

Experimental considerations support the use of artificial sentinel prey—a comment on Rodriguez-Campbell et al

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Predation is a crucial interaction in ecosystems, transferring energy between trophic levels, enhancing ecosystem stability through its impact on herbivore populations, promoting species diversity, and exerting evolutionary pressure. Predation may also provide benefits for humans: preying on pest species translates into biological control with enormous economic value. Ecologists have long sought to quantify predation, which is challenging, especially by and on invertebrates.

One of the approaches is the sentinel method, when a known number of prey items is exposed under field conditions for a specific period (usually 24 h), and subsequently recording the numbers of prey that have been attacked, consumed or disappeared. Such prey can be real or artificial. Speight and Lawton (1976) exposed *Drosophila* pupae and from their rate of disappearance attempted to quantify beetle predation in wheat fields. Turner (1961) used artificial sentinels made of a mixture of flour and lard to quantify insectivorous bird predation on differently coloured prey. The use of real sentinels creates a more “natural” set of conditions, while artificial sentinels may allow the identification of predators from attack marks (Howe et al., 2009). Since the formal description of the method (Howe et al., 2009), there have been numerous articles from virtually all continents that sought to identify various aspects of predation (Lövei & Ferrante, 2017). The use of sentinel prey has also been recommended to characterise natural pest control as an ecosystem service (Meyer et al., 2015).

In an international experiment, Rodriguez-Campbell et al. (2024) measured predation rates using live and dead *Zophobas morio* larvae

and artificial caterpillars made of modelling clay with the intention of “validating” the efficacy of artificial sentinel prey. They found no significant differences in predation rates measured with live and dead larvae, whereas patterns of predation measured with artificial sentinel prey were not always consistent with those recorded using real ones. The authors concluded that artificial sentinel prey are inadequate for comparing predation rates across sites and suggested that this approach should be abandoned for biogeographical studies. In this commentary, we aim to highlight certain logical fallacies in the study by Rodriguez-Campbell et al. (2024), discuss some misunderstandings regarding the use of artificial sentinel prey, and provide recommendations for best practice.

The first fallacy is the assumption that by using real prey we can quantify actual predation levels because “Live prey should most closely reflect predation on wild prey; dead prey have realistic visual and scent cues but lack movement and behavioural cues; and clay prey have only coarsely realistic visual cues.” Rodriguez-Campbell et al. acknowledge that “clay prey only appeal to a subset of potential predators”. However, the same is valid for any sentinel prey, including real ones. Once a sentinel is chosen, its features can either help or hinder predators in recognising it as prey and deciding to attack. One obvious feature is the status of the sentinel (dead, alive or artificial), as different prey types possess distinct chemical profiles, and many predators rely on chemical cues for hunting. However, the sentinel will also have a specific size, shape, texture, colouration, behaviour (or lack of it, if artificial or immobilised/dead) and taste. Because of such differences, inconsistent predation patterns have

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been, unsurprisingly, observed also in studies employing multiple real prey (McHugh et al., 2020). Comparing results obtained from different prey types assuming that one can serve as a true baseline to validate the other is futile; Rodriguez-Campbell et al. provide evidence that different sentinel types can result in different predation levels and patterns (see, for example, their Figure 2d–f for invertebrate predators). It is worth stressing, though that the sentinel prey method does not aim at obtaining absolute estimates of predation levels (Howe et al., 2009) but intends to provide *relative* comparisons (more on this later). Using multiple types of sentinel prey can provide additional information because they could appeal to different (non-overlapping or partly overlapping) subsets of predators. Finally, using dead sentinel prey may be interpreted as a measure of scavenging rather than predation and using live and dead prey can produce different patterns, even if in the study by Rodriguez-Campbell et al. it did not.

The second fallacy is that we must record actual predation levels to be able to draw any useful conclusion. According to the authors, “*clay models should be abandoned for comparing predation rates among sites...but that a possible exception is when studies target an appropriate predator group*” and “*clay prey may still be useful for assessing predation differences within sites...However, caution should be used...whenever the relative importance of predator guilds is likely to vary*”. If artificial sentinel prey are attacked by a specific guild (e.g. insectivorous birds), they can be used to compare predation rates by them in different sites, habitats, or treatments. Predator

