The importance of determining species sensitivity to environmental change: a tree frog example

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Abstract. Conservation schemes typically place species into categories, ranging roughly from rare to common or from vulnerable to least concern. Although a species may not be categorized as rare or vulnerable, it still may be sensitive to environmental change and, therefore, at risk of decline. To illustrate the importance of determining species sensitivity, we documented the relative sensitivities of four species of tree frogs, none of which have been designated particularly rare or vulnerable, to environmental change. Our research was conducted in central Florida, where prior research had suggested that Dryophytes femoralis and Dryophytes gratiosus were more sensitive to environmental change than Dryophytes cinereus and Dryophytes squirellus. A comparison of species characteristics and a field study of local abundances supported the dichotomy. Particularly, important environmental factors affecting the two more-sensitive species adversely appear to be habitat selectivity, at a smaller scale, and nearness to the species’ southern range limits, at a larger scale. We suspected that one of the more-sensitive species, D. gratiosus, is particularly sensitive to environmental change and that the cause is related to reproduction and/or growth. A series of laboratory analyses suggested strongly that the realized production of adults was less for D. gratiosus than for D. femoralis. Despite their identical placement in categorization schemes, the four species exhibited substantial differences in sensitivity to environmental change. A strong case can be made that the degree of sensitivity of D. gratiosus warrants increased conservation attention for the species. Our results indicate the potential value of field work and the generation of species-specific information in improving categorization schemes, of broadening the categorization criteria typically employed, to accommodate sensitivity to environmental change, and of considering geographical scale in categorization schemes more than is done at present.

Key words: anurans; breeding habitat types; dispersal ability; environmental change; hylid tree frogs; population size; reproductive potential; species abundance; species sensitivity.

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Introduction

Species with severely restricted abundances and/or distributions traditionally have been viewed as rare species (e.g., Rabinowitz 1981, McCoy and Mushinsky 1992, Gaston 1994, Yu and Dobson 2000). Describing degree of rarity in an objective way has proven difficult, however (e.g., Davis et al. 1986, Munton 1987, Gaston 1994, Henle et al. 2004). One way to circumvent the problem is to substitute degree of vulnerability (e.g., endangered, threatened) for degree of rarity and then employ a suite of objective criteria—most still related to abundance and distribution—for categorizing species (e.g., http://www.iucnredlist.org/technical-documents/categories-and-criteria). Objective criteria admit only species proven to be most in need of conservation.
attention into the categories of higher vulnerability. Species not so proven are relegated to categories of lower vulnerability because they are not in trouble, but also because data adequate for addressing the criteria are not available. Although the problem of possible mis-categorization because of data insufficiency is well-recognized (e.g., Regan et al. 2000, Ocampo-Penuela et al. 2016, Fox et al. 2018), we are hard-pressed to list examples of dedicated attempts to follow up on such observations. Once species are relegated to categories of lower vulnerability, such as the IUCN (International Union for the Conservation of Nature) category Least Concern, we suggest that they are likely to be treated as all-alike and then largely ignored. Yet, highly vulnerable species could be hidden in categories of lower vulnerability simply because we do not know enough about them. Furthermore, some of the species hidden in these categories could be increasing in vulnerability because they are particularly sensitive to environmental change, and such increases are likely to go unnoticed. Categories of lower vulnerability should not be places where species go to be buried, metaphorically. One might conclude that the true location of the conservation graveyard is the Data-Deficient category; but we suggest that this categorization at least should prompt data gathering, whereas Least Concern is tantamount to writing a species off because we know it is safe. It would be a useful exercise to test this assertion.

Knowing which species are sensitive to environmental change—which is mostly the result of human development—even if they do not appear to be in trouble at present, would promote timelier and potentially more effective conservation actions (e.g., Hockey and Curtis 2008, Lees and Peres 2008, Smiley et al. 2012). If some species are particularly sensitive to environmental change because evolutionary choices have restricted their abilities to respond to the ultimate effects of change, such as habitat fragmentation, then we should be able to partition sets of species into functional types or trait guilds with different sensitivities (Lavorel et al. 1997, Davies et al. 2000, Henle et al. 2004, Williams et al. 2010, Jokimäki et al. 2014). But how can species that are particularly sensitive to environmental change be recognized? The answer to this question is central to maintaining biological integrity in a steadily developing world. Although much research has focused on this question—particularly on the effects of fragmentation—no clear answer is yet to emerge (e.g., Lees and Peres 2008, Williams et al. 2010, Quesnelle et al. 2014). Of the dozens of possible reasons for the differential sensitivities of species, only a few have broad empirical support. Building upon previous work, Henle et al. (2004) suggested, for fragmentation effects, that sensitive animal species should be characterized by some combination of low natural abundances (high individual area requirements), large population fluctuations, low reproductive potentials, intermediate or low dispersal powers, and specialized habitat requirements. These characteristics apply well to sensitivity in general (e.g., Davies et al. 2004, Julliard et al. 2004).

