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Identifying Sources of Error in Surveys of Devils Hole Pupfish (Cyprinodon diabolis)

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Abstract
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Disciplines
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Comments
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IDENTIFYING SOURCES OF ERROR IN SURVEYS OF DEVILS HOLE PUPFISH (*CYPRINODON DIABOLIS*)

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ABSTRACT—We assessed four potential sources of error in estimating size of the population of Devils Hole pupfish (*Cyprinodon diabolis*): net, time of day, diver, and order of diver. Experimental dives (3/day) were conducted during 4 days in July 2009. Effects of the four sources of error on estimates from dive surveys were analyzed using a split-split plot ANOVA. Diver and order of diver had no significant influence on estimates, whereas the effect of presence or absence of a net was significant. Effects of time of day and presence or absence of a net showed a significant interaction with depth of water. Results indicated that pupfish may move upward during the dive, and as a result, the standard methods of dive surveys may underestimate abundance.

RESUMEN—Evaluamos cuatro posibles fuentes de error al estimar el tamaño poblacional del cachorrito del agujero del diablo (*Cyprinodon diabolis*): la red, la hora del día, el buzo, y el orden de los buzos. Buceos experimentales (3/día) se hicieron durante cuatro días en julio del 2009. Los efectos de estas cuatro fuentes de error sobre las estimaciones provenientes de los muestreos del buceo fueron analizados usando un ANOVA de split-split plot. El buzo y el orden de los buzos no tuvieron ninguna influencia significativa en las estimaciones, mientras que la presencia o ausencia de la red fue significativa. Los efectos de la hora del día y presencia o ausencia de la red demostraron una interacción significativa con la profundidad del agua. Los resultados indicaron que los cachorritos puedan subir durante el buceo, y como consecuencia, los métodos estándares de muestreos de buceo pueden subestimar la abundancia.

According to a recent compilation of the conservation status of fishes developed by the American Fisheries Society, ca. 39% of taxa of freshwater and diadromous fishes in North America are imperiled, with 230 vulnerable, 190 threatened, 280 endangered, and 61 presumed to be extinct in the wild (Jelks et al., 2008). Rate of extinction in freshwater ecosystems is estimated to be five times greater than in terrestrial ecosystems (Ricciardi and Rasmussen, 1999). In assessing loss of diversity of native fishes in California, Moyle and Williams (1990) determined that some leading factors characterizing imperiled species were endemicity, small area of habitat, habitat in only one drainage basin, low diversity of the native assemblage, occurrence in isolated springs, or a combination of these. Many of these characteristics are exhibited by aquatic environments in deserts. Although aquatic habitats in deserts may appear depauperate of aquatic fauna when viewed individually, they exhibit much greater diversity when viewed collectively because geographic isolation of habitats has resulted in speciation and endemicity.

The Devils Hole pupfish (*Cyprinodon diabolis*) is the poster child for preservation of aquatic ecosystems in deserts because it has been pivotal to legislation to conserve freshwater fishes and endangered species (Deacon and Williams, 1991). Despite extensive research, population dynamics are poorly understood because researchers cannot physically handle pupfish due to the risk of mortality to the fish. As such, estimates of size of the population of adult Devils Hole pupfish must be obtained using SCUBA-diver, visual-survey methods. Routine monitoring of abundance of adult Devils Hole pupfish started in 1972 and continues to present. During surveys, habitat in Devils Hole is divided into strata: the shallow shelf, a boulder ca. 2 by 5 m in area submersed under 0.2–0.7 m of water, and the deep pool or deeper waters of the cavern. Surveys include dive and shelf surveys; two SCUBA divers count pupfish in the deep-pool habitat (i.e., dive survey) while observers count fish from a platform overlooking the shallow shelf (i.e., shelf survey). Counts from the dive and shelf surveys are summed to estimate size of the population of adult pupfish. Surveys are conducted in April and September when the population is at its annual minimum and maximum, respectively.

