PITFALLS AND PRESERVATIVES: A REVIEW

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Abstract

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An extensive review of the factors that affect the performance of arthropod pitfall traps is given. Liquid preservatives are discussed in a separate section because the choice affects the quality and composition of taxa collected in pitfalls.

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Introduction

Pitfall traps are a popular method for collecting ground beetles, spiders, ants and other epigeal arthropods (Westberg 1977; Niemelä *et al*. 1992; Bestelmeyer *et al*. 2000; Southwood & Henderson 2000; Phillips & Cobb 2005). While many shorter, general overviews exist (e.g., general techniques: Balogh 1958; Duffey 1972; Bestelmeyer *et al*. 2000; Southwood and Henderson 2000; Woodcock 2005; issues with pitfalls: Adis 1979), none have exhaustively examined the published literature recently. Herein we present such a review with the hope it will provide a sound base for those incorporating pitfall traps into research.

While the choice of preservative will affect the quality of specimens in any type of trap, it is a critical decision in pitfalls for several reasons. Chiefly, preservatives differentially attract and repel select arthropod taxa, which will affect the composition of taxa collected (Weeks & McIntyre 1997). Additionally, pitfalls are often set without covers in open fields, so lose more preservative through evaporation than other traps and are affected to a greater degree by rain and dilution by rainwater (Porter 2005). Therefore, we include a section detailing possible positives and negatives of preservatives used in pitfall traps.

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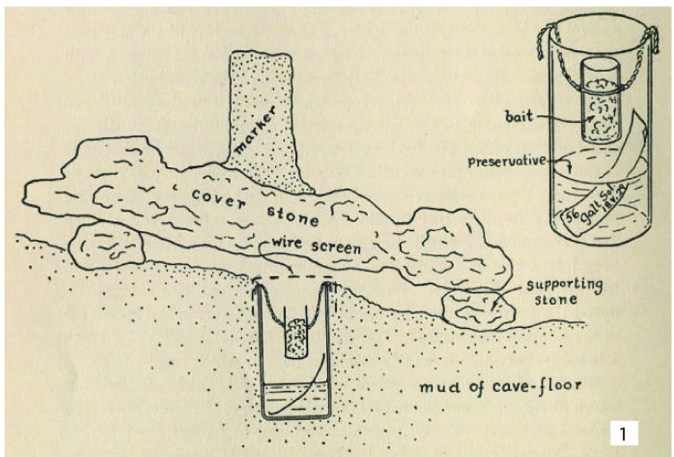
Pitfall Traps

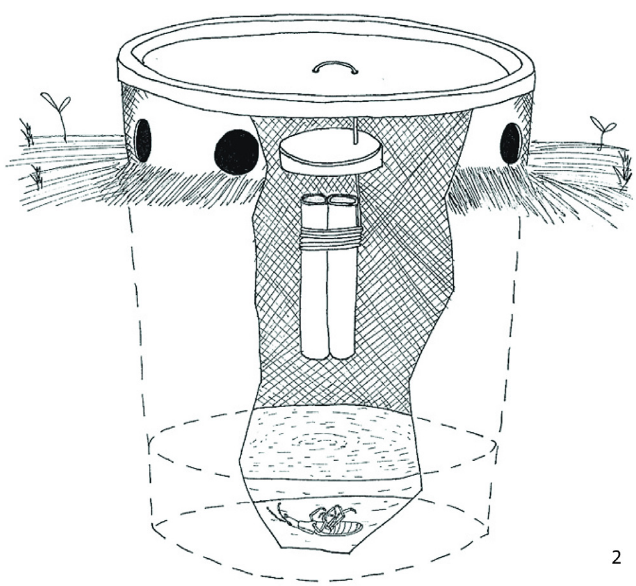
Pitfall traps were first described by Hertz (1927) and shortly thereafter by Barber (1931) (Fig. 1) for collecting cave-inhabiting insects. A pitfall trap is simple in design, consisting of a collecting container buried flush with the ground that passively collects epigeal organisms that accidentally fall into the trap. It may be constructed from any container large enough to hold the target organism, including a large bucket for reptiles or small mammals (Ellis 2013), small plastic cup for larger insects such as Carabidae and large Formicidae (Luff 1975; Abensperg-Traun & Steven 1995), or a glass test tube for small insects such as most Formicidae and small Carabidae (Luff 1968; Abensperg-Traun & Steven 1995). Pitfall traps are widely used in biodiversity surveys as they are cost-effective, ecologically sensitive, collect large numbers of arthropods (Gist & Crossley 1973; Ekschmitt *et al*. 1997; Southwood & Henderson 2000; Work *et al*. 2002), and collect nocturnal species missed by other methods (Törmälä 1982; Samways 1983; Donnelly & Gilmee 1985; Huusela-Veistola 1996).

