

Estimating Occupancy of Rare Fishes Using Visual Surveys, with a Comparison to Backpack Electrofishing

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Abstract - There is an ongoing need to monitor the status of imperiled fishes in the southeastern United States using effective methods. Visual surveys minimize harm to target species, but few studies have specifically examined their effectiveness compared to other methods or accounted for imperfect species detection. We used snorkel surveys to estimate detection probability and site occupancy for rare fishes in the Toccoa River system of north Georgia. We also carried out backpack electrofishing at a subset of sites to compare detection probabilities for both methods. The probability of detecting *Percina aurantiaca* (Tangerine Darter) and *Etheostoma vulneratum* (Wounded Darter) while snorkeling was relatively high, averaging 30% and 22%, respectively, and naïve and estimated occupancy rates (i.e., corrected for incomplete species detection) were almost identical for both species. The probability of detecting *Erimystax insignis* (Blotched Chub) while snorkeling was relatively low (9%), and their estimated occupancy rate (86%) was much higher than we detected in our survey. Detection was negatively related to depth and substrate size for Blotched Chub and positively related to depth for Tangerine Darter. Compared to snorkeling, the probability of detecting a species while backpack electrofishing was higher for Wounded Darter (40%) and comparable for Blotched Chub (11%). Tangerine Darter, however, were never captured while electrofishing even though they occurred at all four sites where both methods were used. Our study demonstrates the successful use of snorkel sampling to estimate occupancy rates of rare fishes in a large, clear southeastern river and illustrates the importance of accounting for imperfect species detection.

Introduction

The southeastern United States is a well-recognized hotspot for fish diversity, but also contains more imperiled fishes than any comparably sized region in North America (Jelks et al. 2008, Warren et al. 2000). Imperilment results from a myriad of historical and modern threats, including large-scale land conversion for agriculture, impoundment of free-flowing rivers, navigation projects that result in direct habitat destruction, industrial pollution, urbanization, invasive species, and climate change (Helfman 2007, Jelks et al. 2008). Because of past and current threats, there is an ongoing need to assess and monitor the status of fish populations, particularly for endangered fishes or species vulnerable to future imperilment. Accurate information on distributional status is needed to help prioritize species and habitats for conservation and to measure the effectiveness of management actions (Wenger et al. 2010).

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Obtaining accurate information on the status and distribution of rare species presents special challenges. Foremost, rare species may be difficult to detect during surveys, which may result in biased status assessments (Bayley and Peterson 2001, MacKenzie et al. 2002, Peterson and Dunham 2003). This problem was ignored in the past, but it is becoming increasingly more common to account for incomplete species detection in surveys for rare fishes (e.g., Albanese et al. 2007, Burdick et al. 2008, Wenger et al. 2008). Another challenge is the need to minimize handling stress and the risk of mortality for legally protected fishes (Jordan et al. 2008), which may restrict or preclude the use of effective but potentially harmful sampling methods such as electrofishing (Bohl et al. 2009).

Visual observation techniques reduce harm to target species and may be an appropriate method for surveying imperiled fishes in rivers with high water clarity. Other advantages include lower cost and the ability to target habitats that may be too deep or structurally complex to sample by seining or electrofishing (Thurrow et al. 2006). While visual observation techniques are routinely applied, only a few studies have specifically examined their effectiveness compared to other methods (e.g., Ensign et al. 1995, Jordan et al. 2008, Thurrow et al. 2006) or accounted for incomplete species detection (Peterson et al. 2002). This latter issue could be particularly problematic for rare southeastern fishes because they are typically small-bodied and cryptobenthic (Jenkins and Burkhead 1993).

Here we illustrate the use of visual observation techniques (snorkeling) to assess the status of rare fishes in a large, clear river in north Georgia. The primary objective of our study was to estimate the proportion of sites occupied (i.e., site occupancy) for our target species. The methods we used also allowed us to estimate detection probability for snorkel sampling, account for imperfect detection in our estimate of site occupancy, and to examine environmental covariates of occupancy and detection. In addition, we also compared our snorkel surveys to results from backpack electrofishing carried out at a subset of sites.

Methods

Study area and sample site selection

We carried out our surveys in the Toccoa River in north-central Georgia. The Toccoa River begins in the Blue Ridge physiographic province near Suches, GA and flows 65 km before entering Tennessee, where its name changes to the Ocoee River. The watershed has high forest cover (86%), in large part due to Chattahoochee-Oconee National Forest property in the headwaters and along an 18-km section of the mainstem river (National Land Cover Database 2001). Only a small proportion of landcover is classified as agriculture (5%) or developed (5%), but the latter category includes an increasing number of cottages being built along the river (B. Albanese, pers. observ.). Blue Ridge Dam impounds the Toccoa River 23 km upstream of the state line, forming a 1335-ha (3300-acre) impoundment that is managed by the Tennessee Valley Authority for flood control and recreation. The dam itself, completed in 1930, is operated for hydropower generation. Compared to the river upstream of the dam, the tailwater is characterized by depressed stream temperature, rapid increases in stream flow during generating periods, and increased

flows during winter reservoir drawdowns. An injection system is used to elevate oxygen levels, and a small, secondary hydroelectric turbine is used to maintain minimum flows during non-generating periods (Tennessee Valley Authority 2010).

The Toccoa River watershed contains important populations of several state-protected fish species, including Georgia's only known populations of *Etheostoma vulneratum* (Cope) (Wounded Darter), *Percina squamata* (Gilbert and Swain) (Olive Darter), and *Percina aurantiaca* (Cope) (Tangerine Darter). It also contains one of only three populations of *Erimystax insignis* (Hubbs and Crowe) (Blotched Chub) in the state (Georgia Department of Natural Resources 2010). The Olive Darter and Wounded Darter are considered vulnerable across their range (Jelks et al. 2008), and populations are ranked as critically imperiled or imperiled in each state in which they occur (Nature Serve 2010). All of these species are associated with benthic substrates for feeding and/or reproduction, making them vulnerable to sedimentation and other forms of stream habitat degradation (Burkhead et al. 1997). Despite the importance of Toccoa River populations, their status has never been formally assessed. Before the onset of our survey, each species had only been documented at 5 (Wounded Darter) to 14 (Tangerine Darter) sites, with last collection dates ranging from 1994 (Olive Darter) to 2005 (Blotched Chub) (Georgia Department of Natural Resources 2008).

