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APPLIED ISSUES

Assessing researcher impacts from a long-term sampling program of wetland communities in the Everglades National Park, Florida, U.S.A.

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SUMMARY

1. Long-term monitoring requires repeated visits to a study site, greatly increasing the potential for cumulative visitation effects. For ecological studies in general, and for monitoring in particular, data must be evaluated for confounding artefacts from researcher presence. We compared aquatic communities at long-term sampling plots (nine sites, each with three plots, studied continuously from 6 to 22 years) in the Everglades National Park to previously unsampled reference plots adjacent to them to assess the effects of researcher visitation on the flora and fauna.

2. We identified two criteria that are sensitive to local habitat heterogeneity for assessment of visitation impacts. First, the long-term plots must differ from adjacent reference plots by a magnitude that exceeded variation among plots separated by equal or greater distance (i.e. the difference is greater than expected by scaling of community change proportional with distance); and second, multiple reference plots must consistently differ in direction (e.g. greater abundance or less abundance) from adjacent long-term plots. We also tested for increased heterogeneity among samples from long-term plots compared with those not previously visited.

3. We found no evidence of researcher effects on fish or macroinvertebrates, and only weak evidence for alteration of emergent plants and periphyton floating mats. Our failure to document visitor impacts may result from either low visitation rate or the dynamic nature of the wetlands studied.

Keywords: impact assessment, long-term monitoring, visitation damage, visitor impacts

Introduction

Scientists can alter ecological systems simply by their presence through alteration of animal behaviour (Burley, Krantzberg & Radman, 1982; Metz & Weatherhead, 1991), attraction of predators (Westmoreland & Best, 1985; MacIvor, Melvin & Griffin, 1990; Mayer-Gross, Crick & Greenwood, 1997) or herbivores (Cahill, Castelli & Casper, 2001), and transmission of pathogens (Agrios, 1997). Motivated by such concerns, Cahill *et al.* (2001) proposed the 'herbivore uncertainty principle' that botanists cannot study plants without altering herbivory simply by their presence. This implies the need for experimental controls for the effects of plant handling in studies of herbivory. While a recent test failed to support the

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generality of this 'uncertainty principle' (Schnitzer *et al.*, 2002), the concerns it raises about bias and uncontrolled artefacts remain. Long-term ecological monitoring is an area with a seldom tested but key assumption that researcher activities do not alter the environment and processes being studied (Fourqurean & Rutten, 2003; Sauer, Link & Nichols, 2003). However, by its nature long-term monitoring requires repeated visitation and measurement in a circumscribed area to track temporal trends (Urquhart & Kincaid, 1999). Such repeated contact may yield cumulative effects from subtle impacts that are minor on the time scale of most experimental studies. Thus, monitoring is an area of ecology where unintended researcher impacts are most likely to be manifested.

The simplest approach to avoid damage from repeated visitation to a study site – expand the area where sampling takes place – may add variance to the data by confounding temporal and spatial heterogeneity. Sampling designs that avoid repeated visitation (Thornton, Saul & Hyatt, 1994), are appropriate for landscape-scale monitoring (Stoddard *et al.*, 1998; Urquhart, Paulsen & Larsen, 1998) but diminish the statistical power to detect temporal trends at a local spatial scale. Not all situations are served by such low-density sampling at a local scale. Clearly, a trade-off of spatial and temporal resolution must be reached in designing a monitoring effort, in addition to selecting field techniques that minimise habitat disruption (McDonald, 2003). However, important indicators of ecosystem status and trends, especially biotic parameters, cannot be measured without biologists entering the study area. In such cases, a protocol to periodically assess the status of monitoring sites relative to that of adjacent habitat should be considered part of the effort.

We propose that contemporary measurements must be made in 'reference areas' adjacent to long-term study plots but not previously visited by samplers, to assess the impact, if any, of repeated measurement. However, such measurements alone are not adequate to indicate an impact, because habitat heterogeneity renders no two locations the same in a natural ecosystem. Instead, results considered evidence of an impact include:

1 The long-term plots differ from adjacent reference plots by a magnitude that exceeds variation among plots separated by equal or greater distance (i.e. the difference is greater than expected by scaling of community change proportional with distance).

2 Multiple reference plots consistently differ in direction (e.g. greater abundance or less abundance) from adjacent long-term plots.

