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Underwater video surveys provide a more complete picture of littoral fish populations than seine samples in clear Florida springs

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Abstract. Traditional fish-sampling methods may be problematic because of public use or safety concerns. In this study, we compared one common sampling method with video assessment of fish abundance and diversity in three springs that differed in water clarity and structure. At each of four or five sites per spring, we placed one GoPro camera on each bank for 12 min and followed the filming with seine sampling. On the video, we counted the maximum number of individuals of each species observed within one frame (MaxN) and summed these counts to produce an estimate of fish abundance (SumMaxN). Then we compared abundance (SumMaxN), species richness and diversity between seine and video samples across all three springs. Video produced higher estimates of abundance (SumMaxN), species richness and diversity differences between sample methods in the structurally complex spring; differences were subtle or non-existent in the low-structure spring and in the turbid spring. In all three springs, video captured relatively more centrarchids; these taxa were captured only rarely in seine samples. Therefore, video sampling performed as well or better than did seine sampling for fish-assemblage assessment in these clear springs.

Additional keywords: diversity, macrophyte structure, species richness, water clarity.

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Introduction

Every sampling technique has its biases. Traditional methods of sampling freshwater fish include various nets and traps, electrofishing and direct visual observation (Portt et al. 2006; Bonar et al. 2009). These methods have been used effectively in monitoring fish assemblages and in showing changes in fish populations over time and space (e.g. Marsh-Matthews and Matthews 2000; Oberdorff et al. 2001; Kennard et al. 2006). However, some fish are too agile to be caught in a seine, may avoid traps, or be galvanonegative and, thus, avoid electrical fields or stay on the bottom when shocked (Portt et al. 2006; Bonar et al. 2009), producing a somewhat skewed picture of the fish assemblage. Sampling accuracy can be improved with the addition of a second sampling method that possesses a different bias. For example, Kennard et al. (2006) measured higher catch rates of Australian stream fish with multiple passes of an electroshocker, but they achieved even higher catches with the addition of seine sampling as well. Ideally, fish populations would be assessed with multiple techniques, but this approach often is not viable for logistical reasons, such as the time required to use more than one sampling technique (Ebner and Morgan 2013; Ebner et al. 2014), particularly when time required to travel from site to site is substantial. In some situations, traditional methods of fish sampling may not be viable

at all. For example, Ebner *et al.* (2015) cited concerns of potential crocodile attacks during traditional fish sampling in lowland areas of Australia and King *et al.* (2018) cited concerns of injury during climbing with heavy fish-sampling equipment to highland tropical streams. Furthermore, in systems with significant conservation concerns, traditional techniques for sampling aquatic assemblages may harm sensitive species and ecosystems (Ebner *et al.* 2009; Ellender *et al.* 2012; Fulton *et al.* 2012).

Underwater video presents an alternative to traditional methods of sampling fish, one that may reduce field effort and make sampling more nimble, while, at the same time, reducing organism disruption and providing additional information on behaviour (Butler and Rowland 2009; Ebner et al. 2009, 2014). Many marine fish and crustacean researchers have turned to underwater video to sample marine systems that are logistically challenging, such as deep or complex areas (Harvey et al. 2012; Lowry et al. 2012; McIntyre et al. 2015; Stobart et al. 2015). Freshwater fish research, which has been dominated by electrofishing in many regions, presents its own set of challenges (Ellender et al. 2012). In Australia, concerns about harm to sensitive species, the difficulty of working in remote locations, and the potential of saltwater crocodile attack, have led several researchers to undertake evaluations of underwater video for freshwater fish surveys (Butler and Rowland 2009; Ebner et al. 2014). Similar to the marine studies, direct visual observation by snorkelling produces a higher species richness than does underwater video (Ebner *et al.* 2015), and underwater video produces a higher species richness than does beach seining (Ebner and Morgan 2013). King *et al.* (2018) found that fixed underwater video produced a species richness comparable to that produced by traditional sampling methods, which included direct visual observation and pop nets. In South Africa, Ellender *et al.* (2012) detected target species more often with underwater video than with electrofishing (Ellender *et al.* 2012). In the lone North American study of underwater video, Frezza *et al.* (2003) had only moderate success in surveying fish in Ontario lakes as a result of high-flow, shallow water, and coarse substrate obstruction of video.