We adopted a stratified-random sampling design to select sample sites along the Toccoa River between its headwaters and the Tennessee state line. Although some of our target species have been collected in the downstream reaches of larger tributary streams (e.g., Coopers Creek), this section of river includes almost all of the potential range of our target species within the Georgia portion of the system (Fig. 1). In addition, tributary streams have received much more sampling effort than the mainstem river because they are wadeable and more easily accessed by

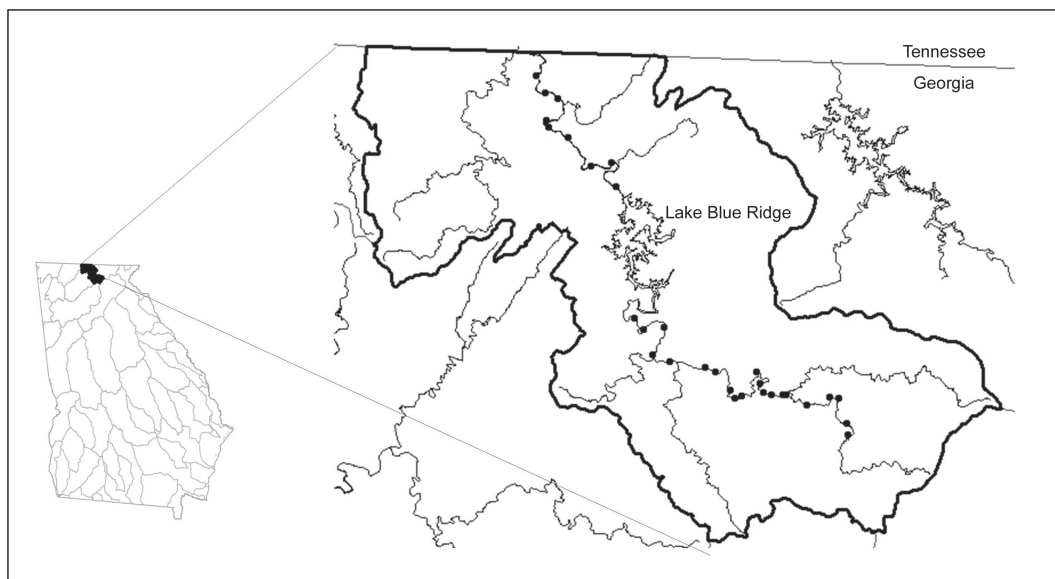


Figure 1. Distribution of sampling sites along the Toccoa River in north-central Georgia. Sites were randomly selected from approximately 10-km strata. The inset shows the Toccoa River watershed highlighted among other large watersheds in Georgia.

road. We divided the river into six approximately 10-km strata and randomly selected five 1-km reaches within each. We then sampled the first riffle-run habitat unit encountered within each selected reach as we traveled downstream through each stratum by kayak. We chose the first riffle-run unit encountered because we had no prior knowledge about the number of riffle-run units in each reach (i.e., we did not want to float past a riffle-run unit that could have been the only potential sampling site within the reach). To avoid excessive travel among strata, all sites within a stratum were sampled consecutively, within a 1–2 week period. The order that strata were sampled was determined randomly, except that we alternated between strata upstream and downstream of the dam to ensure coverage of both areas throughout the sampling season. We sampled 29 sites between 28 May and 8 August 2008 using our snorkel sampling protocol (see below); one site downstream of the lake could not be sampled because of high turbidity after a rainstorm.

Sampling methods

Snorkel surveys were carried out using systematic sampling with a random start. We first estimated the downstream boundary of the riffle-run unit and then paced 0–9 randomly determined meters upstream to the downstream boundary of our first sampling transect. While we did not include deep-slow pools in our study, downstream boundaries of our sites always extended into deep runs. Four snorkelers were then spaced at 15%, 40%, 60%, and 85% of stream width to capture lateral heterogeneity in fish habitat. At sites less than 15 m wide, we used 3 snorkelers spaced at 25%, 50%, and 75% of stream width. Each snorkeler was separated by at least 2.5 m to reduce the probability of disturbing fishes or observing the same fish more than once. Snorkelers recorded fish occurrence data along 15-m long transects oriented parallel with stream flow. The width of each transect varied according to water visibility, which was estimated using a laminated darter image pulled out to the limits of detectability (Thurow et al. 2006). Presuming that our most drably colored target species would be the most difficult species to detect, we used a life-size (13 cm) color copy of an Olive Darter for our image. We measured visibility for each snorkeler at the bottom and top of each sample site and averaged all values for a site-level measure of visibility.

Snorkelers slowly crawled upstream and scanned from side to side in search of target species. Weight belts were worn to maintain contact with the bottom, which was particularly helpful in swift and/or deep transects. Non-embedded cobbles and small boulders were flipped to search for Wounded Darter, which are known to forage and reproduce within the cavities formed by rocks. We used a halogen dive light to illuminate crevices or dimly lit portions of the stream bottom when necessary. The number of occurrences of each target species was recorded on a wrist slate. After the first set of transects was completed, 3 or 4 additional sets of transects were then sampled upstream until 12 transects were sampled at each site. These additional sets of transects were systematically spaced 5 or 10 m upstream of the upstream boundary of the first set of transects to increase independence among transects; the longer separation distance was used to capture more longitudinal habitat heterogeneity in longer riffle-run units. Steel washers with flagging tape were dropped at the bottom

of each transect to facilitate habitat data collection. The same four snorkelers collected data at all sampling sites, but their position within the stream channel (i.e., 15%, 40%, 60%, and 85% of stream width) was haphazardly varied among sites to ensure that any differences in observer skill were not confounded with stream channel position.

Habitat data were collected within each transect at the conclusion of snorkel sampling. Depth and dominant substratum were measured at 1.5-m intervals along each transect, for a total of 10 measurements. Surface current velocity was measured by floating a ping-pong ball through the transect at least twice. Our sample size for habitat variables was determined after examining CV values for 10 vs. 15 depth and substratum measurements and 2 vs. 3 velocity measurements collected during preliminary sampling. These values differed little, and final CVs averaged less than 26% across sample sites for all three variables. Dominant substratum was visually classified into the following categories: silt, sand, gravel (2–16 mm), pebble (16–64 mm), cobble (64–256 mm), boulder (>256 mm), and bedrock (Gordon et al. 1992). The modal dominant substratum category was determined for each transect and converted to an ordinal scale (1 = smallest, 7 = largest) for subsequent analyses. Depth and surface current velocity measurements were averaged for each transect, for a total of 12 measurements per site. Habitat data were not collected at one site upstream of Lake Blue Ridge because of time constraints.

