NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS
SCHOOL OF SCIENCE
FACULTY OF BIOLOGY
DEPARTMENT OF BOTANY

Conservation of priority forests and forest openings in "Ethnikos Drymos Oitis" and "Oros Kallidromo" of Sterea Ellada
LIFE11 NAT/GR/1014 - "ForOpenForests"

ACTION A.4.

Study of population dynamics of the priority plant species *Veronica oetaea**

DELIVERABLE A.4.1.

Population dynamics of *Veronica oetaea*

Pinelopi Delipetrou, Ilias Dimitriadis, Katerina Koutsovoulou, Costas Thanos, Kyriacos Georghiou

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ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
ΣΧΟΛΗ ΘΕΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ
ΤΜΗΜΑ ΒΙΟΛΟΓΙΑΣ
ΤΟΜΕΑΣ ΒΟΤΑΝΙΚΗΣ

Διατήρηση δασών και ανοιγμάτων προτεραιότητας στον "Εθνικό Δρυμό Οίτης" και στο "Όρος Καλλίδρομο" της Στερεάς Ελλάδας
LIFE11 NAT/GR/1014 - "ForOpenForests"

ΔΡΑΣΗ Α.4.
Μελέτη της δυναμικής των πληθυσμών του είδους προτεραιότητας *Veronica oetaea**

ΠΑΡΑΔΟΤΕΟ Α.4.1.

Δυναμική πληθυσμών της *Veronica oetaea*

Πηνελόπη Δεληπέτρου, Ηλίας Δημητριάδης, Κατερίνα Κουτσοβούλου, Κώστας Θάνος, Κυριάκος Γεωργίου

Field work: Delipetrou P., Dimitriadis I., Koutsovoulou K., Kapetanakis G., Georgiadou D., Kalogeropoulos E.

Proposed reference: Delipetrou P., Dimitriadis I., Koutsovoulou K., Thanos C., Georghiou K. 2015. Population dynamics of *Veronica oetaea*. Deliverable A.4.1. for the project LIFE11 NAT/GR/2014 - ForOpenForests. National and Kapodistrian University of Athens - HSPN, 20 p.

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SUMMARY

Population dynamics of the priority plant species *Veronica oetaea* were studied for 2 years at the ponds Livadies, Greveno and Alykaina using a 2x2 grid covering the whole area of the ponds. The results were the following:

1. Phenology. The plant appears after the snow melts in May to early June, depending on the year and pond. The life cycle is completed by late July.
2. Spatial distribution. The spatial distribution of the plant varied between the 2 years regarding both the position and the number of cells occupied but differently in each pond. In Livadies the plant is restricted to dephts c. 10–30 cm.
3. Population size. Population size varied greatly between the 2 years. A total of 16975 and 68976 individuals were counted in the 1st and the 2nd year respectively. This corresponds to a respective estimated total population size of 271600 and 1103616 individuals.
4. Soil seed bank. Soil samples were collected in both years but the determination of the methodology of their study was delayed due to the time consuming procedure for determining an efficient germination method. After a preliminary test, the study is under way.

ΠΕΡΙΛΗΨΗ

Η δυναμική πληθυσμών του είδους προτεραιότητας *Veronica oetaea* μελετήθηκε για 2 έτη στα λιμνία Λιβαδιές, Γρεβενό και Αλύκαινα με τη χρήση πλέγματος 2x2 που κάλυπτε ολόκληρη την περιοχή των λιμνίων. Τα αποτελέσματα ήταν τα ακόλουθα:

1. Φαινολογία. Το είδος εμφανίζεται αφού λιώσουν τα χιόνια τον Μάιο ή νωρίς τον Ιούνιο ανάλογα με τη χρονιά και το λιμνίο. Ο κύκλος ζωής του ολοκληρώνεται μέχρι το τέλος του Ιουλίου.
2. Χωρική κατανομή. Η χωρική κατανομή του είδους διέφερε μεταξύ των 2 ετών και ως προς τη θέση και ως προς τον αριθμό των κελιών παρουσίας του, αλλά με διαφορετικό τρόπο σε κάθε λιμνίο.
3. Μέγεθος πληθυσμού. Το μέγεθος του πληθυσμού παρουσίασε μεγάλη διακύμανση μεταξύ των 2 ετών. Μετρήθηκαν συνολικά 16975 άτομα και 68976 άτομα κατά το 1^{ο} και 2^{ο} έτος, αντίστοιχα. Αυτό αντιστοιχεί σε εκτιμώμενο συνολικό μέγεθος πληθυσμού 271600 ατόμων και 1103616 ατόμων.
4. Εδαφική τράπεζα σπερμάτων. Συλλέχθηκαν δείγματα κατά τα 2 έτη μελέτης, αλλά ο καθορισμός της μεθοδολογίας της μελέτης καθυστέρησε λόγω της χρονοβόρας διαδικασίας του καθορισμού μιας αποτελεσματικής μεθόδου για τη φύτρωση του είδους. Μετά από προκαταρκτικές δοκιμασίες, η μελέτη της τράπεζας σπερμάτων είναι τώρα υπό υλοποίηση.

1. Introduction

Veronica oetaea is an annual dwarf plant species restricted to the vernal pools of Mount Oiti. It is an ephemerous, amphibious species specialised to temporary ponds (vernal pools) and it requires the alternation of a wet and a dry phase in order to complete its life cycle. It occurs in three small high altitude ponds, named Livadies, Greveno and Alykaina.

Veronica oetaea is a priority plant species of Annex II Directive 92/43/EEC and it is also protected by the Bern Convention. It is a threatened plant, characterised as Critically Endangered according to the IUCN Criteria (Καρέτσος 2009). Its habitat, is also a priority habitat of Annex I Directive 92/43/EEC entitled 3170* - Mediterranean temporary ponds.

The study of population dynamics is essential in order to understand the ecology of the species and assess its conservation status and the factors that affect it. Interannual variation is a common feature both in temporary pond community floristic composition (Grillas et al. 2004a, Deil 2005) and in the population size of annual plant species (Kiviniemi and Lofgren 2009). Meteorological conditions as well as sedimentation procedures and also the soil seed bank are the factors that affect interannual variation in temporary pond communities (Grillas et al. 2004a, Deil 2005). At species level, the soil seed bank is a means of perennating for annuals (Begon et al. 1996) and of persistence despite environmental stochasticity (Kalisz and McPeek 1993).

The study of the population dynamics of *Veronica oetaea* was based on phenological observations, spatial distribution determination, population size measurements and soil seed bank assessment coupled with hydrogeological data.