Pitfall traps have been used to sample many arthropod groups, including Scorpionida (Tourtlotte 1974; Margules *et al*. 1994); Isopoda (Hamner *et al*. 1969; Hayes 1970; Paoletti & Hassall 1999; Hornung *et al*. 2007); Diplopoda (Van der Drift 1963; Kurnik 1988; Mesibov *et al*. 1995; Kime 1997; Snyder *et al*. 2006), Chilopoda (Kurnik 1988; Fründ 1990; Adis 1992; Shear & Peck 1992; Voigtlander 2003), and Symphyla (Adis 1992; Shear & Peck 1992; Clark & Greenslade 1996); Araneae (Duffey & Millidge 1954; Muma 1973; Uetz 1977; Corey & Taylor 1988; Bultman 1992; Koponen 1992; Bauchhenss 1995; Buddle *et al*. 2000); Acari (Zacharda 1993; Wickings 2007; Kłosin´ska *et al*. 2009; Mayoral & Barranco 2009; Wohltmann & Mąkol 2009; López-Campos & Vázquez-Rojas 2010; Clark 2013); Collembola (Joosse-van Damme 1965; Pedigo 1966; Budaeva 1993; Cole *et al*. 2001; Frampton *et al*. 2001); Coleoptera (Backlund & Marrone 1997; Simmons *et al*. 1998; Arbogast *et al*. 2000) including Carabidae (Anderson 1985; Kálás 1985; Cameron & Reeves 1990; Epstein & Kulman 1990; Togashi *et al*. 1990), Tenebrionidae (Ahearn 1971), Staphylinidae (Anderson 1985; Braman & Pendley 1993; Ekschmitt *et al*. 1997), Scarabaeoidea (Young 1981; Peck & Howden 1985; Martínez *et al*. 2009; Anlaş *et al*. 2011; Thakare *et al*. 2011), and certain Latridiidae (Hartley *et al*. 2007); Formicidae (Van der Drift 1963; Greenslade 1973; Anderson 1991; Abensperg-Traun & Steven 1995; Bestelmeyer *et al*. 2000); and even terrestrial Amphipoda (Craig 1973; Margules *et al*. 1994) and Decapoda(Williams *et al* 1985; Smith *et al*. 1991; Hamr & Richardson 1994; McGrath 1994; McIvor & Smith 1995). Of these taxonomic groups, ground-dwelling Araneae and Coleoptera have been the most studied (Westberg 1977).

Variations on the basic trap have been developed, including more elaborate traps for use under snow (Kronestedt 1968; Steigen 1973); live traps with a layer of gauze that keeps trapped organisms from drowning in rainwater (Duffey 1972); modifications that allow excess rainwater to drain before overflowing the trap (Duffey 1972; Porter 2005); integrated internal funnel and rain cap (Fichter 1941); collecting cup integrated into a larger structure with a base or ramp (Muma 1970); use of holes or slits in the side of a container so an integrated cap can be used (Fig. 2) (Nordlander 1987; Lemieux & Lindgren 1999); modifications to facilitate emptying (Rivard 1962), including automated devices for segregating trap catch over time (Williams 1958; Blumberg & Crossley, 1988; Buchholz

2009); designs to reduce mortality of vertebrate bycatch including floating shelters and wire mesh (Kogut & Padley 1997; Pearce *et al*. 2005); and inexpensive designs using commonly discarded household materials (Morril 1975; Clark & Bloom 1992). Other techniques, such as using an auger bit to drill placement holes for small diameter traps, and equipment, such as a device that can pull traps out of placement holes without kneeling or disturbing the surrounding soil, have been developed to make pitfall trapping easier (Vogt & Harsh





FIGURES 1–2. 1, Pitfall trap described by Barber for collecting cave-inhabiting insects. After Barber (1931). 2, Pitfall trap modified with entrances in the side of the collection cup, which discourages vertebrates from entering the trap and allows the use of an integrated rain cap. Modified from Nordlander (1987) with permission.

2003).

Barrier fences have been employed, either with a single pitfall situated in the middle of the fence or with pitfalls at the end of the fence (Fig. 3) (Haeck 1971; Meijer 1971; Reeves 1980; Durkis & Reeves 1982). Linear pitfall traps constructed from house gutters have been employed with success in certain situations, such as investigating the speed and timing of insect populations moving between habitats (Pamanes & Pienkowski 1965; Goulet 1974; Pausch *et al*. 1979).

