

Predation or scavenging? Thoracic muscle pH and rates of water loss reveal cause of death in arthropods

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SUMMARY

The difficulty of directly observing predatory events hinders a complete understanding of how predation structures food webs. Indirect approaches such as PCR-based and isotopic analyses clarify patterns of resource consumption but fail to distinguish predation from scavenging. Given that facultative scavenging is a ubiquitous and phylogenetically widespread foraging strategy, an improved ability to discriminate prey from carrion is needed to enhance an understanding of the demographic effects of consumption and the true nature of trophic interactions. Using physiological properties of muscle tissue – specifically pH and rate of water loss – we develop a novel method to discriminate prey from carrion collected by scavenging hymenopteran predators. Our focal system is the western yellowjacket (*Vespula pensylvanica*), a common scavenging predator in Hawaii and western North America. Prior to consumption, the physical properties of hymenopteran muscle tissue change in a quantifiable and deterministic manner post mortem and can be used to estimate the time and putative cause of death of diet items. Applying this method in laboratory and field situations resulted in the correct identification of prey and carrion in 49 out of 56 cases (88%). Although further investigation is needed to determine how post-mortem physiology of diet items changes in the guts of consumers, the approaches developed in this study can be used to distinguish predation from scavenging by central-place foragers (particularly arthropods). Such information will provide a more definitive characterization of species interactions and food webs.

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Key words: *Apis mellifera*, *Bombus impatiens*, central-place foraging, invertebrate carrion, *Vespula pensylvanica*.

INTRODUCTION

Predation can play an important role in structuring ecological assemblages (Paine, 1966; Schmitz et al., 1997; Post et al., 2008). Observing predatory events *in situ*, however, poses numerous logistical challenges (Retana et al., 1991; Miller et al., 2006; Sloggett et al., 2009). When predators are small or highly mobile, for example, direct observation of predation becomes nearly impossible (Hagler et al., 2004). Furthermore, the effort required to detect even a few direct instances of predation, which typically involve multiple species of predators (Heimpel et al., 1997; Rosenheim et al., 1999), makes it difficult to obtain sample sizes large enough for meaningful statistical inference. For these reasons, ecologists rely heavily on PCR-based methods (Symondson, 2002; Sheppard et al., 2004; Foltan et al., 2005; King et al., 2008) and stable isotope analyses (Kelly, 2000; Fiedler et al., 2007; Hood-Nowotny and Knols, 2007) to characterize species interactions and to infer trophic level. A major shortcoming of such methods, however, is their inability to differentiate killed prey from scavenged carrion for consumers that engage in both behaviors (Morris et al., 1999; Foltan et al., 2005; Juen and Traugott, 2005; Tillberg et al., 2007; King et al., 2008).

Refining such distinctions will improve the interpretation of diet analyses because predation and scavenging differ in their ecological effects. Consumption does not necessitate direct demographic changes in the taxa consumed. While predation directly depresses prey populations (Schmitz et al., 1997), scavenging involves consuming individuals already removed from the population and consequently has no specific demographic effects on prey taxa (Abrams, 1987). Diet analyses that fail to discriminate between

carrion and prey can thus substantially overestimate predatory effects and lead to invalid assessments of trophic level (Calder et al., 2005; Sheppard and Harwood, 2005). Distinguishing and quantifying the relative contribution of these ecological processes will provide more accurate characterizations of species interactions (King et al., 2008) and energy flow through food webs (DeVault et al., 2003).

Facultative scavenging is one of the most common foraging strategies of both vertebrates (DeVault et al., 2003; Hunter et al., 2007) and invertebrates (Sunderland and Sutton, 1980; Foltan et al., 2005). Considering that invertebrates comprise much of the global terrestrial animal biomass (Williams, 1960; May, 2000), the importance of invertebrate carrion as a resource for scavenging predators has received surprisingly little attention. Many predatory invertebrates can engage in facultative scavenging, including Amphipoda (Ide et al., 2006), Coleoptera (Sunderland and Sutton, 1980; Juen and Traugott, 2005), Decapoda (Wolcott, 1978), Dermaptera (Sunderland and Sutton, 1980), Diptera (Foltan et al., 2005), Hemiptera (Velasco and Millan, 1998), Hymenoptera (Wilson et al., 2009), Isopoda (Barradas-Ortiz et al., 2003), Litobiomorpha (Sunderland and Sutton, 1980), Neuroptera (Foltan et al., 2005) and Opiliones (Sunderland and Sutton, 1980). Several studies have identified ants as key scavengers of small invertebrate carrion (Fellers and Fellers, 1982; Fellers, 1987; Retana et al., 1991). However, these observational studies capture only a fraction of scavenging dynamics because they used carrion baits that individual ant workers could carry by themselves, while much of insect diversity falls into larger size classes (Finlay et al., 2006). Just as larger prey require larger predators (Cohen et al., 1993), medium to large items of carrion often

attract bigger scavengers (e.g. wasps or beetles) (Seastedt et al., 1981), or multiple individuals cooperating through nestmate recruitment or group retrieval (Hölldobler and Wilson, 1990).

