

SIZE, TEMPORAL AND SPATIAL DYNAMICS  
OF A NATTERJACK TOAD (*BUFO CALAMITA*) POPULATION  
IN SCANIA

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## Abstract

*The breeding season of the natterjack toad (Bufo calamita Laurenti 1768), a „prolonged breeder“, lasts up to three months in southern Sweden. However, not all reproducing individuals are present at the breeding sites during the full time span. Thus, some populations can be sub-divided into temporally isolated subpopulations using earlier or later periods of the season for reproduction. The aim of this Capture-Mark-Recapture (CMR) study on male toads was the investigation of three contiguous Bufo calamita populations in southern Sweden concerning potential temporally isolated subpopulations within each site, spatial isolation of each site from the others, the total population size compared to former estimations and differences among the three groups between the patterns of presence at the breeding sites. While there was strong evidence for spatial isolation (no migration between the sites could be observed), no clearly isolated temporal groups could be detected (high individual overlap between groups breeding earlier and later during the season). The detected different patterns of presence can be attributed to different age classes being present at the sites respectively, according to body length and weight comparison. CMR based calculations reveal more accurate population size estimates (between 94 and 104 individuals in total) than spawn string or calling counts from earlier monitoring of this area. As the maximum number of male individuals observed during one night (41 ind.) is considerably higher than the counts of former years (20-25 ind.) a population growth can be assumed.*

## Introduction

In comparison with other European toads, the natterjack toad (*Bufo calamita*) shows an extraordinary reproduction behaviour: For mating and spawning these amphibians choose shallow pools, often just temporal puddles with little or no vegetation, that desiccate frequently during drier periods. The risk of the pool drying up before the metamorphosis is completed is compensated for by the lack of competition in terms of other amphibians breeding at the same place or bigger predators living in the waters. Additionally, tadpoles develop comparatively quickly if the temperature is sufficiently high. In southern Sweden it usually takes ten to eleven weeks, though (Andrén 1985).

As a prolonged breeder the natterjack toad has an extended breeding season of up to three months in Sweden – and even more in Central Europe (Sinsch 1998) – during which several breeding bouts take place. A bout is characterised as a period of a frequently high calling activity and the presence of females at the site, framed by times without breeding activity. However, Sinsch (1998) assumes that the individual's breeding period is much shorter than the total breeding period, because some individuals become reproductively active early in the season and leave after a successful mating and

others do not appear before the second half of the season. Thus, the whole population can be split at least into "early" and "late breeders" (Sinsch 1992, 1998) and sometimes even more distinct groups have been suggested (Sinsch 1988). While the population as a whole is present at the breeding site during several months, the duration of the presence of individual males is rarely longer than 70 days, whereas individual females tend to be present for only one to five days (Sinsch 1992, 1998).

Furthermore, Sinsch's study on population dynamics suggests that there is temporal and spatial isolation between populations of natterjack toads living in a common area: The overlap and exchange of individuals between the different temporal populations, separated by different breeding strategies, is expected to be limited but detectable (Sinsch 1992). Especially in Sweden and Great Britain, females were observed to spawn twice during one breeding season (Sinsch 1998, Banks and Beebee 1986). Together with males staying over the duration of several bouts, they may cause an exchange of individuals between the subpopulations.

Also a partial spatial separation between the sub-sites of a population's area has been observed: Whereas male toads show a high pond fidelity with low exchange rates between neighbouring sites (Husté et al. 2006, Sinsch 1988), females tend to migrate more frequently, connecting the isolated subpopulations. Natterjack toads are known to overcome distances of maximum 1100m between breeding sites (Sinsch 1992).

The aim of this study is to monitor a small Scanian natterjack population, consisting of three breeding sites within an individual's potential action distance (i.e. 1000 m), during the breeding season 2012 with regard to estimation of each subpopulation's size as well as migration between them to analyse spatial isolation of the sites. Individual tagging and recording of absence and presence at the breeding pools also allows the study of temporal isolation. The male toads (Illustration 1) were captured, marked and recaptured throughout the whole breeding season in 2012, for 83 days from the end of April till the middle of July. As the tagging method involved keeping the toads in a field station over night, female toads were not part of the study because they each attended the site only during a very short period for mating and should not be disturbed.



*Illustration 1: Male natterjack toad in a breeding pool at site 3.*

## Material & Methods

### Study site

The study area (Illustration 2) was composed of three sub-sites within the Revinge military area close to Krankesjön in southernmost Sweden, 17 kilometres east of Lund, Scania.



