

Sampling procedures and adult sex ratios in spruce budworm

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Abstract

Unbiased estimates of sex ratios that reflect local abundance of adult insects are practically difficult to obtain because many gender-specific behavioural adaptations differentially influence the catchability of males and females in commonly applied sampling procedures. Historic data on outbreak populations of spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae), the major pest of conifers in Nearctic boreal forests, include dozens of sex ratio observations for 10 different sampling procedures; these data illustrate the importance of understanding the reproductive ecology of adults to contextualize sex ratio assessments. Sex ratios of resident adults (assessed by rearing field-collected pupae to adulthood or fogging host trees with insecticide) were not different from 1:1. Sex ratios of in-flight adults collected using Malaise traps or light traps deployed in tree canopies were consistently male-biased, which presumably reflects the higher level of flight activity for males relative to females. Sex ratios of moths captured outside the forest canopy (presumed migrants), in contrast, were consistently female-biased, a trend which is expected because females seeking oviposition sites are more likely to undergo migration than are males. The sex ratio among adults that died from natural causes (collected on drop trays) was not distinguishable from 1:1. In pre-outbreak (endemic) populations, sex ratios estimated by light trapping were much more strongly male-biased than in outbreak populations. This surprising result should, however, be interpreted with caution because little is known of reproductive ecology in endemic budworm populations.

Introduction

Sex ratios of reproductive adults are difficult to quantify in field populations of insects (Ehrlich et al., 1984), in part because of behavioural differences between males and females. Such differences are expected because the two sexes have distinct reproductive interests: for males, primarily increasing the number of mates, and for females, increasing mate quality, probability of mating, achieving an optimal number of copulations, and locating suitable resources for progeny (Parker, 1970; Scott, 1975; Kokko & Rankin, 2006). Males and females may differ, for example, in habitat use (Showers et al., 1976; Elliott, 1977; Tabadkani et al., 2012), dispersal response (Rhainds & Shipp, 2003), vertical flight profile (Byers, 2011), or attraction to

light (Feng et al., 2003; Altermatt et al., 2009; Aguiar & Santos, 2010) or chemical baits (Loeb et al., 2011; Knight et al., 2013). In extreme examples, these differences can frustrate attempts to collect one gender or the other (and in the process greatly frustrate the entomologist; e.g., Gibson, 1878). Mark-recapture studies (Cronin et al., 2000; Stoks, 2001) have illustrated the occurrence of sampling artefacts in sex ratio assessments and the importance of understanding the distinct behaviour of males and females for the interpretation of field data.

Whereas these ideas may seem unsurprising, the possibility of behavioural influence on estimated sex ratios is frequently downplayed or ignored in the literature. The result is studies that draw broad conclusions related to mating patterns, dispersal, pest management strategies, or population dynamics based on a single sampling technique that may over-collect one gender or the other (e.g., White et al., 2012; Kallioniemi et al., 2014; Takeda et al., 2014; Lessio et al., 2014). Conclusions from such studies may be

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robust to the sampling issues, or they may not be – without careful consideration of the behaviour behind sampling biases, it is difficult to be sure.

The tortrix moths (Lepidoptera: Tortricidae) include extremely important economic pests in agricultural and forest landscapes, and as a consequence their reproductive ecology has been well studied. Sex ratio is an important population parameter because it strongly influences population growth rate and mating probability, and as a result the literature is rich in measurements of tortrix sex ratios for different sampling procedures (e.g., Knight, 2007; Loeb et al., 2011; Wearing et al., 2012; Lu et al., 2012; Knight et al., 2013). This is particularly true for the spruce budworm, *Choristoneura fumiferana* Clemens, for which assessments of sex ratio include at least 10 distinct sampling procedures (Table 1) that are likely to interact differently with male and female behaviour in determining sex ratio of captured moths.

Spruce budworm (henceforth ‘budworm’) is the most severe defoliator of conifers across the Nearctic boreal forest (Morris, 1963), attacking primarily balsam fir and spruces. Adults emerge in late June and July. Shortly after emergence and mating, females lay their eggs in clusters on the foliage. Gravid females with their full egg complement have limited flight ability because their heavy abdomens increase wing load (Outram, 1973), but they become capable of sustained flight after a 2-day oviposition period (Rhains & Kettela, 2013). Both males and females undergo long-range migrations by ascending above tree canopies and out of the atmospheric boundary layer, whereupon they get carried downwind for distances sometimes exceeding 100 km (Greenbank et al., 1980).