Differentiating prey from carrion may be most crucial for ecosystems with high net primary productivity (NPP) that generate substantial amounts of arthropod biomass. Because higher NPP is associated with increased social insect abundance (Kaspari et al., 2000; Kaspari, 2001), social insect colonies probably serve as important point sources of dead biomass in such environments. Reliable and abundant supplies of carrion, for example, are produced by the large, perennial colonies of *Apis mellifera* (Visscher, 1983; Coelho and Hoagland, 1995). Dead workers of this and other social insect species represent an important and largely underappreciated food source for scavenging predators. Moreover, the production of dead individuals may magnify top-down effects of predation as scavenging predators subsidize their diets with carrion (Polis and Strong, 1996; Rand et al., 2006).

To distinguish between predation and scavenging in arthropods, we require quantitative information about time since death (TSD). Forensic pathology (Bate-Smith and Bendall, 1949) and related fields (Lawrie and Ledward, 2006) have established how TSD and peri-mortem stress (stress that occurs at or near death) influence post-mortem muscle pH. In vertebrates, muscles remain metabolically active after death (Lindahl et al., 2006), causing a decrease in muscle pH. Although the processes driving this drop in pH are well studied in vertebrates (Lindahl et al., 2006), and recent work has shown that just the risk of predation elicits stress responses in some insects (Slos and Stoks, 2008), little is known about how the properties of insect muscle change post mortem. Because energy is produced through anaerobic metabolism after death in invertebrates, we may thus expect changes in muscle pH after death as oxidative phosphorylation ceases and glycolysis continues (Crabtree and Newsholm, 1972; Vishnudas and Vigoreaux, 2006), given that the specific substrates and end products involved will determine the net H^+ yield (Portner, 1987).

In the present study, we examine how invertebrate muscle pH changes post mortem. After estimating TSD from water loss rate (measured as mass loss), we demonstrate that thoracic muscle pH and other physiological properties can be used to differentiate prey from carrion. Because the pH of invertebrate thoracic muscle decreases predictably post mortem and is sensitive to stress, we can evaluate the peri-mortem stress level experienced by diet items and distinguish prey from carrion. Our study uses social insects as a model system, because ants and social wasps represent ecologically dominant scavenging predators in many terrestrial ecosystems (Jeanne, 1979; Hölldobler and Wilson, 1990; Snyder and Evans, 2006; Wilson et al., 2009). Nonetheless, it often remains unclear whether these consumers are serving as predators or scavengers (Tillberg et al., 2007). Furthermore, our approach could be extended to other scavenging predators, especially those that exhibit central-place foraging and for which diet items can be obtained prior to consumption, such as ants, wasps and some birds. Isolating specific mechanisms by which diet items are collected (predation or scavenging) will clarify the nature of species interactions and the structure of food webs.

MATERIALS AND METHODS

We employ a series of physiological measurements to determine whether insect diet items are prey or carrion. The western yellowjacket, *Vespa pensylvanica* de Saussure, serves as our model scavenging predator because of its broad diet, predatory ability and propensity for scavenging (Jacobson et al., 1978). Because

yellowjackets consume bees, which are of economic and ecological importance, and engage in cannibalism (Gambino, 1992; Wilson, 2009), we examine how the physiology of bumblebee (*Bombus impatiens* Cresson), honeybee (*Apis mellifera* L.) and yellowjacket carcasses changes post mortem and in response to predation stress. To estimate TSD, we first identify measurable and predictable time-dependent parameters (Henssge and Madea, 2007). We then develop general approaches to discriminate prey from carrion by estimating TSD from rate of water loss (a time-dependent but stress-independent parameter) and by comparing observed pH (a time and stress-dependent parameter) to estimated pH for a given TSD. Because these parameters may vary between tissues, we examine thoracic muscle, which is typically protected by a sclerotized exoskeleton and consists of concentrated and relatively homogenous muscle. Moreover, foraging yellowjackets often retrieve insect thoraces (Wilson et al., 2009). Because higher ambient temperatures (T_a) can lead to increased rates of water loss (Atmowidjojo et al., 1997), metabolism (Brockington and Clarke, 2001; Brown et al., 2004), and hence rates of pH decline, we controlled ambient conditions in the laboratory to simulate common field temperatures (18–23°C) in order to create reliable and accurate baseline curves.

