Sex Ratio and Breeding Population Size in Fowler's Toad, Anaxyrus (5Bufo) fowleri

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David M. Green[1]

Sex ratios among anurans at breeding sites are routinely observed to be skewed toward males, which has implications for the strength of sexual selection in these animals. However, the relative numbers of males and females observed at breeding sites also depends upon their relative conspicuousness and the physical sex ratio of the adult population as a whole, which includes animals not present at breeding sites. Using intensive capture–recapture methods, I estimated abundances of both sexes of Fowler's Toads in a population at Long Point, Ontario, over a span of 14 years (1998 to 2011, incl.). Although males greatly outnumbered females at breeding sites, persisted there for longer periods of time, and were more readily re-captured, both sexes could be found in their lakeshore non-breeding habitat with equivalent reliability. Estimates of abundance were calculated for each sex based on 3,162 total captures of 686 females and 982 males. The abundances of males and females each year were not significantly different (*P* = 0.738), which was consistent with a 1:1 physical sex ratio. Both sexes also exhibited large, but strongly correlated (*P* << 0.001; *R*² = 0.838), variations in their abundance over the 14 yrs. Only 39% of total estimated males were found at breeding sites. The ratio of males at breeding sites to total males declined significantly (*P* = 0.002; *R*² = 0.542) over the 14-year study, in parallel with a diminishing availability of breeding sites.

COMPETITION for mates is the central driver of sexual selection in animals and is expected to be intensified when the ratio of males to females among individuals that are ready to mate, i.e., the operational sex ratio (Emlen, 1976), is biased toward one sex or the other (de Jong et al., 2012; Kokko et al., 2012). Understanding how accurately the sex ratio observed at a breeding site reflects the operational sex ratio and how much each may differ from the population's physical adult sex ratio is important information for testing hypotheses about mating strategy and for understanding population viability. It is an assumption that the relative numbers of males and females observed at breeding sites is a reasonable estimator of the operational sex ratio, provided that both sexes can be observed with equal consistency.

Anuran breeding congregations invariably appear to be dominated by males who compete with each other for space to call from, time to call in, and, ultimately, access to mates (Wells, 2007; Loman and Madsen, 2010). This male-biased operational sex ratio may be due largely to differing behavior of the two sexes. Among pond-breeding anurans, males are usually the first to arrive at breeding sites and the last to leave (Davies and Halliday, 1979; Reading and Clarke, 1983; Kuhn, 1994). They may spend many days and nights at breeding sites and are capable of, though not usually successful at, breeding multiple times (Davies and Halliday, 1979; Kruse and Mounce, 1982). Their calling behavior and conspicuousness makes them easy to observe, record, and capture. Females appear to behave very differently. They generally spend only enough time at a breeding site to find a mate and deposit their eggs, which they tend to do only once a season. With few exceptions, females are silent, cryptic, and difficult to find compared to the males, with the result that the probabilities of capturing males vs. females may not be equal.

Operational sex ratios in pond-breeding anurans, moreover, may be affected by reproductively mature individuals of one sex or the other failing to appear at breeding sites. Skipping a breeding season as an element of reproductive strategy (Hemelaar, 1988; Kuhn, 1994; Loman and Madsen, 2010) may be favored insofar as the animal is likely to grow larger and/or more fecund in the interim, provided it survives. This is not likely to be a profitable strategy in short-lived animals. Animals may also fail to breed when breeding sites disappear or simply because they get lost, both of which may be presumed to affect both sexes equally.

Factors other than behavior may also contribute to biased operational sex ratios. The primary sex ratio established at fertilization is invariably 1:1 in normally reproducing bisexual amphibians (Sakisaka et al., 2000; Alho et al., 2010), but by the time of sexual maturity, the physical sex ratio of the population may become imbalanced due to sex-biased differences in age of sexual maturity (Berven, 1990; Reading, 1991; Miaud et al., 1999) or annual survival rate (Elmberg, 1990; Friedl and Klump, 1997). If the unbalanced sex ratio observed in anuran breeding choruses is due only to differential behaviours of males vs. females, and not to differential reproductive readiness and/or mortality, then the physical sex ratio of the population as a whole should be 1:1.