Here, we conduct an analysis of relative sensitivity to environmental change for a group of four congeneric tree frogs, none of which is listed as a species of special concern by any government or private conservation organization. Although the four species do not receive much conservation attention, previous studies (Wilson and Forras 1983, Delis et al. 1996, Guzy et al. 2012) have indicated that two of the species, *Dryophytes (Hyla) femoralis* (Pine Woods Tree Frog) and *Dryophytes gratiosus* (Barking Tree Frog), are more sensitive to environmental change than the other two species, *Dryophytes cinereus* (Green Tree Frog) and *Dryophytes squrelius* (Squirrel Tree Frog). Sensitivity in this case was determined by local population size reduction and/or local extirpation in response to both fragmentation itself (decreasing fragment size, increasing fragment distance) and the indirect consequences of fragmentation (e.g., reduction of water levels in remaining waterbodies). Potential indirect consequences of fragmentation are many and are variously important, depending upon the biology of the species of interest (Henle et al. 2004). Quesnelle et al. (2014), for example, suggested that factors such as landscape complementarity and road mortality may be more important for wetland amphibians than the loss of wetlands itself.

Using the characteristics of sensitive species listed by Henle et al. (2004)—natural abundance, habitat requirements, reproductive potential, dispersal ability, population fluctuation—we
conducted a comparison of the differences between the pairs of less- and more-sensitive species noted above. A targeted comparison is needed to elucidate the specific characteristics of these four species that underlie their differences in sensitivity (see Cushman 2006, Hamer and McDonnell 2008, Scheffers and Paszkowski 2012). We acknowledge, however, that the conclusions reached will depend somewhat upon the list of characteristics chosen. To determine if the results of the comparison of species’ characteristics were reflected in the field, we conducted an extensive study designed to document abundances and distributions of the species. Although the results of previous studies of sensitivity suggested a partitioning of the four species into pairs, we had reason to suspect that finer divisions could be made. Species accounts suggest that D. gratiosus possesses characteristics that make it even more sensitive to environmental change than D. femoralis. Based upon our observations of the two species, we hypothesized that the underlying cause of any difference in sensitivity of the two species lay in reproductive potential. Reproductive potential, which is the number of offspring that an individual can produce under optimal conditions, appears to be an especially important characteristic determining the sensitivity of frogs to environmental change (see Quesnelle et al. 2014). We conducted a detailed laboratory study of the reproductive biologies of D. femoralis and D. gratiosus to test our hypothesis.

METHODS

Study species

The four tree frogs are all within the genus Dryophytes and are, to a lesser or greater extent, all denizens of the Coastal Plain of the southeastern United States. Geographical ranges of D. cinereus and D. squirellus are larger and extend farther south than those of D. femoralis and D. gratiosus (Conant and Collins 1998). Two of the species, D. squirellus (2.2–4.1 cm) and D. femoralis (2.5–3.8 cm), tend to be smaller (SVL) across their ranges than either D. cinereus (3.2–5.7 cm) or D. gratiosus (5.1–6.7 cm; Conant and Collins 1998). Molecular phylogenetic analysis (Pyron and Wiens 2011) groups D. cinereus and D. gratiosus as sister taxa, D. squirellus as a sister taxon of that pair, and groups D. femoralis with the more northern species D. avivoca, D. chrysoscelis, and D. versicolor. Early species accounts in central Florida, where our studies were conducted, indicated that the ranking of local abundance, from highest to lowest, was, in general, D. squirellus, D. cinereus, D. femoralis, and D. gratiosus (Carr 1940, Wright and Wright 1949). More recent accounts from the region (e.g., Ashton and Ashton 1988, Conant and Collins 1998, Bartlett and Bartlett 1999, IUCN 2012) describe local abundance within native habitats of the two less-sensitive species and of D. femoralis as common to abundant and of D. gratiosus as less certain—because of its strong fossorial and arboreal tendencies (e.g., Delis et al. 2020)—but probably lower.

Species characteristics

We ranked the focal species on each sensitivity characteristic—natural abundance, population fluctuation, reproductive potential, dispersal power, and adult and breeding habitat requirements—using the best available information. For natural abundance and adult habitat requirement, we used information from Enge (1997), which compiled the Florida records of habitat distribution and relative abundance (common, uncommon, rare) in those habitats. The compilation spans 31 habitat types across three geographic regions (panhandle, northern peninsula, southern peninsula). Overall relative abundance of a species was calculated by adding together instances in which it was listed as common (=3), uncommon (=2), or rare (=1) for all habitats in which it occurred, and then dividing by 3 × the number of habitats. Adult habitat requirement (habitat specificity) was calculated as the proportion of habitats occupied. The focal species were ranked on natural abundance from high to low, and on adult habitat requirement from broad to narrow.

For breeding habitat requirement (habitat specificity), we used information from several sources (Gunzburger and Travis 2004, Rieger et al. 2004, Mitchell 2005a, b, Mitchell and Lanoo 2005, Redmar and Brandon 2005, Gunzburger 2007, Dodd 2013; R. Altig, personal observation). The ranking of species was determined from four inter-related characteristics and preferences: length of larval period and pond depth (which
indicate the risk of pond drying before metamorphosis, pond permanency (which often indicates the risk of predation on larvae), and surrounding vegetation (which may indicate the risk of predation on calling males). We do not know how these characteristics and preferences might correlate with reproductive success across the breadth of types of available breeding sites, however (see Gunzburger and Travis 2004). Length of larval period is highly variable, depending upon density of individuals and other factors (e.g., Leips and Travis 1994, Dodd 2013), and we used the longest reported period for our ranking. The focal species were ranked on breeding habitat requirement from broad to narrow. We did not use records of larval occupancy as an indicator of breeding site specificity, because it incorporates other factors, such as relative abundance of both adults and adult habitats in the area. Comparison of two studies in central Florida illustrates this problem. One study (Babbitt and Tanner 2000) ranked larval species D. squirellus, D. cinereus, D. gratiosus, D. femoralis, from highest to lowest abundance, but the other study (S. Gonzalez, unpublished data) ranked larval species D. femoralis, D. gratiosus, D. cinereus. The second study was of ponds associated with cypress domes, which did not meet preference criteria as breeding sites for some of the species, including the otherwise ubiquitous D. squirellus.