3 The scale of patchiness of species density is altered in long-term study plots relative to adjacent reference plots.

To illustrate these criteria, we present data on long-term study plots in wetlands of the Everglades National Park, along with comparative data from previously un-sampled reference plots adjacent to them. We present these comparisons as a model for assessing the impact of long-term sampling programs that accounts for local spatial heterogeneity in the resources being monitored.

Methods

Study sites and sample design

In October 2001 and July 2002, we sampled fish and aquatic macroinvertebrates, counted emergent plants, and estimated periphyton floating-mat volume at six sites located in northern Shark River Slough and three sites in Taylor Slough, Everglades National Park, Florida, U.S.A. The study sites were located in wet-prairie slough habitats dominated by spikerush, primarily *Eleocharis cellulosa* (Torr.). Other plants commonly found were maidencane (*Panicum hemitomon*, Schult.), arrowhead (*Sagittaria* spp.), and Tracy's beakrush (*Rhynchospora tracyi* Britton). Wet prairies in the Everglades have a distinctive floating mat of periphyton and, at times, bladderwort (mostly *Utricularia purpurea*, Walter). Three plots (A, B, and C; 'long-term study plots') comprised a study site that was sampled for at least 6 years prior to the 2001–2002 assessment. Plot sizes ranged from 35×35 to 70×100 m, depending on available wet-prairie habitat, and were separated from each other by distances ranging from 111 m to 2.62 km. Sites 06 (Plot A) and 23 (Plot A) were sampled monthly between 1977 and 1985, and all three plots at sites 06, 23, and 50 were sampled five times annually (February, April, July, October, and December) from 1985 to present. The other six plots were sampled five times annually since either 1994 (sites 07, 08, and 37) or 1997 (sites CP, MD, and TS). For each sampling event, seven random x–y coordi-

nates were selected for each plot and a single 1-m^2 throw-trap sample was collected at each. Thus, seven locations were sampled in each plot from 1225 to 7000 possible sampling locations and the chances of re-sampling the same 1-m^2 area were minimal. However, the field crew entered the long-term study plots during each sampling event, potentially trampling the sediment and surrounding vegetation. To evaluate the impact of these visits on the plots, new plots ('reference plots') were selected in an available wet-prairie habitat adjacent to one long-term study plot at each site as permitted by environmental managers. These reference plots were located between 46 and 560 m from their adjacent long-term plot. Seven random locations in each of these plots were sampled; these reference plots had not been visited in previous sampling efforts. We selected different reference plots in the 2 years, adjacent to different long-term plots at each site, to prevent possible impacts of re-sampling.

We estimated fish and aquatic macroinvertebrate densities, emergent plant stem density, and floating mat volume using a 1-m^2 throw trap (1.6 mm mesh size) following standard procedures (Jordan, Coyne & Trexler, 1997). After the trap was thrown, all emergent plant stems were identified to the lowest possible taxonomic level and counted, the floating mat was removed and its volume was measured in a 2-L graduated cylinder after draining excess water. Fish and macroinvertebrates were then removed using a 0.95 m by 0.45 m bar seine (1.6 mm mesh size). A minimum of five bar-seines were performed, and bar-seining continued until the process yielded three consecutive seines without capturing a fish, with the final seine containing no fish and no invertebrates. After completing bar seining, researchers used two dip nets to complete sampling of the throwtrap. The first was a D-ring net (1.2 mm mesh size) that was used to sweep through the water column. The second net (4.8 mm mesh size) was used to quickly scrape the benthic area of the throwtrap. Each net was used a minimum of five times; if an invertebrate was found on any of the five sweeps both researchers took one additional sweep. If a fish was found in any of the sweeps, five additional sweeps were performed using both nets. All fish and macroinvertebrates were killed by immersion in a concentrated solution of MS-222 (Tricaine Methanesulfonate, Finquel) and preserved.