Ramp traps collect arthropods similarly to pitfall traps, but rather than being sunk into the ground target taxa are directs upwards into the trap via ramps; this allows

FIGURES 3–4. 3, Pitfall traps (modified from Nordlander 1987) on either side of a barrier fence. 4, Ramp trap.

them to be employed where conventional pitfalls cannot, such as where digging is difficult (e.g., on rocks or in caves) or prohibited by law (Bouchard *et al*. 2000; Campbell *et al*. 2011). Bostanian *et al*. (1983) proposed the first ramp trap design, which is constructed from metal, making it rather bulky and expensive and biased towards large ground beetles. Bouchard *et al*. (2000) proposed a revised design that utilizes plastic sandwich containers and plastic ramps, rendering it light-weight and inexpensive (Fig. 4). Ramp traps have been successfully employed in caves (Campbell *et al*. 2011), areas polluted due to industrial mining (Babin-Fenske & Anand 2010), orchards (Smith *et al*. 2004), and vineyards (Goulet *et al*. 2004). Ramp traps capture a higher abundance and diversity of epigeal spiders than conventional pitfall traps, though when comparing other taxa (e.g., beetles) they collect a different species composition, thus making direct comparison between the trap types difficult or impossible (Pearce *et al*. 2005; Patrick & Hansen 2013). Additionally, ramp traps capture fewer vertebrates than conventional pitfall traps (Pearce *et al*. 2005).

Colored pan traps, sometimes referred to as water traps, are generally used to collect flying insects via visual response to color cues (e.g. yellow, blue, purple or red) (Kirk 1984; Aguiar & Sharkov 1997; Leong & Thorp 1999; Pucci 2008; Gollan *et al*. 2011). While pan traps are generally set on or above the ground, they may be sunk into it, effectively becoming pitfall traps that also attract and capture flying insects.

Issues with pitfall traps

Objections have been raised to the use of pitfall traps in ecological studies (Adis 1979; Majer 1997; Southwood & Henderson 2000) because they do not evenly catch different taxa for several reasons:

1. Different taxa react differently at the lip of the trap. Gerlach *et al*. (2009) found that millipedes show the most trap-avoidant behavior (20–60%) and carabids show the least (10–25%); overall they found an average of 28% of taxa that encountered a trap were caught, with a range of less than 5% (*Enantiulus nanus* (Latzel, 1884) (Julidae)) to 70% (*Pterostichus burmeisteri* Herr, 1838 (Carabidae)). Luff (1975) found approximately 75% of Carabidae that encounter the edge of a pitfall are collected. In mark-recapture studies, some species become trap-shy if they have been caught previously while other species do not (Benest 1989).

2. Activity level (Ekschmitt *et al*. 1997), which is affected by variables such as species-specific behavior (Greenslade 1964; Curtis 1980; Anderson 1991; Topping 1993; Spence & Niemelä 1994; Obrist & Duelli 1996); differences between gender and age (Hayes 1970; Benest 1989; Topping & Sunderland 1992; Thomas *et al*. 1998) including mate-searching (Tretzel 1954), post-copulatory dispersal of females (Merrett 1967) and searching for oviposition sites (Duffey 1956); weather (Williams 1940; Briggs 1961; Greenslade 1961; Juillet 1964; Ericson 1979; Drake 1994); vegetation (Deseo 1959; Greenslade 1964; Novák 1969; Baars 1979), habitat structure (Melbourne 1999; Melbourne *et al*. 1997; Thomas *et al*. 1998), and habitat type (Melbourne *et al*. 1997); size (Luff 1975; Thiele 1977; den Boer 1981; Franke *et al*. 1988) and speed (Braune 1974; Adis 1976); and hunger and prey density (Grüm 1971; Müller 1984; Henrik & Ekbom 1994), also affect the number of organisms trapped, both within and between taxa (Southwood, & Henderson 2000) and are more influential factors than population size (Briggs 1961) in determining trap catch.

3. Larger species are caught in significantly higher numbers than smaller species

(Carabidae: Franke *et al* 1988; Spence & Niemelä 1994). Several reasons have been suggested for this. Larger, faster beetles are successfully caught a higher percentage of the time than smaller, slower beetles (Braune 1974; Adis 1976) – though some authors have found size and speed do not affect the ability to be caught (Luff 1975; Halsall & Wratten 1988). Smaller beetles may escape more readily from traps because scratches and soil on trap walls may be enough to support their mass as they try to climb out whereas larger beetles fall (Spence & Niemelä 1994).

4. Species-specific morphology can affect escape ability; e.g., *Demetrias atricapillus* (L.) has adhesive setae on the underside of the tarsi that allow it to climb out of pitfalls more easily than other similarly sized carabids (Halsall & Wratten 1988).

