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C15 Action (*Calotriton arnoldi*). Introduction of the Montseny newt and expansion of its distribution area

LIFE Tritó Montseny (LIFE15 NAT/ES/000757)

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Summary

The Montseny newt (*Calotriton arnoldi*) is an endemic amphibian to the Montseny massif considered to be the most threatened amphibian in Europe. The main goal of the life project (LIFE15 NAT/ES/000757) is improve the situation of the Montseny newt, for example by broaden and expand the species' range. The main crucial consequence of this strategy of conservation is the increase of the number of newts in the wild, reducing the risk of extinction of the species. Given the low number of individuals, restricted ability of dispersal and the existence of biogeographic barriers this can be only achieved by introduction of captive-born newts in favourable streams. For this reason is very important have a model of the potential species distribution to know what environmental variables influences positively and negatively the presence of the species, and importantly determine optimal sites to create new populations. Data for modelling of species habitat consisted on 159 highly precise geolocations obtained during the monitoring of the natural populations during 2016 and 2017 (113 and 46 from western and eastern distribution ranges). This cartographically represents 19 hydrologic basins from a total of 1493 placed in the PN-RB Montseny (13 and 6 western and eastern distribution ranges). The species distribution within each stream is very contagious. Thus, to avoid problems of multicollinearity derived from the autocorrelation of data, only one data by basin was used for the process of modelling. Variables used to build the models include those topographic (altitude, slope, standard deviation of slope from elevational digital maps), forest characteristics (biomass, canopy cover), aquatic habitat (Ph, oxygen concentration, temperature) and lithology.

Ecological modelling was performed using the Maxent algorithm upon two different schemes. First, data on the two geographic ranges of the species (western and eastern was joined into one), building a common model for both ranges. Second, a specific model for each range was built, using only data from each range. Potential distributions achieved by the models have been projected on the whole massif of Montseny, taking into account only the areas where lithologic conditions (availability of schistous rocks) were favourable for the species, due that this characteristic has been proved as obligate for the presence of the species. Standard procedures of modelling has been done, performing 10 replies and evaluating the accuracy of each model by calculation of the area upon the AUC.

Results showed a good fit of the models (mean AUC: 0.976, 09.64 and 0.966 from western, eastern and total ranges). The area of potential habitat within the overall basin of the Tordera river ranges from 120 to 320 ha according to the model. However, potential habitat was also been detected in the basin of Besos river in four brooks, comprising a maximum area of 154.4 ha.

The projection of the models based on the joined dataset of western and eastern populations on the Montseny cartography, allowed to map the species potential distribution. The examination of results confirm that the four sites where the species was previously introduced were minimally potential for the implantation of new populations. The potential area in the eastern side of the Tordera river (the natural subdivision between the two species geographic ranges) is smaller and is less optimal than the western. Interestingly, optimal potential habitats has been predicted in the Besos

main basin, where the species is not actually present. Some of the potential sites predicted in the eastern range of the species should be theoretically placed on schistose rocks. Nevertheless, visits to these places revealed that their geology really consist in a mix of granite with schists.

Map of potential distribution projected using data separately from each of the species ranges showed a smaller area than those achieved by the total data. The models by each species range were different than obtained by from the total indicating thus, that the habitat characteristics between ranges were different. The potential area for the eastern range is in this case smaller and less optimal than for the western, and included also sites in the main basin of the Besos river.

The differentiation of potential areas corresponding to each ranges was not absolute. A few potential sites with characteristics within those of the eastern range laid in the western range of the species and to the reverse. In summary, results derived from preliminary analysis showed that there are potential area for creation of new populations in the PN-RB Montseny. Based on the results achieved the following considerations can be stated;

a) The amount and habitat quality is lower for the eastern species range and this is very worrisome because the state of conservation of the newt population in this range is worse.

b) Promisingly, the potential area for species introduction is not limited to the Tordera main basin, but also to the neighbouring Besós river headwaters of the river.

c) Some improvements need to be made regarding the environmental data. Specifically, more detailed geological maps and maps of hydroperiods of the streams.

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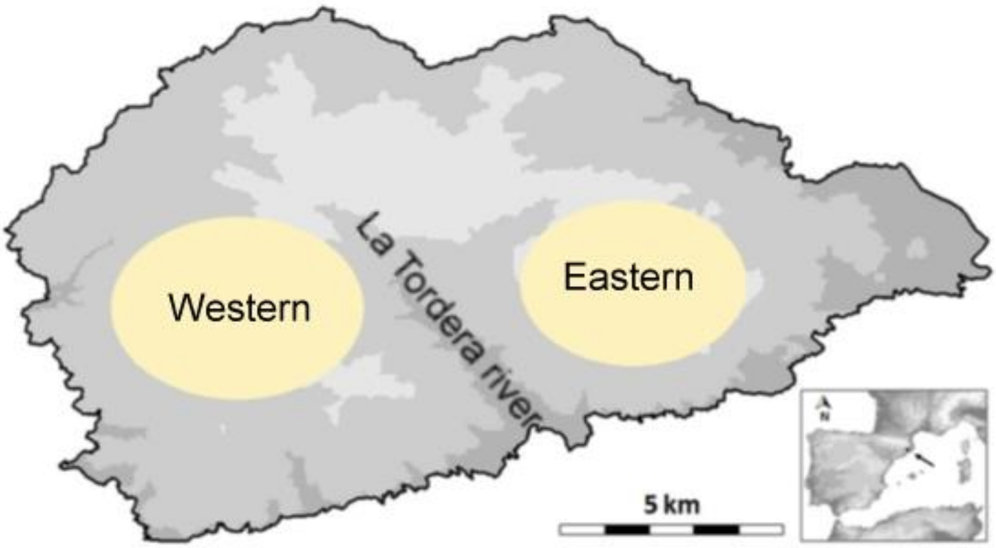
1.- Introduction

1.1.- Background

The presence of the brook newt in the Montseny Massif was detected in 1981. Its presence was detected in two streams and became the southern limit of the Pyrenean endemism *Euproctus asper*. After several years of ecological, morphological and genetic studies, reached the populations of *Euproctus asper* in the Montseny massif and argued that a new specific level (*Calotriton arnoldi*), which suggested the change of genera to *Calotriton* following the systematic recommendations. It was at that moment that the conservation status for the populations of Montseny Brook Newt from PNRBM changed due to the very few locations it could be found in while the scarcity of specimens, worryingly suggested that *C. arnoldi* could be a severely endangered species.

C. arnoldi presents a fragmented distribution in two areas located on the Tordera river's eastern and western slopes (Figure 1). Both subpopulations are separated genet-ically and morphologically and recent studies estimated that this isolation occurred about 180,000 years ago during the Riss glacial period



Localization of PNMRB and the two isolated subpopulations of *C. arnoldi* into La Tordera river basin.

Water catchments in streams are one of the most dramatic and immediate threats to this species. Large amounts of water are being extracted from PNRBM for human consumption and livestock along all the river secondary basins. It is likely that water over-exploitation is a severe threat because of the species' ecological requirements. Moreover, the fact that the current environmental legislation is not enforced efficiently with regard to the maintenance of ecological flows and their monitoring constitutes a significant difficulty when dealing with this threat. Another cause behind water flow reduction in the La Tordera basin could be the presence of forest plantations and more specifically those that consist of fast-growing allochthonous conifers. They requre large amounts of water and take up 11,40 ha within the natural range of *C. arnoldi*, which is 12% of the secondary basins' forest area and where the species lives. Thus, the disappearance of the autochthonous riparian forest and the fact it has been replaced by species that are more economically profitable has also increased the hydric stress.

Global warming is another threat affecting the species. For instance, the beech (*Fagus sylvatica*) forest, an excellent habitat for *C. arnoldi*, has shifted upwards by 70 m at the highest altitudes (1,600-1,700m asl) since 1945, and is being replaced by holm oak (*Quercus ilex*) forest at lower altitudes (800-1,400 m). Consequently, the most favorable woodland habitat is moving up into areas where the streams are drying out more and will reduces stream connectivity and habitat availability. Finally, the recent appearance in Europe of emerging amphibian diseases associated with the genera *Batrachochrytium* forces us to be very strict with regard to preventive biosecurity measures, since an isolated focus of *B. salamandrivorans* has been detected very close to the *C. arnoldi* populations. Based on experimental evidence, this pathogen has been shown to be lethal for the Montseny Brook Newt.

The main goal of this action was to increase the species geographic range by releasing captive born newts. This strategy was based on the two most important conditioning factors of the reintroduction projects, the production capacity of captive breeding centers and the availability of new potential habitats to create new populations.