Evidence of impact and statistical analysis

Solely noting that species composition of the reference plot differed from its adjacent long-term plot was not considered evidence of damage as a result of the long-term study because no two plots were expected to have identical species composition. We identified two patterns among plots A, B, C and X as evidence of potential impacts from repeated sampling. We denoted comparison of the reference plot (X) to its adjacent long-term plot as the 'local comparison' and comparisons among long-term plots at the same site as 'long-term plot comparisons'. At each site, if the local comparison was different from zero, we tested if the magnitude of difference exceeded inter-plot variation among the three long-term plots (Fig. 1a). Also, we considered a consistent direction of difference in the local comparison at most or all sites to be evidence of impact. We considered results consistent with both patterns as strong evidence of impact, while results consistent with only one pattern were suggestive of impact. Finally, Cahill, Castelli & Casper (2002) suggested that visitor impacts might increase the variance among replicates in experiments. We interpret this as affecting patterns of spatial patchiness for studies at the community level, probably increasing it, but the converse is also possible. Thus, we tested for evidence of difference in inter-sample variance for long-term plots compared with reference plots (local comparisons) to indicate this third form of visitor impact.

We analysed fish density and relative abundance separately to differentiate abundance patterns from community structure (relative abundance). All species-level data (fish, invertebrates, and emergent plants) were counts with many zeros, and we used fourth-root transformation to balance the influence of relatively rare and abundant taxa (Clarke, 1993). We created a dissimilarity matrix separately for fish, macroinvertebrates, and emergent plants from the species-by-sample data matrix. This dissimilarity matrix was created by calculating the amount of agreement at the species level for individual comparisons of plots following Clarke & Warwick (1994); dissimilarity between pairs of plots was

$$\delta_{jk} = 100\frac{\sum_{i=1}^{p}|y_{ij} - y_{ik}|}{\sum_{i=1}^{p}(y_{ij} + y_{ik})},$$

where 'j' and 'k' are the plots being compared and 'i' is the species. In other words, y_{ij} is the fourth-root

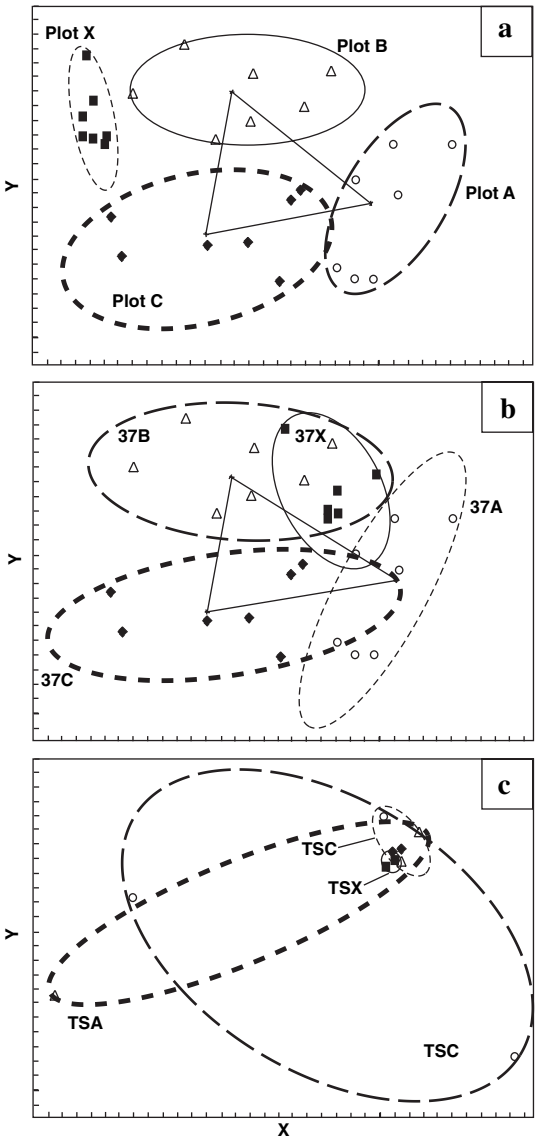

Fig. 1 An illustration of patterns considered indicative of effects by long-term sampling at our study plots. We produced these plots with non-metric multidimensional scaling (NMDS), which permits visualisation of comparisons made by ANOSIM. (a) Hypothetical NMDS plot illustrating evidence of impact of long-term study by a local comparison, in this case A versus X. Few examples of this pattern were found in our study. The solid lines indicate the differences between group means and the line connecting A and X is longer than those connecting A to B, B to C, or A to C. (b) MDS plot of the vegetation community at site 37 from 2001; the local comparison is A versus X. Note that samples from plot X are more similar to plot A, than A is to B or C, or B is to C. Although the local comparison is significant, it does not exceed the local spatial heterogeneity observed at the site. (c) MDS plot of the invertebrate community at site TS during the first year (2001) of the damage assessment; the local comparison is C versus X. Note the large inter-sample variance at plots A and B.