Estimating the physical sex ratio of the adult population requires accurate census information that is unaffected by the differential breeding behaviors of males and females. The challenge is to find the animals reliably outside of the breeding season and census them over many years to compensate for any temporal shifts in relative abundance. Fowler's Toads, *Anaxyrus* (=*Bufo*) *fowleri* at Long Point, Ontario, breed in marshes and pools but spend their summers on the sand dunes and beaches along the shore of Lake Erie (Green, 2005; Greenberg and Green, 2013). At night, as they forage on the open sand, both males and females can be captured easily. These ecological characteristics enable an intensive capture–recapture study both at breeding sites and in non-breeding habitats, where sufficient captures of both sexes can allow highly accurate estimations of their relative abundance. If the physical population sex ratio is 1:1, then the abundances of adult males and females in the population estimated over many years should not be significantly different from each other. If not all of these animals breed in any given year, then the estimated

abundances of both sexes should be significantly higher than the numbers of animals estimated to be visiting breeding sites. Finally, if the observed sex ratio at breeding sites is primarily affected by behavioral differences between males and females, then capture probabilities should differ between the sexes. To test these hypotheses, I analyze relative abundances and capture probabilities of adult male vs. female Fowler's Toads, as well as observed vs. population sex ratios over a period of 14 years.

MATERIALS AND METHODS

I conducted an intensive capture–recapture study of Fowler's Toads annually from 1998 through 2011, inclusive, along 8.4 km of the Lake Erie north shore at the western base of Long Point, Ontario, between 42°34′33″–42°35′3″N and 80°22′15″–80°28′24″W. The study area consists of sandy beach and shallow marshes, which are separated by sand dunes (Greenberg and Green, 2013). The animals are opportunistic in selecting open water breeding sites, which are located in the marshes.

I searched all breeding sites, beaches, and adjacent dunes on foot for all active toads each evening over a period of ca. 40 nights beginning 1 May until ca. 10 June each year to encompass the toads' entire breeding season from the date of the toads' emergence from hibernation to the cessation of calling activity. I employed crews of two to four people to search for all active toads from sundown to as late as 0300 hrs, depending on the toads' abundance and activity. The intensity of the survey protocol ensured a maximum number of possible recaptures and increased the likelihood of obtaining the high capture probabilities required for a reliable census (Freckleton et al., 2006). I did not employ drift fences and pitfall traps to capture toads entering and leaving breeding sites in the marshes, firstly because the edges of the marshland breeding sites were ill-defined and impossible to surround with fencing and, secondly, because the evidence of differential behaviors of the two sexes that I was looking for may be expected to be reflected in the probabilities of hand-capture. All toads were measured with dial callipers to record snout–vent length to the nearest 0.1 mm and marked individually using toe-clipping. I recorded every capture of every individual so as to compile individual capture histories, as well as the numbers of adult males and females found at each breeding site, the total number of individuals identified and the total number of all captures per season. I noted all individuals found in amplexus. All procedures with the animals were authorized by the Ontario Ministry of Natural Resources, Environment Canada and McGill University Animal Use Protocol No. 4569.

As described by Greenberg and Green (2013), I used the program MARK (White and Burnham, 1999) to estimate the toads' annual abundances, $\hat{N}$, from individual capture histories with a closed capture model that allowed capture probability, $\hat{p}$, to vary with time. Estimates of $\hat{N}$ were made separately for adult males and adult females. I readily identified adult males by their dark throats and the release calls they made when handled. However, adult females could not be distinguished *a priori* from similarly white-throated juveniles of either sex other than by relative size, unless they were found in amplexus with a male. I therefore had to differentiate them from the juveniles before I could use their capture histories to estimate their abundance.