5. Pitfall traps do not accurately reflect absolute density of the organisms sampled. This has been demonstrated in the field (Grüm 1959; Briggs 1961; Mitchell 1963; Marsh 1984; Topping & Sunderland 1992) and experimentally in a caged system (Lang 2000) – though caution should be exercised interpreting caged results as they may be skewed by "trap-happy" beetles that prefer dry pitfalls as refugia (Adis 1979, citing Thomas & Sleeper 1977) and may suffer from "Kreb's effect" (Mac Arthur 1984). However, it should also be noted that some studies have recorded 73–96% capture rates of marked beetles in caged systems (Bonkowska & Ryszkowski 1975; Dennison & Hodkinson 1984; Desender *et al*. 1985; Desender & Maelfair 1986; Clark *et al*. 1995; Holland & Smith 1999) and one study found no difference between population estimates of millipedes, spiders, and beetles based on hand collecting or pitfalls in a caged system (Gist & Crossley 1973), suggesting such systems may accurately reflect absolute density in certain situations with specific taxa.

In response to these criticisms, various calculations have been proposed to correct for the differences between taxa collected and true population density based on locomotory activity and motility range (Heydemann 1953; Tretzel 1955; Braune 1974; Thomas & Sleeper 1977; Kuschka *et al*. 1987; Stoyan & Kuschka 2001; see also Seifert 1990), though these have been rejected by others (Adis 1979; Müller 1984; Franke *et al*. 1988; Gerlach *et al*. 2009).

Additionally, it has been argued that samples pooled over an entire season correctly represent local species abundance as variations due to weather and other factors that affect activity level are averaged out (Baars 1979; den Boer 1986; Luff 1982). Results of other studies are conflicting, with some showing a large amount of variation between sampling periods in similar habitat when the sampling periods are short (Niemelä *et al*. 1986), and others showing that traps set for short periods caught all species accumulated by longer trapping periods (Niemelä *et al*. 1990; Borgelt & New 2006). In addition, much of the cited research has only examined carabids caught by pitfalls. When collecting other taxa, pitfalls may estimate absolute population density relatively well (ants: Andersen 1991; Vorster *et al*. 1992; Lindsey & Skinner 2001; cursorial spiders: Muma & Muma 1949; Duffey 1962; Huhta 1971; Uetz & Unzicker 1976; tenebrionids: Thomas & Sleeper 1977).

Certain ecological questions, such as comparing taxa along a successional gradient (Bultman & Uetz 1982) or between similar plots (Koivula *et al*. 1999), may be answered as taxa will be equally biased to pitfall traps along the gradient or between plots.

Pitfalls can be used to answer non-ecological questions, such as investigating the phenology (Maelfait & Baert 1975), seasonal and circadian activity (Williams 1959a, b; Williams 1962; Breymeyer 1966a, b; Doane & Dondale 1979), and lifespan

(Goulet 1974) of commonly collected taxa, estimating the timing of movement of epigeal species between habitats (Pamanes & Pienkowski 1965; Pausch *et al*. 1979), and estimating dispersal using mark-release-recapture methods (Ericson 1977; Best *et al*. 1981). They also can be employed in taxonomic surveys, though should be paired with other sampling techniques that complement the deficiencies of pitfalls (Majer 1997)

Pitfall trap design

If pitfall traps are to be employed, several considerations must be made as there are many factors that can affect the taxa collected.

<u>Effects of shape, size, and material of receptacle</u>. The shape of the trap affects the composition and number of taxa collected (Cheli & Corley 2010). Pitfalls may be straight-sided or round (Southwood & Henderson 2000), depending on the container used; however, round and straight-edged traps with the same perimeter length catch different numbers of specimens (Braune 1974; Luff 1975; Adis 1976; Spence & Niemelä 1994).

Different diameters of pitfall trap collect different taxa at different rates. When examining ants, larger diameter pitfalls catch more species, though differences are primarily due to differential capture rates of rare species (Abensperg-Traun & Steven 1995). Work *et al*. (2002) compared catch rates and species richness of Carabidae, Staphylinidae, and Araneae across five diameters (4.5, 6.5, 11, 15, and 20 cm) of pitfall traps; they found that, after standardizing circumference, small traps caught more small carabids and staphylinids and large traps caught more wolf spiders. Luff (1975) found that small traps (2.5 cm dia.) were the most efficient at catching small species of carabids, while large traps (10 cm dia.) caught relatively more large beetles; however, their small traps were made of glass and large traps made of metal, which probably had a confounding effect on the results. Brennan *et al*. (1999) found the largest and second largest traps (17.4 and 11.1 cm dia.) they tested caught the most diverse assemblage of species, though considered the smaller of the two traps more appropriate for sampling spiders as it may decrease the potential of capturing non-target species. One option when using larger traps is to add a funnel to the trap in order to increase trap retention (Vlijm *et al*. 1961).

Another aspect of size is the depth of the trap. Shallow (8 cm) and deeper (15 cm) pitfalls do not effect ant diversity capture (Pendola & New 2007), therefore, when targeting ants, shallow pitfalls are preferred as small vertebrates, such as skinks, may escape more easily from them, thus reducing vertebrate bycatch. However, this has only been demonstrated in ants and may not hold true for large insects, such as some carabids, which are bigger than some small vertebrates.