Colony maintenance

Three *B. impatiens* (Hymenoptera: Apidae) colonies (Biobest Canada Ltd, Leamington, Ontario, Canada) were maintained in the lab at constant temperature (mean \pm s.e.m.: 26.7 \pm 1.2°C) and humidity (31.4 \pm 1.0% humidity) under a photoperiod of 12h:12h L:D. Each colony was provided with water, sucrose solution and pollen *ad libitum*. We also maintained two freely foraging colonies of *A. mellifera* (Hymenoptera: Apidae) at the University of California, San Diego Biology Field Station. All *V. pensylvanica* (Hymenoptera: Vespidae) used in this study were foragers captured from feral colonies in Haleakala National Park, part of the introduced range of this species.

To minimize peri-mortem stress, female bees and wasps were captured, sedated on ice and then killed by decapitation while anesthetized. In this way, all individuals experienced minimal stress at the time of death but still died by decapitation, which is a common killing behavior of predatory wasps. Each thorax was exposed to the ambient lab environment (22.6 \pm 1.1°C and 32.4 \pm 1.0% humidity). Changes in T_a , thoracic temperature (T_{th}), body mass and thoracic pH were measured at discrete intervals after death (Table 1), except for *V. pensylvanica* for which only T_a and thoracic pH were measured. In this study, we focused on thoracic tissue because it consists of concentrated and relatively homogenous muscle.

We measured surface T_{th} to the nearest 0.1°C with an infrared thermometer (Radio Shack, Fort Worth, TX, USA, cat #220-0325, spot size of 1.6 mm). Temperature measurements were accurate to 2.0°C but were precise and repeatable to within 0.2°C as compared with a reference thermometer. Because the difference between ambient and thoracic temperatures ($T_a - T_{th}$) did not differ from zero, we used T_a in statistical analyses. We measured thoracic pH by inserting a pH probe (Oakton Eutech Instruments™, Vernon Hills, IL, USA, model #PHSPEAR, accuracy: 0.01 pH units) into the thorax of each decapitated bee or wasp. This pH device is designed for measuring pH of solids and semisolids. Each forager was only measured once.

Evaluation of TSD and stress-level estimators in a blind, no-stress experiment

To evaluate how rates of water loss ($mg\ h^{-1}$) change post mortem, we measured the rate of mass loss at 34 time points ranging between

Table 1. Post-mortem variables measured for each of three focal species

Species	Wet mass (mg) at time of death	Time since death (h)											Variables measured
		0	1	2	4	6	8	12	14	16	18	24	
<i>Bombus</i>	134.2±3.3; n=120	7	12	11	17	–	15	16	15	17	10	–	T_a , T_{th} , mass, pH
<i>Apis</i>	106.4±3.5; n=89	12	–	–	10	11	12	13	10	10	11	–	T_a , T_{th} , mass, pH
<i>Vespula</i>	52.8±2.0; n=30	52	–	–	62	26	26	55	27	36	26	29	T_a , pH

The number of individuals measured at each time interval is given. – indicates no foragers were measured at that time interval. T_a =ambient temperature. T_{th} =thoracic temperature. Each forager was measured once.

1 h and 120 h after death for 35 *B. impatiens* foragers. This sampling allowed for the estimation of mass loss rates (mg h^{-1}) in the first hour after a diet item had been collected. We used this approach because under field conditions information about the peri-mortem mass of diet items is unavailable. Ten bees were killed by decapitation to simulate death by predation (hereafter referred to as 'prey' bees), and 25 bees were freeze-killed and decapitated within 2–31 h after death to simulate death by natural causes (hereafter referred to as 'carrion' bees). Freezing was used to simulate natural death because extreme temperatures and their related injuries commonly kill insects (Colhoun, 1960; Wigglesworth, 1972). Decapitation of 'carrion' bees occurred just prior to pH measurements, which were taken under controlled conditions ($23.4\pm 0.5^\circ\text{C}$, $41.7\pm 2.0\%$ humidity). We described the exponential rate of mass loss over TSD for 'prey' bees (Fig. 1) and time since decapitation (TS_{decap}) for 'prey' and 'carrion' bees.

To test the accuracy of water loss rate as a TSD estimator, we collected and killed an additional 45 *B. impatiens* foragers as previously described. In a blind, no-stress experiment, each individual bee was assigned a time (0–18 h after death) to have its mass and thoracic pH measured. Referencing Fig. 1 and TS_{decap} of 'carrion' bees, we estimated TSD and TS_{decap} from rates of water loss exhibited by the 45 *B. impatiens* foragers and then compared the two time estimates. Because TSD and TS_{decap} were equivalent for all bees, TSD was used in subsequent analyses. Using TSD and by referencing Fig. 2A, we then examined whether observed pH measurements fell along the no-stress baseline. To determine the accuracy and precision of both estimators, we compared estimates with actual values and then evaluated the percentage of bees that exhibited the following: (1) estimated and actual TSDs that differed by no more than ± 1 h and ± 4 h, and (2) observed pH values that fell within the 99% confidence interval (CI) of the baseline pH curve.