Potential sources of error associated with visual assessments make attaining replicate counts challenging and may limit detection of trends in populations. Among potential sources of error are effects of movement of fish, time of day, order of diver, and diver. The effect of movement of fish is likely a source of error (Watson et al., 1995) due to tendency of fish to move as a school and linger at the boundary between the shelf and deep pool. Fish at the boundary can be double-counted or missed completely by both dive and shelf surveys. Managers have proposed placing a block net at the shelf-deep-pool boundary during the survey to impede movement of fish between the shelf and the deep pool and improve accuracy of counts. Time of day when the survey is conducted also may influence estimates. While surveys of adults that are conducted at different times of day during the same sampling date are meant to serve as replicated estimates, James (1969) noted that Devils Hole pupfish avoid sunlight during midday. Likewise, order of diver may have an effect on estimates, as the first diver may influence the count of the second diver by disturbing fish. Finally, variability in estimates also may be attributed to differences between divers. In ecological monitoring, multiple observers can produce dissimilar results based on differing abilities to detect organisms (Thompson and Mapstone, 1997; Bue et al., 1998; Nichols et al., 2000; Diefenbach et al., 2003). For example, Thompson and Mapstone (1997) reported that bias of observers in dive surveys in the Great Barrier Reef of Australia varied with species of fish, and that while biases for some species could be corrected by calibration-training the diver, biases for other species persisted despite calibration-training efforts. The extent to which these four potential sources of error affect replicated samples needs to be either corrected, quantified, or have its presence acknowledged. Thus, the objective of this study was to identify and minimize (if possible) sources of error in surveys of adult Devils Hole pupfish.

Materials and Methods—Devils Hole, a fissure formed by tectonic activity, is adjacent to Ash Meadows National Wildlife Refuge, an oasis in the Amargosa Desert in Nye County, Nevada. Devils Hole is the hydrologic head of a deep and extensive carbonate aquifer.

Adult pupfish are typically in the upper 25 m of the deep pool and density of fish is greatest in the upper 10 m. In the deep pool, temperature of water, pH, and conductivity remain fairly constant at 32–33°C, 7.1–7.5, and 820 μS/cm, respectively (Shepard et al., 2000). In contrast, the shallow shelf is more dynamic (Lyons, 2005). While temperature of water along the shallow shelf typically is similar to that of the deep pool, temperature of water on the shelf has reached extremes of 9 and 38°C (Lyons, 2005). Concentrations of dissolved oxygen typically are ca. 2 mg/L until the shelf is seasonally illuminated by the

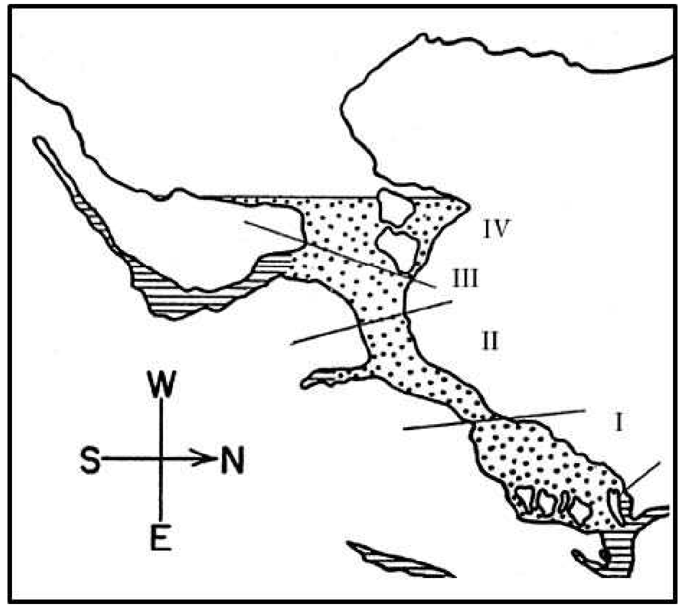

Fig. 1—Side view of Devils Hole, Nye County, Nevada, illustrating the four levels used in SCUBA-dive surveys.

sun when concentrations increase to ca. 8.0 mg/L in the afternoon (Shepard et al., 2000).