Pitfall traps used to collect insects have been constructed out of glass (Briggs 1961; Greenslade 1964; Borgelt & New 2006; Pendola & New 2007), plastic (Luff 1973; Morrill 1975; Clark & Blom 1992; Spence & Niemelä 1994), or metal (Ahearn 1971; Hinds & Rickard 1973; Clark & Blom 1992). Choice of material can affect the taxa sampled in live traps as escape rates differ. One study on carabids found 0% escape from glass traps, 4% escape per day from plastic traps, and 10% escape per day from metal traps (Luff 1975). Other studies have also found glass pitfalls retain more arthropods than plastic or metal (Vennila & Rajagopal 2000), though one found no difference between glass and plastic traps (Waage 1985). Similarly, Topping and Luff (1995) found plastic traps with rough surfaces caught fewer linyphiid spiders than similar traps with smooth surfaces.

Finally, color of the pitfall trap affects the taxa collected: white and yellow traps catch higher numbers of Apidae, Araneae, Carabidae, Diptera, and Formicidae, while brown and green traps catch higher numbers of Isopoda (Buchholz *et al*. 2010).

Effects of trap design, layout, and site selection. Some studies have found that covers do not affect the composition of arthropods trapped by pitfall traps (Work *et al*. 2002; Buchholz & Hanning 2009; Cheli & Corley 2010) while others have found they do (Briggs 1961; Baars 1979; Spence & Niemelä 1994). Some of this may be due to the material used as a cover. Man-made covers, such as metal or ceramic tile, are generally used. Suggestions have been made to use natural material such as bark or rock for covers (van der Berghe 1992), though this has not been systematically investigated.

Pitfall traps that have an integrated cap and circular entrances in the sidewall of the trap (first proposed by Nordlander 1987) caught 80% of the same common carabid species as conventional pitfalls in one study (Lemieux & Lindgren 1999), but otherwise have not been thoroughly investigated and compared to conventional traps.

Pitfall traps must be level with the soil surface as excessive inclination of the soil ringing the traps may direct some arthropods away from the trap (Heydemann 1953). Similarly, a plastic disc surrounding the trap will influence sample size (Adis 1976).

Subterranean pitfall traps have been employed to trap hypogaeic ants (Yamaguchi & Hasegawa 1996; Anderson & Brault 2010; Berghoff *et al*. 2003; Schmidt & Solar 2010), though these preform no better than conventional pitfalls (Pacheco & Vasconcelos 2012).

Use of a barrier fence consistently increases the number of ground beetles collected (Winder *et al*. 2001; Hansen & New 2005). However, the length of the fence influences trap catch (Durkis & Reeves 1982; Morrill *et al*. 1990), with longer fences catching higher diversity of families and species (Brennan *et al*. 2005), making it difficult to compare trap catch between studies. Location and number of the traps along the fence and fence material may also affect trap catch, though these variables have not been specifically investigated.

Spacing between traps is an important consideration as populations, especially of larger taxa such as carabids, can become locally depleted if traps are placed closely together; this can affect trap catch and skew results. Snider and Snider (1986) found no difference in trap catch between pitfalls spaced 0.5, 1, 2, and 4 meters apart. Similarly, Ward *et al*. (2001) found no difference in trap catch between pitfalls spaced 1, 5, and 10 meters apart. However, Digweed *et al*. (1995) found that carabid populations were depleted when pitfalls were placed 10 meters apart but not 25 meters; in addition, traps spaced at 10 meters had the most similar species assemblages and fewest rare species.

The optimum number of pitfall traps depends on the environment of the trapping site. As few as five traps aresufficient in an arid steppe environment (Cheli & Corley 2010), whereas ten to twenty pitfall traps effectively collected the majority of species in temperate areas (Formicidae: Santos *et al*. 2003; Coleoptera: Obrtel 1971; Isopoda Paoletti and Hassall 1999; Araneae: Niemelä *et al*. 1986), and at least twenty five are needed in tropical areas (Vennila & Rajagopal 1999). Various non-parametric estimators have been tested to estimate species richness based on as few as five traps per site (Brose 2002).

Finally, pitfall traps may not be the most efficient method for sampling epigeal arthropods in environments with rugged, steep slopes and a high density of rocks or roots in the soil where the traps are difficult to set or at high elevation where the mean body size of taxa is generally smaller, and thus more difficult to trap (Nyundo & Yarro 2007).

Additionally, some studies have found pitfalls trap more ants in drier areas and seasons (Delsinne *et al*. 2008; Nunes *et al*. 2011), though others have found annual rainfall has no effect (Delsinne *et al*. 2010).