The consolidation and dissemination of budworm sex ratio data, and an exploration of how these data may be sensitive to sampling methodology, should be of value to both forest managers and researchers, especially in context of the looming outbreak in Atlantic Canada (Johns & Pureswaran, 2013). From a broader perspective, the diversity of methods used to collect adult budworm and the wealth of capture data available make budworm an excellent illustration of potential biases in sex ratio assessments when reproductive ecology is ignored.

Materials and methods

Sources of historic data

We consolidated data originating from multiple sources, including published references and unpublished data archived at the Canadian Forest Service – Atlantic Forestry Centre in Fredericton (NB, Canada). The study sites cover a broad geographic range in Atlantic Canada and Maine

(USA) (Table 1). All sites consisted of forest stands with hosts of budworm as dominant or co-dominant tree species. Budworm populations were high at all sites (peak or declining phase of outbreaks), as indicated by time series of egg mass densities (Royama et al., 2005). We included data only for sites and sampling protocols for which budworm had been collected for ≥ 8 days. We included light trap data only for sites where we could compare traps deployed in forest clearings and tree canopies, because trap location greatly influences the sex ratio of adults. Trap location is frequently not specified (Rhains & Kettela, 2014), and this led us to exclude several datasets (including, for example, light-trap data for the 1950s Green River experimental sites in New Brunswick).

The data originated from a broad range of investigative contexts, including studies on dispersal and migration behaviour (Greenbank, 1957, 1973; Outram, 1973; Greenbank et al., 1980; Rhains & Kettela, 2013, 2014), reproductive performance of adults (Thomas et al., 1980), and disease epidemiology (Eveleigh et al., 2007). Some data were part of large-scale trials aimed at interfering with reproduction by killing adults (aerial applications of insecticides: Thomas et al., 1979) or preventing mate location (pheromone-based mating disruption: Miller, 1979; Diamond et al., 1984; Seabrook & Baskerville, 1988); in such cases, we included only data from control (untreated) plots. Although the studies were not explicitly designed to evaluate variation in sex ratio, they consistently used the same sampling procedures and thus provide comparable data.

Sampling procedures used to collect adults

The sampling procedures targeted five groups of adults, defined by behaviour in combination with location (inside or outside the forest canopy):

A Resident adults

- 1 Pupae were collected from foliage and reared to adulthood in the laboratory.
- 2 Host trees were fogged with insecticide (pyrethrins and piperonyl butoxyde) and dead adults collected on tarpaulins placed below tree canopies.

B Adults in flight within forest canopy

- 3 Adults were collected using Malaise traps deployed in tree canopies.
- 4 Adults were collected with Coleman light traps deployed in tree canopies (Figure 1 in Hurley & Titus, 1987).

C Adults in flight outside forest canopy

- 5 Adults were collected manually with sweep nets from observation platforms erected above tree canopies. Moths were collected as they flew

Table 1 Relative abundance of male and female spruce budworm collected using different sampling procedures. Resident adults were collected by rearing field-collected pupae to adulthood or by fogging trees with insecticides. In-flight females were collected with malaise traps as well as light traps deployed at tree canopies or forest openings. Migrants were collected on observation platforms while ascending above the tree canopy, on tarmacs early in the morning, in pyramid traps above the plant canopy, or in the atmosphere using aircraft. Dead adults were collected using drop trays. Locations are in New Brunswick (NB) and Nova Scotia (NS), Canada, and Maine (ME), USA