To discern females from juveniles, I plotted the snout–vent lengths of all white-throated toads captured in any one year as a frequency distribution histogram with 1 mm intervals. This invariably produced a bimodal histogram that I could analyse with the FAO-ICLARM Stock Assessment Tools computer program, FiSAT II, vers. 1.2.2 (FAO, 2005). I first applied Bhattacharya's (1967) log-differences method to decompose the bimodal frequency distribution into two Gaussian components corresponding to the snout–vent length distributions of juveniles vs. adult females. This procedure provided the initial estimation required for Hasselblad's (1966) NORMSEP maximum likelihood algorithm to determine the mean, variance, and size of each distribution of snout–vent lengths within the sample. To verify the results of this procedure, I employed the same method with all 1,832 white-throated toads I had captured and measured and compared the resulting size-frequency distribution of 686 adult females against the size-frequency distribution of 95 females captured at breeding sites, including 41 females captured in amplexus, over the same time period.

I used the results of analyses using FiSAT II to select appropriate capture histories for estimations of female abundance using program MARK. Although FiSAT II cannot identify the maturity status of individual animals, MARK requires individual capture histories to estimate population abundance. Therefore, I used the point of intersection of overlapping frequency-distribution curves as a cut-off value between the two size classes representing juveniles and females, respectively. Assuming that the number of juveniles that were larger than this value equaled the number of adult females that were smaller and that $\hat{p}$ among these animals did not differ significantly, I selected the capture histories of all animals larger than the cut-off size for use in estimating overall female abundance using MARK. This ensured that the number of individual capture histories I used to estimate abundance equaled the number of adult females in the size-frequency distribution.

I calculated sex ratio as the observed or estimated numbers of males divided by the observed or estimated numbers of females.

RESULTS

From 1998 through 2011, I captured and marked 1,668 adult Fowler's Toads, consisting of 982 males and 686 white-throated toads I recognized as adult females following analysis with FiSAT II (Table 1), plus 1,146 toads I recognized as juveniles (Fig. 1). There was no significant difference in SVL between 41 females captured in amplexus with a male and 54 females captured at breeding sites that were not in amplexus (*t*-test: *P* [two-tailed] = 0.098). There was also no significant difference in SVL between these 95 toads and the other 575 animals I recognized as females (*t*-test: *P* [two-tailed] = 0.0.411).

I based estimates of population abundance on 2,039 total captures of males and 1,123 total captures of females and. Within-year $\hat{p}$ values averaged 0.085 for males and 0.061 for females (Table 1) and were significantly different for the two sexes (*t*-test: *P* = 0.004) in reflection of the tendency for males to remain calling night after night at breeding sites. Average $\hat{N}$ over all 14 years (1998–2011, incl.) was 89±10 (SE) males and 86±26 (SE) females but both sexes exhibited large differences in abundance from year to year (Fig. 2A). The toads were most abundant in 2002 (240±23 [SE] males

Table 1. Survey Results and Sex Ratios for All Adult Female and Male Fowler's Toads Captured at Long Point, Ontario, Including Numbers of Individuals, Total Captures, Capture Probabilities, and Estimates of Abundance.

	Individuals captured			Total captures ($\hat{p}$)				Estimated abundance±SE		
Year	♂♂	♀♀	ratio*	♂♂		♀♀		♂♂	♀♀	ratio*
1998	180	142	1.27	494	(0.092)	327	(0.073)	191±4	156±5	1.22
1999	65	48	1.35	143	(0.083)	74	(0.043)	74±4	72±9	1.03
2000	50	32	1.56	92	(0.065)	36	(0.012)	63±5	129±56	0.49
2001	60	13	4.62	125	(0.084)	17	(0.030)	69±4	24±8	2.88
2002	139	44	3.16	197	(0.035)	49	(0.011)	240±23	198±79	1.21
2003	85	40	2.13	128	(0.049)	47	(0.019)	134±14	127±41	1.06
2004	152	130	1.17	336	(0.064)	122	(0.041)	174±6	173±10	1.01
2005	77	60	1.28	162	(0.090)	101	(0.060)	89±5	81±7	1.10
2006	38	32	1.19	72	(0.090)	40	(0.030)	48±4	71±19	0.68
2007	39	50	0.78	87	(0.110)	120	(0.122)	42±3	54±2	0.78
2008	32	34	0.94	51	(0.085)	51	(0.073)	42±6	51±8	0.82
2009	34	25	1.36	79	(0.092)	54	(0.086)	38±2	28±2	1.36
2010	16	24	0.67	28	(0.080)	54	(0.113)	20±3	26±2	0.77
2011	15	12	1.25	45	(0.176)	31	(0.148)	15±0	12±0	1.25
average	70.14	49.00	1.62	146	(0.085)	80	(0.061)	89±10	86±26	1.12
total	982	686	1.43	2039		1123		1239	1202	1.03

