Trends in Ecology & Evolution

CellPress

Opinion

Trophic Cascades by Large Carnivores: A Case for Strong Inference and Mechanism

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Studies on trophic cascades involving large carnivores typically are limited by a lack of replication and control, giving rise to a spirited debate over the ecological role of these iconic species. We argue that much of this debate can be resolved by decomposing the trophic cascade hypothesis into three constituent interactions, quantifying each interaction individually, and accommodating alternative hypotheses. We advocate for a novel approach that couples the rigor characterizing foundational work on trophic cascades (i.e., from studies carried out in mesocosm and whole lake systems) with the conservation relevance of large carnivore-dominated food webs. Because of their iconic status, it is crucial that inferences about the ecological role of large carnivores rise to meet the same rigorous standards to which other studies in community ecology are held.

Trends box

Large carnivores are believed to play a key role in determining ecosystem properties via trophic cascades. While the recovery of large carnivore populations is generally heralded as a conservation success story, the common assertion that such recoveries alter plant communities and other ecosystem properties is currently not supported by the standards of evidence expected in other scientific disciplines.

There have been very few replicated and controlled experiments documenting evidence for trophic cascades involving large carnivores, leaving a knowledge gap with crucial implications for ecology and wildlife conservation.

The absence of a mechanistic understanding of food web dynamics and weak inference in many studies combine to obfuscate the mechanisms underlying if and how large carnivores affect ecosystems.

Carnivores, Cascades, and Controversy

Large carnivores are hypothesized to shape ecosystems both by consuming and scaring herbivores, thus indirectly affecting plants and abiotic processes through trophic cascades [1–3]. It has recently been suggested that trophic cascades are '. . .a universal property of ecosystem functioning, a law of nature as essential and fundamental to ecology as natural selection is to evolution' ([4], p. 354). Moreover, and because of their potential to trigger trophic cascades, some argue that the conservation of large carnivores is a moral obligation of humanity [5]. Notwithstanding the scientific or conservation promise that trophic cascades, the majority of evidence for this 'law' comes from studies on the dynamics of small and short-lived species observed in mesocosm or whole lake experiments [3,6–11]. Typically, these studies are characterized by strong inference, quantification of underlying mechanisms, and multiple hypothesis testing.

By contrast, evidence for the role of large carnivores as drivers of trophic cascades is ensconced in controversy, partly because of a reluctance to entertain multiple alternative hypotheses [12], partly because of a reliance on correlative methods [13], and partly because of a lack of clarity over the appropriate responses to measure [14,15]. Perhaps the most high-profile example of this controversy occurred in the Greater Yellowstone Ecosystem, where changes to the landscape that had been widely attributed to the reintroduction of wolves coincided with other sources of environmental change. As a result of these potential confounds, ecologists have questioned the mechanisms and the strength of the indirect effect of wolves in Yellowstone [15–23]. Similarly, variation in land use, rainfall, and soil conditions coincided with the extirpation of dingoes in Australia [24,25], confounding the reported impact of this apex predator on ecosystem function [26]. In the Eastern Pacific, environmental toxins and predation by sharks [27] obfuscated the putative cascading effects of orcas on nearshore ecosystems [28]. Given that the distribution of large carnivores is changing rapidly across the earth [5,29,30], it is crucial to better predict how, where, and when these iconic species generate trophic cascades.

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Here, we illustrate the salient limitations and opportunities in the approaches used by ecologists to test the trophic cascade hypothesis in food webs comprising large carnivores, herbivorous prey, and autotrophic plants. We then present a novel framework for testing cascades that is based on strong inference, quantifies the constituent interactions giving rise to indirect effects, and tests alternative hypotheses for each interaction. We highlight the particular challenge of assigning causation to a focal carnivore (i.e., relating to the focus of a particular study, such as a species reintroduction) in a speciose food web.

The Gold Standard: A Mechanistic Approach under Controlled and Replicated Conditions

A mechanistic and strongly inferential approach to studying trophic cascades has been used by ecologists for several decades, typically in systems with plankton, arachnids, insects, fish, and other small fauna as the top carnivore [3,6–10]. Taxonomy aside, the approach used in many of these studies might be viewed as the 'gold standard' by which the trophic cascade hypothesis is tested for both small and large carnivores. Through at least three key developments, the use of the gold standard approach generated a body of work that is convincing evidence for trophic cascades.