We re-sampled the exact same transects at 6 of our 29 sites (one per stratum) using a DC backpack electrofisher (Model 12B POW, Smith-Root Inc., Vancouver, WA). Cathode and anode poles held about 1 m apart were bumped along the stream bottom as the transect was sampled in an upstream to downstream direction. Fishes were collected in a 3.7-m x 1.8-m seine with a 1.8-m x 1.8-m x 1.8-m bag, 4.7-mm-mesh, and 15-cm lead spacing. The seine was held at the downstream end of the transect, and captured fishes were identified, counted, and released. Paired sampling was also conducted opportunistically at three additional sites in the most downstream stratum. We attempted to always sample sites by snorkeling before shocking, but high turbidity required us to shock 5 of the 9 sites first. For sites sampled by snorkeling then shocking, paired sampling occurred 1–24 hours after the initial sample. For sites sampled by shocking then snorkeling, paired sampling occurred 7–13 days after the initial sample. This time interval was a compromise between allowing enough time for recovery from electrofishing, but not enough time for seasonal changes in fish occupancy patterns.

Data analysis

We estimated detection probability and site occupancy for each target species using the single-season models described by MacKenzie et al. (2002). Site occupancy (ψ) is the proportion of sites occupied within the overall study area, corrected for incomplete detection; it can also be considered the probability that an individual site is occupied. Detection probability (p) is the probability of detecting a target species within a single transect when the species is present within the site. Instead of re-sampling each site on multiple occasions, we used our transect data to estimate detection probability. In other words, we substituted spatial subunits for repeated

temporal sampling (Albanese et al. 2007, Kendall and White 2009). A critical assumption of single-season occupancy models is that sites are closed to changes in occupancy during the entire survey season. Our spatial subunit approach helped satisfy this assumption because all transects were sampled within a single day. The model also assumes independence among detections both within and between sites. We attempted to satisfy these assumptions by allowing sufficient spacing between transects within each site and by randomly selecting sample sites. We guarded against false detections of target species by training each snorkeler in target species identification using photo cards depicting diagnostic characters of males, females, and juveniles. We also practiced identifying species underwater before beginning the formal survey.

Occupancy models account for variation in occupancy and detection using environmental covariates, which can help improve model fit and detect important relations between target species and habitats. We hypothesized that detection probability would vary with average depth, current velocity, and dominant substrate type in each transect. These variables are often associated with capture probability and abundance, both of which affect species detection (Bayley and Peterson 2001). We predicted that occupancy would vary with river location, which was represented as the distance of the site from the Tennessee state line (DTN) in river kilometers. This variable is correlated with a suite of variables that could affect occupancy patterns, including depth (Pearson's $r = -0.28$), stream width ($r = -0.92$), and percent of open canopy ($r = -0.80$; B. Albanese, unpubl. data). Finally, we included visibility as a covariate of occupancy to make sure that differences in water clarity were not affecting observed occupancy patterns. Visibility also could affect detection probability, but these data were not available for every transect.

Models were built using the occupancy-estimation procedure in Program MARK (White and Burnham 1999). All species were modeled simultaneously, and differences among species were examined using three group variables and modeling ψ and p with Tangerine Darter as the baseline species. We built a global model with all covariates, models with no covariates, and models with all possible combinations of covariates ($n = 32$ models). All covariates were standardized to a mean of zero and standard deviation of one by Program Mark, which facilitates comparison of parameter estimates. We used a parametric bootstrap goodness-of-fit test (MacKenzie and Bailey 2004, MacKenzie et al. 2006) with 100 iterations to evaluate the relative fit of the global model. If the global model fit was adequate ($c\text{-hat} \leq 1$), we used Akaike's Information Criterion (AIC) as corrected for small sample size (AIC_c ; Burnham and Anderson 2002) to compare the relative fit of models. If there was evidence of lack of fit (i.e., overdispersion, $c\text{-hat} > 1$), models were ranked using Quasi-Akaike's Information Criterion ($QAIC_c$), which accounts for overdispersion (Burnham and Anderson 2002, MacKenzie et al. 2006).

Program Mark also calculates model weights that range from 0 to 1, with the most plausible candidate model having the highest weight (Burnham and Anderson 2002). We selected models with weights (w_i) within 10% of the highest ranked model and included them in a confidence set for further interpretation. We compared different models within the confidence set by calculating the ratio of

w_i values, which summarize the degree of evidence for one model over another (Anderson et al. 2000). The magnitude and direction of covariate relations was assessed using odds ratios and their 95% confidence intervals. Odds ratios were calculated as $\exp(B_i)$, where B_i is the parameter estimate for the covariate from the highest ranking model in which it occurs. Odds ratios greater than one indicate an increase in the probability of occupancy or detection with each 1 unit increase (i.e., 1 SD increase because our covariates were standardized) in the predictor variable. Odds ratios less than one indicate a decrease in the probability of occupancy or detection with each 1 unit increase in the predictor variable (MacKenzie et al. 2006).

The analysis described thus far focused on covariate relations for our target species overall, but did not address covariate relations for individual species. A priori, there was no strong basis to expect these riffle-run inhabiting fishes to exhibit different relations with covariates. In addition, testing for all combinations of species-level effects in our initial analysis would have required 160 models. Thus, we built a second set of models (hereafter species interaction models) to explore covariate relations for individual species. We restricted this analysis to covariates that appeared important in the first set of models, as determined by odds ratios and parameter estimates. These covariates were included in a global model that tested for general relations across species (i.e., as in the first set of models). Four alternative models (1 per species) that included an interaction between each covariate of interest and detection/occupancy of the individual species were then constructed to evaluate differences among species. We examined parameter estimates to determine if the relations detected in the first set of models were consistent across individual species. All other modeling procedures were identical to those described for the first set of models.

We calculated cumulative detection probabilities for the number of transects (n) made at survey sites using the following equation: $1 - (1 - p)^n$. We used estimates of p from models without covariates to determine cumulative detection because these estimates reflect average detection over the range of habitats we encountered.

Finally, we built two additional models to compare detection probabilities of electrofishing and snorkel surveys. To do this, we added the electrofishing transect data to the snorkeling data and modeled detection probabilities as a function of sampling method and species using Program Mark. We then evaluated the relative support for two models. In the first model, we estimated p and ψ for each species and an overall effect of electrofishing on detection (i.e., the effect of electrofishing was similar across species). In the second model, we also tested for interactions between detection and electrofishing for each species. No other covariates were included in these models, but all other modeling procedures were as described above.