abundance of the *i*th species at the *j*th plot from the species-by-sample matrix and y_{ik} is the abundance of the same species at the *k*th plot. The dissimilarity matrix was subjected to an analysis of similarities (ANOSIM), followed by a similarity percentage routine (SIMPER) to identify, which species accounted for the observed assemblage differences (Clarke, 1993; Clarke & Warwick, 1994). The same analysis was performed using all plots at each location, to permit pairwise comparisons among long-term study and reference plots. Finally, to determine whether dissimilarity was induced by proximity between plot locations, we performed an ANCOVA with distance as a covariate to determine if the long-term plot regression differed from that generated from local comparisons (Fig. 2a). If plots B and X were the local comparison, then dissimilarity/distance values between A and X and, C and X were used for comparisons of revisited and virgin plots. Also, the dissimilarity/distance values between A and B as well as C and B were used as comparisons of revisited plots only. This created a balanced ANCOVA design for our analysis of distance as well as the type of comparison (revisited-revisited or revisited-virgin).

Our analyses of community composition identified species in each group (fish, macroinvertebrates, and plants) that were responsible for the majority of difference between long-term and reference plots. The density of each species identified in this way was log-transformed and subjected to a nested analysis of variance (ANOVA) with type III sums of squares to determine patterns of densities. We used Tukey's HSD (honestly significant difference) to identify pairwise differences. The difference of specific comparisons did not have to be statistically significant to be considered important if the direction of differences were consistent across sites. We used the Wilcoxon signed rank test (Hollander & Wolfe, 1973) to determine whether more comparisons were consistent in direction of difference than would be expected by chance.

We also investigated patterns of spatial patchiness in the long-term and reference plots for the species identified by our analysis of community composition. Deviation from the Poisson distribution is a common indicator of patchiness in ecological samples (Magurran, 1988). The variance and mean are equal in Poisson-distributed data, so a variance-to-mean ratio greater than one is a measure of aggregation of individuals. We estimated the variance-to-mean ratio from individual samples collected at a plot, and tested for equality

