

Beer, brains, and brawn as tools to describe terrestrial gastropod species richness on a montane landscape

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Abstract. Terrestrial gastropods are part of one of the most vulnerable taxonomic groups, mollusks, but receive relatively little conservation attention. This is partially due to the paucity of peer-reviewed statistical evaluations of common survey techniques. From 2010 to 2014, we conducted a massive survey for terrestrial gastropods in a montane region centered on northern Idaho and including portions of northeastern Washington and northwestern Montana, USA. We fused several commonly used gastropod survey techniques (cover board traps baited with beer or water, pitfall traps, visual search, and leaf litter sorting) into a single survey transect which we deployed at 991 survey sites across our 22,975-km² study area. We used a variety of variables, including air temperature (collected at each site for ≥ 12 calendar months) and relative humidity to evaluate the effects of seasonality, observer bias, and repeated site visits on collection rate of individual specimens and detection of numbers of species. We found a combination of timed searches and leaf litter to be most effective in describing the maximum number of species with the least amount of effort. Although re-visiting sites significantly increased the number of species detected, more time spent at each site likely would have a similar effect and preclude the need for additional expense to visit remote survey locations. Observer bias was determined not to be a factor of concern for within-group observers. But when grouped by observer type, different classifications of observers performed quite differently. Beer, regardless of brand, was clearly a superior trap bait to water. However, because traps outperformed timed searches only slightly for one gastropod sub-group (small slugs) we do not recommend trapping, beer baited or otherwise, be used as part of major landscape-level survey efforts. Our study is the most extensive evaluation of survey techniques available in the literature to date and provides a framework for other practitioners implementing landscape-level surveys for terrestrial gastropods.

Key words: biodiversity; cover board trap; inventory; leaf litter; montane; observer bias; pitfall trap; Species of Greatest Conservation Need; State Wildlife Action Plan; terrestrial gastropod; timed search.

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INTRODUCTION

The conservation and management of molluscan species pose a formidable challenge; despite being among the taxonomic groups with highest conservation need, they receive relatively little

conservation attention. Mollusks have the highest documented extinction rate of any major taxonomic group, with 42% of the 693 recorded animal species extinctions since the year 1500 (Lydeard et al. 2004). Globally, mollusks are listed as the third largest group of International

Union of Conservation of Nature (IUCN) threatened animal species and are the most numerous group of IUCN threatened species in North America (<http://www.iucnredlist.org/>). In the United States, mollusks are listed as Species of Greatest Conservation Need (SGCN) in 54 of 56 states and territories with federally approved State Wildlife Action Plans and comprise 13.5% ($n = 1209$) of all animal SGCN in the nation (<https://www1.usgs.gov/csas/swap/index.html>).

Despite non-marine mollusks being one of the most imperiled groups of animals, resources dedicated to their conservation and management are limited compared to other species (Lydeard et al. 2004). Vertebrates in particular (Clark and May 2002) receive disproportionately greater attention and, in the context of number of described animal species, only arachnids and insects receive less scientific attention than mollusks (Titley et al. 2017). Lydeard et al. (2004) outlined four areas of need for molluscan conservation including identifying biodiversity hotspots, research, management, and education. Although successful implementation of these needs depends on inventory data, there remains a striking paucity of survey techniques available in the peer-reviewed literature. This is particularly true for terrestrial gastropods.

The limited studies available focus on comparison of visual searches and leaf litter sorting (Emberton et al. 1996, Oggier et al. 1998, Cameron and Pokryszko 2005, McDade and Maguire 2005) and provide some of the only technical comparisons of survey techniques. Visual searching is a primary technique used to survey for gastropods with inclusion of a timed or spatial element for standardization (Pearce and Orstan 2006). Leaf litter searches involve sieving and sorting of leaf litter and/or soil samples for specimens (Cameron and Pokryszko 2005). Other commonly used techniques include cover board (Oggier et al. 1998, McDade and Maguire 2005) and pitfall traps (McDade and Maguire 2005). Each method targets gastropod sub-groups of different sizes and life histories (Emberton et al. 1996), making it problematic to select a single method for all species. Multi-species inventory techniques require technical evaluation (Robinson et al. 2017), and an evaluation of the effectiveness of combining these techniques to detect a wide variety of gastropod species across time and space is lacking.

To address this issue, we developed a hybrid survey protocol that fuses several commonly used gastropod inventory techniques. From 2010 to 2014, we deployed 991 hybrid sampling plots across a large and diverse study area covering portions of northern Idaho, northeastern Washington, and northwestern Montana, USA. The objective was to evaluate the effectiveness of these techniques in the context of number of terrestrial gastropod specimens collected and number of species detected.

MATERIALS AND METHODS

Study area

The study area consists of a 22,975-km² area centered on the Idaho Panhandle and containing portions of northeastern Washington and northwestern Montana (Fig. 1). It comprises portions of the Selkirk, Purcell, West Cabinet, Coeur d'Alene, and Saint Joe mountain ranges. The topography is mountainous, ranging in elevation from 702 to 2326 m. The climate is characterized by warm and dry summers and wet and moderately cold winters. Winter snowpack is variable. Low elevation snowpack ranges from non-existent to persisting for several months. Higher elevations typically are characterized by a deep snowpack which persists late into the spring. The heavily forested area is dominated by a diverse mix of conifer species and is characterized as supporting inland temperate rainforest (DellaSala et al. 2011). The vast majority of survey sites were dominated by coniferous trees.

Sampling design

We stratified our study area into 5 × 5 km sampling cells and surveyed 991 sites for terrestrial gastropods in 879 cells. We used ArcGIS 10.1 (Environmental Systems Research Institute, Redlands, California, USA) to generate a buffer around each road and trail from 50 to 150 m. We then generated a random point within this buffer for the survey location. This resulted in survey sites that were randomly located but biased to roads and trails ($n = 842$) to improve field efficiency. Additional sites were sub-selected from randomly selected Forest Inventory Analysis (FIA) plots ($n = 149$; Bechtold and Patterson 2005) based on site characteristics described in Lucid et al. (2016). The FIA plots were not biased to roads (Fig. 2).