Results

None of our species were detected downstream of Lake Blue Ridge, during either the snorkel or electrofishing surveys. Accordingly, we restricted all subsequent analyses to sites upstream of the lake. Including the downstream sites

would potentially confound relations with covariates if suitable microhabitat conditions occur downstream of the lake, but were not accessible to target species due to some unmeasured factor (e.g., altered flow and temperature regime, extirpation, etc.). Our habitat data indicate similar depths, velocities, and substrates upstream and downstream of Lake Blue Ridge, but higher visibility downstream of the lake (Table 1). After excluding these downstream sites and one upstream site where time constraints precluded collection of habitat data, 19 sites remained in the data set.

We detected Blotched Chub at 11 sites, a single Olive Darter at 1 site, Tangerine Darter at 16 sites, and Wounded Darter at 9 sites during snorkel sampling upstream of Lake Blue Ridge (Fig. 2, Table 2). The total number of transect detections and the total number of fish observed during snorkel surveys varied substantially across species (Table 2). We observed more total individuals and more transects occupied by Tangerine Darter compared to the other species. However, within individual transects, the maximum number of individual fish observed was greatest for Blotched Chub and Wounded Darter.

The bootstrap goodness-of-fit test indicated lack of fit for our global model ($c\text{-hat} = 1.05$), so models were ranked according to $QAIC_c$. The model without covariates for ψ and p was the lowest ranked in the entire model set ($n = 32$) and had virtually no model weight ($w_i < 0.001$). Ten models were retained in the confidence set (Table 3). The model containing DTN, depth, and substrate was

Table 1. Mean, standard deviation (SD), and range of habitat characteristics measured at sample sites on the Toccoa River, both upstream downstream of Lake Blue Ridge. DTN = distance to Tennessee. Dom. sub. = dominant substrate.

Statistic	DTN (km)	Visibility (m)	Depth (cm)	Surface current velocity (m/sec)	Modal dom. sub.
Upstream					
Mean	65.8	0.96	47.5	0.52	5.7
SD	11.1	0.17	11.2	0.16	1.4
Range	47–83	0.71–1.34	28.8–68.7	0.17–0.94	2–7
Downstream					
Mean	12.1	1.3	53.4	0.5	5.8
SD	6.5	0.3	9.6	0.1	1.2
Range	4–23	1.0–2.1	36.3–72.2	0.3–0.7	4–7

Table 2. Number of sites and transects where target species were detected during snorkel surveys of 29 sites sampled along the Toccoa River during summer 2008. Twelve transects were sampled at each site. The number of fish observed is also reported. Totals were summed across all sites. Max = the maximum number of transect detections or fish within any individual site.

Species	Site detections	Transect detections		Fish observed	
		Total	Max	Total	Max
Blotched chub	11	18	3	84	41
Olive Darter	1	1	1	1	1
Tangerine Darter	16	65	7	115	13
Wounded Darter	9	25	7	53	28

most strongly supported by the data and was 2.14 times more likely (0.232/0.108) than the next best approximating model. All of the lower ranked models in the

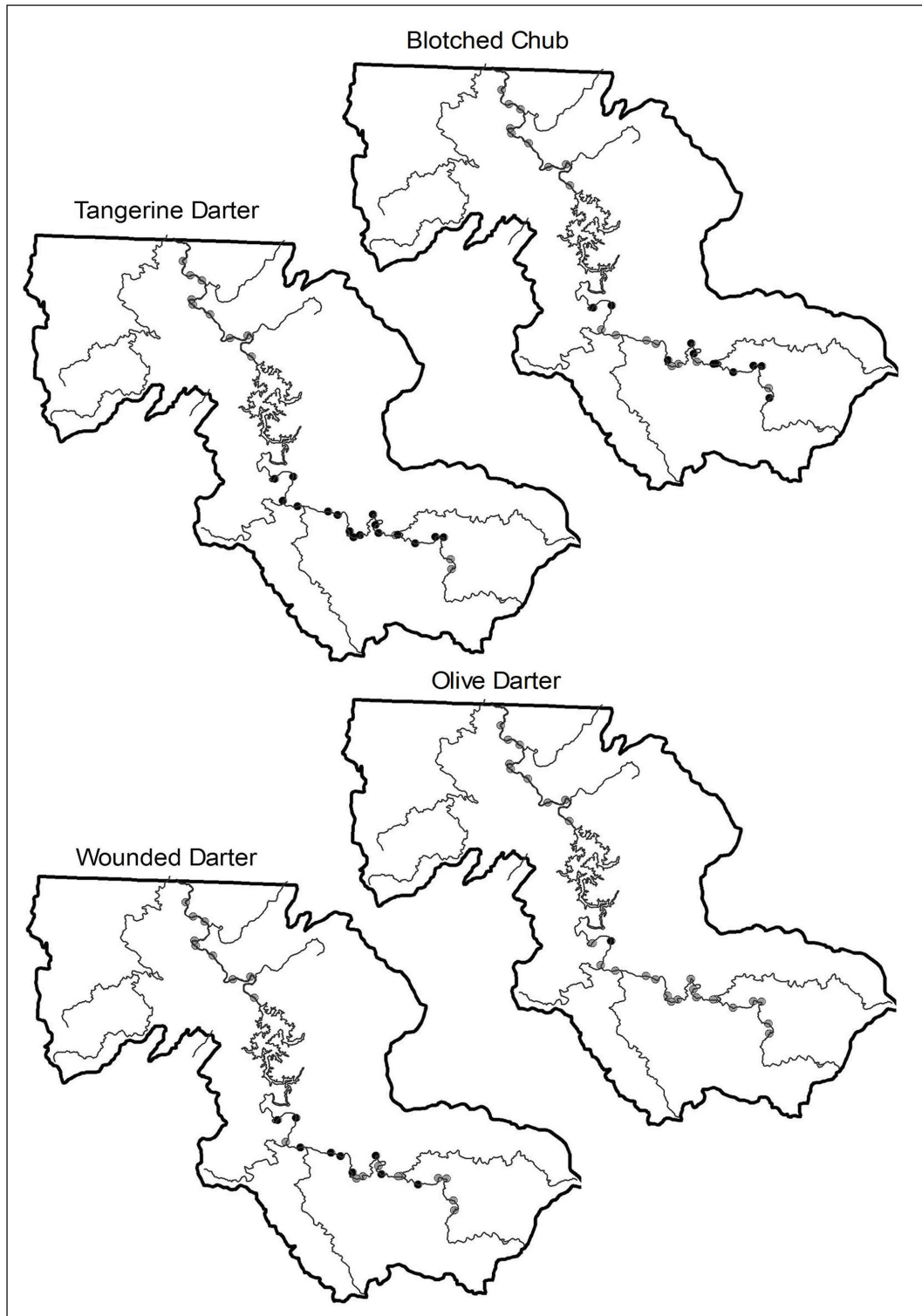


Figure 2. Sites where target species were detected (black circles) and were not detected (grey circles) during snorkel surveys of 29 sites sampled along the Toccoa River during summer 2008.