The first development was that of methodological advances to quantify the constituent interactions giving rise to trophic cascades. In its archetypal form [1], a trophic cascade results from the dampening of herbivory caused by carnivores' numerical suppression of herbivorous prey (but see Box 1), leading to plants' enhanced growth, biomass, cover, reproduction, or survival (hereafter referred to as 'plant abundance'). Thus, a trophic cascade requires demonstration of at least three interactions: (i) reduction of herbivore abundance by carnivores; (ii) reduction of plant abundance by herbivores; (iii) indirect facilitation of plants by carnivores via suppression of herbivory. The most powerful test of these three interactions occurs through species-removal

Box 1. The Special Case of Behaviorally Mediated Trophic Cascades

Trophic cascades occur through two nonexclusive pathways – density mediation and behavioral mediation. Density mediated trophic cascades (DMTCs) require a numerical reduction of herbivores caused by predation. Behaviorally mediated trophic cascades (BMTCs) involve nonlethal, antipredator responses of herbivores to the risk of predation [54]. BMTCs are believed to have stronger effects on plant abundance than DMTCs, particularly when antipredator behavior manifests as risk-averse habitat selection [7]. Understanding how predator hunting mode and the nature of herbivore resource limitation shape herbivore–plant interactions will determine if the indirect effect of carnivores on plants manifests as DMTCs, BMTCs, or not at all [35].

Unlike an archetypal DMTC [1], BMTCs arising from risk-averse habitat selection can lead to either positive or negative effects on plants. For instance, plants in areas that are risky for herbivores are browsed less often than plants occurring in safe habitats [35,39]. Consequently, a BMTC can generate positive indirect effects of carnivores on plants in risky areas and negative indirect effects of carnivores on plants in safe areas. Compared with food chains confined to the spatial boundaries of a mesocosm [9], these habitat shifts by the herbivore can be particularly important in large carnivore-dominated systems. In these open systems, large herbivores and their predators move over vast areas and spatial heterogeneity in landscape structure can greatly alter the direction and distribution of trophic cascades [39].

The framework for quantifying trophic cascades involving large carnivores (Figures 1 and 2) is identical for DMTCs and BMTCs in that evidence for the predicted direction and strength of interactions between trophic levels needs to be quantified individually. The occurrence of BMTCs requires that prey are risk-averse and that this aversion translates into a perceptible change in plant abundance [15,17]. Of these two requirements, researchers have struggled more with ways to quantify perceptions of risk in large mammals. In particular, researchers attempting to quantify how risk shapes habitat selection might first identify if 'risk' entails areas where predators are most abundant (increased probability of encounters between carnivores and herbivores), where prey are least abundant (loss of dilution and the 'many eyes' effect or because prey avoid risky areas), where probability of detecting a carnivore is lowest (increased probability of attack), or where probability of being captured following an encounter is highest [63]. In the few systems where the distribution of risk from large carnivores has been measured, these different types of 'risk' do not always coincide in space and time [16,64,65]. Thus, in testing for a BMTC, researchers must first identify which types of risk are avoided by the prey, and then whether this avoidance has a meaningful effect on plant abundance.