Multi-species Baseline Initiative: Gastropod Survey Overview

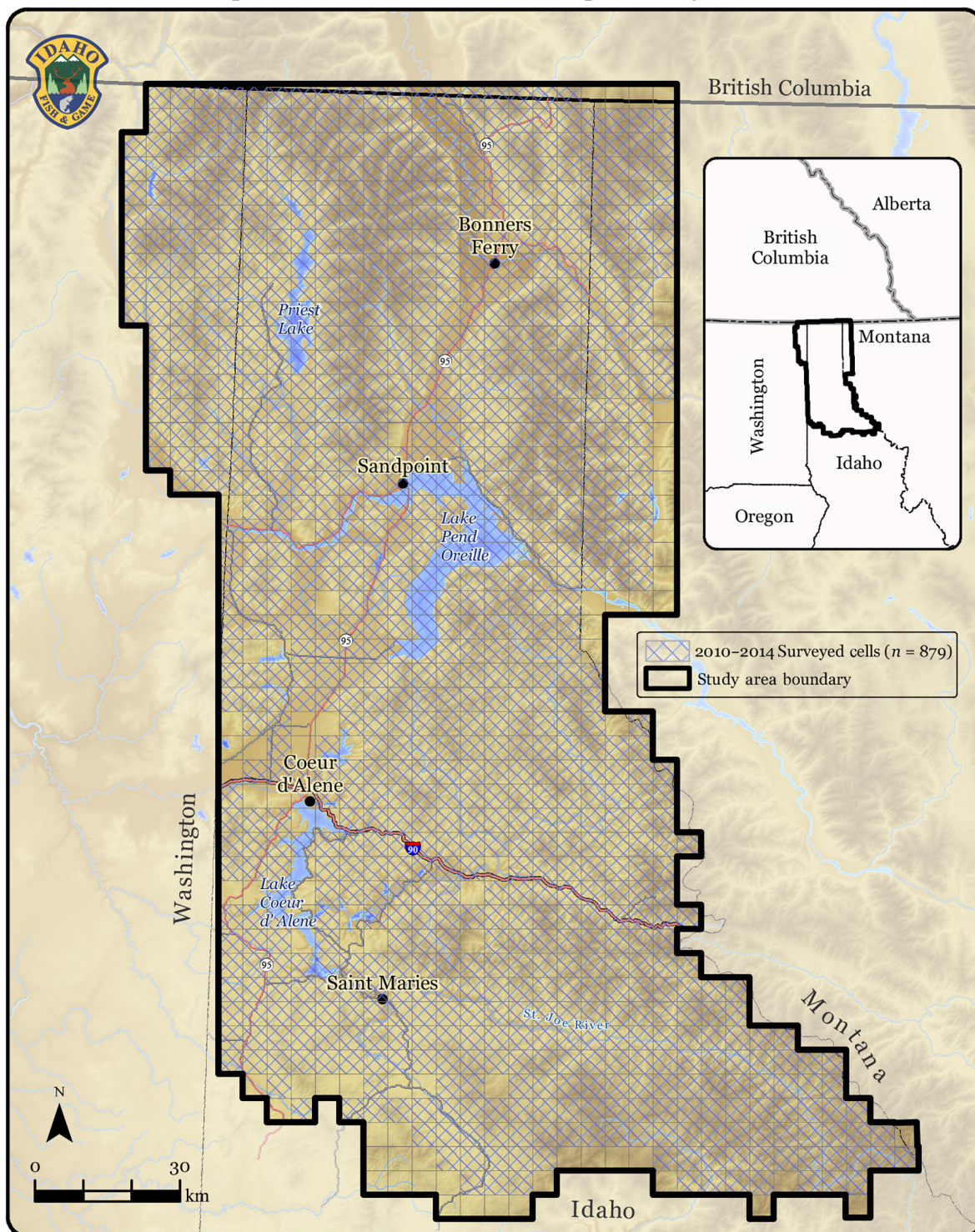


Fig. 1. Study area where 991 fused gastropod inventory transects were deployed in the 879 shaded 5 × 5 km survey cells from 2010 to 2014.

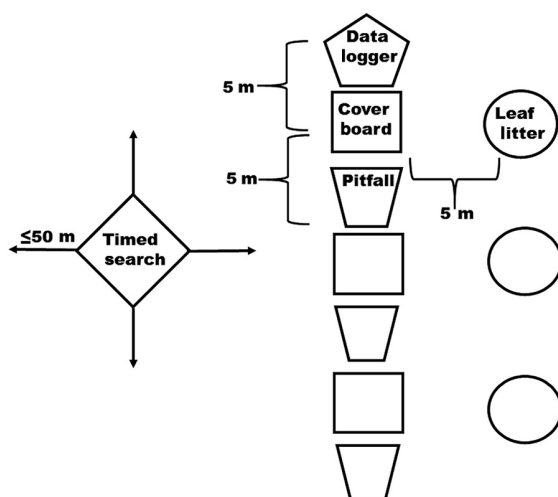


Fig. 2. Fused terrestrial gastropod survey transects. Cover board trap consisted of 30×30 cm square multi-layer cardboard. Pitfall trap was an un-covered 237-mL disposable drinking cup. Leaf litter was collected at three locations along the transect and mixed to form a single 1 litter sample. Data logger station collected air temperature data every 90 min for 12 consecutive months. Some loggers also collected relative humidity. Visual gastropod timed searched occurred within 50 m of the weather station.

Microclimate data logger.—TRIX8, TRIX16, and HAXO8 LogTag Transit data loggers were deployed within plastic radiation shields (Holden et al. 2013) designed to protect the logger from direct sunlight. The radiation shield was attached with nails to a conifer tree >30 cm in diameter (see Lucid et al. 2016 Chapter 5 for more details). Data loggers collected air temperature data every 90 min for 12–48 months. To determine mean annual site temperature for this study, we used air temperature collected from each data logger for a continuous 12-month period when all site with data loggers ($n = 941$) were being monitored (1 September 2013–31 August 2014). Beginning at the data logger, an observer used a compass to set a 45° true-north bearing on which to set up the survey transect.

Cardboard cover boards.—Three 30×30 cm cardboard cover board traps were placed 5 m apart from each other (Lucid et al. 2016: Appendix II-a) at each site. In 2010, traps were initially deployed dry and un-baited (6%, $n = 63$ transects). After the first round of trapping, we

began baiting traps to improve capture rates. Gardeners have long considered beer to be an effective slug attractant. Beer has been shown to be a more effective slug attractant than water (Piechowicz et al. 2014) and some commercially available molluscicides (Dankowska 2011). We tested the effectiveness of dry, water-baited, and beer-baited traps.

The majority of transects deployed in 2010 (11%, $n = 109$) had one dry control trap, one trap baited with 12 oz. of water, and one trap baited with 12 oz. of Natural Ice beer. In 2011, all transects (32%, $n = 322$) had one trap each baited with 12 oz. of water, Natural Ice beer (henceforth pilsner) trap, or Laughing Dog beer (henceforth microbrew). In 2013, each of the three traps in all transects (50%, $n = 497$) was baited with 12 oz. of Natural Ice beer. We chose Natural Ice beer because it was the cheapest commercially available.

