KARST INVERTEBRATE SPECIES SURVEY PROTOCOLS AND TREND ANALYSES AT BALCONES CANYONLANDS PRESERVE

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EXECUTIVE SUMMARY

1. Biologists with the City of Austin and Travis County monitor invertebrate fauna inhabiting Karst features (caves) on the Balcones Canyonlands Preserve. Two protocols are used: one to survey cave crickets and another to survey 6 endangered and 25 species of concern. The survey for cave crickets is conducted at dusk and counts cave crickets that exit caves (cave cricket exit counts). Counts of cave crickets are used as an indication of cave conditions for endangered species and species of concern. The abundance and presence of endangered species (ES) and species of concern (SOC) are surveyed by the ES & SOC protocol. The data from both protocols will be used to estimate time trends of the targeted species.
2. I evaluated the protocols and conducted preliminary analyses to estimate time trends and assess whether survey effort can be shortened without compromising data integrity.
3. Both protocols result in standardized surveys and are conducted in such a manner that analysis of the collected data can be done with statistical methods that can adjust for errors that may be present (e.g., a false negative).
4. The cave cricket exit survey protocol can be shortened by 1 hour because most cave crickets are counted during the second hour of the 2 hour survey period.
5. Counts of crickets exiting caves were not influenced by temperature, relative humidity, and cloud cover at the start of surveys. I detected a slight, negative decline in cave cricket exit counts (sum of nymph, juvenile, and adults) and in counts of juvenile but not nymph and adult cave crickets.
6. There was sufficient data for 1 endangered species (*Texella reyesi*) and 2 species of concern (*Cicurina bandida* and *C. travisae*) to conduct an occupancy analysis to estimate detection probabilities (probability of detecting species during a survey). Detection probabilities were high (0.81 – 1.0) indicating that target species are usually detected when they occupy caves.

7. There were few seasonal patterns to the presence of most endangered species or species of concern in caves. If a species was absent it usually was during fall or winter. Conducting ES & SOC during 1 cool (fall or winter) and 1 warm (spring or fall) season is suggested so that time trends in the presence of endangered species and species of concern can be estimated.
8. There was sufficient data to estimate time trends in counts of the endangered species *T. reyesi*. Counts of *T. reyesi* were influenced by relative humidity at the start of surveys and there was a slight, negative decline in counts of *T. reyesi*.
9. Given the number of personnel trained to conduct surveys, number of caves presently surveyed, and new caves that are likely to be added to the expanding Balcones Canyonlands Preserve, conducting cave cricket exit surveys and ES & SOC surveys twice a year should be sufficient.

INTRODUCTION

Proactive conservation of endangered species and species of concern requires population monitoring. Information gathered from monitoring can then be used to estimate population changes over time (time trends), assess whether goals of a conservation program are met, and implement actions to improve the likelihood of population persistence. Fundamental to monitoring is the protocol used to collect information on population states: abundance, density, or species presence. A protocol is merely a set of instructions that dictate the collection of data so that it is standardized, has sufficient survey effort, and surveys are conducted in such a manner that analysis of the data can be done with statistical methods that can adjust for errors that may be present. Standardization in how the data is collected is fundamental to allowing comparisons to assess trends across time or space. Survey effort refers to sampling with sufficient intensity so that the data gathered reflects the actual state of the population. Because of logistical and monetary constraints, monitoring programs rarely census–enumerate the population. Instead, a subset of the targeted population is sampled. Analysis of sample data can be sufficient when steps are taken to insure that the sample is representative. A sample is likely to be unrepresentative when the number of places or amount of time to survey is insufficient to encounter enough of the inherent heterogeneity (individuals differing in age, sex, variation in spatial preference of individuals, etc.) present in the population.

A common error when species are cryptic or difficult to find is the false negative. A false negative occurs when individuals of the targeted species are not found when surveying (the negative), but the negative is false. The species was present but animals were missed because they were under rocks, in crevices, or not in the area that was surveyed at the time it was searched. If suspected errors are considered in the design of a protocol, and there are statistical methods that can account for errors, then the protocol and analysis can be planned so that the collected data can be analyzed appropriately.

Biologists with the City of Austin and Balcones Canyonlands Preserve have 2 protocols for surveying invertebrate species inhabiting Karst caves (Balcones Canyonlands Preserve Land Mangement Plan Tier II-A, Chapter IX, Karst Species Management, 2007). Surveys are conducted for cave crickets (*Ceuthophilus*), 6 endangered invertebrate species (ES), and 25 additional invertebrate species that are listed as species of concern (SOC). The ES & SOC protocol consists of searches by 2-3 surveyors in 1 – 5 zones in a cave. Zones are designated

areas that are usually 5 meters in length and span the width of the cave; however, the area of zones necessarily depends upon the topographical features of the cave. Surveyors search the zones in a cave and count all individuals of ES, SOC, and cave crickets. The time it takes to conduct the survey is recorded as is the relative humidity and temperature inside and outside the cave. Caves are surveyed 1 – 4 times a year. Counts of cave crickets that exit caves at dusk (CC exit counts) are also conducted using a separate protocol. Cave cricket exit counts are conducted to assess overall condition of the cave, the more crickets that exit the cave at dusk, presumably the 'healthier' the environmental conditions for the ES and SOC that inhabit the cave. Exit counts are done shortly after sunset and last 2 hours. At 10 min intervals (10, 20, so on, to 120 min), the number of cave crickets exiting the cave are recorded by 2 – 3 surveyors on warm (>5°C) evenings with no rain. Relative humidity, temperature, and percent cloud cover is also recorded. Exit counts are conducted 1 – 4 times a year at each cave.

The 2 protocols are designed such that they are standardized, have considerable survey effort, and the data is collected in such a way that statistical methods can be used to adjust for false negatives and influences of environmental conditions (i.e., humidity and temperature) on counts and species presence. There is, however, a need to assess survey effort. As more land with Karst features (caves) are added to the Balcones Canyonlands Preserve, monitoring of these newly added features will be needed. Yet, the crew of biologists that conduct the monitoring is not going to expand. Can survey effort be curtailed without compromising the integrity of data?

There are two possibilities for curtailing survey effort. For CC exit counts: can the 2 hour survey time be shortened to 1 hour? For ES & SOC surveys: are 2 surveys a year sufficient? If false negatives are prevalent then more surveys in a year would be warranted. Conversely, if false negatives are not prevalent then 2 surveys in a year should be adequate.

The data gathered from ES & SOC and CC exit count protocols is designed to estimate time trends. Time trends are summaries of population states over a specified period of time via a regression. The slope coefficients from the regression are interpreted to determine the trend; over a specified period of time populations increased, declined, or remained stationary. To date, a time trend analysis has not been conducted on endangered species, species of concern, or cave crickets.

There were 5 objectives to this analysis.

1. Estimate the counts of cave crickets at 10 minute intervals and determine if more cave crickets are counted in the second hour than in the first hour.
2. Estimate time trends in cave cricket exit count data.
3. Assess the number of cave cricket exit counts that should be conducted annually in caves.
4. Determine whether ES & SOC surveys should be conducted in 2 or more calendar seasons of the year.
5. Estimate time trends in the counts of the most common ES encountered during surveys, *Texella reyesi*.

