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**Report concerning green frogs near Hannäs (enligt ert beslut 2004-04-15, 522-1923-04)**

Dear Mr. Jonsson

After our preliminary report (June 2004) concerning the green frog population near Hannäs, we herewith send you the final report on these frogs.

We have caught a total of 40 Ätlig grodor (*Rana esculenta*) at Lindalsgölen near Hannäs, Östergötlands Län in the nights of June 6 and 7, 2004. The animals were measured and a tissue and blood sample was taken. Some animals were photographed by Jan Pröjts, Ekologgruppen i Landskrona AB. Within 12 hours, the frogs were released again at Lindalsgölen.

Here are in short our main findings:
Contrary to our expectations when measuring the animals and judging from looking at them in 2004, there were no triploid animals in this population. According to our results, this population is a mixed *R. esculenta* / *R. lessonae* system. This makes it a unique population in Sweden, since near Uppsala there are only pure *R. lessonae* populations and in Skåne we find only pure *R. esculenta* populations. We also performed genetic analyses, concerning the origin of this population. Genetic diversity was very low, but comparable to other populations in Skåne. A possible introduction of these animals from Western Europe (France or the Netherlands) can be excluded. Genetically these frogs are similar to individuals from the Baltic States and North Germany. Unfortunately genetic data did not confirm the link-status of this population between Skåne and Uppsala. Summarizing these results, we regard this population as very interesting and advise to take good care of it (possible habitat management for dispersal).

We investigated this population together with Jan Pröjts, Ekologgruppen i Landskrona AB and he knows very much about it already including our results. We therefore suggest getting in touch with him for possible improvement of the habitat or additional information. The completion of our PhD-theses has been slightly delayed, but they will be published in the next few months. The data on this population will be part of a chapter in each of the theses. Both theses focus on the pure *R. esculenta* populations in Skåne, but if you wish, we are happy to send you a copy of them once it is published.

But since it has not been published yet, we kindly ask you not to publish the specific data without contacting us first. Otherwise it can lead to problems when we're trying to publish the data in a scientific journal. But of course it's no problem to publish „unspecific" data, such as the finding of water frogs in Östergötland.

Attached you will find a detailed report on the findings. If you have questions about some of the results or further interest please don't hesitate to contact us. We would like to thank you again for the permission to catch these frogs and are confident that it was very well worth it. We are also very grateful to Jon Loman, who helped us with the application for permission and shared his knowledge about the green frogs with us.

Best wishes,

Christian Jakob & Martina Arioli
Dipl. natw. ETH Dipl. zool.
PhD student PhD student

Detailed information about green frogs found near Hannäs

Population composition:
In Lindalsgölen, east of Hannäs, a total of 40 green frogs were caught: 15 adult females, 2 adult males, and 23 juveniles. A tissue sample was taken by clipping a part of the animal's second toe, which was used for genetical analyses using microsatellites. Also, a blood sample was taken from between the toes (<20 microliters), which was used to determine genotype and ploidy (pure populations of *Rana esculenta* consist of diploid and triploid animals). The results are shown in Table 1. Among the females 10 individuals were LR and 5 were LL and both males were LL. Among the juveniles most individuals were LR (19 individuals) and 4 frogs were LL.
Based on the population composition, this population seems extraordinary. This is to our knowledge the only *R. esculenta* / *R. lessonae* - system found in Sweden. Interestingly, this system (LE) is also the most common population composition found at about the same latitude in the Baltic States. This could indicate that it is well adapted to conditions at this latitude.
As suggested earlier, the abundance of juvenile green frogs at Lindalsgölen indicates that the pond supports a reproducing population of green frogs. We are not aware of further visits to this pond since 2004, but it would be very interesting to know how the population has developed since. For such information a visit during mating season would be necessary. Males are calling during the night in this time and are therefore relatively easy to be counted. Additionally, observations of tadpoles or metamorphs could give information about the viability of this population. Considering that we did not find any other ponds in the adjacent area with green frogs, although the ponds' conditions would have been similar at first look and although distances between the ponds would be appropriate for spreading, makes this population nonetheless vulnerable. We therefore strongly recommend the consider revaluation of surrounding areas in order to facilitate dispersal to other ponds. Attached you will also find the results of the water chemistry analyses (Table 2 & 3).