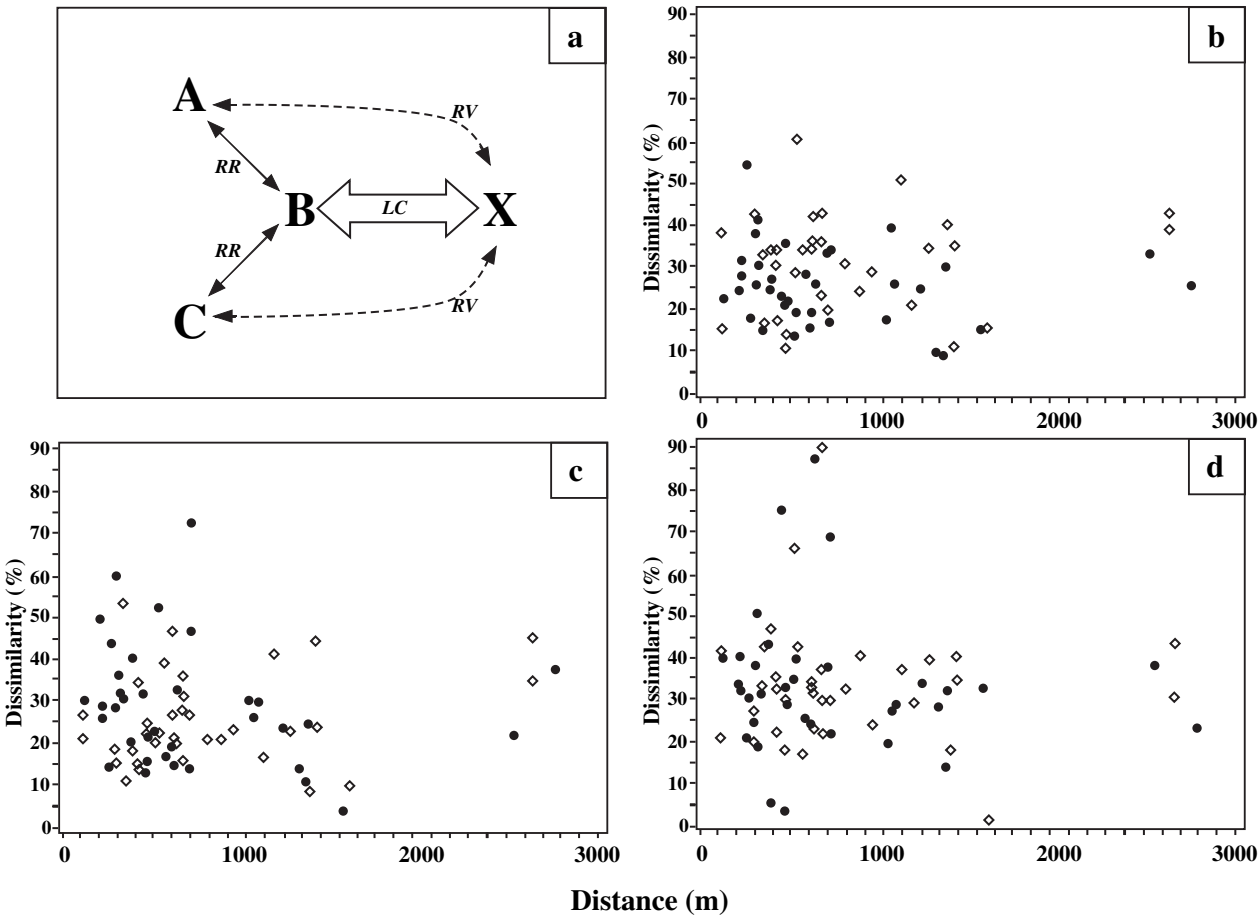

Fig. 2 (a) Illustration of balanced ANCOVA design. LC denotes the local comparison, RR denotes revisited plot comparisons and RV denotes revisited-virgin plot comparisons. (b–d) Comparison of distance versus dissimilarity between plots for the plant [b], fish [c] and macroinvertebrate [d] groups. RV comparisons are indicated by open diamonds (◇) and RR comparisons are indicated by filled circles (●).

between long-term and reference plots (local comparisons) using the Wilcoxon matched-pairs test (Hollander & Wolfe, 1973).

Results

Community composition

Emergent plants. The omnibus test of the local comparisons indicated that community composition (pattern of relative abundance) of emergent plants differed between the reference plots and their adjacent long-term study plots on average. Pairwise comparisons revealed that this difference resulted from three of nine sites in 2001 and five of the nine sites in 2002. In only two of these eight cases did the dissimilarity between the local comparisons exceed the maximum dissimilarity in the long-term plot comparisons (Table 1; Fig. 1b). The remaining ten comparisons displayed no difference between the adjacent long-term and reference plots. There was no indication that distance influenced the amount of dissimilarity between plots, and the ANCOVA did not indicate a difference of slopes between local comparisons and long-term plot comparison dissimilarities ($F_{1,71} = 1.05$, $P = 0.310$; Fig. 2b). There was a slight difference (0.5% dissimilar) between the revisited-revisited and revisited-virgin comparisons, but it was not significant ($F_{1,71} = 0.00$, $P = 0.953$).

Seven species were identified as contributing approximately 85% of the observed variance of emergent-plant stem density: *Eleocharis* spp. (spikerush); *Sagittaria* spp. (arrowhead); *P. hemitomon* (maidencane); *R. tracyi* (Tracy's beakrush); *Cladium*

Table 1 Summary of local comparisons of community structure indicated to be significant by ANOSIM

Group	Year	Site	R_{LC}	*P*
Plant	2001	7	0.589	0.002*
Plant	2001	37	0.301	0.010
Plant	2001	MD	0.333	0.003
Plant	2002	7	0.567	0.001
Plant	2002	37	0.33	0.016
Plant	2002	MD	0.462	0.004*
Plant	2002	TS	0.352	0.002
Plant	2002	23	0.352	0.004
Invertebrate	2001	MD	0.196	0.016
Invertebrate	2001	TS	0.229	0.028
Invertebrate	2002	6	0.399	0.001
Invertebrate	2002	8	0.165	0.033*
Invertebrate	2002	23	0.374	0.003
Fish	2002	6	0.454	0.001

Records marked with an asterisk indicate comparisons in which the dissimilarity of the local comparison exceeded the maximum dissimilarity among long-term plots at the same site.

