- 1 Title: A 117 year retrospective analysis of Pennsylvania tick community dynamics
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### 1 Abstract

## 2 Background

3 Tick-borne diseases have been increasing at the local, national, and global levels. Researchers

- 4 studying ticks and tick-borne disease need a thorough knowledge of the pathogens, vectors,
- 5 and epidemiology of disease spread. Three surveillance approaches are commonly used to
- 6 provide insight into tick-borne disease risk: human disease case surveillance, active tick
- 7 surveillance, and passive tick surveillance. Long-term passive surveillance can provide up-to-
- 8 date data on the spatial variability and temporal dynamics of ectoparasite communities and
- 9 shed light into the ecology of rarer tick species. We present a retrospective analysis on

10 compiled data of ticks from Pennsylvania over the last 117 years.

11

## 12 Methods

13 We compiled data from ticks collected during tick surveillance research, and from citizen-

14 based submissions to the Penn State University Department of Entomology (PSUEnt).

15 Specimens were deposited at the PSUEnt arthropod collections that eventually became The

16 Frost Entomological Museum. While most of the specimens were submitted by the public, a

17 subset of the data were collected through active methods (flagging or dragging, or removal of

- 18 ticks from wildlife). We analyzed all data from 1900-2017 for tick community composition, host
- 19 associations, and spatio-temporal dynamics.

20

# 21 Results

22 In total there were 4,491 submission lots consisting of 7,132 tick specimens. Twenty-four

1	different species were identified, with the large proportion of submissions represented by five
2	tick species. We observed a shift in tick community composition in which the dominant species
3	of tick ( <i>Ixodes cookei</i> ) was overtaken in abundance by <i>Dermacentor variabilis</i> in the early 1990s,
4	and then replaced in abundance by <i>I. scapularis</i> . We analyzed host data and identified overlaps
5	in host range amongst tick species, suggesting potential hubs of pathogen transfer between
6	different tick vectors and their reservoir hosts.
7	
8	Conclusions
9	We highlight the importance of long-term passive tick surveillance in investigating the ecology
10	of both common and rare tick species. Information on the geographic distribution, host-
11	association, and seasonality of the tick community can help researchers and health-officials to
12	identify high-risk areas.
13	
14	Keywords: ticks, passive surveillance, museum collections, community composition
15	
16	
17	Background
18	
19	The Centers for Disease Control reported a 3.5× increase in vector-borne diseases in the USA
20	between 2004–2016, with 76.5% of cases caused by tick-borne pathogens [1]. The increase in
21	tick-borne disease is attributed to multiple abiotic and biotic factors. Changes in geographic
22	variability and the temporal dynamics of tick species may influence tick-borne disease

outcomes. Several endemic tick species have been expanding into new habitats across North
America, posing novel risks to local communities [2]. Although there are many tick-borne
pathogens, the vast majority of tick-borne disease cases are caused by *Borrelia burgdorferi*[1,3], the main etiological agent of Lyme disease in the USA. Pennsylvania has had the highest
number of total Lyme disease cases since 2000, with increasing numbers of annual cases
across several counties (Figure 1).

7

Surveillance can be a powerful tool for detection of introduced species (transient or 8 9 established), emergent arthropod-borne pathogens, and disease risks due to increases or 10 changes in vector community composition. Active tick surveillance approaches such as 11 dragging, flagging, CO<sub>2</sub>-trapping, or live animal capture, can be very effective for assessing tick 12 load by habitat [4,5]. However, active surveillance is labor-intensive, costly, and difficult to implement over a wide geographic area. Passive surveillance, in which citizens submit ticks for 13 14 identification and/or pathogen testing, is more cost-effective and less labor-intensive and can 15 provide insight into ectoparasite abundance, host associations, or habitat associations across a wider geographic area [6]. Even when tick samples may be in less-than-ideal conditions (e.g. 16 17 missing taxonomically diagnostic), or data, citizen-submitted tick can accurately represent tick encounter frequency and potential risk of tick-borne disease exposure [7]. 18 19 20 Passive surveillance data collected over decades may reveal spatio-temporal changes in 21 ectoparasite communities [8]. Data such as spatial distribution and occurrence of both

abundant and rare species of ticks can be correlated with landuse (e.g. habitat loss,

1	fragmentation, management), fluctuating environmental conditions, or changes in human or
2	animal behavior (e.g. encroachment may bring reservoir hosts such as groundhogs in closer
3	proximity)[9,10]. Additionally, long-term surveillance data can also reveal shifts in temporal
4	dynamics of tick populations and communities [2]. The seasonality of known tick species have
5	been described, but year-to-year distribution of tick species may be influenced by inter-annual
6	variability (e.g. local climate) and biotic factors (e.g. local reservoir species abundance). These
7	data can be used to create predictive models that accurately measure risks of tick-borne
8	zoonotic agents.
9	
10	We present a retrospective analysis of tick collection data in Pennsylvania from the early 1900s
11	to June of 2017. Some of the data prior to 1968 had been published in a USDA report on ticks
12	from Pennsylvania, but were presented in a format that included anecdotes and overall
13	percentages rather than raw number breakdowns by species (Snetsinger 1968). In this
14	manuscript we revisit these specimens and utilize the raw data from both these specimens
15	(1900-1960s) as well Pennsylvania ticks submitted from 1968-June 2017 to identify shifts in tick
16	community composition, phenological patterns, and host associations. We used our database
17	to map the distribution of major tick species at the county level, investigate tick community
18	spatiotemporal dynamics, and explore host and vegetation associations by tick species.
19	
20	Methods
21	
22	Study locations

1

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2	The state of Pennsylvania (PA) is located in the mid-Atlantic region of the United States
3	(latitude: 39° to 42°N and longitude: -80° to -74° W). The climate varies across Pennsylvania
4	depending on the region and altitude, but it typically consists of hot, humid summers and cold
5	winters with heavy snowfalls in certain areas. The majority of Pennsylvania's land-use is
6	dedicated to agriculture (both croplands and pastures), forestland, and dense urban areas.
7	There have been significant changes in the human populations of PA from 1960 to 2010, but a
8	large proportion of the PA population has remained heavily clustered around Philadelphia and
9	Pittsburgh, which are located in south-eastern and in south-western PA respectively (Figure 2).
10	While most specimens were collected within state boundaries, a few were declared from
11	people either visiting or returning from visiting other states. Tick specimens identified as
12	species that are not commonly found in Pennsylvania were later discovered to have been
13	imported or on exotic animals. These non-PA data were not included in state-wide analyses,
14	but were included in the Supplemental Tables (Table S1).
15	
16	Submissions
17	
18	The PSU Frost Entomological Museum (hereafter 'Frost Museum') houses arthropod samples
19	collected by researchers, teaching collections, and samples submitted by the public for
20	identification. Although some of these samples date as far back as the late 1800s, we only
21	present our analysis of the tick specimens from 1900 to June 2017. Because tick samples were

- submitted to the Department of Entomology or the Frost Museum over a period of 117 years,

1 they represent multiple collection/submission periods (early 1900–1959, 1960s, 1970–1988, 2 Tick Research Lab (TRL) submissions from 1990–1993, and 1995–present). Two public 3 campaigns account for the majority of the specimens. The first campaign (between 1963-1967) 4 was conducted by Dr. Robert Snetsinger. Dr. Snetsinger collected approximately 500 ticks 5 using a combination of dragging, sweeping, live animal trapping, and roadkill examinations of 6 mammals and birds to assess tick abundance in localized areas (Snetsinger, 1968). He enlisted 7 the help of the public through advertisements in radio, television, and newspapers to obtain 700 additional specimens (Snetsinger, 1968). A second funded campaign dedicated to 8 9 estimating tick abundance and species diversity was launched by Steven B. Jacobs (second 10 author) from 1990-1993 (TRL). In total 1357 submission lots made up of 3561 tick individuals 11 were contributed by members of the public or by physicians requesting identification.

12

Tick submitted prior to 1960 reflect the sporadic nature of citizen-based submissions. An active 13 14 advertisement campaign was used to enlist the help of citizens in the 1960s and again in the 15 early 1990s. Subsequently, post-funding submission rates decreased in volume, but tick submissions are still received regularly by PSU Entomology for identification. Data from the 16 17 ticks submitted prior to 1989 were identified by museum staff and stored in alcohol at the Frost 18 Museum. Submissions from the general public received from 1989 until June of 2017. TRL 19 submissions were identified by SBJ and stored in alcohol. All data were combined into a single 20 dataset for our analyses. We compiled these data to identify how species abundance have 21 changed over the years. We defined a submission lot ('submission') as a vial or lot containing 22 one or more ticks. For analysis of host-association, we used "submissions" versus total tick

1	counts by host. We chose to use this more conservative measure rather than the numbers of
2	ticks submitted to avoid a skew in abundance by host. For example, a submission lot of 1 tick
3	versus 50 ticks from a host were both treated as one 'submission'. For analysis on the
4	distribution of tick species over time, we used total tick counts.
5	
6	Identification
7	
8	Ticks were morphologically identified to species and life stage using the following taxonomic
9	keys: Argasidae, Ixodidae east of the Mississippi, Dermacentor, nymphal Ixodes, and nymphs of
10	<i>Amblyomma</i> [11–17]. Identification of samples to species is crucial since at least 3 <i>Dermacentor</i>
11	species, 3 species of Amblyomma, and 9 different Ixodes species have been reported in
12	Pennsylvania. If diagnostic characters were missing due to damage to the specimen, the next
13	level of taxonomic identification was used ( <i>e.g.</i> samples with missing mouthparts that were
14	clearly Prostriata were identified as " <i>lxodes</i> spp."). In a few cases, samples were not identified
15	beyond "tick" and were designated "Ixodidae" for hard ticks or "Argasidae" for soft ticks.
16	Difficult-to-identify, or unusual specimens were sent to the National Tick Collection, Georgia
17	Southern University for confirmation (by Dr. James Oliver at the time of confirmation).
18	
19	Spatial distribution of ticks
20	
21	To get an overview of the spatial variability of the tick community from 1900 to 2017, we
22	summed the total tick counts by species across the counties. Rarer tick species with less than

150 individual specimens were grouped by genus. We then plotted the total individuals with a
 dot-density map with each dot representing a single individual. As the associated geographic
 data for each submission was at the county-level, the placement of the dots within each
 county's boundary was randomized.