We designed a series of 12 experimental dives to examine effects of time of day (3 times/day), order of diver (two orders), diver (two divers), and net (two treatments) on estimates of size of the population of Devils Hole pupfish. Experimental dives consisted of 4 days of dives during 10–13 July 2009. Dives occurred each day at 0800 h (morning), 1100 h (midday), and 1330 h (afternoon) and lasted ca. 1 h each. On 2 days of dives, we placed a mesh block net at the boundary between the shelf and deep-pool habitats to prevent movement of fish. On dives with a block net, we stretched the net across the shelf-deep-pool boundary before divers began descending into the deep pool. Between dives, we drew ends of the net inward to allow movement of fish between habitats. The other 2 days of dives did not include a block net (no-net dives). Similar to standard dive surveys, the route for all dives was divided into four levels that corresponded with consistent changes in depth (Fig. 1). Divers recorded number of fish encountered at each level. Unlike standard surveys, divers switched order three times during the dive at transition points between levels in Devils Hole. Switching orders during the dive provided greater replication for testing effects of diver and order of diver. The same two divers counted fish on every dive; both divers were experienced and had participated in surveys for multiple years. As with historical surveys, divers swam down to ca. 30 m, waited 10 min, and then ascended to count fish.

Design of the experimental dive also included experimental shelf surveys to assess effects of time of day and presence or absence of a net. All experimental shelf surveys followed the standard surface-survey method, with exception of the 2 days when a block net was placed at the boundary between the shelf and deep pool. Shelf surveys occurred concurrently with dive surveys and began ca. 10 min after divers started their descent. The shelf was divided into three sections on the N-S axis, and three different observers counted fish in their section of the shelf. Shelf counts were not timed and ended when counters finished counting fish in their section. Typically, each shelf count lasted 2–3 min. Shelf counters were allowed to communicate with one another to prevent double-counting fish that swam into multiple sections. Each shelf survey consisted of four counts; one practice count and three subsequent official counts. Each observer counted the same section of shelf for all four counts in a survey, but observers were assigned randomly to different sections of the shelf between surveys. Official counts were summed across sections and averaged for each shelf survey.

We conducted statistical analyses using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina) and we set $\alpha = 0.05$ for analyses. As a preliminary analysis, we examined historical records of estimates of size of population (1972–2009) to assess effects of diver (three divers), order of diver (two orders; first and second), and time of day (two times of day; morning and midday). To help meet assumptions of normality and homogeneity of variances, estimates of abundance were ln-transformed prior to analysis. We used a mixed model (PROC MIXED) with time of day (morning and midday), diver (three divers), and order of diver (two orders) designated as fixed effects and date $\times$ time of day designated as a random effect to analyze sources of error from estimates in historical surveys. Designating date $\times$ time of day as a random effect accounted for variation across individual dives and allowed for assessment of time of day, diver, and order of diver. We neither assessed sources of error in shelf surveys in historical analysis, nor did we assess interactions between factors due to limited samples.

Due to differences in sizes of experimental units across factors, we used a split-split plot ANOVA to assess effects of net, time of day, diver, and order of diver on estimates from surveys in experimental dives for Devils Hole pupfish (Littell et al., 2002). In the split-split plot analysis, number of days represented whole plots, because block-net-present and no-net treatments were set up for the entire day (net, $n = 4$). Dives signified the first subplots, as time of day was constant for all four levels in each dive (time of day, $n = 12$). The second subplots were represented by each level, as diver and order of diver changed according to level (level, $n = 96$; diver, $n = 96$; order of diver, $n = 96$). Level was added as a fixed effect to account for differences in density of pupfish at different levels. Because there were differences in density among levels, excluding level from analysis would have hindered our ability to test effects of the other four factors. Counts were $\log(x + 1)$ transformed to help equalize variances and to enable assessment of multiplicative effects.

Similarly, a split-plot ANOVA was used to assess effects of net and time of day in $\log(x + 1)$ transformed shelf surveys with days representing the whole plot and dives signifying the first subplot. Due to the limited sample, only certain interactions were assessed in dive surveys. Interaction terms included in the model were those presumed to be important (i.e., a priori). For example, we assumed that the difference in abilities of divers to count fish was not influenced by time of day and excluded time of day $\times$ diver from the model.

Because official estimates have been attained from both dive and shelf surveys in the past, we conducted additional analyses to evaluate effects of time of day and net on combined estimates of dive and shelf surveys. For each dive, estimates of abundance in all four levels were summed and estimates from the two divers were then averaged and added to the average of the three official shelf counts. A split-plot ANOVA was then used to assess effects of time of day and net on the combined estimate from dive and shelf surveys. The same combined estimates were used to compare variances between the net-present and no-net treatments, because one of our goals was to determine how to increase precision in surveys. We hypothesized that estimates from dives with net present would have lower variability than estimates from dives with no net because we speculated that variability in estimates was partly attributed to movement of fish. As such, we tested influence of the net on variability by comparing residuals between treatments. Residuals were calculated by subtracting the mean combined estimate from surveys with nets from each survey with nets, and the mean combined estimate from surveys with no net from each survey with no net. Absolute values of residuals were then used in Levene's test to evaluate differences in variability between treatments.