Florida springs are heavily used by recreationalists, American alligators (Alligator mississippiensis) and, in some cases, Florida manatees (Trichechus manatus latirostris). As a result, in many springs, electrofishing is impossible and seining is discouraged, making remote underwater video attractive. The water in many Florida springs is quite clear, but many springs have abundant macrophytes, algae or snags that may obstruct a camera's field of view. In this study, we compared the relative abundance, species richness and diversity of fish between traditional seine samples and fixed underwater-video samples in three springs that varied in water clarity and structure. We hypothesised that underwater video would produce higher values of all three measures of fish abundance and diversity than would seining under the optimal conditions of clear water and little structure, but that the benefit of video would disappear when the video was compromised by poor water clarity or structure that may reduce the field of view of the camera.

Materials and methods

Study sites

Volusia Blue Spring is a large spring that produces a high discharge ($\sim 4 \text{ m}^3 \text{ s}^{-1}$) into a wide and moderately deep spring run ($\sim 30 \text{ m}$ wide, $\sim 1-3 \text{ m}$ deep). The substrate of the run is a mixture of exposed limestone, sand and organic material, including seasonal thick algal beds, leaves and decomposing material (Fig. 1). The banks of the run are lined with a mixed live oak (*Quercus virginiana*) and sabal palm (*Sabal palmetto*) forest. These trees regularly fall into the run, providing the majority of the structure in the run. Although some of these logs, as well as algae and some emergent shrubs, could obscure the view of the camera, much of the view is undisturbed for wide expanses.

In contrast, Rock Springs has a much smaller discharge $(\sim 1.6 \text{ m}^3 \text{ s}^{-1})$ and the run is confined by the banks to a narrower run than that of Volusia Blue Spring $(\sim 10-20 \text{ m wide}, \sim 0.8-1.5 \text{ m deep})$. Unlike Volusia Blue Spring, which has a large underground vent, the water at Rock Spring leaves a cave in a small limestone bluff. The substrate of the run is sand and gravel, with large limestone rocks dotting the upper portion of the run. Abundant eelgrass (*Valisneria americana*) lines the run along its length, along with emergent macrophytes (Fig. 1), such as pickerelweed (*Pontederia cordata*) and spadderdock (*Nuphar luteum*). The riparian vegetation is a mixture of wax myrtle (*Morella cerifera*) and willow (*Salix caroliniana*), as well as some larger trees. The run was widened to produce a pool

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Fig. 1. Bluegill (*Lepomis macrochirus*) and redear sunfish (*Lepomis microlophus*) at the headspring of Volusia Blue Spring (top), a blue tilapia (*Oreochromis aureus*) in the run of Rock Springs (middle), and bluegill and largemouth bass (*Micropterus salmoides*) in the run of Gemini Springs (bottom).

 \sim 500 m from the headspring, but the run narrows again into the original spring run \sim 800 m from the headspring.

Finally, Gemini Springs has the lowest discharge $(\sim 0.3 \text{ m}^3 \text{ s}^{-1})$ and is the most modified of the three springs. Two small vents provide the flow into a narrow, sandy run $(\sim 10 \text{ m} \text{ wide}, 0.2-0.5 \text{ m} \text{ deep})$, which widens into a large pool maintained by a human-made weir. Much of the riparian vegetation has been removed from the banks of the run; mixed live oak and sabal palm forest remains set back from the banks of the spring. The small natural run, but not the pool, contains beds of macrophytes. Instead, the pool and the run just below the weir are silty and turbid with little vegetation or structure other than the weir (Fig. 1).

Comparisons between seine and video samples

At each spring, we sampled four or five sites, ~ 150 m apart down the length of each spring run, starting at the headspring. At each of the sites, we deployed one GoPro camera on each bank for 11–15 min. We placed the cameras in areas that were near structure, but that had reasonably open fields of view. At the end of the video period, we seined from ~ 3 m from the bank (3×1 m with $\times 3$ -mm mesh) towards each camera, capturing the fish within the field of view of the camera. We identified the fish and released them on site. We visited each spring twice and collected all regular paired video and seine samples in July and August of 2017, with the exception of the second sample at Rock Springs (collected in February 2018) because of high human use of the narrow spring that restricted our access in summer.