It must be noted that:

- The study of reproductive biology by marking of individuals and by counting of seedlings was not possible due to the small size (0.5 – 2 cm, rarely up to 5 cm) and fragility of the plants and to the extremely small size of the seedlings (1 mm).
- The relationship of population counts to meteorological data by comparison of the data for the first 2 years was not possible because the meteorological station of Mt Oiti was established after the first year. This relationship will be explored using the counts of the subsequent years.
- The study of the soil seed bank has not been completed due to reasons mentioned in the respective section.
- The species presented

Due to the above and due to the very large population size fluctuations obeserved, Deliverable A.4.1. will be updated for the final report of the project with data spanning the 5 year period. This will be done partly within action D1 and with no additional expenses.

2. Phenology of *Veronica oetaea*

Methodology

The phenology of *Veronica oetaea* was recorded by observations of the plant at the three ponds, Greveno, Livadies and Alykaina, for 2 years (2013, 2014) twice a month in May, June, and July and monthly in September and October. There have been no visits in August when the ponds are completely dry and the plant has completed its life cycle. Visiting the ponds is not possible or very diffiicult from November to April.

The plant starts to be visible in the field only when the shoot has grown, just before flowering or in flower and it is also visible in fruit. Seedlings, i.e. plants soon after germination with cotyledons, are too small to be visible in the field. Thus, seedling emergence could not be observed and the timing of the germination phase was deduced from germination experiments and the onset of flowering.

Results

The phenological cycle (according to Grillas et al. 2004b) of *Veronica oetaea* at each of the three ponds is presented in Figure 1a-c.

Veronica oetaea appears when the ponds start to dry up (Figure 2a) and the soil is waterlogged but not flooded (i.e., the water level of the ponds is 0 – 0.5 cm). The plants do not appear simultaneously throughout the area of the ponds, but asynchronously depending mainly on water depth. Each plant flowers and sets fruit (Figure 2b,c) within 2 – 3 weeks. The fruits mature and seed dispersal takes place approximately 2 – 4 weeks after fruit setting.

Germination experiments (action C7) indicate the plant germinates in spring and temperature is the main regulator of the timing of germination. It seems that temperature increase after the winter temperature lows or a certain long period of low temperature along with water level decrease trigger germination. It also seems that, after germination the plant soon grows, flowers and fruits irrespective of the subsequent temperature and water level fluctuations. In some years, the ponds start to dry in early to late May and *Veronica oetaea* appears but subsequent heavy rain may cause the ponds to flood again, at least partly. This was observed only in 2015. Apparently, it does not stop the development of the plant (from flower to fruit) but it is not known whether it triggers a second germination event.

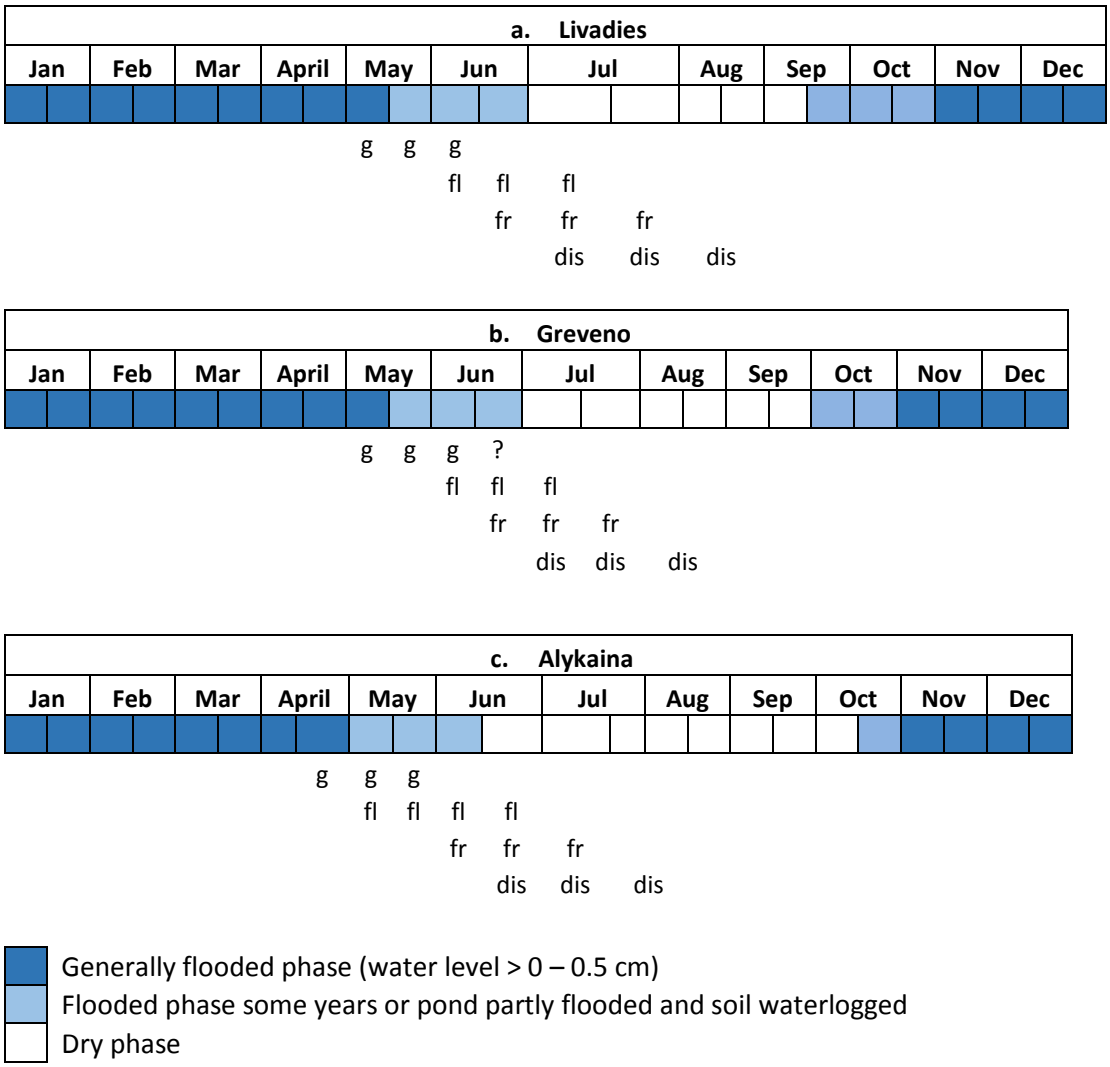


Figure 1. Phenological cycle of *Veronica oetaea*. g: germination, fl: flowering, fr: fruiting, dis: seed dispersal.

Figure 2. *Veronica oetaea*. **a**: young plant in May· **b**: flower· **c**: fruit. Livadies pond. Photo: P. Delipetrou.