The development of this action does not start from scratch, but since 2010 and therefore prior to the implementation of the Life Tritó Montseny project (LTM from now), there have been several pilot experiences of creating new populations, divided between both populations, eastern and western. The individuals released came in most cases from the Torreferrussa captive breeding center -Generalitat de Catalunya, which supplied larvae, immatures and adults from the eastern and western core populations. These first experimental introductions revealed the need to improve the planning, development and monitoring of this process in order to optimize the probability of success, learning from the process, correcting errors and adapting to unexpected contingencies.

On this basis, Action C15 aims to improve the process of creating new populations, in addition to actions intended to improve the production capacity of newts from captive breeding.

With regard to this two aspects, and in order to provide effective decision-making tools, a specific process was carried out from the beginning to define a long-term Plan to create new populations and a reintroduccions expert workshop was created to asses in this process. The goals proposed by the LifeTM project in C15 actions are: 1. To expand its geographic distribution. 2. Creating new populations in optimal streams and 3. Monitoring new created populations.

2.- New population decision making

The creation of *Calotriton arnoldi* new populations it is not an easy process and there are many factors involved that must be taken into consideration.

- To decide the context, that is to say frame the problem and identify all relevant stakeholders.
- To identify goals and objectives to be achieved and that these are achievable.
- To decide what actions are necessary to achieve these goals.
- To plan a methodology for evaluating the effect of the actions and consider the uncertainty.
- To monitor the results of the actions and decide which actions are the most effective in relation to the objectives and establish a protocol for self-learning and making new decisions.

2.1.-Methodology

2.1.2.- Biosecurity measures.

In order to avoid the spread of emerging infectious diseases, biosecurity protocols were implemented throughout the sampling (Fernández-Guiberteau & Montori, 2020; Fernández-Guiberteau et al. 2020a, b). The materials on the field were disinfected by using Virkon^{S}, before and after the visits for each population. Gloves are used in all handling captured, measured and released newts.

Biosecurity measures (disinfection) applied before and after the survey.

2.1.3.- Planning new population process

Due to the species' critical situation shortly after their description, in 2007 a captive stock of newts was started by placing 20 newts from the two subpopulations in the facilities of the Wildlife Center Recovery of Torreferrusa (Generalitat de Catalunya) (Carbonell-Buira et al. 2014).

Within the LTM project the creation of new populations could not be done without careful planning of the decision-making process. Taking advantage of the knowledge acquired with the other actions and following the advice of experts, the process of creating new populations had to include the following items:

• Use a structured decision process framework

• Involve key stakeholders to better frame specific objectives for the creation of new populations.

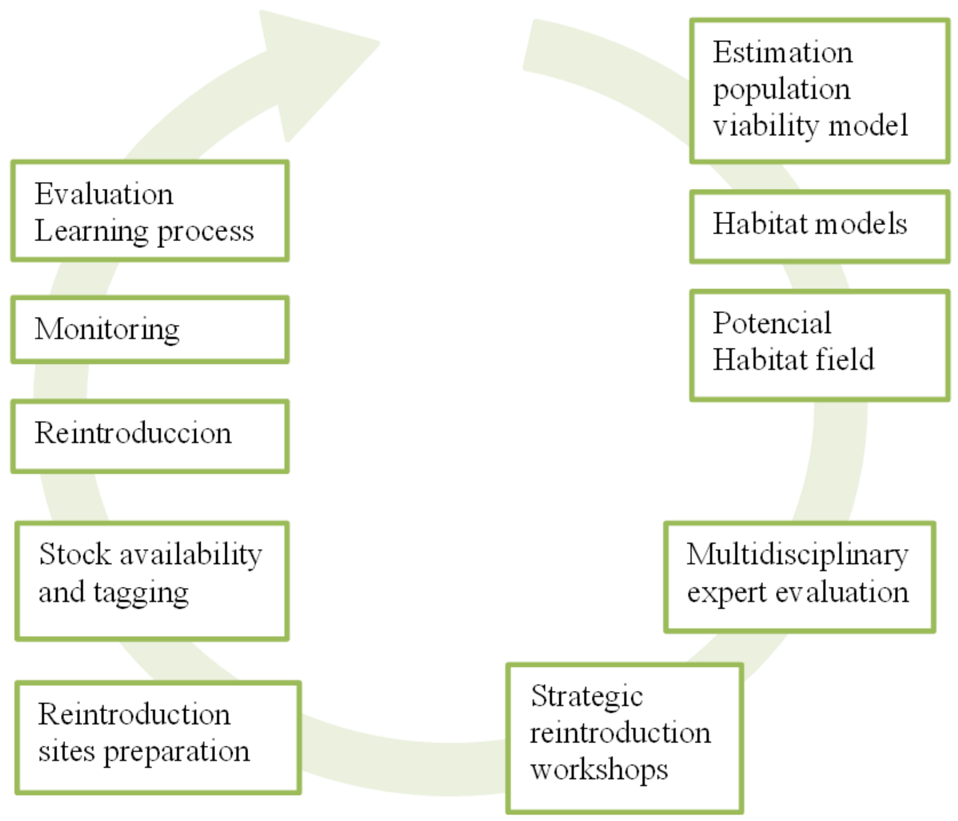
• Identify new potential sites through SDM and expert knowledge and subsequent field validation.

• Build population models based on expert assessments.

• Integrate all in an optimization analysis in order to identify the better strategy to create new populations.

• Give a strong basis for a future learning process (Adaptive management and contingency plan).

After several multidisciplinary meetings of the expert and new population committees, it was agreed that the process of creating new populations should conform to the following flow diagram.



Flow diagram actions carried out

2.1.4.- Creating and monitoring new populations and stream selection

The species' potential distribution area was modeled by employing the Maxent maximum entropy method by the Centre Tecnologic Forestal (CTF) team (Villero et al. 2018, 2019). The lack of biological and environmental representativeness in the available data was also taken into account when calibrating the models (Montori et al. 2008). Comparative multidisciplinary studies were performed between the streams which the newt inhabits and those which it does not. The plant structure, hydrology, trophic availability, geomorphology and the presence of predators, among others, were analyzed (Avila, 2018; Gomà & Sabater, 2018; Martí & Ribot, 2018; Mata & Puiguriguer, 2019).

Extraction of the data collected by the sensors located in the torrents.

Neighbouring and similar brooks without newt populations have been classified as well, with the aim of finding possible newt-excluding hydromorphological factors. The data was obtained from a two-season sampling (late spring and late summer 2017) of a set of 8 streams where the newt lives as well as from 5 streams of similar characteristics with catchments where the newt has never been found. Stretches of 30 metres were es-tablished in each brook to perform the hydrological description (Avila, 2018, Gomà & Sabater, 2018; Martí & Ribot, 2018). To compare streams innabited by *C. arnoldi* with streams without newts Generalized Linear Model (GLM) with logistic function for each variable has been applied. Registered variables are: Altitude, Slope, LBOM (leaf), FBOM, Bed structure (Rocks, stones, pebbles, gravel, sand), Flow, Depth, Wet width, Maximal water speed, Stream structure (Runs, Falls, Pools, dry streaches) and hydraulic state (dry, hiporheic, arheic, oligorheic with some subterranean flow streaches, oligorheic, eurehic, hiperheic).

To analyze the water's chemical components, a total of 59 samples were obtained (40 in streams without newt), and 13 chemical parameters were analyzed: pH, H+, alkalinity, Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}, NH^{4+}, NO^{3-}, $SO4^{-2}$, Cl^{-}, Cu and conductivity (Martí & Ribot, 2018).

In 2019, a geomorphologic, geotectonic and hydromorphologic study on the river courses where C. *arnoldi* is located compared to where it is not present was conducted to outline the parameters that define the fluvial habitat of the newt, and, to identify if there are any geological or geomorphological factors conditions its absence. Sixteen streams of the upper La Tordera basin have been analyzed while in each river course there have been 1, 2 or 3 control stations, resulting in a total of 29 sections of ten meters of length (Mata & Puiguriguer, 2019).

In order to determine the stream candidates to release a new population, a "New Populations Analyses Commission" was created working in parallel with the experts' commission to decide, after several field surveys in optimal candidate streams, on how, where and when to release newts as well as the number and which age-classes should be released. A cost evaluation was also important to determine the project's effort capacity and the viability of its objectives.

2.1.5.- Demographic model

This consisted of constructing a model based on the geographical variables that were most likely to be decisive, using data on the presence of the species. To verify the results obtained, a commission of experts visited the potential localities proposed by the model and prioritized them.

At the same time and based on the available biological data, a demographic model was constructed to establish a reintroduction program. This model defined for each potential habitat several scenarios that combined the periodicity of reintroductions with the number of adult individuals to be released, in order to provide a guide when establishing a release protocol.