confidence set also included DTN, which suggests that this variable was an important covariate of occupancy. Summed across all models in the confidence set, models with DTN had 87% of the total model weight. The total weight of models with depth (67%) and substrate (53%) suggest that they were important covariates of detection, whereas the total weight of models including velocity (29%) suggested less support for the hypothesis that species detection was related to velocity. Visibility was only included in three models, which included 20% of total weight. Parameter estimates and odds ratios indicate that occupancy was negatively related to DTN (Table 4). Species detection was positively related to depth and substrate size, but negatively related to velocity. However, confidence intervals for parameter estimates and odds ratios suggested that the effect of substrate and velocity on species detection was not strong. There was no evidence for a relationship between visibility and occupancy.

Based upon these results, DTN, depth, velocity, and substrate were included in the species interaction models. There was no evidence for lack of fit (bootstrap goodness-of-fit test), and models were ranked according to AIC_c . Specifying interactions resulted in substantial improvement in model fit relative to a model

Table 3. Model structure and weights for the 10 models within the confidence set; an additional 22 models were also built but are not shown because of low model weights. Models are ranked by Quasi- AIC_c ($QAIC_c$), which is corrected for small sample size and accounts for overdispersion of the data. Covariates of occupancy (ψ) include the distance of the sample site from Tennessee (DTN) and the average underwater visibility for the site. Covariates of detection (p) were measured at the individual transect scale and include average depth, average surface current velocity, and modal dominant substrate (sub) category.

Model	$QAIC_c$	Weight	Number of parameters
ψ (DTN) p (depth, sub)	502.3	0.232	11
ψ (DTN, visibility) p (depth)	503.8	0.108	11
ψ (DTN) p (depth)	503.8	0.106	10
ψ (DTN) p (velocity, sub)	503.9	0.101	11
ψ (DTN) p (depth, velocity)	504.2	0.090	11
ψ (DTN) p (depth, velocity, sub)	504.7	0.070	12
ψ (DTN, visibility) p (depth, sub)	505.0	0.061	12
ψ (DTN) p (sub)	505.9	0.038	10
ψ (DTN) p (.)	506.1	0.035	9
ψ (DTN, visibility) p (velocity, sub)	506.6	0.026	12

Table 4. Parameter estimates, standard errors (SE), 95% confidence intervals (CI), and odds ratios for predictor variables in occupancy models. Parameter estimates are from the highest ranked model within the confidence set that contained the variable listed. An odds ratio of one indicates no change in the probability of detection or occupancy as the predictor variable changes.

Parameter	Estimate (SE)	95% CI of Estimate		Odds	95% CI of odds	
		Lower	Upper		Lower	Upper
p (depth)	0.32 (0.13)	0.07	0.57	1.38	1.07	1.78
p (velocity)	-0.28 (0.13)	-0.54	-0.02	0.75	0.58	0.98
p (sub)	0.18 (0.14)	-0.10	0.46	1.20	0.90	1.58
ψ (DTN)	-2.20 (0.95)	-4.05	-0.34	0.11	0.02	0.71
ψ (visibility)	0.30 (1.05)	-1.76	2.37	1.36	0.17	10.69

with the same variables but without interactions (Table 5). A model specifying interactions between Blotched Chub and covariates was most strongly supported by the data and was 1.45 times more likely than the next best approximating model. The model specifying interactions for Olive Darter also was strongly supported relative to models for Tangerine Darter and Wounded Darter.

Parameter estimates and odds ratios from the species interaction models (Table 6) indicated that the overall relations identified in the first set of models were not consistent across species. This lack of consistency was most evident for Blotched Chub, where the probability of detection was negatively related to depth and substrate size and the probability of occupancy was positively related to DTN. Odds ratios indicate particularly strong effects of depth and DTN. For example, the probability of detecting a Blotched chub, on average, was 5.2 times (1/0.19) less likely for each 1 SD (11.2 cm) increase in depth. Similarly, the probability of occupancy, on average, was 26.9 times greater for every 1 SD (11.1 km) increase in

Table 5. Model structure and weights for the species-interaction models, which tested for interactions between individual species and important covariates of occupancy and detection from the initial set of models. One model from the initial set that did not include any interactions was also included for comparison. BC = Blotched chub, OD = Olive Darter, TD = Tangerine Darter, and WD = Wounded Darter.

Model	AICc	Weight	No. of parameters
ψ (DTN) p (depth, velocity, sub) x BC	508.5	0.678	16
ψ (DTN) p (depth, velocity, sub) x OD	510.3	0.274	16
ψ (DTN) p (depth, velocity, sub) x TD	513.8	0.048	16
ψ (DTN) p (depth, velocity, sub) x WD	526.0	<0.001	16
ψ (DTN) p (depth, velocity, sub)	528.5	<0.001	12

Table 6. Parameter estimates, standard errors (SE), and odds ratios from species interaction models. Covariates shown indicate the interaction between the target species and occupancy (ψ) or detection (p); other model parameters are not reported. Program Mark indicated that the estimates for all Olive Darter covariates and estimates of the DTN covariate for Tangerine and Wounded Darters were unreliable and are not reported.

Parameter/Species	Estimate (SE)	95% CI of Estimate		Odds	95% CI of odds	
		Lower	Upper		Lower	Upper
Blotched Chub						
p (depth)	-1.66 (0.42)	-2.49	-0.83	0.19	0.08	0.44
p (velocity)	0.02 (0.35)	-0.67	0.70	1.02	0.51	2.02
p (sub)	-0.76 (0.34)	-1.43	-0.10	0.47	0.24	0.91
ψ (DTN)	3.29 (1.41)	0.53	6.06	26.91	1.69	428.00
Tangerine Darter						
p (depth)	0.89 (0.28)	0.34	1.43	2.43	1.41	4.19
p (velocity)	0.22 (0.28)	-0.32	0.77	1.25	0.72	2.17
p (sub)	0.37 (0.29)	-0.20	0.93	1.44	0.82	2.54
Wounded Darter						
p (depth)	0.14 (0.34)	-0.53	0.80	1.15	0.59	2.23
p (velocity)	-0.44 (0.35)	-1.12	0.23	0.64	0.33	1.26
p (sub)	0.40 (0.38)	-0.35	1.16	1.50	0.71	3.18

DTN (i.e., as you move upstream). The probability of detecting Tangerine Darter was positively related to depth, with an odds ratio suggesting a stronger effect than in the initial models. Based on odds ratios and parameter estimates, none of the other relations appeared meaningful. Model diagnostics in Program Mark indicated that the parameters for Olive Darter were unreliable (i.e., very large standard errors) and are not reported.