Reproductive potential is particularly difficult to estimate. At a minimum, it should include estimates of length of reproductive season, clutch size, clutch frequency, and clutch fertility, all of which vary under field conditions. Information on these variables is especially sparse for most frog species (e.g., Dodd 2013), and a surrogate, reproductive rate (mean clutch size × clutch frequency), typically is employed (e.g., Quesnelle et al. 2014). Available clutch size estimates for the focal species generally are correlated with body size, but are highly variable by location (e.g., Gunzburger and Travis 2007, Gunzburger 2015). Estimates, therefore, should come from a geographically proximate locations, and only eastern Virginia satisfies this requirement (Mitchell and Pague 2014). We assumed that, although the clutch sizes in eastern Virginia would not be the same as those in central Florida, the rank-order of clutch sizes would be. The focal species were ranked on reproductive potential from high to low. We did not incorporate clutch frequency into the ranking, because it is poorly known for the focal species. Any influence of clutch number on reproductive rate would depend on the precise number of clutches per species and the reduction in mean egg number of subsequent clutches, for which we have no information. Multiple clutches have been reported for D. cinereus and D. gratiosus. (Perrill and Daniel 1983), and our data suggest that multiple clutching is at least possible for D. femoralis in central Florida, based upon the time available. Because multiple clutching has not been confirmed for D. femoralis, however, the best available evidence suggests that if multiple clutching does occur in central Florida, it occurs predominantly for the species with greater energy reserves, the larger species. If so, then multiple clutching would simply reinforce the ordering of species resulting from clutch sizes alone.

Dispersal power (sensu lato) is the ability to find and occupy available living and breeding sites. Once again, a surrogate typically is employed (e.g., Quesnelle et al. 2014), this time home range, estimated from movement distances. Movement distances have been estimated by tracking individuals only for a few species of tree frogs (e.g., Pellet et al. 2006, Johnson et al. 2007), but these studies indicate that adults are capable of movements of hundreds of meters between breeding ponds and upland habitats (also see Lemckert 2004). Virtually, no tracking information is available specifically for adults of the focal species (Mitchell 2005a, b, Mitchell and Lanoo 2005, Redmar and Brandon 2005), and information on other potentially critical movements, such as dispersal of juveniles from natal sites, consists only of basic observations that juveniles can disperse widely (e.g., Mitchell 2005b, Redmar and Brandon 2005). As far as we know, only two studies have tracked adults, one of D. gratiosus individuals moving between two breeding sites (Murphy et al. 1993) and the other of D. gratiosus individuals moving (migrating) away from a breeding site (Delis et al. 2020). Dodd (1996) used capture distances of adults from the nearest potential breeding sites to estimate movement distances for the other three species. These latter kinds of data are not independent of other species characteristics, such as local abundance, and are accompanied by a
substantial number of additional caveats (e.g., Dodd 1996, Lemckert 2004). Nevertheless, the data on mean and maximum distances recorded by Dodd (1996) constitute the best available evaluation of dispersal ability for the three species. Data on movement distance of *D. gratiosus* came from the only source available (Delis et al. 2020). The focal species were ranked on dispersal power from high to low.

Documenting population fluctuation properly requires extensive time-series of population size estimates (e.g., Green 2003). Population size estimates obtained with rigorous methods largely are unavailable for the focal species (but see Pham et al. 2007, for *D. cinereus*), and no extensive time-series are available. Although not without potential problems (e.g., Bridges et al. 2000, Corn et al. 2000), the best available data come from records of the species identities and abundances of calling males at 21 sites in the southern Florida peninsula (North American Amphibian Monitoring Program, NAAMP; https://www.pwrc.usgs.gov/naamp/). The NAAMP calling index has been shown to correlate with mark-recapture population size estimates for several frog species (e.g., Nelson and Graves 2004). We chose the records for sites at which a focal species had been recorded and monitoring had taken place during the height of the breeding season in at least two years (range 2–6 yr): *D. femoralis*, one site; *D. cinereus*, seven sites; *D. squirellus*, five sites. No sites met the criteria in the case of *D. gratiosus*. For each species × site combination, we used \( \Sigma \) (chorus size per run) to estimate population size per year, and then calculated the mean and 95% CI across all years. We determined the lowest lower limit of a confidence interval for which any single run did not approach or exceed zero by simple inspection. We then counted the number of times, for all records in the database, that a species fell below the lowest lower limit of a confidence interval for which any single run did not approach or exceed zero, and the number of times that a species was expected to have been recorded at a site—because it was recorded there at least once—but was not. These two measures contributed equally to the ranking of population fluctuation from small to large.

