Ever-increasing human pressures on cave biodiversity have amplified the need for systematic, repeatable, and intensive surveys of cave-dwelling arthropods to formulate evidence-based management decisions. We examined 110 papers (from 1967 to 2018) to: (i) understand how cave-dwelling invertebrates have been sampled; (ii) provide a summary of techniques most commonly applied and appropriateness of these techniques, and; (iii) make recommendations for sampling design improvement. Of the studies reviewed, over half (56) were biological inventories, 43 ecologically focused, seven were techniques papers, and four were conservation studies. Nearly one-half (48) of the papers applied systematic techniques. Few papers (24) provided enough information to repeat the study; of these, only 11 studies included cave maps. Most studies (56) used two or more techniques for sampling cave-dwelling invertebrates. Ten studies conducted ≥10 site visits per cave. The use of quantitative techniques was applied in 43 of the studies assessed. More than one-third (42) included some level of discussion on management. Future studies should employ a systematic study design, describe their methods in sufficient detail as to be repeatable, and apply multiple techniques and site visits. This level of effort and detail is required to obtain the most complete inventories, facilitate monitoring of sensitive cave arthropod populations, and make informed decisions regarding the management of cave habitats. We also identified naming inconsistencies of sampling techniques and provide recommendations towards standardization.

Keywords: systematic sampling, repeatability, conservation, pitfall trapping


INTRODUCTION

With mounting anthropogenic threats to cave ecosystems, it is increasingly important to systematically and efficiently collect data on cave-dwelling arthropods, so that informed management decisions can be made or adjusted on a regular basis. Cave ecosystems face numerous human impacts globally including land cover conversion (Culver, 1986; Trajano, 2000; Howarth et al., 2007; Silva et al., 2015), mining (Elliott, 2000; Silva et al., 2015; Sugai et al., 2015), groundwater pollution (Aley, 1976; Notenboom et al., 1994; Graening & Brown, 2003; Whitten, 2009), water extraction and water impoundments (Lisowski, 1983; Ubick & Briggs, 2002; Olson, 2005), invasive species (Elliott, 1992; Reeves, 1999; Taylor et al., 2003; Howarth et al., 2007; Wynne et al., 2014), global climate change (Chevaldonné & Lejeune, 2003; Badino, 2004; Mammola et al., 2018), and recreational use (Culver, 1986; Howarth & Stone, 1993; Pulido-Bosch et al., 1997). These threats have significant implications for conservation because caves are highly sensitive habitats, often serving as hotspots of endemism and subterranean biodiversity (Culver et al., 2000; Culver & Sket, 2000; Mammola et al., 2018). These threats have significant implications for conservation because caves are highly sensitive habitats, often serving as hotspots of endemism and subterranean biodiversity (Culver et al., 2000; Culver & Sket, 2000; Mammola et al., 2018).
small populations (Mitchell, 1970). Thus, effectively sampling caves to detect troglobionts should be a priority of cave biological inventories.

The fauna of most of the world’s caves remain unknown or, at best, incompletely surveyed (Howarth, 1983; Whitten, 2009; Gibert & Deharveng, 2002; Deharveng & Bedos, 2000; Encinareas & Lit, 2014; Gilgado et al., 2015). In addition, accurate information on the taxonomy, genetics, distribution, and environmental requirements of cavernicolous will be necessary to make rigorous ecological inference, as well as to develop appropriate recommendations for monitoring and protecting cave animals (Wynne et al., 2018).

Numerous researchers (e.g., Weinstein & Slaney, 1995; Howarth et al., 2007; Krejca & Weckerly, 2007; Zagnajster et al., 2008; Wynne et al., 2018) have emphasized the difficulties of sampling terrestrial cavernicolous arthropods, which present challenges for effectively inventoring, and managing sensitive cavernicolous arthropod communities. Caves are highly diverse habitats with constricted, maze-like interconnected passageways, uneven terrain, loose rocks and boulders, deep fissures and pits. This diverse array of habitats often requires technical climbing and rope work for access. Additionally, temporal and spatial heterogeneity of cave habitats (Kane & Poulson, 1976; Chapman, 1983; Pellegrini & Ferriera, 2013; Trontelj et al., 2013) often requires considerable pre-planning and on-site evaluations prior to sampling.

Furthermore, research emphasis should be placed on identifying and surveying nutrient resource sites that support troglomorphic animals (Howarth et al., 2007; Wynne, 2013; Wynne et al., 2018). For example, Peck & Wynne (2013) showed a cave cricket roost (Family Rhaphidophoridae), within the type locality of the troglomorphic leiodid beetle (Ptomaphagus paraschant), provided an important substrate (frass and decaying carcasses) for the growth of fungi – a primary food source for this beetle. Additionally, Stone et al. (2012) and Wynne (2013) underscored the importance of root curtains as both microhabitats and a nutrient source for subterranea-adapted animals in Hawai’i and New Mexico, respectively. Wynne & Shear (2016), Wynne et al. (2014), and Benedict (1979) identified vegetation and moss within entrances and beneath cave skylights as key habitat for relictual species.

In this study, we (i) examine how cave-dwelling invertebrates have been sampled (from 1967 to 2018); (ii) provide both a summary of techniques most commonly applied and their appropriateness, and; (iii) make recommendations for sampling design improvement. We also identify naming inconsistencies of sampling techniques and provide recommendations towards standardization.

