# ACTION D2. Montseny Brook Newt wild population monitoring

## LIFE Tritó Montseny (LIFE15 NAT/ES/000757)

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Summary

We monitored the eighth natural populations of the Montseny Brook newt (*Calotriton arnoldi*) during 2017-2022 in the frame of the D2 action of the LIFE15 NAT/ES/000757 (LifeTritoMontseny) in order to evaluate the conservation status of the species. Night surveys were carried out by a team of a technician and two rural agents of the mountain group of the Generalitat de Catalunya, counting and geolocalizing newts, wherever possible sexed and classified as adults, subadults or larvae. We mapped geolocalizations to analyse the species range and compute indicators of maximum adult abundance, percentage of stream occupation and quotient between them as a measure of relative abundance. These calculations were performed for each population and population groups inhabiting the two second order hydrographic basins of the species geographic range. However, we used part of this information in combination of results of demographic and genetic analyses to estimate population sizes and the connectivities among them. The demographic study of the eastern A2 population by means of capture and recapture samplings marking newts using elastomers gave us interesting information about the species ecology that was useful for the species management. From 2018 to 2022, 81 adult newts were captured and allowed to estimate a mean displacement of 5.5m with maximums of 56 and 20m ups and downs. Thus, we conclude that *Calotriton arnoldi* is highly philopatric. Based on an adult recapture rate of 29.3% the estimated population size was 93 newts (80-119, 95% confidence interval) leading to a mean density of 0.63 adults/m. The main conclusion derived from the inferences of the analyses we performed is that Montseny Brook newts are, after almost 20 years of its discovery, continue to be a critically endangered species and significantly we found evidence that the situation of the natural populations have slightly deteriorated and worsened since this time. In concrete, we find evidence of the dramatic decline of one of the three populations of the eastern basin, which is the most endangered, that might have already become extinct. Another population, in this case in the western basin showed signs of decreases in their initially reduced range and population decline. Evaluation of the characteristics of the populations in relation to the negative impacts experienced by the habitats indicates that only three populations - all of them in the western basin - have possibilities to successfully face the severe droughts that each time more frequently affects the Montseny. On the basis

of this conclusion, the main hope for the species persistence in the future is the success of the program of reintroduction and implantation of new populations to compensate for the loss of geographic range and individuals. Nevertheless, the monitoring of the natural populations is a very important tool providing critical information for the species management and should be continued during the next years.

## 1.- Introduction

### 1.1.- The Montseny Brook Newt

The presence of rheophilic newt populations living in the streams of the Montseny massif was reported by science in 1981 (Montori and Pascual, 1981) and classified as belonging to the *Euproctus asper* species. Currently called *Calotriton asper* (Carranza and Amat, 2005), this species - Pyrenean newt - is distributed along the Pyrenean ridge, including the pre-Pyrenean slopes located to the north and south of the mountain range, reaching its southern limit at the southern end of the Serralada Transversal Catalana. This newt mainly inhabits streams, creeks and brooks, but also high mountain lakes and caves with flooded parts. The progress in the knowledge of the situation of the "Pyrenean newt" of Montseny made it possible to better define its distribution, morphological characteristics and population size (Montori and Campeny, 1991; Amat, 2004; Amat and Roig, 2004; Amat, 2005). Finally, a careful genetic and morphological study of the Montseny populations in the context of the

Pyrenean newt and the other rheophilic newts of Europe (Carranza and Amat, 2005) led to the recognition of these newts as a well-differentiated species, the Montseny newt *Calotriton arnoldi*. The two newts, *C. arnoldi* and *C. asper*, are the only current descendants of a species that adapted to the life in running waters from semi-aquatic habitats. Thus, this ancestral species combined a terrestrial life phase in humid environments as an adult or immature, with one in stagnant waters as an egg, larva and reproductive adult. The evolutionary process of adaptation to running waters allowed the ancestral *Calotriton* to occupy a new ecological niche with respect to its next sibling lineage, the *Triturus* newts (Carranza and Amat, 2005). This adaptation, which happened nearly 8 million years ago - estimated on the basis of molecular studies - involved changes in morphology and anatomy, behaviour and reproduction.

The current geographical distribution of *Calotriton arnoldi* with respect to its sister species allows us to hypothesise that the isolation of an ancestral *Calotriton* population in Montseny massif - which is the southern margin of the its distribution- led to the appearance of the species during one of the first glacial cycles about 1.7 million years ago (Valbuena et al., 2017). The other climatic event that affected the distribution of the species took place during the Riss glaciation (180,000 years ago), which produced a reduction in the distribution range of the species. During this period, *Calotriton arnoldi* remained isolated in two refuges located on both sides of the Tordera into two different second order basins without connection between them. This resulted in the separate evolution of these two groups of populations which is reflected in the development of different morphological and genetic characteristics (Valbuena et al., 2015-2017). The Montseny newt is a micro-endemism of the Upper Tordera Basin, which is delimited by the ridges of the Montseny mountains - the main peaks are les Agudes, Matagalls, Sui and Turó de l'Home (more than 1000m high) - that delimit the inverted U shape of the massif. It is a species of small size (maximum length of the snout at the cloacal opening, 68 mm and total, 110 mm: Amat et al., 2015) and of strictly aquatic habits, because only on very rare occasions we have observed individuals on land and always less than two meters of distance from the water.