Location	Year	Females	Males	% (females)	References
<i>Resident adults</i>					
<i>Pupal collections</i>					
Green River, NB	1953	2123	2044	50.9	4
	1956	78	64	54.9	4
	1957	100	122	45.0	4
Fredericton, NB	1976	440	560	44.0	5
Dunphy, NB	1987	688	795	46.4	6
Mean \pm SE		685 \pm 377	717 \pm 359	48.2 \pm 2.0	
<i>Insecticide fogging</i>					
Fredericton, NB	1976	390	361	51.9	1
Saint-Quentin, NB	1977	605	853	41.5	2
Fredericton, NB	1977	1167	1112	51.2	1
Saint-Quentin, NB	1978	3481	3391	50.7	3
Amherst, NS	1978	1475	1924	43.5	3
Mean \pm SE		1424 \pm 550	1528 \pm 530	47.8 \pm 2.2	
<i>Adults in flight within forest canopy</i>					
<i>Malaise traps</i>					
Fredericton, NB	1977	142	713	16.6	7
Heath Steele, NB	1977	74	230	24.3	1
Saint-Quentin, NB	1978	229	348	39.7	3
Amherst, NS	1978	407	926	30.5	3
Bare Island, ME	1980	90	950	8.7	8
Fredericton, NB	1985	2902	4555	38.9	9
	1986	668	842	43.7	9
	1987	18	26	40.9	9
Dunphy, NB	1987	477	804	37.2	6
Mean \pm SE		556 \pm 302	1044 \pm 452	31.2 \pm 4.1	
<i>Light traps in tree canopy</i>					
Fredericton, NB	1972	2380	18393	11.5	10
Chipman, NB	1973	3666	16511	18.2	10
	1974	7319	38139	16.1	10
	1975	10305	28711	26.4	10
Juniper, NB	1975	8501	22708	27.2	10
Fredericton, NB	1976	2333	8229	22.1	10
Saint-Quentin, NB	1977	5048	31917	13.7	2
	1978	533	3351	13.7	3
Amherst, NS	1978	763	4678	14.0	3
Mean \pm SE		4539 \pm 1164	19182 \pm 4105	18.1 \pm 1.9	
<i>Adults in flight outside forest canopy</i>					
<i>Observation platforms</i>					
Fredericton, NB	1971	45	3	93.8	11, 12
	1972	52	2	96.3	11, 12
Chipman, NB	1973	158	18	89.8	12, 13
	1974	493	145	77.3	13
	1975	255	113	69.3	12, 14
Fredericton, NB	1976	89	12	88.1	14
Mean \pm SE		182 \pm 70	49 \pm 26	85.8 \pm 4.2	

Table 1. Continued

Location	Year	Females	Males	% (females)	References
<i>Tarmac</i>					
Chipman, NB	1973	41	17	70.7	12
	1974	300	30	90.9	12
	1975	273	126	68.4	14
Mean \pm SE		205 \pm 82	58 \pm 34	76.7 \pm 7.1	
<i>Aerial samples</i>					
Fredericton, NB	1971	30	2	93.8	13
	1972	63	20	75.9	13
Chipman, NB	1973	40	23	63.5	12, 13
	1974	659	412	61.5	12
Renuous, NB	1974	267	81	76.7	12
Juniper, NB	1975	41	28	59.4	1
Chipman, NB	1975	149	184	44.7	1
Mean \pm SE		178 \pm 86	107 \pm 56	67.9 \pm 5.9	
Mixed (within and outside canopy)					
<i>Light traps in forest opening</i>					
Fredericton, NB	1972	26	32	44.8	10
Chipman, NB	1973	831	645	56.3	10
	1974	218	411	34.7	10
	1975	503	601	45.6	10
Juniper, NB	1975	3420	4901	41.1	10
Fredericton, NB	1976	51	60	45.9	10
Saint-Quentin, NB	1977	5016	7669	39.5	2
	1978	71	360	16.5	3
Amherst, NS	1978	205	615	25.0	3
Mean \pm SE		1149 \pm 601	1699 \pm 890	38.8 \pm 4.0	
<i>Above-canopy traps</i>					
Fredericton, NB	1976	65	166	28.1	1
	1980	8892	6539	57.6	1
	1981	4185	7988	34.4	1
	1983	6290	6979	47.4	9
	1984	6440	4169	60.7	9
	1985	5152	4064	55.9	9
	1986	1931	1594	54.8	9
	1987	34	47	42.0	9
Mean \pm SE		4124 \pm 1133	3943 \pm 1097	47.6 \pm 4.2	
Dead adults					
<i>Drop trays</i>					
Chipman, NB	1975	26	29	47.3	1
Fredericton, NB	1976	456	498	47.8	1
Heath Steele, NB	1977	68	232	22.7	7, 14
Fredericton, NB	1977	564	1496	27.4	1
	1983	158	440	26.4	9
	1984	250	566	30.6	9
	1985	263	541	32.7	9
	1986	101	165	38.0	9
Mean \pm SE		236 \pm 67	496 \pm 159	34.1 \pm 3.3	

