrats and number of quadrats with sluggish or no perceivable current velocity, moderate to swift current velocity, <25% aquatic vegetation coverage, and >25% aquatic vegetation coverage. Data are summarized separately for quadrats where *Pteronotropis welaka* (Bluenose Shiner) was and was not captured. Data for additional non-random sites sampled in 2005 are not included.

		Current velocity		Vegetative cover		
Captured	Max depth (SD)	Slow	Swift	< 25%	> 25%	Total
Yes	46.3 (20.9)	11	0	5	6	11
No	42.6 (22.1)	536	317	673	180	853

Table 3. Model structure, relative difference in AIC_c (ΔAIC_c), and model weights for *Pteronotropis welaka* (Bluenose Shiner) occupancy models fit with Program Mark. Periods indicate model parameter estimated without covariates. DM = distance to largest stream in the watershed (i.e., the mainstem).

Model	ΔAIC_c	Weight
psi (DM, conductivity) *p* (current velocity)	0.00	0.872
psi (DM, conductivity) *p* (.)	5.38	0.059
psi (.) *p* (.)	6.77	0.029
psi (DM, conductivity) *p* (vegetative cover)	7.23	0.023
psi (DM, conductivity) *p* (maximum depth)	8.03	0.015

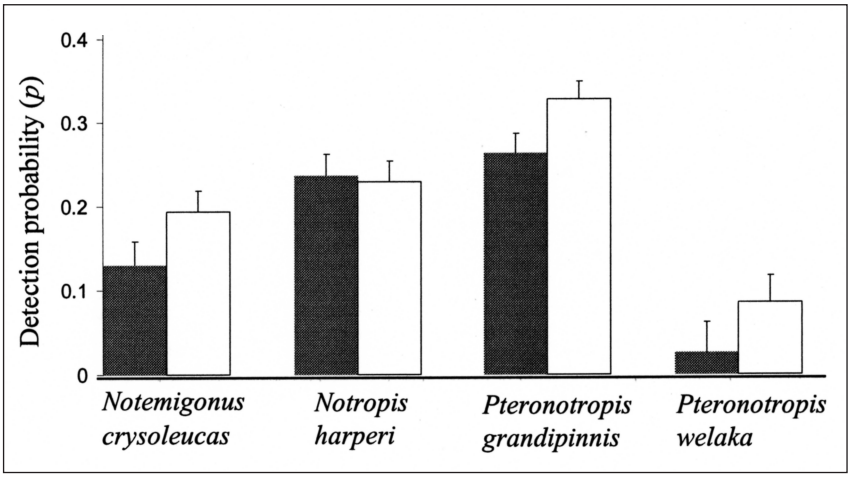

Figure 2. Estimates and standard errors of detection probability from occupancy models generated in Program Mark. Models with covariates (filled bars) always provided the best description of the data (i.e., lowest AIC_c value) compared to models without covariates (empty bars). Estimates reflect the probability of capturing the target species when making a single seine haul in sites where they are present.