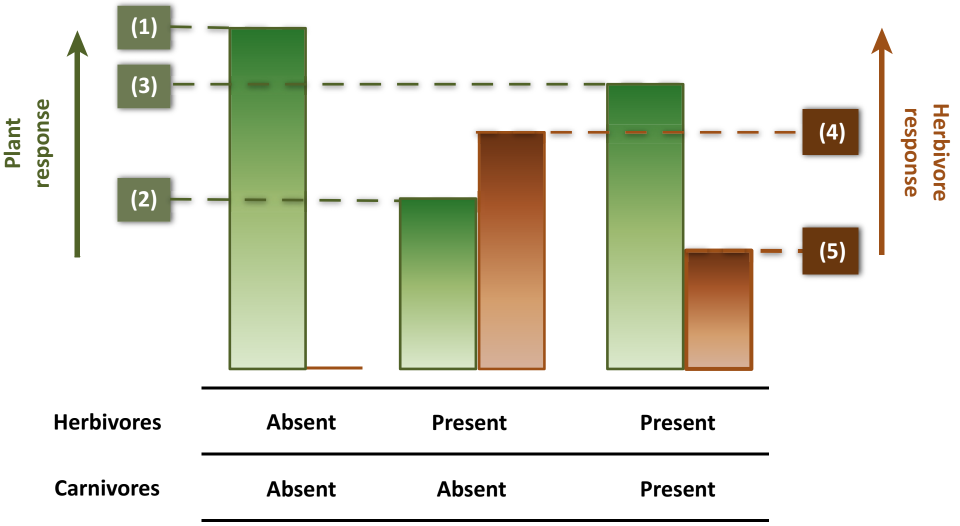

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Figure 1. The Proposed Gold Standard Approach to Identifying the Occurrence of Trophic Cascades. Archetypal trophic cascades arise from three constituent interactions: herbivores reduce plant abundance, carnivores suppress herbivores, and carnivores have an indirect, positive effect on plants. The alternative hypotheses of resource limitation or donor control generate testable predictions that contrast with the trophic cascade hypothesis for each interaction. To quantify the effect of herbivores on plants, plant responses (e.g., abundance, biomass, growth, cover, productivity) in the absence of herbivores (height of far left, green bar) should be compared with those of plants exposed to herbivores (height of middle, green bar). Resource availability limits plant responses in the absence of herbivores [plant response (1)] and plant resistance to (or tolerance of) herbivory determines the degree to which plants persist in the presence of herbivores [plant response (2)]. The trophic cascade hypothesis predicts a strong effect of herbivory, which is observed as the difference between plant responses (1) and (2). The difference between plant responses (1) and (2) will be minimal in the case of a bottom-up driven food web. To quantify the effect of carnivores on herbivores, herbivore responses (e.g., density, biomass, risk avoidance) to the absence of carnivores (height of middle, brown bar) should be compared with those of herbivores exposed to carnivores (height of far right, brown bar). Resource limitation constrains the response of herbivores in the absence of carnivores [herbivore response (4)], while the efficacy of risk avoidance behavior determines the degree to which herbivores persist in the presence of carnivores [herbivore response (5)]. The trophic cascade hypothesis predicts a strong effect of carnivory, which is observed as the difference between herbivore responses (4) and (5). To quantify the indirect, positive effect of carnivores on plants, plant responses to herbivory in the absence of carnivores (height of middle, green bar) should be compared with those of plants exposed to herbivory in the presence of carnivores (height of far-right, green bar). The trophic cascade hypothesis posits that plant responses to herbivores will be dampened in the presence of carnivores; that is, plant response (2) will be demonstrably less than plant response (3). In classic food web theory [1], the trophic cascade hypothesis also predicts that plant response (1) and plant response (3) should be equivalent; however, unless herbivores are completely extirpated by carnivores, plant response (1) is expected to exceed plant response (3) as a result of consumption by herbivores that persist in areas where carnivores are present, that is, the effect of herbivore response (5) on plant response (3).

experiments. In these experiments, plant (or another basal trophic level) responses are quantified in one- (plant-only), two- (plant and herbivore), and three-trophic level (plant, herbivore, and carnivore) food webs, while herbivore responses are quantified in two- and three-trophic level food webs (Figure 1).

The second development was the articulation and testing of alternative hypotheses that, in addition to trophic cascades, explain the dynamics of food webs (Figure 2). These alternatives include (but are not limited to): (i) donor control, resource limitation, or bottom-up effects – three properties of food webs where consumers have little regulatory effect on their prey; and (ii) reticulate food webs, in which trophic levels comprise multiple species with similar resource requirements, leading to weak interactions among pairs of species [31–33]. Testing these alternative hypotheses usually involves manipulation of resource availability, productivity [11], and the length of food chains [9].