**Field studies**

Field studies were conducted at three locations known to support all four of the focal species. Pine Flatwoods Wilderness Park (28°06′ N, 82°23′ W) is approximately 22 km² of managed pine flatwoods interspersed with areas of sandhill (see Myers and Ewel 1990 for descriptions of these two habitat types). County Line Road (28°06′ N, 82°26′ W) is approximately 10 km² of mixed-usage lands, including some residences and pasture, but mostly pine flatwoods. Green Swamp Wildlife Management Area (28°23′ N, 82°03′ W) is a large managed reserve, of which we surveyed approximately 10 km². The uplands, once again, mostly are pine flatwoods. Wetlands, which include freshwater marshes, temporary ponds, and cypress domes (see Myers and Ewel 1990 for descriptions of these habitat types), are numerous at all three locations. Because they occupy areas of low relief, these wetlands retain standing water for extended periods of time, especially during the summer and fall rainy seasons. We use the abbreviations PF, CL, and GS, respectively, for the three locations.

We determined relative abundances in two ways: by visual observation of individuals and by estimation of breeding chorus sizes, both along transects. Sampling of individuals was conducted by one of the authors and assistants for three consecutive breeding seasons at PF. The transect employed was a 20-km loop road through the location. Sampling began at or near dusk, 17–21 h, and continued through the night. Sampling effort was 427 total hours on 146 occasions. Representative individuals were captured whenever possible, to confirm visual identifications. When local densities were too high to allow individual counts, we estimated numbers of individuals from 6 × 3 m samples. We did not include individuals less than 20 mm snout-vent length (SVL), because they cannot be identified reliably. The smaller body sizes of *D. squirellus* and *D. femoralis* made it more likely that their numbers were underestimated.

Sampling of breeding choruses was conducted by one of the authors and assistants for one to three breeding seasons at the three locations. Sampling was conducted as much
as possible during or immediately following rains. Choruses were located by driving on roads traversing the locations at low speed and stopping when a chorus was heard. Sampling effort was 628 total hours on 156 occasions at a total of 83 waterbodies. We focused on each chorus heard for at least two minutes, and then recorded species and relative chorus size. When individual calls within a chorus were distinct, we recorded chorus size exactly; but, when individual calls overlapped and could not be discriminated, we classified a chorus as small, medium, or large, depending upon our perception of its intensity (see Zimmerman 1994, Bishop et al. 1997). We roughly scaled the classification using data from transect sampling: small chorus ≤10, medium chorus = 10 to a several dozen individuals, large chorus = several dozens to hundreds of individuals. Waterbodies at which at least one species was heard calling were subsequently searched for larvae of species that were not heard calling there by dip-netting.

**Laboratory studies**

We collected 56 amplexing pairs of *D. gratiosus* and 120 of *D. femoralis* from four breeding sites at PF and CL to obtain clutches of eggs. Each pair was transported to the laboratory, where we emptied the female’s bladder by applying light pressure over the ventral abdomino-pelvic surface, and then recorded her mass to the nearest tenth of a gram. The pair was then placed in a 4-L ventilated plastic box containing 1 L of water from the breeding site until the female had oviposited. All *D. gratiosus* females and all but one *D. femoralis* female oviposited. After oviposition, females were palpated to determine if eggs remained in their reproductive tracts, but retained eggs were never detected. Once a female had oviposited, her bladder was again emptied, and her mass recorded. All individuals were measured (SVL, to the nearest millimeter, and mass, to the nearest tenth of a gram) prior to release. Individuals were released at their points of capture within 48 h. Clutch mass was determined as the difference in female body mass before and after oviposition. Number of eggs per clutch was determined within 10 h of oviposition, as eggs were transferred to a shallow, wide plastic container, and total number of larvae hatched was determined three to four days later.

We raised larvae in the laboratory to estimate time to sexual maturity. Approximately two hundred larvae of each species were selected from multiple clutches and placed in shallow plastic pans. The larvae were maintained at 25°C under normal lighting conditions and fed rabbit chow ad libitum. When the larvae had successfully initiated metamorphosis (Stage 41; Gosner 1960), we selected twelve individuals of each species at random and transferred them to 9 cm diameter × 2.5 cm deep plastic pools of aged tap water contained within 18 × 10 × 14 cm plastic boxes. All larvae completely metamorphosed and moved out of the water within about 24 h, at which time we placed them into cages. We fed froglets suitably sized crickets ad libitum and measured SVL and mass each month. The time required to reach the minimum SVL of amplexing adults (*D. femoralis* males = 21 mm, females = 22 mm; *D. gratiosus* males = 53 mm, females = 52 mm) was taken as the estimated time to sexual maturity. Individuals of *D. femoralis* reached the minimum SVL within the time frame of the study, but individuals of *D. gratiosus* did not; therefore, minimum time to sexual maturity for *D. gratiosus* was estimated from known growth rates of other tree frog species (Jameson 1956, Ritke et al. 1991, Relyea and Hoverman 2003).

Because of the large number of total clutch failures of *D. gratiosus* in the laboratory, we repeated the analysis of clutch viability under conditions more like those at the breeding sites, and with as little manipulation as possible. We collected amplexing pairs of both species during a single month at one site at PF. We placed each pair in a 25-L bucket containing water from the breeding site and covered the bucket with screening. Pairs were removed from the buckets the following day, and the eggs were left undisturbed to develop. We determined total number of larvae hatched and number of undeveloped eggs three-four days after oviposition.