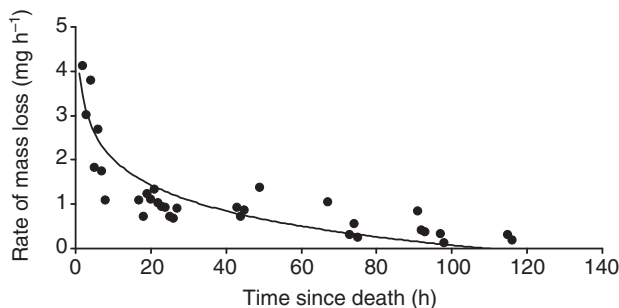


Fig. 1. Rate of post-mortem water loss in *Bombus impatiens* killed by decapitation decreases exponentially with time. Each data point represents the mean rate of water loss for 10 bees.

Effect of stress on TSD estimates: pH as an indicator of cause of death

Peri-mortem stress probably depresses pH and would thus truncate the pH curve such that pH at death is lower and ultimate pH is attained more quickly. Thus, muscle pH should indicate high peri-mortem stress levels when the observed pH falls below the no-stress baseline curves at the estimated TSD. To test this hypothesis under controlled conditions, we collected 21 *B. impatiens* foragers; eight were killed as previously described under no-stress conditions, and 13 were subjected to one minute of stress (e.g. aggressive prodding) prior to decapitation. A minute roughly corresponds to the duration of natural predation by yellowjackets, where mean handling time is 106 ± 14 s ($N=30$ predation events). Changes in T_{th} , body mass and thoracic pH were measured immediately following death.

To assess the extent to which stress reduces muscle pH in the field, we presented freshly killed versus live honeybee foragers in

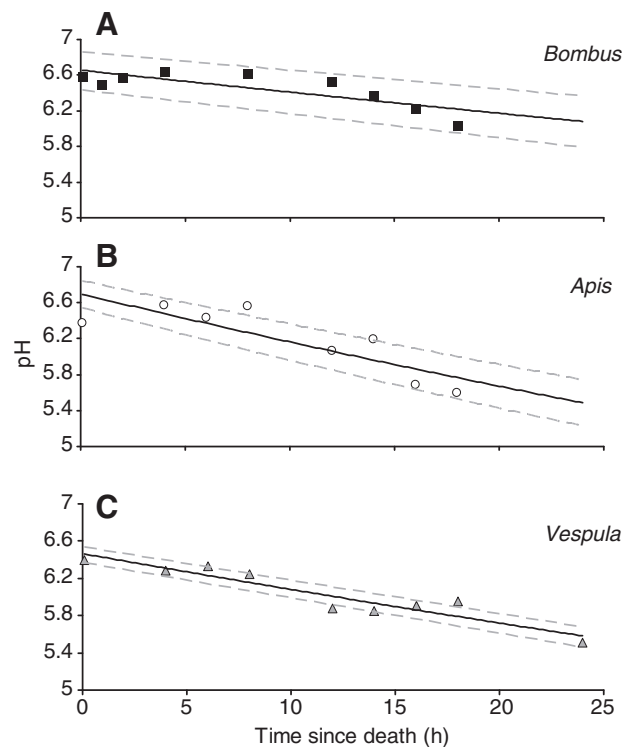


Fig. 2. Thoracic muscle pH decreases predictably after death in three hymenopteran species in no-stress baselines. (A) *Bombus*: $\text{pH}=6.781e^{-0.00469\times\text{TSD}(\text{h})}$, (B) *Apis*: $\text{pH}=6.6753e^{-0.008\times\text{TSD}(\text{h})}$, (C) *Vespula*: $\text{pH}=6.458e^{-0.006162\times\text{TSD}(\text{h})}$. Each data point represents the mean of multiple individuals (see Materials and methods for sample sizes). TSD, time since death.