jamaicense Crantz (sawgrass); *Paspalidium geminatum* Forssk. (Egyptian paspalidium); and *Potamogeton illinoensis* Morong (pondweed). Pairwise comparisons revealed numerous differences among plots within sites, although the three most abundant taxa accounted for most differences (*Eleocharis* spp., *P. hemitomon*, and *R. tracyi*). For all three species, local comparisons were more likely to be significant than those among long-term plots (Table 2). Higher densities of *Eleocharis* were observed at the reference plot in three of those five local comparisons (site 07 [2001], 23 [2002], & TS [2002]). For *P. hemitomon*, a higher density was noted at one reference plot with a significant local comparison (MD [2001]); the local comparison was significant for the same site in 2002, but with more *P. hemitomon* at the long-term plot in that year (note that we used different reference plots in the 2 years, see Methods). At all three sites, the density of *R. tracyi* was higher at the long-term plots. At the three sites where these differences were observed, there was at least one other significant difference among pairs of long-term plots, indicating marked inter-plot variation at this site. *Rhynchospora tracyi* was the only species indicating some consistency in the direction of change (more abundant in five of the six long-term plots where it was present). However, this did not yield a significant result because of low power (Wilcoxon signed rank test $Z = 1.44$, $P = 0.15$). This species was generally rare, comprising approximately 1.5% of the plant community overall and was not observed at 12 of the 18 study sites.

For the three most abundant plant species, we found no evidence that the within plot patchiness differed between long-term and reference plots. None of the Wilcoxon matched-pair comparisons were significant.

Floating mat. In addition to investigating emergent-plant stem counts, we analysed the volume of periphyton floating mat observed at each plot to test for effects of repeated sampling. Of these 18 local comparisons, 22% were significant, whereas 11% of the comparisons were significant at the long-term plots (Table 2). The local comparison was significant both years at site 23, whereas none of the long-term plot

Table 2 Planned comparisons of species indicated by SIMPER to display marked inter-plot heterogeneity

Group	Species	Significant local comparisons (%)	Significant long-term plot comparisons (%)	Significant local comparisons with higher densities at reference plot
Plant	*Eleocharis* spp.	5 (28)	14 (25.9)	3
Plant	*Panicum hemitomon*	3 (17)	11 (20.3)	1
Plant	*Rhyncospora tracyi*	3 (17)	10 (18.5)	3
Periphyton	n/a	4 (22)	10 (18.5)	1
Invertebrates	*Palaemonetes paludosus*	2 (11)	6 (11.0)	1
Invertebrates	*Pelocoris femoratus*	1 (5.5)	8 (14.8)	0
Invertebrates	*Procambarus* spp.	2 (11)	6 (11.0)	1
Fish	*Fundulus chrysotus*	1 (5.5)	0 (0.0)	1

Local comparisons were compared with long-term plot comparison variance estimated at the same site. There were 18 possible local comparisons (2 years at nine sites) and 90 long-term plot comparisons for each species. Numbers reported are total number of significant comparisons followed by the percentage of the total number of comparisons. The number of local comparisons with higher density at the X plot than the adjacent local plot is indicated in the last column.

comparisons were significant either year; site CP yielded the same pattern, but only in 2002. In all three of these cases, floating mat volume was greater at the long-term plot than at the reference plot. The remaining significant local comparison (site 08, 2002) revealed greater floating mat volume at the reference plot.

We found no evidence that the within plot patchiness differed between long-term and reference plots in the volume of floating mat. The Wilcoxon matched-pair comparison was not significant.

Fish. The omnibus test of the local comparison (ANOSIM) indicated that fish species-composition differed between the reference plots and their adjacent long-term study plots. The pairwise analysis indicated this difference resulted from only one of the 18 local comparisons. However, that comparison did not exceed pairwise comparisons among the long-term plots (Table 1). Seven species of fish were responsible for the majority of variance in our data: *Jordanella floridae* Goode & Bean (flagfish); *Fundulus chrysotus* Günther (golden topminnow); *Gambusia holbrooki* Baird & Girard (mosquitofish); *Fundulus confluentus* Goode & Bean (marsh killifish); *Lucania goodei* Jordan (bluefin killifish); *Heterandria formosa* Agassiz (least killifish); and *Poecilia latipinna* Leseuer (sailfin molly). Only one local comparison was significant of 126 tests (18 plot-site combinations for seven species). That significant result was observed for *F. chrysotus* in 2002, when the reference plot had more *F. chrysotus* than the adjacent long-term plot (0.51 individuals (ind.) m^{-2} at the reference plot and 0.0 at the long-term plot). The ANCOVA test did not indicate a difference of slopes between local comparisons and long-term plots dissimilarities ($F_{1,71} = 2.85$, $P = 0.09$; Fig. 2c) or difference between the type of comparison ($F_{1,71} = 2.11$, $P = 0.15$) and there was no indication that distance influenced the amount of dissimilarity between plots.