5

6 We focused on the geographic distribution of the five most abundant species in our database, 7 which are of significant public health and veterinary importance: Amblyomma americanum (Linnaeus), Dermacentor variabilis (Say), Ixodes cookei (Packard), Ixodes scapularis (Say), and 8 9 *Rhipicephalus sanguineus (Latreille)*. Working on the assumption that counties with higher 10 populations would submit more specimens than less populated counties, we estimated the 11 incidence rate (the total numbers of individual ticks per 100,000 people). This was done by 12 adjusting the total tick count of each species by the county's total population. Because we did not have the county population data for each year, we looked at relevant time periods during 13 14 the surveillance program: 1960-1970, 1990-2000, 2000-2010, and 2010-2020. For each time 15 periods, we used the 1960, 1990, 2000, and 2010 United Census data respectively to calculate the incidence rate (Figure 2) (U.S. Census data from 1960 to 1990: retrieved from 16 17 <a href="https://www.census.gov/population/cencounts/paigoogo.txt">https://www.census.gov/population/cencounts/paigoogo.txt</a>>. 2000 U.S. Census data: U.S. Census Bureau; Table DP-1, Census 2000, Profile of General Demographic Characteristics 18 19 Summary File 1 using American FactFinder <a href="http://factfinder2.census.gov">http://factfinder2.census.gov</a>, Table retrieved: 20 July 1, 2017). 2010 US Census data: U.S. Census Bureau; Table DP-1, Census 2010, Profile of 21 General Demographic Characteristics Summary File 1 using American FactFinder 22 <a href="http://factfinder2.census.gov">http://factfinder2.census.gov</a>>, Table retrieved: July 1, 2017).

2	For the tick species with less than 150 submissions across 1900 to 2017, we aggregated the
3	submissions by genus. We excluded the five major tick species (Amblyomma americanum,
4	Dermacentor variabilis, Ixodes cookei, Ixodes scapularis, and Rhipicephalus sanguineus) from this
5	analysis. We then mapped presence or absence of each genus by county.
6	
7	Temporal analysis
8	
9	To investigate how the annual submissions changed over the course of the passive tick
10	surveillance program, we first aggregated all individual tick specimens by year. Because there
11	were few submissions in the beginning of the program, we grouped the annual submissions
12	into decades starting from 1900–1910 (the years included would be from 1900 to 1909) to
13	2010-2020.
14	
15	We analyzed the temporal dynamics of the five most abundant taxa (A. americanum, D.
16	variabilis, I. cookei, I. scapularis, and R. sanguineus). We did not evaluate total counts by year as
17	it varied drastically during active campaigning for citizen submissions or introduction of
18	identification fees. Therefore, we looked at the proportional contribution of each species to
19	the annual summed counts of the five major species. To detect if there have been any
20	monotonic trends ( <i>i.e.</i> gradual shifts in abundance), we ran a non-parametric, two-sided Mann-
21	Kendall trend test on the yearly proportion of each of the species from 1900–2017.
22	

# 1 Seasonality

3	For analyzing seasonal patterns of submissions in the passive-surveillance program, we looked
4	at the months of when the tick specimens were received for identification. For some
5	submissions, there were no dates of when the ticks were found by the citizen, so we used the
6	date that the specimens were received. We first looked at the frequency of the months in
7	which the ticks submissions were received for the collective tick community across 1900–2017
8	and then by decades. We then investigated the seasonality of the five most abundant tick
9	species individually. For some of the submissions, information of the life-stage (larvae,
10	nymphs, and adults) was included. We then explored if the distribution of the monthly
11	submissions for the life-stages for the major tick species.
12	
13	Associated data
14	
15	Where available, we utilized host-association data for our analyses. Submissions generally
16	included date of tick discovery, location, vegetation associated with tick encounter, from
17	vertebrate the tick was collected (if any), and any additional comments that might provide
18	further insight. The data on human hosts such as gender and age were not included.
19	
20	Vegetation type
21	
22	For a small subset of these data (1989-1990, TRL) the vegetation associated with the tick

1	encounter were also recorded. We defined the dominant vegetation types as brush, forest
2	(inclusive of mixed, hardwood, and evergreen), pasture (land intended for grazing), managed
3	(residential or urban landscapes), and ecotone (used broadly here to refer to any zone of
4	transition between two plant communities). We used R (Version 3.4.1, RStudio version 1.1.383)
5	to performed a two-way analysis of variance (ANOVA) test to determine whether total tick
6	counts were dependent on the different types of vegetation by tick species.
7	
8	Host Associations
9	
10	Host information was available for many of the tick specimens (combined by family, except for
11	dog, cat, human, and groundhog). Host data were classified as either domestic or wildlife. We
12	summarized the host-tick data by summing the total submissions by both the tick species and
13	the host groups. We constructed a circular network map to visualize the relationships between
14	tick species and hosts. All host association analyses were done with R (Version 3.4.1, RStudio
15	version 1.1.383) with the packages 'MannKendall' for the Mann-Kendall test and the 'circlize'
16	package for chord diagrams of host association mapping [18,19].
17	
18	Availability of data and materials section
19	
20	The dataset(s) supporting the conclusions of this article is(are) available in the
21	passive_tick_surveillance_2018repository,
22	https://github.com/pakdamie/passive_surveillance_tick_2018/tree/master/MAIN_DAT

1

## 2 Results

3

4 General observations

5	From 1900 to 2017, PSU Entomology handled a total of 4,491 submission lots consisting of
6	7,132 tick specimens from twenty-three species (Table 1). There were five species of ticks that
7	accounted for the majority (91%) of the total number of tick: <i>Dermacentor variabilis</i> (n = 3172),
8	Ixodes scapularis (n = 1899), Ixodes cookei (n = 897), Rhipicephalus sanguineus (n = 332), and
9	<i>Amblyomma americanum</i> (n=196). Other tick species that were represented in at least 100
10	submissions were Dermacentor albipictus (Packard) (n = 107), Ixodes dentatus (Marx) (n = 120),
11	and <i>Ixodes texanus (Banks)</i> (n= 111). The remaining ticks had < 100 submissions/species and
12	included both hard and soft tick species.
13	
14	Spatial Analysis:
14 15	Spatial Analysis:
	<b>Spatial Analysis:</b> Ticks were submitted from all 67 counties in Pennsylvania (Figure 3). We hypothesized that
15	
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15 16 17 18 19	Ticks were submitted from all 67 counties in Pennsylvania (Figure 3). We hypothesized that more tick submissions would come from areas with higher human populations and as expected, tick submissions were heavily clustered around Allegheny and Philadelphia County where Pittsburgh and Philadelphia are located respectively. When we adjusted the total tick

1 Forest and Cameron counties also had high submissions of I. scapularis with 116.64 and 589.97 2 individuals per 100,000 respectively. Other counties with high *I. scapularis* incidence rates 3 included Northumberland (299.56 individuals per 100,000), Snyder (198.98 individuals per 4 100,000), Union (171.31 individuals per 100,000), and Clearfield (99.21 individuals 100,000) 5 (Figure 4). 6 7 Dermacentor variabilis distribution was largely localized to southern portions of the state, in 8 1990–2000, the highest proportion of *D. variabilis* submissions came from Greene County, the 9 most southeastern county of Pennsylvania (865.45 submissions per 100,000). Other southern counties with significantly high rates included Fulton County (350.60 per 100,000) and Franklin 10 11 County (117.26 per 100,000). 12 *Ixodes cookei* was more evenly distributed throughout Pennsylvania, although similar to *Ixodes* 13 14 scapularis, it was more highly abundant in the northern counties. In 1990-2000, Forest County 15 had the highest incidence rates of *Ixodes cookei* with 80.87 per 100,000. *R. sanguineus* and *Am. americanum* had very few submissions and their distribution was mostly scattered across 16 17 Pennsylvania. 18 19 Multiple species within the genera *Ixodes* and *Dermacentor* were widely distributed across 20 Pennsylvania (I. scapularis, I. cookei, D. andersoni, and D. albipictus) (Figure 5). Other species in 21 the genera Amblyomma, Argas, Carios (Ornithodoros), and Haemaphysalis were not as widely

22 distributed, possibly because these species are not commonly encountered or because the

- 1 specimens were introduced from their native geographic ranges. For example, we only had
- 2 four submissions of Argas cooleyi and A. persicus.
- 3
- 4 Temporal
- 5
- 6 Temporal shifts in species abundance
- 7

Prior to the 1990s, the majority of the tick submissions were identified as *I. cookei* and *R.* 8 9 sanguineus (Figure 6). The spike in the number of submissions 1990 were largely due to D. 10 variabilis, but gradually, I. scapularis became the dominant taxon submitted. Results from the 11 Mann-Kendall test supports these observations with an upward trend in the *I. scapularis* counts 12 (tau = 0.288, p = 0.02) and a significant downward trend in *D. variabilis* (tau = -0.408, p = 0.002). The Mann-Kendall also indicate that the proportional contributions of *I. cookei* (tau = -0.607, p 13 14 < 0.001) and *R. sanguineus* (tau = -0.377, p = 0.005) to the total count have also significantly 15 shifted over a century. 16 17 Seasonality 18 19 Overall, we find that the majority of tick specimens were received in the months between April 20 and July with May being the month with the highest proportion of tick submissions (Figure 7).

21 However, there was significant variation in the seasonality of the submissions during the

22 program (Figure 8).