Table 2. Multiple regression statistics for thoracic muscle pH for focal three hymenopteran species

	<i>Bombus impatiens</i>			<i>Apis mellifera</i>			<i>Vespa pensylvanica</i>		
	F	d.f.	P	F	d.f.	P	F	d.f.	P
Time since death (TSD)	7.93	1,117	0.0057	12.01	1,83	0.0008	305.52	1,335	<0.0001
Ambient temperature (T_a)	2.33	1,117	0.13	0.085	1,83	0.77	0.39	1,335	0.53
Final mass	0.38	1,117	0.54	0.0516	1,83	0.82			
$T_a \times$ TSD	0.18	1,117	0.67	0.074	1,83	0.79	2.54	1,335	0.11
Final mass \times TSD	1.34	1,117	0.25	2.41	1,83	0.12			
Overall model	$F_{5,117}=4.14, P=0.0017$			$F_{5,83}=16.22, P<0.0001$			$F_{3,335}=103.0, P<0.0001$		

choice tests to free-foraging yellowjackets from six different wasp colonies. Bees in the freshly killed experimental group consisted of feral honeybee foragers that were caught in the field and killed by momentary immersion in ethanol to minimize stress. All bees were restrained by pins through the thorax; this procedure prevented freshly killed bees from blowing away and live bees from flying away. Live bees retained the ability to sting and fight attacking yellowjackets. We retrieved the honeybee thoraces processed by *V. pensylvanica* in these trials and subsequently measured thoracic muscle pH to quantify the effects of predation stress (freshly killed: $n=11$ bees; live: $n=24$ bees). This approach reasonably mimicked natural predatory events; pinning live bees ($n=10$) did not decrease pH relative to control bees ($n=10$) (mean $\text{pH}_{\text{pinned}}=6.47\pm 0.06$, mean $\text{pH}_{\text{control}}=6.42\pm 0.05$, $t_{17}=0.56$, $P=0.58$).

Statistical analyses

All statistical analyses were performed with JMP v. 8.0.1 (SAS Institute, Cary, NC, USA). Models of pH change over time were evaluated by comparing AIC values (Bozdogan, 1987); the best model was an exponential curve (see Table S1 in supplementary material for values). For *B. impatiens* and *A. mellifera*, we performed multiple regressions to assess how thoracic muscle pH changes with (i) TSD (h), (ii) T_a , and (iii) body mass. For *V. pensylvanica*, we performed a multiple regression to assess how (i) TSD (h), and (ii) T_a affected thoracic pH. For linear regressions of pH over time, we calculated 99% CI. To evaluate pH as an indicator of cause of death, we performed two-sample *t*-tests to determine the effect of stress on the thoracic pH of *B. impatiens* and *A. mellifera*.

RESULTS

Our results demonstrate that thoracic muscle pH and rates of water loss decrease predictably post mortem and can thus be used to infer time and cause of death in groups of aculeate Hymenoptera (Figs 1 and 2). Thoracic muscle pH can furthermore provide evidence of whether death was caused by predation or by natural reasons excluding predation.

Rates of evaporative water loss for bumblebees killed by decapitation decreased exponentially post mortem [rate of water loss

(mg h^{-1})= $-0.8443 \times \ln(\text{TSD})+3.9631$, $R^2=0.80$]. Rates of water loss for 'prey' bees illustrated in Fig. 1 are consistent with water loss observed in no-stress baseline experiments: decapitated *B. impatiens* individuals lost 1% of their body mass per hour, while decapitated *A. mellifera* lost 0.75% of their body mass per hour. Qualitatively lower rates of water loss were observed in 'carriion' bumblebees where TS_{decap} ranged between 2 h and 31 h after death; 'carriion' bees also lost water exponentially [rate of water loss (mg h^{-1})= $-0.5121 \times \ln(\text{TS}_{\text{decap}})+2.4492$, $R^2=0.87$]. Perhaps because decapitation increased the surface area of tissue exposed to ambient environment, 'prey' and 'carriion' bees exhibited different rates of mass loss for the first 10 h following death or decapitation, after which time rates of mass loss converged.

In the no-stress baseline experiments, the three focal species exhibited significant post-mortem decreases in pH (Table 2). In the absence of peri-mortem stress, the rate of change in thoracic pH varied by species (Fig. 2). Overall, bumblebees exhibited $0.024 \text{ pH decrease h}^{-1}$ (Fig. 2A), while thoracic muscle of honeybees declined at a higher rate of $0.050 \text{ pH decrease h}^{-1}$ (Fig. 2B). *Vespa* exhibited an intermediate rate of $0.037 \text{ pH decrease h}^{-1}$ (Fig. 2C).

Microscopic inspection of thoracic muscle demonstrated that post-mortem muscle changed qualitatively over time. Under laboratory conditions, thoracic muscle of bumblebees remained moist for 8 h following death and was characterized by a predictable sequence of color change (Fig. 3A–C). As time passed, thoracic muscle became increasingly dry and stringy, and its color deepened (Fig. 3D,E). After a week, thoracic muscle was dry and brittle (Fig. 3F). The predictable sequence of muscle appearance provides additional confirmation of TSD.