Three species were collected in greater abundance at the site 06 reference plot in 2002 than in the adjacent long-term study plot (*F. chrysotus*: plot C = 0.00 ind. m^{-2}, plot X = 2.71 ind. m^{-2}; *L. goodei*: plot C = 0.43 ind. m^{-2}, plot X = 1 ind. m^{-2}; *H. formosa*: plot C = 0.00 ind. m^{-2}, plot X = 0.86 ind. m^{-2}). *Jordanella floridae* was the only species more abundant at the long-term study plot than the adjacent reference plot at that location and time (plot C = 2.86 ind. m^{-2}, plot X = 2.14, 33.4% decrease). However, none of these species displayed a consistent direction of difference in abundance in the local comparisons at the remaining sites (no signed rank tests were significant).

For these seven species of fish, the Wilcoxon matched pairs test indicated no differences of patchiness between the long-term and reference plots. Also, there was no consistency in the pattern of patchiness recorded.

Macroinvertebrates. The omnibus test of the local comparison indicated that macroinvertebrate species composition differed between the reference plots and their adjacent long-term study plots. Pairwise tests indicated that two of the sites contributed to this difference in 2001, and three sites in 2002 (Table 1; Fig. 1c). In only one of these cases did the dissimilarity between the local comparisons exceed the maximum dissimilarity in the long-term plot comparisons (Table 2). The remaining 13 comparisons displayed no difference between the adjacent long-term and reference plots. In addition, the ANCOVA test did not indicate a difference of slopes between local comparisons and long-term plots dissimilarities versus distance ($F_{1,71} = 1.34$, $P = 0.84$; Fig. 2d).

Crustaceans [*Palaemonetes paludosus* Gibbes (grass shrimp), *Procambarus* spp. (crayfish)], insects [belastomatids, coenagrionids, coleopterans, *Pelocoris femoratus* Palisot & Beavois (alligator flea), larval *Coryphaeschna ingens* (Rambur), *Libellula needemi* (Westfall), and *Pachydiplax longipennis* Brumeister (dragonfly naiads)], and snails (*Planorbella duryi* Weatherby) contributed approximately 85% of the total variance among plots in our data. Our nested ANOVA indicated that the density of seven of these ten taxa differed among plots within sites and only three yielded significant local comparisons (*P. paludosus*, *P. femoratus*, and *Procambarus* spp.). For *P. paludosus*, local comparisons were more likely to be significant than those among long-term plots, although the number of significant comparisons was low (11%; Table 2). Furthermore, two significant local comparisons did not differ in the same direction. For *P. femoratus*, 5.5% of local comparisons were significant, while 8.9% of the long-term plot comparisons were significant. The frequency of significant local and long-term plot comparisons observed for *Procambarus* spp. was identical to that of *P. paludosus*, although the locations where significant results were noted were not the same and the two significant local

comparisons did not differ in the same direction. Finally, the variance-to-mean ratios were not consistently different between long-term and reference plots and no Wilcoxon matched pair test was significant. In summary, we observed no patterns of abundance for macroinvertebrates that was consistent with our criteria for researcher impacts.

Discussion

We have proposed a protocol that accounts for the spatial scale of heterogeneity at sampling locations when estimating the effect of researcher impacts on long-term study plots. We tested for evidence that the difference between a long-term plot and an adjacent reference plot exceeded the difference observed among pairs of long-term study plots. As an alternative design, one could take samples at multiple reference plots to estimate background spatial heterogeneity. Ideally, the reference plots would be separated in space by a similar distance and habitat matrix as the long-term study plots. As this study was restricted to one reference plot per site by National Park Service managers, we investigated patterns of distance versus dissimilarity between long-term plot comparisons and compared them to patterns from the local plot comparisons. We used an ANCOVA to compare the two patterns for the plant, fish, and invertebrate groups and found that the patterns of distance versus dissimilarity between plots did not differ.