Use of attractants in pitfall traps. The choice of preservative can affect the taxa collected in pitfall traps (Weeks & McIntyre 1997). For instance, bark beetles (Curculionidae: Scolytinae), certain Staphylinidae, and Nitidulidae are caught in higher numbers in pitfalls that use ethanol as the preservative (Drift 1963; Greenslade & Greenslade 1971). In one study, some Carabidae, especially *Bembidion*, were caught in higher numbers in ethylene glycol than water, though the effect varied by sex and time of year (Holopainen 1990, 1992); another study, however, found no difference between ethylene glycol and water when trapping four species of Diplopoda, one species of Chilopoda, and two species of Carabidae (Gerlach *et al*. 2009), suggesting that any effect is species dependent. Formaldehyde has been found to be repellant to Opiliones and Diplopodaand attractive to Carabidae and Staphylinidae (Luff 1968; Pekár 2002; Gerlach *et al*. 2009), though one study found no difference between water and formaldehyde when collecting Carabidae (Waage 1985). Differences have been found between commercially available antifreeze and diluted ethylene glycol (Koivula *et al*. 2003). Efficacy of preservatives can vary with trap size – one study found vinegar to be more effective in large traps but propylene glycol more effective in small traps (Koivula *et al*. 2003). Brine and an ethanol-glycerin mix have lower capture efficiency than other fluids such as pure water, ethanol-water, and ethylene glycol-water, possibly due to the high specific gravities of these fluids, which may allow captured arthropods to float and escape (Schmidt *et al*. 2006). Brine is also attractive to Lepidoptera (Cheli & Corley 2010). Additionally, attraction and repulsion to preservatives can vary due to sex (Adis 1976), season (Dethier 1947; Adis & Kramer 1975; Adis 1976), and environment (Koivula *et al*. 2003). Thus, careful consideration should thus be used in order to avoid or account for the influence of preservative on the taxa collected.

A drop of detergent is often used to break the surface tension of the preservative in wet pitfalls. This does not seem to affect the rate of capture of most arthropods, though Linyphiidae are caught in higher numbers (up to 1000%) in traps with detergent (Topping & Luff 1995; Pekár 2002), whereas Staphylinidae are caught in higher numbers in traps without detergent (Pekár 2002).

Some Coleoptera naturally aggregate using pheromones to locate conspecifics (Greenslade 1963; Wautier 1970, 1971; Ahearn 1971), which can affect trap catch distribution as the first specimen captured may artificially attract others to the same trap (Luff 1968; Thomas & Sleeper 1977; Luff 1986).

Digging-in effects have been recorded among Formicidae (Greenslade 1973), Carabidae (Digweed *et al*. 1995; Schirmel *et al*. 2010) and other Coleoptera (Schirmel *et al*. 2010), Collembola (Joosse-van Damme 1965; Joosse & Kapteijn 1968), Linyphiidae and other Aranaea (Topping & Luff 1995; Schirmel *et al*. 2010), and Isopoda (Schirmel *et al*. 2010). These effects consist of high capture of certain taxa immediately after pitfall traps are established followed by a subsequent decline. A variety of explanations – such as an increased level of CO2 (Collembola: Joosse & Kapteijn 1968), decreased barriers to movement (Carabidae: Greenslade 1964), increased number of prey that attract predators (Adis 1979), and decreasing number of foraging Formicidae workers (Romero & Jaffee 1989) – have been suggested, though no consensus has been reached. If digging-in effects are to be avoided, it has been suggested either to place pitfalls inverted for one week before operating them as traps (Greenslade 1973; Schirmel *et al*. 2010) or to install a tube or second container in which the pitfall can be placed in order to avoid disturbing the soil when it is serviced (Schirmel *et al*. 2010). Alternatively, if the goal is to catch large numbers of arthropods without regard to comparing between-trap catch, traps may be serviced more frequently in order to take advantage of digging-in effects (Schirmel *et al*. 2010).

Disturbance of leaf litter and vegetation around the traps can cause increased catch of highly mobile taxa, such as Gryllidae (Sperber *et al*. 2007). Areas around active pitfalls should therefore not be visited unless the traps are being serviced. Alternatively, regularly scheduled visits to the trap area will increase the catch of certain mobile taxa, though care should be taken in designing and executing such visits in order to provoke the same disturbance between traps (Sperber *et al*. 2007).

If attraction is desired, baits can be used to purposely affect the taxa collected (Greenslade & Greenslade 1971). Dung and carrion can used to collect Scarabaeidae, Staphylinidae, Silphidae, Ptiliidae, Histeridae, Hydrophilidae, and Leiodidae. Carnivore and omnivore dung provide good results – with human dung being among the most effective and readily available – while herbivore dung is generally poor (Newton & Peck 1975). Meat, tuna, and honey can be used as baits for ants (Romero & Jaffee 1989). Though not intentional, previously trapped insects may begin to rot in traps in which the preservative is ineffective due to dilution from rain or large numbers of trapped insects, thus attracting carrion feeding taxa (Holland & Reynolds 2005). Vegetable oils have been shown to increase the catch of ants in the tropics (Pacheco & Vasconcelos 2012), especially army ants (Weissflog *et al*. 2000; Berghoff *et al*. 2002; Berghoff *et al*. 2003), although this has not been studied in temperate regions.