2	Submissions of <i>D. variabilis, Am. americanum, I. cookei</i> and <i>R. sanguineus</i> were most abundant
3	during the period between May and July. By species, D. variabilis and Am. americanum were
4	most abundant from March to October. <i>I. cookei and R. sanguineus</i> samples were submitted
5	throughout the year, but had peak abundance in June. Samples of <i>I. scapularis</i> were also
6	submitted year-round, but the peak abundances were bimodally distributed, with a large peak
7	in between May to June, and a second peak between October to November. Our data tracks
8	these peaks, although nymphal <i>I. scapulari</i> s were also submitted during late spring to early fall.
9	
10	Life stage abundance by species
11	
12	There were 6,233 specimens for which the lifestage data were available. Four percent of the
13	submissions were larvae (n=237), 20% of the were nymphs (n = 1271), and 75% of the
14	submissions were adults (n = 4725).
15	
16	For <i>Dermacentor variabilis</i> , the submissions included 32 larvae, 33 nymphs, and 3059 adults
17	from 1960 to 2017(Figure 9). While we only had larval specimens from 1990–2000, we found
18	that the peak submissions were in September (90%). For the nymphal submissions, there was
19	a more unimodal distribution with the peak centered around June. Finally, the adult
20	submissions of both 1990–2000 and 2000–2010 showed similar patterns with the peak in
21	submissions between May and June.
22	

1	Before 1990, there were only six submissions of adult <i>Ixodes scapularis (Figure 10</i> ). In 1990–
2	2000, the nymphal and larval submissions show a unimodal pattern with the highest
3	proportion of submissions received in June. For the adult submissions during this decade, there
4	are prominent bimodal peaks in May and October with similar proportion of submissions
5	received in both seasons. There were fewer submissions in 2000–2010 with only 23 larvae, 2
6	nymphs, and 486 adults. The monthly submissions of both the larvae and adults in this decade
7	were consistent to the seasonal patterns found in 1990–2000. Finally, in 2010–2020, the adult
8	submissions (n = 31) show a shift with a number of submissions found earlier in March.
9	
10	The majority of <i>Ixodes cookei</i> submissions were identified as nymphs with a total of 521
11	submissions followed by adults (n = 182) and larvae (n= 88) (Figure 11). Submission patterns
12	indicate that <i>lxodes cookei</i> specimens are found all year-round specifically in the nymphal
13	stages (Figure 11). Across all life-stages, we see that the distributions are unimodal with peaks
14	in early summer between May and June.
15	
16	There were a total of 154 of 183 specimens identified to lifestage for Amblyomma americanum
17	(17 larvae, 64 nymphs, and 73 adults). Between 1990 and 2010, the nymphal and adult
18	submission patterns were similar with peak submissions occurring between June and July.
19	
20	The only decade with high enough counts of <i>Rhipicephalus sanguineus</i> to evaluate seasonal
21	variation was the 196os. In total, we received 59 adult submissions, 17 nymphs, and no larvae.

- 1 Submissions were mostly from July and August. The nymphs were submitted in October and
- 2 adults were submitted between February and September.
- 3

# 4 Vegetation type

6	There were 677 submissions for which we had data on tick associations with vegetation type
7	(from 1989 to 1990). The large proportion of vegetation data was of ecotone (n = 190) which
8	made up 27 percent of the data. This was followed by forest (n = 178) and managed (n=176).
9	ANOVA f indicated that habitat was a significant predictor for the tick counts (F =3.1997, p =
10	o.oo7). We found that ecotone had a weakly significant correlation with tick abundance (F=
11	2.097, p= 0.036*) and using a pairwise Tukey comparisons in the vegetation types, the greatest
12	difference in the vegetation type was between managed and ecotone.
13	
14	Host association
15	
16	One of our assumptions about passive surveillance is that there is an inherent bias toward
17	humans as the hosts, particularly since most specimens submitted by the humans on
18	
	themselves, their pets, or other domestic animals. Figure 12 is a visual representation of the
19	themselves, their pets, or other domestic animals. Figure 12 is a visual representation of the quantitative data on ticks associated with different hosts. By far the majority of submissions
19 20	
	quantitative data on ticks associated with different hosts. By far the majority of submissions
20	quantitative data on ticks associated with different hosts. By far the majority of submissions were associated with humans and their domestic animals and this reflects the fact that many

1	associated with cats or dogs, 20 from other domestic animals, and 168 were submissions
2	pooled from multiple hosts (mixed). There were 11 additional submissions found on various
3	exotic animals. There were 689 submissions for which there was no host record or the ticks
4	were not attached to a host. The remaining 275 were found on various wildlife.
5	
6	Discussion
7	
8	Our data adds to the current understanding of tick community composition and spatio-
9	temporal dynamics over 117 years. With the caveat that our dataset contains gaps in years of
10	submissions, we were able to detect shifts in tick community composition, seasonality, and
11	host associations that have not been well-documented in a quantitative manner. To our
12	knowledge this is the first time that these data have been compiled in their entirety and
13	analyzed in this format. Since 1993 (~25 years) there have been 28 publications on ticks from
14	Pennsylvania, and 22 of them were focused on <i>I. scapularis</i> and/or the microbiota (mostly on
15	pathogens)[20–48]. Subsets of our data had been reported as percentages or combined with
16	data from other museums and literature reviews to assess known distribution of one or more
17	tick species across the state of Pennsylvania [43,49].
18	
19	Without any data for comparison of other tick species, we can only speculate why there were
20	shifts in the abundance of certain tick species. For instance, <i>I. cookei</i> abundance was greatest
21	prior to the 1990s, but has since declined our submission dataset. In contrast, the abundance of

22 *I. cookei* in the Maine passive surveillance program has been constant, even as *I. scapularis* 

submissions have increased [8]. It is possible that Pennsylvania *I. cookei* abundance has also
 remained relatively stable, but perhaps we lack sufficient power to detect *I. cookei*.

3

Apart from one recent fine-scale analyses on the phenology of *I. scapularis* by Simmons et al 4 [42], much of the literature on PA ticks reflects presence/absence data of *I. scapularis* and/or 5 6 the pathogens they harbor. There have been 2 exceptions: a study of river otters that found 3 7 female, 1 nymphal and 1 larval *I. cookei* on 3 otters, and a study guantifying the ectoparasites of 8 bats in Blair County, PA that identified 5 specimens of soft tick Ornithodoros kelleyi Cooley & 9 Kohls [40, 28]. Certainly *I. scapularis* warrants the scrutiny it has received, but other tick species 10 have not been studied closely enough to detect the shifts in tick biodiversity, the potential 11 causes, or epidemiological consequences of these shifts. Further, neither expanding tick 12 ranges nor presence of introduced or established species have been closely monitored. Thus, 13 although we have only recently discovered the presence of the newly invasive longhorn tick (H. 14 longicornis) in Pennsylvania, we do not know whether it had been introduced previously. H. 15 *longicornis* is a tick with a wide host range, potentially capable of harboring and transmitting 16 multiple pathogens, may induced meat allergies, and may be capable of reproducing 17 parthenogenically [50–52]

18

19 Temporal comparisons of tick community compositions

1 In the 1960s, PA tick communities consisted predominantly of three species: I. cookei, D. 2 variabilis, and R. sanguineus. The most abundant species at that time, I. cookei, is often referred 3 to as a groundhog tick, but has been known to multiple mammalian hosts and will readily bite 4 humans and dogs [53]. The second most abundant species, D. variabilis, was widely distributed and eventually became the dominant species submitted over *I. cookei* in the 1990s. By 1991 D. 5 6 variabilis had been identified from all but 4 counties [43]. After 1995, D. variabilis annual 7 submission rates declined as *I. scapularis* submission rates increased. Although we cannot directly infer a causal negative relationship between these two species from our data, this 8 9 pattern was also observed in neighboring Ohio. The Ohio State passive surveillance program 10 (started in 1978) did not detect *Ixodes scapularis* (=formerly *I. dammini*) until 1989 [54]. At that 11 time, the dominant species were D. variabilis (~97% of submissions) and Ixodes cookei 12 (1.2%)[54]. Between 1989 until 2008, I. scapularis accounted for less than 1% of the total 13 submissions, but after 2009, the abundance began to increase until in 2012, they accounted for 14 24.8% of ticks submitted to the Ohio Department of Health [55]. 15 The fourth most abundant species of note in 1968 was *R. sanguineus*, the Brown Dog tick. *R.* 16 17 sanguineus originated in Africa, but has since become a cosmopolitan urban pest species found 18 worldwide in association with humans and their canine companions [56]. Snetsinger suggested 19 in 1968 that *R. sanguineus* had established breeding populations in Pennsylvania, but according 20 to our records, we have had very few submissions since then and none since 2002. Since both 21 R. sanguineus and D. variabilis are competent vectors of Rocky Mountain Spotted Fever 22 (RMSF), the recognition of either of these species is important for diagnosis and treatment

- 1 after a bite (ProMed-mail. Rocky mountain spotted fever USA (03): (PA) fatal. ProMED-mail
- 2 2014; 8 Sept; 20140908.2757657.<u>http://www.promedmail.org</u> July 2018 [57].
- 3

4	The last species that accounted for more than 150 submission lots was Am. americanum.
5	Although this species was not common in the 1960s, there was an increase in submissions from
6	1990s-2000s. Springer et al used predictive modeling to determine that the southern border of
7	Pennsylvania encompassing the Appalachia Mountains were unsuitable for supporting Am.
8	<i>americanum</i> , but that western Pennsylvania might experience expansion from the west [44].
9	We have identified specimens from as recent as October 2016 in our dataset, although we
10	cannot say for certain whether this specimen represents transient introductions or established
11	colonies since the lifestage was not available. <i>Am. americanum</i> is a competent vector of
12	ehrlichiosis, tularemia, RMSF, multiple <i>Borrelia</i> species (Including <i>B. lonestari, B. andersonii,</i>
13	and <i>B. americanum</i> ), and competent vector of heartland virus)[2,58,59]. Am. americanum has
14	also been implicated in inducing meat allergies in certain people [60].
15	
16	One additional species of note is <i>Haemaphysalis leporispalustris (Packard) (=Hlp)</i> , a congeneric
17	of <i>H. longicornis.</i> This species abundantly distributed throughout North America [49,53]. <i>Hlp</i> is

18 often commonly referred to as a rabbit tick, although it will readily parasitize any mammal or

19 bird and has been known to parasitize humans (Parker et al., 1951; Snetsinger, 1968; Freitas et

- al., 2009). It can be of public health concern because of its potential role as a vector of
- 21 Tularemia and Rocky Mountain Spotted Fever [49,62]. In 1968 this species was widely

1	distributed throughout the Pennsylvania, but we have no records of this species after 1993.
2	Since the submissions for tick identification after 1990 were mostly limited to ticks removed
3	from humans or their domestic animals, there is insufficient power to detect this species.
4	Farther north and west, <i>Hlp</i> is still found parasitizing a wide range of avian and mammalian
5	hosts [63,64].
6	
7	The seasonality data for the five most abundant tick species inferred by our passive
8	surveillance data is consistent with previous records of seasonality described by other
9	researcher [5,42,65,66], demonstrating that these types of passive data contain biologically
10	meaningful signal.
11	
12	Quantifying host association
13	
14	Vector-host associations are important factors for predicting risk of pathogen transmission
15	and identifying key players in a sylvatic disease cycle. Common names such as the rabbit tick,
16	deer tick, or dog tick are often used when describing ticks to the public, but can sometimes
17	lead to misinformation about host preferences. For example, many tick resources for the
18	public refer to <i>I. cookei</i> as the "Groundhog" or "Woodchuck tick" (e.g. CDC
19	https://www.cdc.gov/ticks/tickbornediseases/tickID.html). Yet, previous literature on I. cookei
20	clearly describes it as a cosmopolitan species that parasitizes a wide range of medium-sized
21	mammals [53,67]. Our data support the observations that this species can be found
22	parasitizing human, domestic animals, and wild animals. Although I. cookei is important as a

vector is of the very rare, but potentially fatal Powassan Encephalitis Virus, it is not a
competent vector of *B. burgdorferi* [68,67,69]. It therefore remains a sometimes ignored
species for which little is known about its microbiota (including pathogens, commensals, or
symbionts). Because of its broad host range and co-parasitism with other tick species, *I. cookei*may be a potential bridge vector that can transmit pathogens from one reservoir host to
another.