**METHODS AND MATERIALS**

We reviewed the literature (from 1967 to 2018) by obtaining articles through a Web of Science search using combinations of the following search terms: ‘cavernicolé’, ‘troglobiont,’ ‘troglobite,’ ‘cave arthropod,’ ‘cave invertebrate,’ ‘inventory,’ and ‘ecology.’ We augmented our search using Google Scholar (with the same search terms), examining titles and abstracts of all papers published in both the International Journal of Speleology (1967-2018) and National Speleological Society Bulletin, now Journal of Cave and Karst Studies (years 1967-1995 and 1996-2018, respectively), and working ‘backwards’ into the literature by reviewing the literature cited of all of the articles considered. As most of the work in cave biology has been published in English and to a lesser extend in French, Spanish, and Portuguese, we assert the papers assembled in this review are representative of the work conducted over the past ~50 years.

Papers were selected for inclusion or exclusion using the following decision rules: (1) papers focused on inventorying cave-dwelling terrestrial invertebrate communities and/or investigating an aspect of cave arthropod community ecology; (2) for studies using an all taxa approach (i.e., terrestrial and aquatic invertebrate sampling and vertebrate sampling), only terrestrial invertebrate techniques were examined; (3) only multi-taxon inventories were included; single species or single taxonomic group studies were excluded; and (4) because reviews and synthesis papers of specific geographic regions rarely include sampling methods, these papers were not included. When possible, we examined the original studies that included field methods.

We evaluated each article on cave-dwelling invertebrates using the following questions and criteria.

(1) Were systematic techniques (i.e., techniques consistently applied throughout a given cave or across cave study sites) employed (yes, no, or not known)? (2) Was sufficient information provided to enable repeatability of data collection and/or the experiment (yes or no)? (3) To further facilitate repeatability, did the researchers include cave maps with plotted sample locations and/or use the information from cave maps as part of their experimental design (yes or no)? (4) Did the workers apply multiple sampling techniques (yes, no, not stated)? If yes, how many? (5) Did the researchers conduct multiple site visits (yes, no; if yes, how many)? (6) Were the data analyzed using statistical techniques? (7) Finally, did the authors provide conservation and management implications for their work (yes or no)? We also summarized the techniques encountered in the literature, discussed the functional groups each technique is best suited for capturing, and provided recommendations for best practices.

**RESULTS**

We assessed nearly 300 papers on terrestrial cave-dwelling arthropods. Of these, 110 articles (Appendix I, Supplemental Information) met our decision rules and were included in this review. More than half of these articles (67) were based upon work conducted in the Western Hemisphere - United States (36), Latin America, the Galapagos, and the Caribbean (31; Fig. 1).

Papers reviewed were parsed into four categories (inventory, ecology, techniques, and conservation).
Fifty years of cave arthropod sampling
International Journal of Speleology, 48 (1), 33-48. Tampa, FL (USA) January 2019

We used these categories to frame how the evaluative criteria were applied. Slightly over half of the studies (56) examined were biological inventories, 43 advanced ideas and hypotheses on various aspects of cave ecology, seven examined the efficacy of sampling/analytical techniques, and four studies were conservation focused. For the ecological studies, 17 examined the distribution and assembly of communities within caves, 11 investigated the influence of habitat on arthropod diversity, eight probed how nutrients affected community structure, four explored the influence of seasonality on diversity, and three analyzed the evolution and colonization of subterranean-adapted arthropods (Fig. 2).

Fig. 1. Summary of 110 studies reviewed per geographic region. Number totals 111, as Wynne et al. (2018) had study areas in both the United States and Polynesia.

We used these categories to frame how the evaluative criteria were applied. Slightly over half of the studies (56) examined were biological inventories, 43 advanced ideas and hypotheses on various aspects of cave ecology, seven examined the efficacy of sampling/analytical techniques, and four studies were conservation focused. For the ecological studies, 17 examined the distribution and assembly of communities within caves, 11 investigated the influence of habitat on arthropod diversity, eight probed how nutrients affected community structure, four explored the influence of seasonality on diversity, and three analyzed the evolution and colonization of subterranean-adapted arthropods (Fig. 2).

Fig. 2. Pie chart describing the reviewed articles by study type. Numbers associated with each “pie slice” represent the number of papers per study type. Subdivisions of ecology (community, habitat, nutrients, seasonality and evolution) are depicted in hues of blue. Legend reads left to right; pie chart reads clockwise starting with the largest slice.

Five studies addressed all seven of our evaluative criteria. For ecological studies, four (Chapman, 1982; Martín & Oromí, 1986; Ferreira et al., 2000; Iskali & Zhang, 2015) of 43 papers addressed all of the criteria. Schneider et al. (2011) met all but one criterion – the inclusion of cave maps with plotted sample locations to further enhance repeatability. An additional four ecological studies (Chapman, 1983; Herrera 1995; Prous et al., 2004; Tobin et al., 2014) met all criteria with the exception of discussing conservation implications. One techniques paper (Wynne et al., 2018) met all evaluative criteria. None of the conservation studies or biological inventories met all evaluative criteria. However, one inventory study (Northup et al., 1994) used a systematic and repeatable sampling design, applied multiple sampling techniques, and provided conservation implications.

Systematic sampling
Overall, 48 studies incorporated systematic sampling into their study design, 50 did not, and 12 studies did not provide enough information to make this determination. Of the 43 ecologically focused papers, 26 of these studies applied systematic techniques, 15 did not, and two studies did not provide enough information to make this determination. For the 56 inventory studies, 15 applied systematic techniques, while 32 did not; for nine inventory studies, this could not be determined. Five of seven techniques studies applied systematic techniques. Two (Borges et al., 2012; Howarth et al., 2007) of four conservation papers applied systematic techniques.