Traps were baited in the field by placing the trap and bait in a two-gallon zip-top bag and allowing the cardboard to become saturated. Traps were placed 5 m apart along the transect. Leaf litter was moved aside, the trap was placed directly on the soil, any remaining bait was poured on the trap, and the trap was covered with leaf litter to slow drying. Traps were re-visited after approximately 14 d when an observer used a magnifying glass to view the trap and remove all gastropods both adhering to the trap and those sheltering under or within it.

Leaf litter.—Leaf litter was collected during the second visit to the survey transect. We limited our sampling to the top 10 cm of leaf litter and collected 333 mL of leaf litter from each of three locations along the transect and perpendicular to each trap. If leaf litter was not 10 cm deep, we gathered the proper amount by brushing surrounding litter into a pile. Leaf litter samples were placed in zip-top plastic bags and remained in the field with observers for 1–8 d and were then frozen upon return from the field. Samples were later removed from the freezer and placed in paper bags which were stapled shut to prevent contamination by other organisms, while they dried at room temperature. Litter was then sifted through a series of three filters (0.635, 0.508, and 0.254 cm; Lucid et al. 2016: Appendix II-a) by wildlife technicians (38%, $n = 374$ samples), paid workers from a temporary job service (18%, $n = 179$ samples), college students (6%, $n = 57$

samples), and volunteer citizen naturalists (39%, $n = 382$ samples). Gastropod shells were preserved in separate dry vials.

Timed searches.—During each site visit, an observer conducted at least one gastropod timed search (GTS). Beginning at the climate station, one observer spent 15 min searching under rocks and logs for gastropods, traveling no farther than 50 m from the climate station. Forest Inventory Analysis sites received two additional GTS in the fall of 2014.

Pitfall traps.—Three 8-oz. plastic cups with a 4-cm² piece of Hot Shot No-Pest fumigant strip (Spectrum Brands, Middletown, Wisconsin, USA; to prevent the escape of non-gastropod arthropods) were placed 5 m apart along the transect to act as pitfall traps. A trowel was used to dig a small hole, and then, the rim of the cup was placed level with the ground. The pitfall trap was not covered in any way. During subsequent visit(s), collected rainwater was poured from the trap (amount of water was measured in 2013) through a strainer with approximately 0.1 cm mesh. Gastropods were handpicked from the strainer and placed in a vial of 95% ethanol.

Statistical methods

Summary.—We summarized survey method effectiveness by species and species group. We grouped species as large slug (>10 mm length), small slug (<10 mm), large snail (>10 mm diameter), and small snail (<10 mm). We grouped solely by species size, not specimen size. We compared the number and percentage of surveys that detected each group.

Bait type.—We used mixed-effects ANOVA with PLOT as a random factor to determine whether bait type or transect type affected number of animals and number of species detected. A post-hoc Tukey multiple comparison test was used to determine the extent that bait types or transect types differed from each other.

Seasonality.—For trap transects and leaf litter detections, we independently evaluated the effect of five variables on number of species detected per transect. Temp_13 (mean air temperature for 13-d trap deployment period), precipitation (rain-fall collected during trap deployment period), and RH_13 (mean relative humidity for 13-d trap deployment period) were available for 772, 398, and 140 transects, respectively. Additionally, we

removed from analysis the trap transects or leaf litter survey where all traps were un-baited and dry for the analysis (which explains why the total seasonality sample size changes from 992 to 912). We first used generalized linear model (GLM) Poisson regression for Julian date and elevation because those variables were available for all transects. We then subsetting the data three times to run regressions for remaining variables.

For GTS, we used 2222 searches to evaluate the effect of eight variables on number of species detected per GTS. We used the same variables as above and added temp_2 (mean air temperature for 2 d prior or after survey) and RH_2 (mean relative humidity 2 d prior or after survey). Julian date and elevation were available for all transects. Sample size of the remaining variables was as follows: temp_13 and temp_13² ($n = 1549$), temp_2 ($n = 1013$), RH_13 ($n = 277$), RH_2 ($n = 421$), and ppt ($n = 420$). As above, we ran a series of regressions, sub-setting the data each time to reduce to the records containing the newly included variable. As a separate test, we then used a mixed-effects ANOVA to test whether collection season (summer or fall, independent of the preceding variables) affected number of animals or number of species detected with GTS. This test was done only on the 149 FIA plots.

Observer bias GTS/leaf litter.—We ran a mixed-effects ANOVA using our best observer as the relative class to test whether there was a significant observer bias in number of animals or species detected in GTS. Each of the 21 observers conducted 25–120 GTS. For leaf litter, we ran a mixed-effects ANOVA using our best observer type (paid college student) as the relative class to test whether there was a significant observer bias in number of animals or species detected. Observer classes included paid college student ($n = 57$ searches), paid wildlife technician ($n = 352$), community volunteer ($n = 379$), and paid temporary job-service employee ($n = 179$). There were 25 sorted samples that were removed from the analysis due to their larger size (3.8 L sample vs. 1 L). Gastropod timed search and leaf litter observers were randomly assigned plots or samples.

Re-sampling.—To test trap re-sampling, we used the 109 dry-water-pilsner traps deployed in 2010 because they were visited three times. To avoid seasonal bias in testing GTS re-sampling, we used only summer GTS ($n = 948$). For both

Table 1. Number of species/species group detections per survey type (values in parentheses are percentages of species/survey type).