Evaluation of TSD and stress-level estimators in a blind, no-stress experiment

To confirm that water loss data provide a true metric of TSD and not just TS_{decap} , we took the two time estimates for individual bees, determined the expected pH on the *Bombus* no-stress baseline (Fig. 2A) for each estimate, and then calculated the difference in pH for the two estimates. Because the mean difference in pH ($0.08\pm 0.03 \text{ pH units}$) was substantially smaller than the CI around

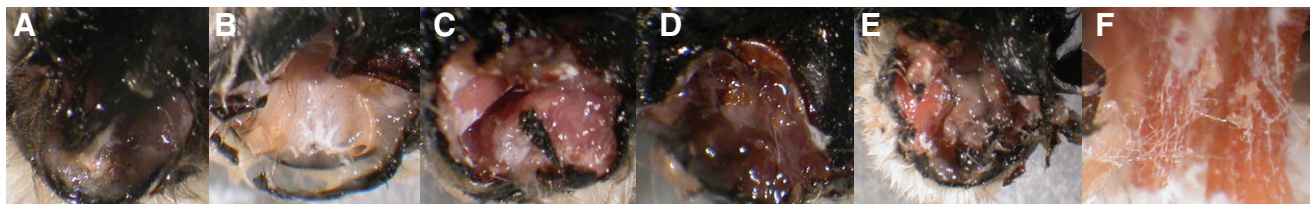


Fig. 3. Post-mortem thoracic muscle in *Bombus* under laboratory conditions. (A) Immediately after death, muscle is moist and appears deep gray with pink undertones. (B) At 6 h after death, moist muscle changes color to white with pink undertones. (C) At 8 h after death, moist muscle begins to turn pink with white undertones. (D) At 18 h after death, muscle turns dark pink. (E) At 24 h after death, muscle begins to dry out and becomes increasingly brittle. (F) At one week after death, muscle is completely dried out and appears fibrous.

the baseline curve (± 0.24 pH units), any discrepancy between time estimates is unlikely to result in a misclassification of diet items as prey *versus* carrion. For all analyses, we compared TSD and TSD_{decap}; however, we report only TSD for the remaining experiments because of the similarity of these time values to one another.

Using post-mortem rate of water loss (mg h^{-1}) alone to estimate TSD, 87% of estimates were accurate to within ± 1 h and 100% fell within ± 4 h of the actual TSD. If water loss data were combined with blind visual assessments of muscle quality, then accuracy within ± 2 h of the actual TSD increased to 95% of estimates. As an indicator of stress, 82% of observed pH values fell within the 99% CI of the no-stress baseline curve. An additional 9% of observations could be classified as no-stress because they slightly exceeded the upper CI. Thus, 91% of pH measurements confirmed that no stress was experienced.

pH as an indicator of cause of death

We tested the hypothesis that muscle pH can indicate the level of peri-mortem stress experienced by putative prey. For all individuals in the stress experiment ($n=21$ *B. impatiens*), rates of water loss in the first hour after initial measurement were insensitive to stress and resulted in 86% of TSD estimates falling within ± 1 h and 100% of estimates within ± 1.05 h of actual TSD. Visual assessment of muscle confirmed that measurements occurred shortly after death and before muscle coloration changed for the *Bombus* stress experiment and *Apis* field predation trials.

Peri-mortem stress caused an immediate decrease in muscle pH. Bumblebees subject to intermittent stress exhibited a mean decrease of 0.2 pH units (Fig. 4A), a drop detectable within minutes of death ($t=-2.25$, d.f.=19, $P=0.036$). Predation on *Apis* foragers caused a larger pH decrease of 0.4 pH units (Fig. 4B; $t=-7.395$, d.f.=33, $P<0.0001$). Using TSD estimates derived from water loss rates and referencing the no-stress baseline pH curve (Fig. 2A), we established whether observed pH measurements fell along or below these curves for the *Bombus* stress experiment. Due to the sensitivity of pH to stress, observed pH values fell along no-stress baselines only when peri-mortem stress was low or absent (75% of estimates for control

Bombus, and 89% of estimates for scavenged *Apis*). For bumblebees experiencing intermittent stress for one minute prior to decapitation, observed pH measurements fell below the no-stress baseline curve in Fig. 2A at TSDs estimated from the rate of water loss curve. Similarly, for honeybees killed by predatory yellowjackets, at TSD estimated from rates of water loss from Fig. 1, 100% of observed pH values were below the no-stress baseline curve in Fig. 2B.