3. Spatial distribution and population size of *Veronica oetaea*

Methodology

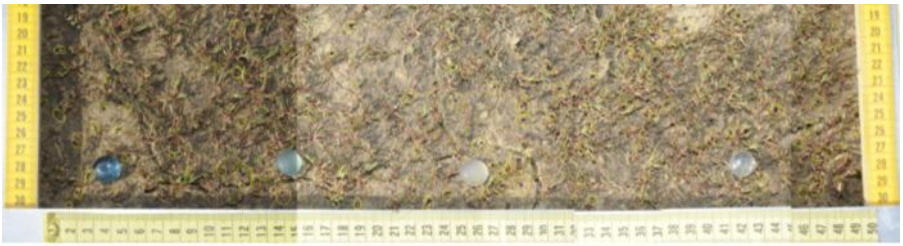
Spatial distribution and population size were estimated at the 3 ponds of Livadies, Greveno and Alykaina in June 2013 and 2014. Permanent transects at distances of 2 m were established throughout the area of each pond (Figure 3) and a 2x2 grid was created (see Action A3). Counting the whole population was not possible due to the extemely small size of the plant and the extremely large size and density of the population. Population counts were made at 50x50 cm plots systematically placed within the 1x1 m plots of action A3 in each 2x2 cell (Figure 3, Figures 5, 6, 7). Thus, in total 1/16 of the area of the lakes was surveyed. In addition, the presence of the plant was recorded in each 2x2 grid, even if it was absent from the 50x50 cm plot.

Counts were made either by photos at the plots with large numbers of plants (Figure 4) or by counting the individuals at the plots with small numbers of plants. The images were connected with the software PtGui and the plants of each image were counted with the software imageJ. The total number of individuals was estimated for each 2x2 cell based on average density in the 50x50 cm plots.

Figure 3. Transects at the pond of Livadies. Photo: P. Delipetrou.

a.

b.



c.

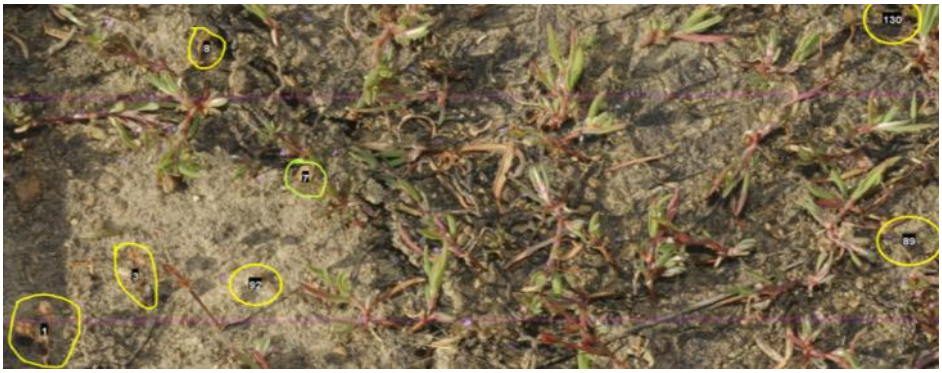


Figure 4. Population count of *Veronica oetaea*. **a**: field work, 50x50 cm plot· **b**: office work, connection of images· **c**: office work, population count. Livadies pond. Photo: I. Dimitriadis.

Results

Population counts at each pond are presented in Table 1. A total of 16975 and 68976 individuals were counted in the 50x50 cm plots in 2013 and 2014, respectively. This corresponds to an estimated total population size of 271600 individuals in 2013 and of 1103616 individuals in 2014. Based on the plot counts, population size increased in all the ponds in 2014 and was in total quadraple compared to 2013. In both years there was a large variation of the number of individuals between plots (as indicated by the large standard errors of mean values) in each pond, especially at the ponds of Greveno and Alykaina.

The spatial distribution pattern of *Veronica oetaea* in each pond in 2013 and 2014 is presented in Figures 5, 6, and 7. In Livadies, the larger size of 2014 corresponds to a larger population density in each plot since the number of plots with presence of the plant actually decreased compared to 2013. In 2014, the plant established in 5 new plots but receded from 30 plots. In Greveno, the spatial distribution of the plant did not actually change, so again population increase was due to the larger density. In Alykaina, on the other hand, population increase was mainly due to the establishment of the plant at 6 more plots.

Spatial distribution in relation to pond depth based on mean depth at the 1x1 plots for the pond Livadies (where the necessary detailed data were available) is presented in Figure 8. *Veronica oetaea* started appearing at the very shallow depth of 10 cm and was sporadic up to c. 30 cm. The main part of the population appeared at depths between c. 30 and 41 cm. At larger depths, *Veronica oetaea* was not present, it was actually replaced by *Ranunculus lateriflorus* and *Eleocharis palustris*.

In conclusion, the population of *Veronica oetaea* presented significant interannual variability in total size, in density and in spatial distribution.

Table 1. Population counts and population size estimation of Veronica oetaea.

Year	2013				2014			
Pond	**Livadies**	**Greveno**	**Alykaina**	**Total**	**Livadies**	**Greveno**	**Alykaina**	**Total**
Number of Plots	170	51	45	266	170	51	45	266
Number of Plots with plant presence	103	45	32	180	78	46	38	162
Number of individuals (50x50 cm plots)	6578	6440	3957	**16975**	11174	48120	9682	**68976**
% increase					70	647	145	306
Mean number of individuals (50x50 cm plots)	64	143	124	94	143	1046	276	426
Standard Error	11.4	69.0	53.0	20.7	38.3	323.2	105.5	100.1
Total number of individuals (2x2 m cells)	105248	56624	63312	**271600**	178784	769920	167580	**1103616**
Mean number of individuals (2x2 m cells)	1022	2290	1979	1509	2292	16737	4077	6812
Standard Error	181.7	381.7	847.9	331.0	612.5	5171.5	1430.9	1601.6