Data were analyzed with t-tests and Pearson product-moment correlations. Assumptions for these parametric analyses were met. Analyses were carried out with SAS software.
RESULTS

Species characteristics (D. cinereus, D. squirellus, D. femoralis, D. gratiosus)

Information from Enge (1997) showed that adults of *Dryophyes squirellus* are most widespread across habitats (99% of habitats in Florida, 100% of habitats in the southern peninsula), followed by *D. cinereus* (90%, 84%), *D. femoralis* (78%, 64%), and *D. gratiosus* (62%, 52%). The species were ranked on adult habitat requirements (habitat specificity) in this order (Table 1). Relative abundance estimates, also from Enge (1997), followed a slightly different trend: *D. squirellus* (82%, 85%), *D. femoralis* (72%, 77%), *D. cinereus* (69%, 70%), *D. gratiosus* (57%, 52%). Note that we could not discriminate effectively between two of the species; and in this case, and other, similar cases, we gave both species the higher ranking. The species were ranked on natural abundance in the order of the relative abundance estimates (Table 1). Incidentally, this ranking was reinforced by records from the NAAMP, which showed that *D. cinereus* and *D. squirellus* were heard at more sites (20 and 17, respectively) than *D. gratiosus* or *D. femoralis* (13 and 7, respectively). Records of relative abundance (Σ [chorus size per run per site], averaged over all sites at which it occurred) followed a similar trend, but with estimates for the latter two species virtually indistinguishable.

Based upon the four inter-related characteristics and preferences that we used to rank breeding site requirements (habitat specificity), the ranking, from least to greatest, was *D. squirellus* (shortest maximum larval period [50 d, 40 d minimum]; preference for shallow, temporary ponds; little vegetation preference), *D. femoralis* (relatively long maximum larval period [75 d, 40 d minimum]; preference for somewhat less shallow, temporary ponds; little vegetation preference), *D. cinereus* (relatively short maximum larval period [63 d, 24 d minimum]; preference for deeper, permanent ponds; preference for well-vegetated margins), and *D. gratiosus* (longest maximum larval period [160 d, 41 d minimum]; preference for deeper, temporary ponds; preference for well-vegetated margins; Table 1).

In eastern Virginia, *D. gratiosus* has the largest clutch size (2728 ± 66 eggs, n = 4), followed by *D. femoralis* (1290 ± 44, n = 65), *D. cinereus* (1272 ± 55, n = 41), and *D. squirellus* (1076 ± 230, n = 8). Sources referenced by Mitchell (2005b) indicated mean clutch size of *D. gratiosus* ranges from 1500 to 4000, but that lower numbers may reflect shortened amplexus. Gunzburger and Travis (2007) reported a similar mean clutch size for *D. gratiosus* in North Carolina as in Virginia, but a substantially reduced mean clutch size in northwestern Florida (1008 ± 249, n = 17). We found a mean clutch size in central Florida of 1990 ± 690 (n = 56), for *D. gratiosus* and 924 ± 432 (n = 120) for *D. femoralis*. Sources in Redmar and Brandon (2005) indicated mean clutch size of *D. cinereus* ranges from 700 to 2152, but that fecundities may be substantially higher because of multiple clutching. Gunzburger (2015) reported a similar mean clutch size for *D. cinereus* in northwestern Florida as in eastern Virginia. Sources in Mitchell

### Table 1. The four hylid species ranked on six characteristics potentially contributing to sensitivity to disturbance and on two estimates of abundance in the field.

| Characteristic/abundance                  | Ranking | Species            |
|------------------------------------------|---------|--------------------|
| Habitat requirement (adult)              |         |                    |
| Broad to narrow                          | 1       | D. squirellus      |
| High to low                              | 2       | D. cinereus        |
| Broad to narrow                          | 3       | D. femoralis       |
| High to low                              | 4       | D. gratiosus       |
| Natural abundance                        |         |                    |
| High to low                              | 1       | D. squirellus      |
| High to low                              | 2       | D. cinereus        |
| High to low                              | 3       | D. femoralis       |
| High to low                              | 4       | D. gratiosus       |
| Habitat requirement (breeding)           |         |                    |
| Broad to narrow                          | 1       | D. squirellus      |
| Broad to narrow                          | 2       | D. cinereus        |
| Broad to narrow                          | 3       | D. femoralis       |
| Broad to narrow                          | 4       | D. gratiosus       |
| Reproductive potential                   |         |                    |
| High to low                              | 1       | D. squirellus      |
| High to low                              | 2       | D. cinereus        |
| High to low                              | 3       | D. femoralis       |
| High to low                              | 4       | D. gratiosus       |
| Dispersal power                          |         |                    |
| High to low                              | 1       | D. squirellus      |
| High to low                              | 2       | D. cinereus        |
| High to low                              | 3       | D. femoralis       |
| High to low                              | 4       | D. gratiosus       |
| Population fluctuation                   |         |                    |
| Small to large                           | 1       | D. squirellus      |
| Small to large                           | 2       | D. cinereus        |
| Small to large                           | 3       | D. femoralis       |
| Small to large                           | 4       | D. gratiosus       |
| Abundance of individuals                 |         |                    |
| Many to few                             | 1       | D. squirellus      |
| Many to few                             | 2       | D. cinereus        |
| Many to few                             | 3       | D. femoralis       |
| Many to few                             | 4       | D. gratiosus       |
| Abundance of breeding choruses           |         |                    |
| Many to few                             | 1       | D. squirellus      |
| Many to few                             | 2       | D. cinereus        |
| Many to few                             | 3       | D. femoralis       |
| Many to few                             | 4       | D. gratiosus       |

Note: Species are ranked 1 (low sensitivity or high abundance) to 4 (high sensitivity or low abundance).
and Lanoo (2005) indicated mean clutch size of *D. squirellus* ranges from 950 to 1059. The ordering of clutch sizes reported from eastern Virginia appears to be the general case; so, we ranked the species on reproductive potential accordingly (Table 1).