*Illustration 2: The Map shows the three main study sites 1, 2 and 3 and the two additional subsites 1b and 3b as well as distances and obstacles between the sites. Source: Google Inc. (2012)*

Altogether, site 1 represents the typical natterjack toad habitat: It consists of small and shallow pools with nearly no vegetation, the result of military tanks driving on the sandy soil. The number of pools depends on rainfall and this year there were up to 8 pools. The maximum water depth was between 5 and 25cm, the diameters between <1m and 4m. Due to their small size all of the pools were dried out around May 23<sup>rd</sup> (Illustration 3) and some filled up again around June 23<sup>rd</sup>. Southwards, the site's border is defined by shrubs, north of the pools there is open, non-grazed grassland. Site 1 is the only one where no newts or cattle were found. *Rana temporaria* L., 1758, was breeding in one of the larger pools. As it is used as a road by military vehicles, the area had to be protected by a fence during the toads' breeding season. During the last days of the sampling period, a new pool was filled by heavy rain at 1b, another place highly frequented by tanks, and used by the toads as a breeding site as well.



Illustration 3: typical pool at site 1(left), dried-up pool at site 1(right).

In contrast, site 2 is located in a small valley with up to 6 larger but still shallow pools with densely growing submerged vegetation (Illustration 4). The water was at the most 60cm deep and the pools' lengths up to 10m. They were inhabited by other amphibians – *Rana temporaria* and *Triturus cristatus* Laurenti, 1768 – as well as by different (including predatory) insect larvae and diving beetles. No other anurans than *Bufo calamita* used these pools for reproduction purposes. Except for the largest, all pools fell almost dry in the period between the end of May and the end of June. The surroundings are dominated by grazed grassland. Fences shielded the pools from cattle as they can cause severe damage to the littoral zone and the bottom by walking through the water, aside from influencing the water quality with their manure.



Illustration 4: Left: pool at site 2 after the first bout. Right: natterjack tadpoles from the first bout.

All in all, including the larger pool at 3b, there were 8 to 10 pools containing water at site 3. They differed in size and conditions from sandy shallow basins without vegetation, similar to the ones at site 1, and temporal puddles in the woods (Illustration 5) to weed-infested, larger pools in meadows which did not fall dry. Non-breeding common and moor frogs (*Rana arvalis* Nilsson 1842) were found in and close to the pools, as well as diving beetles and crested newts that might have used the pools for reproduction. Manure was also a problem there, thus an electrical fence was installed to

keep the cattle away. However, a couple of new pools could be found after every rain period and it was not practicable to protect all of them.



*Illustration 5: a pool at site 3.*

The sub-sites 1b and 3b, being used for calling or breeding only during a very short period, are included in sites 1 and 3 respectively when it comes to statistical analysis.

### ***Field procedures***

The sampling took place during 42 nights between April 26<sup>th</sup> 2012, when the first calling male could be detected, and July 16<sup>th</sup> 2012, after which no more toads could be seen or heard at the sites. In random order the three sites were checked successively, starting just before sunset when male natterjack toads usually begin to call. Notes were taken regarding calling males and number of encountered marked and unmarked males, females and pairs

close to or in the pools.

An encountered toad was checked for a tag while it was sitting on the ground or in the water, i.e. controlled with the Oregon RFID portable reader (Illustration 6). The device was prolonged with a stick antenna that was held close to the toad's body to inspect if it had already been marked. If the toad had an injected tag, the tag number appeared on the reader's display (Illustration 7). In this case, the individuals identity was noted down. If an unmarked toad was found, i.e. the reader did not



*Illustration 6: Oregon RFID portable reader extended with stick antenna.*

give any number when being held close to the toad, it was taken to the field station and tagged. Body mass and total body length (snout-to-vent length) were measured and the animal was kept there for one night before being released after the next night's sampling.

The individual toads were detected by acoustical and visual cues with headlamps. Searching time was not limited or standardised as the efficiency depended on conditions and

searching was considered exhaustive. Lighting conditions degraded after sunset. Therefore, the searching conditions differed between the site first sampled (twilight; the toads behave more wakefully) and the one last sampled (darkness; toads keep calling when a searching person approaches). Additionally, the catching process was more time-consuming during nights of high toad activity, whereas on occasions with no calling checking could be done more quickly.

By default, the pools were controlled every night. However, if no toads could be detected during a night and the weather conditions did not change, the next night was skipped.

## ***Tagging***

Adult males were tagged with a 12mm 16-digit HDX PIT tag, which was placed directly under the loose skin on the back, about one centimetre alongside the spine on the lower half of the body. The toad was fixed with the left hand and with the right the injector (Illustration 8) was carefully pressed against a skin fold until the sharp tip produced a small cut. Then, the tag was quickly pushed in through the injector and placed in a sufficient distance from the entrance cut. It is possible to move the tag under the skin, once it is injected, until it sits firmly. The small cut was disinfected with a cotton bud immersed in iodine. The individuals were kept in a plexiglass pet box for the next 20 hours to make sure the wound was healing and the tag still sat firmly. Although the animals showed a slight discomfort during the injection process, no injuries or open wounds could be detected on the next day. No tag losses could be detected, either. The toads were released the next night at the same spot they had initially been found.



*Illustration 8: PIT tag and injector.*



*Illustration 7: 16-digit identity number of a tag on the portable reader.*

## **Statistics**

The term "population size" (N) refers to the number of toads that were present at their breeding site at least once during the total breeding period 2012 (which is assumed to be the time between April 26th and July 16th). It is known in several anuran species that adult individuals, that have previously bred, do skip breeding altogether in some years (Loman and Madsen 2010). If there were such toads in the studied natterjack populations, they would therefore not be included in the population size, N.