METHODS

Cave crickets

Data from CC exit count surveys were obtained from the City of Austin and Travis County for 2006 – 2010 at 13 caves: Amber, Coterell, District, Flint Ridge, Spider, Gallifer, Kretschmarr, Kretschmarr Double Pit, McDonald, North Root, Root, Tardus Hole, and Tooth. Because most caves had multiple surveys conducted across multiple years I used a mixed effect model to test whether cave cricket emergence from caves differed across the 10 minute intervals within the 2 hr survey (Pinheiro and Bates 2000). The response variable was count at a 10 minute interval divided by the sum of counts across all 10 minute intervals. The cave was the random effect and the fixed effects were each 10 minute interval (10, 20, 30, to 120 min), temperature at the start of the survey, relative humidity at the start of the survey, and percent cloud cover at the start of the survey. To assess the variability in counts among caves I calculated the intraclass correlation coefficient (Pinheiro and Bates 2000). The intraclass correlation coefficient ranges from 0 to 1.0; the higher the coefficient the greater the variability among caves than within caves.

I also used mixed effect models to analyze time trends in cave cricket exit counts (sum of counts at 10 minute intervals). The random factor was the cave and fixed effects were time, temperature at the start of the survey, relative humidity at the start of the survey, and percent cloud cover at the start of the survey. Time was coded to include year and month that the survey was conducted. For example, a survey conducted in September, 2007, was coded 7.75 (.75-9th month of the year divided by number of months in a year [12]). For the time trend analysis I analyzed 2 mixed effect models, an intercept model and an intercept and slope model. I selected

1 of these 2 models based on computational instability (Cheng et al. 2010). Computational instability is when some of the parameters in a mixed effect model are not estimable. A problem that occurs when the model does not match the patterns in the data or there is insufficient data to estimate all the parameters in the model (Cheng et al. 2010). I considered these 2 mixed effect models because of how time trends are likely to vary across caves. Across caves the counts probably differ, at least in the years when the first surveys were conducted. Counts in the first years of surveys are estimated by the intercept coefficient of a regression (Fig. 1). Change in counts across the time surveys were conducted (trend) was estimated by the slope coefficient (Fig. 1). If initial abundance varies across caves but trends do not, then the intercept model should fit the data. If initial abundance and trend varies across caves then the intercept and slope model should fit the data.

In 3 caves, Kretschmarr, Kretschmarr Double Pit, and McDonald, the exit counts may not reflect cave cricket abundance. These caves have multiple openings and crickets may emerge from openings that are not surveyed. Consequently, we estimated time trends from the cave crickets counted in the cave during ES & SOC surveys conducted from 2007 to 2010. The response variable was counts/number of surveyors/minutes surveyed to adjust for changes across surveys in time to search and number of surveyors. Because there were only 3 caves, time trends were analyzed using the intercept model with fixed effects of time, relative humidity as the start of surveys, temperature at the start of surveys, and cave as the random factor.

Cave crickets during exit counts were also aged as nymphs, juveniles, and adults. Time trends in age classes of cave crickets may reveal changes in productivity that influences population dynamics. The time trend analyses of each age class of cave crickets was conducted identically to the time trend analysis of cave cricket exit counts.

ES & SOC

Since counts of ES and SOC were sparse there is concern that the ES & SOC survey protocol may not result in detecting species that are actually present: a false negative. To assess this possibility I conducted an occupancy analysis (MacKenzie et al. 2006). Occupancy models estimate the probability of detection, the probability that the species was detected when it actually occupied a cave. A detection probability ranges from 0, the species was present in the cave but it was detected in any survey, to 1; the species was detected in all of the surveys. We used data from surveys conducted in 2009. In 2009, more caves (25) were surveyed in more

seasons than in any other year. These caves were Airman, Amber, Arrow, Cotterell, District, Flint Ridge, Gallifer, Geode, Goat, Jest John, Jester Estates, Kretschmarr, Kretschmarr Double Pit, Little Bee, Maple Run, McDonald, New Comanche, North Root, Pond Party, Root, Seibert, Slaughter, Spider, Tartus, and Tooth. The count data was coded as 0 when no individuals of a species were detected and 1, at least 1 animal of a species was counted in the cave. There was sufficient data (>9 detections) to conduct an occupancy analysis for 3 species: *Texella reyesi* (TR, an endangered species) and 2 species of concern (*Cicurina bandida* [CB] and *C. travisae* [CT]). I analyzed 2 occupancy models, a pooled model and a seasonal model. The pooled model estimated probability of detection for survey data pooled across seasons. The seasonal model estimated probability of detection for fall and winter survey data and spring and summer survey data. Comparing these 2 models allowed me to assess if detection probabilities varied across seasons. If so, then surveys should be conducted in seasons with higher detection probabilities. Both models also estimated occupancy, the proportion of caves that were occupied by a species. I calculated Akaike weights for both models to assist in selecting the pooled or seasonal model (Pinheiro and Bates 2000). Akaike weights range between 0 and 1. The higher the Akaike weight, the more likely it is that the model summarizes the data relative to the other model. When the two models had similar Akaike weights, I adhered to parsimony and selected the model with fewer parameters, the pooled model.

A time trend for the endangered species, *T. reyesi*, was estimated. There were sufficient data collected from 2004 – 2010 in 3 caves: Coterell, McDonald, and Tooth. Because there were insufficient numbers of caves to estimate parameters for an intercept and slope model, I analyzed the intercept model. The random factor was cave, and fixed effects were time and relative humidity and temperature at the start of ES & SOC surveys. To account for changes in survey effort across caves from differing number of surveyors and minutes surveyed, the response variable was count/number of surveyors/minutes surveyed.

RESULTS

Cave crickets

Relative humidity (t = -0.30, P = 0.766), temperature (t = 0.62, P = 0.537), and cloud cover (t = 1.53, P = 0.127) at the start of the CC exit surveys had little detectable influence on counts at 10 minute intervals. With the exception of the first 10 minute interval (t = 0.14, P =

0.886), the percentage of cave crickets counted at each 10 minute interval was greater than 0 (t >3.16, $P < 0.002$). The counts progressively increased until 80 minutes (Fig. 2). Thereafter counts declined slightly but were markedly higher than 10 minute intervals in the first hour of surveys (10 – 60). The intraclass correlation coefficient was 0.32, indicating that when cave crickets emerged did not differ substantially across caves.

To summarize time trends of exiting cave crickets, the intercept and slope model was computationally unstable, therefore I chose the intercept model. All parameters of the intercept model were estimable. Because of computational instability of the intercept and slope model, the slopes of time trend regressions do not change substantially across caves. A slope estimated from data of all caves is, therefore, good enough for each cave regression. Counts of exiting cave crickets were not influenced by relative humidity ($t = 0.71$, $P = 0.482$), temperature ($t = 0.99$, $P = 0.327$), and cloud cover ($t = 1.34$, $P = 0.185$) at the start of surveys. There was a slight, decreasing time trend in exiting cave cricket counts (slope = -32.07, $t = -1.79$, $P = 0.076$). Across caves there were considerable differences in intercepts (Fig. 3) because the intraclass correlation coefficient was 0.57.