<u>Pests of pitfall traps</u>. Occasionally, traps will be regularly disturbed by mammals between collections. Van der Berge (1992) presented three situations with the possible culprits and associated solutions. For traps where the cup is still in the hole but pushed up "just enough so that the rim is no longer flush with the soil" he suggests moles or voles whose passage has been obstructed are to blame and moving the cup a short distance usually resolves the problem. When one or a few cups, but not the entire trap line, are completely out of the hole, spilled clean, but not chewed on he suggests squirrels are attempting to burry or dig up nuts. Unfortunately, "one is helpless against squirrel disturbance". The third case is when many, and often the whole line, of cups are out of the hole and chewed or mangled. This, he suggests, is the work of raccoons, opossums or deer that are interested in consuming the preservative. Raccoons are intelligent and will continue to harass a line of pitfall traps if they are reset, so it is best to abandon the line or add a distasteful substance to the preservative. If deer are molesting the traps, it is best to switch from a salt-based preservative which is probably drawing their attention.

Preservatives

Pitfall traps can be used to collect insects to be kept alive or killed in preservative. If live specimens are required, such as for rearing experiments (as is common in parasitengone mites to correlate life stages) or in cases where the taxon of interest is endangered, e.g.

the American burying beetle (*Nicrophorus americanus* (Olivier, 1790)), traps are run dry without preservative. In such cases, traps must be checked at least daily, and often more frequently, so captured individuals do not succumb to heat, desiccate, drown in accumulated rain water, or become predated on by other captured organisms (Mitchell 1963; Luff 1968; Weeks & McIntyre 1997; Bestelmeyer *et al*. 2000; Moreau *et al*. 2013).

When collecting specimens to be killed, the choice of trap preservative is an important consideration as it will affect the quality of specimens, cost of trap maintenance, and how frequently traps must be serviced. Many authors have investigated the preservation properties of different chemicals and solutions, which are summarized herein.

Ethylene glycol was once used as a preservative, especially in pitfall and pan traps, as it has low volatility compared to ethanol and other alcohols (Martin 1977), is relatively inexpensive, and is readily available as antifreeze. When used in the field it has been reported to not preserve internal organs well and causes specimens to deteriorate to the point of breaking when pinned (Aristophanous 2010), though other studies report sufficient preservation (Sasakawa 2007; Cheli & Corley 2010). Because ethylene glycol is toxic to vertebrates (Thrall e al. 1984) and is readily ingested due to its sweet taste (Grauer & Thrall 1982), its use has been discouraged (Hall 1991).

The addition of bitter agents, such as quinine, to ethylene glycol has been suggested as a way to deter vertebrates from drinking the fluid (Hall 1991). Quinine added to ethylene glycol, propylene glycol, and formalin has been shown to have no effect on the number of spiders caught in pitfall traps; in addition, it improves the preservation quality of specimens collected in ethylene glycol (Jud & Schmidt-Entling 2008). Alternatively, a red marking flag placed next to the trap may deter large vertebrates from investigating the trap and drinking the ethylene glycol (Cheli & Corley 2010).

An alternative to ethylene glycol but with similar characteristics is propylene glycol, which is sold as recreational vehicle and boat antifreeze. It also has low volatility and is inexpensive. Propylene glycol is nearly non-toxic as it is metabolized into constituents of the Krebb's cycle and extremely large quantities must be ingested over a short period of time before acute toxicity is reached (Yu 2007). In the field, propylene glycol preserves insects similarly to ethylene glycol (Jud & Schmidt-Engling 2008; Aristophanous 2010). However, Moreau *et al*. (2013) found no detectable difference in the quality of DNA preservation between propylene glycol and ethanol when undiluted chemicals were used in a lab setting. One reason for the difference between field and lab studies may be due to the fact that ethylene glycol and propylene glycol are hygroscopic; when humidity is moderate to high, both substances will absorb water from the air and dilute naturally (Aristophanous 2010).

Salt brine and saturated borax solution are inexpensive and easy to make as the constituent materials are readily available in grocery stores. The ability of these solutions to preserve insects is extremely poor, however, and not outweighed by cost-savings (Lemieux & Lindgren 1999; Sasakawa 2007; Aristophanous 2010) (though see Schmidt *et al*. 2006 for a counter opinion).