Repeatability
Most of the studies (86) did not provide enough information to replicate the study. The twenty-four repeatable studies included 15 ecological projects, five biological inventories, and four techniques papers. For nearly half of the repeatable studies, maps were included or referenced; this included seven ecological studies (Chapman, 1982, 1983; Martín & Oromí, 1986; Herrera, 1995; Tobin et al., 2014; Iskali & Zhang, 2015; Lunghi et al., 2014), two techniques papers (Kozel et al., 2017; Wynne et al., 2018) and two biological inventories (Lamprinou et al., 2009; Dumnicka et al., 2015). Additionally, a total of 36 studies (which included both repeatable and unrepeatable) provided cave maps with plotted sampling locations or employed maps to establish sampling intervals. The combination of both repeatable sampling techniques and cave maps enables future workers to replicate those studies with the highest level of accuracy.

Multiple techniques
Multiple sampling techniques were applied in ~51% of the studies (56 of the 110), while 11 papers did not provide information on number of techniques used. Of the 56 studies, 32 applied two techniques, 18 used three techniques, four employed four techniques, and two studies applied six techniques (Fig. 3).

Notably, three techniques papers (Weinstein & Slaney, 1995; Encinares & Lit, 2014; Wynne et al., 2018) found that applying multiple methods maximized the completeness of the survey. Weinstein & Slaney (1995) descriptively compared four sampling techniques: pitfall trapping (bailed and unbailed), leaf litter traps (wet and dry), timed searches with interval spacing on transect, and timed direct intuitive searches in a tropical Australian cave. When comparing the performance of each technique against total diversity and abundance values, they found wet leaf litter traps to be most effective and uniquely detected two species. Encinares & Lit (2014), when sampling a tropical Philippine cave by environmental zone, discovered that a combined wet and dry leaf...
litter trap approach was required to maximize the number of species detected.

Wynne et al. (2018) applied three techniques (live capture baited pitfall trapping, timed constrained searches around traps before and after deployment, and opportunistic searches) across 26 study caves in the American Southwest and Easter Island, and applied three additional techniques in selected caves (bait sampling and timed searches within a 1-m² grid established within cave deep zones, timed searches in nutrient resource sites – moss-fern/ moss gardens in cave entrances and root curtains in cave deep zones). They revealed that each method uniquely detected species, and thus applying multiple techniques (with multiple site visits) optimized the number of species detected – in particular, management concern species.

Multiple site visits

Overall, seven studies conducted one site visit, 43 studies applied two or more site visits, eight studies used a non-standardized approach whereby the number of site visits varied per cave, and 52 studies did not disclose the number of site visits. For studies applying multiple site visits, these were: 15 studies at two visits, 18 studies between three to eight visits, nine studies between 10 and 36 visits, and one study with more than 100 site visits.

For the 43 studies specifically addressing ecological questions, three studies applied one site visit, 12 conducted two site visits, 10 studies between three to eight visits, three studies between used 10 and 23 visits, and one study with more than 100 site visits. Additionally, two studies employed a non-standardized approach where the number of visits varied across the caves sampled, while 12 studies did not disclose how many site visits were conducted.

Quantitative techniques

We found 43 of the 110 studies included some sort of quantitative analytical framework. Most of the ecological studies (34 of 43), three of four conservation papers, six of seven techniques studies applied quantitative techniques. None of the 56 inventory papers included quantitative analysis.

Conservation and management

Most studies (68) did not mention conservation or management. The 42 papers that discussed conservation were: 16 of 43 ecological studies, all four conservation papers, five of seven techniques studies, and 17 of 56 inventory papers. When we examined this by decade, we found that none of the papers from 1967 through 1979 discussed conservation; however, for the last two decades, most of the papers (per decade) addressed conservation issues and impending human impacts (Fig. 4).

Study design

Cave biologists applied a wide array of techniques for sampling invertebrate populations. We examined the most commonly published study designs and techniques. Based upon our experience, we also provided information on advantages and disadvantages of each technique.

Dividing the total length of the cave into sampling increments occurred in three forms: environmental zones, predefined intervals, and quadrats. As caves are strongly zonal habitats, this is often a useful approach for dividing the cave into more manageable sampling units. Four principal zones are recognized: two photic (light and twilight) and two aphytic (transition and deep; Howarth, 1980). Howarth & Stone (1990) described a fifth environmental zone, the “bad air” zone, which is beyond, and technically a subdivision of, the deep zone. Overall, 25 studies applied a zonal approach – concentrating on three or more zones, a variation on this theme, or specifically on the dark (i.e., deep) zone, which breaks down as follows: 10 of 43 ecological studies, one of four conservation studies, five of seven techniques studies, and 9 of 56 inventory studies (Table 1). Only two studies (Howarth
Table 1. Summary of publications where sampling was conducted by environmental zone.