7

Host associations of more rarely encountered tick species can sometimes lead to incorrect 8 9 assumptions about host preferences. While some tick species may be presumed to hold strict 10 host preferences, they may bite humans if given the opportunity. For instance, *I. dentatus* 11 biting humans in cabins that had been inhabited by their squirrel hosts in Maine and Vermont, 12 and I. marxi was found (Hall et al., 1991, Lubelczyk et al 2010). In our dataset so-called "generalist" tick species (D. variabilis, I. scapularis, I. cookei) were found parasitizing a wide 13 14 range of vertebrate hosts. Similarly, we found that 'specialist' tick species (I. dentatus, I. marxi, 15 *I. muris*) that were mostly associated with a single host or limited to host size (*e.g.* small mammals or birds). Some species known to be multi-host species, however, were limited to 16 17 one host or few in our collections (e.g. *I. texanus* was only found on raccoons, yet it has been found parasitizing small rodents and medium sized mammals [5,70,71]. 18 19 Importance of tick species identification 20

1	Merten and Durden (2000) described 84 tick species naturally occurring in the United States,
2	40 species of which will are known to bite humans (11 species of soft ticks and 29 hard
3	ticks)[72]. Nine of the 40 human biting species are classified by the CDC as important vectors
4	of zoonotic disease website (https://www.cdc.gov/ticks/tickbornediseases/tickID.html): 1)
5	American dog tick ( <i>D. variabilis</i> ), 2) Blacklegged tick ( <i>I. scapularis</i> ), 3) Brown dog tick ( <i>R.</i>
6	sanguineus), 4) I. cookei (groundhog tick), 5) Gulf Coast tick (A. maculatum), 6) Lone star tick (A.
7	americanum), 7) Rocky Mountain wood tick (D. andersoni Stiles), 8) Soft ticks (specifically
8	<i>Ornithodoros</i> spp.), and 9) Western blacklegged tick ( <i>I. pacificus</i> Cooleyi and Kohls). In the last
9	20 years, Ixodes scapularis has become the most abundant tick species in Pennsylvania.
10	Distinguishing <i>Ixodes</i> from other genera of ticks is fairly simple, but <u>species</u> level identification
11	requires more detailed morphological examination, since there are 6 endemic species of <i>Ixodes</i>
12	and 3 exotic species that could potentially be misidentified as <i>I. scapularis</i> . More generally,
13	although many tick species are incompetent vectors of <i>B. burgdorferi</i> , they may be vectors
14	and/or reservoirs of other pathogens/parasites, or acquire pathogens during co-feeding
15	[5,21,73,74]. It is therefore important to correctly identify tick species, not only for
16	determination of disease risk, but also because the treatments for the pathogens they transmit
17	may differ significantly.
18	
19	Finding context for exotic specimens: the hidden gems in retrospective analyses

20

Our data encompasses a large timeframe and includes several collection periods. While such a
 rich dataset held much promise, the reality was that cleaning up and standardizing the data

1 into an analyzable format was a laborious task. Additionally, without context, it was difficult to 2 understand how exotic specimens arrived onto Pennsylvania soil. Much of the literature on the distribution of ticks other than *I. scapularis* in Pennsylvania was found in lists published either 3 4 in before or during 1940s or after the 1990s. Fortunately, we were able to locate a USDA report on ticks and tick-borne disease by Dr. Robert Snetsinger [49]. In it were explanations for 5 the presence of exotic specimens, but also contained were snapshots into the PA tick 6 7 community composition as well as the public health concerns of tick experts at that time. Although the report did not have actual counts (only percentages) with associated metadata 8 9 (and in fact contained data collected from other museums as well as references to data from 10 previous papers or unpublished data), we were able to cross-reference the existing tick 11 specimens from this time period with associated data contained in handwritten notes found at 12 the Frost. The Snetsinger report provided a snapshot the epidemiological focus of the time with respect to tick vectors and the pathogens they could spread. For instance, it was known, 13 14 but not published at that time, that the rare and deadly Powassan Encephalitis Virus (PEV) was 15 present in groundhogs in southern PA (Snetsinger, 1968). I. scapularis is now responsible for more arthropod-borne disease outbreaks in the continental US, but in the 1960s *I*. scapularis 16 17 populations (which had been decimated by deforestation in the early 1900s) had not yet re-18 established a foothold in most of the commonwealth[49,76]. The tick-borne diseases of 19 concern from 1963 to 1993 were Tularemia (caused by *Francisella tularensis*) and Rocky Mountain Spotted Fever (caused by Rickettsia rickettsii) (Snetsinger, 1968; Snetsinger et al., 20 21 1993).

22

1	In addition to 19 specimens that were from out-of-state, we also identified several exotic tick
2	specimens within Pennsylvania. One specimen of Amblyomma cajennense (the Cayenne tick
3	generally limited to neotropic regions) was collected from a capybara in 1913 from the
4	Philadelphia Zoological Garden [49]. A century later, Brazilian researchers documented
5	spotted fever-infected A. <i>cajennense</i> on capybaras [77]. Perhaps if this specimen has been
6	maintained well enough, we might be able to extract DNA and assay for spotted fever
7	rickettsial species.

Another specimen, a single European species of tick, *I. ricinus* (the castor bean tick), and sister
taxon to *I. scapularis*, was found on lizards in the Pittsburgh Zoo [49]. A third oddity was a
reptile-associated tick, *A. dissimile*, known to be imported through the pet trade (snakes) and
on research animals. In one humorous, but almost hidden anecdote, one of the tick specimens
was described to come from a snake that was used as part of the costume by a "night club
ballerina" [49].

14 Some exotic specimens suggested periodic introduction, but subsequent lack of establishment. In 1967 there was one specimen of the Gulf Coast Tick (A. maculatum (Koch)) 15 16 identified after being removed from the ear of a child who had never left Pennsylvania 17 (Snetsinger 1968). At that time, A. maculatum was only known from the neotropics and nearctic regions along the coasts of southern Atlantic states, so the presence of this specimen 18 19 was "a mystery" (Snetsinger 1968). We identified 4 additional submissions of this species in our 20 database since 1968 from 1969, 1990, 1994, and 2004. In the last several decades, the range of 21 this species has expanded westward into the Mid-west and northward into the Mid-Atlantic on

1	migrating birds, including into neighboring states (Sonenshine 2018). Their establishment
2	northward has been attributed to climate change, but because they require high humidity and
3	higher temperatures, what has driven their movement inland and westward is not yet clear. It
4	is possible that successful breeding populations are more tolerant of cooler and/or drier
5	conditions, or alternatively, because they are localizing to warmer and/or more humid areas
6	along riparian ecotones [3]. Am. maculatum is a vector of Rickettsia parkeri, a mild fever-
7	causing sickness in the Mid-Atlantic states, but its epidemiological significance is amplified
8	should it share its rickettsial load to <i>A. americanum</i> through co-feeding on the same host [3].
9	
10	
11	There were two soft ticks that were most either introduced or very rare. The first is <i>Argas</i>
12	persicus, an Old World species associated with poultry [78]. This is a species rarely collected in
13	the United States and it is presumed to have been imported on poultry. The only reported
14	sightings confirmed by Glen Kohls came from California, Georgia, Maryland, and Pennsylvania
15	[78–80]. Our two confirmed instances of <i>A. persicus</i> from Pennsylvania chickens came from
16	York and Adams county [78].
17	The other argasid species found was Argas cooleyi. A. cooleyi is mostly associated with cliff
18	swallows in southwestern states of the USA, so humans do not normally encounter them
19	unless they disturb the nests or if the nests are in close proximity to human dwellings [81]. It is
20	therefore unlikely that this specimen is commonly encountered in Pennsylvania unless
21	imported by humans or through bird movement.
22	

#### 1 Impact of free versus per-submission charges for tick identification on submission rates

2

Rates of tick submissions from the public varied depending on the extent of advertisement and 3 4 whether there was a cost associated with tick identification. In the case of the 1960s Snetsinger campaign, submission rates were high largely due to a multimedia advertisement 5 6 campaign that targeted principally housewives, although other citizens also submitted 7 specimens during that period. Similarly, during the first year of the TRL campaign, submissions 8 rates were initial high, but the submission rates declined over the next two years. Subsequent 9 lack of funding to support a free tick species identification meant that to identifications had to 10 be done at cost to continue to provide the service. This meant that submissions were mostly 11 limited to those willing to pay 25 dollars per specimen or to medical professionals who 12 removed the ticks from patients. In addition to the cost of tick identification, there was no available funding for either standardized pathogen screening or database management, which 13 14 greatly hampered efforts to provide a more robust dataset. This is in contrast to states such as 15 Maine, where the state Department of Health was directly involved in collection of tick data and screening for tick-borne pathogens [8]. In just under 2 decades, the Maine DOH program 16 17 (after engaging in a sustained outreach campaign to encourage public submissions) obtained 24,519 ticks were submitted for identification free of charge. We suspect that the submission 18 19 numbers for a statewide tick identification program would likewise be high enough to detect 20 lower abundance ticks and potentially detect invading species.