Carnoy's fixative (60% ethanol, 30% chloroform, 10% acetic acid) and white vinegar (10% acetic acid) do not preserve DNA and cause specimens to become brittle, though they generally keep the specimens from rotting (Sasakawa 2007; Aristophanous 2010; Moreau *et al*. 2013). If DNA extraction is not intended, these may be acceptable preservatives.

Methanol and chloroform do not preserve specimens in a way that allows DNA extraction and amplification (Post *et al*. 1993; Fukatsu 1999). In addition, chloroform is difficult to acquire, especially in the large quantities required for use as a trap preservative.

FAACC solution (formaldehyde 4%, acetic acid 5%, calcium chloride 1.3%) and 4% phosphate buffered formaldehyde (4%PBF) both preserve internal organs well, with 4%PBF being the superior of the two (Aristophanous 2010). However, specimens become excessively stiff and although DNA can be extracted from specimens preserved with formaldehyde solutions, DNA amplification is impossible with standard kits (such a Qiagen DNEasy) because formaldehyde causes DNA to cross-link with proteins (Schander & Halanych 2003). Protocols using prolonged extraction times (up to 7 days) (France & Kocher 1996; Chatigny 2000; Schander & Halanych 2003) and chemical agents (Johnson *et al*. 1995; Chatigny 2000) can be successful.

Amyl acetate is sometimes used in insect jars as the killing agent. This banana-smelling liquid keeps specimens relaxed, unlike other killing agents such as chloroform (Woodward 1951). It is commonly used as a water-removing solvent in industry and can be purchased through specialized suppliers. Amyl acetate has been used for preservation of anatomical dissections (Saunders & Rice 1944) and insects "may be kept stored almost indefinitely between cotton-wool impregnated with this agent" (Woodward 1951), though it has not been tested for DNA preservation (Nagy 2010). Additionally, it has not been tested as a preservative in pitfall traps, can be a skin irritant, and is probably attractive to some insect groups so other, more proven preservatives may be a better choice.

Ethanol is probably the most widely used preservative. It maintains the integrity of internal organs and allows DNA to be easily extracted and amplified (Gurdebeke & Maelfait 2002; Aristophanous 2010; Moreau *et al*. 2013). In the United States, price may be prohibitive for individuals who do not qualify for ethanol tax exemption; however, fuel ethanol has been shown to preserve specimens as well as pure ethanol, so this will provide an alternative source as fuel ethanol becomes more widespread (Szinwelski *et al*. 2012). In addition, ethanol is the most volatile commonly used preservative. In open containers such as pitfall traps ethanol can lose ¾ of its volume in fewer than 5 days (Aristophanous 2010). Depending on the trap location this may have implications on how often the traps must be serviced.

Isopropanol, commonly known as rubbing alcohol, is a cheap alternative to ethanol. Similar to ethanol, it preserves DNA well (Rake 1972), so it can be extracted with little difficulty. One drawback is that isopropanol often discolors specimens, which is a hindrance to identification and morphological studies involving color.

Acetone has shown promise as a preservative. It is relatively inexpensive and readily available as a paint solvent. DNA has been extracted and successfully amplified from acetone-preserved Copepods (Goetze & Jungbluth 2013), pea aphid (*Acyrthosiphon pisum* (Harris, 1776)) (Fukatsu 1999), and Zygoptera (Logan 1999). Additionally, acetone is used to preserve adult Odonata as it dissolves fat, dehydrates the specimen, and reduces decomposition of enzymatic color pigments (Abbott 2008).

Other preservatives require more testing as contradictory results have been reported. Fukatsu (1999) reported DNA amplification after specimens were stored in 2-propanol, ethyl acetate, and diethyl ether, though Post *et al*. (1993) and Reiss *et al*. (1995) reported poor results with 2-propanol and ethyl acetate, respectively.

Summary

Pitfall traps are often used to sample epigeal arthropods as they are inexpensive and easy to use. However, many factors influence the taxa so collected. Abiotic factors, such as weather, season, slope and aspect, degree of rockiness, and trap characteristics (color and material of the trap, diameter of the opening, spacing between traps, and number of traps at a site) affect the composition of collected taxa, often by affecting behavior of the target arthropods. Biotic factors affecting trap catch include species-specific factors (activity level, size, aggregation to conspecifics, and behavior at the edge of the trap), response to digging-in effects, and habitat structure, including the density of low-growing vegetation. The choice of preservative affects not only the level of preservation of specimens, but also the composition of specimens collected because various compounds differentially repel and attract different taxa. Taken together, these factors make comparisons between studies difficult.

While there have been calls to standardize pitfall trapping, the design employed in individual studies will continue to be based on the research question and materials available. An effort, however, should be made to report all of the factors that might influence the composition of specimens collected. While this may not be immediately useful, comparisons may be made in the future after further studies elucidate the effects various factors have upon trap catch.

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