Results—Inconsistencies in historical records limited our ability to detect sources of error in dive surveys. Specifically, dives were excluded from historical analysis if dives included only one diver counting fish or if records did not indicate which of two divers dove first and which dove second. As such, only 88 observations from 22 days were used in historical analysis. In the mixed-effects model, order of diver was the only factor that had a significant effect on estimates ($F_{1,41} = 10.21$; $P < 0.01$; Table 1), with the second diver seeing 16% fewer fish than the first diver.

Similarly, there was no effect of diver ($F_{1,56} = 0.01$; $P = 0.92$) in analysis of experimental dive surveys; however, effect of order of diver was not significant ($F_{1,56} = 0.83$; $P = 0.37$; Table 1). Statistically significant interactions between time of day $\times$ level ($F_{6,56} = 7.11$; $P < 0.01$) and net $\times$ level ($F_{3,56} = 9.25$; $P < 0.01$) complicated assessment of time of day and net as main effects in analysis of experimental dives. We determined that time of day could not be assessed as a main effect due to opposing directionality of time of day $\times$ level interactions at levels III and IV. Specifically, there were more fish on level IV during dives in morning and midday and less fish on level IV during dives in afternoon; whereas, there were more fish on level III during dives in afternoon and fewer fish on level III during dives in morning and midday (Fig. 2a). Estimates for level IV, the uppermost level, were greater for dives with net present than for dives with no net ($t_{56} = -6.72$; $P < 0.01$; Fig. 2b). Because estimates of size of the population were reported as the sum of fish on all four levels, we assessed net as a main effect. Evaluation of net as a main effect averaged the effect of presence of net over all four levels; thus, allowing us to determine how the net affected the final estimate. As such, net was significant as a main effect ($F_{1,2} = 24.10$; $P = 0.04$); dives with a net produced higher estimates than dives without a net. The significance of net as a main effect was most influenced by greater abundance of pupfish on level IV during dives with nets present.

Analysis of experimental shelf surveys showed the effect of time of day was nearly significant ($F_{2,4} = 6.60$; P

Table 1—Evaluation of sources of error in estimates of size of the population of Devils Hole pupfish (*Cyprinodon diabolis*) in Nye County, Nevada. Test statistics are shown for mixed-effects model assessing historical records of estimates, split-split plot ANOVA evaluating experimental dives, split-plot ANOVA examining experimental shelf surveys, and split-plot ANOVA analyzing combined estimates from experimental dives and shelf surveys.

Variable	Numerator *df*	Denominator *df*	*F*-value	Probability > *F*
Historical analysis				
Day	22	20	53.29	<0.01
Time of day	1	20	0.07	0.79
Order of diver	1	41	10.21	<0.01
Diver	2	41	0.73	0.49
Dive survey				
Net	1	2	24.1	0.04
Time of day	2	4	5.64	0.07
Level	3	56	213.14	<0.01
Order of diver	1	56	0.83	0.37
Diver	1	56	0.01	0.92
Net × time of day	2	4	1.84	0.27
Net × order of diver	1	56	0.43	0.51
Order of diver × diver	1	56	0.05	0.82
Net × level	3	56	9.25	<0.01
Net × time of day × level	6	56	1.73	0.13
Time of day × level	6	56	7.11	<0.01
Net × order of diver × level	6	56	0.51	0.8
Shelf survey				
Net	1	2	0	0.99
Time of day	2	4	6.6	0.05
Time of day × net	2	4	0.03	0.97
Combined (dive + shelf survey)				
Net	1	2	4.24	0.18
Time of day	2	4	3.38	0.14
Time of day × net	2	4	0.30	0.76

= 0.05) and effect of net was not significant ($F_{1,2} = 0.00$; $P = 0.99$; Table 1). Summing average estimates from dive and shelf surveys helped determine the effect of these potential sources of error on a coarser scale. Effects of net ($F_{1,2} = 4.24$; $P = 0.18$) and time of day ($F_{2,4} = 3.38$; $P = 0.14$) were not significant after combining shelf counts with dives. Levene's test showed that residuals from net and no-net treatments were not different for combined estimates ($F_{1,10} = 0.18$; $P = 0.68$). Because residuals between treatments were not significantly different, there was no evidence that precision differed between net and no-net treatments; however, comparing estimates of the variance for net and no-net dives is tenuous due to the small sample.