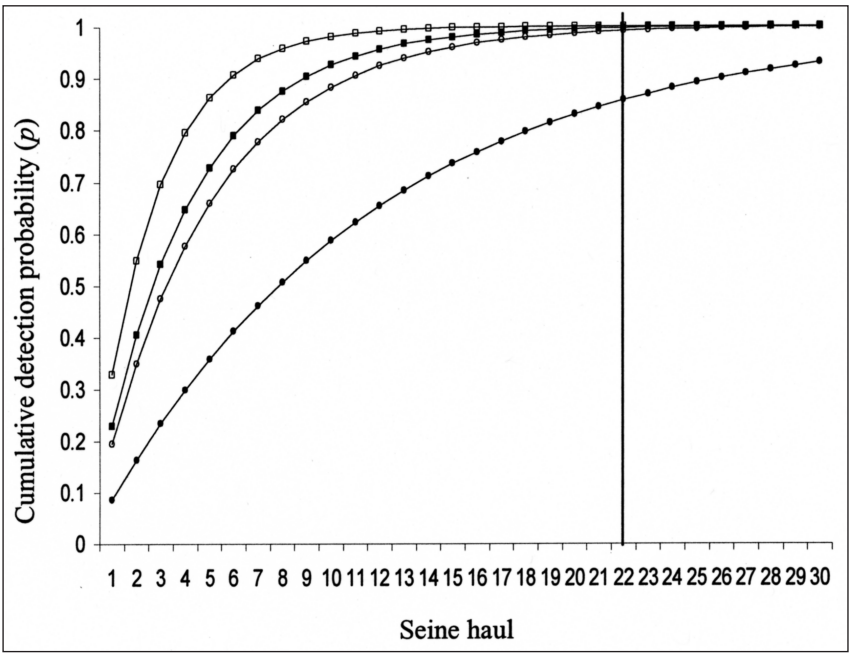

Figure 3. Detection probability as a function of the number of seine hauls sampled within a site for *Notemigonus crysoleucas* (Golden Shiner; open circles), *Notropis harperi* (Redeye Chub; filled squares), *Pteronotropis grandipinnis* (Apalachee Shiner; open squares), and *P. welaka* (Bluenose Shiner; filled circles). The solid vertical line indicates the average number of seine hauls we made per site during this survey.

critical to the conservation of this species in Georgia because of the relatively large number of extant sites. In addition, the two largest collections of Bluenose Shiner (20 and 12 fish) made during our survey were at two sites in Spring Creek. Because of the small number of known sites, demographic isolation (i.e., all three stream systems are isolated from each other by impoundments), and general threats to aquatic habitat in the region, additional monitoring and protection efforts are warranted.

Incorporating site- and quadrat-specific covariate data into our occupancy models resulted in substantial improvements in model fit for all species. Covariates can be used to target sites and microhabitats in future surveys. Our follow-up sampling in 2005, although limited, suggests that such an approach would be fruitful. Two of the four sites we sampled in sites within or near mainstem creeks represented new occurrences of Bluenose Shiner. This association with larger streams is in contrast with their distribution in Mississippi, where they are more commonly collected in streams with small drainage areas (Ross and Baker 1981). Streams with high conductivity, which usually indicates the presence of groundwater discharge in our study area, and microhabitats with slow current velocity should also be targeted during future surveys. The mainstems of Muckalee and Kinchafoonee Creeks lie between two of the known creek systems occupied by Bluenose Shiner and should be a very high priority for additional survey work (Fig. 1).

We caution, however, that our analysis should not be considered a definitive test of habitat relationships for this species. Foremost, the relatively

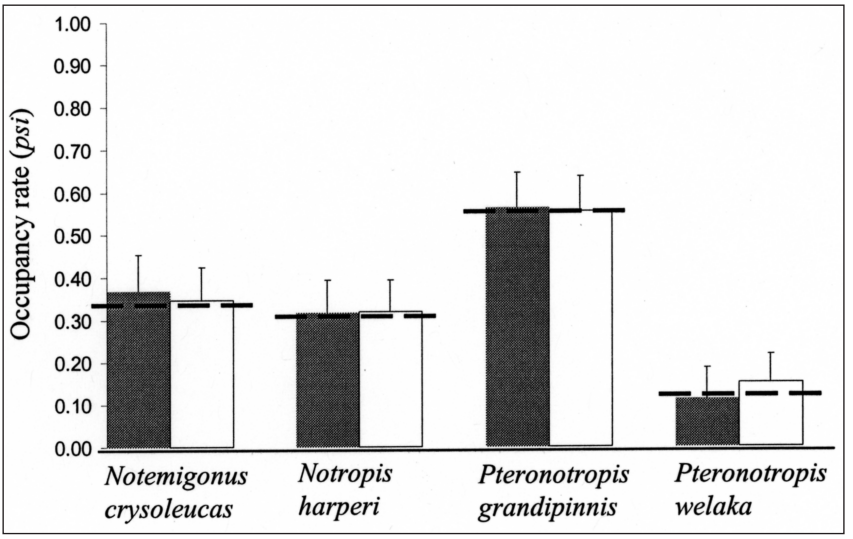

Figure 4. Estimates and standard errors of occupancy rate from Program Mark. Models with covariates (filled bars) always provided the best description of the data (i.e., lowest AICc value) compared to models without covariates (empty bars). Estimates reflect the proportion of sites occupied within the survey area and have been adjusted for incomplete detection. Observed occupancy (i.e., actual number of detections/number of sites surveyed) is indicated by a dashed line.

small number of sites we sampled prevented us from including a large number of predictor variables in the analyses. Our habitat data suggests that Bluenose Shiner may be less common in turbid streams, but we did not include this variable in analyses because of sample-size constraints. In addition, the small number of detections for Bluenose Shiner makes it difficult to fully characterize their habitat use. For example, although vegetation was not included in our best-fitting model, Bluenose Shiner was disproportionately collected in this rare microhabitat type.