21

22 Multi-faceted approach to tick surveillance

1

2	Passive surveillance is sometimes criticized for under-representation of certain taxa or bias
3	toward certain host associations. However, citizen-submitted tick collections can provide
4	valuable baseline data on prevalence and likelihood of tick encounters ([82–84,7]. In fact, there
5	is evidence that passive tick surveillance data is more strongly correlated with reported human
6	cases of tick-borne diseases than active surveillance [7]. A community engagement program
7	that actively recruits ticks submitted by citizens should be coupled with support for a rigorously
8	curated database of tick submission.
9	

While passive surveillance is able to capture a wide geographic range, active surveillance is able 10 to detect fine-scale population estimates and host associations. Utilizing complementary 11 12 strategies can help fill in knowledge gaps about tick prevalence. For example, in an exclusively passive surveillance-based study, only 17 of 77 Oklahoma counties were identified as having 13 established lonestar tick populations (A. americanum) [85]. In contrast, in a study using a 14 15 combination of retrospective literature review, data compilation of specimens from archival 16 collections, and active collection (dry ice, dragging, and flagging) in counties presumed to be 17 free of *A. americanum*, 68 of 77 counties of Oklahoma were identified as colonized [83]. One of 18 the limitations of active collection methods is that while overall population sizes may be high, sampling may not reflect population abundance, particularly if the distributions are spotty. 19 20 Tick populations are not static and may be highly mobile, depending on the host upon which 21 they alight. Nevertheless, active surveillance can fill in the gaps in tick distribution throughout 22 each state.

1

2	Thus, an ideal surveillance program for vector-borne disease epidemiology would utilize both
3	active and passive collection strategies. Both passive and active collections provide
4	complementary data for accurate assessment of tick-borne disease risk that can be combined
5	with reported human cases of tick-borne disease. The metadata associated with both active
6	and passive tick surveillance (assuming it has been curated and well-managed) can provide
7	insight into tick-host association, vegetation, seasonality, and shifts in population structure
8	that can be used for modeling disease risk. Archival tick samples (or their DNA) can be useful
9	for retrospective mining for research on the population genetics of ticks to detect gene flow,
10	host shifts, or on their microbial inhabitants.
11	
12	Conclusion
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13 14 15 16 17 18	When we combine citizen-based passive surveillance data with museum collection data, we find that hybrid datasets are a powerful tool for mining past ecological and epidemiological events. Many states maintain county records on passive tick submissions to veterinary or medical health officials, but there may be other cryptic collections (and associated data) housed in museums, universities, government institutions, or with private individuals. In the

22 correlated with increasing human encroachment on natural habitats, 2) what are some

1	phenological reasons for the increase in <i>Ixodes scapularis</i> abundance; or 3) if displacement of a
2	dominant tick community species occurs, what are the implications for tick-borne disease risk?
3	We anticipate that participating in such a study will fill in the gaps of knowledge about less
4	well-studied tick species.
5	
6	
7	DECLARATIONS
8	Steve Jacobs is a retired Senior Extension Specialist with the Department of Entomology at
9	Penn State.
10	
11	Acknowledgements
12	The authors would like to thank Dr. István Mikó for critical manuscript review, the public and
13	researchers who have contributed ticks to PSU, The Frost Entomological Museum for access to
14	the collection, and critical suggestions from anonymous reviewers. This work was supported
15	by funds from NSF GRFP DGE1255832 (to support Damie Pak), the Huck Institutes of Life
16	Sciences, and the Penn State College of Agriculture.
17	
18	Competing interest
19	The authors declare no conflicts of interest.
20	
21	Author Contributions
22	SBJ compiled that data into a digital database, collected metadata, identified tick specimens from

1	1989 until 2017, and manuscript revisions. DP generated data visualizations, conducted statistical
2	analysis, and contributed to manuscript writing and revisions. JMS curated the database, directed
3	analyses, and contributed literature review, manuscript writing, and revisions
4	
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### 1 Figures

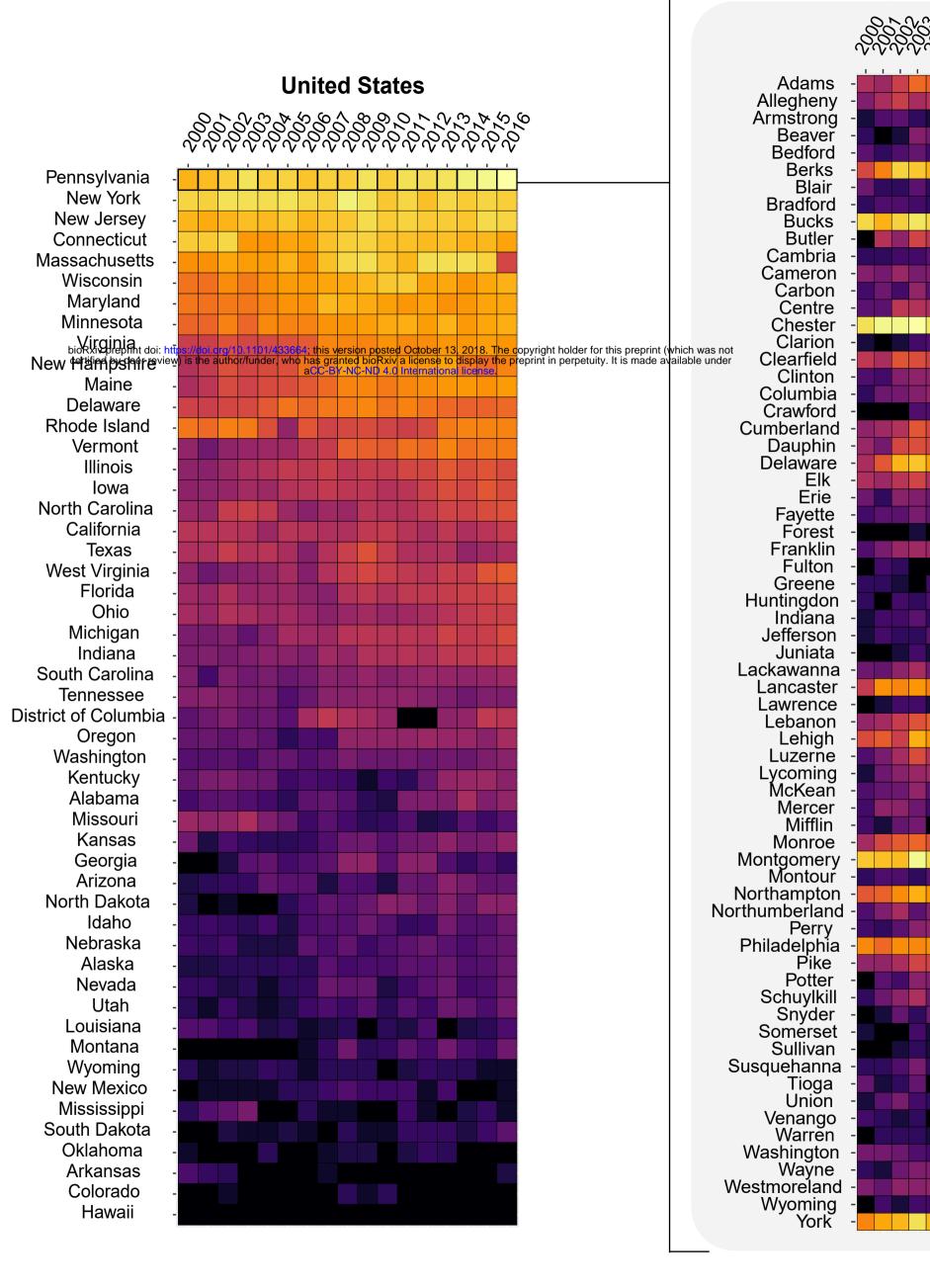
2	Figure 1: Annual reported cases of Lyme disease by state from 2006—2017 (Left) and the
3	annual reported cases of Lyme diseases by counties in Pennsylvania from 2006–-2017. Public
4	data from the Center of Infectious Disease.
5	
6	Figure 2: Total population in Pennsylvania counties in 1960, 1990, 2000, and 2010.
7	
8	Figure 3: Dot-density map of all individual tick specimens across Pennsylvania from 1900–
9	<b>2017.</b> Each point represents an individual specimen with its placement randomized within the
10	county.
11	
12	Figure 4: Incidence rates of the five most abundant tick species across Pennsylvania at
13	different time periods from 1960–2018 . On the left, is cumulative number of individuals.
14	
15	Figure 5. Presence or absence map of the tick genera excluding Ixodes scapularis, Ixodes cookei,
16	Dermacentor variabilis, Amblyomma americanum, and Rhipicephalus sanguineus.
17	
18	Figure 6: On the left is the annual sum of tick specimens (log-transformed) from 1900 to 2017.
19	On the right are the proportional contribution of the major tick species to the total tick counts
20	(1900–2017). The grey shaded area represent area where there was no tick submissions.
21	

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1	Figure 7: On the left is the total proportion of tick specimens received at different months of
2	the years from 1900 to 2017. On the right are the proportion of the five major tick species
3	received at different months of the years from 1900–2017.
4	
5	Figure 8: The monthly proportion of the tick specimens received at different months of the
6	years aggregated at different decades.
7	
8	Figure 9: The monthly proportion of <i>D. variabilis</i> specimens at the larvae, nymphal, and adult
9	stages across 1960 to 2010
10	
11	Figure 10: The monthly proportion of <i>I.scapularis</i> specimens at the larvae, nymphal, and adult
12	stages across 1990 to 2017
13	
14	Figure 11: The monthly proportion of <i>I. cookei</i> specimens at the larval, nymphal, and adult
15	stages across 1980 to 2017
16	
17	Figure 12: Visual representation of the quantitative data on ticks associated with different
18	hosts.
19	
20	Figure S1: The monthly proportion of A. americanum specimens at the larvae, nymphal, and
21	adult stages across 1990 to 2017
22	

- 1 **Figure S2:** The monthly proportion of *R. sanguineus* specimens at the larvae, nymphal, and
- 2 adult stages across 1990 to 2017
- 3
- 4 **Table 1:** The total submissions to the PSU Department of Entomology/Frost Entomological
- 5 Museum from 1900 to 2017. Generic names that have been changed since the submission date
- 6 are shown in parentheses. Specimens which were not identified to species were listed under
- 7 the genus and "sp."
- 8
- 9 **Supplementary Table S1:** Specimens submitted from outside of the state of Pennsylvania.