Population genetics:
We investigated the population with eight microsatellite loci. In all loci but one, the alleles were fixed (i.e. only one allele was found throughout all animals). So, genetic diversity is rather low in this population. However, this is not surprising, since in most other Swedish populations the genetic diversity is also rather low. None of the detected alleles in Hannäs were population-specific. Results from the population genetic analysis indicated that frogs from Hannäs clusters on one hand with individuals from the Baltic States as well as with individuals from the coast of northern Germany. This might seem a bit odd on first looks, but the whole area around the Baltic Sea was genetically not as structured as we have expected, implying that there has been genetic exchange for quite along time.
Additionally, we also sequenced two genes of the mtDNA, which are less variable than the nuclear DNA microsatellites. Interestingly, the frogs from Hannäs showed a unique haplotype which was differing only slightly from the haplotypes found in Southern Sweden.

In conclusion, the results from our analyses suggest that the Hannäs frogs are native. However, introduction from somewhere around the Baltic Sea can not be ruled out completely, although Skåne is definitely not the place of origin and they are certainly not introduced from Western Europe. We therefore recommend protecting this population, based on its status as a remaining population of a larger distribution in the area along the Eastern Coast of Sweden.

| Date | Frog No | Sex | SVL | Tibia | F-Hoecker | Zehe | Weight | Genotype |
|---|---|---|---|---|---|---|---|---|
| 06.06.04 | Östergötl-401-001 | F | 81.06 | 37.62 | 5.40 | 10.00 | 50.5 | LR |
| 06.06.04 | Östergötl-401-002 | F | 82.86 | 39.24 | 5.20 | 10.76 | 67.5 | LR |
| 06.06.04 | Östergötl-401-003 | F | 64.71 | 27.40 | 4.37 | 8.12 | 25.5 | LL |
| 06.06.04 | Östergötl-401-004 | F | 75.32 | 36.81 | 5.02 | 8.79 | 48.5 | LR |
| 06.06.04 | Östergötl-401-005 | F | 86.79 | 40.94 | 5.90 | 11.10 | 76.0 | LR |
| 06.06.04 | Östergötl-401-006 | F | 81.35 | 39.46 | 5.29 | 10.33 | 62.0 | LR |
| 06.06.04 | Östergötl-401-007 | F | 60.93 | 26.86 | 4.59 | 7.60 | 21.5 | LL |
| 06.06.04 | Östergötl-401-008 | F | 79.31 | 37.98 | 5.29 | 10.08 | 57.5 | LR |
| 06.06.04 | Östergötl-401-009 | F | 71.12 | 29.52 | 4.97 | 8.90 | 31.5 | LL |
| 06.06.04 | Östergötl-401-010 | F | 91.00 | 43.45 | 6.15 | 11.94 | 81.0 | LR |
| 06.06.04 | Östergötl-401-011 | F | 58.43 | 25.62 | 4.07 | 8.07 | 19.5 | LL |
| 06.06.04 | Östergötl-401-012 | M | 61.31 | 26.03 | 4.76 | 7.71 | 21.0 | LL |
| 06.06.04 | Östergötl-401-013 | Juv | 42.52 | 21.05 | 2.69 | 5.52 | 7.5 | LR |