DISCUSSION

We provide the first evidence for how rate of water loss, pH of thoracic muscle and muscle color can distinguish freshly killed prey from decomposing arthropod corpses. For scavenged carrion (subject to little to no stress), observed pH values fall along no-stress baseline curves at the TSD estimated from baseline water loss rates; in this situation, diet items with a low pH have been dead for many hours or days (Fig. 5). For prey (typically subject to high stress), observed pH lies well below no-stress baselines at the TSD estimated from baseline water loss rates. Thus, the responsiveness of pH to stress may provide important information about peri-mortem conditions and can be used to distinguish prey from carrion (Fig. 5). Use of pH measurements alone to estimate TSD would lead to accurate estimates for non-stressed individuals but overestimates for individuals experiencing strong peri-mortem stress. By combining information derived from rates of water loss, muscle pH and no-stress baselines for these parameters as described in this study, one can examine diet items of scavenging predators and infer the ecological process at work.

In the absence of peri-mortem stress, muscle pH decreases predictably and at species-specific rates within 24 h of death. Although the three focal species differed in body size, this variable does not predict rates of pH decline. Bumblebees had the lowest rate of pH decline and were the largest of the species tested. Honeybees were intermediate in size but they exhibited the fastest rate of pH decrease. *Vespula* were the smallest species measured but yellowjacket muscle pH declined at an intermediate rate compared with the bees. Although further research is needed to determine the factors influencing rates of post-mortem pH decline, our results illustrate in a general sense how muscle physiology can be used to discriminate predation from scavenging prior to consumption. Applications of this approach to other prey taxa will require separate quantification of species-specific post-mortem pH responses.

We conducted our analyses under a narrow range of environmental conditions that reflected conditions under which yellowjackets actively forage and retrieve Hymenoptera from the field. However, if the microclimates experienced by diet items are beyond the ranges used in this study, the approaches described here can still be used to distinguish recent predatory events – prey that experienced peri-mortem stress and have little water loss regardless of the ambient microclimate. Furthermore, the approaches developed in this study can be tailored to specific environmental conditions by controlling the conditions under which baseline data are collected. Using post-mortem muscle pH to infer cause of death may be most useful when temperature and humidity fluctuations are moderate.

The methodologies described in this study promise to provide important insights into the probable cause of death of items recovered in a scavenging predator's diet. However, any definitive conclusions should incorporate relevant knowledge about local conditions (e.g. large temperature fluxes or disease outbreaks). While some modes of natural death may be stressful to individuals, this study demonstrates that events that are short, intense and stressful (e.g. predation) cause an immediate and relatively large drops in

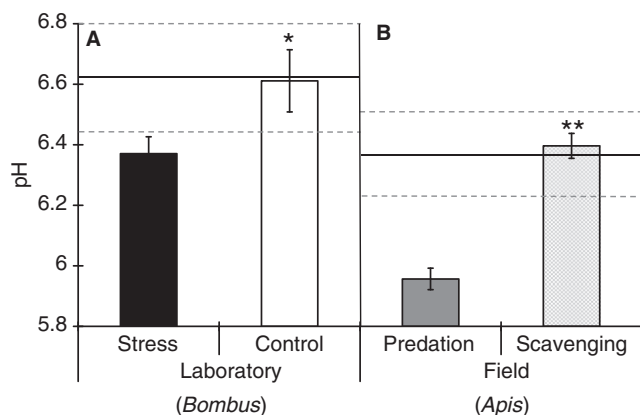


Fig. 4. Peri-mortem stress lowers pH in laboratory and field situations, and these decreases become evident immediately after death. (A) *Bombus impatiens* foragers exhibit lower thoracic muscle pH after experiencing intermittent stress. Control measurements are comparable with baseline *Bombus* pH measurements (solid lines) and 99% CI (broken lines) at time since death (TSD)=0 h from Fig. 1. (B) *Apis mellifera* foragers exhibit significant drops in thoracic muscle pH as a result of predation. The pH values of scavenged bees are comparable with baseline *Apis* measurements at TSD=0 h from Fig. 1. * $P<0.05$. *** $P<0.0001$.