1: Unpublished data. 2: Rhinds & Kettela (2013). 3: Miller (1979). 4: Greenbank (1957). 5: Miller et al. (1978). 6: Seabrook & Baskerville (1988). 7: Thomas et al. (1979). 8: Dimond et al. (1984). 9: Eveleigh et al. (2007). 10: Rhinds & Kettela (2014). 11: Greenbank (1973). 12: Greenbank et al. (1980). 13: Outram (1973). 14: Thomas et al. (1980).

- vertically in rapid ascent above the canopy (exodus flights) (Figure 2 in Greenbank et al., 1980).
- 6 Adults were collected from airport runways early in the morning (no moths having been present the evening before).
 - 7 Adults were collected high in the air with aircraft.
- D Mixed (including adults in flight within or nearby the forest canopy)
- 8 Adults were collected in pyramid traps deployed above tree canopies.
 - 9 Adults were collected with light traps deployed in forest clearings.
- E Dead adults
- 10 Adults that died of natural causes were collected using drop trays placed on the ground below tree canopies.

For all sites and collection procedures, adult budworm were classified as male or female based on the shape of the last abdominal segment. Details on sampling protocols are available in the references listed in Table 1.

Statistical analysis of historic data (outbreak populations)

Our data include 69 moth collections (a 'collection' referring to the moths taken by one sampling procedure at one site in 1 year) applying 10 sampling procedures to estimate sex ratio for five target groups. We had between three and nine collections (mean 6.9) per sampling procedure and between eight and 18 collections (mean 13.8) per target group; collections included between 32 and 39 000 individual moths (mean 5 070; Table 1).

Our statistical design envisions estimated sex ratio as potentially dependent on target group (the fixed effect of interest), sampling procedure (a random effect nested within target), site (random effect), and year (random effect). However, when seen in context of this full model, the data matrix has many empty cells, because the combination of sampling procedures varied among sites and years (67% of site*method combinations are empty cells). Therefore, we were unable to run a single analysis of the entire dataset including all factors (target group, sampling procedure, site, and year). Instead, we took a 3-step approach to exploring our sex ratio data.

We began by considering only Fredericton data, for which all targets and all methods except 'tarmac' were represented. We modelled sex ratio with a generalized linear mixed model in the 'glmer' procedure of package 'lme4' (Bates et al., 2011) in R 2.12.0, assuming binomial error (this procedure is appropriate for sex ratio data; Wilson &

Hardy, 2002). Independent variables were target and sampling procedure (nested within target), with collections from different years as replicates, and we structured the analysis so that the intercept term tested the deviation from 1:1 sex ratio for our 'residents' group, with other target groups compared to residents. This parameterization makes sense because the resident group best represents local sex ratio of emerging adults free of behavioural effects. We checked suitability of this model for our data by examination of plotted residuals for heteroscedasticity, bimodality, and other non-random pattern. We conducted a test for significance of 'target' using a log-likelihood comparison of models including and excluding target as a factor, where $G (= 2 \times \Delta\text{-log-likelihood})$ is ca. χ^2 distributed.

Next, we used simple G-tests to compare sex ratio across site*year combinations for each sampling procedure. We calculated confidence intervals for sex ratio for each site*year combination and inspected them to gauge the strength of sex ratio variation among sites and years.

Finally, because our G-tests suggested that sex ratio variation within sampling procedures was similar among sites and among years, we conducted a single large analysis of all our data treating collection (site*year combination) as the replicate. This analysis is only approximately correct, because it assumes no important effects of site or year, but it allows us to assess whether the sex ratio pattern evident in the Fredericton-only analysis is present in the full dataset. The model and testing procedure were the same as for the Fredericton-only analysis. We also ran an equivalent analysis using data for all sites except Fredericton, to ensure that the Fredericton data were not entirely driving the all-sites analysis.