<table>
<thead>
<tr>
<th>Purpose of study</th>
<th># (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecological</td>
<td>9/43 (21%)</td>
<td>Howarth &amp; Stone, 1990; Ashmole et al., 1992; Reeves &amp; McCreadie, 2001; Dao-Hong, 2006; Silva et al., 2013; Sendra et al., 2014; Iskali &amp; Zhang, 2015; Arauco &amp; Peixoto, 2015; Růžička et al., 2016</td>
</tr>
<tr>
<td>Conservation</td>
<td>1/4 (25%)</td>
<td>Borges et al., 2012</td>
</tr>
<tr>
<td>Techniques</td>
<td>5/7 (71%)</td>
<td>Weinstein &amp; Slaney, 1995; Schneider &amp; Culver, 2004; Krejca &amp; Weckerly, 2007; Encinares &amp; Lit, 2014; Wynne et al., 2018</td>
</tr>
</tbody>
</table>

Cave biologists applied an array of methods for capturing arthropods including direct intuitive searching, opportunistic collecting, visual searching, timed and untimed searches, several types of pitfall trapping, substrate sampling, and using a variety of baits and leaf litter to attract arthropods (Fig. 5; Appendix II and III, Supplemental Information; Table 2). We also provided information on studies that applied each technique, their methodological limitations, and functional groups each technique was most likely to target (Appendix III, Supplemental Information).

Direct Intuitive Searching (DIS): Direct intuitive searches (i.e., specifically targeting a microhabitat and/or environmental zone to address a research question(s) and/or increase the likelihood of maximizing number of species detected) were applied in 34 studies. These microhabitats included flood detritus, penetrating tree roots hanging from ceilings/walls, guano deposits, edges of drip pools and ponds, muddy banks, animal and/or insect carcasses. These areas were targeted because they were likely to support high diversity or contained specific functional groups (e.g., guanophiles). Additionally, researchers applied this approach to specific environmental cave zones (typically, the cave deep zone). This method may be either timed or untimed DIS with defined or undefined search radius.

Of the 34 studies applying DIS, 16 examined specific microhabitats, seven studies sampled bat guano deposits, seven studies searched for subterranean-adapted arthropods in deep zones, and four studies used DIS across multiple environmental zones. Eight studies were timed DIS, while 26 were untimed DIS. For timed DIS, Ferreira et al., (2000) searched each bat guano pile encountered within a cave for 30 minutes; search radius was not defined. Additionally, Wynne et al. (2018) applied a one-hour DIS in moss-fern/moss gardens and root curtains without defining a search radius, and one timed DIS within selected cave deep zones (10 minutes within an estimated 1-m² area).

Seventeen of 34 studies employed DIS within selected habitats as their only technique. Of these, six were designed to address ecological questions (Hill, 1981; Trajano, 2000; Silva et al., 2011, 2013; Zampaulo, 2015; Bento et al., 2016), two studies were conservation focused (Simões et al., 2014; Silva & Ferreira, 2015), one was a techniques paper (Gallão & Bichuette, 2015), and four studies were inventories (Barr & Reddell, 1967; Holsinger, et al., 1976; Edington, 1984; Drost & Blinn, 1997). Visual Searches: Studies applying this technique explicitly stated “hand collection,” “visible searches,” “collecting,” and “direct searches;” visual searching was employed in 29 studies (22 inventory and seven ecological studies). Overall, hand collecting and/or using instruments (e.g., aspirators) to facilitate collection was applied in 13 studies, visual search, direct search or visual inspection (in none of the cases was this clearly defined) was applied in six studies, and some variation on hand collection or visual search (e.g., “make collections”, “basic collecting”, “collecting,” etc.) was applied in seven studies, and visual counting was used in three studies. This method was combined with other sampling techniques in four ecological studies and 12 inventory studies.
Table 2. Descriptions of the nine primary cave-dwelling arthropod sampling techniques within four methodological groups (hand sampling, trapping, substrate sampling, and attractants). We recommend standardizing to this terminology and providing more complete descriptions of all techniques used. Note: Multiple sampling techniques were applied in half the studies reviewed; thus, the “Times Applied” will total more than 110 (i.e., the number of papers reviewed).

<table>
<thead>
<tr>
<th>Group / Method</th>
<th>Description</th>
<th>Times applied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hand Sampling</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct Intuitive Search (DIS)</td>
<td>Surveys targeted to habitats likely to yield highest diversity, which include flood detritus, edges of pools, streams and flowstone, bat guano and other animal feces, carrion and/or cave deep zones; applied in conjunction with a grid/quadrat system or without defining size of search area; may be timed or untimed; arthropods collected by hand, aspirator, forceps, or paint brushes.</td>
<td>34</td>
</tr>
<tr>
<td>Visual search</td>
<td>Category created due to a lack of information provided; this category is probably DIS, opportunistic collecting or both; workers described this approach as “visual searching”, “hand collecting”, “direct searching”, or simply “collecting” with no additional information provided.</td>
<td>29</td>
</tr>
<tr>
<td>Opportunistic collecting</td>
<td>Collecting arthropods as encountered while walking through the cave and/or conducting other tasks.</td>
<td>5</td>
</tr>
<tr>
<td>Timed search</td>
<td>Searches were timed and centered around pitfall trapping, leaf litter trap-like structures (with or without defining search area around traps), or within grids/quadrats; arthropods were collected via same methods as DIS by examining the cave floor and/or adjacent wall, and searching beneath rocks and other objects.</td>
<td>16</td>
</tr>
<tr>
<td>Untimed search</td>
<td>Same as timed searches, but without allocating or reporting a standardized time spent searching per area.</td>
<td>10</td>
</tr>
<tr>
<td><strong>Trapping</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pitfall trapping</td>
<td>A container or tube-like apparatus counter sunk into the cave floor, left in situ for a specific period of time (typically no more than several days), then traps and contents are retrieved. The four primary pitfall trap types are baited with or without a preservative (e.g., alcohol, ethylene, or propylene glycol) and unbaited with or without a preservative; various baits may be used, refer to text for more information.</td>
<td>41</td>
</tr>
<tr>
<td>Leaf litter</td>
<td>Cleaned (autoclaved recommended), arthropod-free brown leaves from surface placed upon a wire mesh/window screen or directly on cave floor, and typically in damp areas; water delivery systems may be used for xeric areas within caves.</td>
<td>4</td>
</tr>
<tr>
<td><strong>Extraction</strong></td>
<td></td>
<td></td>
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<tr>
<td>Substrate sampling</td>
<td>Direct removal of cave sediment, bat guano, leaf litter, and/or flood detritus; arthropods are subsequently extracted using Berlese/Tullgren funnels, sorting and removing by hand, sieving, or a combination thereof.</td>
<td>36</td>
</tr>
<tr>
<td><strong>Attractants</strong></td>
<td></td>
<td></td>
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<tr>
<td>Bait</td>
<td>Deployed in specific habitats (typically in cave deep zones to attract troglobionts), left in situ for a few days, then baits and arthropods selectively removed; baits typically placed on the cave floor and within cracks and crevices of walls and ceiling; a variety of baits may be used to attract different feeding guilds, refer to text for more information.</td>
<td>14</td>
</tr>
</tbody>
</table>