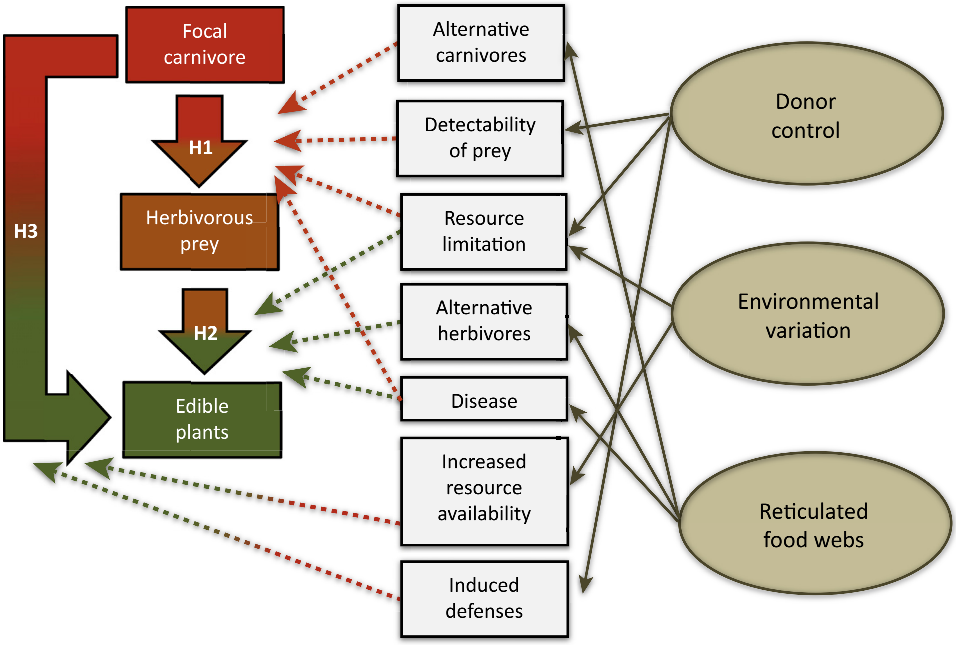

Figure 2. Conceptual Framework for Evaluating Alternative Hypotheses for a Three-Level Trophic Cascade Involving a Large Carnivore, its Herbivorous Prey, and Plants. Trophic cascades are a composite hypothesis, entailing three constituent interactions: (H1) suppression of the herbivore by a focal carnivore; (H2) suppression of plants by the prey of the focal carnivore; (H3) suppression of herbivory by the focal carnivore. These hypotheses are in contrast to a number of alternatives (gray boxes), which are supported by food web theory (brown ovals). Predictions under H1 might include a negative correlation between focal carnivore and herbivorous prey biomass. Evidence of lower recruitment or adult survival of herbivorous prey in areas or times of high carnivore abundance support the hypothesis of top-down control. Alternative hypotheses (gray boxes) to H1 include heightened predation by alternative (nonfocal) carnivores, reduced detectability of herbivores, reduced resource availability for herbivores, and increased prevalence of disease or pathogens in the herbivore population. These alternatives would confound and thus reduce support for trophic cascades if they occurred in areas or times of high focal–carnivore abundance relative to areas or times of low focal–carnivore abundance. Predictions under H2 include a negative correlation between herbivore and plant biomass. Evidence in support of H2 might include higher abundances of edible plants in areas or times from which herbivores were excluded, relative to areas that are accessible to herbivores. Alternative hypotheses to H2 include increased availability of resources for plants in the absence of herbivores or increased abundance of nonprey herbivores. These alternatives would confound and thus reduce support for trophic cascades if they occurred in areas or times in which plants are accessible to the herbivorous prey, relative to areas or times in which plants are inaccessible to the herbivorous prey. Similarly, such confounding variation would arise if the prevalence of plant pathogens and herbivore biomass are positively correlated. Predictions under H3 include reduced herbivory in areas or times where carnivores are abundant, relative to areas where carnivores are rare. Alternative hypotheses to H3 include heightened plant resource availability or increased plant defenses in areas with abundant carnivores relative to areas where carnivores are rare.

The third development occurred as ecologists clarified both the vocabulary and response variables inherent to testing the trophic cascade hypothesis. This clarification includes the distinction between species- and community-level cascades, and emphasizes biomass or abundance as principal response variables [32]. Moreover, species interactions are measured over a long-enough period to quantify the stability [32,34] and identity of the indirect effect (Box 1), and have sufficient magnitude to be both statistically and biologically meaningful [32,35].

Approaches to Quantify the Cascading Effects of Large Carnivores

In contrast to the application of the gold standard in experiments, the cascading effects of large carnivores have typically been assessed in conjunction with the extirpations, serendipitous recolonizations, or planned reintroductions of a single species of carnivore. Under these *ad hoc* settings, a positive correlation between carnivore and plant abundance often is interpreted as sufficient evidence for a trophic cascade.

By itself, a positive correlation between carnivore and plant abundance neither supports nor disproves the existence of a trophic cascade, for at least three reasons: (i) bottom-up driven systems can also produce a positive correlation between carnivore and plant abundance [36,37]; (ii) top-down driven systems can produce a negative (rather than positive) correlation between carnivore and plant abundance [37–39]; (iii) the reduction of herbivory by carnivores need not generate positive carnivore–plant correlations [40–42]. Without experimental control and replication, the extent to which correlations in species abundance constitute a rigorous test of the trophic cascade hypothesis will depend on consideration of multiple competing hypotheses (Figure 2). Next, we discuss applications, assumptions, and limitations of five, nonexclusive approaches that have been used to identify trophic cascades (Table 1).