The objective was to design an optimal release strategy to maximize the overall population size of *C. arnoldi* meeting the annual constraints of budget and number of captive-bred individuals available for release (Canessa et al., 2022). The management team selected a total of 17 candidate sites (stream reaches) to establish new populations, all within the existing species' range in the Montseny massif, 10 in the western part of the species range and 7 in the eastern part.

Parameters used in the demographic model for each population. Mean and range values were obtained from literature or averaged across the expert group, then used to fit PERT distributions to reflect uncertainty. All estimates reflect 'ideal' conditions for Calotriton arnoldi, assuming sites would be restored adequately prior to any release. (Canessa et al. 2021).

Parameter	Mean (range)	Source
Age at first breeding (years)	3.5 (3–4)	Montori (1988)
Age at last breeding (years)	13.5 (12–15)	Expert elicitation
Larval survival	0.023 (0.02–0.026)	Montori and Herrero (2004)
Clutch size	35 (30–40)	Montori et al. (2002)
Sex ratio	0.5	Montori (1988)
Juvenile survival	0.8	Colomer et al. (2014)
Adult survival	0.95 (0.92–0.98)	Montori (1990); expert elicitation
Adult survival (first year post-release)	0.179 or 0.678[a]	Expert elicitation
Density (N/m of stream)	0.87 (0.29–2.85)	Expert elicitation

2.1.6.- Making decisions: Releasing and Monitoring

The number of newts to be released depends on several factors: the availability of specimens from the breeding centers, the characteristics of the stream chosen and the population models developed based on the estimated ecological parameters of the species. The methodological procedures involved in the modelling process have a main limitation, the lack of digital information on the hydroperiod of the Montseny streams. The models obtained, thus, could select a stream based on climate, forest structure and other relevant variables, but these potential habitats, actually, may have not enough water flow to allow the persistence of introduced populations. Therefore, it was essential, once the models were available, to carry out field surveys to validate the results obtained. Visit the different locations and to inspect and verify if they are, in fact, optimal to create new populations. Other information limitation, as biodiversity and abiotic (geology and soil) data on a very detailed scale, in the specific habitat of the Montseny newt, was contracted to specialists, in the field of action D5 or D6.

The results obtained above, were evaluated by a multidisciplinary team of experts (biologists, ecologistst , geologists, herpetologist, etc.) and through multiple workshops, and both, the target localities and the most favourable range of individuals to release were defined by them. All releases and reinforcements carried out during the LTM have been previously agreed by the reintroduction workshop group.

Meeting of new populations committee.

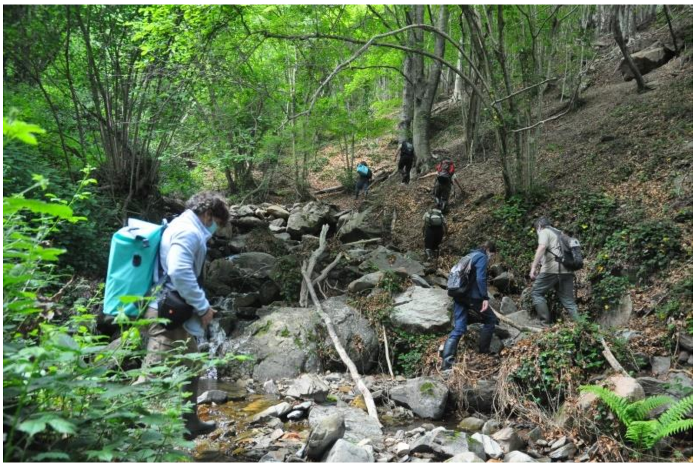
Once the available locations and the individuals available for release have been established, two parallel processes start:

1. to prepare the locality: georeferencing, mapping and marking the available ponds in each stream for reintroduction, assessing the carrying capacity of each release point.
2. To prepare the individuals in the rearing centers: separate, mark (Trovan® microchips or elastomer color code) and analyze the individuals to rule out emerging diseases before their release (Carbonell-

Buira et al. 2014).

Release points were marked with iron flags, while GPS coordinates were also registered for each point. Once the specimens were released, two active surveys per year were carried out.

Considering meteorological and hydrological conditions, the date of reintroduction is chosen and all the teams involved are coordinated to facilitate the transfer of the individuals to the field, reduce transport time and document the whole process.



The transport of the specimens must be fast from the breeding centers. The release of the newts always took place within a few hours after leaving the breeding centers. They are transported in individualized boats inside portable coolers.

2.1.7.- Monitoring

Of course, each reintroduction experience necessarily involves a posteriori monitoring aimed at tracing the evolution of the population over time, in order to assess its success. This is carried out through visits to the reintroduction areas during periods of favorable activity for the species and sampling of the population by capture-recapture. New population surveys employ a similar methodology to that of an intensive survey (view D2 action).

Surveys are always carried out at night through an active search for the specimens.

In the case of adult individuals, the identification procedure consists of the implantation of microchips or elastomers, while in the case of immature it is the marking with elastomer in at least one point of the body. The first populations that were created (before 2018), adults were also marked with elastomer. The marking and subsequent recapture allows an estimation of the minimum survival of individuals and population size, in addition to assessing their movement pattern. In addition, an inspection of the physical, sanitary and reproductive conditions of the individuals is carried out, which helps to evaluate the success of the reintroduction.

2.1.8.- Evaluation/Learning process

Establishing a specific reintroduction methodology and documenting it, has allowed us at the end of each reintroduction to evaluate the process, with its successes and failures. Detect and learn from the mistakes made and unforeseen events to improve the process.

3.- Results

3.1.- Creating and monitoring new populations

The field studies (Avila, 2018; Gomà & Sabater, 2018; Martí & Ribot, 2018; Mata & Puiguriguer, 2019), confirm that streams where *C. arnoldi* is absent have similar biotic and abiotic characteristics when compared to streams where the newt is present. These results make it more difficult to select the streams in which newts could be released to create new populations. The absence of *C. arnoldi* before LifeTM project in apparently optimal streams could be a consequence of intensive human activity during the first half of the 20th century (coal production from oaks, mining activity, creation of grazing lands, forest wood extraction, slope erosivity, water catchments …). For this reason, the old anthropic activities in the first selection streams must be taken into account. Table shows the new populations created prior and during to the LifeTM project. Only in populations A4, A5 and B7 out of the seven that were newly created, is there no evidence of newt presence since 2016, 2020 and 2019 respectively. However, *C. arnoldi* fossorial behavior and an extremely dry period in 2021 and 2022 could be the reason behind these cases where there was no detection of newts.

From 2010 until now, 8 experiences of creating new populations have been carried out. In all the new populations releases the morphological and genetic structure of the species has been respected. In total, *Calotriton arnoldi* newts have been released into 3 new streams from the eastern slopes and five from the eastern slopes. There is little potential of new habitats that does not require improvement actions, especially in the case of eastern populations and the private ownership of a large part of the areas of the Montseny Natural Park is a problem.

New populations established in the *C. arnoldi* potential area of distribution. Only population codes are written because of conservation policies for this CR species. Range: slope location in La Tordera river basin. First release: year when first release was made. Last released: last booster. Last recapture: year when *C. arnoldi* was captured in the stream. Property: Land property where the new population and stream stretch are located.

Code	Range	Number of released newts	First released	Last released	Last recapture	Property
A4	Eastern	166	2011	2014	2016	Private
A5	Eastern	153	2020	2022	2020	Public
A6	Eastern	127	2014	2020	2021	Public
B6	Western	436	2010	2020	2021	Private
B7	Western	106	2014	2015	2019	Private
B8	Western	327	2019	2022	2021	Public
B9	Western	1318	2021	2022	----	Public
B10	Western	219	2022	2022	----	Public

Released individuals by year and new population during and previously to LTM. OCC: Western populations (B..). OR: Eastern populations (A..).

Population/year	2010	2011	2012	2014	2015	2019	2020	2021	2022	Total
OCC	112	181	81	79	44	136	107	814	852	**2406**
B6	112	181	81	17			45			436
B7				62	44					106
B8						136	62	63	66	327
B9								751	567	1318
B10									219	219
OR		123		84	21		128		90	**446**
A4		123		43						166
A5							63		90	153
A6				41	21		65			127
Total	**112**	**304**	**81**	**163**	**65**	**136**	**235**	**814**	**942**	**2852**

3.1.1.- Eastern new Populations

3.1.1.1.- A4 new population

This stream, located on privately owned land, faces northwest and is approximately 900 m long. The riparian vegetation consists mainly of *Platanus hybrida* and *Castanea sativa*. It presents a poor conservation of the terrestrial habitat as a result of the planting of exotic trees, the existence of water catchments, drainage paths and strong soil erosion. On the other hand, the habitat is very open. The aquatic habitat is characterized by an excessive discharge of sediments despite the permanent hydroperiod.