Visual searching was employed as a single technique in nine inventory studies and one ecological study. Additionally, in three cases, arthropods were “visually counted” (two ecological and one inventory); most of these studies applied visual counting in combination with other techniques.

Phrases such as “hand collecting”, “visual searching”, “direct searching”, or simply “collecting” were used to describe this technique. This category likely represents studies applying direct intuitive searches, opportunistic collecting, or a combination of the two; unfortunately, there was not sufficient information provided to confidently make this determination. This approach is particularly useful for targeting some predators (in particular, spiders and harvestmen). Additionally, some negatively phototactic arthropods will retreat from the observers’ light and may not be detected.

**Opportunistic collecting**: Five studies applied what we considered ‘opportunistic collecting’. With the exception of two cases (Wynne & Pleytez, 2005; Ferreira et al., 2000), the remaining studies specifically stated...
arthropods were collected opportunistically (refer to Reeves et al., 2000; Wynne & Voyles, 2014; Wynne et al., 2018). This technique involves researchers walking through the cave, examining rock walls, floors, and ceilings, and collecting arthropods as they are encountered (e.g., Wynne et al., 2018).

**Timed searches:** This adds a systematic component to visual searches for cave-dwelling arthropods. Timed searches (TS) may be applied to improve the thoroughness of trapping or baiting, as well as for sampling arthropods by cave environmental zone, at predetermined intervals or within quadrats. When applied in concert with trapping or baiting, we recommend further standardizing this approach by using a fixed grid or radius around the trap or bait.

Timed searches were applied in 16 of the 110 papers examined. This technique included several variations viz: coupled TS with pitfall trapping (3 studies); conducted TS within defined quadrats (5 studies); applied TS by cave environmental zone (4 studies); conducted TS at standard intervals (1 study); applied a total amount of time spent per cave searching (1 study); and two studies did not provide enough information to determine how the TS was applied. For the studies using pitfall trapping, two (Campbell et al., 2011; Wynne et al., 2018) used fixed radius TS around the traps prior to deployment and removal, while Peck (1976) applied an undefined radius, one-minute search prior to trap deployment and removal.

For 11 studies, timed searches were employed within study designs using cave environmental zones, quadrat, or interval sampling approach. Wynne & Voyles (2014) and Oromi et al. (1990) used TS in the three primary environmental zones (entrance, twilight, and "dark"), while Ashmole et al. (1992) applied TS at selected locations within the twilight and "dark" zones. Prous et al. (2004) searched for ≥25 minutes at 2-m intervals. Lunghi et al. (2014), Sharratt et al. (2000) and Tobin et al. (2014) performed TS using quadrat sampling at 7.5 minutes, 10 to 25 minutes, and 30 minutes per quadrat, respectively. Weinstein and Slaney (1995) employed a TS approach along five transects, which encompassed the twilight, transition, and deep zones; their results were compared to the results of other systematically applied techniques. Christiansen & Bullion (1978) applied TS most broadly; whereby they searched for 30 to 120 minutes along the length of each of their 58 study caves. Both Sendra & Reboleira (2012) and Sendra et al. (2014) performed one-hour searches within selected areas, but were not specific about where these searches occurred.

An added benefit of this technique, when combined with baited pitfall trapping, is detection of animals attracted by the bait but not ensnared by the trap. If consistently applied, it also allows comparisons of relative population density between caves – at least for species that are common and whose behavior is well known (e.g., Wynne et al., 2018).

**Untimed searches:** Untimed searches (UTS) were employed in 10 studies and applied in similar circumstances as timed searches. For five cases, this protocol was applied in conjunction with either pitfall or leaf-litter trapping, two studies employed UTS within a multi-technique sampling frame (not related to pitfall or leaf litter trapping), and three studies used UTS as a single technique. For studies coupling this technique with trapping, three of these studies used this method both before trap deployment and prior to trap removal (Poulson & Culver, 1969; Martin & Oromi, 1986; Wynne & Voyles, 2014), while two studies (Schneider & Culver, 2004 and Humphreys, 1991) applied this technique to pitfall traps and leaf litter traps, respectively, upon trap removal only. Martin & Oromi (1986) were the only study to define a search radius (1 to 5-m) around trapping stations.