Dodd (1996) listed the mean (maximum) capture distance from the nearest potential breeding site as 594 ± 188 (914) m for *D. squirellus*, 545 ± 181 (914) m for *D. cinereus*, and 266 ± 318 (815) m for *D. femoralis*. Delis et al. (2020) recorded mean (maximum) distances for radio-tracked individuals of *D. gratiosus* as 164 ± 119 (474). Again, we ranked the species on dispersal power accordingly (Table 1).

We obtained 13 time-series of population estimates for three species, *D. squirellus*, *D. cinereus*, and *D. femoralis*, from the NAAMP database, from which we calculated means and 95% CIs. Mean abundance (total chorus size) was correlated strongly with the position of the lower limit of the confidence interval, relative to zero ($r_S = 0.81, P < 0.001$). In general, variance that brings the lower limit near or below zero is associated with low abundance. The lower limit tended not to approach zero when the total chorus size exceeded 10. Abundances were lower than 10 only 23% of the time for *D. cinereus*, but 81% for *D. squirellus*, 89% for *D. femoralis*, and 92% for *D. gratiosus*. Percentages of occasions on which a species was expected to be recorded, but was not, were 3% for *D. cinereus*, 16% for *D. squirellus*, 26% for *D. gratiosus*, and 44% for *D. femoralis*. Combining the results of these analyses yielded the final ranking of the species on population fluctuation (Table 1).

**Abundance and distribution** (*D. cinereus*, *D. squirellus*, *D. femoralis*, *D. gratiosus*)

During the three years of study, the breeding season for *D. femoralis* tended to be the longest (5–8 months; maximally, February–October). The breeding seasons for the other three species were roughly similar in length (*D. squirellus*, 3.5–4 months, maximally June–September; *D. cinereus*, 3–4 months, maximally June–September; *D. gratiosus*, 2–5 months, maximally, April–August). Because of the species’ low abundance, the length of the breeding season of *D. gratiosus* could have been underestimated.

About 52% of the individuals encountered by transect sampling during the breeding season were *D. squirellus*, 31% *D. cinereus*, 16% *D. femoralis*, and 1% *D. gratiosus* (Table 1). Numbers of individuals of the other three species exceeded number of individuals of *D. gratiosus* on every sampling occasion. For *D. femoralis* and *D. gratiosus*, the mean number of individuals encountered per year was 819 ± 91 (range 757–924) and 39 ± 26 (range 9–56), respectively. After scaling numbers of individuals by mean mass (*D. femoralis* = 1.79 ± 0.52 g, n = 105 males and 106 females; *D. gratiosus* = 15.60 ± 2.79 g, n = 25 males and 25 females), total biomass of *D. femoralis* was estimated to exceed that of *D. gratiosus* by 2.5x, on average. *Dryophyes femoralis* formed the largest number of breeding choruses (550 recorded over three years), followed by *D. squirellus* (337), *D. cinereus* (203), and *D. gratiosus* (53; Table 1). Of 83 breeding sites that were monitored, *D. femoralis* was heard calling at least once at 78 of them, *D. squirellus* at 53 of them, *D. cinereus* at 38 of them, and *D. gratiosus* at three of them. Percentages of medium and large choruses ranged from 95% for *D. femoralis* to about 56% for *D. squirellus* and *D. cinereus* to 0% for *D. gratiosus*. Isolated calling males of *D. gratiosus* were sometimes detected at sites with no choruses. Larvae of a species were not detected by dip-netting at sites where males of that species were not heard calling.

**Reproductive biology** (*D. femoralis*, *D. gratiosus*)

Clutch sizes were larger for *D. gratiosus* than for *D. femoralis*. Mean clutch size for *D. gratiosus* was 1991 ± 690 and for *D. femoralis* was 924 ± 432 ($t = 13.1, P < 0.001, n = 176$). The largest clutches contained 4034 eggs and 1948 eggs, respectively. Although mean clutch size and mean female SVL could be shown to differ on occasion between locations and years, the relative differences between species were maintained. Within species, clutch size (log scale) could not be shown to be related strongly to female SVL for either species (*D. femoralis*, $r = 0.13, P = 0.14, n = 120$; *D. gratiosus*, $r = 0.14, P = 0.30, n = 56$; Fig. 1A). Clutch mass, on the other hand, comprised a larger percentage of body mass for *D. femoralis* than for *D. gratiosus*. Clutch mass comprised 29 ± 8% of the body mass.
mass of *D. femoralis* females and 21 ± 7% of the body mass of *D. gratiosus* females (*t* = 5.95, *P* < 0.001, *n* = 161). Body mass (log scale) tended to increase linearly with SVL for females of both species (*D. femoralis*, *r* = 0.55, *P* < 0.001, *n* = 120; *D. gratiosus*, *r* = 0.57, *P* < 0.001, *n* = 56; Fig. 1B). Hatching rates were lower for *D. gratiosus* than for *D. femoralis*. Of 56 *D. gratiosus* clutches maintained in the laboratory, 15 (29%) did not show any signs of embryonic development. Eggs in these clutches appeared to be arrested in early developmental stages (1–12; Gosner 1960). On
the other hand, of 120 *D. femoralis* clutches, only 3 (2%) did not show any signs of embryonic development. So, clutch viability rates for *D. femoralis* and *D. gratiosa*, respectively, were 98% and 77%. Mean hatching rate for viable clutches was 34 ± 32% for *D. gratiosa* and 73 ± 25% for *D. femoralis* (*t* = 7.77, *P* < 0.001, *n* = 151). In the field, 3 of 13 clutches (23%) of *D. gratiosa* did not show any embryonic development, but all 15 clutches of *D. femoralis* were viable. Hatching rates of viable clutches were similar between laboratory and field for *D. femoralis* but increased to 65 ± 27% in the field for *D. gratiosa* (*t* = 0.95, *P* = 0.241, *n* = 28).