The population size was calculated for each site independently with 3 different methods including different assumptions about the data concerning population dynamics:

If the population can be assumed to be closed, i.e. no immigration or emigration took place during the breeding period, and the samples from the first and second bout were pooled as one sample respectively, it is possible to estimate N with the Lincoln-Petersen estimator  $N = n1 * n2 / m2$  where  $n1$  is the number of toads captured during the first bout,  $n2$  the number captured during the second bout and  $m2$  the number of toads captured in both (Schwarz 2005).

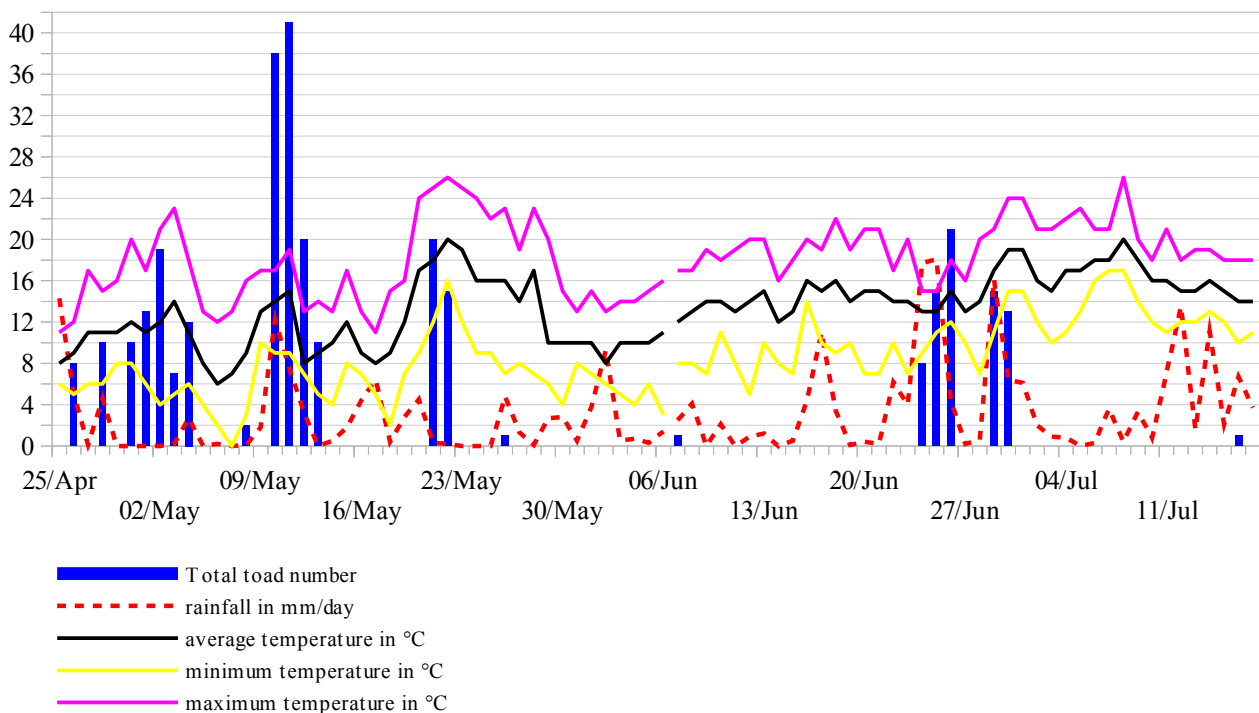
To get an alternative approach the POPAN model was run in MARK (White and Burnham 1999) in case the population was open during the whole season: The model is applicable if catchability and survival from that occasion to the next are equal for all individuals (marked and unmarked) at one sampling occasion and if the study area stays constant. In contrast to other Jolly-Seber models, POPAN reveals estimations of N (Pollock and Alpizar-Jara 2005).

Additionally, the population size was calculated with a frequency of capture model. As catchability is assumed to be equal, the Poisson method (Caughley 1977) was chosen: The observed frequencies of individuals were compared to expected frequencies according to a Poisson distribution to be able to estimate the "zero class", which is the number of individuals that were present at the site within the breeding season but never captured. With an equal catchability, the distribution of the data can be expected to be close to a zero-truncated Poisson distribution (Caughley, 1977). Therefore N is calculated as the ratio of the total number of captures ( $\sum f_i$ ) and the mean number of captures per head in the total population (X):  $N = \sum f_i / X$

## Results

### *Sampling/breeding period*

Male natterjacks could be detected in or close to the pools between April 26<sup>th</sup> and July 16<sup>th</sup>. The first breeding bout took place from the beginning until the end of May, the second from the 24<sup>th</sup> till the 16<sup>th</sup> of June (Illustration 9). The onset of breeding activity can be partially explained by a rise in temperature, partially by heavy rain falls (Illustration 9): During the earlier period, both temperature and rainfall were crucial. When the pools had been filled after the precipitation in late April, mean temperature had to be close to or above 10°C for males to start calling. In the late season, when the mean temperature was constantly above 10°C, rainfall was the limiting factor. Only after two days in a row of more than 15mm precipitation (June 24<sup>th</sup> and 25<sup>th</sup>, Illustration 9) there was enough water in the former dried up pools for the males to start calling again. All spawn strings produced during the first bout or tadpoles hatched from those were lost in the dry period in the first half of June. Late July and August were dry as well and most pools including all pools containing tadpoles dried up during the second bout (Jon Loman, personal comment).