| 06.06.04 | Östergötl-401-014 | Juv | 46.94 | 22.72 | 3.12 | 6.25 | 9.5 | LR |
| 06.06.04 | Östergötl-401-015 | Juv | 47.87 | 22.39 | 2.91 | 6.05 | 9.5 | LR |
| 06.06.04 | Östergötl-401-016 | Juv | 49.09 | 22.52 | 2.86 | 5.99 | 9.5 | LR |
| 06.06.04 | Östergötl-401-017 | Juv | 43.13 | 21.86 | 3.17 | 6.31 | 9.0 | LR |
| 06.06.04 | Östergötl-401-018 | Juv | 43.96 | 20.62 | 2.39 | 5.83 | 7.0 | LR |
| 06.06.04 | Östergötl-401-019 | Juv | 44.96 | 22.25 | 2.98 | 6.26 | 8.5 | LR |
| 06.06.04 | Östergötl-401-020 | Juv | 45.72 | 22.15 | 2.75 | 6.18 | 8.0 | LR |
| 06.06.04 | Östergötl-401-021 | Juv | 41.44 | 20.29 | 2.31 | 5.92 | 6.5 | LR |
| 06.06.04 | Östergötl-401-022 | Juv | 44.67 | 21.08 | 2.53 | 6.15 | 7.5 | LR |
| 06.06.04 | Östergötl-401-023 | Juv | 46.39 | 21.71 | 2.83 | 6.28 | 8.5 | LR |
| 06.06.04 | Östergötl-401-024 | Juv | 40.73 | 18.98 | 2.47 | 5.37 | 5.0 | LR |
| 06.06.04 | Östergötl-401-025 | Juv | 43.33 | 20.66 | 2.28 | 5.95 | 6.5 | LR |
| 06.06.04 | Östergötl-401-026 | Juv | 41.98 | 19.83 | 2.42 | 5.94 | 6.5 | LR |
| 06.06.04 | Östergötl-401-027 | Juv | 40.93 | 19.29 | 2.52 | 5.65 | 5.5 | LR |
| 06.06.04 | Östergötl-401-028 | Juv | 39.35 | 18.90 | 2.54 | 5.45 | 6.0 | LR |
| 06.06.04 | Östergötl-401-029 | Juv | 38.88 | 18.80 | 2.43 | 5.55 | 5.0 | LR |
| 06.06.04 | Östergötl-401-030 | Juv | 37.05 | 17.51 | 2.43 | 4.84 | 4.0 | LR |
| 06.06.04 | Östergötl-401-031 | Juv | 34.66 | 15.11 | 2.33 | 4.36 | 3.5 | LL |
| 06.06.04 | Östergötl-401-032 | Juv | 36.11 | 15.67 | 2.33 | 4.85 | 4.0 | LL |
| 06.06.04 | Östergötl-401-033 | Juv | 35.48 | 15.08 | 2.19 | 4.63 | 4.0 | LL |
| 06.06.04 | Östergötl-401-034 | Juv | 31.24 | 14.44 | 2.16 | 4.33 | 3.5 | LL |
| 06.06.04 | Östergötl-401-035 | Juv | 35.00 | 15.77 | 2.17 | 4.65 | 3.5 | LR |
| 07.06.04 | Östergötl-401-036 | F | 85.75 | 39.37 | 5.76 | 10.68 | 70.5 | LR |
| 07.06.04 | Östergötl-401-037 | F | 85.27 | 39.37 | 5.43 | 10.25 | 61.5 | LR |
| 07.06.04 | Östergötl-401-038 | F | 63.90 | 29.29 | 5.56 | 8.62 | 29.5 | LL |
| 07.06.04 | Östergötl-401-039 | F | 74.77 | 37.55 | 5.22 | 9.95 | 49.5 | LR |
| 07.06.04 | Östergötl-401-040 | M | 58.28 | 26.22 | 4.16 | 7.96 | 20.0 | LL |

**Table 1 Specific frog data** PLEASE DO NOT PUBLISH WITHOUT AGREEMENT BY THE AUTHORS

Explanation of the table header:
Date: Date of catching
Frog No.: Internal frog number, arbitrary
Sex: F=Female (adult), M=Male (adult), Juv=Juvenile
SVL: Snout-Vent length (body size) in mm
Tibia: Tibia length in mm
F-Hoecker: length of callus internus in mm
Zehe: length of digitus primus in mm
Genotype: Assignment by flowcytometry and genetic analysis
Genotypes: LL = *Rana lessonae*, LR = diploid *Rana esculenta*