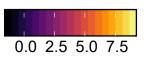
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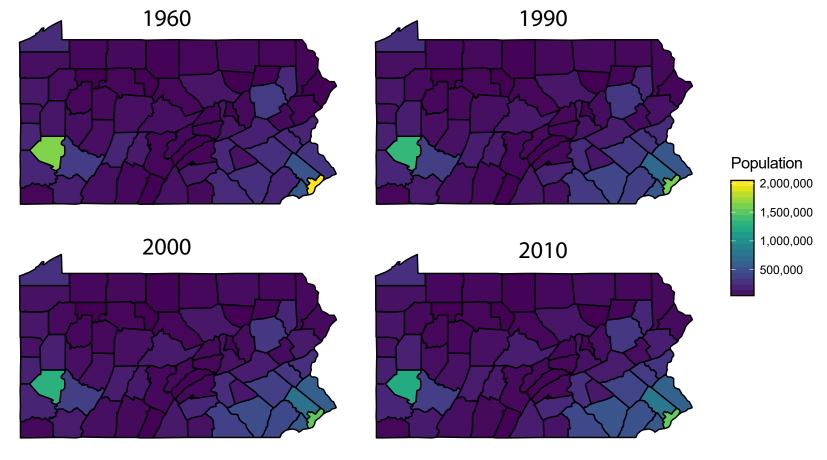


## Pennsylvania counties

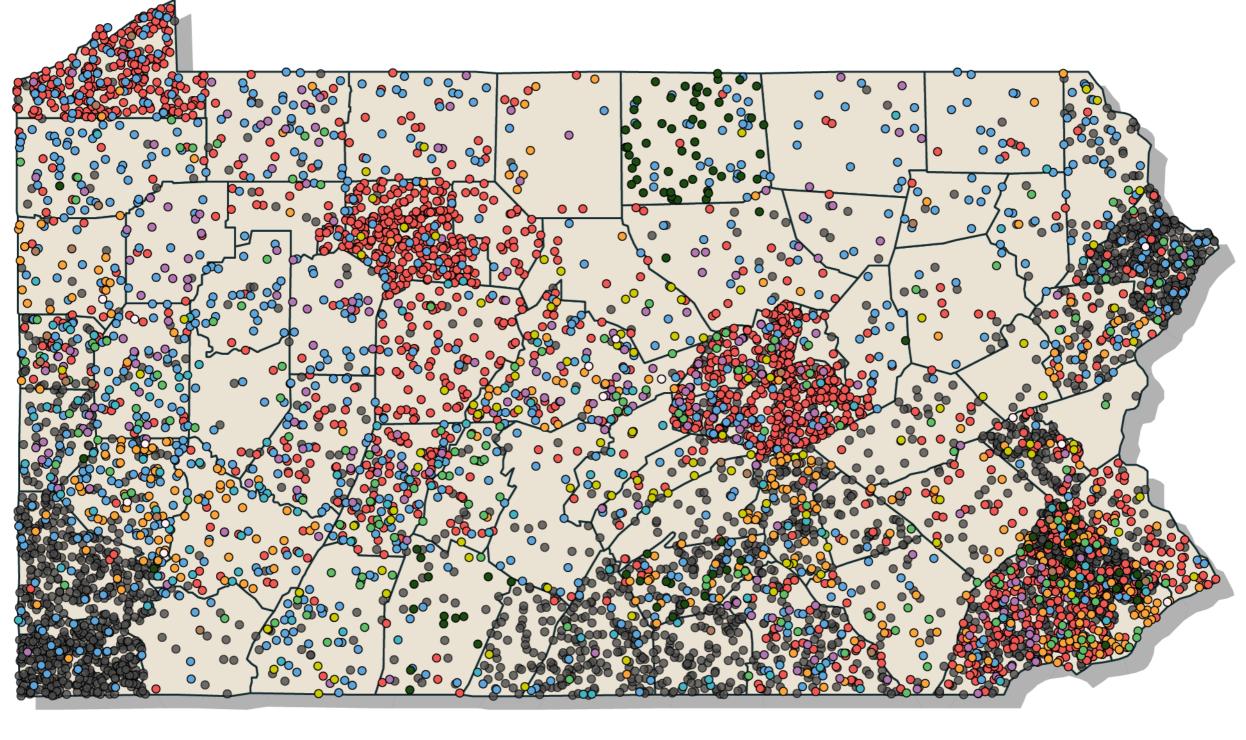
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Tick submissions across Pennsylvania (1900–2017)



Species

A.americanumD.albipictus

D.variabilis

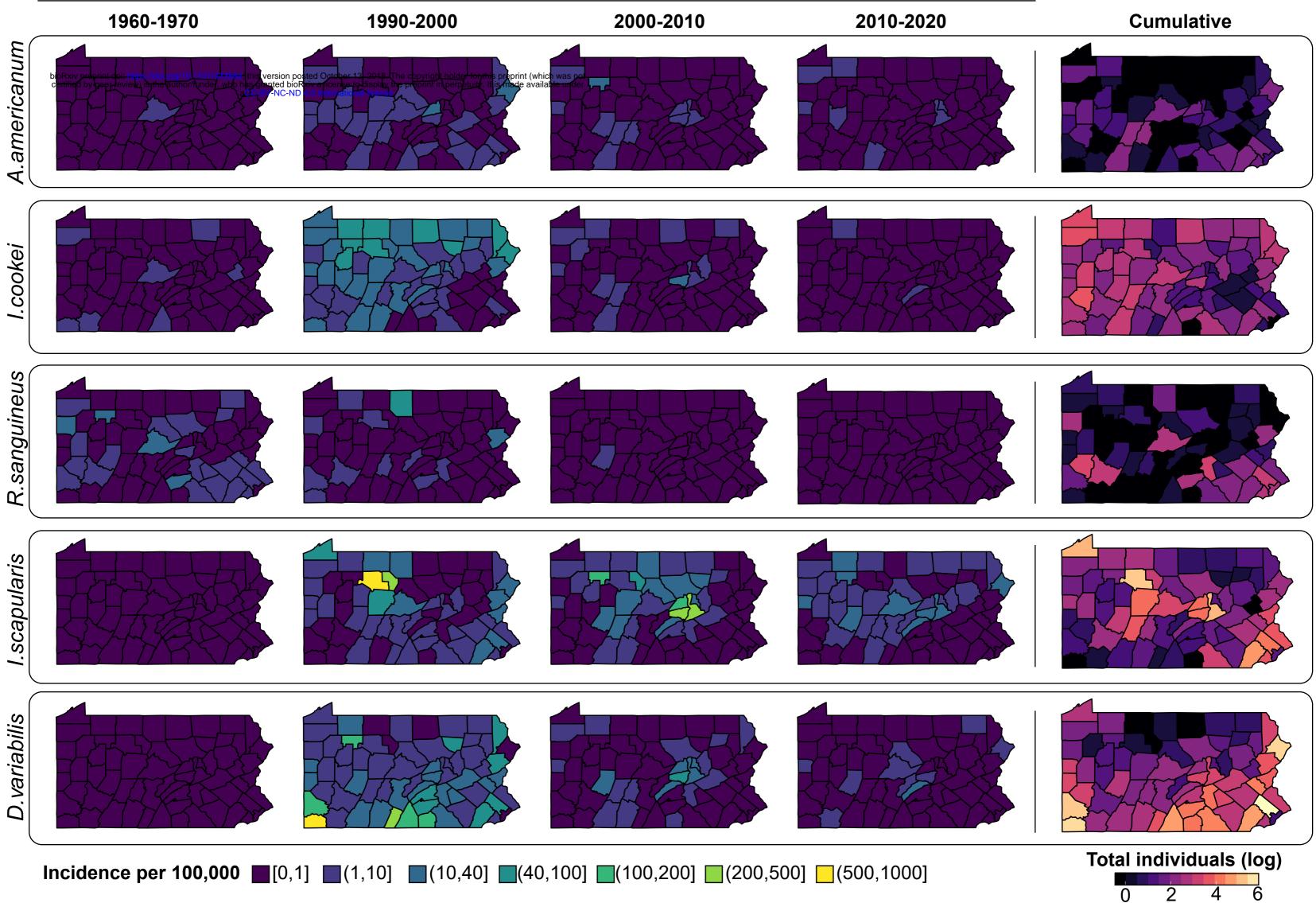
I.cookei
 I.dentatus

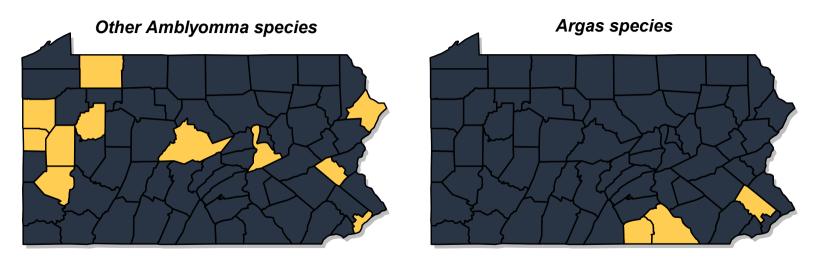
• I.scapularis

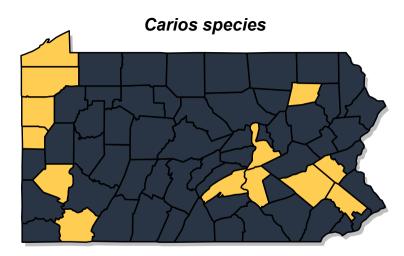
• I.texanus

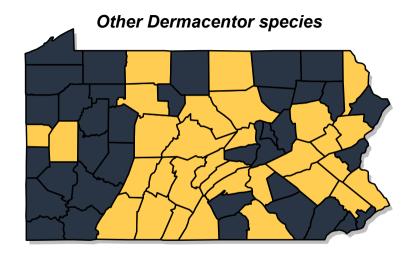
- Other Amblyomma species
- Other Dermacentor species
- Other Ixodidae species
- R.sanguineus
- Soft ticks