*Illustration 9: rainfall, temperature and the total number of male toads seen during each night of sampling throughout the breeding season.*

### *Population size*

The overall number of captured male toads was 25 for site 1, 20 for site 2 and 40 for site 3. All methods for population size estimations suggest that these numbers are close to the size of the total population (Illustration 1). The differences between the results for each site's population size (maximum 4 individuals between the Lincoln-Petersen and the Poisson estimate for site 2) can be explained by the difference in the models' assumptions and weightings. Thus, the size of the whole population in 2012 can be estimated between 94 and 104 males.

*Table 1: Number of captured toads and population size estimates for the three sites.*

	Total number of captured male toads	Population estimate after		
		Lincoln-Petersen	POPAN	Poisson <sup>1</sup>
Site 1	25	29	26	26
Site 2	20	26	23	22
Site 3	40	46	48	49

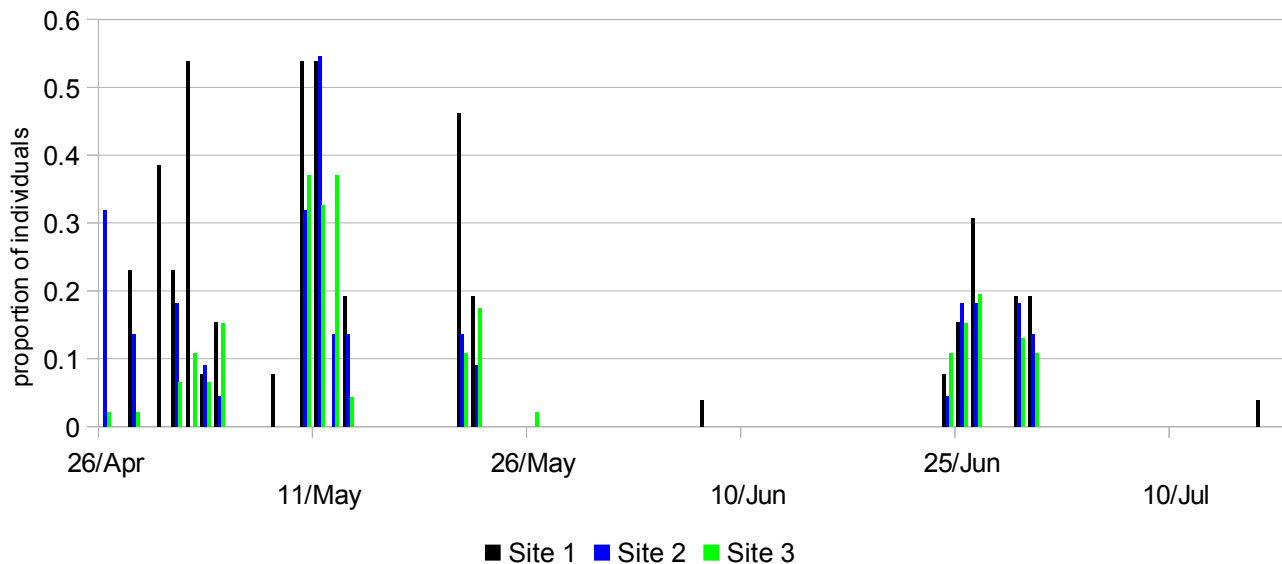
### *Patterns of presence at the breeding sites*

Comparing the proportion of the individuals of one subpopulation present at each sampling occasion reveals some differences (10): At site 3, there were never more than 40% of all toads present at the same night. Only during mid-May more than one third attended the breeding pools, at other times the number was much lower. At site 2, the largest number of males was present during the first night of activity and again around the 11<sup>th</sup> of May with more than 50% of all male toads in the population present at one night. During the other occasions, there were never more than 20% present. At site 1, however, ca. 50% of the male toads were present during 4 nights in May and had the highest proportion of presence in 13 of 19 nights with high toad activity.

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<sup>1</sup> Testing the fit of the distribution does not find it significantly different from the Poisson distribution

(site 1:  $\chi^2=4$ ,  $df=2$ ,  $0.5 < p < 0.9$ ; site 2:  $\chi^2=3.65$ ,  $df=2$ ,  $0.5 < p < 0.9$ ; site 3:  $\chi^2=3.01$ ,  $df=2$ ,  $0.5 < p < 0.9$ ).