Approach 1: Carnivore Occurrence and Age–Class Distributions in Plants

This approach employs a space-for-time substitution to compare contemporary age and/or size class distributions of plants in a carnivore-absent area with plants in an area where carnivores are

Table 1. A Summary of Five Approaches Used to Test for Trophic Cascades in Food Webs Dominated by Large Carnivores[a]

	Evidence for Interactions				
Approach	Carnivore–Herbivore	Herbivore–Plant	Carnivore–Plant	Other Strengths	Weaknesses
1	Assumed; not directly measured	Assumed; not directly measured	Plant recruitment or survival (estimated through size or age distributions) enhanced in the presence of carnivores	• Spatially expansive	• Uncontrolled • Unreplicated • Carnivory is treated as binary • Restricted to plants with measurable age (e.g., through annuli)
2	Assumed; not directly measured	Assumed; not directly measured	Higher plant abundance where carnivores occur	• Spatially expansive • Replicated	• Uncontrolled • Carnivory is treated as binary
3	Carnivore and herbivore abundance negatively correlated	Herbivore and plant abundance negatively correlated	Carnivore and plant abundance positively correlated	• Temporally expansive • Top-down control is continuous	• Uncontrolled • Spatially restricted
4	Carnivore and herbivore vital rates and abundances are negatively correlated	Herbivore and plant abundance negatively correlated	Carnivore and plant abundance positively correlated	• Spatially expansive • Temporally expansive Replicated	• Uncontrolled
5	Carnivore and herbivore vital rates and abundances negatively correlated	Herbivore and plant abundance negatively correlated; plants increase with herbivore exclusion	Carnivore and plant abundance positively correlated; plants decrease with carnivore exclusion	• Spatially expansive • Temporally expansive • Replicated Controlled	• Duration of experiments can be short relative to the lifespans of the organisms involved

[a] We reiterate that these five approaches need not be mutually exclusive. For example, it is possible to use a space-for-time substitution that is replicated, accounts for demographic responses, and addresses alternative hypotheses to explain variation in species abundance. Indeed, we expect that integrating the strengths of these different approaches will prove fruitful in future research in the ecology of large carnivores and encourage further development of a framework for quantifying trophic cascades (Figure 2).

still present or have recovered [21,43–46]. Compared with the carnivore-present area, the trophic cascade hypothesis predicts that a range of plant age (or size) classes will be missing from or suppressed in the carnivore-absent area. The missing or suppressed classes correspond to a temporal interval during which carnivores were extirpated, and herbivory was presumed to be prolific.

Approach 1 makes at least three assumptions: (i) that the loss of the focal carnivore is sufficient to account for increases in herbivore abundance; (ii) that heightened herbivore abundance led to the loss of the age and/or size classes; (iii) that other drivers (e.g., weather, disease) had comparable effects over time on both plant and herbivore abundance, across carnivore-absent and carnivore-present areas. The strength of inference in approach 1 hinges on the extent to which these assumptions are both true and tested, with the latter being rare in the published literature. For example, Ripple and Beschta [46] acknowledge that both variation in weather and multiple sources of predation were possible drivers of deer populations, yet they attribute variation in deer abundance to predation by a single species of carnivore, mountain lions. Moreover, for food webs involving plant species that do not leave annuli (e.g., trees in the tropics or herbaceous plants), it would be nearly impossible to quantify the indirect effects of carnivores over the multidecadal interval required of this approach.

Approach 2: Replicated Correlation between Plant and Carnivore Abundance

Similar to approach 1, this approach examines the legacy of a historical change in carnivore abundance. Approach 2 improves upon approach 1 through replication, which can be challenging at the expansive spatial scales over which large carnivores interact with their prey. For example, Callan *et al.* [47] used long-term (decadal) spatial stability of wolf territories in 32 areas, Letnic *et al.* [26] took advantage of an enormous (5614 km) dingo-exclusion fence spanning eight study areas, and both Croll *et al.* [38] and Terborgh *et al.* [48] studied trophic interactions in archipelagos where the constituent islands (n = 18 and n = 14 sites, for each study, respectively) varied in carnivore occurrence. The replication employed by these studies is a necessary step towards matching the inferential strength of the gold standard approach.