Discussion—Although there was no significant effect of diver on estimates of size of population in both the historical and experimental analyses of dives, both analyses included only a small subset of divers that had participated in past surveys. Thus, neither analysis tested effects of other divers in the historical records. In particular, experience of a SCUBA diver can affect estimates (Williams et al., 2006) and was not assessed in this study. In addition, effect of order of diver was significant in the historical analysis and not significant in analysis of experimental dives despite a similar estimate of precision in both tests. Whether these contradictory results can be attributed to divers switching orders between levels is unknown. The effect of order of diver can be further assessed during subsequent surveys of adult pupfish.

Laboratory and field studies have shown that different species of fish may select habitat based on temperature of water (Barlow, 1958; Podrabsky et al., 2008; Rydell et al., 2010). Our results from assessment of time of day support the conclusion of James (1969) and Baugh and Deacon (1983) who observed that Devils Hole pupfish tend to move into deeper waters in afternoon during summer to avoid thermal stress in shallow water. Discovering that the time of day × level interaction was significant in dive surveys and nearly significant in shelf surveys indicates that fish were more likely to be in deeper water during dives in afternoon compared to dives in morning and midday. However, temperature on the shelf is lower in autumn and spring, the two seasons when surveys of adults typically occur. Accordingly, dive surveys conducted during autumn and spring may be a better test for assessing effect of time of day, because it is less likely that there will be a significant interaction between time of day × level during these seasons. Because preference of fish

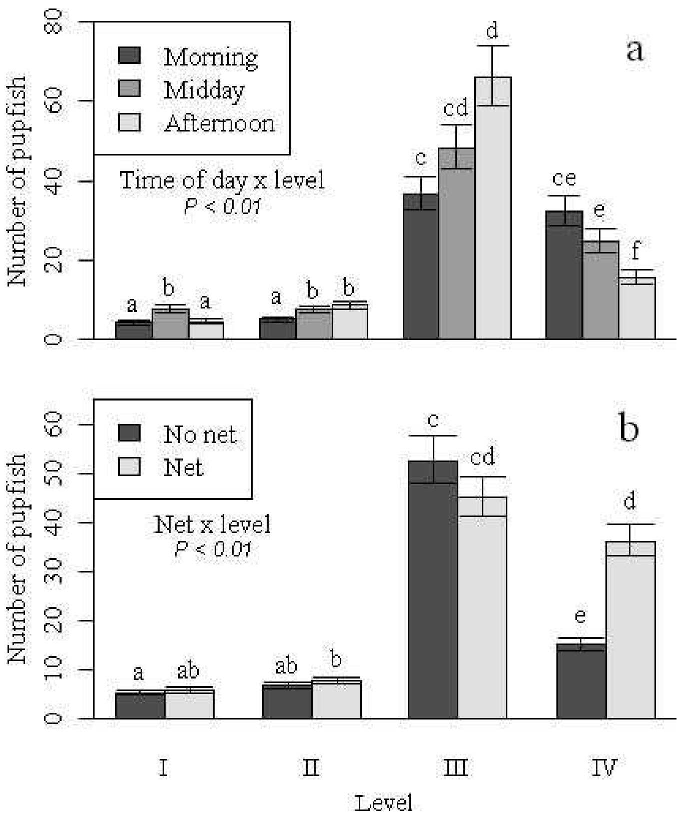

FIG. 2—Mean number of Devils Hole pupfish (*Cyprinodon diabolis*) in Nye County, Nevada, by a) time of day × level and b) net × level. Error bars represent *SE* estimated from ln-transformed estimates of size of population. The interval of the ln-transformed mean ±1 *SE* was then back-transformed so that it could be applied to estimates of abundance. Different letters represent contrasts that were statistically different ($P < 0.05$).

for shelf or deep-pool habitat may depend on time of day, combining the two surveys may provide a more precise assessment of the effect of time of day on estimates.