*Illustration 10: proportion of the smallest estimated population size that was encountered at each site at the sampling occasions*

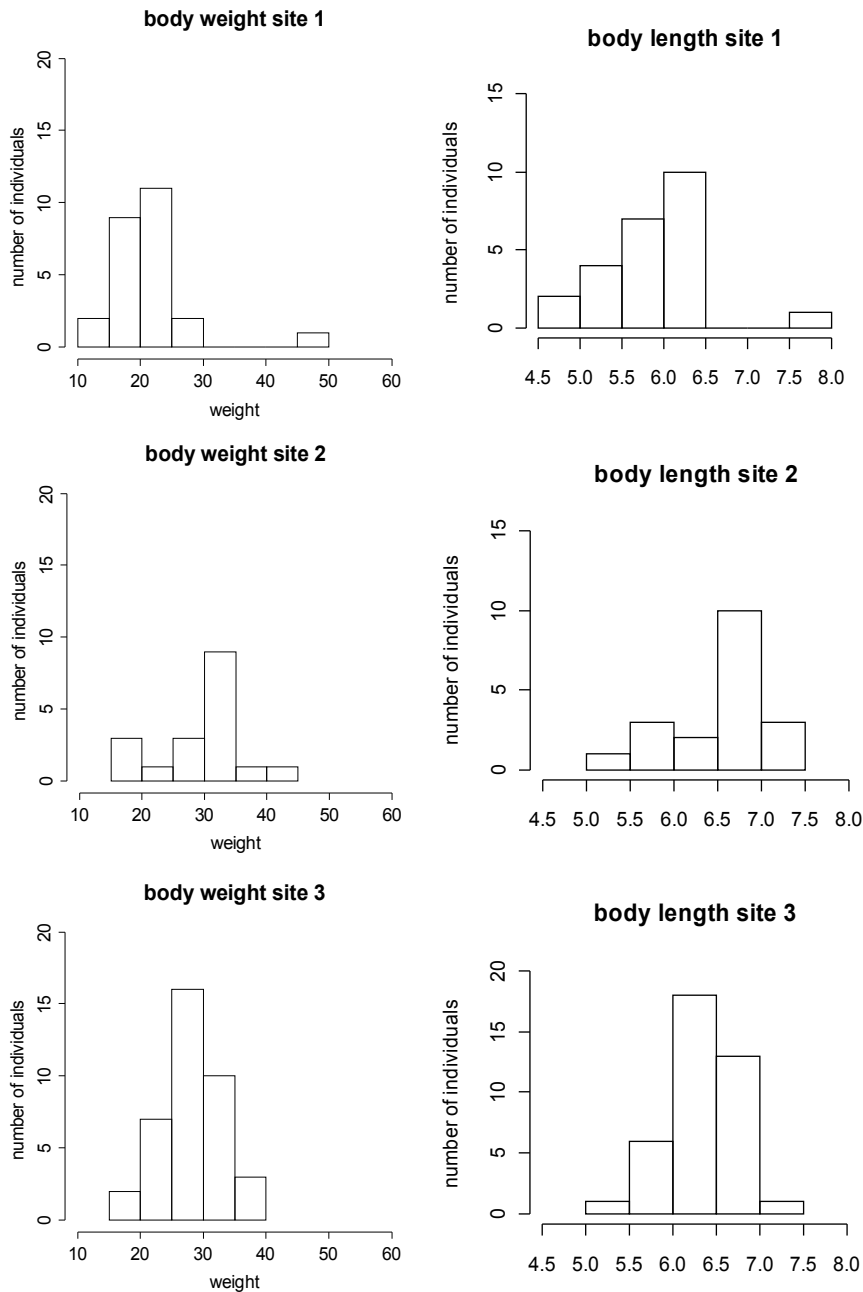
#### *Body mass and body length distribution*

The data for site 3 are almost normally distributed. The toads are smaller and lighter than at site 2 but larger and heavier than those at site 1 (Illustration 11). There is only one toad heavier than 30g and only 2 larger than 6.5cm at site 1, whereas this is the case for most of the toads at site 2. Especially the median differences between sites 1 and 2 for body mass and size each are remarkable (21.0g vs. 32.25 g; 5.93cm vs. 6.7cm).

#### *Spatial separation of potential subpopulations*

No direct migration of male toads between the three sites could be detected, although calling males from site 2 could be heard at site 3 and vice versa. However, a vivid exchange of individuals could be observed between sub-site 3b and main site 3 within and between the two breeding bouts: 11 of 40 individuals were encountered at both places within the breeding season. The distance was approximately 210metres on a grazed meadow (Illustration 2).

The sub-site 1b was not used during the first bout. Only during the last weeks of breeding activity, calling toads were heard here. While most of them had not been encountered before, one individual (ca. 4% of the site 1 population) had migrated there from the main site 1.