Type	Species	Trap (n = 912)	Ground timed search (n = 2222)	Leaf litter (n = 912)	Pitfall (n = 803)
Large slugs	<i>Arion</i> spp.†	7 (0.77)	8 (0.36)	0 (0.00)	2 (0.25)
	<i>Hemphillia</i> spp.‡	34 (3.73)	91 (4.10)	0 (0.00)	8 (1.00)
	<i>Limax maximus</i>	3 (0.33)	16 (0.72)	0 (0.00)	0 (0.00)
	<i>Magnipelta mycophaga</i>	7 (0.77)	27 (1.22)	0 (0.00)	8 (1.00)
	<i>Prophysaon</i> spp.§	41 (4.50)	184 (8.28)	0 (0.00)	8 (1.00)
	Large slugs total	92 (10.09)	326 (14.67)	0 (0.00)	26 (3.24)
Small slugs	<i>Deroceras</i> spp.¶	12 (1.32)	18 (0.81)	0 (0.00)	1 (0.12)
	<i>Kootenaia burkei</i>	51 (5.59)	39 (1.76)	0 (0.00)	1 (0.12)
	<i>Securicauda hermani</i>	0 (0.00)	2 (0.09)	0 (0.00)	0 (0.00)
	<i>Udosarx lyrata</i>	12 (1.32)	39 (1.76)	0 (0.00)	0 (0.00)
	<i>Zacoleus idahoensis</i>	76 (8.33)	120 (5.40)	0 (0.00)	3 (0.37)
	Small slugs total	151 (16.56)	218 (9.81)	0 (0.00)	5 (0.62)
Total slugs		243 (26.64)	544 (24.48)	0 (0.00)	31 (3.86)
Large snails	<i>Allogona pychophora</i>	10 (1.10)	63 (2.84)	2 (0.22)	4 (0.50)
	<i>Anguispira kochi</i>	89 (9.76)	341 (15.35)	22 (2.41)	81 (10.09)
	<i>Cryptomastix mullani</i>	5 (0.55)	122 (5.49)	3 (0.33)	17 (2.12)
	<i>Cryptomastix sanburni</i>	1 (0.11)	19 (0.86)	0 (0.00)	2 (0.25)
	<i>Haplotrema vancouverense</i>	13 (1.43)	167 (7.52)	8 (0.88)	11 (1.37)
	<i>Oerohelix strigosa</i>	1 (0.11)	4 (0.18)	0 (0.00)	1 (0.12)
Small snails	Large snails total	119 (13.05)	716 (32.22)	35 (3.84)	116 (14.45)
	<i>Cochlicopa lubrica</i>	6 (0.66)	7 (0.32)	3 (0.33)	2 (0.25)
	<i>Columella</i> spp.#	1 (0.11)	1 (0.05)	3 (0.33)	0 (0.00)
	<i>Discus</i> spp.	11 (1.21)	18 (0.81)	1 (0.11)	0 (0.00)
	<i>Euconulus fulvus</i>	22 (2.41)	36 (1.62)	24 (2.63)	0 (0.00)
	<i>Helicodiscus salmonaceus</i>	0 (0.00)	1 (0.05)	0 (0.00)	0 (0.00)
	<i>Microphysula ingersolli</i>	14 (1.54)	51 (2.30)	9 (0.99)	1 (0.12)
	<i>Nesovitrea</i> spp.††	3 (0.33)	4 (0.18)	3 (0.33)	0 (0.00)
	<i>Planogyra clappi</i>	2 (0.22)	2 (0.09)	3 (0.33)	0 (0.00)
	<i>Polygyrella polygyrella</i>	1 (0.11)	16 (0.72)	0 (0.00)	0 (0.00)
	<i>Pristiloma idahoense</i>	4 (0.44)	19 (0.85)	2 (0.22)	2 (0.25)
	<i>Pristiloma wascoense</i>	2 (0.22)	0 (0.00)	7 (0.77)	0 (0.00)
	<i>Punctum</i> spp.‡‡	6 (0.66)	7 (0.32)	100 (10.96)	0 (0.00)
	<i>Radiodiscus abietum</i>	17 (1.86)	204 (9.18)	22 (2.41)	6 (0.75)
	<i>Striatura pugetensis</i>	7 (0.77)	2 (0.09)	46 (5.04)	0 (0.00)
	<i>Vallonia</i> spp.§§	4 (0.44)	4 (0.18)	7 (0.77)	0 (0.00)
	<i>Vertigo</i> spp.¶¶	4 (0.44)	5 (0.23)	13 (1.43)	0 (0.00)
	<i>Vitrina pellucida</i>	3 (0.33)	10 (0.45)	8 (0.88)	1 (0.12)
	<i>Zonitoides</i> spp.##	35 (3.84)	255 (11.48)	11 (1.21)	2 (0.25)
	Small snails total	142 (15.57)	642 (28.89)	262 (28.73)	14 (1.74)
Total snails		261 (28.62)	1358 (61.12)	297 (32.57)	130 (16.19)
Total gastropod		504 (55.26)	1902 (85.60)	297 (32.57)	161 (20.05)

† *A. circumscriptus*, *A. rufus*, and *A. subfuscus*.‡ *H. camelus*, *H. skadi*.§ *P. andersoni*, *P. coeruleum*, *P. dubium*, and *P. humile*.¶ *D. laeve* and *D. reticulatum*.# *C. columella* and *C. edentula*.|| *D. shimekii* and *D. whitneyi*.†† *N. binneyana* and *N. electrina*.‡‡ *P. californicum*, *P. minutissimum*, and *P. randolphi*.§§ *V. cyclophorella*, *V. excentrica*, and *V. pulchella*.¶¶ *V. concinnula*, *V. cristata*, and *V. modesta*.## *Z. arboreus* and *Z. nitidus*.

trap and GTS re-sampling testing, we ran mixed-effects ANOVAs with site as a random factor to evaluate new species detections during the first visit, redetected on the second visit, and new detections on the second visit.

RESULTS

Overall comparison

Overall, we detected the most gastropods (66.41%, $n = 1902$) via GTS followed by traps (17.60%, $n = 504$), leaf litter (10.37%, $n = 297$), and pitfall (5.62%, $n = 161$; see Table 1). We did not detect any slugs in leaf litter. All groups were detected in similar ratios with the exception of small snails which were detected more often in leaf litter (24.72%, $n = 262$) than on traps (13.40%, $n = 142$). Leaf litter varied in importance by snail size, with small snail species detected more frequently in litter. Additionally, some species or species groups were predominately detected in leaf litter. For example, 88.50% ($n = 100$) of *Punctum* spp. detections were reported from leaf litter searches.

Bait type

Trap bait type.—For both traps and transects, we found significant effects of bait type on number of animals (trap, $P < 2.2 \times 10^{-16}$; transect, $P = 1.1093 \times 10^{-7}$) and number of species (trap, $P = 1.146 \times 10^{-6}$; transect, $P = 0.0001826$) detected. We calculated likelihood ratios that represent the weight of evidence for the effect of bait type for both number of individuals (trap = 160.5243, transect = 37.76) and number of species (trap = 35.177, transect = 19.98), which indicate very large effects of trap bait type.

The trap Tukey test (Table 2, Fig. 3) showed that dry traps detected fewer animals ($P < 0.001$)

and species ($P < 0.004$) than all baits. There was not a significant difference between pilsner or microbrew baits for animal ($P = 0.0936$) or species detections ($P = 0.9921$). Both pilsner ($P = <0.001$) and microbrew ($P = 0.0032$) outperformed water in individual detections. However, pilsner marginally outperformed water ($P = 0.0437$) in species detections, while microbrew ($P = 0.2039$) did not outperform water in species detections.