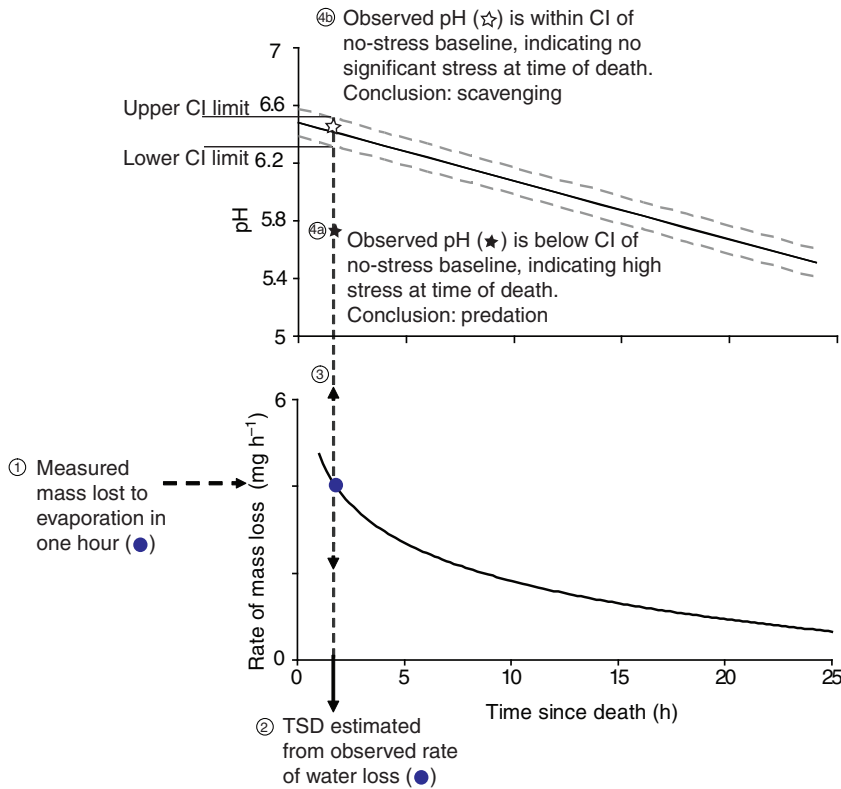


Fig. 5. Conceptual diagram depicting how water loss and pH data can be used to classify diet items as prey or carrion. First, no-stress baseline curves are created for water loss and pH. When a diet item is collected, rate of water loss in one hour (filled circle) is determined and located on the no-stress water loss curve (1) to provide an estimate of time since death (TSD) (2). Using this TSD estimate (3), we find the corresponding expected pH on the no-stress baseline. The observed thoracic muscle pH of the diet item (star) is plotted on the pH graph at the estimated TSD and compared with baseline and its confidence intervals (CI). For the filled star (4a), the observed pH is well below the lower 99% CI. For the open star (4b), the observed pH falls within the 99% CI of the baseline.

muscle pH compared with events associated with less severe stress. We thus expect that an organism experiencing a naturally stressful death (perhaps due to disease) will have more time to adjust and regain homeostatic muscle pH levels than would prey that are physically struggling to escape moments before death.

It is important to note that we did not assess the pH and water loss properties of other body tissues. Flight muscles were chosen given their high metabolic activity compared with non-flight muscles (Crabtree and Newsholm, 1972). Because these properties probably vary in the magnitude of their response to death or stress, baseline data must be collected for the same muscle type that is measured in the diet samples. If the putative prey is commonly dissected in multiple ways by predators, then this would require additional baselines (one for each muscle type).

Confirming how diet items enter a scavenging predator's diet has broad applications in food web studies (King et al., 2008), which require reliable information about who is eating whom, in what quantities and *via* what mechanisms (Hagler et al., 2004; Wilson et al., 2009). The importance of carrion in the diets of scavenging predators cannot be determined solely through gut content analysis. Although consumed carrion can be detected in gut contents (Foltan et al., 2005; Juen and Traugott, 2005), PCR-based methods cannot distinguish this class of resource from prey. Although information about the availability of fresh carcasses in the field might improve estimates of how much carrion predators consume (Foltan et al., 2005; Juen and Traugott, 2005), measuring predator number, carcass removal rates and carrion availability in the field poses logistic difficulties (Foltan et al., 2005), and may be infeasible for small taxa such as arthropods. The approach developed in this study could indicate the relative frequency of scavenging *versus* predation by central-place invertebrate foragers (Kasper et al., 2004; Tillberg et al., 2007; Wilson et al., 2009) because diet items can be obtained from returning individuals prior to consumption. Further

investigation into how muscle physiology of prey and carrion change in the gut of consumers is needed to assess the applicability of this method to non-central-place foragers.

With appropriate baseline data, water loss rates and muscle pH of diet items can be used to gain insight into the relative roles of predation and scavenging in the diet of ecologically dominant generalists. Knowing whether a species is experiencing high levels of predation can be of vital importance when direct observation of predator behavior is impossible or when focal species are of conservation or economic importance. Assessing the relative importance of predation *versus* scavenging can also clarify the role of consumers in food webs. If predators respond numerically through consumption of both carrion and prey, for example, then subsidies resulting from scavenging can decouple numerical responses of predator populations from their prey (Roth, 2003) and magnify predatory impacts of generalist predators (Rand et al., 2006). In conjunction with ecological studies that quantify predator and prey populations, the method developed in this study allows for the detection and evaluation of such carrion supplementation.

LIST OF ABBREVIATIONS

CI	confidence interval
NPP	net primary productivity
T_a	ambient temperature
T_{th}	thoracic temperature
TSD	time since death
TS_{decap}	time since decapitation

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