For the 3 caves where exit counts of cave crickets were questioned, Kretschmarr, Kretschmarr double pit, and McDonald, counts (adjusted for number of surveyors and minutes surveyed) conducted during the ES & SOC surveys showed a decreasing trend (slope = -0.12, $t = -2.82$, $P = 0.012$) and neither temperature ($t = -0.08$, $P = 0.936$) nor relative humidity ($t = 1.31$, $P = 0.206$) influenced time trends. There was virtually no variability among caves in the intercepts of time trends (intraclass correlation coefficient < 0.001). Therefore, the time trends averaged across caves reflects the time trends in each cave ($\hat{Y} = 0.31 – 0.12X$).

Time trends from exit counts in nymph, juvenile, and adult cave crickets were summarized using the intercept model because the intercept and slope models for each age class displayed computational instability. A time trend was not detected for nymphs ($t = -1.51$, $P = 0.134$) and adults ($t = -0.45$, $P = 0.631$). Juvenile cave crickets displayed a weak declining time trend ($t = -1.87$, $P = 0.065$). Counts of juveniles were positively influenced by the percent cloud cover ($t = 2.36$, $P = 0.020$), but there was no influence from temperature ($t = 0.79$, $P = 0.434$) and relative humidity ($t = 0.44$, $P = 0.659$) at the start of surveys. Across caves the differences in intercepts was not great (Fig. 4) as the intraclass correlation coefficient was 0.22.

ES & SOC

The ES & SOC survey protocol appeared to detect CB, CT, and TR species when they were present in caves. The seasonal occupancy models for all three species were not selected (Akaike weights < 0.56). Detection probabilities ranged from 1.0 for CR to 0.81 for TR (Fig. 5). Occupancy of caves ranged from 0.32 for CT to 0.5 for TR. These 3 species are not widely distributed across the 25 caves surveyed, yet when they were present in a cave the chances were high that they were found during surveys.

To facilitate decision making about seasons to conduct ES & SOC surveys, I generated bar charts of the seasons when species were found. There was data for 5 endangered species and 8 species of concern. Because the occupancy analysis suggested high detection probabilities, using the information from the count data should provide useful summaries on seasons when ≥ 1 individual of a species were found. For endangered species, seasonal patterns in presence were not substantial for any ES (Fig. 6). *Texella reddelli* was not found in winter or fall and *Texella texana* was not found in fall. For the species of concern (Fig. 7), there also were not strong seasonal patterns in when they were found.

There was a declining time trend in counts of *T. reyesi* (Fig. 8). The slope coefficient was -0.013 and the coefficient was less than 0 (t = -2.1, P = 0.047). Counts of *T. reyesi* were not related to temperature at the start of surveys (t = -0.1, P = 0.974), but greater relative humidity at the start of surveys had lower counts (t = -3.1, P = 0.007).

DISCUSSION

There were 5 objectives of this analysis:

1. Estimate the counts of cave crickets at 10 minute intervals and determine if more cave crickets are counted in the second hour than in the first hour.
2. Estimate time trends in cave cricket exit count data.
3. Assess the number of cave cricket exit counts that should be conducted annually in caves.
4. Determine whether ES & SOC surveys should be conducted in two or more calendar seasons of the year.
5. Estimate time trends in the counts of the most common ES encountered during surveys, *Texella reyesi*.

For objective 1: there were considerably more cave crickets counted during the second hour than in the first hour of exit counts. A consistent pattern across the 13 caves included in this analysis. Therefore, shortening the cave cricket exit count surveys to 1 hour (beginning 1 hour after sunset) is acceptable.

For objective 2: time trends in CC exit counts (sum of age class counts) and juvenile counts suggested a weak, declining time trend. A weak, declining time trend in total counts and juveniles are suggestions of deteriorating conditions in caves for its inhabitants, a cause for concern. The declining time trend from the exit counts was also detected from cave cricket counts in the interior of 3 caves. Yet, a call for actions to improve cave conditions may be premature for 2 reasons. One reason has to do with a limited number of data points. In a mixed effect model analysis the data set is the number of random units (caves) and the number of data points in each cave (repeated surveys across years). The number of repeated surveys across years is still rather limited (2006 to the present). Hence, the weak, declining trend may be an artifact of the limited data set. My second reason is; what do cave cricket exit counts measure about condition of caves for their inhabitants? If abundant cave cricket counts indicate productive ecosystems for ES and SOC that inhabit caves, then there should be positive correlations between cave cricket and ES and SOC trends. After more surveys are conducted in the future, it would be useful to estimate whether trends in cave cricket exit counts are correlated with trends in endangered species and species of concern.

For objective 3: I recommend that 2 CC exit counts be conducted annually at caves. Variability from count to count within a cave can be considerable; hence conducting 2 counts in a cave each year is warranted.

For objective 4: the high probability of detections estimated in this analysis suggests that 2 ES & SOC surveys a year is sufficient. Because ES and SOC are 'spotty' in distribution across caves monitored by City of Austin and Travis County, the use of presence-nonpresence data instead of counts is suggested. Obtaining reliable estimates of time trends when counts are small and the species is found in few (<50%) caves is problematic. If 2 surveys a year are conducted in 25 caves then time trends in the presence-nonpresence data can be estimated using multi-seasonal occupancy models (MacKenzie et al. 2006). If 2 surveys are conducted a year in 25 caves there should be sufficient data to estimate all parameters that are needed to have confidence that the trends detected actually reflect the time trends of the species.

The detection probabilities estimated for the 3 species in this study are markedly higher than the detection probabilities reported for 3 endangered karst invertebrates by Krejca and Weckerly (2008). Those detection probabilities ranged from 0.06 to 0.38. The disparities in detection probabilities between Krejca and Weckerly (2008) and this analysis may be due to species, caves, and units of analyses. The study by Krejca and Weckerly (2008) occurred in Bexar County with a different set of species and the unit of analysis differed. In this analysis the unit of analysis was the cave and in the other study the unit of analysis was the zone in a cave.

The 2 seasons chosen to conduct ES & SOC surveys needs to be carefully considered. Conducting surveys in fall and winter is not recommended because some endangered species and species of concern were not found in one or both of these seasons. Based on the seasonal patterns of presence of endangered species and species of concern, 1 cool season (fall or winter) and 1 warm season (spring or summer) should be selected to conduct ES & SOC surveys.

For objective 5: the endangered species *Texella reyesi* had a declining time trend. The declining time trend of *T reyesi* is similar to the declining time trend detected for exiting cave crickets.