Detection probabilities varied substantially across the species in our study and were extremely low for Bluenose Shiner. Within occupied sites, the large number of non-detections likely reflects the patchiness of suitable microhabitats. Their association with mainstem creeks probably makes Bluenose Shiners more difficult to catch than species that are common in shallower, tributary streams. In addition, low abundance may have also accounted for low detection probability in this study (Bayley and Peterson 2001). Finally, many of our occurrences were represented by small, young-of-year fish that could be easily overlooked or confused with other young-of-year cyprinids (e.g., Redeye Chub or *N. chalybaeus* (Cope) [Ironcolor Shiner]). We mitigated this problem by retaining voucher specimens for laboratory confirmation and recommend this protocol for future surveys. We do not believe that Bluenose Shiner is particularly elusive to our capturing methods compared to the other species, and Albanese (2000) found that this species is very vulnerable to seining in south Mississippi streams, where it is more abundant.

Interspecific variation in detection probabilities has important implications for future surveys. Although our observed and estimated occupancy rates were similar for all species, these rates would have differed substantially if we had not completed so many seine hauls at each site. For example, if we had only completed 10 seine hauls at each site, we would have had a high probability (i.e., > 80%) of detecting Golden Shiner, Redeye Chub, and Apalachee Shiner, but not Bluenose Shiner. We found that at least 19 seine hauls were needed to have an 80% chance of detecting Bluenose Shiner. Even greater sampling effort and the use of multiple gear types (e.g., dipnets) would be required when more definitive assessments of species occurrence are needed (e.g., for a site-specific environmental impact assessment). A more conservative and potentially cost-effective alternative to these intensive surveys would be to assume presence based upon nearby occurrences or the presence of suitable habitat (Peterson and Dunham 2003). The cost savings associated with the forgone survey could then be invested into better habitat-protection measures.

If the objective of a survey is to document new populations, the modeling approach we followed could be used with an initial data set to identify target habitats and the amount of sampling effort required at each site. For example, if we wanted to identify new populations of Bluenose Shiner in Georgia, we would carry out about 20 seine hauls at sites with the habitat characteristics described above. While additional seine hauls would increase the probability of detection at each site, this would come at the expense of the number of sites that could be surveyed. MacKenzie et al. (2006) indicate

than an optimal survey design for species with low occupancy rates is to sample more sites rather than expending more effort at individual sites.

The approach we followed can also be used to design a long-term monitoring program. The proportion of sites occupied is a good index of overall population status and is typically less expensive to estimate than abundance (MacKenzie et al. 2002). Again, the initial data set can be used to gauge sampling effort for future monitoring samples. Since the model will adjust occupancy rate for incomplete detection, it is not necessary to detect the species at every site. Thus, a modest sampling effort can be carried out at each site (e.g., 20 hauls in the case of Bluenose Shiner), which can result in significant cost savings over surveys where a more definitive assessment of site-specific occupancy is needed. Because our sample sites were randomly selected and thus representative of habitat conditions throughout historical watersheds, they could be resurveyed to document changes in the proportion of sites occupied over time and to estimate colonization and local extinction probabilities (MacKenzie et al. 2006). Resurveys that only focused on currently occupied sites would not be able to detect colonization of new sites and would thus be biased toward detecting a decline (Strayer and Smith 2003, MacKenzie et al. 2006).

Our study was successful at documenting the occurrence of Bluenose Shiner at new and historical sites within southwest Georgia. Although detection probabilities for this species were very low per seine haul, our observed and estimated occupancy rates were similar because of the large number of seine hauls we carried out at each site. The use of covariate data in our models improved model fit and will help identify sites and microhabitats to target in future surveys. Similarly, our estimates of detection probability will help determine how much sampling effort will be needed in future surveys for Bluenose Shiner and other coastal plain minnow species. The modeling approach we followed increases the confidence in our survey results and has broad application to future survey and monitoring efforts for southeastern aquatic fauna.

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