One of the novel findings of the experimental dives is that fish may move nonrandomly during the dive survey. Greater density of fish at level IV during dives with a block net suggests that fish may be avoiding divers, and as a consequence, they may escape both dive and shelf surveys. Other studies have reported different responses of fish to SCUBA divers. While some studies have shown that abundance decreases with presence of SCUBA divers (Stanley and Wilson, 1995), others detected no effect of SCUBA divers (De Girolamo and Mazzoldi, 2001), that abundance increases with presence of SCUBA divers (Chapman et al., 1974; Cole et al., 2007), or that the effect of presence of SCUBA divers varies by species (Watson and Harvey, 2007; Dearden et al., 2010). If Devils Hole pupfish avoid SCUBA divers, timing of shelf and dive surveys is such that fish may be missed by both surveys. Specifically, fish could be present in the deep pool during the first 15 min of the dive while counters at the shelf are counting fish and divers are counting fish at lower levels. These fish may then move onto the shelf later during the dive, after counters at the shelf have finished their counts and divers were in the process of counting fish in upper levels. Such trends in movement would cause historical surveys to underestimate abundance. There are at least two other possible explanations for the increased number of fish on level IV during dives with a net present. First, fish may have used the net as cover; however, lack of a significant interaction between time of day × net × level would indicate otherwise. Second, the white background of the net may make fish more visible to divers, but both divers indicated that they did not believe that the net increased detectability.

Although analysis of dive surveys indicates that presence or absence of a net had a significant effect on estimates, this effect disappeared after estimates from dive surveys were combined with shelf surveys. Assessment of combined surveys may provide valuable insight as to how dive and shelf surveys interact. Accordingly, more research is needed to determine the effect of net on combined estimates. If combined estimates from dives with nets present are statistically different from no-net dives, estimates from dives with nets present are not comparable to those of no-net dives. As a result, estimates from dives with nets present are not comparable to estimates from historical records. Moreover, if fish avoid divers, as is supported by our results, dives with nets present may produce more accurate estimates of abundance because it is less likely that fish can escape both dive and shelf surveys.

Based on our study, managers must decide whether to use the net on future surveys for adult Devils Hole pupfish. Importantly, because distribution of fish throughout the four levels in Devils Hole differs in spring and autumn compared to summer, the block net must be evaluated as an alternative method during September and April to determine how the net affects accuracy and precision of estimates during the times of year when surveys of adults occur. As such, we suggest that subsequent surveys include 2 days of dives, with at least two dives conducted with a net present and two dives conducted with no net. This will provide additional data as to whether dives with a net result in larger estimates and whether dives with a net improve precision of surveys occurring in September and April. If agencies decide to implement dives with a net as the standard sampling method, estimates from dives with a net and dives with no net could be calibrated, as calibration has been used in sampling fisheries to facilitate comparison of methods (Peterson and Paukert, 2009). However, calibration should proceed with caution because such efforts are, at best, rough comparisons. Simulation of estimates from when a net is present and dives with no net also may be a useful tool to compare the two sampling methods (Peterson and Paukert, 2009).

Nichols and Williams (2006) described differences between two types of environmental monitoring: surveillance monitoring, a general monitoring used to assess trend, and targeted monitoring, or monitoring designed to assess a priori hypotheses, prioritize conservation needs, and evaluate management actions. We believe assessment of sources of error in dive and shelf surveys for adult Devils Hole pupfish represents an important first step toward targeted monitoring. Further, monitoring should be evaluated regularly for ability to assess trends, because routine assessment will help monitoring efforts avoid getting stuck with a poor design because it has years of historical precedence. The philosophy behind the monitoring protocol for Devils Hole pupfish has been to control observational error by maintaining the highest degree of consistency possible among surveys. Unfortunately, maintaining consistency among surveys decreases the sample available to estimate mean and variability in estimates, while also limiting ability to detect sources of error. In particular, past estimates have been obtained exclusively from the estimate of the first diver on the morning survey; thereby, excluding estimates from the second diver and the survey in midday. The limited sample hinders differentiation of observational error from trends in populations, and as a result, there is little confidence in estimated trends. Managers recognize pitfalls of the current survey protocol, yet they are understandably reluctant to change methods because comparisons between future estimates and 38 years of historical records may become obscured. As such, future surveys of adult Devils Hole pupfish will continue to evaluate sources of error; thereby, helping determine which estimates can serve as replicate counts. Increasing replicate counts will improve the power of dive and shelf surveys to assess trend, ultimately helping evaluate management actions and aiding conservation of this unique species.

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