Sex ratio in budworm populations with low to moderate densities

In 2012–2013, we estimated sex ratio for budworm populations in the early phase of outbreak development. Two sites were located in New Brunswick and had low budworm density (<0.1 L2 per 45-cm branch section): one near Kedgwick (2012; five plots within a 50-km² area) and one near Juniper (2013; six plots within a 20-km² area). A third site was located near Mont-Joli, QC (2013; three plots in a 30 km² area) and had moderate density of budworm (>1 L2 per 45-cm branch section). In each plot, one stainless steel vane light trap (Leptraps, Georgetown, KY, USA) with a 15-W neon tube as light source was suspended on a rope between two host trees about 4 m above ground. Each trap was wired to a marine battery for power. In Kedgwick and Mont-Joli, we used ethyl acetate placed in two 250-ml aluminium cans to kill captured adults, which we collected daily. In Juniper, we used strips of

dichlorvos (Vapona) to kill the moths and we emptied the traps at intervals of 3–4 days.

We analyzed the low-to-moderate density data using a generalized linear mixed model assuming binomial errors, as above. Independent variables were site and plot (nested within site). We conducted a test for significance of 'site' using a log-likelihood comparison of models including and excluding site as a factor. However, this test is constrained to be exploratory, because in the dataset site is confounded with budworm density (low densities in NB and moderate densities in QC). We also calculated confidence intervals for sex ratio in each plot.

Results

Analysis of sex ratio in outbreak populations

In the Fredericton-only analysis, sex ratio varied strongly among target groups ($G = 19.9$, $d.f. = 4$, $P = 0.0005$; Table 2). Examination of residuals indicated good model fit. For resident adults, sex ratios were very close to 1:1 (Figure 1A) and we could not detect any deviation from that null expectation (Table 2). Adults in flight within the forest canopy showed strong male bias (Figure 1B) and adults in-flight outside the forest canopy showed strong female bias (Figure 1C), with both easily distinguishable from the resident 1:1. Mixed adults (those sampled by methods that trap moths from within and nearby the forest canopy) had variable sex ratios (Figure 1D) that overall could not be distinguished from the residents' 1:1 ratio. Adults that died and were collected in drop trays tended to show male bias (Figure 1E), but without a significant difference from the residents' 1:1 ratio (Table 2).

For every sampling procedure, there was significant variation in sex ratio among collections (all $P < 0.0001$; $23.8 \leq G \leq 4641$, $2 \leq d.f. \leq 8$; Figure 1). However, there was nearly as much variation among sites as there was among years for a given site. Furthermore, where data were available for multiple sampling procedures at a single site*year combination (for instance, Saint Quentin in 1978), there was no tendency for those collections to be consistently more female- or male-biased than collections taken by similar sampling procedures in other sites or years. This suggests that observed variation among sites and years can reasonably be pooled, and so we proceeded with an analysis combining data for all sites, treating each collection as a replicate. The result was entirely consistent with the Fredericton-only analysis: significant sex ratio variation among target groups ($G = 21.0$, $d.f. = 4$, $P = 0.0003$), a resident sex ratio very close to 1:1, male bias for adults flying within the canopy, female bias for adults flying outside the canopy, and mixed adults indistinguishable from the residents' 1:1 (Table 2). Dead adults again showed a trend

Table 2 Generalized linear mixed-model analyses of spruce budworm sex ratio, based on data for Fredericton only and data for all sites, using collection (site*year combination) as the replicate. For each factor, a negative parameter estimate suggests male bias, a positive one female bias

Target	Parameter estimate	SE	z	P
Fredericton				
Resident (intercept)	-0.091	0.254	-0.36	0.72
In flight	-1.024	0.358	-2.86	0.004
Migrants	2.022	0.397	5.09	<0.0001
Mixed	0.034	0.365	0.09	0.93
Dead	-0.636	0.438	-1.45	0.15
Target effect: $G = 19.9$, $d.f. = 4$, $P = 0.0005$				
All sites				
Resident (intercept)	-0.058	0.203	-0.29	0.78
In flight	-0.978	0.286	-3.42	0.0006
Migrants	1.082	0.264	4.10	<0.0001
Mixed	-0.115	0.287	-0.40	0.69
Dead	-0.686	0.351	-1.95	0.051
Target effect: $G = 21.0$, $d.f. = 4$, $P = 0.0003$				

toward male bias, this time nearly reaching statistical significance (Table 2). Results of the all-sites-except-Fredericton analysis were very similar, except that the male bias of dead adults was now significant ($P = 0.002$). Examination of residuals again indicated good model fit.