* sex ratio = males/females
† $\hat{p}$ = capture probability

and 198±79 [SE] females) and least abundant in 2011 (15±0 [SE] males and 12±0 [SE] females). Values of $\hat{N}$ for females and males per year were not significantly different (*t*-test: $P = 0.731$) and were highly correlated (Fig. 1; $P \ll 0.001$; $R^2 = 0.838$). There were also significant, parallel trends toward lower abundance over the course of the study (Fig. 2A) for both males ($P = 0.016$, $R^2 = 0.248$, $n = 14$ years) and females ($P = 0.007$, $R^2 = 0.439$, $n = 14$ years).

The majority of animals present, particularly among females, were never found at the breeding sites. The 561 individual males I encountered in total at breeding sites (Table 2) comprised 57% of all 982 males encountered and 45% of the 1,238 total estimated males over all years. The proportion of males captured at breeding sites to the estimated number of males varied among years from 5% in 2010 to 72% in 2001. Values of $\hat{p}$ for males at breeding sites

averaged 0.089 and ranged from 0.021 in 2005 to 0.194 in 2011 (Table 2). These were not significantly different from $\hat{p}$ values for males on the beaches (*t*-test, $P = 0.808$). Estimated male abundance at breeding sites averaged 80±8 [SE] individuals, ranging from 2±0 [SE] in 2010 to 168±18 [SE] in 2002. There was a significant trend of reduction in the ratio of males at breeding sites compared to total males present (Fig. 3; $P = 0.002$; $R^2 = 0.542$) over the 14 years of study, parallel to the declining trend in total male abundance after 2002 (Fig. 2A).

Many fewer adult females than males were encountered at breeding sites (Table 2). Ninety-three females accounted for 14% of all 686 females encountered and 8% of the total estimated 1,202 females over all years. In 2005, 2007, 2009, and 2010, I could not find any females in the breeding sites although, in 2007, I did find three amplectant pairs on the
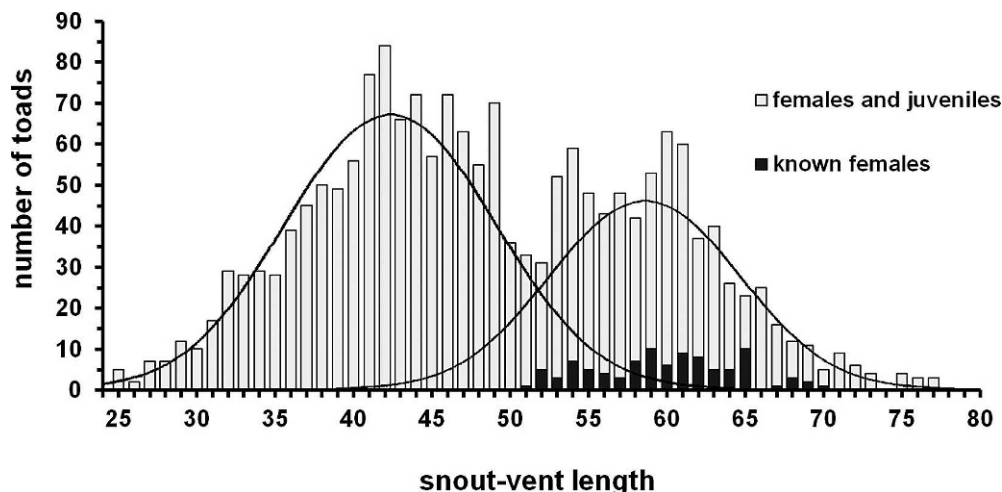