In summary, the 2 protocols used by the City of Austin and Travis County appear to be designed well. The protocols are standardized, survey efforts are adequate, and the data is collected in such a manner that errors that are likely to be present can be accounted for when estimating time trends. Data collections using these 2 protocols, however, have not been implemented for long (mostly 2006 onward). Consequently, it is still too soon to estimate, with confidence, time trends for cave crickets, endangered species, and species of concern. A simulation study conducted by Ogutu and Piephu (2002), with a similar number of random units (herein caves) and 10 surveys conducted in each random unit, indicated that the true time trends were detected at least 95 percent of the time. If the City of Austin and Travis County continue to collect data using these protocols, and the adjustments outlined in this report, there should be >10 surveys in every cave presently surveyed in 2 more years.

ACKNOWLEDGMENTS

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FIGURE LEGEND

Fig. 1. An example of the time trend estimated in Root cave. The time trend is a linear regression. There are 2 coefficients, intercept, which reflects the counts of the initial surveys, and slope, the change of time in counts. Time was coded by the year and month surveys were conducted: August 2006 (6.67 [8/12]), March 2007 (7.25), August 2007 (7.67), October 2007 (7.83), February 2008 (8.17), August 2008 (8.67), and September 2009 (9.75). The count was the sum of cave crickets tallied at 10 minute intervals throughout the 2 hour survey period.

Fig. 2. Line chart of means and 95% confidence intervals of the percent of cave crickets that emerged from 13 caves at 10 minute intervals during a 2 hour cave cricket exit survey.

Fig. 3. Time trend regressions in counts of exiting cave crickets at 13 caves plus the time trend averaged across the 13 caves (thick line).

Fig. 4. Time trend regressions in counts of exiting juvenile cave crickets at 13 calves plus the time trend averaged across the 13 caves (thick line).

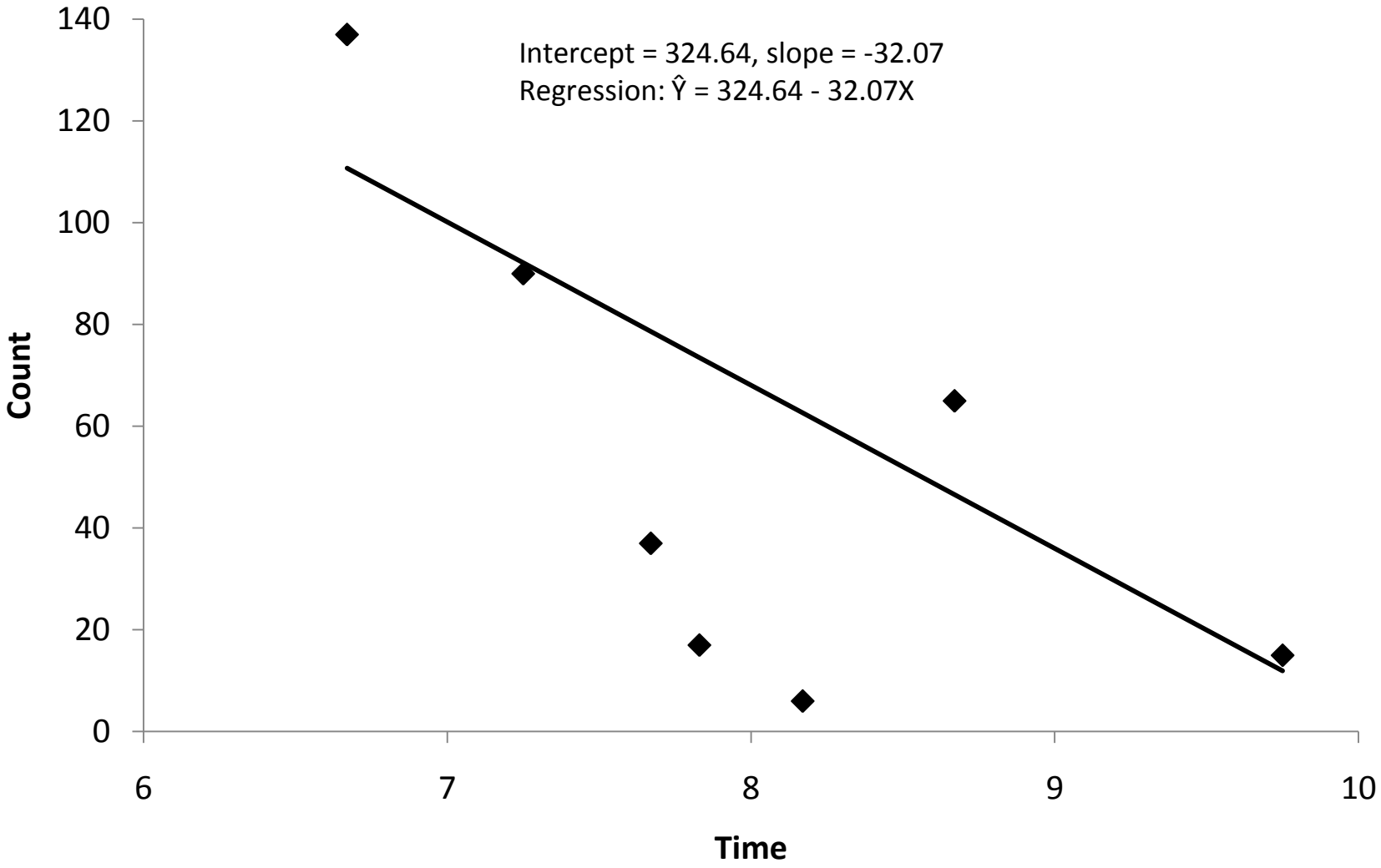
Fig. 5. Bar charts of means with 95% confidence interval error bars for probabilities of detection and occupancy for 2 species of concern (*C. bandida*, *C. travisae*), and an endangered species (*T. reyesi*). There is no error bar for *C. travisae* detection probability because the detection probability is 1.0, the highest possible value.

Fig. 6. Bar charts of the proportion of surveys a species was found during ES & SOC surveys in the 4 calendar seasons (winter-win, spring-spr, summer-sum, fall) for 5 endangered species.

Fig. 7. Bar charts of the proportion of surveys a species was found during ES & SOC surveys in the 4 calendar seasons for 8 species of concern.

Fig. 8. Time trend regressions of counts of an endangered species (*Texella reyesi*) adjusted for number of surveyors and minutes surveyed in 3 caves plus the time trend averaged across the 3 caves (thick line).