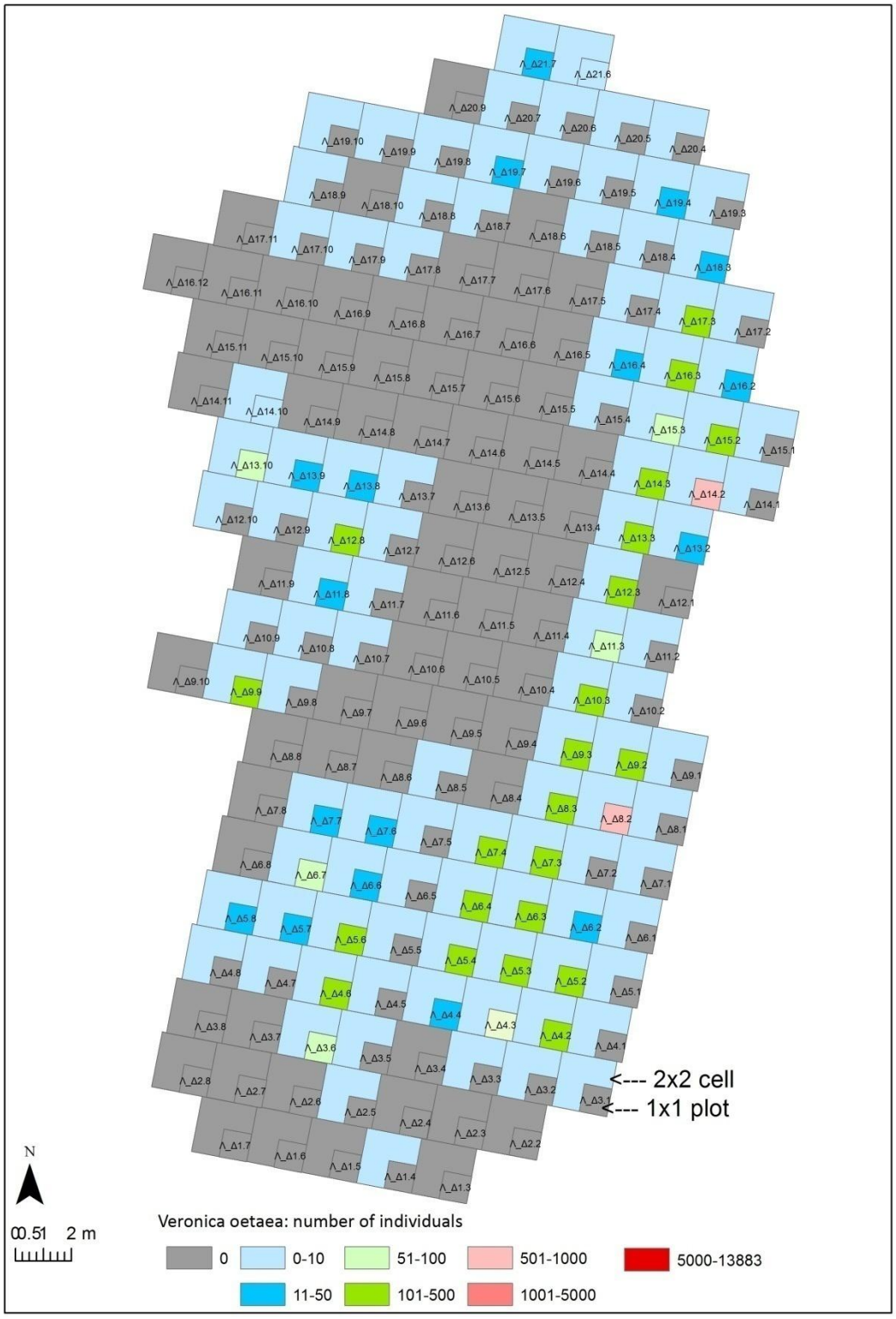


Figure 5a. *Veronica oetaea* spatial distribution in Livadies, in 2013.

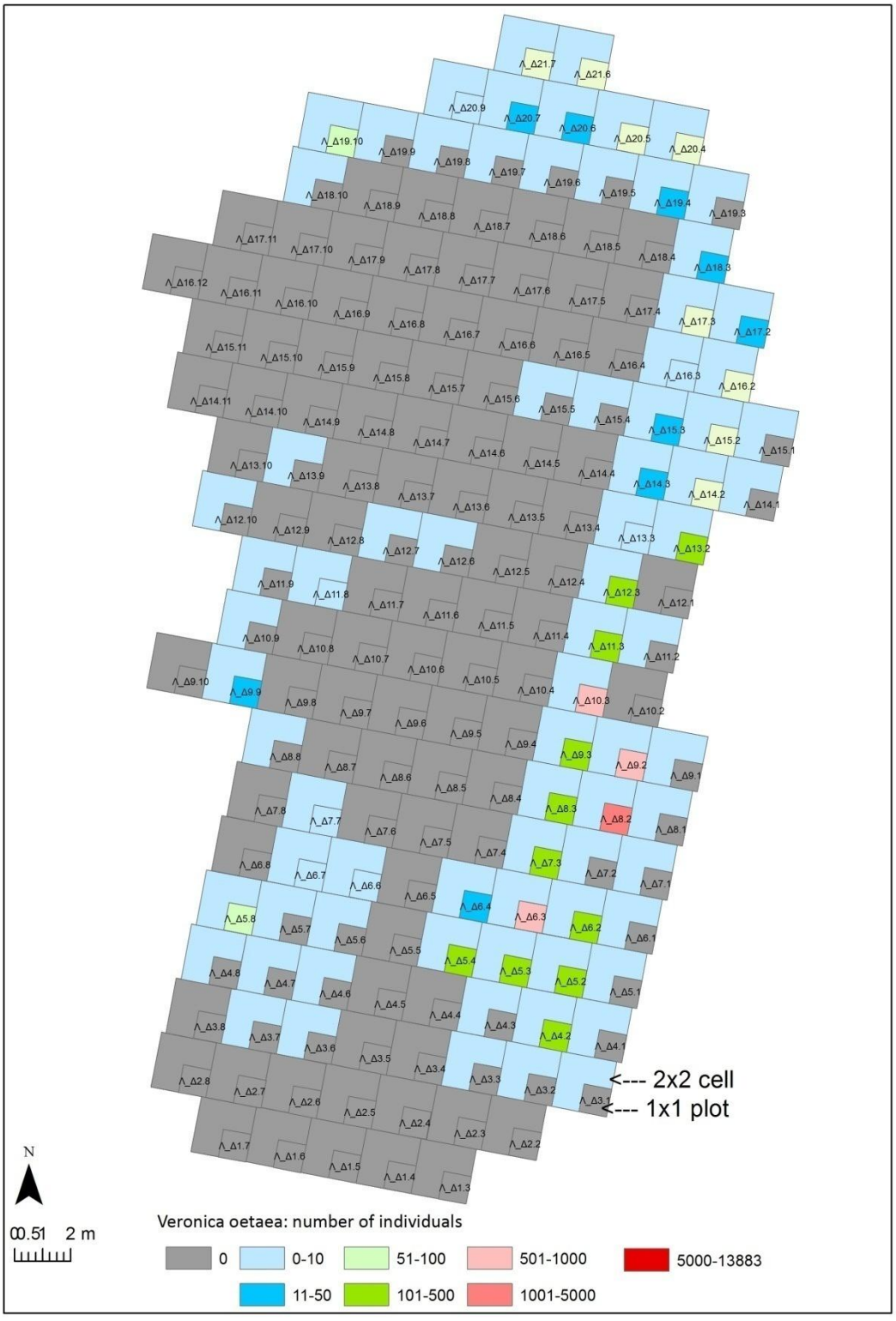


Figure 5b. *Veronica oetaea* spatial distribution in Livadies, in 2014.