Occupancy estimates were very similar to naïve occupancy rates for Tangerine and Wounded Darter, but were substantially higher and had wider confidence intervals for Blotched Chub (Table 7). The probability of detection within a single snorkeling transect was highest for Tangerine and Wounded Darter, relatively low for Blotched Chub, and extremely low for Olive Darter. These interspecific differences become much more apparent as cumulative detection is plotted against the number of transects surveyed (Fig. 3). Cumulative detection

Table 7. Parameter estimates, standard errors (SE), and 95% confidence intervals (CI) of occupancy (ψ) and detection probability (p) for 19 Toccoa River sites located upstream of Lake Blue Ridge. Data are from the model with no covariates and reflect average detection and occupancy over the range of habitats we sampled. The proportion of sites where species were actually detected (i.e., Naïve estimate) is also reported for comparison. Program Mark indicated that the estimate of ψ for Olive Darter was unreliable and is not reported (NR).

Species	Naïve	ψ (SE)	95% CI	p (SE)	95%CI
Blotched Chub	0.58	0.86 (0.25)	0.10–1.00	0.09 (0.03)	0.04–0.18
Olive Darter	0.05	NR	NR	<0.01 (<0.01)	<0.01–0.03
Tangerine Darter	0.84	0.86 (0.09)	0.59–0.97	0.30 (0.04)	0.24–0.38
Wounded Darter	0.47	0.50 (0.13)	0.27–0.73	0.22 (0.04)	0.15–0.32

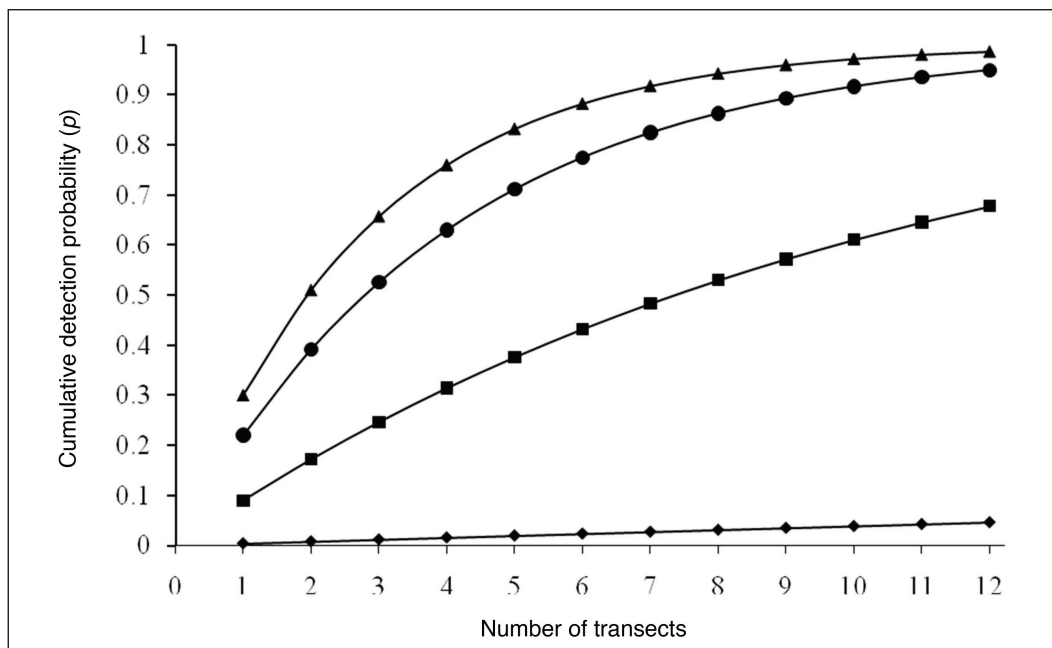


Figure 3. Detection probability as a function of the number of transects sampled within a site for Blotched Chub (*Erimystax insignis*; squares), Olive Darter (*Percina squamata*; diamonds), Tangerine Darter (*Percina aurantiaca*; triangles), and Wounded Darter (*Etheostoma vulneratum*; circles).

approaches an asymptote near one if 12 transects are sampled for Tangerine and Wounded Darter. In comparison, obtaining a comparable level of detection (e.g., 95%) would require sampling at least 30 transects for Blotched Chub and over 700 transects for Olive Darter.

Four of the 19 sites upstream of Lake Blue Ridge were sampled by both methods. Snorkeling preceded electrofishing at three of these sites, but followed shocking at one site. The bootstrap goodness-of-fit test did not suggest lack of fit, and models were compared by AIC_c . There was no support for the model that included only an electrofishing effect with no species interactions ($w_i = 0.00$, $\Delta AIC = 28.3$), and detection probabilities differed widely across species and methods (Table 8). Blotched Chub exhibited no meaningful difference in detection between snorkeling and electrofishing, and the number of sites, transects, and individual fish observed/captured was almost identical for both methods. While both methods resulted in the same number of site-level detections, the probability of detecting Wounded Darter was much higher for electrofishing. Twice as many transects and more than twice as many individual Wounded Darter were detected by electrofishing. Although we detected Tangerine Darter during snorkel surveys at all four sites where the electrofishing method was also carried out, we never captured them by electrofishing. Similarly, we only observed an Olive Darter during one of the snorkel surveys.

Discussion

Status of target species

Our study provides the first quantitative assessment of the status of our target species in the Toccoa River system. All of our target species were either absent or very rare in the reach downstream of Lake Blue Ridge, which likely reflected the effects of habitat alteration and fragmentation associated with Blue Ridge dam (Pringle et al. 2000). Three of our four target species were broadly distributed upstream of Lake Blue Ridge, suggesting that conservation efforts be focused in these areas. This free-flowing reach of the Toccoa retains high habitat quality, but was recently affected by the construction of hundreds of vacation homes along the river (B. Albanese, pers. observ.). Other potential threats include loss of *Tsuga Canadensis* (L.) Carr (Eastern Hemlock) due to *Adelges tsugae* (Annand)

Table 8. Estimates (standard error, SE) of detection probability (p) for our snorkel survey method carried out at 19 sites upstream of Lake Blue Ridge and for electrofishing surveys (shock) carried out at a subset ($n = 4$) of these same sites. For the four sites where both methods were used, we also report the number of sites, transects, and fish detected. BC = Blotched Chub, OD = Olive Darter, TD = Tangerine Darter, and WD = Wounded Darter.