In 2012, 123 specimens were experimentally released and in 2014 the population was reinforced with 43 new newts. The releases are formed for both juvenile and adult individuals. Adults' individuals and larger juveniles were tagged with elastomers.

During the years following the releases, very few individuals were observed, intermittently and for a very short period of time. The last detection of newts in this stream occurred in 2016, so the population is considered not established due to this worsening habitat.

3.1.1.2.- A5 new population

This stream is located in the domain of the beech (*Fagus sylvatica*) with chestnut (*Castanea sativa*). It has a length of 738 meters with optimal conditions for the release of newts and is located on public owned land. Schists are the dominant rocks and occasional outcrops of granite without sedimentation of sauló are observed. A5 stream faces to sud-west.

This population were created in 2013 and reinforced in 2014 by larval newts. For geological and hydroperiod reasons the section of stream favorable for the species is short, but presents very good environmental conditions. Because it takes at least four years for larvae to reach sexual maturation, monitoring was not carried out until 2017, and since then the results have been negative.

In 2020, 63 adult individuals (22 females and 41 males) were released, marked by micro-chips from captive breeding at the Torreferrusa CRF and Barcelona Zoo. The release section measured 125 m and is located below the section where newts were released in 2013 and 2014. The sex ratio in this case is very favorable to males (♂♂/♀♀ = 1.83). In fact, that does not correspond to the sex-ratio of wild populations but is determined by the availability of specimens in the breeding centers. Subsequently, two sampling campaigns were carried out.

During monitoring, 5 individuals were detected, capturing 4 (3 males and 1 female). The body size measured ranges from 63-71 mm. Of these 4 newts, displacement data are available for 3 of them, two below and one above the point of release. One of the captured specimens did not carry a microchip and for this reason is likely to be one of the larvae released during 2013 and 2014. No data can be given yet on displacements since only three specimens have been positioned, of these two have moved downriver and one upriver. Taking into account the characteristics of the habitat, the populations have possibilities to prosper if periodic reinforcements should be carried out. It is also observed that the average size of the released individuals is higher than that of the wild population A2.

Stream located in the domain of the beech (*Fagus sylvatica*)

3.1.1.3.- A6 new population

This eastern population was created in 2014 by releasing 41 larvae and reinforced in 2015 also with 21 larvae. The conditions of this stream are very similar to A5, since they are in the same area and in fact both converge in the same watershed. It is located in the domain of the beech (*Fagus sylvatica*) with chestnut (*Castanea sativa*). They have optimal conditions for the release of newts and is located on public owned land. However, the stretch stream selected in very short and does not exceed 110 meters. In this zone 17 release points are characterized.

In 2020, 65 adult specimens (22 females and 41 males) were released from the Torreferrusa CRF in a 110m stretch where adults were released during 2014 and 2015. Subsequently, two sampling surveys were carried out. The sex-ratio in this case is favorable to the males (♂♂/♀♀ = 1.32) as in the A5 torrent. In fact, that does not correspond to the sex-ratio of wild populations which is favorable for the females.

During the surveys, 4 individuals were detected, of which 3 could be captured (1 male and 2 females) of body length between 55-65 mm. One of the captured specimens could not read the chip number and a genetic sample was taken to determine if it was a specimen that had lost it or was an individual from the 2014 and 2015 releases. The results obtained confirmed the latter hypothesis. Both individuals with previous positioning have moved below the release point. No data can be given yet about displacements since only two specimens have been positioned. No more data can be given yet about displacements since only two specimens have been positioned.

3.1.2.- Western new Populations

3.1.2.1.- B6 new population

Introduction and Background

The stream B6 is part of the eastern high basin of the Tordera river and is formed by the confluence of two small streams below a Turó at 1273m of altitude. This stream is faced to the north-east joining the Tordera river at 370 m of altitude and is constituted by a succession of cascades, small ponds and riffles. The geology is formed by phyllites and slates at high and medium altitudes that are replaced by limestones in the lower section. The vegetation is dominated by oaks that in the immediacy of the stream is accompanied by a tiny riparian forest of hazelnuts.

Middle section of the B2 stream where the reintroduced population was successfully implanted.

Upon expert criteria this stream was chosen to host the first experience of generation of a new population by releasing newts born in the captive reproductive program started in 2007. Since 2010 four releases of larvae, subadults and adult newts has been done in order to create and reinforce this population: 2010, 112 immatures; 2011, 180 immatures and larvae; 2012, 81 larvae and immatures; 2014, 17 larvae and immatures. Initially, in 2010 and 2011, newts were released along a long stream stretch (estimated in 708 m length) in the upper and middle sections. After these two experiences and following the evolution of the reintroduced population we found surviving newts only in the middle section of the B6 stream. Given the large number of released newts we assumed that the failure of implantation of newts in the other stream sections could be caused by deficient habitat features, maybe by the lack of an appropriate inner rocky matrix. For this reason, subsequent newt releases were exclusively centered in the section where the reintroduced population survived.

Montseny Brook newts are at a higher risk of extinction because of the combination of small and fragmented range, and ecological specialization. The best way to overcome this situation is to increase the number of populations and logically the number of newts, taking into account the geographically-based genetic structure. Natural range expansions are unlikely to happen in the case of Calotriton arnoldi because of the high philopatry of the species, the low number of newts and the dramatic isolation effect generated by the habitat alteration derived by human exploitation of the natural resources. For this reason the only way to expand the species is by generation of new populations in favourable habitats by releasing newts born in captivity. This is the rationale justifying the launch of a program of reintroduction and therefore the monitoring of the new populations in order to evaluate their success in the mission of increasing species range and population size.

Goals and methodology

The main goal of the monitoring of the B6 population is to determine whether the reintroduced newts have survived leading to the successful implantation of a new population. In order to achieve this, we examined several indicators:

a) number of newts through the period of the Life project implementation.

b) physical condition of the individuals.

c) evidence of reproduction.

Our methodology consisted of sampling the medium section of the B6 during spring and autumn nights, investing a variable number of visits per year depending on the environmental conditions, basically adequate temperatures and water availability, and on logistic limitations. Night samplings were performed by one field researcher performing an itinerary searching visually newts and trying manually to catch them. Newts were classified as larvae, subadults and adults, based on their morphologic characteristics defining the

transition to metamorphosis and sexual maturity. When possible, length to the tip of the snout to the rear end of the cloacal protuberance (SVL), the length of the tail and the weight were recorded using digital callipers and scales with the excpetion of the larvae. We also examined externally the newts in order to evaluate the level of reproductive capacity of the newts by recording whether they had sexually dimorphic traits or ovarian follicles. This latter is possible because the translucent venter of the females allows us to observe it. The released newts were previously marked using a visible implant elastomer with the exception of the larvae. Initially, we tried to combine five positions in the ventral side of the newts with different colors, but unfortunately these marks moved in the subadults and for this reason we decided to implant only one mark in the gular area of different color depending on the year of release. When recaptured at adult size the individuals were remarked using a complex code of marks in nine points of the ventral side. We also recorded the place where newts were observed by geolocalization and during the last three years by positioning them in intervals of approximately 10m marked by means of labels on the trees or rocks.
We mapped the geolocalizations of Montseny Brook newts using digital cartography of the PNRB Montseny. We used descriptive statistics, parametric correlations and ANCOVA and ANOVA in order to characterize the morphology of the reintroduced population by population classes and evaluate whether there are differences among them.

Results

Location of the reintroduced population

We found newts during all the years of monitoring distributed along the middle stream section where in previous years we observed the species, demonstrating the persistence of the reintroduced population. A large geographic scale the population seems to be uniformly distributed, but detailed examination on the locations by stretches revealed that newts tend to concentrate in the upper part of the stream section, with the exception of the larvae. One individual was recaptured five meters up the point of release out the area of reintroduction.

Population structure, ecological observation and morphology

During the period 207-2022 we collected 66 observations of *Calotriton arnoldi* in the B6 population inverting 5 - 3 nights by year (median 3) during spring and autumn excepting the two last years because of the superficial drying of the stream allowed only to perform spring samplings. Apart from an unidentified individual, 42 were adults (16 males, 25 females and one not sexed adult), 18 subadults and 5 larvae. Thus, the proportion of adults / subadults was 2.3:1 and the sex ratio was favorable to the females 0.64 males: 1 females.

Number of newts observed and classified by population classes found in each stream stretch (10 m of length). The stream section was marked starting by the upper part (letter A) from the lowest (Letter I).