So, best estimates of egg viability rates (of viable clutches in the field) for *D. femoralis* and *D. gratiosa*, respectively, were 74% and 65%.

Individuals of *D. femoralis* reached minimum adult body mass more quickly than those of *D. gratiosa*. Under ideal conditions in the laboratory, *D. femoralis* and *D. gratiosa* displayed mean post-metamorphic growth rates of 0.63 ± 0.06 and 0.42 ± 0.05 g/month, respectively (*t* = 6.31, *P* < 0.001, *n* = 15), after larval periods of about 44 and 55 d, respectively. All juveniles of *D. femoralis* reached minimum adult body mass, 1.1 g, by the fourth month after metamorphosis, and nearly all reached the maximum adult body mass observed in the field, 3.0 g, by the sixth month. By the sixth month after metamorphosis, juveniles of *D. gratiosa* had reached only 25–30% of minimum adult body mass (females = 8.4 g, males = 11.1 g). Using conservative growth projections, these results suggest that females require more than a year to reach minimum reproductive size. Although newly metamorphosed individuals of *D. gratiosa* were larger than newly metamorphosed individuals of *D. femoralis* (0.47 ± 0.04 g and 0.18 ± 0.04 g, respectively; *t* = 16.30, *P* < 0.001, *n* = 24), juveniles of *D. femoralis* had surpassed juveniles of *D. gratiosa* in body mass by the second month of growth.

We used the resulting data on clutch size, clutch viability, egg viability, and time to maturity to derive estimates of realized production of adults. Beginning with mean clutch sizes of 1991 and 942 for *D. gratiosa* and *D. femoralis*, respectively, we first applied clutch viability rates of 77% and 98% and egg viability rates for viable clutches of 65% and 74%. After application of these rates, mean effective clutch sizes were 996 and 661, respectively. Then, to account for time to maturity, we used the estimate that it takes 2–4 times longer for *D. gratiosa* to reach maturity and assumed that survival was about the same over time and between species. Because we do not know absolute survival rates, we can derive only a relative estimate of realized production of adults, which our results suggest is 1.3–2.7 times greater for *D. femoralis* than for *D. gratiosa*. This difference could be modified, however, by factors such as difference in juvenile survival rates, difference in propensity for multiple clutching, and the proportion of juveniles of *D. femoralis* reaching maturity in the same year as hatching.

**DISCUSSION**

The goal of the research was to determine if any of the four species of tree frog could be considered particularly sensitive to environmental change and, therefore, in need of special conservation attention. Using a three-pronged approach—documentation of relevant species’ characteristics, local field studies of abundance and distribution, and laboratory studies of reproduction—we were able to reveal important differences in sensitivity among the species. We found that the difference in sensitivity between two species pairs—*Dryophytes squirellus* and *D. cinerea* vs. *D. femoralis* and *D. gratiosa*—derives from a combination of ecological differences, salient among them being habitat specificity. Adults of the species discriminate variously in selecting both breeding and non-breeding sites. Calculation of Simple Matching (Jaccard Similarity) using the data compiled by Enge (1997) showed greater distributional similarity in Florida between *D. squirellus* and *D. cinerea*—the less-sensitive species (86%) and between *D. femoralis* and *D. gratiosa*—the more-sensitive species (85%) than between any of the other possible pairs (55–78%). The two more-sensitive species tend to co-occur in forested habitats, habitats that are among the most easily and commonly affected negatively by development (Homan et al. 2004, Gamble et al. 2006, Todd et al. 2009). Changes at breeding sites brought on by development, such as in hydrology and chemical composition, affect breeding sites directly (Adolfo et al.

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Brodman et al. 2003); but, because forested habitats need to be juxtaposed with breeding sites in particular arrangements, changes at breeding sites also affect the use of the forested habitats indirectly (Gagné and Fahrig 2007, Semlitsch et al. 2009). Other ecological differences between the two species pairs, such as apparent dispersal power, abundance, and population size variability, measure the potential of species to respond to these direct and indirect habitat changes. Species possessing multiple sensitivity characteristics are likely to be hypersensitive to environmental change (cf. Henle et al. 2004) and more prone to population decline (e.g., Collins and Storfer 2003, Hayes et al. 2010, Blaustein et al. 2011).