Species	p (SE)		No. sites		No. transects		No. fish	
	Snorkel	Shock	Snorkel	Shock	Snorkel	Shock	Snorkel	Shock
BC	0.10 (0.03)	0.11 (0.05)	3	3	3	4	7	7
OD	<0.01 (<0.01)	<0.01 (<0.01)	1	0	1	0	1	0
TD	0.30 (0.03)	<0.01 (<0.01)	4	0	12	0	17	0
WD	0.22 (0.04)	0.40 (0.08)	3	3	7	14	9	22

(Hemlock Woolly Adelgid; Roberts et al. 2009) and bank destabilization/nutrient enrichment from cattle access (B. Albanese, pers. observ.). Priority conservation actions we recommend include protection of existing riparian forest on private lands, riparian zone reforestation in agricultural areas and on residential lots, and continued monitoring of fish populations.

The observation of only a single Olive Darter during the survey prevented us from reliably estimating occupancy and was cause for concern. In addition to the surveys reported here, we also searched unsuccessfully for Olive Darter at the three historic sites known from Coopers and Wilscot Creeks (Toccoa River tributaries). Although Olive Darter are considered difficult to capture or observe because of their occurrence in deep, rocky areas with moderate to very swift currents (Etnier and Starnes 1993), we believe our extensive survey data utilizing two different methods suggests a rare population in the Toccoa River.

Occupancy and detection during snorkel sampling

Our initial models were useful in identifying factors that had an overall effect on occupancy and detection and also minimized the number of models needed to evaluate all possible relations between individual species and covariates. However, the relations we documented in the initial models were not consistent across species, which emphasizes the importance of evaluating interactions between individual species and covariates. Furthermore, accounting for interactions between individual species and covariates resulted in substantial improvement in model fit despite the inclusion of a greater number of model parameters. Therefore, we focus our discussion on the relations documented in the species interaction models.

Occupancy of Blotched Chub was strongly and positively related to DTN, indicating a higher probability of occupancy as you move further upstream along the Toccoa River. This species was documented further upstream than any of the other target species and also was absent from several of the downstream sites that were occupied by the other target species. We don't understand the underlying mechanism, but note that several ecological variables decrease as you move upstream (e.g., width, depth, water temperature, percent of open canopy, etc.). Blotched Chub are known from the lower reaches of Cooper's Creek (Georgia Department of Natural Resources 2008), and the relation with DTN suggests that this species also may utilize the lower reaches of other tributary streams that are comparable in size to the upstream reaches of the Toccoa River.

Blotched Chub had a relatively low probability of detection, which was negatively related to stream depth and substrate size. We believe that low detection was due, in part, to habitat use and schooling behavior. While our transects were spaced across the width of the channel, we may have failed to detect some Blotched Chub by not sampling the shallowest habitats along shorelines. Similarly, the effect of substrate size on detection may reflect a true microhabitat preference or the ease at which groups of Blotched Chub were observed over smaller substrates (e.g., sand, gravel) relative to larger substrates that can obscure the snorkeler's field of view. Blotched Chub were observed in groups ranging from 2 to 22 individuals (mean = 4.7, SD = 6.1), which also could have

decreased their probability of detection because individuals were clumped in one or a few transects rather than spread uniformly throughout the site. Our data were consistent with this pattern, as we actually observed more Blotched Chub than Wounded Darter, but detected the latter species in more transects. While detection probability increases with abundance when fish behave independently, a school of fish provides only one opportunity for detection (Bayley and Peterson 2001). Therefore, detection probabilities may be lower in rare species that exhibit schooling behavior.

Tangerine Darter had the highest probability of detection in this study, which presumably reflected their large size, bright breeding coloration, and behavior. Leftwich et al. (1997) also considered this species easy to detect using underwater observation techniques. Tangerine Darter are atypical among darters in their habit of swimming in the water column (Jenkins and Burkhead 1993), which likely increases their detection compared to cryptobenthic darters. The detection of Tangerine Darter was positively associated with stream depth in this study, which is consistent with other studies on their microhabitat use (Leftwich et al. 1997) and emphasizes the importance of including deeper habitats in surveys for this species.

In contrast to Blotched Chub and Tangerine Darter, there was little evidence that occupancy and detection of Wounded Darters were related to the covariates we measured. However, Wounded Darter appeared to be over-represented in transects dominated by boulder substrates: 63% of our detections were in transects dominated by boulder substrates, but this substrate type was only dominant in 33% of all transects. The positive, yet imprecise, parameter estimate for substrate size is consistent with this pattern. Like other members of the *Etheostoma maculatum* species group within the subgenus *Nothonotus*, Wounded Darter are known to spawn on the underside of cavity-forming cobbles and boulders (Page 1985). Spawning occurs between late May and late July (Etner and Starnes 1993), which coincided with the sampling period in this study. Stiles (1972) found that optimum spawning habitats consist of layers of cavity-forming cobbles and boulders piled on top of each other, and we also observed our highest counts of Wounded Darter in these habitats.

The spawning behavior and habitat use of Wounded Darter made them very difficult to detect while snorkeling. In our study, Wounded Darter were frequently observed under rock cavities or with only their snout exposed, which emphasizes the importance of targeting these habitats during surveys. If a boulder has to be moved or flipped to see the cavity, we recommend that this is done slowly and carefully to minimize the chances that the Wounded Darter will rapidly swim out of the area and avoid detection. Additional studies are needed to determine if sampling outside of the breeding season would increase detection. The behavior and habitat use of Wounded Darter also has important implications for conservation, as their habitat is lost when cavities are filled by fine sediment (Osier and Welsh 2007).

Snorkel sampling versus electrofishing

Snorkel sampling has an obvious advantage over electrofishing because it greatly reduces handling stress and mortality, which is an important consideration when assessing the status of imperiled fishes (Bohl et al. 2009, Jordan et al. 2008,

Poos et al. 2007). Our study indicated that the relative effectiveness of these two methods differed among species and that snorkel sampling was comparable or in some cases superior to electrofishing for estimating site occupancy. Jordan et al. (2008) found that snorkel sampling was more accurate and precise than seining for estimating the abundance of *Etheostoma okaloosae* (Fowler) Okaloosa Darter. Thurow et al. (2006) documented higher detection rates for single-pass backpack electrofishing compared to daytime snorkeling for *Salvelinus confluentis* (Suckley) (Bull Trout), emphasizing that the relative effectiveness of these different methods varies across species and systems.