Studies using approach 2 often exhibit limitations similar to approach 1, including limited efforts to evaluate alternative hypotheses, the binary representation of top-down control (e.g., carnivores are absent or present, rare or common), uncontrolled distributions of carnivores, and unquantified direct interactions between species. For example, Callan *et al.* [47] conclude that wolves have cascading effects on plants because the preferred forage of deer was more abundant in areas with longer-term wolf occupancy than in areas with shorter-term occupancy. This study assumes that the duration of territory occupancy by wolves is a proxy for the magnitude of top-down control of deer, yet this assumption, as well as the potential effects of deer on plants, was not tested. In fact, the prediction that the preferred forage of deer is more abundant in areas with longer-term occupancy by wolves is equally valid for a bottom-up driven food web: abundant forage could increase the deer population, thereby stabilizing wolf occupancy. As Peterson *et al.* [13] recently identified, the tendency for consumers to aggregate in resource-dense areas is a bottom-up process, antithetical to trophic cascades. Thus, testing predictions for each of the constituent interactions in a trophic cascade remains crucial to improving approaches 1 and 2.

Approach 3: Time Series of Biomass for Three or More Trophic Levels

Unlike the binary representation of top-down control used in approaches 1 and 2, approach 3 tests for continuous correlations between species abundance in adjacent trophic levels. For example, McLaren and Peterson [49] quantified 40 years of covariation in wolf abundance, moose abundance, and tree growth in Isle Royale National Park, Houghton, MI, USA. Similarly, Brashares *et al.* [50] used long-term (ca. 30 years) population monitoring to track changes in large carnivores (lions and leopards), baboons (the prey of these carnivores), and small antelope (the prey of baboons) – in Ghana. Under approach 3, stronger inferences can be made for top-down control than approaches 1 or 2. This is because, statistically, there is more power to detect change in a time series than in a snapshot comparison and, methodologically, there is lower risk of confounding the effects of carnivores with other factors that limit the abundance of herbivores and plants.

A key requirement of approach 3 is that of long-term, consistent efforts to generate sufficient data on the interactions between long-lived species such as large mammals and trees. Moreover, this approach also relies on variation – often natural – in carnivore abundance or resource availability to detect a trophic cascade. For example, at Isle Royale, two low points in the wolf population cycle corresponded to heightened abundance and herbivory by moose [49]. These two events were separated by more than 15 years and were crucial to identifying the response of trees to variation in herbivory [13]. Had the population of wolves remained stable in this system, or if this research was conducted over the typical <4-year period of many field studies, this trophic cascade would have been difficult to quantify without additional studies (e.g., experimental removals).

Approach 4: Comparison of Time Series Data between Sites with and without Carnivores, with Quantification of Direct Interactions

Similar to approach 3, approach 4 uses inherent variation within a time series of species abundance to quantify interactions between adjacent trophic levels, with the additional element of an area in which carnivores are absent. The comparison of plant and herbivore (or prey) responses over time in carnivore-present and carnivore-absent areas provides insight on the relative roles of bottom-up and top-down control in the food web. For example, Estes *et al.* [28] relate the energetic demand of killer whales to demographic rates and population change of one species of their prey, sea otters, over a 17-year period. The authors explore alternative hypotheses to killer whale predation that could drive changes in otter abundance, including otter fertility, pollution, and population redistribution. Further, Estes *et al.* [28] capitalized on natural variation in whale abundance to assess the indirect effects of whales on urchins (otter prey) and kelp (urchin prey). Another example of approach 4 is Hebblewhite *et al.* [51], who took advantage of human-avoidance behavior by wolves to formulate a natural experiment. Over a 14-year period, Hebblewhite *et al.* [51] quantified elk abundance (the primary prey of wolves) in areas that wolves did and did not avoid. Crucially, both Estes *et al.* [28] and Hebblewhite *et al.* [51] related demographic rates of prey to carnivore abundance. Lower adult survival or recruitment associated with increases in carnivore abundance suggests a mechanism by which top-down control of herbivores could arise. Given their relatively slow life histories, quantifying these demographic rates in the prey of large carnivores is difficult without the types of long-term population monitoring used in approach 4.

One limitation of approach 4 is the absence of experiments to reinforce inferences from correlative observations. Such experiments will rarely be feasible across all trophic levels, but their inclusion at any trophic level will strengthen inference. As Underwood *et al.* [52] note, ‘experimental removals of otters have not been done, even though there is overwhelming evidence that properly controlled experiments are necessary to prove any sound basis for unraveling the complexities of indirect interactions’ ([52], p. 107), but see Hughes *et al.* [53].