Fig. 1. Body size frequency distribution of 1,832 juvenile and female Fowler's Toads, *Anaxyrus fowleri*, captured and measured at the study site at Long Point, Ontario, over 14 years (1998–2011, incl.), including 95 adult females captured at breeding sites. The normal curves indicating the two components of the bimodal distribution were calculated using FiSAT II software.
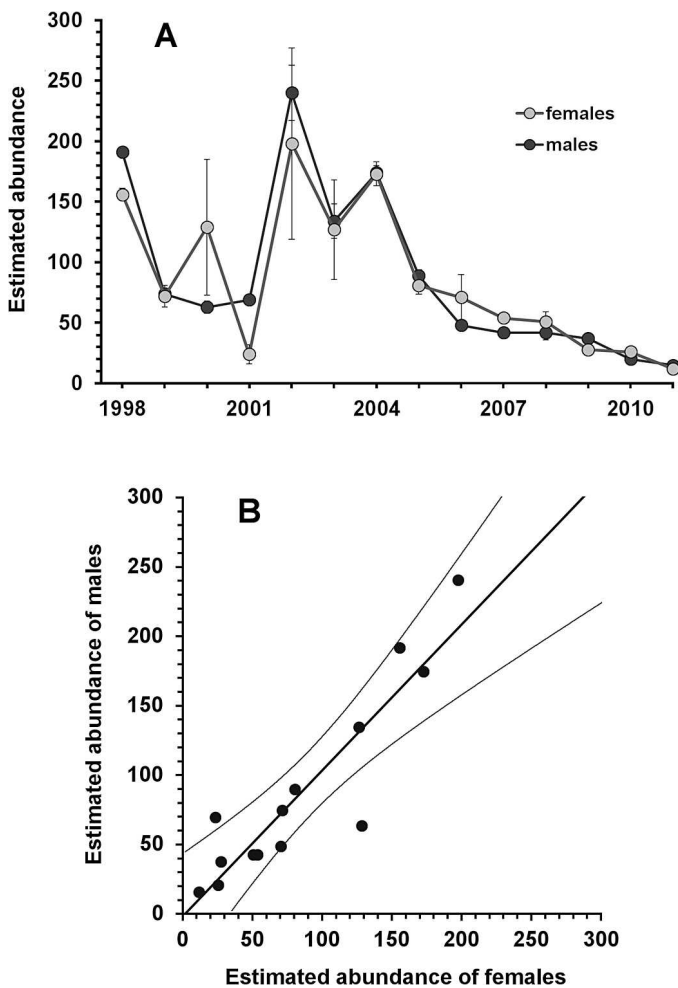
Fig. 2. Estimated abundances of adult male and female Fowler's Toads, *Anaxyrus fowleri*, in the study site at Long Point, Ontario, over 14 years (1998–2011, incl.). (A) Estimated abundances (*N*), with standard errors, of males and females. (B) Relationship between male and female abundance per year, with 95% confidence limits.

beach. These were attempting to breed in beach pools that were subsequently washed away. The proportion of females captured at breeding sites to the estimated number of females present varied among years from 0% in 2005, 2007, 2009, and 2010 to 25% in 2001. Capture probabilities for females at breeding sites could not be calculated for either 2003 or any year following 2004 due to lack of recaptures. Values of $\hat{p}$ that could be calculated averaged 0.022, ranging from 0.005 in 2004 to 0.039 in 1999 (Table 2), and were significantly different from $\hat{p}$ values for females on the beaches during those same years (t-test, $P = 0.002$). Estimated female abundance at breeding sites averaged 23 ± 12 [SE] individuals, ranging up to 153 ± 96 [SE] in 2002, but with such low values of $\hat{p}$, and consequently high standard errors, that none of these estimates of female abundance at breeding sites should be considered accurate.

The year-by-year estimates of $\hat{N}$ for each sex (Table 1) strongly indicated a near 1:1 physical population sex ratio among the adult toads. The slope of the relationship between male and female abundance per year (Fig. 2B) was 1.05, which was not significantly different from a slope of 1 ($P = 0.707$). Though I captured 1.43 individual males for every female overall, the average annual ratio of males to

females based on $\hat{N}$ was 1.12:1 and ratio of total estimated males to females was 1.03:1 (Table 1).