Transect bait type.—The transect Tukey test (Table 3, Fig. 4) showed that the all dry transect detected significantly fewer animals ($P \leq 0.008$) than all other transects. The all dry transect detected significantly fewer species than the all pilsner ($P < 0.001$) and the dry/water/pilsner ($P = 0.019$) transects and fewer species than water/micro/pilsner ($P = 0.0631$), but not at the alpha 0.05 level. The all pilsner transect detected significantly more animals than the water/micro/pilsner ($P = 0.0355$). All pilsner transects also detected more animals than dry/water/pilsner ($P = 0.0590$) but not at the alpha 0.05 level. All pilsner transects detected more species than the water/micro/pilsner transects ($P = 0.0880$) at the alpha 0.10 level but did not outperform the dry/water/pilsner ($P = 0.5592$) transects. We found no differences between water/micro/pilsner and dry/water/pilsner transects for number of animals ($P = 0.9996$) or species detected ($P \geq 0.8770$).

Seasonality

Trap seasonality.—Zero to eight species (mean = 0.55) were detected per transect, but 0 gastropods were detected at the majority of transects ($n = 613$). An average of 1.72 species were detected on the 299 transects that did detect gastropods.

There are highly significant relationships with both elevation and Julian date across the full dataset, with fewer species detected at higher

Table 2. Tukey's pairwise comparisons between trap bait types.

Trap comparison	Animals				Species			
	Estimate	SE	z	Pr > (z)	Estimate	SE	z	Pr > (z)
Water vs. control	1.4502	0.2139	6.781	<0.001***	0.8576	0.2553	3.359	0.0038**
Microbrew vs. control	1.8936	0.2216	8.547	<0.001***	1.1352	0.2717	4.178	<0.001***
Pilsner vs. control	1.7103	0.2121	8.064	<0.001***	1.1729	0.2507	4.678	<0.001***
Microbrew vs. water	0.4434	0.0854	5.193	<0.001***	0.2777	0.1443	1.924	0.2039
Pilsner vs. water	0.2601	0.0765	3.402	0.0032**	0.3153	0.1220	2.584	0.0437*
Pilsner vs. microbrew	-0.1833	0.0808	-2.268	0.0936	0.0377	0.1360	0.277	0.9921

Note: SE, standard error.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

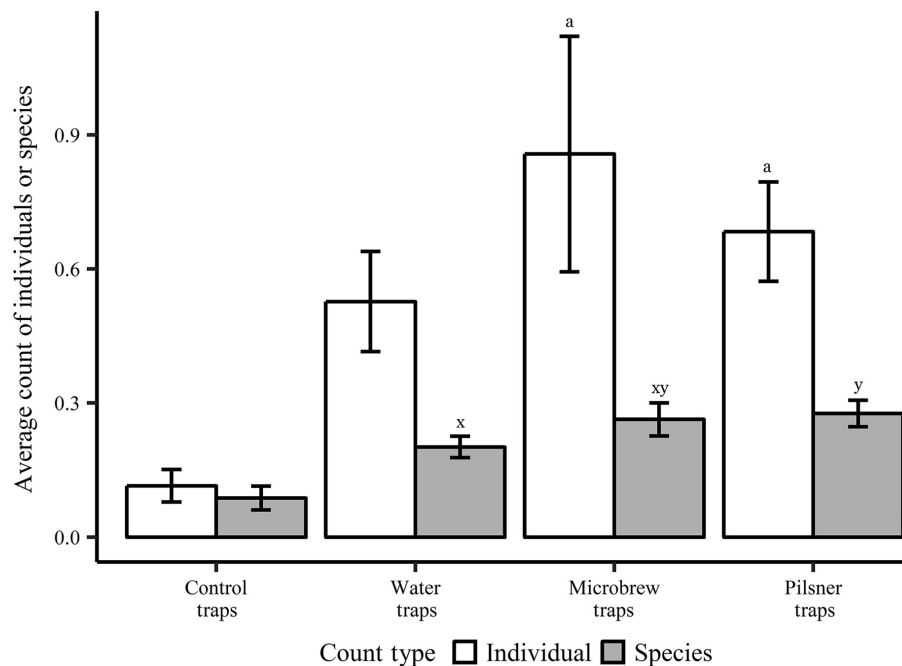


Fig. 3. Tukey's pairwise comparisons between trap bait types. Nonsignificant comparisons are indicated with the same letter. Comparison was made within individual count data and species count data. Error bars are standard error.

Table 3. Tukey's pairwise comparison between trap transect types.

Transect type comparison	Animals				Species			
	Estimate	SE	z	Pr > (z)	Estimate	SE	z	Pr > (z)
All dry vs. dry, water, pilsner	-1.1339	0.3600	-3.150	0.0082**	-0.7553	0.2617	-2.886	0.0190*
All dry vs. water, micro, pilsner	-1.1596	0.3441	-3.370	0.0041**	-0.6225	0.2534	-2.457	0.0631
All dry vs. all pilsner	-1.7105	0.3274	-5.225	<0.001***	-0.9619	0.2402	-4.005	<0.001***
Dry, water, pilsner vs. water, micro, pilsner	-0.0257	0.2557	-0.100	0.9996	0.1328	0.1799	0.738	0.8770
Dry, water, pilsner vs. all pilsner	-0.5766	0.2320	-2.485	0.0590	-0.2066	0.1604	-1.288	0.5592
Water, micro, pilsner vs. all pilsner	-0.5509	0.2061	-2.674	0.0355*	-0.3394	0.1461	-2.323	0.0880

Note: SE, standard error.

elevations ($P < 0.001$) and fewer species detected later in the season ($P < 0.001$). There were a decreasing number of species as the snow-free season progressed, with few plots showing any detection between days 250 and 270 (September 7–27). The spline shows low average detections per site in all dates but declining to near 0 on average after day 205 (July 24).

The all subset Poisson regression model (Table 4)-averaged variable importance value indicates that precipitation and elevation are the strongest predictors of species richness per plot.

Temp_13, Julian_date, and RH_13 were similar in influence to each other and about half as important as elevation and precipitation. Together this suggests that the highest richness is found on plots at low elevations early in the season in periods that have had precipitation and high humidity, with low temperatures.