*Illustration 11: body weight in gram (left) and body length in centimetre (right) of the toads at each site; medians: weight: site 1 (21,00); site 2 (32,25); site 3 (28,50); length: site 1 (5,93); site 2 (6,70); site 3 (6,32)*

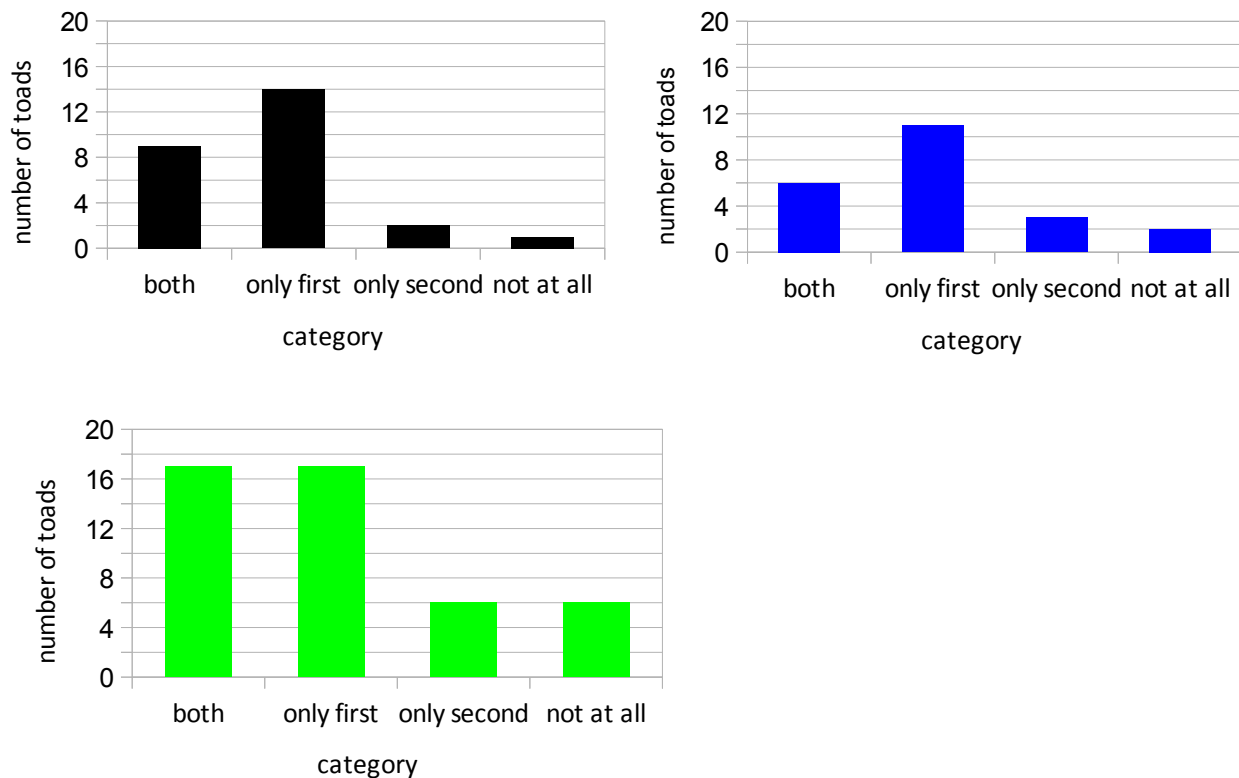
### *Temporal separation of potential subpopulations*

Concerning their temporal breeding strategy, the male toads can be divided into four encounter categories: The individuals, that were encountered during the first bout only, during the second bout only, at both bouts, and those that were present some time during the season but were not encountered during the sampling. The latter group is what makes the difference between the total

population estimate and the number of encountered toads ( Illustration 12). The proportion of individuals belonging to each category is illustrated in Illustration 12, based on the lowest population size suggested according to table 1: At least one third of all toads were present during both bouts at each site. At site 1 and 2 50% of the toads were only present during the first bout whereas just one third of the individuals at site 3 are in this category. The "only second" individuals were least represented among the toads at site 1 (8%) and only a little more common at site 2 and 3 (13% each). At site 3, 13% were never observed at a breeding pool, whereas the corresponding numbers at sites 1 and 2 were lower (4%/10%).

For all three sites the observation of a toad at one bout was independent of the toad's encounter at the other bout (Fisher's test with cell totals based on the minimum estimated population size: site 1:  $P=0.56$ ; site 2:  $P=0.61$ ; site 3:  $P=0.76$ ). The result was the same with the maximum estimated total number of toads (site 1:  $P=1$ ; site 2:  $P=1$ ; site 3:  $P=0.55$ ). Thus, the appearance of a toad at the second bout was independent of if it was part of the first.

Of the estimated total number of toads at a site, however, the proportion of individuals observed at only one bout was higher than the proportion of toads present at both bouts, independent of site (Illustration 12).