Stretch	Adults	Males	Females	Subadults	Larvae
A - B	2	1	1	2	1
B - C	3	2	1		
C - D	7	3	3	4	1
D - E	3	1	2	3	
E - F	1		1	4	2
F - G	1		1	1	
G - H				1	1
H - I	1		1		

We observed larvae in 2020, 2021 and 2022, thus evidencing the reproduction of the released newts, because the latest larvae reintroduced from captive reproduction were in 2014 and it is impossible that these individuals remained in the larval phase after and during all these years without metamorphose. Another evidence of the reproductive capability of the population were the observation of females with enlarged ovarian follicles and males with well-developed sexually dimorphic traits. In addition, and despite the unfavourable hydrologic conditions experienced by the stream in 2022 we observed for the first time a copulation and a male displaying the behaviour of females waiting to capture females for copulation.

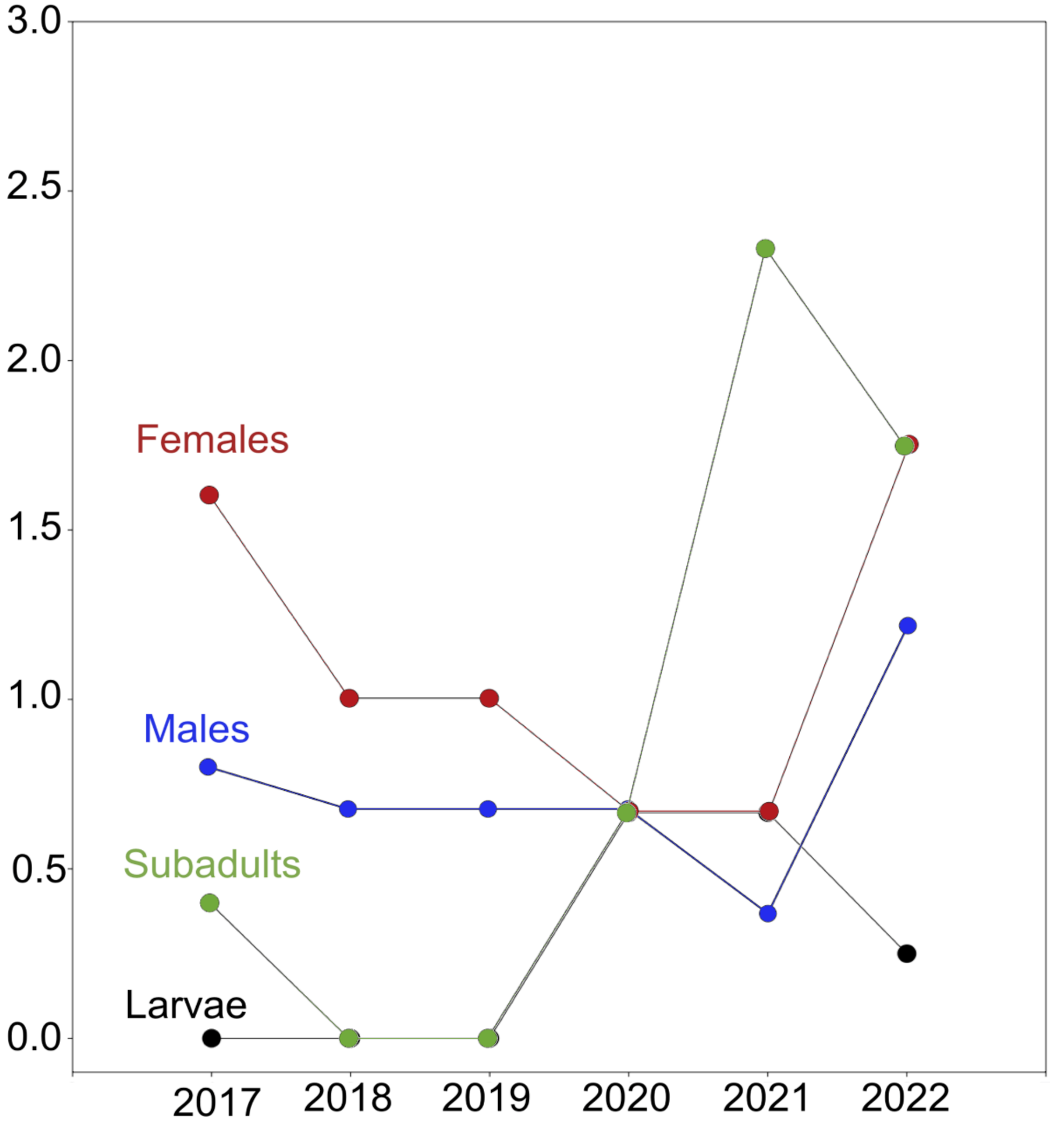

Reproductive behaviour, amplexus of newts observed in the population B6 on 30 march of 2022.

Male of *Calotriton arnoldi* raising its tail and waiting for a female observed in the population B6 on 30 march of 2022.

We collected morphometric data from 43 individuals, 29 adults (17 females and 12 males) and 14 subadults. Snout-vent length (SVL) was significantly larger in males (ANOVA $F_{1\ 27}$=4.045, P=0.0235), although the mean values were not much different: males 62.5 mm, females 62.4 mm. Weights increased positively with size in females (R=0.763, P=0.001) and in subadults (R=0.947, P=<0.001), but not in males (R=-0.345, P=0.327). However, weights were not very much different between males (mean =5.6 g) and females (mean = 5.2 g) and in relation to the body size were not significantly different (ANCOVA $F_{1\ 21}$=0.655, P=0.427). Of course, subadults are smaller (mean = 45.3 mm) and lighter (mean = 2.3 g) than adults.

Population size and dynamics

During the period 2017-2022, we observed an annual mean of 6.8 adults (4.1 females and 2.6 males), 3 subadults and 0.8 larvae. These two latter population classes showed larger oscillations among years than adults. To analyse the dynamics of each population class through the period it is necessary to standardise the number of observations by the number of nights invested in sampling, because it was variable among years. Thus, the number of observations divided by the number of night samplings showed differences between adults and immatures. As stated previously, larvae were found only during the three last years of study, similarly to the immatures which were rarely found during the first half of the study period, but became more frequently observed in the second one. The dynamics of the adults are similar between them. Until the last year of the study they tend to decrease, when males and females increase greatly.



Yearly evolution of the number of newts divided by the number of nights involved in samplings for males, females, subadults and larvae.

Only 13.6% of the detected individuals escaped. In the case of the captures and based on the unique codex of elastomer, we identify 25 adults (16 females and 9 males) which represents a density of 0.4 adult newts / m. In 2017 and 2019 all the newts captured were unmarked, but the number of recaptures showed a tendency to increase in the last years. In adults for example the rate of recaptures were 0.75 in 2020, 1 in 2021 and 0.4 in 2022 and in subadults were 0.5, 1 and 0.4, respectively. Nevertheless the low number of captures precludes us to perform an accurate estimation of population size.

Discussion and conclusions

The main conclusion of the monitoring carried out in the B2 population during 2017-2022 is that the reintroduced population succeeded and likely is stabilizing. We have several pieces of evidence to sustain this affirmation. Newts have been observed repeatedly year by year despite the unfavourable environmental conditions experienced by the B6 stream in some years, as for example accumulation of sediments and severe droughts, especially during the two last years. The reintroduced population seems to evolve towards a natural structure as evidenced by the increases of immature and larvae in the last years. Remarkably, the detection of larvae clearly indicates the reproduction in situ of the released newts in concordance with the observation of ovarian follicles and copulations. In addition and despite the severe drought suffered by the watercourses of the Montseny in 2022, the relative and absolute number of adult newts observation clearly increases breaking the negative tendency of preceding years. Captured newts showed a defensive behaviour typical of the species and lack of externally discernable pathologies. Compared with natural populations the physical condition seems to be normal and individuals were functional and well adapted to the habitat. Another positive evidence we found is that the estimated adult population density (0.40 adults / m) is slightly higher than the minimum inferred in the natural populations (0.32). Based on the results of this experience, we were aware of the importance of the subterranean habitat of the species that could explain why in this section of the stream B6 the reintroduced population survived while in others not. Unfortunately the inaccessibility of this part of the habitat prevents us from evaluating this aspect in other candidate streams. However, the duration of the superficial hydroperiod and the slope could indirectly indicate the optimality of the streams for releasing newts. Abundant and prolonged superficial water implies that the subterranean water is also greatly available whereas stepped slopes give place to fast running and oxygenated water.The section inhabited by the population is very short, but it also happens in a few natural populations of Calotriton arnoldi and for this reason we believe that the B6 could be viable long-term. Unfortunately, this population cannot be extended because of the negative impact of a stone quarry in the upper extreme and the lack of favourable conditions in the lower. Nevertheless, it was the first experience of the generation of a new population and it was useful to learn practical lessons improving the efficiency of the reintroduction program. However, we will continue monitoring the B6 population to evaluate how it evolves during the next years, but investing less time because we prefer to prioritize other reintroduced populations given our logistic and budgetary limitations.