The remaining studies used untimed searches within a quadrat, zonal or zonal sampling design. Krejca & Weckerly (2007), Daz-Hong (2006) and Prous et al. (2015) applied UTS as a single technique. Prous et al. (2015) and Kur et al. (2016) employed this technique in concert with other sampling methods.

**Pitfall trapping:** Pitfall trapping (PT) was the most commonly employed technique (41 of 110 studies; Fig. 5). Four approaches were used including baited with or without a preservative (e.g., alcohol, ethylene, or propylene glycol), and unbaited with or without a preservative. Traps with preservative result in 100% take (i.e., kill) of animals that fall into the trap. Traps without a preservative maintain captured animals alive until examined by researchers. However, captured animals may escape, be eaten by other animals, or die and begin to decompose before retrieval (Weeks & McIntyre, 1997). Weinstein & Slaney (1995) used glass jars with a constricted mouth since the curved neck should limit escape.

Pitfall traps were typically counter-sunk within the cave sediment and/or rocky substrate to minimize an exposed lip that might prevent capture of arthropods. When this was not possible, researchers built ramps around each trap using local materials (e.g., rocks, wooden debris, etc.) to provide invertebrates with easier access to the trap (e.g., Ashmole et al., 1992; Wynne & Voyles, 2014; Wynne et al., 2018). Campbell et al. (2011) developed a ramped PT design (trap was placed on the ground surface with plastic ramps leading to PT). Růžička et al. (2016) applied a free-hanging PT design, which attached to the walls of a vertical deep pit; these traps consisted of a ramp leading from the wall onto a platform with PT at center.

Of the 41 papers reporting on the use of pitfall traps, one study used both baited and unbaited traps (without preservative), 17 studies applied baited traps without preservative, 13 used bait with a preservative, three studies applied unbaited traps, five employed traps with preservative only, and two studies stated only that traps were used (Fig. 6). Various types of bait were used including rotten liver, cheese, banana, and peanut butter (Table 3). Four studies suspended baits (either cheese or liver) over a “Turquin” liquid, which served as both an attractant and preservative. Serrano & Borges (2010) described Turquin as a mixture of 1000-ml of dark beer, 5-ml acetic acid, 5-ml formalin, and 10-g of chloral hydrate. One study used PT with a “variety
of attractants" (Araujo & Peixoto, 2015). Two studies used PT with only a preservative – formalin (Dessen et al., 1980) and a 50/50 water/ethanol mixture (Iskali & Zhang, 2015). Two studies (Reeves, 2001; Deleva & Georgiev, 2015) used ethylene glycol, which served as both an attractant and preservative. One study used cheese and ethylene glycol as a bait/ attractant (Isaia et al., 2011). Wynne & Voyles (2014) used live capture baited and unbaited PT and Wynne et al. (2018) used live capture PT; both studies baited with peanut butter.

Functional groups most often captured in PT include detritivores and omnivores, as well as some predators and other functional groups. However, some cavernicoles, especially many troglomorphic species, do not enter pitfalls (Kuštor & Novak, 1980; Bell et al., 2007). Both Barber (1931) and Valentine (1941) favored baited PT for capturing cavernicolous, omnivorous, and carrion beetles due to its quick return rate. Wynne et al. (2018) reported that most (70% of beetle morphospecies) were detected with baited traps. Conversely, spiders and volant species may escape from live capture pitfalls.

Other considerations include anticipating possible disturbance by rats or other mammals (including humans), as well as mitigating the potential harmful effects of this method on the cave resources. Placing cages around each trap may limit rodent disturbance, but may also prevent access by targeted animals. To limit human disturbance, hiding traps or deploying in cryptic areas may help. Importantly, efforts must be made to prevent disturbance to other cave resources (e.g., archaeological, cultural, geologic, and paleontological) when placing and removing traps. In some cases, physically disturbing the cave floor or sediments to install traps may be prohibited. Additionally, many preservatives applied in the past (e.g., picric acid, choral hydrate, and formalin) are now regulated chemicals and considered dangerous to use in caves. Propylene glycol with the proper mixture of ethanol to break the surface tension is a preferred preservative for most invertebrates and is considered environmentally safe.

**Substrate sampling:** Substrate sampling involves collecting samples of sediment (e.g., soil, guano, or organic material), and then extracting specimens using a variety of techniques. The most common extraction methods include using Berlese or Tullgren funnels, floating in a liquid, and sieving. Thirty-six studies applied this approach including: 16 studies examined bat guano; eight studies sampled sediment; five sampled “organic debris” or “detritus;” two studies sampled leaf litter; one study examined both sediment and bat guano; one study sampled oilbird (*Steatornis caripensis* Humboldt, 1817) seed beds and bat guano deposits; and, three studies did not state clearly what substrate was sampled.