Gastropod timed search seasonality.—Zero to eight species (mean = 0.86, $n = 2222$) were detected per search and, of those, 0 gastropods were detected at 48.65% of searches ($n = 1081$; see Table 5). An average of 1.68 species was detected on the 1141

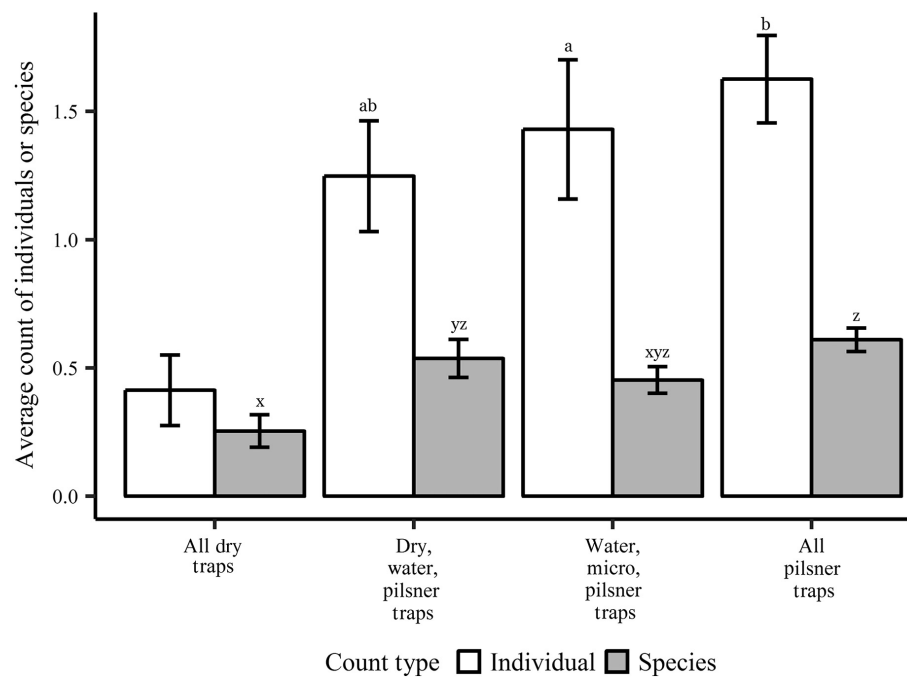


Fig. 4. Tukey's pairwise comparisons between trap transect types. Nonsignificant comparisons are indicated with the same letter. Comparison was made within individual count data and species count data. Error bars are standard error.

Table 4. Poisson regression with model-averaged coefficients to predict the variables that influence number of species captured using traps.

Variable	Estimate	SE	Adjusted SE	z	Pr > (z)	Relative variable importance	No. of models
(Intercept)	0.6713	1.2748	1.2854	0.522	0.6015		
Elevation	0.0009	0.0005	0.0004	2.130	0.0332*	1.00	6
Precipitation†	0.0061	0.0018	0.0019	3.280	0.0010***	1.00	6
Mean temperature‡	0.0146	0.0280	0.0282	0.518	0.6043	0.48	3
Julian date	0.0020	0.0054	0.0054	0.375	0.7078	0.42	3
Mean relative humidity§	0.0019	0.0071	0.0072	0.264	0.7919	0.40	3

Note: SE, standard error.

† Rainfall collected on plot during trap deployment.

‡ Mean air temperature for the 13 d of trap deployment.

§ Mean relative humidity for the 13 d of trap deployment.

* $P < 0.05$, *** $P < 0.001$.

searches that did detect gastropods. Across the full dataset, the only model with Akaike's information criterion (AIC) support was the global model including both variables with highly significant relationships between number of species detected and elevation and Julian date ($P < 0.001$), with more species detected at lower elevations and earlier in the season.

For the second collection season test, we detected nearly twice as many animals per plot in the dates we defined as summer (Julian date = 144–222)

than fall (Julian date = 248–284). There was a highly significant ($P < 2.2 \times 10^{-16}$) seasonal effect with a large likelihood ratio (173.588) in terms of number of animals detected. We detected 1.3 more species per plot in the summer than the fall, and there was a significant difference ($P = 2.09 \times 10^{-7}$) with a large likelihood ratio (35.99) in terms of number of species detected.

Leaf litter seasonality.—Zero to five species (mean = 0.32, $n = 912$) were detected per transect, and 0 gastropods were detected at the

Table 5. Poisson regression with model-averaged coefficients (with shrinkage) predicting the variables that influence number of species detected during timed searches.

Variable	Estimate	SE	Adjusted SE	z	Pr > (z)	Relative variable importance	No. of models
(Intercept)	0.1311	0.8959	0.8973	0.146	0.8838		
Elevation	−0.0003	0.0002	0.0002	1.156	0.2478	0.74	2
Julian date	−0.0045	0.0014	0.0014	3.186	0.0014**	1.00	3
Mean temperature†	0.0475	0.0190	0.0190	2.499	0.0125*	1.00	3
Mean relative humidity‡	0.0040	0.0051	0.0051	0.792	0.4284	0.57	2

Note: SE, standard error.

† Mean air temperature 2 d prior to or after the timed search.

‡ Mean relative humidity 2 d prior to or after the timed search.

* $P < 0.05$, ** $P < 0.001$.

76.21% of transects ($n = 695$; see Table 6). An average of 1.36 species was detected in the 217 samples that did detect gastropods.

The global model including elevation and Julian date was the only model with AIC support in the first step, with negative coefficients for both date and elevation indicating more species detected at lower elevations and earlier in the season.

In the full subset with all variables, elevation, precipitation, and Julian date were all significant. Elevation had a negative coefficient indicating more species detected at lower elevation. Julian date had a positive sign indicating more species detected later in the year. Precipitation had a positive sign indicating more species detected with increasing precipitation. All three of these variables had AIC importance value of 1. In contrast, both RH_13 and temp_13 had nonsignificant coefficients and AIC importance values of 0.21.

Observer bias GTS/leaf litter

Gastropod timed search observer bias.—Observer 1 detected significantly more animals ($P \leq 1.44 \times$

10^{-11}) than all other observers except observer 2 ($P = 0.299$; see Fig. 5). Observer 2 detected significantly more species ($P \leq 0.012021$) than all other observers including observer 1.

Leaf litter observer bias.—College students detected significantly more animals ($P = 0.000872$) and species ($P = 4.33 \times 10^{-11}$) than all other groups (Fig. 6). Paid temporary employees performed the worst, detecting only 3.80% ($n = 42$) of all animals detected ($n = 1107$) even though they performed 18.40% of the surveys.

Re-sampling

Trap re-sampling.—We found significant differences ($P = 0.0031$) and a large likelihood ratio (9.736) between species detected on the first trap check (mean = 0.56, range 0–5 new species), redetected on second trap check (mean = 0.19, range 0–5 species), and new species detections on the second trap check (mean = 0.32, range 0–3 new species, $n = 109$).

Gastropod timed search re-sampling.—We found significant differences ($P = 5.536 \times 10^{-11}$) with a high likelihood ratio (59.11) between species

Table 6. Poisson regression with model-averaged coefficients predicting the variables that influence number of species detected during leaf litter collections.