After we downloaded the videos from the cameras, we recorded all of the species observed over the course of 10 min to estimate species richness. We also recorded the maximum number of individuals of each species observed within one frame of each video (MaxN) during that 10-min period. We used these MaxN numbers as an estimate of abundance for each species to calculate diversity by using a Shannon-Wiener diversity index. We also summed all of the MaxN numbers for each species for each sample as an approximate measure of total fish abundance (SumMaxN) in each sample. We used twofactor ANOVA to test for the main effects of spring and sampling method (seine vs video) as well as the interaction of spring and sampling method on (1) fish abundance (SumMaxN), (2) species richness and (3) diversity of fish. For each of the three ANOVA tests, we nested the sampling site within the variable 'spring' because the sample sites occurred in comparable longitudinal gradients on each spring. Unlike diversity, SumMaxN and species richness deviated from a normal distribution, so we applied a natural log (ln) transformation to these two variables before the ANOVA. To validate the use of parametric ANOVA, we determined that the variances among groups were equal with Barlett's tests and that the residuals of each group were normally distributed with Shapiro-Wilk tests.

To determine whether the composition of the 'catch' differed between video and seine samples, we used principal-component analysis (PCA) to create new variables that represent the composite fish assemblage. We omitted species that occurred in fewer than six samples and applied a ln transformation to the species counts. For this analysis, we used the data of all three springs on both sampling dates and then identified the samples on a biplot. To determine whether PCA scores differed between springs and sample types, we compared the scores of each of the first two axes with a two-factor ANOVA using spring and sample type as the main effects.

To evaluate the ability of the cameras to detect species in a 10-min video, we collected five 30-min videos at the springs on three occasions (one camera at Volusia Blue Spring and two cameras at each of the other two springs) to determine when the detection rate was saturated. Over the course of the 30 min, we recorded when species appeared and kept a cumulative total count of species. From these counts, we produced cumulative species richness curves and the point at which all species were observed was considered the 'saturation' point. Furthermore, we recorded when we detected each species in each video (fifty-one 12-min videos plus five 30-min videos) to determine the average detection time for each species.

To test the performance of the cameras under different circumstances, we also measured the approximate size of the field of view of the camera on 2 days in July 2017. We laid transect tapes perpendicular to each other and walked the length of each transect tape, stopping at every metre on each tape for the width and the depth. On the tape parallel to the bank, we recorded the location on the tape where the walking feet appeared. On the tape perpendicular to the bank, we recorded the point at which the feet became unclear as they walked away from the bank. We also measured the ability of the camera to detect certain colours (particularly red) by filming lures tied to a piece of rebar at 0.5-m increments away from the camera. We selected 8-cm lures that had colour contrasts similar to those of fish that we were identifying in the spring (black and green and tan, counter-shaded grey and white, tan with 3-mm red spots). At increments of 0.5 m from the camera, we recorded whether we could detect the different colours on the lures. From these distances, we determined at what distance the 'fish' were no longer identifiable by colour. We also tested the performance of the video relative to seine samples in direct sunlight relative to under heavy canopy coverage. On three occasions, we collected seine and video samples as described before, except that we collected one set of samples in an area of direct sunlight, and one set directly across the run in an area with thick canopy coverage. We compared the main effects of canopy coverage and sample type on fish abundance (SumMaxN), species richness and diversity of samples with a two-factor ANOVA. We ran all statistics on JMP (ver. 10.0.2, SAS Institute, Cary, NC, USA).

Results

Comparison of the detection of fish among springs

The maximum number of individual fish detected (SumMaxN) differed among springs (P = 0.0004, Fig. 2), although the heterogeneity of fish assemblages did not differ among springs, either in terms of species richness (P=0.34) or diversity (P = 0.23). However, all three measures of the fish assemblage differed significantly between video and seine sample methods. We detected more individuals (P = 0.031) and species (P = 0.0002), and we calculated a higher diversity (P = 0.0005)in video samples than in seine samples. The higher abundance in video samples was consistent across springs (SumMaxN interaction between spring and sample type: P = 0.27), although this effect was slight because none of the multiple comparisons between sample methods in the three springs was significant. In contrast, the identity of the spring influenced the degree to which video samples had a greater richness (interaction between spring and sample type: P = 0.015) and diversity (interaction between spring and sample type: P = 0.028) than did seine samples. Both measures of heterogeneity were higher in video samples from Rock Spring, they were similar between the methods from Blue Spring, and they were virtually identical for video and seine samples from Gemini Springs (Fig. 2).