We have taken measurements of dissolved Oxygen, pH and water temperature in situ at Lindalsgölen, Fallaviken, and at two spots of Kyrkviken. Results are shown in table 2. At the same spots, we have collected water samples to analyse them in our lab. We have checked the filtered water samples on total amount of phosphorous, total amount of nitrogen, total carbon by total inorganic (TIC) and total organic carbon (TOC), as well as water hardness and the amounts of calcium and magnesium. These results are displayed in table 3. We can provide you with a detailed description of material and methods used for water analysis if requested.

| | Datum | Time | T Water (°C) | O2 (%) | O2 (mg) | pH | Weather |
|---|---|---|---|---|---|---|---|
| Lindalsgölen | 06.06.04 | 16:00 | 23.1 | 93.2 | 7.86 | 5.36 | Sunny |
| Vindommen Fallaviken, Pier | 07.06.04 | 15:30 | 19.3 | 94.0 | 8.62 | 6.84 | Cloudy |
| Vindommen Kyrkviken Norr, Vattentag | 07.06.04 | 15:55 | 20.0 | 62.5 | 5.65 | 6.68 | Cloudy |
| Vindommen Kyrkviken Söder, Bro | 07.06.04 | 16:10 | 19.6 | 87.9 | 8.01 | 6.67 | Cloudy |

Table 2 Results of in situ measurements

Water temperatures were quite high, compared to those in Skåne at the same time. Especially Lindalsgölen, which appears to be quite deep and which is surrounded by forest was quite warm. The relative amount of dissolved oxygen in the water was moderate, only at Kyrkviken Norr at the Vattentag it seemed low, probably because of the amount of organic material composing in the Phragmites belt. Relative dissolved oxygen seems to be related to tadpole and egg survival, but the amounts seemed low, but not (yet) too low, even at Kyrkviken Norr. The range of pH is also found in our ponds in Skåne, where we find pH from about 5.3 to over 9 in ponds with *R. esculenta*.

| | Date | Time | Total N (mg/l) | Total P (mg/l) | Total C (mg/l) | TIC (mg/l) | TOC (mg/l) | Hardness (°dH) | Ca2+ (mg/l) | Mg2+ (mg/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| Lindalsgölen | 06.06.04 | 16:00 | 0.94 | 0.043 | 32.8 | 8.5 | 24.3 | 2.51 | 17.9 | 0.00 |
| Vindommen Fallaviken, Pier | 07.06.04 | 15:30 | 1.02 | 0.056 | 18.8 | 9.6 | 9.2 | 2.23 | 15.9 | 0.00 |
| Vindommen Kyrkviken Norr, Vattentag | 07.06.04 | 15:55 | 1.34 | 0.079 | 24.7 | 12.5 | 12.2 | 2.50 | 17.8 | 0.00 |
| Vindommen Kyrkviken Söder, Bro | 07.06.04 | 16:10 | 1.70 | 0.068 | 22.2 | 10.6 | 11.6 | 2.23 | 15.9 | 0.00 |

Table 3 Results of filtered water samples' analyses

As expected, the sampled waters turned out to be low (Fallaviken and Lindalsgölen), or low to moderate on nutrients and showed an also low amount of calcium, so water hardness is very low. Mg2+ ions were only present in traces. Lindalsgölen showed the highest amount of dissolved organic carbon, which is also expectable concerning the nature of this pond. All in all the results range at the lower end of the scale we experience in the ponds we observe in Skåne, but are definitively suitable for green frogs.

Note: Our measurements are only depicting one single point in time and space, and therefore are only of limited power. Repeated measurements would add statistical confidence.