Variable	Estimate	SE	Adjusted SE	z	Pr > (z)	Relative variable importance	No. of models
(Intercept)	−0.3175	0.6713	0.6733	0.472	0.6373		
Elevation	−0.0009	0.0003	0.0003	3.298	0.0010***	1.00	4
Julian date	0.0021	0.0039	0.0039	0.529	0.5968	0.77	3
Precipitation†	0.0010	0.0013	0.0013	0.774	0.4392	0.71	3
Mean temperature‡	−0.0003	0.0186	0.0186	0.018	0.9856	0.65	3

Note: SE, standard error.

† Mean air temperature for the 13 d of trap deployment.

‡ Rainfall collected on plot during trap deployment.

*** $P < 0.001$.

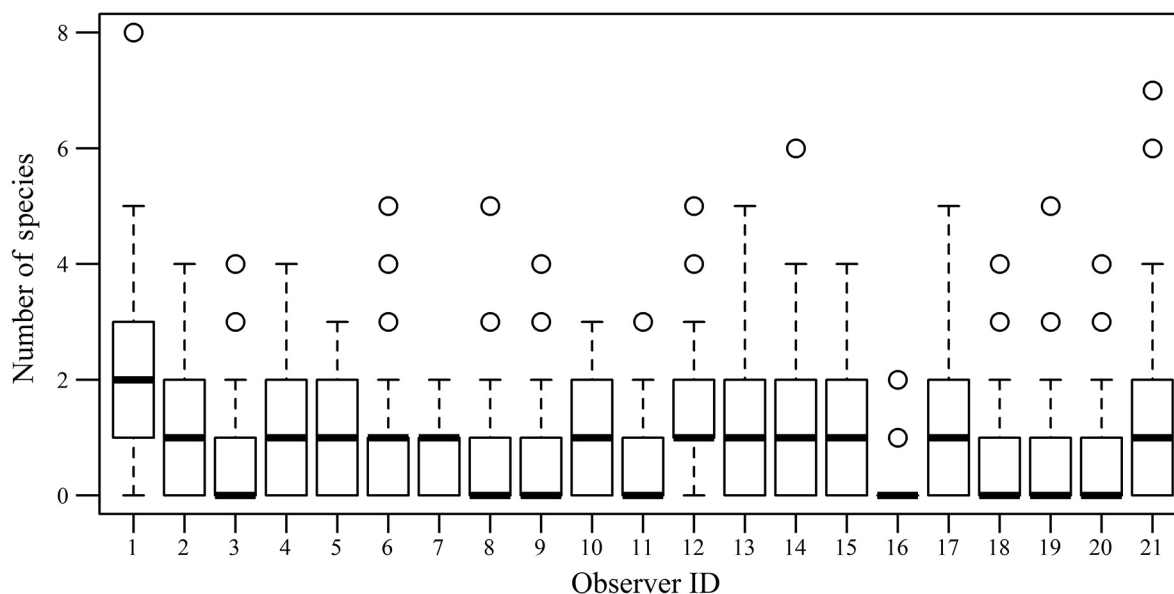


Fig. 5. Mixed-effects ANOVA results for observer bias for number of species detected by observer during visual gastropod timed searches. All observers were paid wildlife technicians.

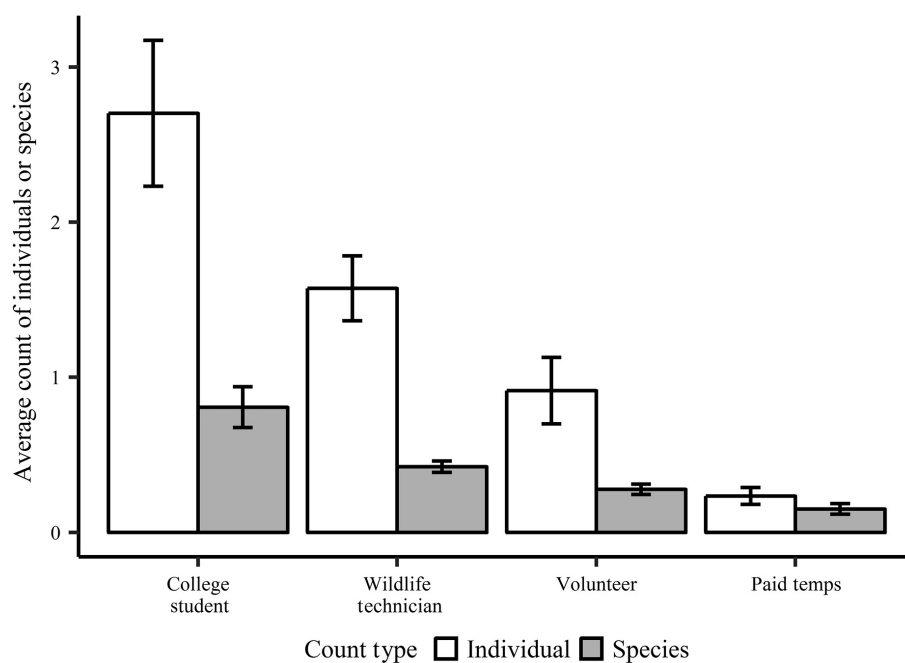


Fig. 6. Mixed-effects ANOVA results for observer bias for number of species detected by observer type during timed leaf litter searches.

detected during the first GTS (mean = 0.89, range = 0–7), redetected on second GTS (mean = 0.27, range = 0–3), and new species detections on second GTS (mean = 0.65, range = 0–6, $n = 948$).

DISCUSSION

We examined a variety of variables affecting gastropod detection by incorporating several

established techniques into a single fused transect. Our detection of 51 terrestrial gastropod species was affected by a wide range of factors both on a species and species grouped-by-size level.

Cover board trapping and bait type

Traps performed reasonably well for most groups but were generally outperformed by GTS. Small slugs were the exception to this rule with traps collecting more small slugs (16.56%, $n = 151$) than GTS (9.81%, $n = 218$). The key to trap performance was keeping the trap moist or baited as dry control traps were nearly a complete failure. Although we did not measure precipitation during the control trapping portion of the study, we suspect gastropods collected on these cover boards likely entered during rain events when the traps were naturally wet.

Although single species studies have shown *Arion lusitanicus* (Piechowicz et al. 2014) and *Arion vulgaris* (Piechowicz et al. 2016) preferentially select certain beer brands, our multi-species study found no difference in beer bait type (micro or pilsner). We suspect the wide range of species in our study in concert with trap location being more important than beer type (Piechowicz et al. 2016) influenced this finding.