communities are rarely static, either between- or within-sites, but this does not make real sentinel prey more suitable than artificial ones, or relative estimates of predation less useful. Imagine an experiment where the researcher was able to simultaneously record predation rates in two habitats (a and b) using two real prey types: (1) by releasing a large number of caterpillars and accurately recording their fate 24 h later (which is of course impossible in real life) and (2) by using immobilised caterpillars (as in numerous real-life cases). Imagine that in both habitats the predator community consists only of two predators, an insectivorous bird and a web-building spider, both having identical killing rate per capita. In Habitat (a), the birds make up 80% of the community and web-building spiders make up 20%, while in Habitat (b), birds make up 20% and web-building spiders 80% (Figure 1). Both predators consume freely moving caterpillars, but only birds consume immobilised caterpillars because web-building spiders do not actively search for prey. After 24 h, predation rates on free caterpillars would be identical in the two habitats (albeit due to the different contributions of each predator guild), whereas predation rates on immobilised caterpillars would be higher in Habitat (a) due to the higher number of birds in this habitat. Of course, in many cases, we have no information about the exact composition of the predator community or the preference of each predator species for different prey types. Yet, the fundamental question is: was the part of the experiment using immobilised prey useless because it did not reflect the predation rates measured using free caterpillars (supposedly “closer to the

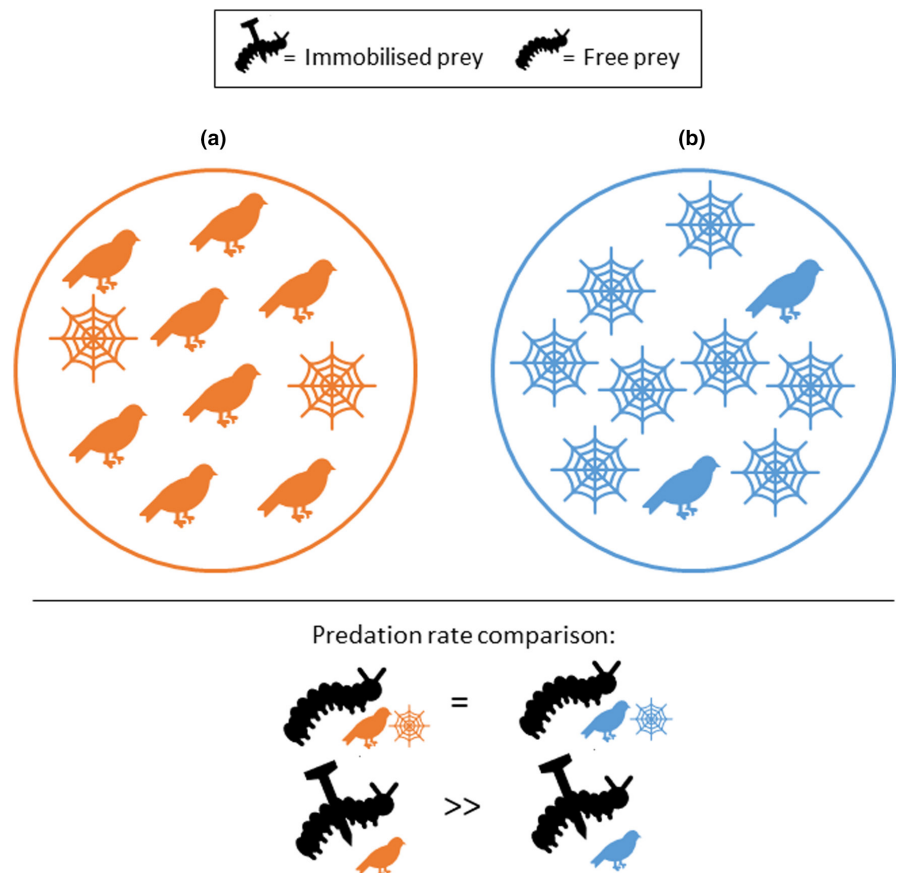


FIGURE 1 Thought experiment where predation was quantified in two habitats using immobilised caterpillars and free caterpillars. In both habitats the predator communities consist only of two predator species, an insectivorous bird and a web-building spider that hypothetically have identical killing rate per capita. However, in Habitat (a), insectivorous birds make up 80% of the community and web-building spiders make up 20%, while in Habitat (b), insectivorous birds make up 20% and web-building spiders 80%. Both predators consume free caterpillars, but only birds consume immobilised caterpillars because web-building spiders do not actively search for prey. After 24 h, predation rates on free caterpillars would be identical in the two habitats (albeit due to the different contributions of each predator guild), whereas predation rates on immobilised caterpillars would be higher in Habitat (a) due to the higher number of birds in this habitat.

true predation")? We claim that the immobilised prey gave useful information on the predation pressure exerted by birds in these two habitats. This can be very useful if the aim was to characterise the efficiency of a management intervention to encourage birds as possible biocontrol agents. This aspect was clearly articulated in the original description of the artificial caterpillar method (Howe et al., 2009).