3.1.2.2.- B7 new population

This stream located on the western side of the La Tordera valley runs in an east direction. The optimal stretch for releases is a very short section of about 450 m. The riparian forest is basically made up of alder (*Alnus glutinosa*) and ash (*Fraxinus* sps).

In 2014, 62 larvae were released and in 2015 the population was reinforced with 44 more larvae. No more individuals were subsequently located in the following surveys until two male individuals were found in 2019, indicating the survival of some larvae from the 2014 and 2015 releases. Despite spending two nights in good hydroperiod and temperature conditions surveying this population, the results were negative the next years.

Beyond the introduction section, the stream presents many conservation problems. It has little slope, there are many remnants of forest management within the bed, they are forest roads, a very open forest and non-continuous hydroperiod. In order to successfully implant a population, it would be necessary to carry out subsequent reinforcements. Moreover, it is located on privately owned land.

3.1.2.3.- B8 new population

Background

Within of action C.15 of the LTM project, several meetings were held to bring together the key actors in the management of the natural environment of Montseny to initially decide by consensus the main criteria related to key aspects for the creation of new populations and finally decide to which streams the introduction was made. In this sense, the B8 stream was one of those chosen for the creation of a new population based on the biotic, abiotic and modeling data generated in the LTM project. Population B8 is the first created during LTM project and which they are more consistent data. This torrent has about 500m of water and within this interval two segments were chosen to release the specimens after visiting the area to see the suitability of the sections, especially where the granite was not dominant.

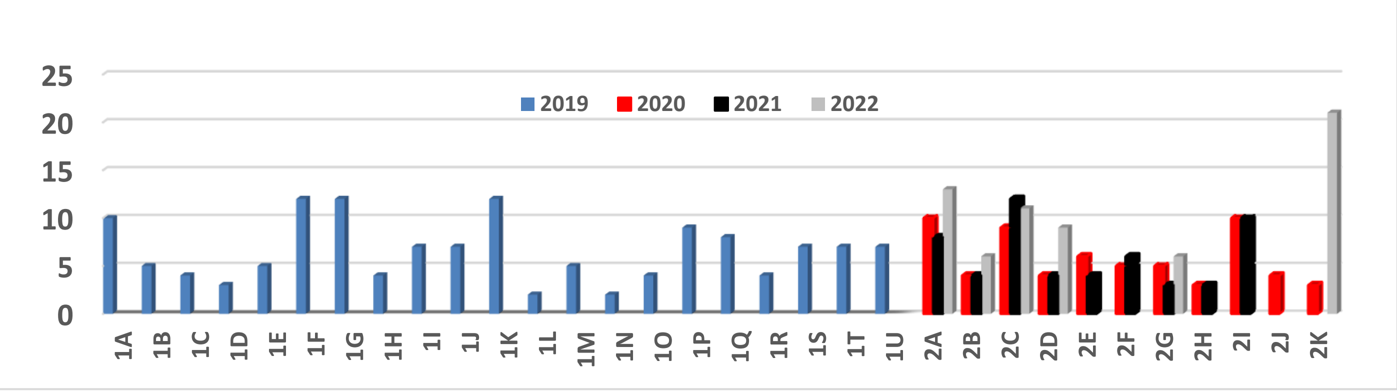
This stream is a western population created in 2019 by releasing adult individuals, in the middle section of a stream with good environmental conditions both in terms of hydroperiod and riparian forest. The stream experiences summer droughts and abundant sediments have been observed one time. The monitoring after the release carried out the same year, gave positive results indicating survival of a part of the individuals.

Results

In 2019, 136 specimens (47 males, 61 females and 28 immatures) were released in 21 points of the stream in a stretch of approximately 120 meters of this stream. All of them were born between 2010 and 2015 and came from the Torreferrussa breeding center (Carbonell et al. 2011). These points are georeferenced and marked with numbered tags.

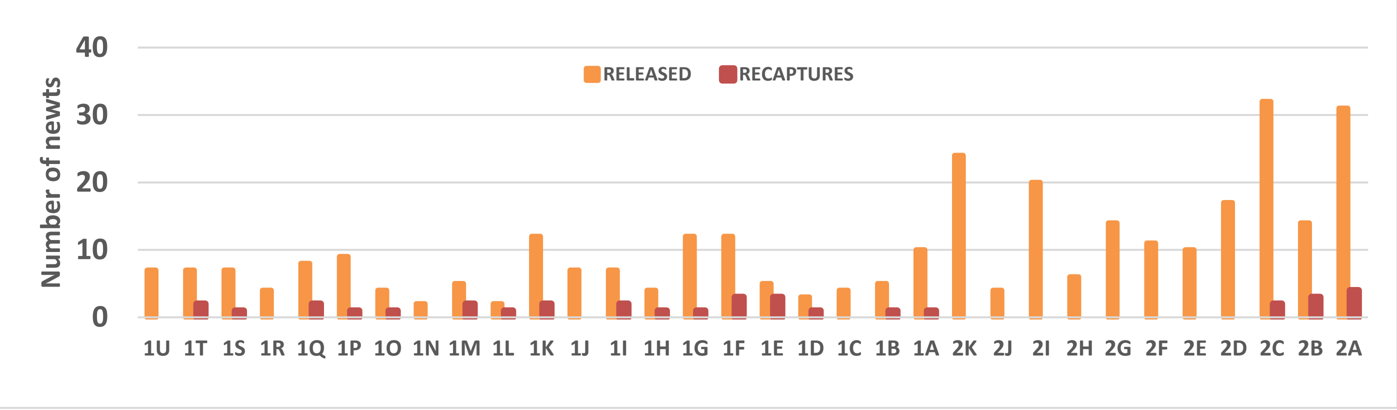
In 2020, 62 adult specimens were released from the CRFS of Torreferrusa and Pont de Suert, marked with micro-chips. The release segment was above the one where the species was reintroduced in 2019 and had a length of 92m. Subsequently, 6 sampling campaigns were carried out in 2019. The sex ratio of the released individuals (♂♂/♀♀ = 0.77), a value very similar to that obtained in the wild population A2 (♂♂/♀♀ = 0.75).

In 2021 63 individuals were released and 66 more in 2022. These last year newts were immature at the time of release. A total of 327 newts have been released in this stream. In 2019, 21 specimens were recaptured between the months of June and October. In 2020, 4 specimens were recaptured, one in 2021 and 10 newts in 2022. The recaptures have been continued and decreased in the years 2020 and 2021 since the monitoring could not be carried out in conditions due to the increase in flow in 2020 as a result of the Glòria and Filomena storms; and due to the restrictions imposed by the Covid-19 pandemic.



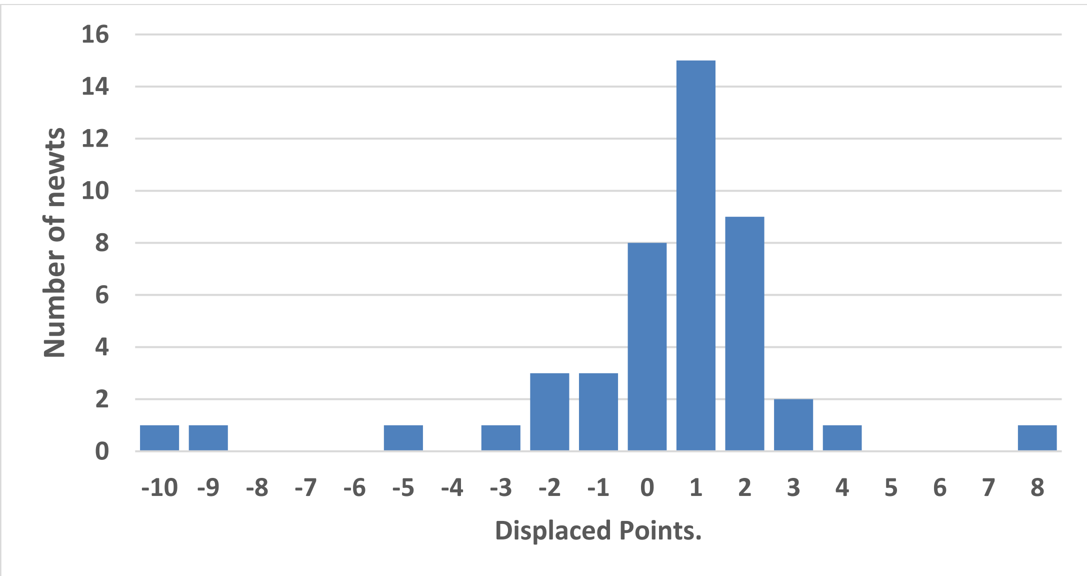
Released newts in torrent A8 by year. 1A to 1U: lower segment of stream. 2A to 2K: upper stretch of stream.