Fig. 1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Regression: Ŷ = 324.64 - 32.07X
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Time
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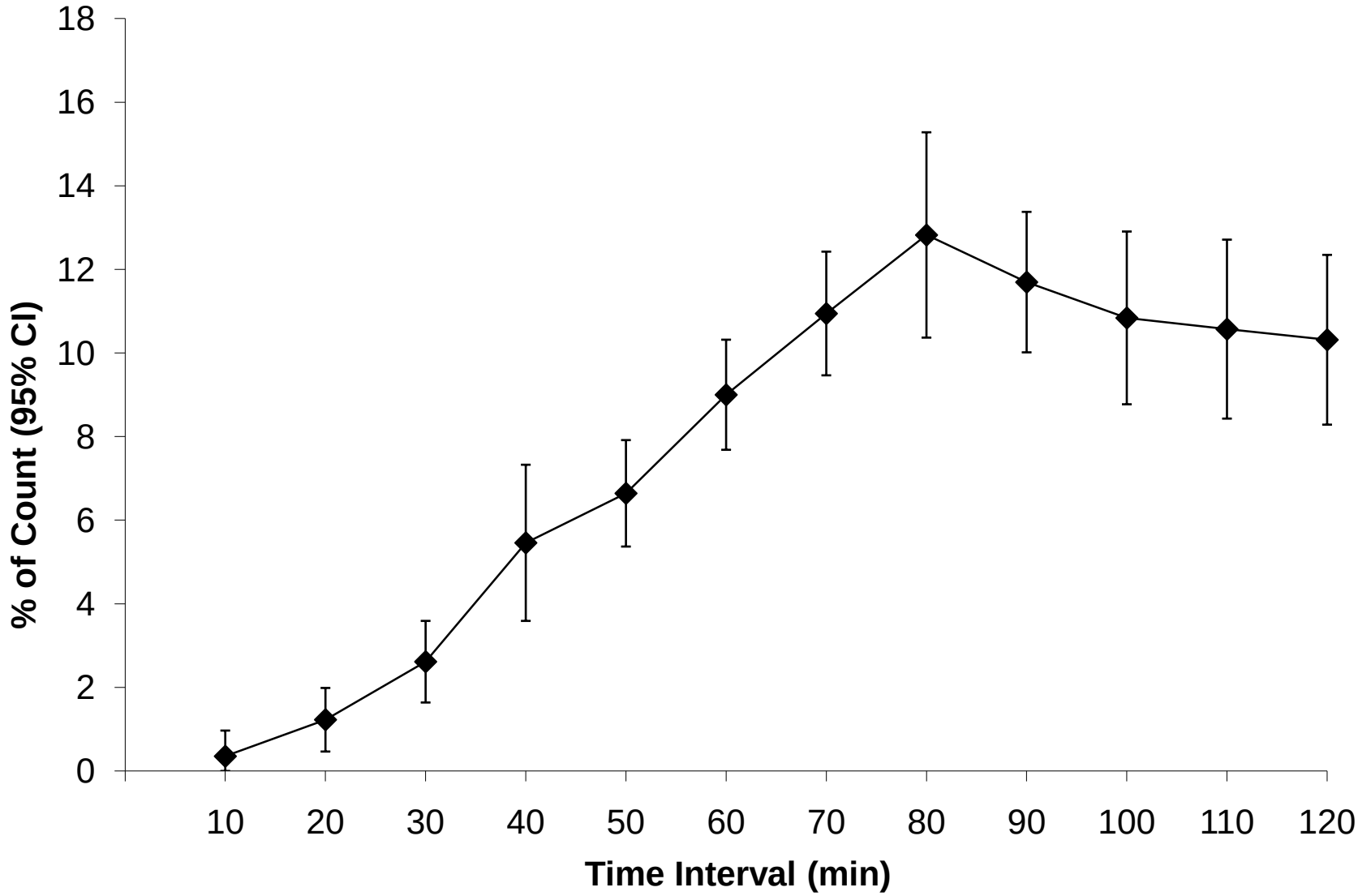
120
100
80
60
40
20
0
6
7
8
9
10

Fig. 2


18
16
14
12
10
8
6
4
2
0
% of Count (95% CI)
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20
30
40
50
60
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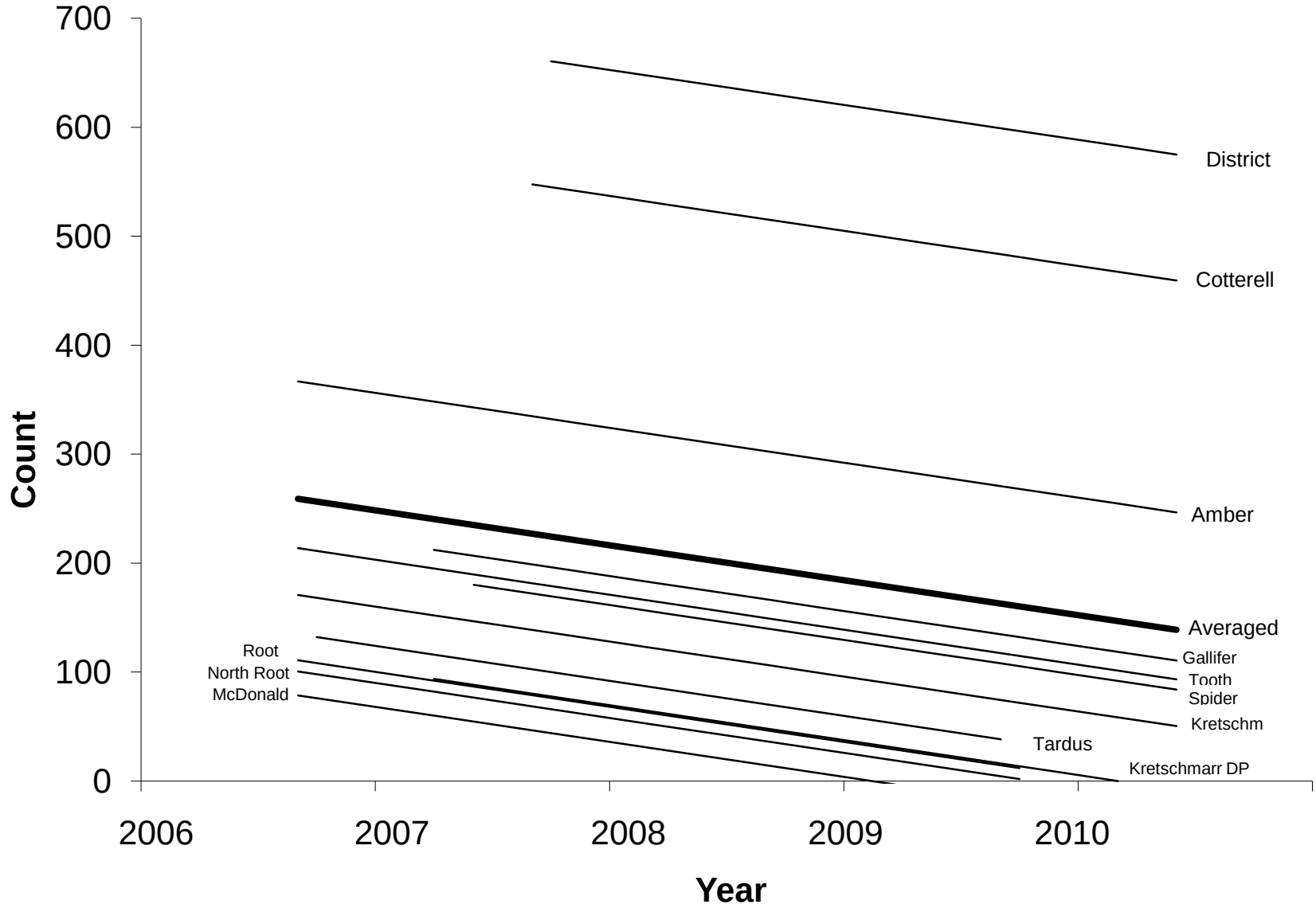
100
110
120
Time Interval (min)