*Illustration 12: Toads at the three sites by their encounter category: site 1 (black), site 2 (blue), site 3 (green). The sum of all four bars adds up to the minimum population size estimate for each site.*

## Discussion

### *Population size*

The limited difference between the three population size estimate approaches indicates that it was possible to capture and mark almost all males that were active in 2012. To make assumptions about the population size in general, including females and individuals that did not take part in the breeding season 2012, a long-term study would be necessary.

All three approaches, Lincoln-Petersen, POPAN and Poisson, provide similar population size estimates. However, they are based on different assumptions. The Lincoln-Petersen estimation requires a closed population, in a sense that no immigration occurs between the two bouts, and that there are equal mortality and emigration rates of marked and unmarked toads. The individual numbers in the encounter category “only second” (Illustration 12) may either indicate a slight immigration or they represent toads that were present but not encountered during the first bout. The latter is unlikely for different reasons. As searching was exhaustive there are only two possibilities for not seeing an individual during a whole bout although it was present:

A) These individuals may have attended the breeding activities only before or after sampling took place at nights during the bout which they were not encountered at – so that they actually took part in both bouts. As the order in which the sites were sampled was changed each night, it is not likely that individuals were missed systematically, though.

B) These individuals were only captured during one bout and were hiding during the nights of the other bout, at least for the time span of sampling. Regarding the fact that encountered toads generally did not become frightened during searching procedures if breeding conditions were good (choruses stopped for a moment but continued even when humans stood right next to the breeding pools), it is most likely that toads which were part of a breeding bout could be encountered at least once during that bout. This is particularly the case for the first bout which lasted one month in contrast to the week-long second bout. Only if there were individuals that took part in the breeding activities just for very few nights there is a possibility worth considering that they were not encountered at all in spite of being part of the population.

Any violation of the assumption of no immigration leads to an underestimate of the population size. Still, the estimates after Lincoln-Petersen are the largest for site 1 and 2. However, the number of potentially immigrated individuals was very low at those sites (2 and 3 respectively, Illustration 12). At site 3, with 6 individuals in the category “only second” (Illustration 12), the Lincoln-Petersen estimate was lower than the other approaches'.

The question concerning an equal emigration and mortality rate of marked and unmarked toads

cannot be decided in detail. Most likely at least a part of the – marked – toads of the encounter category “only first” may have left the breeding site for the rest of the season. However, it is not possible to compare their number to the proportion of unmarked toads having left. Mortality is presumably low during the breeding period; still deaths cannot be neglected completely. Although Husté et al. (2006) detected a high mortality rate in natterjack toads especially during hibernation, they also lost some individuals during summer.

The POPAN approach accepts an open population, which in this case implies that immigration and emigration could take place in the time span between the two bouts, and it requires equal catchability for all toads during one sampling occasion. As potential immigration and emigration are discussed above, the main concern is if marked and unmarked toads are equally catchable. If it was not the case and unmarked toads were less likely to be caught, the proportion of unmarked toads would have been underestimated. If on the other hand marked toads were less catchable, for example due to their unpleasant experience of having been caught and tagged, population size would have been overestimated. Considering the small number of individuals in the category “not [observed] at all” (Illustration 12), this objection is negligible.

Furthermore, as the Poisson frequency-of-capture model also requires equal catchability of marked and unmarked toads and the p-values indicate that data are in accordance with a Poisson distribution, the requirement can be considered fulfilled.

Comparing the population's size to earlier estimates of 15–30 males in the Revinge area (Loman 2010, 2011, Berglund 2000) made on the basis of calling males and spawn strings, CMR gives larger and by far more accurate results. Whereas Grafe and Meuche (2005) and Pellet et al. (2007) state in earlier studies that chorus counts are inferior to CMR techniques when it comes to the estimation of population sizes, chorus and spawn string counts are still used for this purpose regularly (Beebee and Rowe 2001, Rannap et al. 2007). However, a growing population is an additional explanation for higher numbers in 2012. The highest number of male toads encountered during one night was 41 (May 11<sup>th</sup>, Illustration 9), a fraction of less than 50% taken into account that there were at least 94 male individuals present. Thus, an estimate based on the highest number of encountered individuals, as Loman (2010, 2011) provided, would only include less than 50% of the smallest of the population estimates. Based on calling males only, the number would have been even lower as not all 41 males were calling. The population increase could be due to the exceptionally successful recruitment at site 1 two years ago, described by Loman (2010), leading to a large group of first breeders there.

### *Breeding season*

The fact that the toads did not start calling at an average temperature below 10°C fits the observation that natterjack toads are unable to reproduce at temperatures below 10°C (Sinsch, 1998). As there was no reproductive success after the first bout, it seems to be more difficult for the toads to correctly assess a sufficient water depth or amount of rain to avoid the risk of the pools falling dry. Due to the short summer season, the time span for reproduction is relatively brief in Sweden compared to other countries (Sinsch 1998). This might explain why the toads use not quite optimal weather conditions for breeding attempts. Furthermore, it is a reason for focussing on the early part of the potential breeding season. If the natterjacks spawn too late, there is a risk for the offspring not to be able to finish metamorphosis before it gets too cold.