We found no examples where the long-term study plots differed consistently from the adjacent reference plots at any of our long-term study sites. We used the Wilcoxon signed rank test based on counts of pairs of reference and long-term study plots with consistent sign of the direction of difference (but without regard to magnitude). The lack of consistent patterns of difference convinced us that there was no compelling evidence of general or widespread impacts of our visits. A similar lack of consistency at the species level was reported by Cahill *et al.* (2001). Following Cahill *et al's* (2001) results, we also tested for a change in inter-sample variance (and thus species patchiness) between long-term and reference plots, but found no evidence for such an effect in our data.

Only the volume of floating periphyton/bladderwort mat provided evidence of visitor impact in our study. In three comparisons of 18, including two from site 23, the local comparison exceeded the long-term plot comparison; at site 23, the difference was also in a consistent direction both years (the reference plots had less floating-mat volume than the long-term plots). However, even this effect was only observed in both years at one of nine study sites, and in one of 2 years at a second. While suggestive that our sampling may alter the floating mat at these sites, we did not consider this compelling evidence for general concern. *Eleocharis* spp. also provided some evidence of visitor impact by displaying significant local comparisons. However, the magnitude of difference seldom exceeded that observed in the accompanying long-term plot comparisons, the presence of a significant difference was seldom observed at the same site in both sampling events, and the direction of difference was not consistent across sites or time. We interpreted these results as indicating a patchy distribution, rather than as evidence of visitor impact. Finally, *R. tracyi* also gave a hint of impact from visitation by being present at low density in some of our long-term plots, but absent from the adjacent reference plots. However, it was a relatively rare species and this pattern could not be differentiated from a simple effect of chance sampling with the current data. We found no evidence of researcher impact in our macroinvertebrate or fish data.

Is our result of little or no researcher impact at long-term study plots likely to be a general result? We believe that this may be so for dynamic wetland habitats studied at similar intensity. Much of the Everglades ecosystem dries for a portion of the year at least one of 10–20 years, because of periodic droughts (Fennema *et al.*, 1994). These drying events affect both the animal and plant communities by causing extensive mortality (Loftus & Kushlan, 1987; Trexler *et al.*, 2001), but also affect soil and nutrient dynamics. In extreme cases, portions of the ecosystem burn each dry season (although none or our study sites has burned over the course of long-term study). Frequent disturbance may shape plant community structure in some aquatic systems (Capers, 2003). Similarly, we believe physical processes have a greater influence on the ecological dynamics at our long-term plots than did our pattern of visitation, which was relatively low in comparison with other visitation studies (Cahill *et al.*, 2001, 2002; Schnitzer *et al.*, 2002; Hik *et al.*, 2003). Researcher impacts may be more problematic in aquatic systems with greater

temporal stability or experiencing higher visitation rates.

Our plots varied in the length of time they have been sampled, from 6 years (30 visits) to 22 years (approximately 160 visits). However, we found no relationship between the length of study and evidence for researcher impacts. While the site where we found evidence of visitor impact on floating mat volume included one of the longest sampled plots, that plot did not deviate from its partners (A versus B or C). Also, site 06 has experienced a similar length of study as site 23, but displayed no similar evidence of impact; site 50 also has a very long history of study and failed to yield evidence of damage. The other site with some evidence of impact, site CP, has been studied for a much shorter time (6 years).

Although our study indicates that efforts to minimise researcher impacts to long-term study plots in the Everglades are adequate, we support a broad interpretation of the 'uncertainty' concept proposed by Cahill *et al.* (2001). While Schnitzer *et al.* (2002) provide convincing evidence that Cahill et al.'s concern for herbivore attraction is not universal, the broader issue remains. Mechanisms of inadvertent researcher impact are known and cannot be ignored in the absence of proof to the contrary. A prudent researcher will identify sources of artefacts and bias, rank their severity based on past experience, uncertainty and risk of error they pose, and design a study with controls for those considered of pressing concern. While this subjective view is not satisfying for those seeking a 'water tight' study, at some point all research efforts are confronted with limitations of staff, time, or other resources. We point to long-term monitoring as a type of ecological research that may be especially susceptible to cumulative researcher impacts and especially in need of controls to monitor the monitoring.

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