Fig. 3.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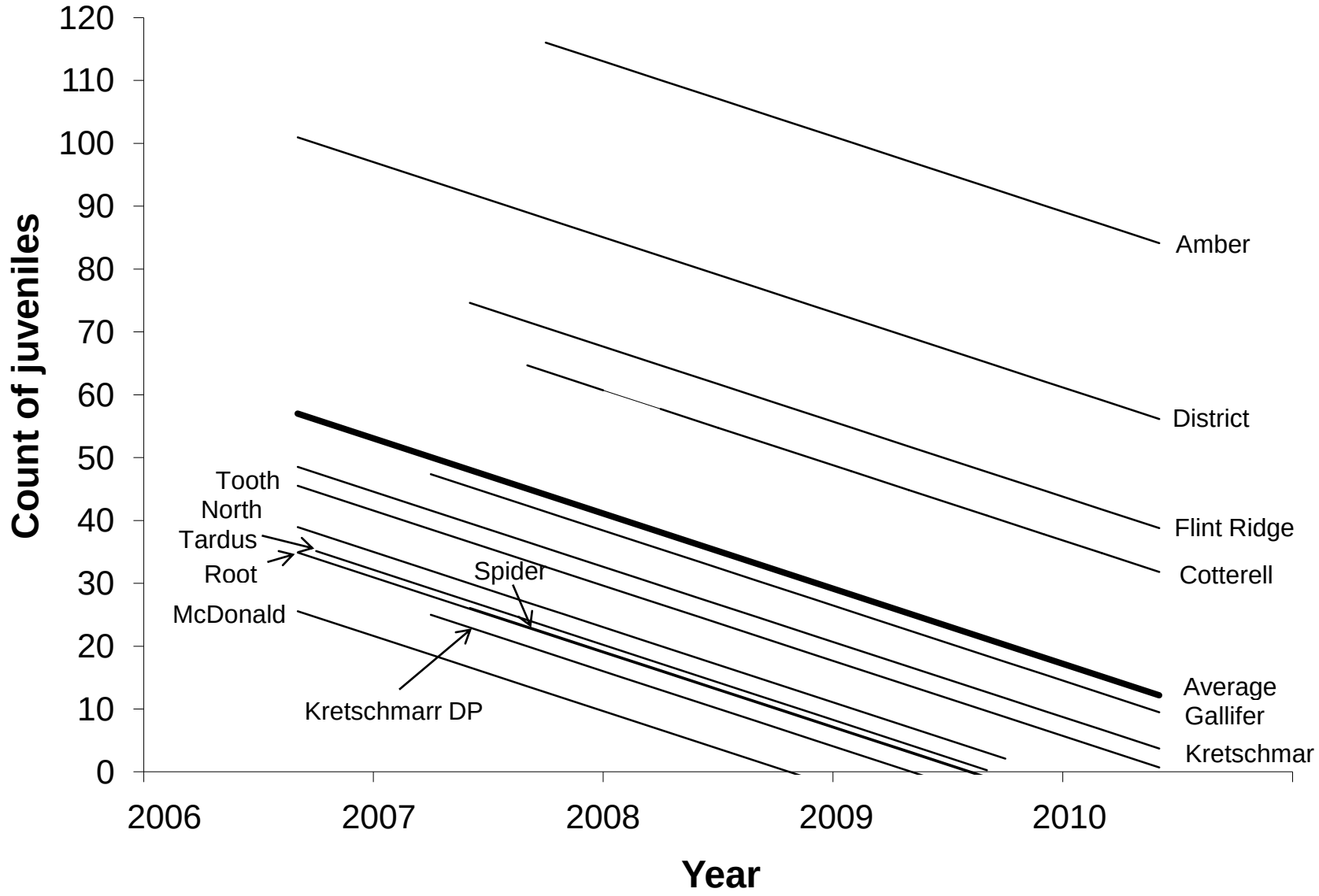
600
500
400
300
200
100
0
Count
2006
2007
2008
2009
2010
Year
District
Cotterell
Amber
Averaged
Gallifer
Tooth
Spider
Kretschm
Tardus
Kretschmarr DP
Root
North Root
McDonald

Fig. 4


Count of juveniles
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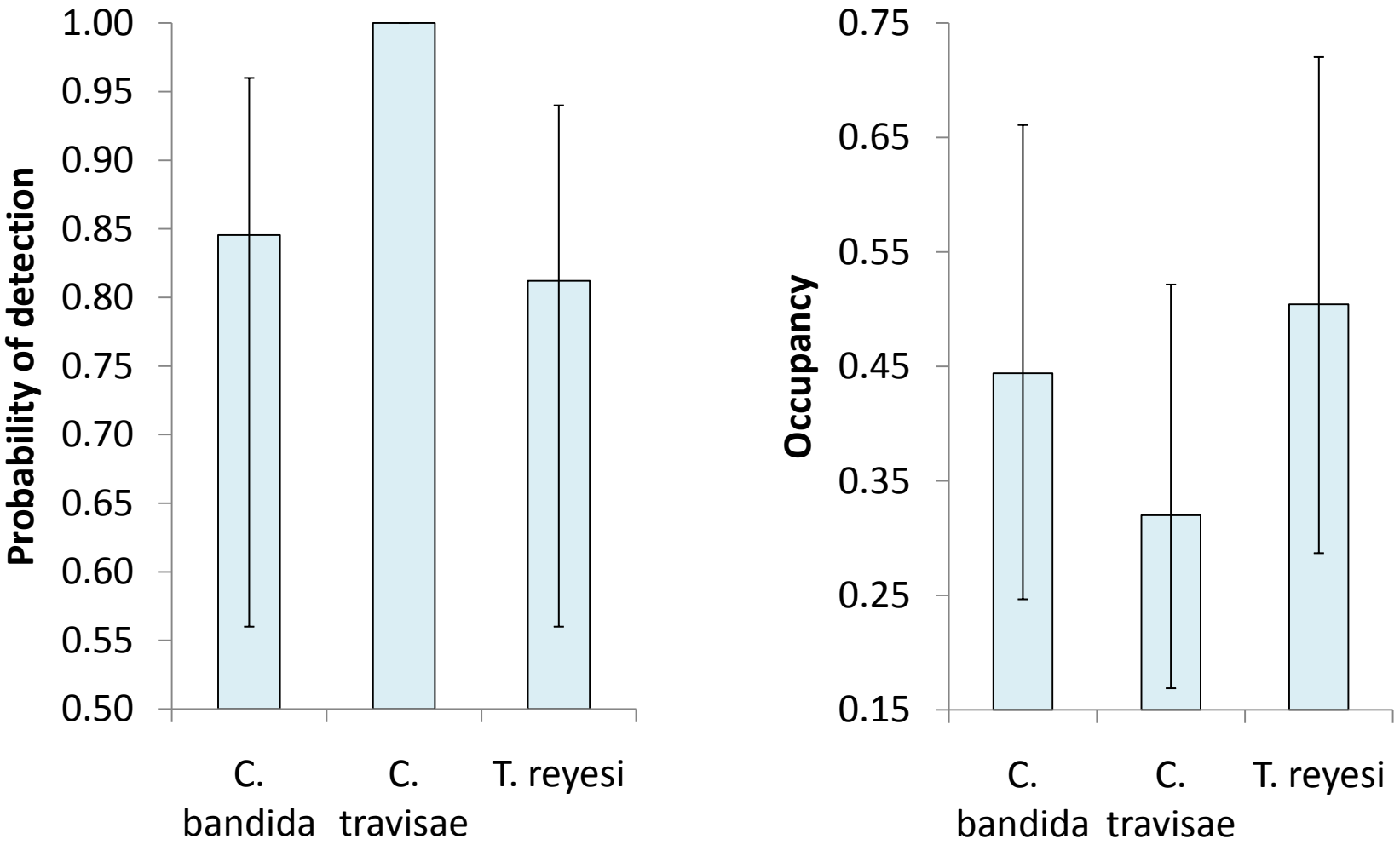
110
100
90
80
70
60
50
40
30
20
10
0
2006
2007
2008
2009
2010
Year
Amber
District
Flint Ridge
Cotterell
Average Gallifer
Kretschmar
Tooth
North
Tardus
Root
McDonald
Spider
Kretschmarr DP