Approach 5: Manipulating the Length of Food Chains

Approach 5 uses field experiments to approximate some of the key elements of the gold standard. At least six studies have used this approach, but with mixed results: four studies did not find evidence of a trophic cascade [17,40–42], while two did [39,53]. These studies used exclusion fences (or cages) to quantify both direct and indirect species interactions. For example, Sinclair *et al.* [41] measured woody plant abundance in plots that excluded snowshoe hares and their predators (one-level food web), allowed hares but excluded lynx (two-level food web), and allowed both hares and lynx (three-level food web). They found that while lynx suppressed hares, and hares suppressed woody plants, the abundance of woody plants was statistically indistinguishable regardless of whether lynx could access plots. Using exclusions similar to those of Sinclair *et al.* [41], two other studies [40,42] found evidence for top-down control through sequential trophic levels, but no subsequent indirect effect of carnivores on the basal trophic level. Intriguingly, of the dozens of studies that have putatively tested for trophic cascades in large carnivore-dominated food webs, only two have adopted approach 5 and found support for a trophic cascade [39,53].

We reiterate that approaches 1 through 5 are not mutually exclusive. For example, it is possible to use a space-for-time substitution that is replicated, accounts for demographic responses, and addresses alternative hypotheses to explain variation in species abundance. Indeed, we expect that integrating the strengths of these different approaches will prove fruitful in future research on the ecology of large carnivores.

A Framework for Quantifying Trophic Cascades Involving Large Carnivores

If trophic cascades are the manifestation of at least three interactions, then ecologists should endeavor to quantify each interaction separately, regardless of whether the apex predator is a wolf spider [54] or a gray wolf [49]. For studies in which experimental control and replication are impossible, stronger emphasis on testing alternative hypotheses is especially important (Figure 2). These alternative hypotheses might assess the role of environmental variation, reticulate food webs, and donor control as drivers of species abundance.

Direct Effect 1: Carnivore Suppression of Herbivores

Of the three constituent interactions in a large carnivore-mediated trophic cascade, quantifying the suppression of herbivores by carnivores often is the least amenable to experimentation. The strongest inference will likely arise through replicated, before–after control–impact (BACI) approaches that demonstrate both a negative correlation between focal carnivore and herbivore abundance, and evidence for bottom-up regulation in areas where the focal carnivore is absent. These replicated BACI approaches often are natural experiments, so additional attention is required to address confounding variation. For instance, quantifying the effect of carnivores on the behavior (Box 1) or demographic rates of herbivores can further illuminate the mechanisms of top-down control [28,35,51]. Finally, scrutiny of the relative roles of the focal carnivore versus other carnivores in the study system will aid in revealing the potential for reticulate food webs (Figure 2). This scrutiny might become especially important in cases where the focal carnivore is of conservation interest.

Direct Effect 2: Herbivore Suppression of Plants

The potential for herbivores to reduce plant abundance is a crucial and often overlooked component in testing the trophic cascade hypothesis. One of the most commonly used means to quantify herbivory is through exclosures: suppression is evident when plants inside an exclosure become more abundant than those in areas accessible to herbivores, when compared over the same period of time.

Similar to top-down control of herbivores by carnivores, the degree of reticulation in herbivore–plant interactions bears consideration. Not all herbivores are equally vulnerable to predation [55], and more than one species of herbivore can suppress the same species of plant [56]. Thus, it is important to quantify the extent to which herbivory by the prey of the focal carnivore alters plant abundance, not just the herbivore guild in aggregate. For example, both elephants and impala dominate the browser community in an East Africa savanna [56]. To quantify a trophic cascade in this area, Ford *et al.* [39] used size-selective exclosures to separate the effect of browsing by impala – a common prey of wild dogs and leopards – from the effect of browsing by elephants, which these carnivores do not typically kill. Additionally, diet preference of herbivores can be particularly important to consider in food webs with strongly donor-controlled interactions, for example, those containing well-defended plants. In the absence of information on herbivore diets and plant defenses, a positive correlation between well-defended plants and carnivores could be mistakenly interpreted as a trophic cascade. Feeding trials [39] or scat analysis [57] can be used to identify diet composition of herbivorous prey, and thus lend support to the interpretation that increased abundance of preferred plants inside exclosures is evidence for top-down control by herbivores.