Of the two more-sensitive species, D. gratiosus displayed stronger indicators of sensitivity than D. femoralis in our comparison. Locally, D. gratiosus was less abundant than D. femoralis and was heard calling at fewer breeding sites and for shorter seasonal durations. The same pattern has been shown for other sites in central Florida (Delis et al. 1996, Babbitt and Tanner 2000). Furthermore, the relatively high level of breeding site discrimination suggested for D. gratiosus by our comparison (also K. Dodd, W. Meshaka, and P. Moler, personal observations) coupled with a relatively high degree of breeding site fidelity (Murphy 1994a, b) suggests that sites lost to environmental change could not be easily replaced. For example, a breeding site at PF used for more than a decade by both D. gratiosus and D. femoralis (personal observation) became useless to both, probably because of changes in hydropo- riod and invasion by predatory fish (personal observation; see Rieger et al. 2004). Dryophytes femoralis also bred at several nearby sites, but the nearest site used by D. gratiosus was about 2 km away.

A major contributor to the difference in sensitivity between D. gratiosus and D. femoralis appears to be reproductive potential. Although measured clutch size of D. gratiosus was more than double that of D. femoralis, the realized clutch size was smaller. The smaller realized clutch size of D. gratiosus can be attributed in part to poor egg viability, including total clutch failure. As well, the time needed to reach sexual maturity was estimated to be substantially longer for D. gratiosus than for D. femoralis, which potentially places froglets of D. gratiosus at greater risk. Relative reproductive potentials could be altered by multiple clutching, however. Although multiple clutching has been confirmed for D. gratiosus, the low abundance of males and the shortness of the breeding season may make multiple clutching rare or nonexistent at our sites. On the other hand, the length of the breeding season for D. femoralis may allow multiple clutching at our sites. Multiple clutching has not been confirmed for D. femoralis, but is known for a species of comparable size, Pseudacris regilla (Perrill and Daniel 1983).

Larger-scale processes may be contributing to the greater sensitivity exhibited by D. femoralis and D. gratiosus than the other two species because our study sites were near the southern limits of the geographical ranges of both species. Consequently, these two species may display reduced abundances and limited fine-scale distributions there, relative to more northern locations (see Cox 1993). Furthermore, if an edge effect exists, and it is stronger for D. gratiosus, then it might explain at least part of the extraordinary sensitivity that we found the species to exhibit. The distribution and abundance of D. gratiosus are reduced in the southern peninsula, compared to the rest of Florida (Enge 1997). Species’ descriptions (Ashton and Ashton 1988, Conant and Collins 1998) list similar lengths of breeding seasons for D. femoralis (April–September) and D. gratiosus (March/April–August), yet we documented a relatively short breeding season for D. gratiosus. Estimates of clutch sizes of D. gratiosus from farther north (e.g., Mitchell and Pague 2014) indicate they are larger, on average, than those in southern peninsular Florida. These differential local responses to geographical location may have provided a useful natural experiment, in which the species’ relative sensitivity to its environment is revealed in southern locations. In more northern and potentially less stressful locations, the species’ relative sensitivity might not have been recognized, or a study of sensitivity even considered worth undertaking in the first place. This point is not a new one (see Connell 1961a, b), but it is easily overlooked.

When sensitivity to environmental change is part of the equation, D. gratiosus appears to merit a closer look as a species of special concern. The strong selectivity of D. gratiosus, coupled with its limited reproductive potential, place it at a severe
disadvantage in the face of environmental change. We should not expect the species to do well when faced with climate change that may cause stress by adjusting distributional limits, or invasive species such as the Cuban Tree Frog (*Osteopilus septentrionalis*) that may cause population declines from which the species cannot recover. The other more-sensitive species, *D. femoralis*, has a higher reproductive potential, which may allow it to compensate for detrimental effects of environmental change such as these to a degree that *D. gratiosus* cannot. The categorization of *D. gratiosus* as a species of little conservation concern does not seem to be warranted. In fact, the species shares many characteristics with other amphibians that are protected (e.g., Sodhi et al. 2008, Roh et al. 2014).

Much of the work that has attempted to improve categorization schemes in general has focused on constructing categories (e.g., Regan et al. 2000) or on using new technologies (e.g., Ocampo-Penuela et al. 2016). Our study, on the other hand, indicates the potential value of field work and the generation of species-specific information in improving categorization schemes. Coordinating these approaches is likely to move the process forward synergistically. For example, the use of new technologies may provide a means of efficiently targeting field work (Brooks et al. 2019). Our study also indicates that some broadening of the categorization criteria typically employed may be warranted, to accommodate sensitivity to environmental change. Fine-scale habitat selectivity and reproductive potential proved important measures of sensitivity in our example, but we do not know how broadly these factors may apply. They could be unique to the environmental conditions found in central Florida or general features of the more-sensitive species. Because sensitivity may be location-specific, consideration of geographical scale may need to play a larger role in conservation schemes than it does at present. Nearness to range limits may have influenced the degree of sensitivity of species in our study, which is a potentially important scale-related issue considering the increasing potential of range contraction. Populations not near the range limits may not show the same degree of sensitivity. Important information about a species often comes from a small portion of its range, and how broadly that information applies is usually unknown. We had to use information from elsewhere to judge sensitivity and had to make assumptions about how it applied locally. We were careful to use this information judiciously and in conjunction with data gathered locally. Extending these ideas about geographical scale from species’ sensitivity to conservation categorization schemes in general suggests that using a single categorization scheme, such as the Red List, locally as well as globally, could promote misapplication of information when switching scales.

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