Although Tangerine Darter exhibited the highest detection and occupancy rates in our snorkel sampling, we never detected this species while electrofishing at sites where they were known to occur. With proper use of weight belts or SCUBA, visual methods allowed sampling of habitats that are too deep to sample effectively with a backpack electrofisher. In addition, the wider streams in which Tangerine Darter occur (Leftwich et al. 1997) make it easy for this large bodied and presumably swift species to escape the electric field. Peterson et al. (2005) documented elevated movement of Bull Trout in response to sampling by electrofishing, day snorkeling, and night snorkeling. While some Tangerine Darter may have escaped our snorkel sampling transects, we were able to detect them with a high probability and we often observed them swimming within close proximity to snorkelers.

Detection rates for Wounded Darter were higher for electrofishing, which likely reflected the difficulty of effectively searching rock cavities during snorkel sampling. Our protocol involved searching only the rock cavities that were visible as the snorkeler moved upstream through the transect, but all rock cavities are presumably sampled by electrofishing. It is tempting to advocate electrofishing over snorkel sampling, because fewer sites would have to be sampled to achieve a comparable level of precision (see below). However, we suspect that electrofishing is particularly stressful to Wounded Darters because of their association with rock-cavity habitats, which likely increases their time exposed to electrofishing (vs. fishes that rapidly flee) and their vulnerability to trampling by a sampling crew.

An important limitation of our snorkel sampling method is that it requires clear water to be effective. Water clarity affects sighting distance (Ensign et al. 1995) and therefore must also affect the probability of detecting a species when present (Thurow et al 2006). We found no evidence that visibility affected occupancy patterns in this study, presumably because visibility was generally good (>0.7 m) at our sample sites. However, there are many rivers and streams where poor water clarity will preclude the use of underwater observation techniques altogether. Electrofishing also may be compromised in these systems, particularly if fishes are actively netted by sight (Poos et al. 2007). Our protocol of electrofishing upstream of a stationary bag seine may be an effective method in these systems, provided that there is enough stream current to carry stunned fishes into the seine. This finding was consistent with Price and Peterson (2010), who found that electrofishing upstream of a seine was more effective than standard electrofishing for capturing benthic species (e.g., sculpins and darters), but was less effective for water-column species (e.g., minnows and bass).

Applications to monitoring

Our study illustrates the importance of accounting for incomplete detection in status assessments and monitoring. As discussed above, detection probability was relatively low for Blotched Chub. Although our estimate of occupancy was not precise for this species, the point estimate suggests that our raw snorkel survey data may have significantly underestimated occupancy. Future monitoring efforts for Blotched Chub would have to increase effort to get a more precise estimate of occupancy (MacKenzie et al. 2006). To help identify an optimal design yielding a precise estimate of occupancy, MacKenzie and Royle (2005) provided a table yielding the suggested number of surveys per site (K) for different combinations of detection probability (p) and occupancy (ψ). For Blotched Chub ($p = 0.10$, $\psi = 0.90$, rounded to closest values), the optimum number of snorkel surveys (transects in our study) per site is 34. Similarly, using our estimated values of ψ , p , K , and equation 6.3 in MacKenzie et al. (2006), the number of survey sites needed to achieve the desired level of precision can be estimated. For example, we estimate that 60 sites need to be sampled with snorkeling (34 transects per site) to achieve a desired level of precision of 5% for Blotched Chub occupancy.

In contrast to Blotched Chub, high cumulative detection rates from snorkel sampling of Tangerine and Wounded Darter resulted in almost identical values of occupancy from the raw survey data and models. While accounting for incomplete detection did not change conclusions about the status of these two species from raw survey data alone, it increased our confidence in the survey results. Furthermore, estimating occupancy and its associated variance provides an unbiased basis for assessing future changes in population status (MacKenzie et al. 2006). Increasing the number of sample sites for both species would result in greater precision for estimating occupancy and a more powerful monitoring protocol. Using the same approach as described above, an optimal snorkel sampling design for estimating Tangerine Darter occupancy requires sampling 10 transects at 59 sites to achieve a 5% level of precision, whereas an optimal design for Wounded Darter requires 9 transects at 135 sampling sites. Utilizing the detection estimate from electrofishing and holding K constant at 9 transects, we estimate that 102 sites need to be sampled to achieve the same level of precision for Wounded Darter. However, this gain in efficiency (i.e., fewer sites) would have to be weighed against the greater risk of electrofishing injury.

While these calculations are based on simplifying assumptions (e.g., p and ψ are constant), they provide a useful approximation for planning future surveys. Clearly, it would be difficult to optimize designs for all species. Based on our experience, we think that samples could be collected at 60 or more sites during a comparable time period if electrofishing surveys were eliminated, surveys were focused upstream of Lake Blue Ridge (decreasing travel time between sites), and a full time survey crew was dedicated to the project (our crew worked on a different project for half of the survey period). It would be difficult to sample the 34 transects needed to obtain a precise estimate of occupancy for Blotched Chub while simultaneously increasing the number of sample sites to 60. Adding more transects also is constrained by the amount of suitable riffle-run habitat within a site and the need to maintain adequate spacing between transects to minimize disturbance to fishes.

Similarly, it may not be feasible to sample enough sites for a precise estimate of occupancy by Wounded Darter based on our estimates. Consequently, it may be necessary to accept a lower level of precision when estimating occupancy of Blotched Chub and Wounded Darter. For example, sampling 9 transects at 53 sites for Wounded Darter would yield an 8% level of precision.

As an alternative to increasing effort, our snorkel sampling protocol could be improved to increase detection so that fewer sites and transects would have to be sampled. Two recommendations suggested by our results include spending more time searching cavities for Wounded Darter and searching shallow shoreline habitat for Blotched Chub. We also noticed that many fishes maintained position downstream of snorkelers, so floating downstream through a transect after completing the upstream search could improve detection for some species. Given our extremely low estimate of detection for Olive Darter, we do not believe that this species could be efficiently monitored in the Toccoa River system using our methods under any realistic scenarios of sampling effort or sampling refinement.

Our study demonstrated the use of snorkel sampling to estimate occupancy rates of rare fishes in a large southeastern river with good water clarity. Detection probabilities varied across our target species, which illustrates the importance of accounting for imperfect species detection when estimating site occupancy. We also identified habitat covariates that explained interspecific differences in detection and suggest improvements to our sampling protocol. Our snorkel sampling method was comparable or more effective than electrofishing for detecting our target species and provides additional advantages for assessing the status of rare or imperiled fishes.

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