The Indirect Effect: Carnivore Facilitation of Plants

Under the trophic cascade hypothesis, the indirect effect of the focal carnivore on plants derives from the dampening of herbivory. One way to test for such dampening is to establish herbivore exclosures paired with controls in areas (or times) of high and low carnivore abundance. If a trophic cascade is operating, the difference in plant abundance between exclosures and controls will be greatest where (or when) carnivore abundance is lowest (Figure 1). These differences should diminish as increasing carnivore abundance reduces herbivory. This approach to quantifying trophic cascades is robust to the effects of environmental variation on plant abundance over time because the relative (rather than absolute) difference between herbivore exclosures and paired controls is considered as the response variable.

When is a Negative Result Informative?

Studies reporting the absence of a trophic cascade should not necessarily be viewed as a negative result – this finding is consistent with a deep body of ecology theory that predicts the conditions through which bottom-up control occurs [37,58] or where top-down control does not lead to the indirect effects of carnivores on plants [35]. Indeed, donor control and other forms of resource limitation, reticulation, and environmental heterogeneity are all predicted to diminish the strength of trophic cascades [31–33,35,59]. These features characterize many large carnivore-mediated food webs, yet such alternative hypotheses seem to have been largely disregarded in the trophic cascades literature surrounding large carnivores.

To explore this point further, we conducted a topic search on the keyword 'trophic cascade' within papers published in the research areas of environmental sciences, ecology, and biodiversity conservation (Web of Science, accessed August 12, 2015). We identified three highly cited papers [31,33,59] for which the central focus was a conceptual discussion on trophic cascades and associated alternative hypotheses (e.g., donor control, reticulate food webs). Next, we conducted a topic search on the keywords 'trophic cascade' AND the names of seven species recently linked to trophic cascades by Ripple *et al.* [5] (i.e., '*Enhydra lutris*' or 'sea otter' or '*Puma concolor*' or puma or cougar or 'mountain lion' or '*Panthera leo*' or 'African lion' or '*Panthera pardus*' or leopard or '*Lynx lynx*' or Eurasian lynx or '*Canis dingo*' or dingo or '*Canis lupus*' or wolf or wolves or 'large carnivore'). Then, we conducted a second topic search using the same rules, except that we replaced 'AND' with 'NOT' to generate a list of trophic cascade studies published for ecosystems in which large carnivores – and particularly the seven species featured prominently in Ripple *et al.* [5] – were not featured. We limited the search to articles or reviews published at least 1 year after the publication of the aforementioned three highly cited papers [31,33,59]. Finally, we compared the proportion of papers from the 'large carnivore list' and 'not large carnivore list' that cited each of the three highly cited papers (see Table S1 in the supplemental information online). For each influential paper, citation rates were significantly lower by large carnivore studies than studies on trophic cascades in which large carnivores were not featured ($P < 0.02$ for all three papers). This systematic underrepresentation suggests that researchers working on the ecology and conservation of large carnivores are either unfamiliar with these foundational papers, or view theory as peripheral to quantifying the indirect effects of large carnivores.

Concluding Remarks

Pinpointing causal links in the dynamics of large carnivores, their prey, and plants across expansive scales of time and space is undoubtedly challenging and raises many questions about how science is conducted (see Outstanding Questions). However, such challenges should not excuse weak inference [12,60], and we believe that many of the controversies surrounding trophic cascades can be resolved by improving the rigor and objectivity with which this hypothesis is tested. Given the recent extirpation [5,30] and expansion [29,61,62] of large carnivore populations around the world, there is an urgency to predict their role as drivers of ecosystem change. The accuracy of these predictions will depend on the capacity of the scientific community – researchers, journal editors, science media, funders – to strengthen the inferences through which the trophic cascade hypothesis is tested.

Outstanding Questions

Reciprocity: Carnivore removal and recovery have been used interchangeably to study trophic cascades, but are these reciprocal approaches or is the strength of indirect effects anisotropic?

Generality: How comparable is the trophic cascade hypothesis between food webs composed of highly divergent organisms? Do we expect wolves, elk, and trees to exhibit dynamics comparable to spiders, grasshoppers, and forbs?

Performance measures: Are performance measures for plants (cover, density, growth, reproduction, biomass) and herbivores (presence/absence, abundance, per capita growth) interchangeable demonstrations of trophic cascades, and to what extent does a trophic cascade occur if only a subset of these measures is affected by carnivores?

Scale: Over what spatial extent and temporal duration should the indirect effects of large carnivores be measured to test for and validate the occurrence of a trophic cascade?

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Supplemental Information

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