Sex ratio in budworm populations with low to moderate densities

At sites early in budworm outbreak, with low to moderate budworm density, sex ratios were consistently male-biased (Figure 2). There was significant variation in sex ratio among sites ($G = 20.7$, $d.f. = 4$, $P < 0.0001$), with male bias particularly strong at our lowest-density sites (Juniper and Kedgwick, NB). Examination of residuals indicated moderate heteroscedasticity, with greatest residual variance at Mont Joli. However, given the strength of the sex ratio pattern, we are confident that significance was not generated only by heteroscedasticity.

Discussion

The extensive data available for outbreak budworm populations reveal a wide range of sex ratio estimates. Each estimate is arguably sound, when considered as applying to an ecologically meaningful 'population' of budworm that is accessed by the sampling procedure in question (what we call target groups). However, without careful consideration of how behaviour and reproductive ecology influence capture success, one could err by conceptualizing instead a single underlying sex ratio for the species that all

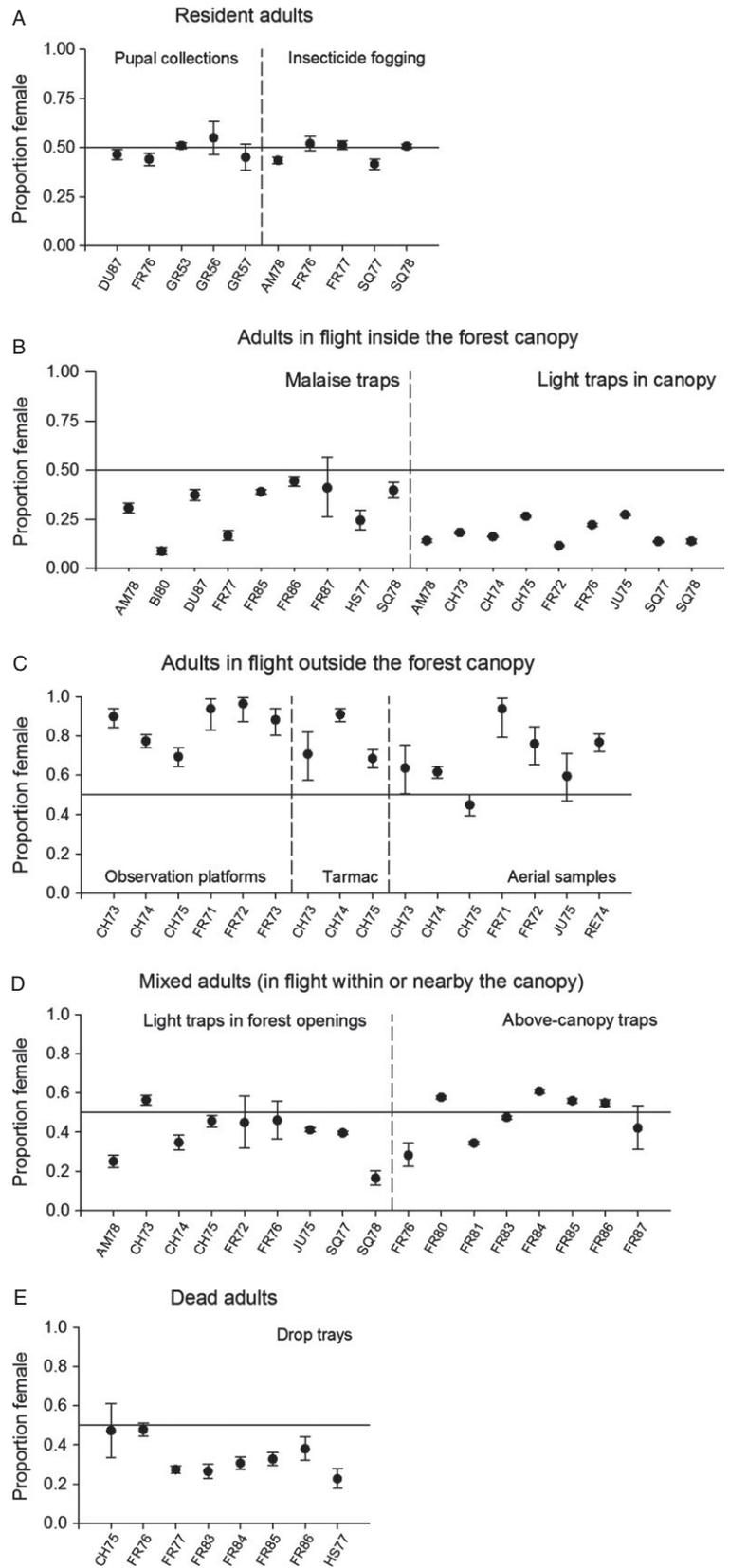


Figure 1 Mean proportion of female spruce budworm collected for five target groups (panels A–E) using 10 sampling procedures. Error bars are exact binomial confidence intervals (where no error bars are evident, they are smaller than the plotting symbols). Collections are labelled by site (two letters) and year (two digits). Site codes are AM (Amherst, NS), BI (Bare Island, ME), CH (Chipman, NB), DU (Dunphy, NB), FR (Fredericton, NB), GR (Green River, NB), HS (Heath Steele, NB), JU (Juniper, NB), RE (Renous, NB), and SQ (Saint Quentin, NB).