At the breeding sites, males outnumbered females by a margin of 6.03:1 and were captured 10.06 times more frequently (1,077 captures of males compared to 107 captures of females). Observed sex ratios among toads captured at breeding sites in any one year ranged from 15.75:1 in 1998 to 2.77:1 in 1999. Sex ratios based on estimates of abundance at breeding sites, if calculable, were in most cases substantially above 1:1, averaging 5.34 males per female (Table 2), but the correlation between male and female abundance at breeding sites per year was not significant ($P = 0.169$; $R^2 = 0.341$).

DISCUSSION

The beachfront existence of the Fowler's Toads at Long Point outside of the breeding season provides a rare opportunity to accurately census the abundances of both sexes year after year. With accurate censuses based on individual capture histories, I have shown that the physical population sex ratio is indistinguishable from 1:1 regardless of variations in overall abundance. This indicates that the male-biased sex ratio observed in breeding choruses is due to differential behavior on the part of males vs. females at breeding sites and not to differential reproductive readiness and/or mortality.

The probability of capturing females at breeding sites is significantly different from, and lower than, capture probabilities either of males at the breeding sites or of both sexes on the beach, but this does not appear to be because the males are unusually conspicuous but, rather, because the females are more cryptic at the breeding sites and thus very difficult to find. The total number of breeding females, furthermore, must be considerably larger than it would seem to be based solely on the numbers of individuals I captured at breeding sites since it is unlikely that the population could be maintained by the reproductive effort of only a handful of females per year. My estimates of abundance based on individual capture histories certainly indicate this. I did tend to observe obviously gravid females along the beach early in the season, soon after the toads' emergence from winter dormancy. Many, though not all, of these individuals were also caught soon after at breeding sites. Later in the season, I tended only to record non-gravid females on the beach, including females I had caught at a breeding site. Clearly females spend little time at the breeding sites and I had missed where and when these animals may have deposited their eggs.

On the beaches, the equal capture probability of male and female Fowler's Toads is consistent with a lack of significant behavioral differences between the two sexes away from breeding sites. There likewise appears to be little difference in difficulty between catching males in the breeding sites compared to the beaches. This is a bit surprising in that breeding males actively attempt to make themselves conspicuous to other toads. The similar values of $\hat{p}$ for males in breeding sites and on the beaches reflect both the lack of any attempts to hide by the animals when foraging on the beach at night and the persistence of the animals at breeding sites during the breeding season.

Accurate estimation of the numbers of adult individuals in anuran populations is not easy. For most pond-breeding species, breeding sites may provide the only opportunity to encounter a reasonably high proportion of a population

Table 2. Survey Results and Sex Ratios for Female and Male Fowler's Toads at Breeding Sites at Long Point, Ontario, Including Numbers of Individuals, Total Captures, Capture Probabilities, and Estimates of Abundance Where Possible.

Year	Individuals captured at breeding sites			Captures at breeding sites ($\hat{p}$)				Estimated abundance at breeding sites±SE		
	♂♂	♀♀	ratio*	♂♂		♀♀		♂♂	♀♀	ratio*
1998	126	8	15.75	264	(0.065)	11	(0.027)	140±5	12±4	11.67
1999	36	13	2.77	100	(0.097)	19	(0.039)	44±2	21±6	2.10
2000	40	8	5.00	79	(0.074)	9	(0.013)	48±4	26±19	1.85
2001	50	6	8.33	121	(0.093)	8	(0.035)	57±3	9±4	6.33
2002	101	20	5.05	144	(0.036)	22	(0.010)	168±18	95±58	1.77
2003	48	5	9.60	77	(0.057)	5	—	66±7	5	13.30
2004	91	26	3.50	153	(0.039)	28	(0.005)	124±10	153±96	0.81
2005	15	0	—	18	(0.021)	0	—	38±17	0	—
2006	15	3	5.00	30	(0.102)	3	—	16±1	3	5.33
2007‡	16	0	—	31	(0.101)	0	—	17±2	0	—
2008	6	2	3.00	13	(0.164)	0	—	6±1	0	—
2009	5	0	—	10	(0.087)	0	—	5±1	0	—
2010	2	0	—	4	(0.118)	0	—	2±1	0	—
2011	10	2	5.00	33	(0.194)	2	—	10±0	2	5.00
average	40.07	6.64	6.30	77.93	(0.089)	8.23	(0.022)	80±8	23±12	5.34
total	561	93	6.03	1077		107		326	617	1.89