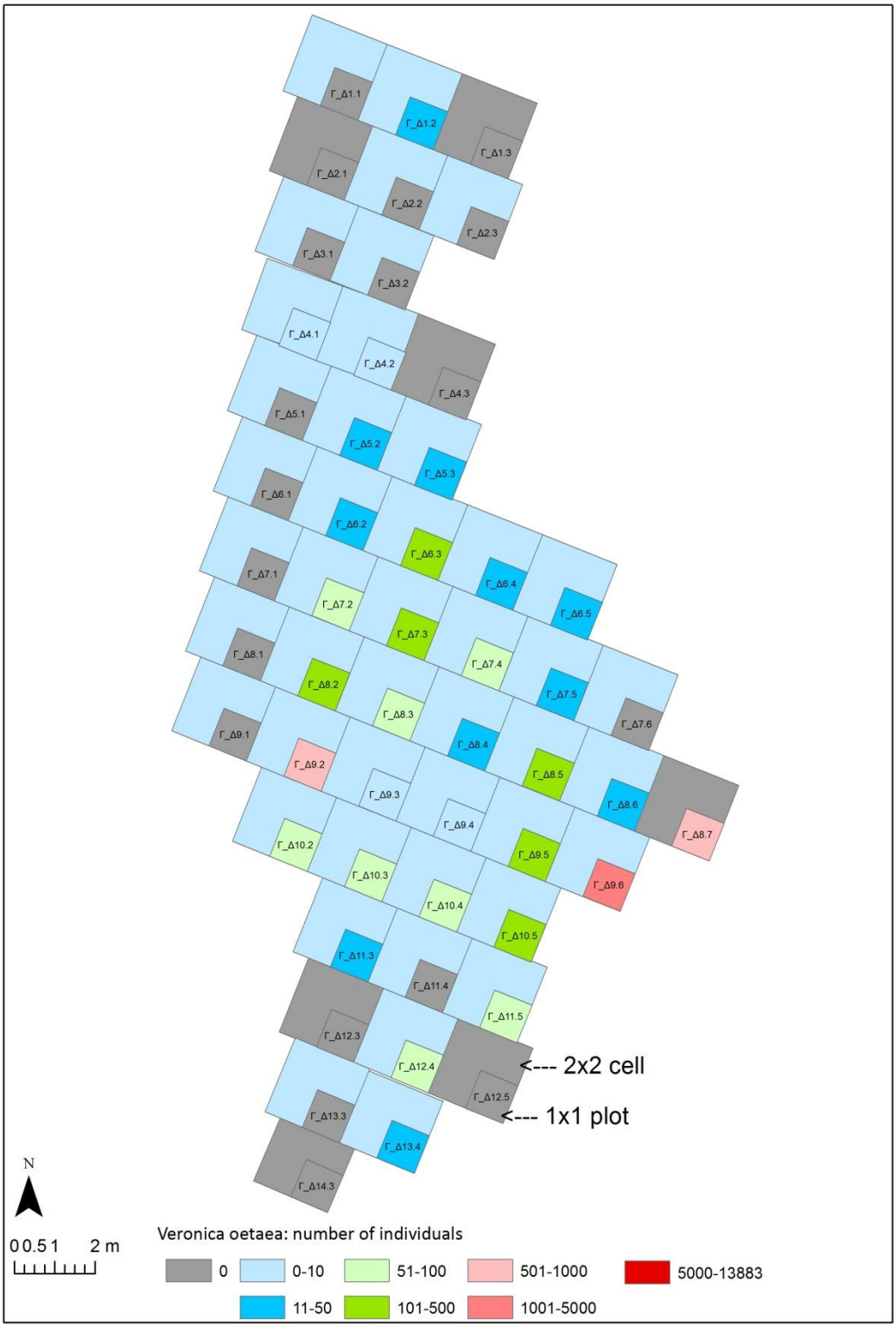


Figure 6a. *Veronica oetaea* spatial distribution in Greveno, in 2013.