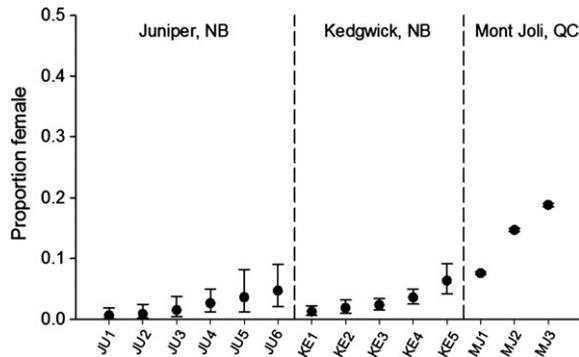


Figure 2 Proportion female spruce budworm collected in areas with low (Kedgwick, NB, 2012; Juniper, NB, 2013) and moderate (Mont Joli, QC, 2013) population density. Error bars are exact binomial confidence intervals (where no error bars are evident, they are smaller than the plotting symbols). Collections are labelled by two letters (site) and one digit (plot number). Sex ratios were significantly different between sites ($G = 20.7$, d.f. = 4, $P < 0.0001$).

sampling procedures are estimating. In that context, most of the estimates we report are biased, and their straightforward interpretation could be misleading.

The similar abundance of males and females among resident adults is consistent with previous findings reporting a 1:1 ratio among budworm pupae, a lack of variation in sex ratio between pupal and adult stages, and non-significant relationships between population density and sex ratio (Miller, 1963). Assuming that the primary sex ratio for budworm larvae is 1:1 (as is usually the case for organisms with diploid sex determination; Hamilton, 1967), the equal abundance of males and females at the pupal stage (secondary sex ratio) suggests similar mortality rates for male and female larvae. That insecticide fogging also yields a sex ratio indistinguishable from 1:1 suggests that net immigration (immigration minus emigration) is small relative to the population of local origin. If it was not, we would expect fogging sex ratios to depart from 1:1 because budworm migrants are primarily female: if emigration greatly exceeded immigration, remaining populations would be male biased, whereas if immigration greatly exceeded emigration, remaining populations would be female biased.