### *Patterns of presence at the breeding sites/Weight and body length distribution*

An explanation for the different patterns of presence at the three sites (Illustration 10) could be that they differ in age structure. Although it is Sinsch's (1998) advice not to use body mass as a factor to draw conclusions from because it depends to a great extent on if the animal is hydrated and if its bladder is filled, body length and mass show a similar distribution. Male toads usually take part in breeding activities for the first time when two or three years old (Sinsch 1997), most of them after their third hibernation (Leskovar et al. 2006). The median body length at site 1 (5,98cm) is close to the size of first breeders in Germany (5,58cm) found by Leskovar et al. (2006), especially if taken into account that some older individuals (one above 6.5cm and one even above 7.5cm, Illustration 11) are included in the former value. In contrast, the vast majority of individuals were above 6 and above 6.5 cm at site 3 and 2, respectively (Illustration 11). This obvious difference can be attributed to age classes: A poor recruitment at site 2 two years ago combined with a successful reproduction at the nearly extinct population at site 1 during the same period is described by Loman (2010). Less experienced or first-time breeders might choose a different strategy than older ones. Whereas the latter seem to sense more exactly when conditions are good enough to appear at the pool and start calling – an energy-sapping behaviour with increased exposure to potential predators – the former use every increase of temperature or rainfall to show up at the pools (10).

### *Temporal separation of potential subpopulations*

Temporal isolation of sub-groups in a population is observed not only in amphibians (Frost 1965, Cooley 2003). If a distinct temporal separation of the sites' populations in “early” and “late” breeders is possible and the exchange of individuals is low, temporally isolated populations can coexist at the same place. However, it is not clear if the concept of different temporal breeding

strategies (Sinsch 1988) applies for the populations in this study.

If a breeding strategy – being an “early” or “late” breeder – is an inherited trait (Sinsch 1997) the overall higher activity during the first four weeks can be explained by a self-perpetuating process. If the proportion of early breeders has been larger from the beginning, they will on average produce a higher proportion of offspring who become early breeders, too.

Note, however, that the breeding season in Scania (83 days in 2012) is shorter than in the studies conducted in Germany (up to 4 months, Sinsch 1998). Therefore, the individuals staying during “both” bouts in Sweden might be present at the breeding sites for the same duration as “early breeders” in Sinsch's studies. There is no indication that a toad's attendance during the second bout was dependent on the presence at the first. This would have been observed if there were clearly distinguishable “early” and “late” breeders. On the other hand, 33–50% of the encountered individuals were classified in the “first only” encounter category (Illustration 12), hence they can be called “early breeders”. Another small number of individuals is captured for the first time around late June and could be identified as “late breeders”. The ratio of individuals being present during both bouts is higher than in Sinsch's studies (33% vs. 12%, Sinsch 1992). Furthermore, Sinsch (1992) observed that of those individuals, none was actually found in amplexus during more than one bout. This raises the question if the temporal breeding strategies should be defined by actual reproduction rather than presence at a breeding site. However, as the spawn strings found during the breeding season 2012 could not be attributed to particular males, they cannot be used as an indicator of their reproductive success. The clearly detectable overlap in male individuals leads at least to the assumption that there is an exchange between the toads breeding in April/May and those breeding in June. To conclude, a clear temporal separation cannot be detected for any of the three sites' populations.

#### *Spatial separation of potential subpopulations*

The spatial isolation was quite obvious in contrast to the temporal. It was observed that natterjack toads are able and willing to overcome distances of more than 600 metres if necessary. Although no direct migration between the main sites could be observed, it has to be taken into account, that the distance between site 1 and 1b, where exchange took place, is not much smaller than the distance between the sites 2 and 3. In both cases it was possible to hear the other site's choruses clearly which might affect the toads' willingness to migrate (Sinsch 1988), but the area between site 2 and 3 is differently structured. Whereas there is just open, flat grassland between site 1 and 1b, a wood and a height gradient can be found between 2 and 3, reducing the strength of sound.

## Conclusion

Despite the failure of all breeding attempts in 2012, there seems to be a growing population of currently about 100 male natterjack toads in the Revinge area. Most likely, the three sites are connected through an occasional exchange of individuals. An investigation of the gene-flow would allow more detailed conclusions about population boundaries (Rowe and Beebee 2007) and a metapopulation structure.

Overall, statements about population dynamics of the underlying group can hardly be concluded with certainty and are not easily transferable from studies in other parts of Europe. Further (long-term) observations, especially including females, could be more illuminating. The use of PIT tags allows long-term observations of the male individuals encountered during this year, which makes it possible to investigate questions about breeding site fidelity, breeding frequency, mortality etc. over several years. Additionally, the hypothesis that the temporal breeding strategy will stay the same throughout an individual's life (Sinsch 1998) could be studied.

Finally, the results of this study emphasise the superiority of the capture-mark-recapture methods over chorus counts or approaches based on the maximum number of individuals encountered during one night. As all of these methods are frequently used for monitoring purposes in amphibians, an evaluation of their preciseness is helpful.

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