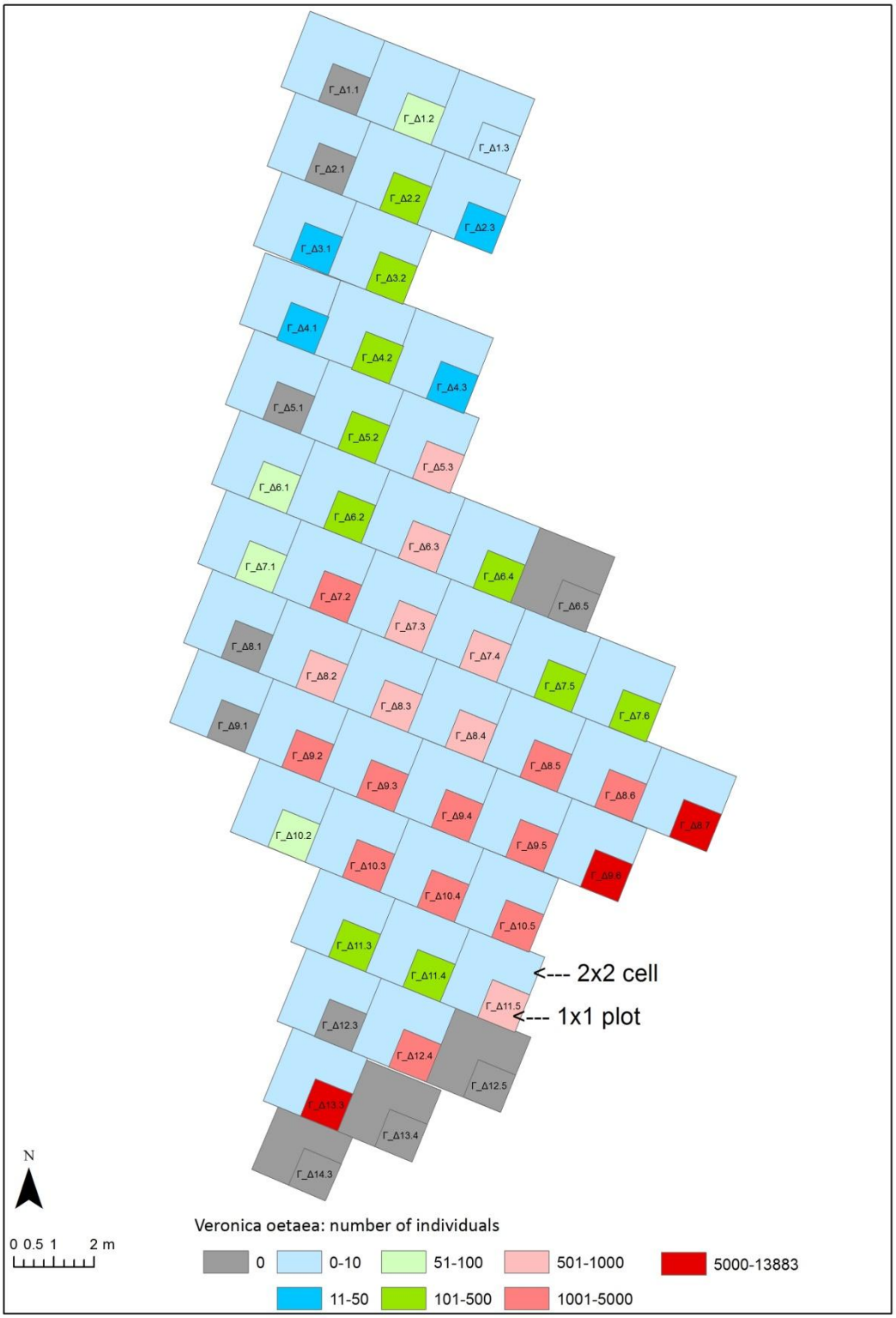


Figure 6b. *Veronica oetaea* spatial distribution in Greveno, in 2014.

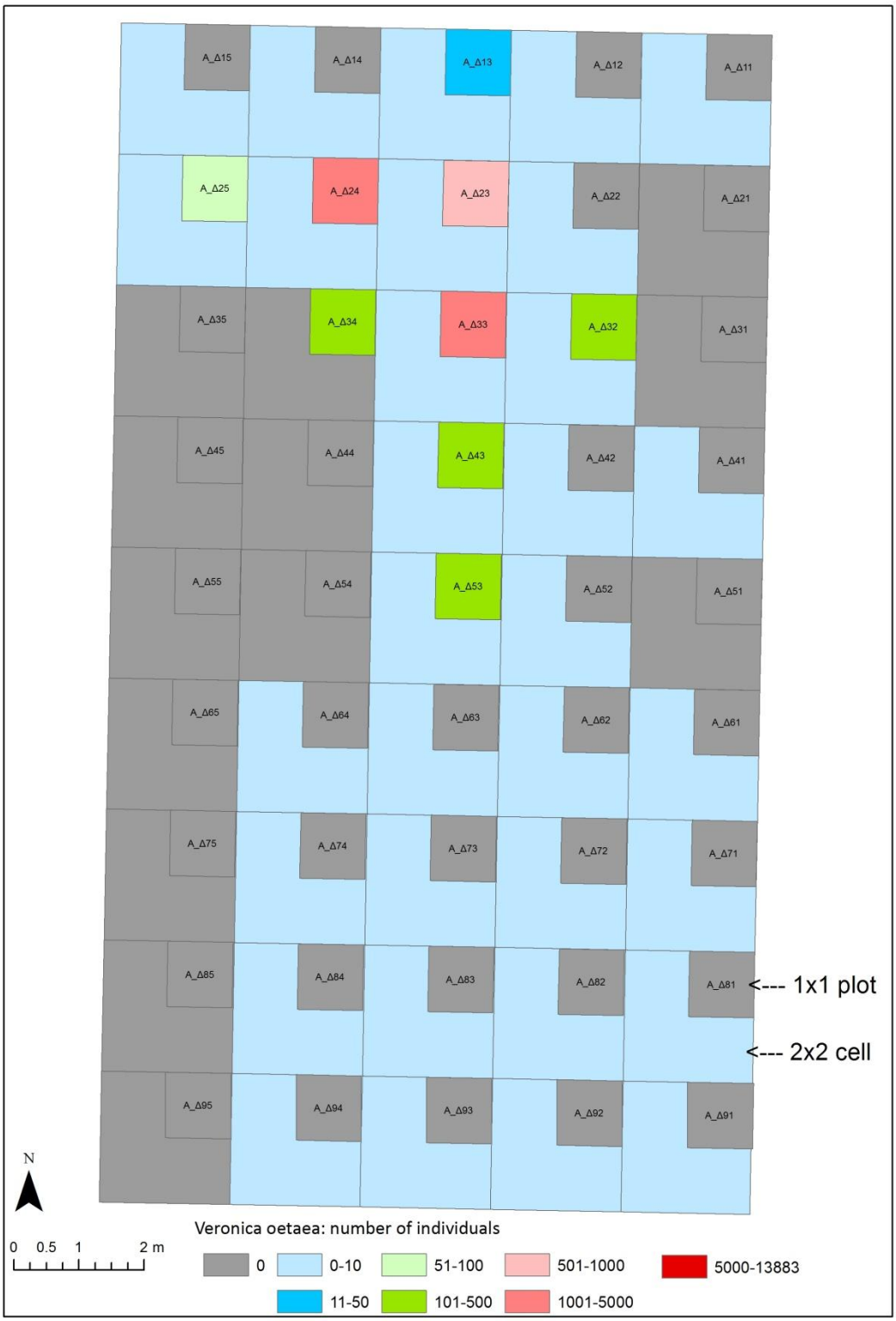


Figure 7a. *Veronica oetaea* spatial distribution in Alykaina, in 2013.

Figure 7b. *Veronica oetaea* spatial distribution in Alykaina, in 2014.