We believe that we do not always need absolute quantification to advance our understanding of ecological processes and that relative information can also be useful (e.g. differences between farming practices or conservation interventions). The sentinel prey method is intended, and should only be used, for comparisons between habitats or treatments *and* within the same predator guild (Howe et al., 2009). When used in combination with biodiversity sampling and/or other types of sentinels, they provide different, complementary information. Most often the challenge is to find techniques that can be used in a standardised way and replicated in sufficient numbers to perform robust statistical analyses. Ultimately, the sentinel approach aims to quantify ecological processes relying on the *applicability* and *repeatability* of standardised monitoring tools. This applies not only to predation, but also to other ecosystem functions that can be measured using the sentinel approach (Ferrante et al., 2022). To fulfil these important requirements, which are the essence of the scientific method and allow us to obtain comparable results, ecologists may use "unnatural" tools. We agree that sentinels do not necessarily reflect the real situation, but experiments are always a simplification of reality in the interest of answering a central question.

Once this premise is accepted, it makes sense to look for a method that allows controlling most variables (e.g. prey type and size, density, distribution, exposure time), is easy to use and replicable in large sample sizes, is cost-effective, and provides information about the identity of the predators. Artificial prey (1) enable standardisation, since they can be produced from the same material with consistent characteristics (e.g. coloration and size), whereas real prey can vary in terms of their chemical composition, taste, smell, size, age, status (live prey can die under field conditions due to abiotic factors, which may cause some of the prey to be dead and some to be alive at the time when the researcher collects the results); (2) they are cost-effective to produce and can be manufactured in large quantities ensuring high replicability and statistical robustness; and (3) allow the identification of predators, even if with varying taxonomic resolution. The artificial sentinel prey method can do this, thus it can be useful despite its (recognised) limitations. If *Z. morio* larvae, a species found only in Central and South America, would have been used for a global predation study, other advantages and limitations would have emerged. Tethering a given sentinel prey to a substrate may also influence predator searching behaviour; attaching sentinel prey using glue, thread, pins or bulldog clips on popsicle sticks (the latter two used by Rodriguez-Campbell et al.) potentially introduce other biases to measurements and should be acknowledged.

There exist several limitations to the use of artificial caterpillars. However, we believe that we should avoid throwing out the baby

with the bathwater: the sentinel caterpillar method is useful to quantify relative predation rates and help to better understand ecosystems, as well as the impact of humans on them. We suggest that the following considerations would improve the quality of information obtained from artificial sentinel prey experiments:

1. When estimating predation rates on a prey species, a real prey is more suitable than an artificial one. Artificial caterpillars should not be used to get "absolute" estimates of predation, as the method is only meant for comparative purposes. Rather, predation rates obtained with artificial prey reflect predator activity or predation risk and are likely underestimated (Lövei & Ferrante, 2017). However, even if predation rates on artificial prey are biased, we can compare such estimates between sites or management types.
2. While there are numerous field studies with artificial sentinel prey, laboratory experiments that could help us understand the behaviour of predators towards different prey types are scarce (but see Ferrante et al., 2017). Moreover, predation rates on sentinel prey may be affected by the nutritional status of the predator or the presence of alternative food sources. Until the reasons why different predators attack artificial caterpillars are clarified, this method should not be used to compare predation rates by different predator groups although groups can be analysed separately based on attack marks by predators from broad taxonomic categories. Several factors determine whether a predator would attack an artificial caterpillar, including the cues on which its hunting strategy relies (e.g. visual, sound, chemical, movement), its ability to detect the prey, and its learning ability. Certain predators do not attack artificial caterpillars at all, while unexpected "predators", such as slugs or woodlice, do (Lövei & Ferrante, 2017, pers. obs.).
3. As recommended in the review of the method (Lövei & Ferrante, 2017), artificial caterpillars should be exposed for no longer than 24 h. This is important to avoid the complication of correctly timing predation events, the resulting decrease in real predation pressure, and a possible distortion due to predator learning (Lövei & Ferrante, 2017) but also to guarantee statistical independence between samples.
4. Sentinel prey are suitable when the aim of the research is to quantify predator activity rather than identifying predators, because after a predation event, real prey typically disappears while artificial caterpillars provide (limited) information about predator identity. Predator identification on artificial caterpillars can and should become more accurate and reach a deeper taxonomic resolution. Scientists with previous experience with this method can still identify marks incorrectly, particularly arthropod and non-predatory marks. However, we Valdés-Correcher et al. (2022) reported identification accuracy of ~80% based on pictures, and it is plausible that this would have been higher with the samples at hand, and if the volunteers had known where the sentinels were exposed and what they looked like before predation.

AUTHOR CONTRIBUTIONS

M.F. and G.L.L. conceived the comment. M.F. wrote the first draft. All authors contributed to the submitted first and the revised version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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