Seventeen studies sampled substrate systematically. Of note, researchers applied the following methods: (i) a percentage or specific quantity of sediment (Welbourn, 1978; Northup et al., 1994; Lamprinou et

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**Table 3. Summary of bait types used in pitfall traps.**

<table>
<thead>
<tr>
<th>Bait type</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotten liver</td>
<td>Poulson &amp; Culver, 1969; Richards, 1971; Peck, 1976; Welbourn, 1978; Martin &amp; Oromí, 1986; Ferreira et al., 2000</td>
</tr>
<tr>
<td>Cheese</td>
<td>Richards, 1971; Chapman, 1980; Oromí et al., 1990; Ashmole et al., 1992; Lewis et al., 2003; Schneider &amp; Culver, 2004; Serrano &amp; Borges, 2010; Sendra &amp; Reboleira, 2012; Sendra et al., 2014</td>
</tr>
<tr>
<td>Peanut butter</td>
<td>Wynne &amp; Voyles, 2014; Phillips et al., 2016; Wynne et al., 2018</td>
</tr>
<tr>
<td>Mixture of rice, fish, and meat</td>
<td>Chapman, 1982</td>
</tr>
<tr>
<td>Combination of meat, cheese, tinned fish, damp biscuits, jam, bird carcasses, human feces</td>
<td>Chapman, 1980</td>
</tr>
<tr>
<td>Ripe banana</td>
<td>Weinstein &amp; Slaney, 1992</td>
</tr>
<tr>
<td>Beef liver, banana</td>
<td>Campbell et al., 2011</td>
</tr>
<tr>
<td>Oats, sugar, margarine</td>
<td>Reddell &amp; Veni, 1996</td>
</tr>
<tr>
<td>Cheese and mushrooms</td>
<td>Howarth et al., 2007</td>
</tr>
<tr>
<td>Rotten beef with apple and cherry/ maraschino essence</td>
<td>Kozel et al., 2017</td>
</tr>
<tr>
<td>“Bone”</td>
<td>Bertolana et al., 1994</td>
</tr>
</tbody>
</table>
he examined the effects of nutrient subsidies to caves. To keep the litter wet and facilitate leaf decomposition, he used leaf litter traps with a water delivery system to attract surface environments. Insect predators (Howarth & Slaney, 1995) and alien species competitors (Wynne et al., 2014) may also be introduced. Failure to apply this cleaning step may also result in captured surface arthropods being incorrectly classified as cavernicolous. For reference, Slaney & Weinstein (1996) provided an illustration of their trap design.

**Light trapping & Dry ice:** Three studies used incandescent white light trapping (McClure et al., 1967; Chapman, 1980; Peck, 1984). Peck (1984) indicated his light suction trap designed to specifically target Diptera was unsuccessful. While this technique may be useful in attracting some arthropods like certain species of Diptera, Lepidoptera, and Coleoptera, using full spectrum lighting to attract arthropods hasn’t resurfaced in the literature (at least based upon our review) in over 30 years. However, Reeves (2001) employed both black lights and dry ice for trapping arthropods, although there was no discussion specifically stating the efficacy of these techniques.

**DISCUSSION**

As cavernicolous arthropod inventories and question-driven research projects are conducted in the future, we recommend structuring these studies in a manner that maximizes scientific inference and provides the information necessary to make evidence-based management decisions. To this end, future studies should include the following elements: systematic experimental design; repeatability (in that the methods are thoroughly reported); use of multiple techniques; and use of multiple site visits (Wynne et al., 2018).

Most of the studies reviewed did not include a clearly discernable a priori systematic study design. Culver & Sket (2002) even questioned the utility of such an approach for both sampling and monitoring cavernicolous arthropods. Certainly, low population densities and the heterogeneous nature in which microhabitats and nutrients are distributed within caves, as well as the seasonal influx of nutrients (e.g., bat guano and flood detritus), have presented researchers with challenges for both optimal sampling and monitoring. Nonetheless, for cave biology to...
progress, systematically applied experimental design and sampling efforts are necessary both to make results comparable across caves and to advance hypothesis-driven studies. For monitoring cave-dwelling arthropod species of concern, the U.S. Fish and Wildlife Service has partially addressed the heterogeneity issue by requiring a suite of environmental conditions be met and surveys conducted during the most appropriate season, as well as providing a checklist of suitable conditions for trogloborphic arthropods (USFWS, 2006). We recommend that sampling also include an intensive systematic approach to optimally detect the greatest number of species, as well as potentially detect more cryptic animals (such as troglobionts; Wynne et al., 2018).

Only 24 studies were repeatable (i.e., workers clearly documented their sampling techniques and experimental design). For cave arthropod studies to more solidly advance our understanding of cave communities both temporally and spatially, researchers should thoroughly document how their field data were collected. Without this step, meaningful quantitative comparisons across caves and regions cannot be made and evidence-based conservation planning and monitoring of sensitive taxa and/or communities cannot be assured.

We recognize that optimal sampling methods change over time as technology and our understanding of cave ecology advances; therefore, we hope that this paper will serve to advance future work. We realize the methods applied must be appropriate to fulfill the objectives of the particular study. Thus, one set of protocols will unlikely be suitable for all cave studies. Furthermore, the complexity of caves often requires that study designs be modified in the field to address local cave conditions.

That said, we recommend further standardization of sampling terminology (refer to Table 2). The most substantial gray areas in our review were the lack of clarity on use of the terms: visual searches, direct searches, opportunistic collecting, and direct intuitive searching. ‘Visual searching’ was either ‘opportunistic collecting’ or ‘direct intuitive searching;’ however, because there was not sufficient information to explain what the workers meant by ‘visual searching,’ we created the visual search category. Furthermore, we also found inconsistencies in how direct searches were described. Thus, when information was lacking, studies using direct searches were included in the visual search category. Providing a sufficient description of the sampling methods applied will be critical to avoiding confusion in the future.