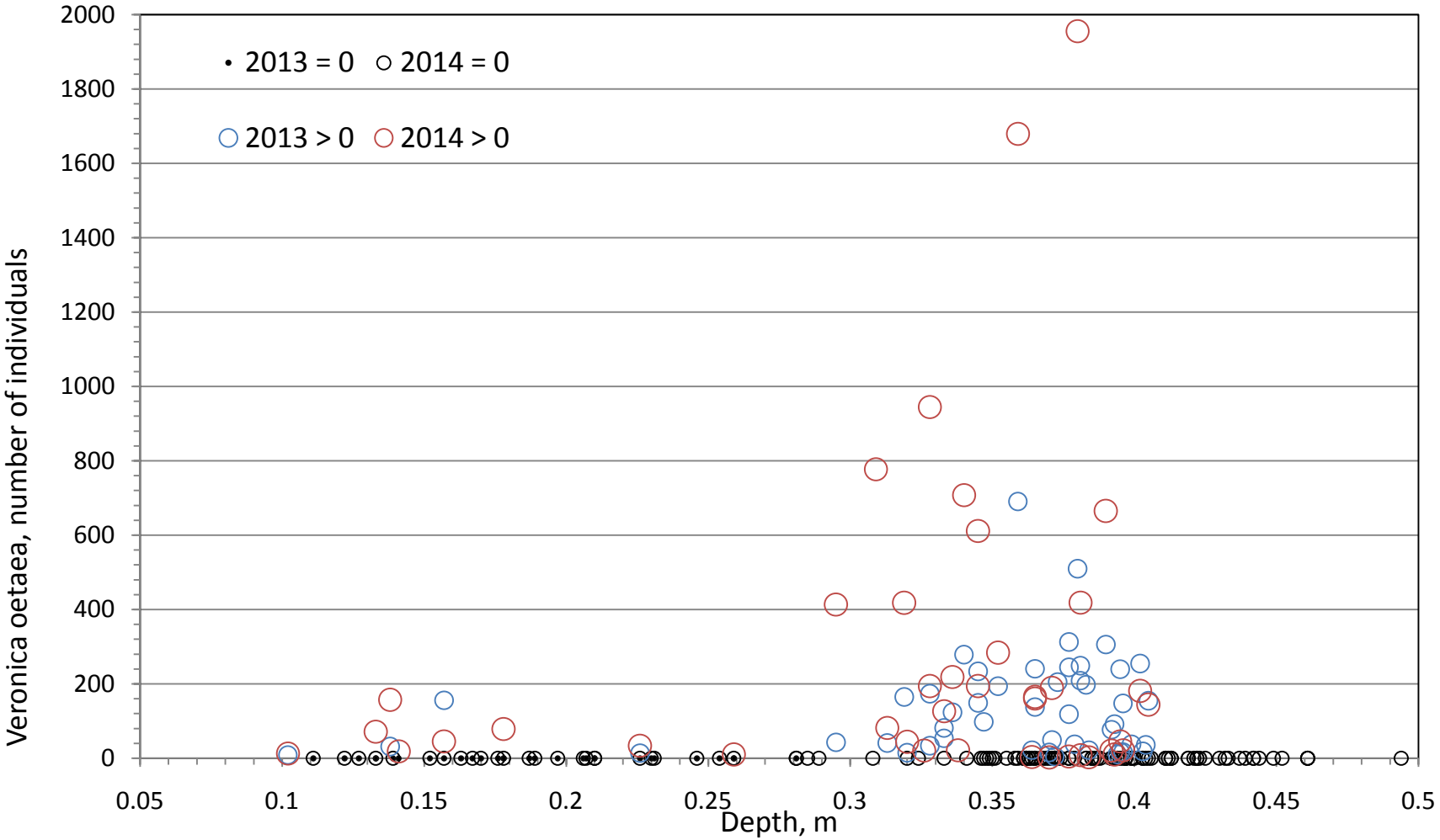


Figure 8. *Veronica oetaea* spatial distribution in relation to pond depth in Livadies.

4. Soil seed bank of *Veronica oetaea*

Methodology

Seeds form a soil seed bank from the time of dispersal until germination (Fenner & Thompson 2005). Seeds that remain viable in the soil for less than a year form transient soil seed banks, whereas seeds that remain viable for over a year form persistent soil seed banks (Thompson 2000, Fenner & Thompson 2005).

The advantage of seed persistence in the soil is due to the variation in establishment success and reproductive output, i.e. 'good' and 'bad' years (Thompson 2000). In a constant environment, immediate germination is always the most stable evolutionary strategy (Rees 1994). In the simplest case of an annual plant with no density dependence, the fraction of seeds that germinate immediately should be approximately equal to the probability of a good year (Thompson 2000). However there are disadvantages since a) seeds that do not germinate at the first opportunity may die and b) due to the nature of population growth, seed germination soon after dispersal results in a greater number of descendants compared to germination at a later stage (Thompson 2000).

One of the primary reasons that seeds do not germinate in the soil and form soil seed banks is light-requirement for germination (Baskin & Baskin 1989, Thompson 2000). Delayed germination is also an important characteristic for seeds that form soil seed banks (Saatkamp et al. 2011). Seeds requiring light for germination are usually small in size (Pons 2000, Fenner and Thompson 2005). Small seed size has been associated with the formation of permanent soil seed banks in various studies (e.g. Thompson et al. 2001, Cerabolini et al. 2003).

Soil seed bank of *Veronica oetaea*

In order to investigate the soil seed bank of *Veronica oetaea*, soil samples from the three temporary ponds Greveno, Livadies and Alykaina were collected in 2013 (before and after seed dispersal, in June and October, respectively) and in 2014 (before seed dispersal, in June). Collection of soil cores in autumn was performed to ensure that seeds in the soil had persisted since dispersal the summer or earlier. Collection of soil cores in summer was performed to count the seeds that did not germinate from the soil seed bank during late spring. Soil samples were collected by using a 2, 10 or 5 cm diameter core at various depths (Table 2). In the laboratory, all soil samples were air-dried at room conditions.

All samples were collected within permanent monitoring plots (1 x 1 m) used for population size estimations and determination of vegetation structure, inside the temporary ponds (Figure 9). The pattern of the soil sample collection differed between the two collection years: in 2013, samples were collected on the basis of floristic composition differences while in 2014, on the presence/absence of *Veronica oetaea* individuals during both 2013 and 2014 (Figure 10).

Table 1. Data of the soil cores sampled in 2013 and 2014 from the three temporary ponds in Oiti.

Temporary Pond	Collection Date	Soil samples	Soil core cylinder diameter, cm	Soil core depths, cm	Vegetation quadrats
2013 before seed dispersal					
Livadies	23-06-13	33	10	0-5, 5-10, 10-15, 15-20, 20-25	11
Greveno	24-06-13	27	2	0-2, 2-5, 5-10	9
Alykaina	07-06-13	15	2	0-2, 2-5, 5-10	3
2013 after seed dispersal					
Livadies	19-10-13	33	2	0-2, 2-5, 5-10	11
Greveno	19-10-13	27	2	0-2, 2-5, 5-10	9
Alykaina	19-10-13	18	2	0-2, 2-5, 5-10	5
2014 before seed dispersal					
Livadies	16-06-14	18	5	0-5, 5-10	9
Greveno	16-06-14	19	5	0-5, 5-10	10
Alykaina	16-06-14	16	5	0-5, 5-10	8

Figure 9. Collection of soil samples within permanent monitoring plots in Alykaina.