* sex ratio = males/females
† $\hat{p}$ = capture probability
‡ In 2007, three pairs were captured in amplexus on the beach, but none at breeding sites. These captures were not included in calculations relating to breeding sites.

when adults are otherwise dispersed and unseen the rest of the year. All survey protocols have their limitations. Call count surveys of males can be highly inaccurate at high densities (Shirose et al., 1997), whereas egg mass counts must make assumptions about the number of egg masses a female may lay (Crouch and Paton, 2000; Brede and Beebee, 2006; Phillipsen et al., 2010). Neither of these methods can account for the opposite sex without assumptions concerning sex ratio, which is generally unknown. Protocols that concentrate solely on animals entering and leaving breeding sites cannot account for animals that are present but do not breed, except for those individuals that may appear to skip breeding but return in later years (Loman and Madsen, 2011). Drift fences, though efficient for counting migrating animals, and thereby estimating relative abundance, may perturb the normal movement behaviors of the animals under study and cannot account for animals that may be
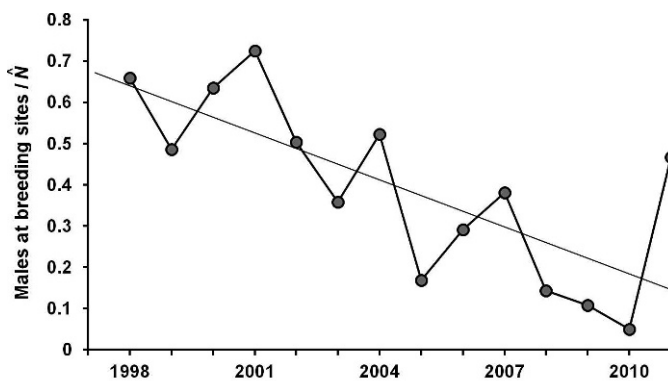

Fig. 3. Ratio of male Fowler's Toads, *Anaxyrus fowleri*, encountered at breeding sites to total estimated number of males present over 14 years (1998–2011, incl.) at Long Point, Ontario.

deflected by the fencing but not caught. The nature of the Long Point site precludes the use of drift fences, yet I was highly successful at repeatedly capturing animals along the beach outside of the breeding sites and at capturing calling males.

It is evident from my results that the assumption that all sexually mature adults appear at breeding sites (Breden, 1988) is almost certainly incorrect as not all of the animals that are present in my study population, of either sex, will breed or even appear at breeding sites. It is unlikely, though, that both sexes of Fowler's Toads would be strategically skipping breeding in the high numbers I recorded. Opting not to breed in certain years is not likely to be a viable reproductive strategy for these toads since they do not appear to live long enough, on average, to make it worthwhile. Very few of the animals in this population survive beyond three years of age (Kellner and Green, 1995; Green, 1997; Middleton, 2012), and so the payoff to females of being able to produce more, higher quality eggs by deferring breeding for a year is not outweighed by the high probability of dying in the interim. The advantage to males of deferring reproduction is difficult to fathom. If skipping breeding is unlikely as a strategy, then there must be other reasons why the adults do not all appear at breeding sites. They may vary in reproductive readiness or physical condition, or they may just get lost and fail to find a site.

At Long Point, a particular factor preventing animals from breeding has emerged in the past decade: loss of breeding sites. Progressive spread of the invasive strain of the common reed, *Phragmites australis*, has obliterated most of the high-quality, open water breeding sites previously used by the toads (Greenberg and Green, 2013). This can explain the diminishing proportion of calling males among total males present over that time. Because the effective population size, rather the census population size, is the relevant

factor related to long-term population viability (Frankham, 1995; Brede and Beebee, 2006), this trend is symptomatic of a population in decline. By extension, therefore, if breeding population size becomes substantially reduced compared to physical population size in a declining amphibian, breeding habitat loss may be a culprit to investigate.

ACKNOWLEDGMENTS

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