During monitoring 2020, 4 individuals were detected in the upper reach and none in the lower reach, all of them captured. The newts, 1 male and 3 females had a body length between 61-68 mm. Most of the individuals did not move but, when they did, they were more frequently moved upwards from the release point. The results obtained suggest that the reintroduction of captive-born newts into the wild accelerates their sexual maturation, produces a slight increase in weight and causes individuals to be more mobile than the natural reference population (A2), which has been studied. It should be noted that during the surveys a potential predator of the species, the water snake (*Natrix maura*), was found, although due to the characteristics of the habitat, its presence seems occasional.



Distribution of released newts in the two segments of B8 population (Orange bars) and recapture newts from 2019 to 2022 (red bars).

The distribution of recaptures in the new population B8 is very similar to the distribution of released individuals in the stream after two years in the low segment. However The dispersion of the recaptured specimens in relation to their release point. The average displacement of newts throughout 2019 was 0.76 in downstream released points. Despite this, one individual was found more than 10 meters downstream after the great flood, outside the marked area. Although wild *C. arnoldi* specimens show very little mobility and dispersal capacity in natural habitats, it is expected that newts that were born in captivity and released into a new and unknown natural stream, will disperse and explore more while seeking suitable microhabitats. Results confirm this. The dispersion observed is much larger than that which was estimated for wild population A2. The average of displacements (3,83 points; STD: 4.49) indicate that most displacements are made upstream. This fact is due to the exploration of the new territory, while looking for the most suitable microhabitats. The weight of the recaptured individuals after one year in relation to the time of release gives an average weight gain of 1.36% with a standard deviation of 10.67%. Preliminary results indicate that the population is well-established and that there have been few changes despite the floods and the summer drought.



Displaced points between recaptures from 2019 to 2022. Negative values indicate downstream displacements. Positive values indicate upstream displacements.

The average displacement of newts throughout 2019 in B8 stream was 2.44m down-stream. However, displaced recaptured newts move in average 14.75m downstream and 7.44m upstream. From 2019 to 2022 the dispersal activity increased probably due to exploratory behavior in a new habitat. Despite wild *C. arnoldi* specimens showing very little mobility and dispersal capacity in natural habitats, it is expected that newts that were born in captivity and released into a stream will disperse and explore more while seeking suitable microhabitats. However, the results don't confirm this. The dispersion observed in the first re-capture in the B8 stream compared to the estimate for the wild population A2 within two consecutive captures is not significant (t-test: -1.21; df: 56; P=0.229). After the surveys made in 2019, we have recaptured 14.7% of the released individuals. From 2019 to 2022, with four years of recaptures, percentage reached the 18.38% in the lower stretches. In the upper stretches (from 2020 to 2022), recaptures reaches only 6,1% with one area between 2D and 2K stretches without recaptures. Probably the extreme drought of the last two years could be the cause of this small recapture percentage.


Releasing newts in one selected point.

Results obtained during surveys indicate that the population is well established. A few individuals have been observed in almost all subsequent surveys, but at the moment, we don't have evidence of breeding. During the years 2023 and 2024 it is expected to locate the first large-sized mature ones. The distribution of recaptures in the new population B8 is very similar to the distribution of recaptured individuals in A2 population (see D2 action).

B8 stream during 2022 extreme drought.

3.1.2.4.- B9 new population

B9 is a western population opened in 2021 by releasing larvae and reinforced in 2022 also with larvae. Because it takes at least four years for larvae to reach sexual maturation, monitoring will not be carried out until 2024-25. The stream flows through a beech forest (Fagus sylvatica) with an undergrowth of ferns in many areas. It is located on public land and is approximately 1.5 km long and in a good state of conservation. The stretch selected for releasing newts measures 247.9 m. It has good potential as a viable population but there are areas where the habitat has little tree cover and areas where bedrock outcrops. Some sections dry out during periods of drought.

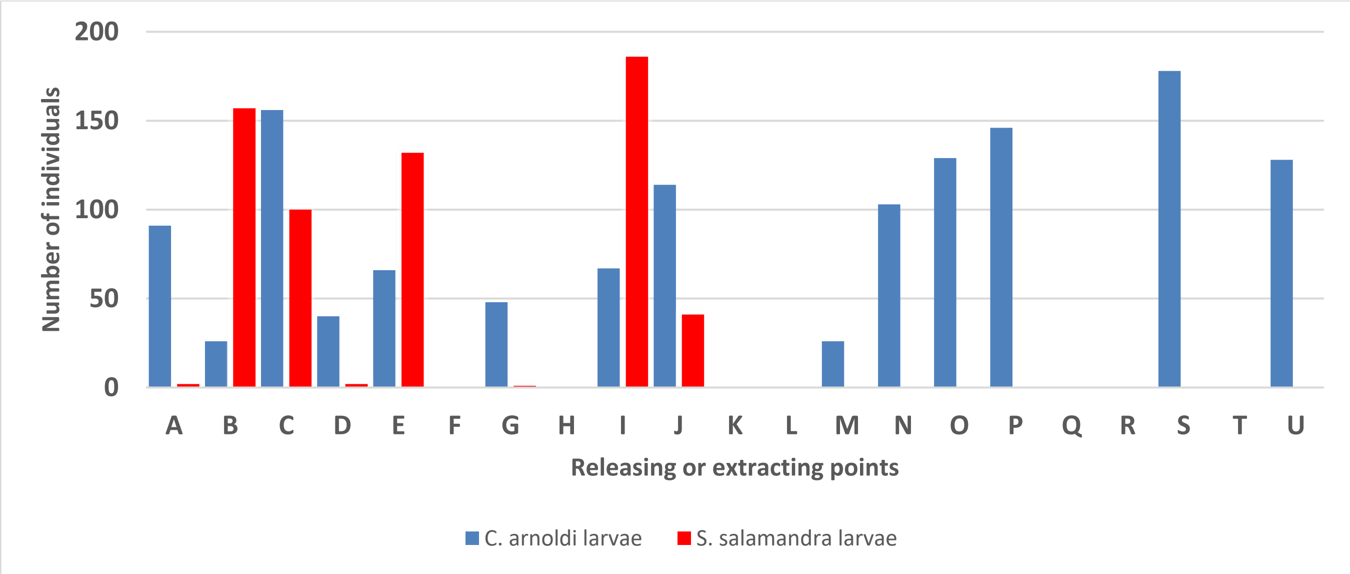
The minimum cost of opening a new population of *Calotriton arnoldi* with immature and adult specimens exceeds 345 euros per newt, mainly due to the costs of breeding in captivity. This amount does not take into account the cost of marking, evaluation of the stream, meetings, travel, monitoring, office work and habitat adaptation, which can multiply the cost of creating a new population by up to 50 times. For this reason, at the meetings of experts and the committee for new populations, the possibility of carrying out a test by introducing larval individuals was considered. At the first meetings of both committees, it was decided that the best option was to introduce immatures or adults because of their higher survival rate.
However, observations in the field, especially in the B6 and A2 populations, confirmed that larval survival could be much higher than previously estimated. In this case, the creation of new populations from larvae could be a much cheaper and quicker option. The main problem for its implementation would be to have a large number of larvae available each year. Furthermore, if the release of larvae were an efficient measure for the creation of new populations, it could increase the number of aquaria for reproduction by releasing those for the growth of immatures and adults. The year 2021 saw a great breeding success that led to a large number of larval individuals in the breeding centers. This situation was discussed at a meeting of the new

populations committee to assess the possibility of opening a new population with larval individuals and it was agreed to start an experimental opening of a population with larvae only. Once the stream had been chosen and visited several times, it was observed that there was a high density of salamander larvae that could compete with those of *C. arnoldi*.

This was seen as a double opportunity to test whether salamander larvae were indeed competing with or predating on newt larvae. It was therefore decided to carry out the introduction using two different protocols. In a lower section of the stream, *C. arnoldi* larvae were introduced without removing salamander larvae (green circles). In the upper section of the stream, the newts would be introduced after extracting as many salamander larvae as possible at the selected points (red circles).

B9 Stream selected stretch. Red cercles: areas with *Salamandra salamandra* larvae extraction. Red and green points: areas where *C. arnoldi* larvae has been released.

Five points will be selected in the lower part of the stream and 5 in the upper one separated by an average of 31 m between them to avoid the effect of drift. In this way we also obtained 5 replicates and five controls of the experiment. However, the result of this experiment will not be able to be assessed for up to 3 or 4 years and depending on many variables that a priori are not under our control (drought periods, torrential rains, survival of the larvae, among others).



Released *C. arnoldi* larvae from May, 2021 to October, 2022 (Blue bars) and extracted S. Salamandra larvae in the same period (Red bars).

From 2021 to 2022, 1328 Montseny newt brook larvae have been released and 621 salamander larvae have been extracted in the upper section of stretch. However, because larvae needs at least four years for to reach sexual maturation, monitoring will not be carried out until 2024-25.