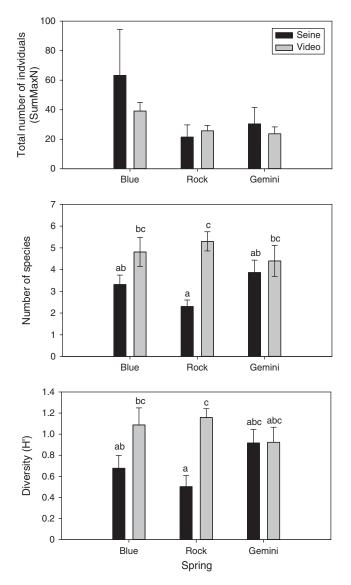


Fig. 2. The difference in the maximum number of individuals counted (SumMaxN), species richness and Shannon–Wiener diversity between seine and video samples in Volusia Blue Spring, Rock Springs and Gemini Springs (mean \pm s.e.). Bars that do not share letters are significantly different in Tukey's multiple-comparison tests.

Variation in fish-assemblage composition between sample types

The first three axes of the PCA analysis explained 58% of the variation in fish-assemblage structure. Centrarchids (sunfish and largemouth bass) loaded highly on the first axis, whereas fundulids loaded highly on the second axis and poeciliids loaded highly on the third axis (Table 1). Across all three springs, the fish-assemblage composition in the video and seine samples differed, even for springs in which the maximum number of fish detected, species richness and diversity did not differ significantly between the sample types (Fig. 3). Across the three springs, PCA Axis 1 differed significantly between video and seine samples (P = 0.0013), but not among springs (P = 0.19,

Table 1. Loadings of fish species on the axes produced by the principal-component analysis (PCA) of seine and video samples at the three springs

These loadings represent correlations of the species with the axes. The first three axes of the PCA explain 58% of the variation in the fish abundance

Species	Axis 1	Axis 2	Axis 3
Gambusia holbrooki	-0.33	0.39	0.51
Poecilia latipinna	-0.25	0.64	0.49
Heterandria formosa	-0.28	0.29	0.60
Lucania goodei	-0.45	0.46	-0.21
Lucania parva	-0.29	0.75	-0.36
Fundulus seminolis	-0.08	0.64	-0.64
Notropis sp.	0.01	-0.11	0.05
Lepomis macrochirus	0.72	0.34	-0.02
Lepomis microlophus	0.73	0.43	0.15
Lepomis auritus	0.75	0.08	0.15
Lepomis punctatus	0.75	0.26	0.10
Micropterus salmoides	0.75	0.06	-0.09
Variance explained by axis (%)	27.4	18.5	12.6

interaction P = 0.22). In contrast, PCA Axis 2 did not differ between sample types (P = 0.75), but it did differ among springs (P = 0.0009, interaction P = 0.081). The positive correlation of centrarchids (sunfish and largemouth bass) with PCA Axis 1 indicated that greater detection of these species largely accounted for the difference in composition between the two types of sample (Fig. 3).

Evaluation of video limitations

We detected most species on video in well under 10 min (Table 2) and, in two of the springs (Volusia Blue and Gemini Springs), we detected all of the species that were observed over a 30-min video in less than 10 min (Fig. 4). At the more structurally complex Rock Springs, one species was detected at 19 min in one 30-min video and two species were detected at 16 and 25 min in the other 30-min video (Fig. 4). All other species were observed in less than 10 min on the Rock Spring videos.

With respect to the area sampled by the two methods, the video-sample area was comparable in size to the seine sample area. We were able to confidently identify 'species' (using lures as models) within an area of 4.5×3 m; this area was slightly larger than our $\sim 3 \times 3$ -m seine sample. Although we could identify the 'species' to a distance of 3 m, some of the species' characteristics were lost farther from the camera; the red dots on one of the lures disappeared within 0.5–1 m.

Finally, the presence of tree canopy cover over the cameras had no significant effect on the estimate of fish abundance (SumMaxN, P = 0.21) or species richness (P = 0.07) and the two sample methods were similar for fish abundance (P = 0.12, interaction P = 0.47) and species richness (P = 0.24, interaction P = 0.27) under both sun and canopy-covered conditions. Similarly, the main effects of tree canopy cover (P = 0.21) and sample type (P = 0.066) had no significant impact on diversity. However, the interaction of light and sample type was significant (P = 0.016); we calculated the highest estimates of diversity from the video samples collected in the light.