Fig. 5.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0.80
0.75
0.70
0.65
0.60
0.55
0.50
C. bandida
C. travisae
T. reyesi
Occupancy
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0.55
0.45
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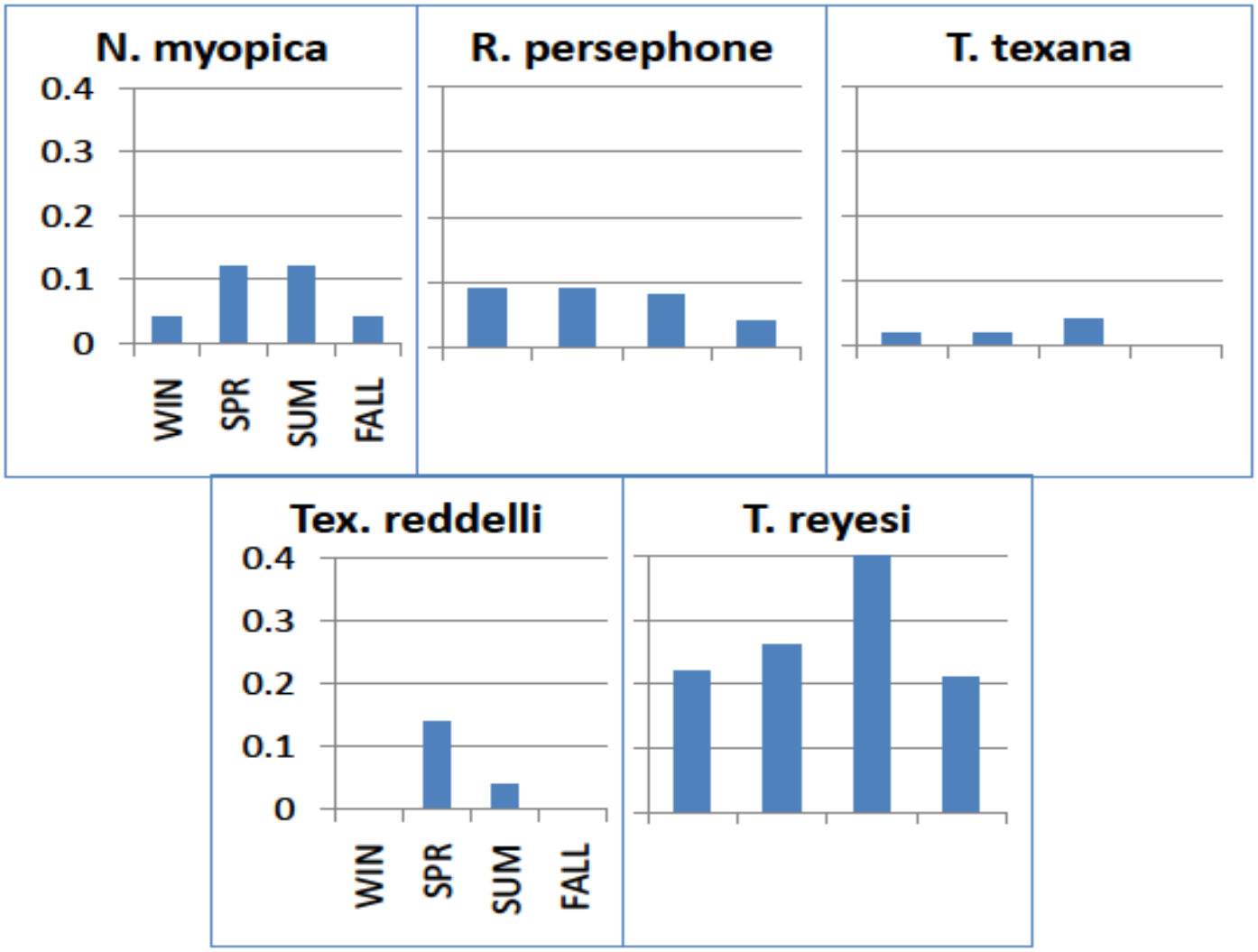
0.15
C. bandida
C. travisae
T. reyesi