We found striking departures from 1:1 sex ratios for adults flying within the forest canopy (male bias) and those flying outside the forest canopy (female bias), and we interpret these as consequences of gender-specific behaviour. The male-biased sex ratio of moths flying within the canopy presumably reflects higher activity of males than of females (Edwards, 1962; Sanders & Lucuik, 1972), as is usually the case in moth species with phero-

none-calling females and mate-seeking males (Greenfield, 1981). In contrast, the female-biased sex ratio of moths flying outside the canopy reflects dispersal behaviour. Female budworm undergo long-range migration after laying their first batch of eggs (because the weight of mature eggs constrains their capacity to fly; Johnson, 1969; Outram, 1973). This represents a bet-hedging strategy in which females lay some of their eggs near the site of their emergence and the remainder at some distant location (Gatehouse, 1997). Inter-reproductive migrations in budworms have evolved in an ecological context (large-scale boreal forests with abundant conifers, but with severe defoliation at the height of outbreaks) which suggests value to searching for host plants both pre-dispersal (because host plants tend to be locally abundant) and post-dispersal (because local hosts may become depleted, and in the expansive forest landscape risk of dispersal may be relatively small). Even though males are less likely than females to undertake long-range migration, they do migrate, probably in search of virgin females in low-density populations (Régnière et al., 2013). This behaviour may be of adaptive value if it allows males to sire offspring with access to underexploited hosts. The wide variation in sex ratio data for migratory moths suggests that the tendency for males to join migrations varies among sites and years.

Two sampling procedures seem to capture a mixture of in-flight (local) adults and migrants: light traps in forest gaps, and pyramid traps above the tree canopy. Our data suggest that the offsetting sex ratios of local and migrant moths can combine to generate sex ratios from male to female bias, with considerable variation across sites and years. These techniques differ only in location from light traps and Malaise traps (respectively) placed in tree canopies, which predominantly capture in-flight moths (Miller et al., 1978; Rhains & Kettela, 2014). Thus, apparently minor details in sampling procedures can interact with insect behaviour to generate striking differences in sampled sex ratio.

Sex ratios of adults that died from natural causes (drop trays) tended toward male bias in most collections, although this trend was not quite significant except when analyzed for just the two non-Fredericton sites; more geographically extensive sampling would be useful. If such sampling were to establish a consistent male bias for drop trays, it might suggest a net impact of migration on sex ratios at late life-history stages. As the sex ratio of emergent and resident adults is ca. 1:1, a male bias at death could result from a net outflow of late-life females (emigration > immigration). This hypothesis deserves testing, although there are other plausible explanations for male-biased drop-tray data – for instance, ovipositing females might be preferentially removed by predators.

If there is male bias in drop trays, and if it reflects female emigration, then we might expect a female bias at sites outside current outbreaks, in which moth populations include an important fraction of received migrants (Stedinger, 1984). Little has been known about sex ratios in low-density populations, though, and surprisingly, our data for such populations instead show very strong male bias. Our argument that behaviour strongly influences sex ratio estimates points to one possible explanation: it is possible that when moths are rare, males are forced into more and longer mate-finding flights and this strengthens the male bias of methods like light traps that sample primarily in-flight individuals. That male bias was weaker at our moderate-density site than at our two low-density sites is consistent with this notion, but because density was confounded with region in our study we emphasize that this is only a hypothesis for future testing. Furthermore, our data compilation makes it clear that sex ratio data are difficult to interpret without a good understanding of reproductive ecology, and it is largely unknown whether adults behave differently in outbreak and pre-outbreak (endemic or low density) budworm populations.

Conclusion

Our analyses show that estimated sex ratio for spruce budworm is likely to depend strongly on the way budworm behaviour interacts with sampling methodology, because different sampling procedures sample moths behaving differently and in different locations with respect to their forest canopy habitat. Sex ratios are near 1:1 among emergent and resident adults, but are male biased among adults flying within the canopy (probably because males are more active than females) and female biased among adults flying outside the canopy (probably because females are more likely than males to undergo long-range migrations). It may be feasible to assess the role of migration in local population dynamics by comparing sex ratios of emergent adults and of adults dying of natural causes, although more work is needed to determine whether such inference is possible. The scarcity of sex ratio estimates at low population density makes it extremely challenging to understand how adult dispersal may affect the transition from endemic to epidemic (outbreak) population levels. As a result, studies of behaviour, reproductive ecology, and movement of moths in low-density populations are urgently needed.

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