Video survey of freshwater fish

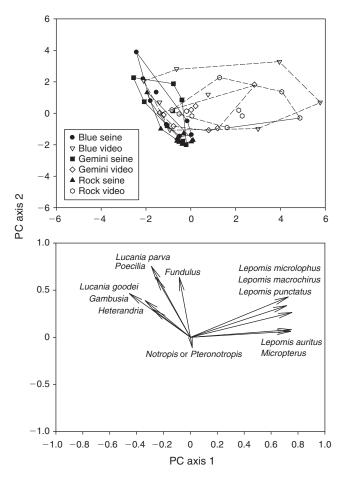


Fig. 3. Plots of the principal-component analysis (PCA) of fish-assemblage structure of Volusia Blue Spring, Rock Springs and Gemini Springs, indicating the locations of the sample types for each spring in principle component (PC) space (top) and the loadings of species on the axes (bottom). The lengths of the arrow represent the magnitudes of the loading, which in turn represent the degrees of correlation with the axes. The first two axes explain 58% of the variability in the data.

Discussion

In all three springs, the video samples produced abundance, species richness and diversity estimates that were comparable to or higher than the estimates from the seine samples. In general, video and seine samples 'captured' similar numbers of individual fish and it appeared that the videos 'sampled' an area comparable to that sampled by our seines. However, the species composition of the two sample types differed, with more centrarchids and the occasional cichlid or other large species (gar, mullet, grass carp) detected on video rather than in seine samples. Large and highly mobile species typically avoid seines (Bonar et al. 2009) and our seine samples missed a portion of the fish assemblage that the video captured. We collected only two species in seine samples that were not observed in our videos, namely, a juvenile exotic loricariid catfish (Hoplosternum littorale) and a silverside (Labidesthes vanhyningi). In almost two decades of Florida springs research, we have observed only a few Hoplosternum littorale in springs, probably because loricariid catfish are extremely behaviourally cryptic (Power 1983). We have observed many *Labidesthes vanhyningi* at Rock Spring, where we observed the species in seine, but not in video, samples; however, their occurrences have been sporadic in our previous seine samples or visual observations. Of the species that occurred on video, but not in seine samples, only grass carp was rarely observed. The other species are common, but simply too large and fast swimming for us to catch in a seine. Overall, we conclude that video performed as well as, or better than, seining in our clear, freshwater springs.

Structure, water clarity, light level, fish colour and fish species all influenced the performance of video in 'capturing' fish in this study. In this study, the structure consisted of a combination of snags and macrophytes, both of which can slow the progress of a seine and pull the leadline off the bottom, such that fish can escape under or around the seine. However, structure also can obscure individuals from the view of a camera. In our high-structure spring (Rock Springs), more species were captured with video, but more individuals of a subset of these species were captured with the seine than with the video, presumably those species that were less manoeuvrable. Water clarity had an effect opposite to that of structure, reducing the effectiveness of the video relative to the seine. Fish were easier to catch, at least the small species with a limited manoeuvrability, but were harder to see in the turbid Gemini Springs. Low light level somewhat mimicked the problem of turbidity; like turbid water, limited light reduced the diversity in video samples relative to seine samples. However, even in turbid water or low light, we were able to identify most of the species, although we found that identifying species with small or subtle defining characteristics was a challenge. For example, the colour red attenuated quickly, so we had to learn to identify redear sunfish (Lepomis microlophus), which we typically identify by the red edge of the operculum, using other characters. Cyprinid identification often requires fin ray counts or observation of subtle differences in pigmentation, both of which are impossible on video, so we could not distinguish among many cyprinids morphologically.

Despite these shortcomings of video sampling, our short clips were sufficient to capture samples comparable to the seine samples in our clear-water springs with abundant fish. Ellender et al. (2012) and King et al. (2018) both suggested that longer films were required to adequately describe freshwater fish assemblages in African streams and Australia billabongs; however, we detected most species in just a few minutes on our 10-min video clips. Structural complexity also may affect the time required to detect fish species. We observed all fish species that occurred in the videos in under 10 min at the two springs that possessed few visual obstructions. However, at Rock Spring, abundant macrophytes produced a more complex structure that likely obscured fish until they were closer to the camera. As a result, we observed one or two species, which represented 20-25% of the total number observed in two 30-min videos, after 10 min in this spring. This difference in species detection time at springs with varying levels of structure suggests that longer videos may produce better estimates of abundance and diversity at more structurally complex springs. For our short videos, the total time invested in collecting video samples for each spring was similar between the two methods. Of course, the video samples required additional time at the computer to