Adult female of *Calotriton arnoldi*, as can be seen from the smaller relative size of the head, longer and lower tail, which presents the typical light brown coloration with yellowish spots of the populations of the eastern basin of the species range.

The habitat occupied by *Calotriton arnoldi* are the headwater streams subject to a hydrological regime clearly influenced by the Mediterranean climate, which gives rise to summer droughts and periods of flow growth resulting from spring or autumn rains. As a result, the streams experienced a permanent hydroperiod fed from an underground source that flows between the bedrock and the stony matrix, and thus becomes the microhabitat refuge of the newts against unfavourable environmental conditions. The surface runoff of rainwater increases the volume of water, which when it emerges and in combination with the topography - characterised by a rather steep slope - gives rise to a system of ponds, riffles and waterfalls. The streams -located between 615 and 1150m altitude- are placed on a substrate of slates and phyllites that generate porosity in the form of rock accumulations, cracks and cavities. The terrestrial habitat is made up of a riverside forest formed by alder and hazel trees which is quite narrow given that it is limited by the reduced available riverbed due to the stepped topography. In the upper reaches of the streams, this vegetation is replaced by beeches. The only exception to this typology of habitat is the existence of a natural population that occupies the head of a large stream, where the lower slope and greater width of the riverbed gives an increase in the size of the ponds and riffles and a more developed riparian forest.

Male of *Calotriton arnoldi*, sexable based on comparatively larger head and higher tail. In this case it is an individual from the populations of the Western basin characterised to the darker coloration, you can observe the whitish tip of the snout, which appears only in this sex.

**1.2.- Threats and state of conservation**

The strict ecological specialisation of the Montseny newt in so specific habitat in combination with its extremely small and fragmented geographical distribution lead that since its discovery, the species was considered "Critically Endangered" by the International Union for Nature Conservation (IUCN, 2022). The use of Montseny's natural resources by the human population has negatively affected this species due to its sensitivity to changes in habitat structure and its fragmentation, and their limited ability to recolonise new or restored habitats. However, in historical times it is possible that *Calotriton arnoldi* had a wider geographical distribution as can be inferred from the genetic structure of the populations (Valbuena et al., 2017).

Stream inhabited by the Montseny newt, in the western basin. The forest cover of riparian forest allows the maintenance of temperate environmental conditions, while the accumulation of rocks on the riverbed creates the fissured microhabitat that the species requires.

The intensive forestry, livestock and agricultural exploitation that took place during the last centuries could be the cause of this decline, although there is no concrete evidence. At present, the territory has been depopulated, reducing traditional agro-livestock activities and being partly replaced by recreational and touristic uses. In the current context, studies carried out have made it possible to identify a series of problems that have a negative impact on the species and its habitats.

**Forest exploitation**. The logging of riparian forest dramatically modifies the environmental conditions of streams, resulting in increased temperatures, loss of water retention capacity and soil erosion. Apart from affecting the activity of *Calotriton arnoldi* on the stream-bed, the accumulation of sediments eliminates the subterranean porosity that serves as a refuge for the species by filling cracks, spaces between rocks and cavities. On the other hand, it is possible that this also affects the fauna of aquatic macro-invertebrates that constitute its diet. This problem increases its impact even more in the case of allochthonous plantations of fast-growing conifers such as Douglas fir or chestnut trees.

**Communication paths**. Forestry activity and the existence of villages have resulted to the development of a network of roads, forestry tracks and clearing tracks along the massif. At the points where these communication routes intercept the streams inhabited by *Calotriton arnoldi*, negative impacts occur for the species. For example, they facilitate access to the habitat, which can allow the capture of newts, the discharge of pollutants, the spread of infectious diseases and the introduction of non-native species. However, the main consequence is the fragmentation of the habitat when the tree cover is removed and the water course is buried in the crossing point. The main consequence of this impact is the disappearance of the newt population around the points where the torrents are intercepted by the communication routes.

**Water exploitation.** This problem occurs on two levels. The first and most direct are the water intakes that local owners install in the streams to ensure a supply of water for domestic use. On a larger scale, the exploitation of aquifers by companies of commercial mineral water product can have a negative effect during the wet season in streams that are partly fed by underground springs. In any case, the final consequence of unsustainable water exploitation is the decrease in flow which can cause stress in populations by restricting their ability to feed and reproduce. This can negatively affect the survival of the newts, reducing the population size and eventually causing the complete extinction of the populations, since *Calotriton arnoldi* is completely aquatic.