Clear descriptions of the study design and the sampling methods can be further enhanced by including figures of sampling locations plotted on cave maps for the sake of repeatability. Given that most journals offer archiving of data as online supplemental information, this is a methodological perk available to most researchers, often at no extra cost. Thus, inclusion of this information will enable future workers to know the precise locations of past sampling efforts, and may use this information for both replicating experiments and establishing future monitoring strategies for resource management. However, researchers must adhere to federal and local agencies and regulations in the countries in which they work regarding the publication of potentially sensitive information (e.g., USC, 1988). Research permits for cave access often include a nondisclosure clause regarding the dissemination of sensitive data (e.g., cave names and in some cases, cave maps). When such guidance is not provided, we recommend using a decision tree like the one developed by Tulloch et al. (2018) to examine the risks and benefits associated with disclosing potentially sensitive information.

Of the papers included in this analysis, 10 studies conducted 10 or more site visits per cave. We recognize many biological inventories are designed to visit as many caves as possible in a short time to establish a baseline for site specific or regional diversity. Unfortunately, in most cases it is unlikely enough site visits were conducted to reasonably characterize arthropod diversity or community structure. For example, Wynne et al. (2018) intensively sampled 26 caves (10 caves each in two southwestern U.S. national monuments and six caves at Rapa Nui National Park, Easter Island, Chile), where they conducted between two to six site visits per cave. For each region, they pooled data across all caves and generated species accumulation curves – none of the curves for any of the regions exhibited signs of asymptotic behavior (Wynne et al., 2018). Thus, while biological inventories are of critical importance in establishing baseline information, as well as being helpful as a hypothesis generating exercise for future work, these data are typically quite limited in their ability to fully characterize arthropod communities.

Multiple site visits may be especially critical to more thoroughly inventory trogloborphic arthropods. For a cave in Williamson County, Texas, Krejca & Weckerly (2007) reported that despite intensive surveys by trained cave biologists an undescribed pseudoscorpion species was discovered upon the 40th visit to the cave. While not directly applicable to terrestrial cave-dwelling invertebrates, Sket (1981) and Culver et al. (2004) reported a new stygobiont (belonging to a new genus) after over one hundred site visits to a well studied cave in Slovenia. Granted it may be impossible for most studies to conduct 40 to 100 site visits per cave, but these examples underscore the need to conduct multiple site visits to most thoroughly define cave communities.

When sampling techniques are applied singly, the study may (a) fail to identify species of potential management concern (e.g., troglobionts and relict species), and (b) not be effective for long-term monitoring to detect changes related to anthropogenic impacts or stochastic events. Through their work, Wynne et al. (2018) found that the six techniques uniquely identified morphospecies; had multiple techniques not been applied, eight new species of presumed cave-restricted arthropods on Easter Island (Wynne et al., 2014), and the range expansions of two species of two tiphiid wasps in west-central New Mexico, would not have been detected (Wynne, 2013). In general, numerous studies (e.g., Muma,
1945; Ashmole & Ashmole, 1987; Basset et al., 1996; Wynne et al., 2018) have shown that applying multiple techniques resulted in the detection of a greater number of individuals and species than studies employing only one technique.

While it is often quite difficult to identify the number of site visits and the suite of techniques required to best capture cave arthropod diversity, Wynne et al. (2018) recommended applying as many sampling techniques and conducting as many site visits as possible. Species accumulation curves and species richness estimators (see Magurran, 2004) are also recommended tools for both gauging the efficacy of sampling efforts, and identifying areas requiring additional inventories. In most cases, Wynne et al. (2018) reported that species accumulation curves were more asymptotic (i.e., flatter) for all techniques combined (they applied a total of six techniques) than for curves generated using data from single techniques. Schneider & Culver (2004), who focused their efforts on troglomorph arthropods, reported none of their species accumulation curves near asymptotic behavior. Reporting similar non-asymptotic behavior, Gallão & Bichuette (2015) emphasized that sensitive subterranean-adapted species may be overlooked due to limited sampling; this could result in making incorrect management decisions based upon incomplete information.

To address the dilemma of incomplete surveys, Howarth & Ramsay (1989) recommended the use of ‘indicator species’ as a proxy for making management decisions. Specifically, discovering a cave passage with suitable environmental conditions associated with one or more significant cavernicolous may be sampled to gain inference into whether the cave warrants protective management or should be more fully studied.

While most of papers examined (~62%; or 68 of 110) did not discuss conservation and management implications, we acknowledge recommendations may have been made directly to resource managers and thus were not reported in the peer-reviewed publications we reviewed. Furthermore, conservation may simply not have been a goal of some studies, especially for those papers published before the amendment to the U.S. Endangered Species Act in 1978, which expanded the Act to include invertebrate species (USC, 1973). Subsequently, our review may underestimate the contributions made by some of these studies to conservation. However, with the rising anthropogenic impacts facing cave ecosystems globally, we maintain that inclusion of this information in the published literature is essential to aid in further developing the field of cave biology and promoting improved management and policy strategies.

Given the sensitivity of most cave communities and troglomorphic species to human disturbance, conservation and management should be at the forefront of cave biology. Through improvements in methodological reporting, systematic sampling designs using multiple techniques, and reliance on species accumulation curves to guide the number of site visits required to establish a reasonable baseline, cave biologists will both strengthen their ability to make more robust statistical inference and develop sound management recommendations based upon the best available data and resultant science.

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