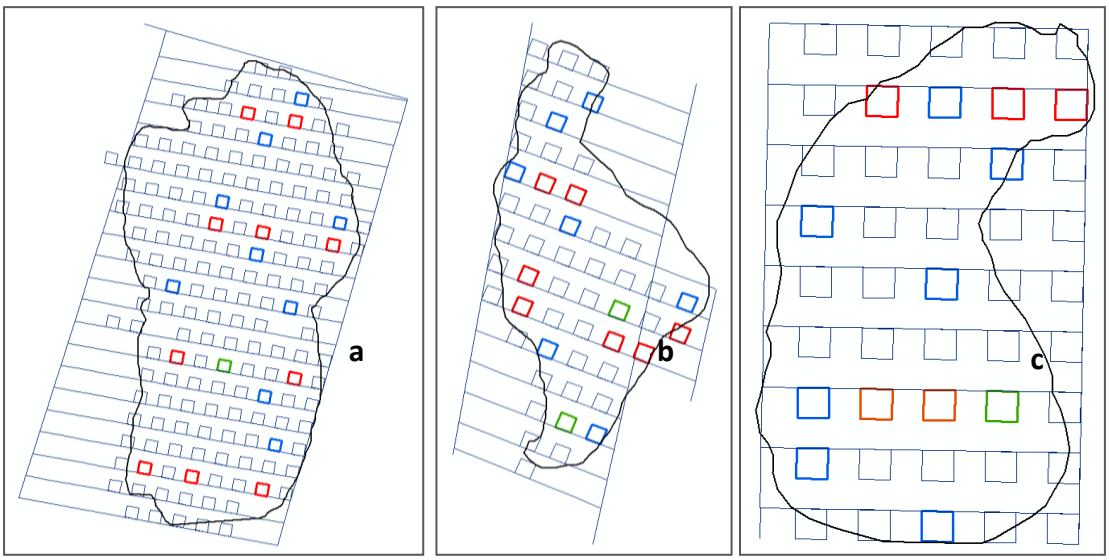


Figure 10. Pattern of soil sampling within permanent monitoring plots in Livadies (a), Greveno (b) and Alykanaina (c). The location of soil sampling for 2013 is marked in red, for 2014 in green and for both 2013 and 2014 in blue. Margins of the three temporary ponds are marked in black.

Veronica oetaea is a small-seeded species (seed mass < 23 μg) and therefore soil samples could not be sieved in order to separate and count seeds. Moreover, germination of *Veronica oetaea* was achieved only in near-freezing temperatures, at 5 °C in the light after c. 4 months (either initially placed in this temperature or transferred from another higher one), to percentages ranging between 29 and 44% (Figure 11). However, with the use of the potent, but artificial, germination promoter gibberellic acid germination percentage exceeds 80%. Thus the application of gibberellic acid in soil samples was selected to promote seedling emergence. In a preliminary test, a small number of soil samples were spread in trays, and water containing gibberellic acid was administered to each tray (Figure 12). The trays were placed in an incubator, at 20/10 °C 12h light/12h dark daily and, after 2-3 weeks, *Veronica oetaea* seedlings emerged and we were able to identify them among other seedlings. All soil samples will be tested as mentioned above in the forthcoming months.

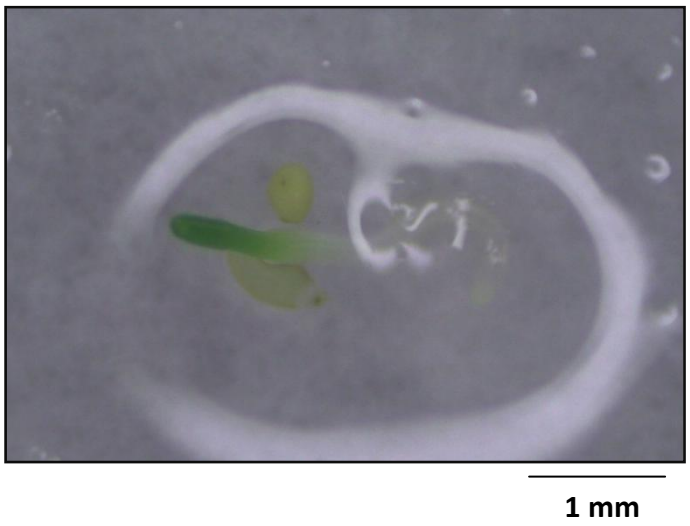


Figure 11. *Veronica oetaea* seeedling. Laboratory. Photo: K. Koutsovoulou.

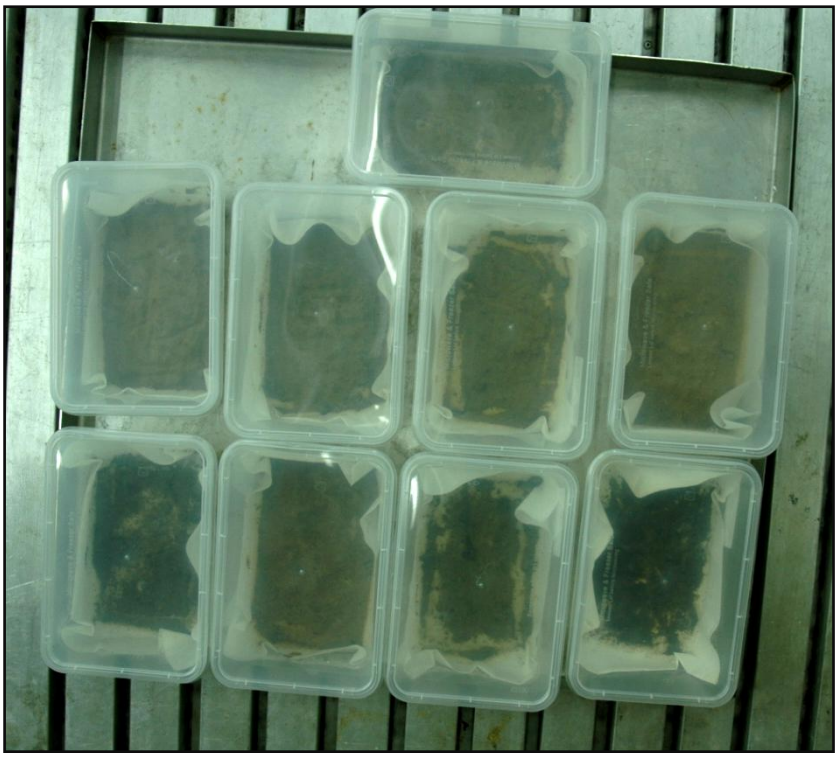


Figure 12. Trays containing soil samples from Greveno. Laboratory. Photo: K. Koutsovoulou.

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