With the assumption that all beers are equal behind us, we are next led to the question of are gastropods attracted to moisture or the beer bait. Piechowicz et al. (2014 and 2016) found beer was clearly more effective bait than water as a slug attractant and our results support and we expand on these findings. In all tests but one, both micro and pilsner outperformed water in detection of animals and species. The confounding exception is, although there was no difference between beer types, the micro did detect more species per trap than water ($P = 0.2$) but not at the 0.05 level. Despite this result, the majority of evidence points toward beer, of any form, being a superior choice to water for terrestrial gastropod trapping.

Seasonality

For all survey methods, except the final leaf litter output, we detected more species earlier in the season, at lower elevation, in periods of higher precipitation. Although higher species richness at lower elevations is not surprising (Baur et al. 2014, Schmera and Baur 2014), we must interpret this result with some caution. We generally

surveyed lower elevation sites earlier in the season as snowpack prevented access to higher elevation sites until late season hotter and drier weather prevailed. We addressed this concern by comparing summer and fall GTS results at the 149 FIA plots. Although other northern hemisphere studies have indicated autumn as a more effective gastropod sampling season (Johnston et al. 2017), our summer vs. fall comparison of GTS results still supported early season sampling detecting more species. Therefore, our recommendation is to survey locations relatively early in the snow-free season. In studies aiming to describe gastropod diversity in temperate mountain ecosystems, it remains important to include higher elevation areas as some species, such as *Magnipelta mycophaga*, occur predominately along higher elevation gradients.

Observer bias

Observer bias is often cited as a confounding factor making quantitative gastropod surveys challenging if not impossible (Pearce and Örsan 2006). Our large observer sample size presented a unique opportunity to assess this commonly held malacological belief. Our 21 GTS observers were all paid wildlife technicians or biologists. They each participated in a short training exercise in how to search for gastropods, and none had prior experience working with gastropods. We were quite surprised to find, with two exceptions, they performed nearly equally.

The two exceptions both developed strategies, within the sideboards of the protocol, which likely influenced success. Observer 1, who performed best in species detections, moved quickly about the defined search area searching in a wide variety of micro-habitats. Observer 2, who detected the most animals, did not move widely during the search and generally sat down and did not leave a certain spot during the search period (thus generally focusing on a single micro-habitat).

The leaf litter results presented an opportunity to evaluate success of different classes of observers. College students, wildlife technicians, and paid temporaries were paid an hourly wage to sort leaf litter samples. Paid temporaries were hired from a temporary employment company, had no background in science or natural resources, and were supervised directly by a paid wildlife technician. Despite extra guidance

and oversight, the paid temporaries performed abysmally. It is difficult to provide reasons for this result but possibilities range from a general lack of investment in the purpose of the work (conservation) or to their being older on average (and perhaps having poorer eyesight) than other observer groups. We may have seen greater success from paid temporaries had we provided a small cash incentive for the most and smallest shells similar to Emberton et al. (1996).

It seems grouping observers by type may be a possible way to achieve quantitative survey goals. For instance, only use paid wildlife technicians or only use un-paid volunteers. Still the occasional observer may possess a knack that could skew results. Regardless, our overall results suggest quantitative measures of gastropod diversity are possible despite potential observer bias, and observer bias may be more of a malacological perception than reality.

Re-sampling

Our results show a clear increase in the number of species detected on subsequent visits of both traps and GTS. Given the predominance of elevation and season as predictive factors in species richness, we may have seen similar results had we put more effort into each site visit (i.e., six traps during the same visit could yield a similar increase to three traps visited twice). Given the amount of effort taken to reach remote survey sites and the decrease in species detections we see over the snow-free season and recognizing the possibility of missing species with short seasons (Emberton et al. 1996, Cameron and Pokryszko 2005, Baur et al. 2014), we suggest more effort be put into individual site visits early in the season rather than visiting the same site multiple times over the course of a season. On the other hand, smaller scale studies or those with less remote sites may find it worthwhile to include a trapping component.

CONCLUSIONS

Were our goal to definitively map the full diversity of terrestrial gastropods in our study area, our protocol could be criticized for not targeting soil (Emberton et al. 1996) or arboreal specialists (Johnston et al. 2017). Indeed, our irregular detection of species from groups such as Pupillidae suggests we may have under-

detected some species, perhaps due to their seasonal nature (Cameron and Pokryszko 2005). However, our goal was to broadly map the range and distribution of the majority of terrestrial gastropods in our study area and it is generally considered acceptable to miss some species at the site level when the goal is broad survey results (Cameron and Pokryszko 2005). Although we did not detect each species at the site level, the broad picture we paint appears reasonably accurate. For the majority of species, we were able to develop range maps which clearly show edges of distributions and contact zones (Lucid et al. 2016, 2018). Additionally, our survey drastically changed the scope of knowledge of this taxonomic group in our study area as evidenced by the substantial influence this dataset had on species status changes during the 2015 state species ranking and Idaho SGCN assignment process (Lucid et al. 2016, IDFG 2017). Data collected as part of this project were partially or wholly responsible in the removal of seven gastropod species from (*Cryptomastix mullani blandi*, *Kootenai burkei*, *Polygyrella polygyrella*, *Prisiloma idahoense*, *Radiodiscus abietum*, *Udosarx lyrata*, and *Zacoleus idahoensis*) and addition of four gastropod species (*Hemphillia skadei*, *Prophysaon coeruleum*, and *Prophysaon dubium*) to the Idaho SGCN list (Lucid et al. 2016, 2018, IDFG 2017).

Our data and analyses leave us with the opportunity to ask what we would have done differently. Given the difficulty and expense of accessing sites in our remote and mountainous study area, we would likely not re-visit survey sites. Although re-sampling clearly increased our detections, we would recommend spending more time during a single site visit and conducting multiple sampling sessions during a single visit. This strategy would effectively remove the opportunity for trapping. This is clearly fine for pitfall traps as they were rather ineffectual. Although the cover board traps did outperform GTS in detecting small slugs, they are still likely not worth the additional effort to re-visit sites. To describe terrestrial gastropod diversity in a large study area, we recommend a combination of GTS and leaf litter collection relatively early in the snow-free period. We would increase the amount of time searched and volume of litter collected. Beer worked well as a bait, and we recommend its use in studies that do include a trapping component.

Understanding species status is a necessary and critical part of resource management. Our survey effort profoundly changed our understanding of terrestrial gastropod diversity in our study area, and we suspect efforts in other geographic areas would provide similar benefits. We hope this work provides a roadmap to others seeking to implement this crucial first step in terrestrial gastropod conservation.

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