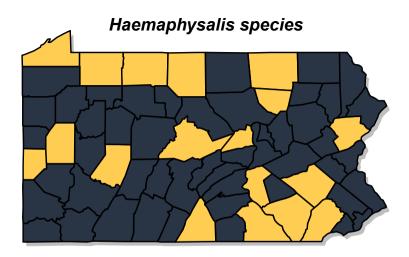
# **Incidence rates**

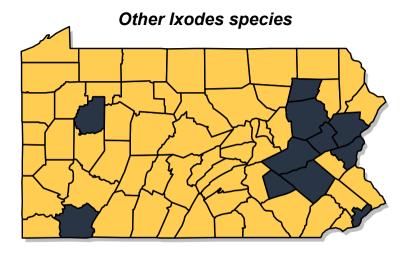




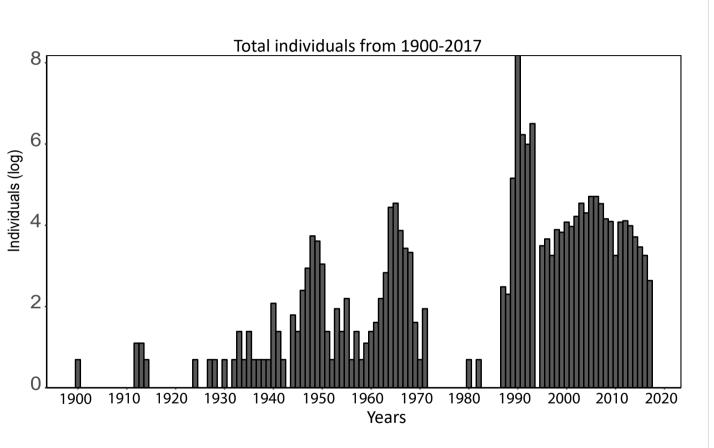


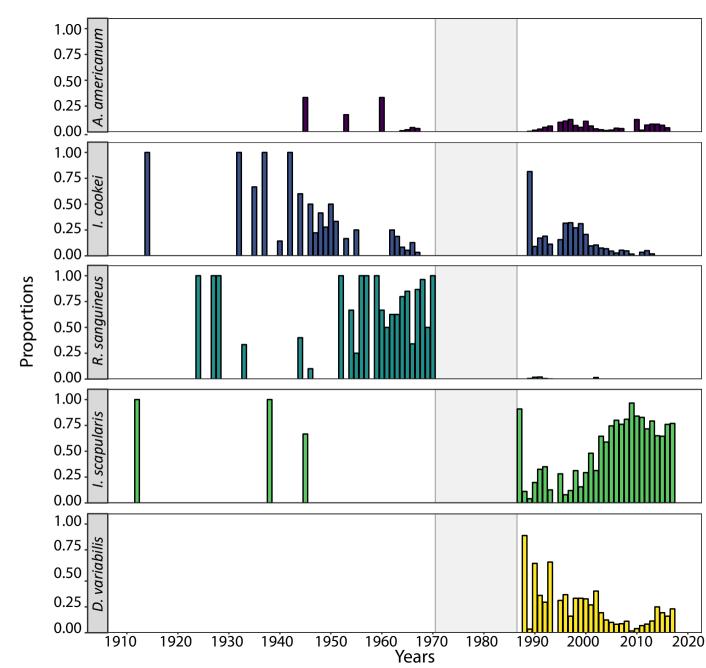


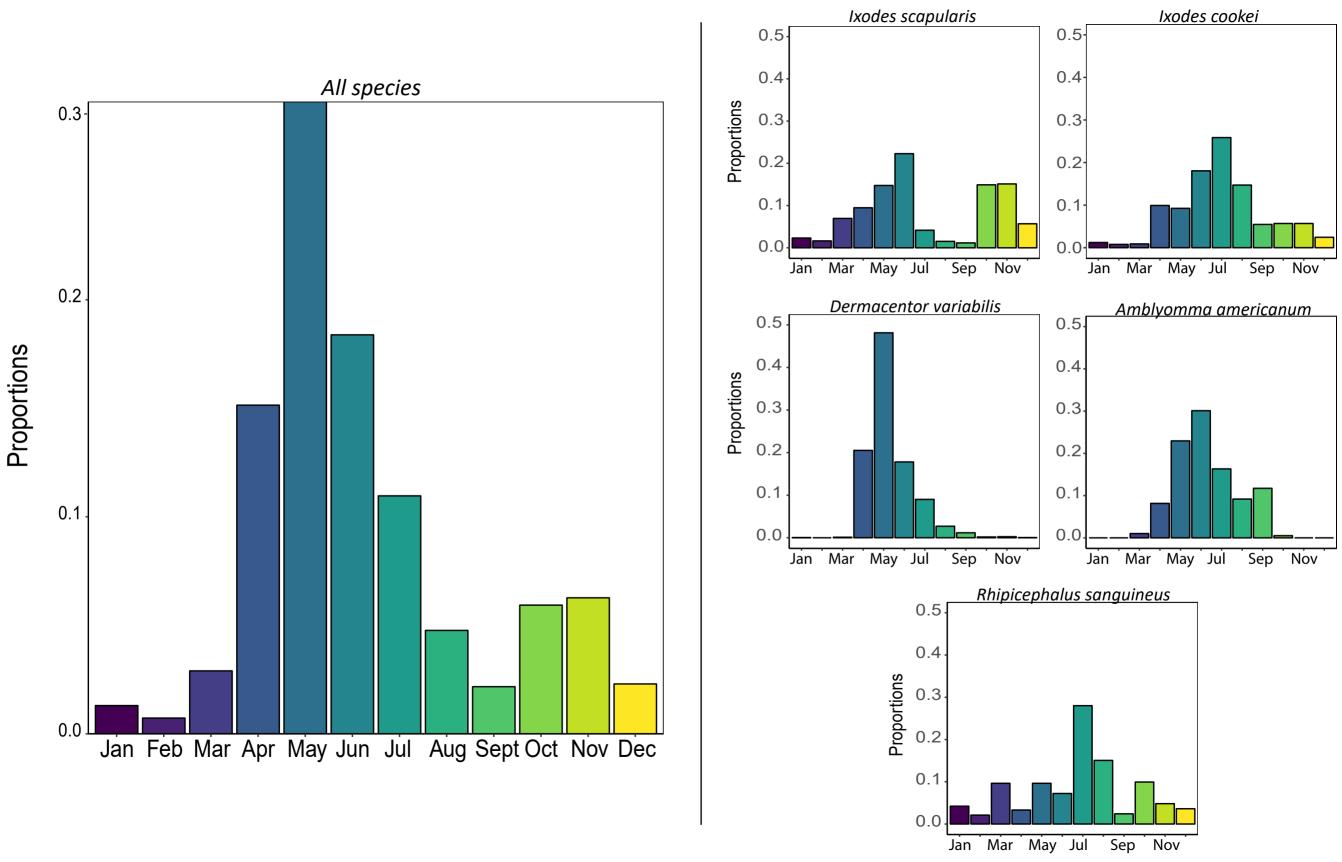




Presence No Yes





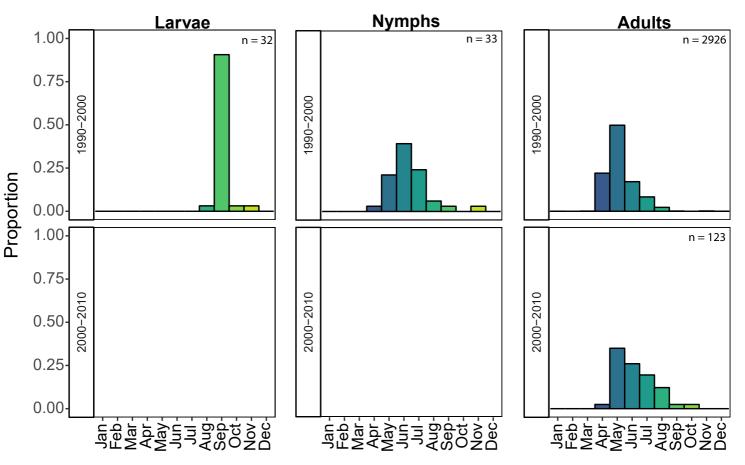


1900-1910 1920-1930 1930-1940 1910-1920 1.00-0.75-0.50-0.25-0.00-1940-1950 1970-1980 1950-1960 1960-1970 1.00-Proportions 0.75 0.50 0.25-0.00 1980-1990 1990-2000 2000-2010 2010-2020 1.00-0.75-0.50-0.25-0.00 Jan Feb Mar Apr May Jun Jul Aug Sept Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sept Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sept Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sept Oct Nov Dec

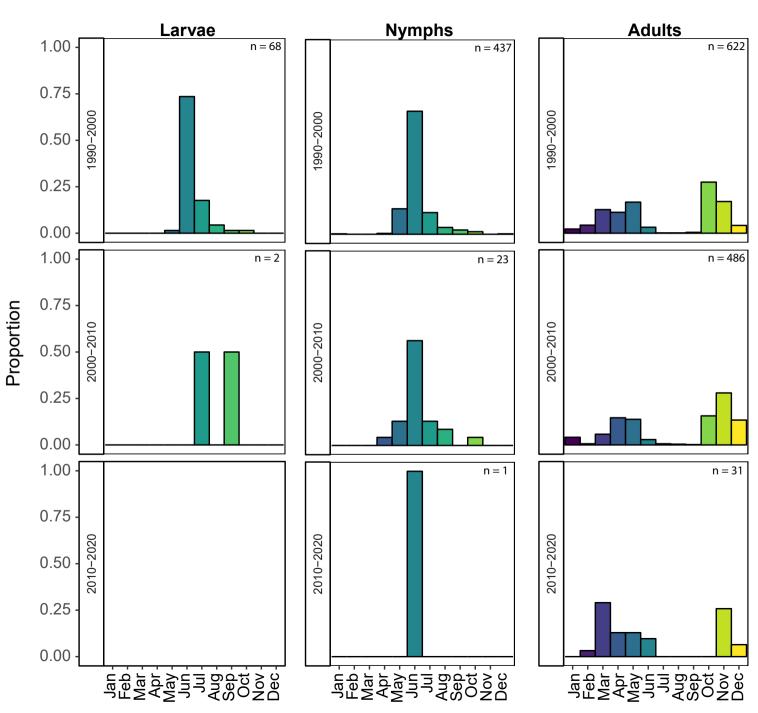
Monthly proportions of ticks submitted by decades (1900–2017)