Fig. 6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WIN
SPR
SUM
FALL
R. persephone
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0.3
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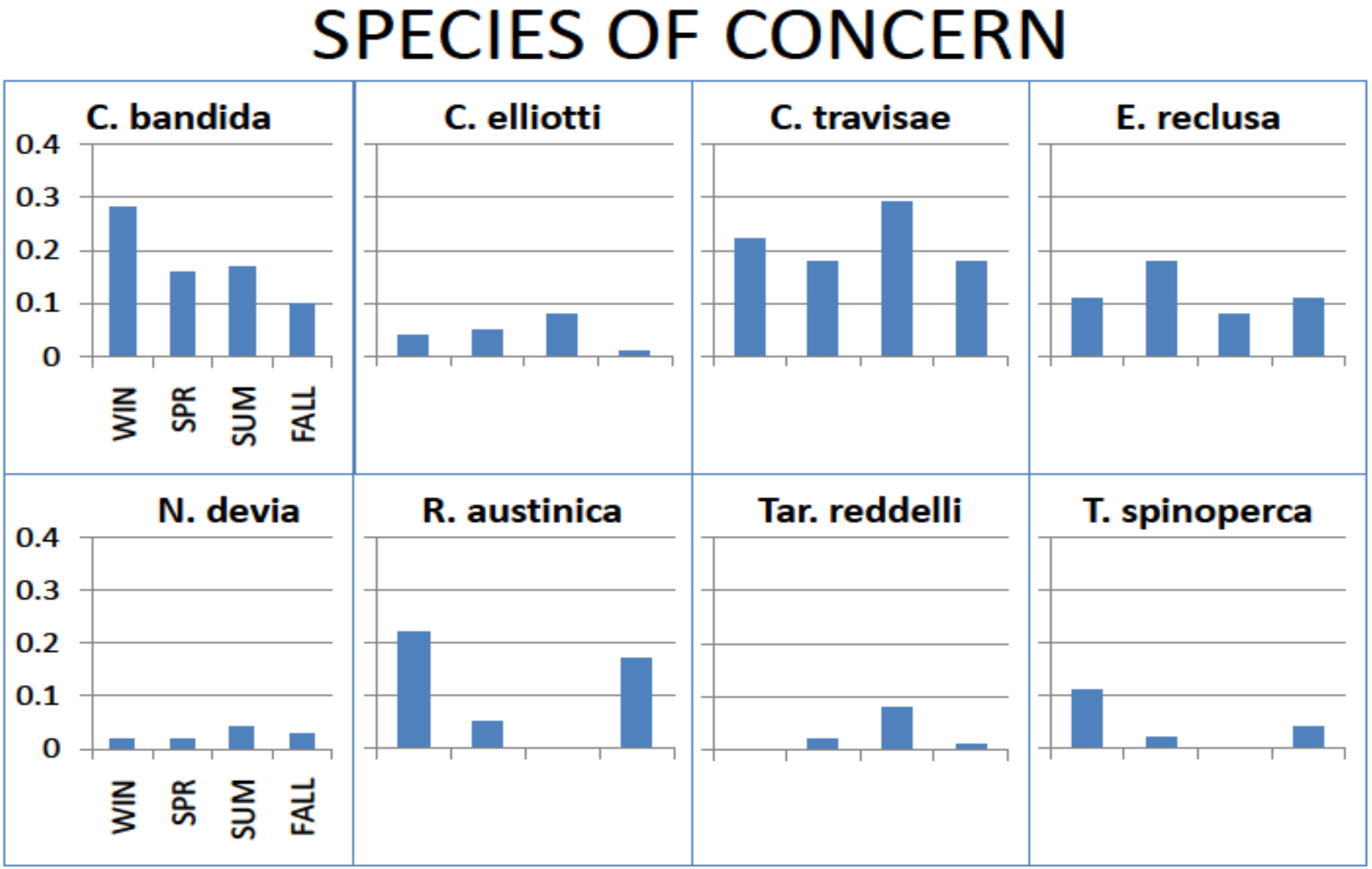
0
WIN
SPR
SUM
FALL
T. reyesi

Fig. 7


SPECIES OF CONCERN
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C. elliotti
C. travisae
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N. devia
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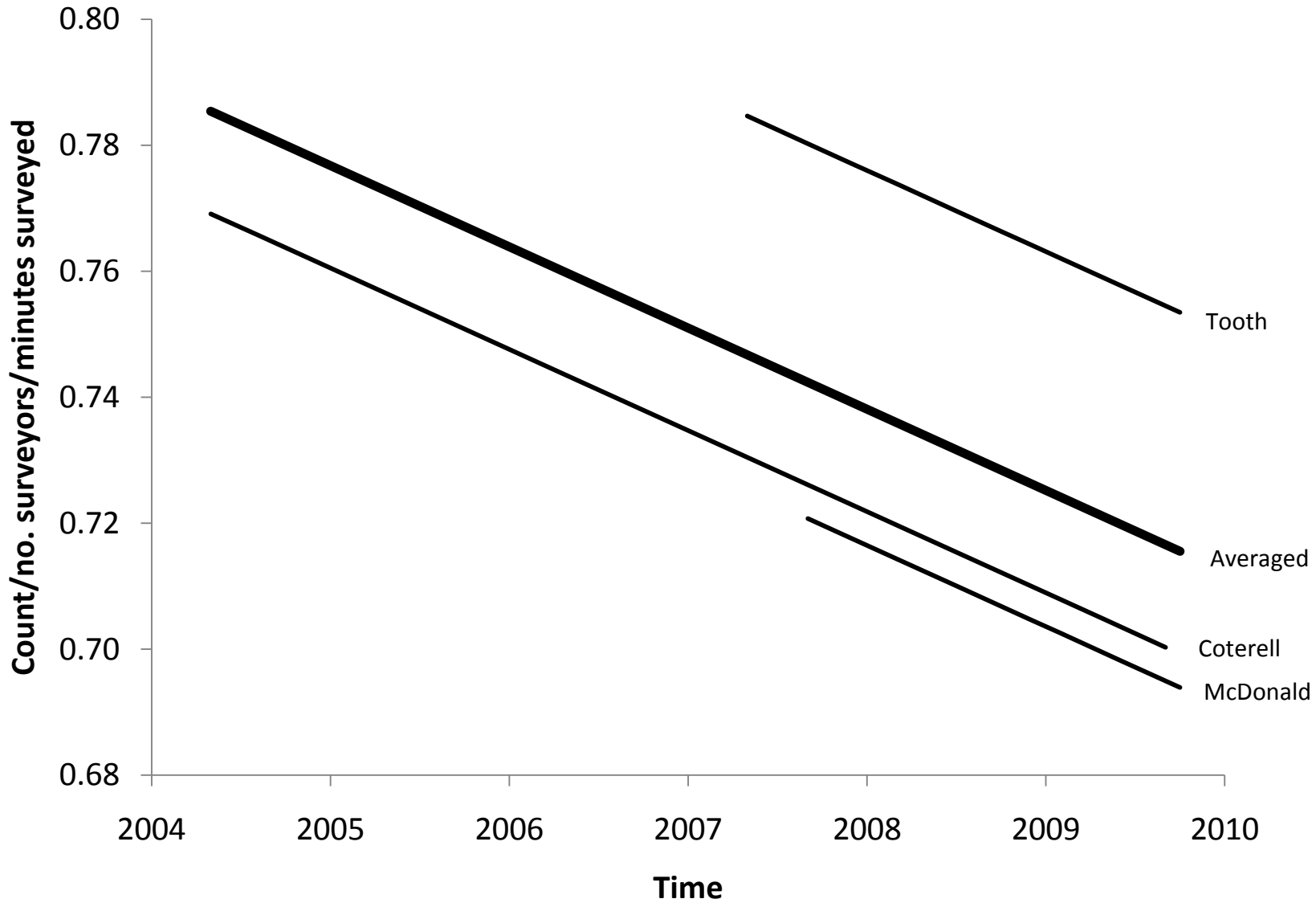
Tar. reddelli
T. spinoperca
0.4
0.3
0.2
0.1
0
WIN
SPR
SUM
FALL

Fig. 8


0.80
0.78
0.76
0.74
0.72
0.70
0.68
Count/no. surveyors/minutes surveyed
2004
2005
2006
2007
2008
2009
2010
Time
Tooth
Averaged
Coterell
McDonald