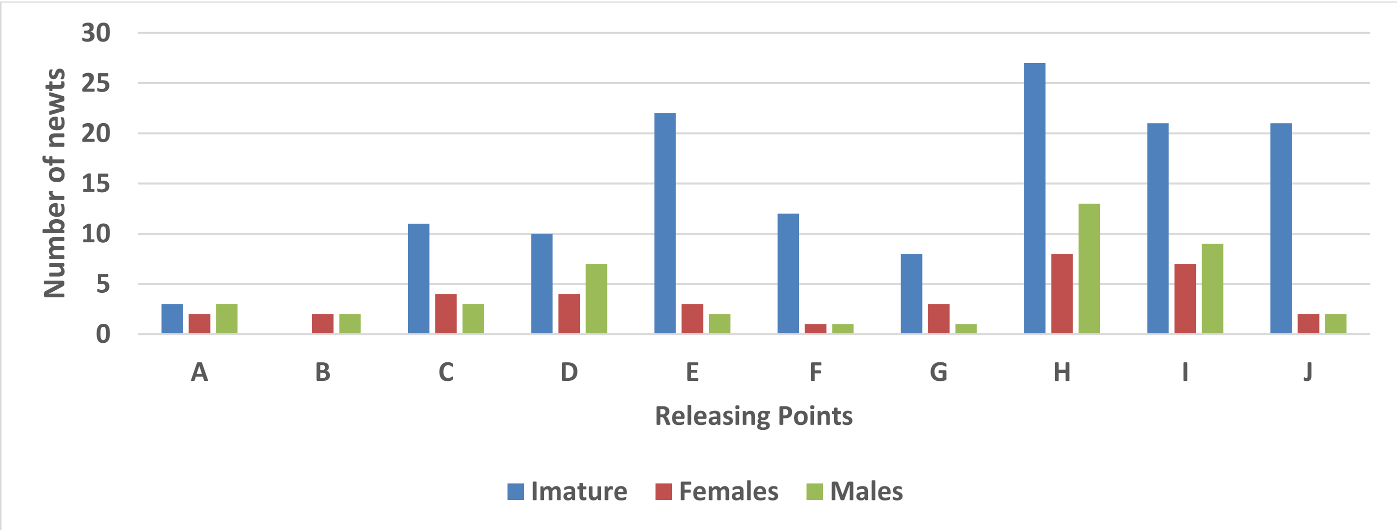
Releasing *C. arnoldi* larvae in B9 stream.

3.1.2.5.- B10 new population

B10 stream is a western population created in 2022 by releasing adult individuals in a section of the stream with good environmental conditions in terms of hydroperiod and riparian forest. Riparian forest not exist and it is mainly formed by oak trees (*Quercus ilex*) and hazel trees (*Corylus avellana*). The land is public owned and there is a total of almost 400 meters of stream in good condition. However, in some sections the water circulates underground and it has been verified that it maintains surface water flow even in periods of drought.

In April 2022, 119 adult newts have been released and in December 2022 population was reinforced releasing 95 adult newts. The newts released came from the CRFS breeding centers in Torreferrussa, Pont de Suert and Barcelona Zoo. Surveys will start in the spring of 2023.

Ten releasing point were selected in this stream and the newts were distributed:



Distribution of newts (males, females and immatures) released in B10 stream.

4.- Discussion and conclusions

One of the LTM project's main successes has been to work with multidisciplinary teams (formed by biologists, foresters, hydrologists, geologists, environmental engineers, civil engineers, environmental educators, journalists, lawyers, …) and the creation of committees based on expertise, working together to evaluate hydrological dynamics and actions to create new populations. These two factors have made it possible to have a cross-sectional view of the various questions and doubts that have arisen throughout the project. Undoubtedly, the sharing of different points of view by different specialists has led to decision-making, which is much more relevant to the actual problem.

Despite the species' very small geographic range, its distinct populations experience environmental spatial heterogeneity, which may drive the differences we found in their demographic parameters. The extensive survey (D2 action), highlights that the estimated survival rate is lower in the eastern population than in the western population, whereas population size follows the opposite pattern. One potential explanation is that larger population density increases competition for resources which leads to higher mortality.

Regarding the success of each of the populations, population B6 has, within the reduced extension of habitat conditions, the possibility of being viable, although it is advisable to carry out periodical reinforcement releases. In the case of populations B8, A5 and A6, a minimum survival leads us to be optimistic about their future, while in B7, this could be much lower. According to what has been observed in population A4, the reunion of individuals even some years later does not represent any guarantee of long-term success, as it depends on the degree of habitat conservation. In addition to monitoring, it would be advisable to carry out periodic and spaced reinforcement releases to try to consolidate them.

The most prominent aspects of the process of creating new populations could be summarized as:

- There are currently 8 new towns open, 5 western and 3 eastern. Of these, two can be considered established and 3 will most likely be so in a few years. The rest is too early to be able to define their future success.
- None of the populations created during the LTM project seem to have failed so far. Despite this, it must be taken into account that the time needed to be able to say that a population is definitely established is very long for this species. Sexual maturity occurs in the fourth year of birth in natural conditions and even a little later in breeding centers with larger sizes than in nature.
- 2852 specimens of *C. arnoldi* have been released into the streams, approximately 50% larvae and the rest adults and immatures. This means having released almost as many adult specimens as are estimated to exist in wild populations.
- We consider that the goal of creating new populations has been achieved since we have doubled the

number of streams in which the species is present.

- Newts spend most of their time within the interstitial hypogeum environment of the stream bed. Moreover, maintenance of a subway water flow during the summer or large periods of drought, ensures the survival of the populations.
- Eastern and western streams' physical environment was different: the stream bed in the eastern population is covered by rocks and it lacks big and deep ponds, whereas the western population inhabits a succession of very small waterfalls, riffles and well-defined pools.
- The Montseny brook newt's presence in streams seems to depend both on the morphology and structure of the stream. Existence of a slope that guarantees a minimum flow and oxygenation, and presence of fissured shale type rocks which favors the interstitial water flow.
- Native riparian forest or beech forest favors the persistence of flowing waters, both superficial and subterranean.
- The demographic data obtained with intensive monitoring are essential to estimate the ecological parameters needed for modelling new populations and to estimate the probability of survival and the carrying capacity of the streams selected to create it.
- Prey availability in streams running over beech forests may be greater, as it was also recorded for the sister species *Calotriton asper*.
- Fish presence determines the absence of *C. arnoldi* because of the special morphology of Montseny streams don't permit the sinthopic coexistence.
- The new population established shows greater dispersal than individuals in the wild population, which may be a consequence of their previous life in captivity or due to the search of optimal environments in a new habitat.
- It is still too early to say that the models developed for the creation of new populations are actually fulfilling their function and that the newly created populations are actually establishing themselves.
- It is important to emphasize that the creation of the new populations commission and the adjusted demographic data obtained in the field studies with regard to the wild populations have allowed us to obtain very precise theoretical models.
- In the species potential distribution area in the upper La Tordera river basin, there are no differences between the torrents where the species lives and where it is not currently found. None of the hydrological, morphogeological, riparian habitat or trophic availability variables show differences between torrents. Therefore, the most plausible hypothesis is that land uses in the last 150 years may have caused the rarefaction and disappearance of the species in many torrents. Mining, carbonization,

the transformation of forest masses into pastures and the consequent increase in the insolation of torrents and the contribution of sediments and chemical pollutants.

- Biosecurity measures and protocols have clearly proven their effectiveness. Despite the few cases detected of Bd, in one of the points it is caused by the illegal release of a non-native amphibian species of the PNRBM (far from *C. arnoldi* distribution area).

***Problems and solutions found*:**

The success of the program of introduction of new populations of Montseny newt is founded in two crucial aspects: release of large number of newts in the nature and the selection of streams keeping an optimal habitat.

Until 2018, the number of released newts was slow because only one of the centers of captive breeding (CRF Torreferrusa) was highly productive. In addition, most of the newts born in this center were stocked to mantain a genetic reserve or to give to the other centers involved. At present time, four more centers are involved in the breeding and growing process, Barcelona ZOO, Pont de Suert, Chester ZOO and most recently CREAC (Center for Research and Environmental Education of Calafell), and more larvae are available to release.

Previous experiences in generating new populations occurred by selecting streams based on an objective point of view of the leading experts in the ecology of the species, but without an objective analysis of the variables involved. To overcome the problem of the subjectivity on election of locations, an external team conducted an environmental modelling of the species distribution, providing a high precision map of the potential habitat of the species, and one of the firsts steps in the reintroduction process.

Due to these preliminary tasks, no newts were released before 2019, although, even considering this delay, the objectives of the action have been fully achieved.

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