Month

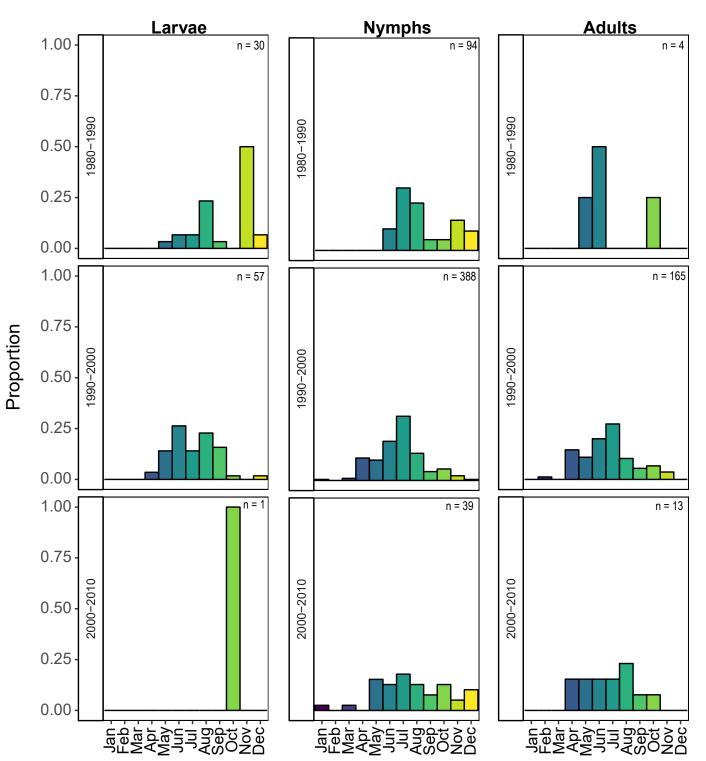
### Dermacentor variabilis

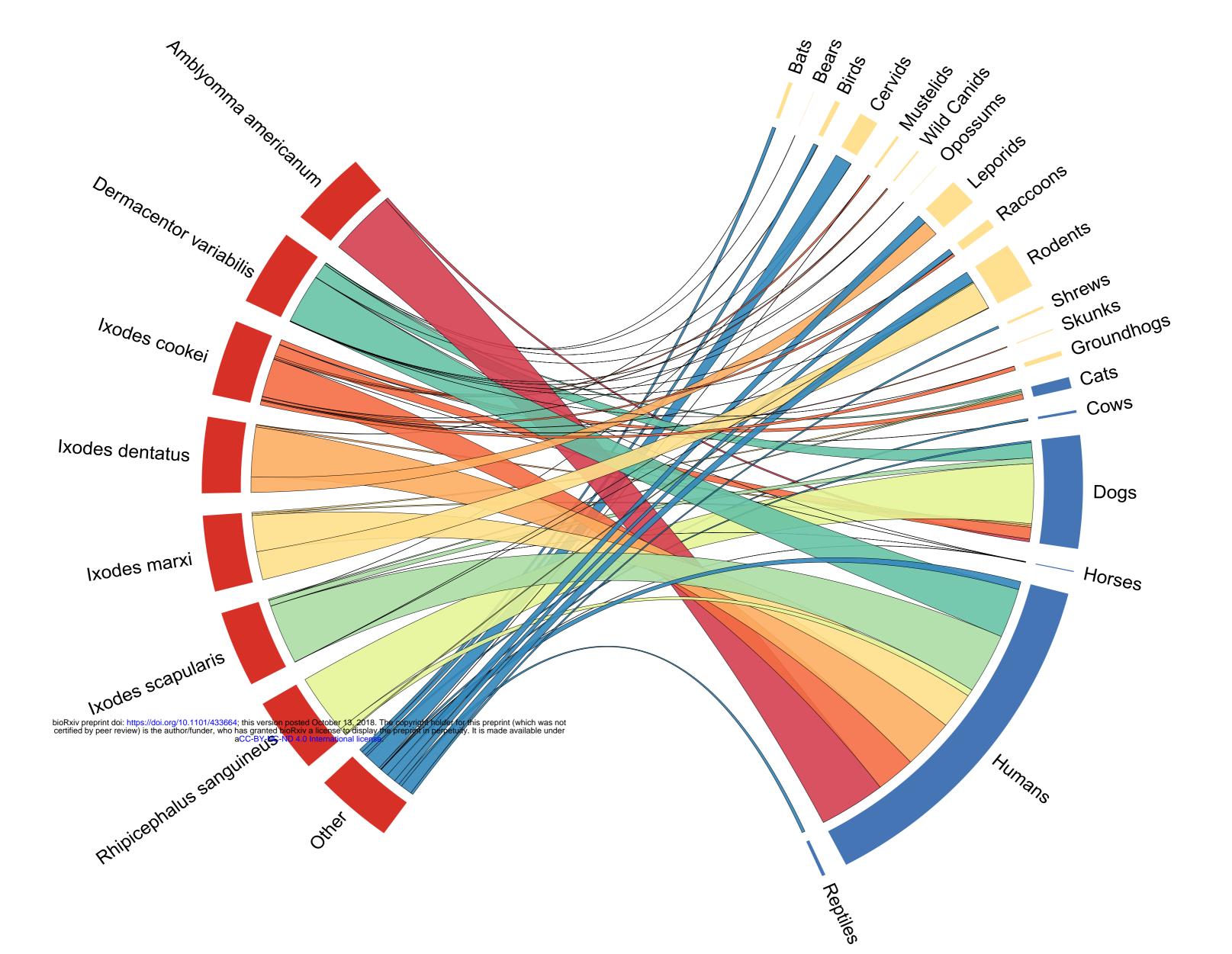


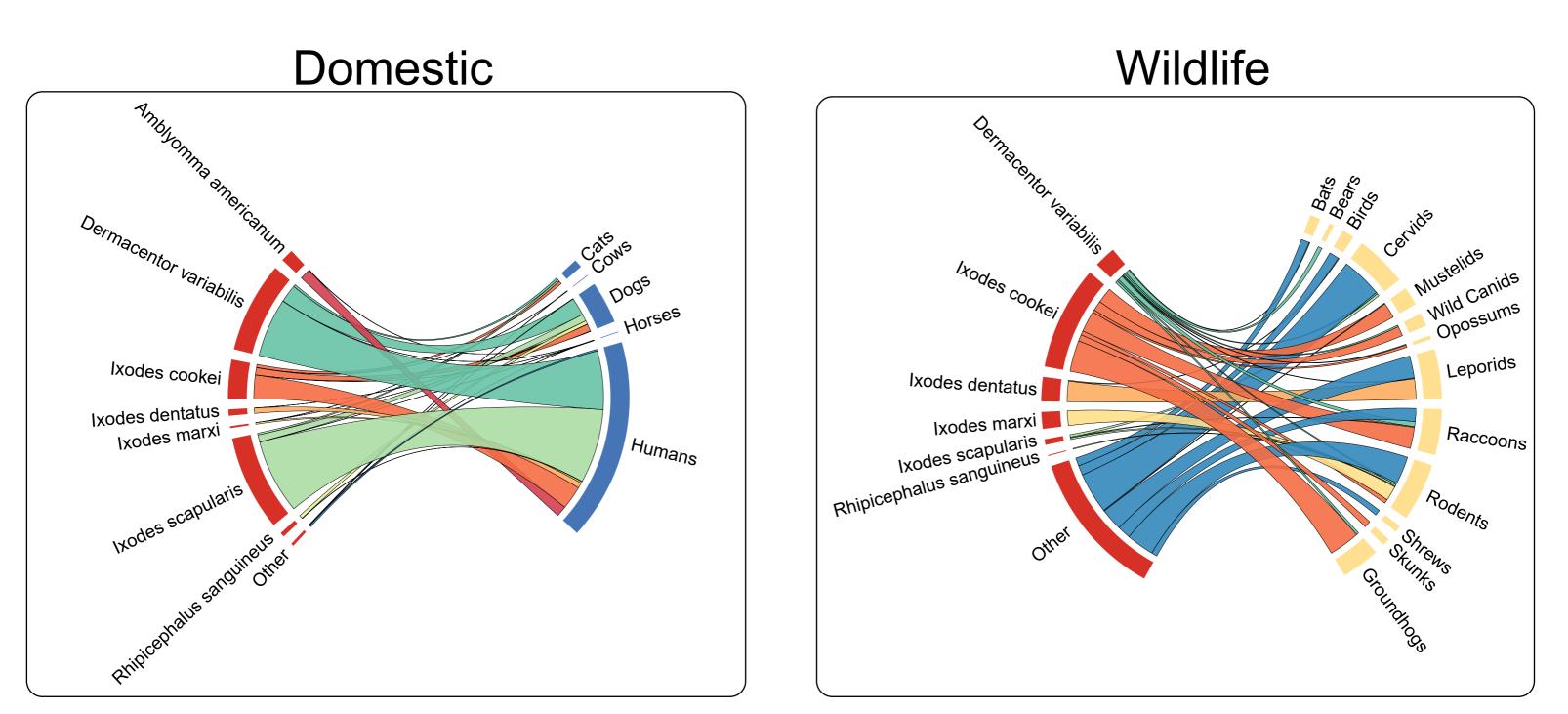
## Ixodes scapularis



## Ixodes cookei







**Table 1:** The total submissions to the PSU Department of Entomology/Frost Entomological Museum from 1900 to 2017. Generic names that have been changed since the submission date are shown in parentheses. Specimens which were not identified to species were listed under the genus.

Species	<b>#Submission lots</b>	#Specimens
Amblyomma americanum	183	196
Amblyomma cajennense	1	1
Amblyomma dissimile	8	8
Amblyomma longirostre	1	1
Amblyomma maculatum	5	5
Amblyomma (Aponomma) transversale	2	2
Amblyomma sp.	2	2
Argas cooleyi	2	2
Argas persicus	3	3
Carios (Ornithodoros) kelleyi	18	18
Dermacentor albipictus	81	107
Dermacentor andersoni	1	1
Dermacentor variabilis	1498	3172
Haemaphysalis chordeilis	1	1
Haemaphysalis leporispalustris	38	78
Ixodes affinis	1	1
Ixodes angustus	39	39
Ixodes cookei	661	897
Ixodes dentatus	115	120
Ixodes marxi	46	48
Ixodes muris	6	6
Ixodes scapularis	1395	1899
Ixodes texanus	16	111
lxodes sp.	79	80
Rhipicephalus sanguineus	287	332
Rhipicephalus sp.	1	1
Not determined	1	1
Total	4491	7132