Species	Volusia Blue Spring	Rock Spring	Gemini Spring
Gambusia holbrooki	<1±<1	<1±<1	<1±<1
Poecilia latipinna	$1\pm < 1$	$1 \pm < 1$	$1 \pm < 1$
Heterandria formosa	9	$<1\pm<1$	$2\pm <1$
Jordanella floridae	ND	ND	2
Lucania goodei	2 ± 2	$3 \pm < 1$	5 ± 2
Lucania parva	$1\pm < 1$	$2\pm <1$	$1 \pm < 1$
Fundulus crysotus	ND	ND	4
Fundulus seminolis	2 ± 1	6 ± 1	2 ± 1
Notemigonus crysoleucas	3	ND	ND
Notropis or Pteronotropis sp.	$<1\pm<1$	1 ± 1	6
Labidesthes vanhyngini	ND	ND	$1 \pm < 1$
Percina nigrofasciata	ND	1	ND
Lepomis macrochirus	1 ± 1	6 ± 2	3 ± 1
Lepomis microlophus	$2\pm < 1$	2 ± 1	$1 \pm < 1$
Lepomis punctatus	$2\pm < 1$	3 ± 1	3 ± 1
Lepomis auritus	$3\pm < 1$	4 ± 1	2 ± 1
Lepomis gulosus	ND	3 ± 1	ND
Lepomis marginatus	ND	<1	ND
Micropterus salmoides	5 ± 3	5 ± 2	3 ± 3
Mugil cephalus	4	ND	ND
Oreochromis aureus	5 ± 4	2	6
Pterygoplichthys disjunctivus	ND	25	ND
Ctenopharyngodon idella	3	ND	ND
Cichlasoma bimaculatum	ND	8	ND

 Table 2.
 The time of first detection (mean ± s.e.) of species that occurred in all 56 videos collected for this study

 ND. no data

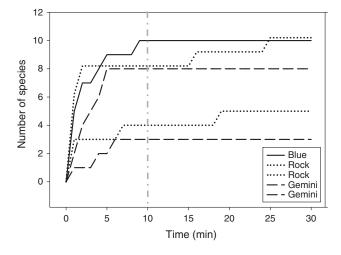


Fig. 4. Species accumulation curves for the clear and low-structure Volusia Blue Spring (one video), the clear and high-structure Rock Spring (two videos) and the turbid Gemini Springs (two videos). The vertical dotted line indicates the length of the videos used in the seine–video comparisons.

enumerate the fish, as has been observed in other studies (Holmes *et al.* 2013; Ebner *et al.* 2014).

However, it is possible that the method of camera deployment matters and the performance of different types of deployment varies with the system. In Australian wetlands, King *et al.* (2018) found that fixed cameras capture more large and fastswimming species than do moving cameras, whereas in South African headwater streams, Ellender *et al.* (2012) suggested that their fixed video captured more cryptic and structure-oriented species than did electrofishing. In marine systems, several studies have suggested that baited cameras capture more species than do unbaited stationary cameras, moving cameras, moving divers and traps (Watson *et al.* 2005; Harvey *et al.* 2007, 2012; Lowry *et al.* 2012; Holmes *et al.* 2013; McIntyre *et al.* 2015). However, Lowry *et al.* (2012) found that baited cameras, in particular, skew the sample towards large, mobile species; they attributed this bias of bait to the attraction of predatory species that might, in turn, scare off some smaller species. In our clear, freshwater springs, fish abundance was sufficiently high so that baiting was not necessary to attract fish.

The better detection of species in video samples produced a more complete picture of the fish assemblage than did seine samples. At the same time, video samples can provide added behavioural information, almost all of which is lost in seine sampling. For example, Ebner and Morgan (2013) found that video provided information on species' depth associations that nets could not. Video can also provide a permanent dynamic record of the fish assemblage at the moment of capture, which can be re-analysed by the same or other researchers as new questions arise (Ebner et al. 2014). For example, in our video samples, we observed more species breathing at the air-water interface than we expected; this observation provides the basis for a hypothesis about fish behaviour in low-oxygen environments that can be tested in the future by using the same, and perhaps additional, videos. We could have detected this behaviour during snorkel surveys, but we would have been unlikely to make this observation while seining.

In conclusion, this study suggests that in the clear, freshwater springs of Florida, video sampling performed as well or better than did seine sampling in terms of fish abundances, the number of species 'captured', and the calculations of diversity from these counts. Therefore, we suggest that, in clear water, video presents a viable alternative to seining that, in fact, can provide additional behavioural and ecological information and a permanent record that can be revisited in perpetuity. Of course, every sampling method has its bias and so complementing the stationary video collection with an additional sampling method, such as a moving camera mounted on a kayak, would increase the precision of the picture of the fish assemblage.

Conflict of interest

The authors declare that they have no conflicts of interest.

Declaration of funding

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