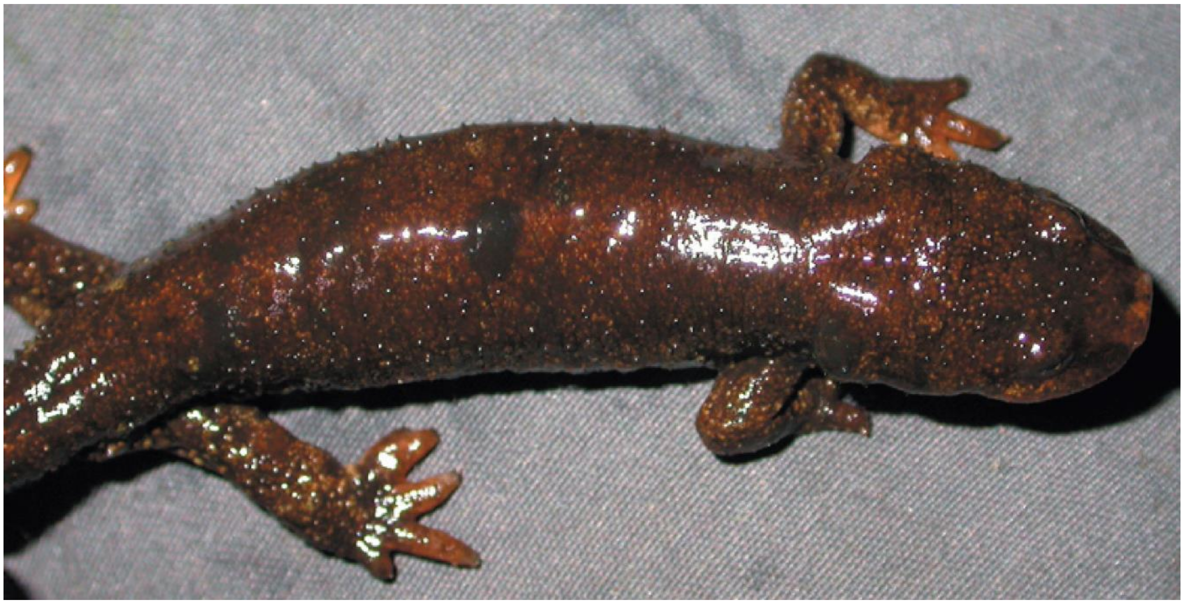
**Climate change.** The consequences of climate change on Montseny newt populations primarily affect the hydrological dynamics of streams. Model predictions for the Mediterranean region indicate an increase in temperatures leading to extremely hot summers and mild winters. At the same time, even though the overall rainfall over the decades is not generally expected to change, precipitation patterns will be more irregular among years, increasing the frequency of extreme events, i.e. droughts and torrential rain concentrated in a few days. The increase in temperatures will affect the environmental humidity given the increase in evaporation and evapotranspiration from the plants, reducing the contribution of water to the streams by runoff. As a result the amount of water in the streams inhabited by the species will decrease, especially during the drier periods. Secondly, the increase in temperatures has given rise to an altitudinal replacement of beech, the type of forest most favourable for *Calotriton arnoldi*, by mediterranean oaks.

Apart from the problems affecting the habitats, *Calotriton arnoldi* is a very sensitive species to the introduction of non-native species, the collection of individuals and emerging infectious diseases such as chytridiomycosis.

### 1.3.- The Montseny Biosphere Reserve and Natural Park

The Montseny massif is the southernmost end of the Transversal Catalan Range, separated from the nearby massifs by the plains del Vallès and Osona, and the Congost valley, generating a mountainous area of nearly 400 $km^2$. The axis of the massif is the Tordera, a river that has its source in the stream of Sant Marçal, and the highest peak, Turó de l'Home (1706m).

The summer drought negatively affects the streams inhabited by *Calotriton arnoldi* (B1 population, western basin), although the species can survive by taking refuge into the rocky matrix of the riverbed, if there is underground water.



The epidermis of amphibians is very vulnerable to the environment stressors. Some individuals in the eastern group of populations develop melanomes due to some unknown environmental factor.

The geographical situation of the massif produces a certain climatic variation, despite being within a typically Mediterranean area. Thus, this combination of altitude and geography made the Montseny a border zone between the Boreo-Alpine and Mediterranean regions with a large variety of forests and habitats, and supports a high biodiversity and endemicity in comparison with surrounding areas.
The consequence of this is that Montseny has high floristic and faunal richness, and because of the threats to the conservation of the massif, in 1978, Montseny was declared a Biosphere Reserve by UNESCO and subsequently, in 1987 it was declared Natural Park managed by the Provincial Councils of Barcelona and Girona (PN-RB Montseny).

**1.4.- The LifeTritoMontseny**

The discovery of *Calotriton arnoldi* and subsequently the cataloging of the species as critically endangered, generated the interest of the PN-RB Montseny in deepening the knowledge of the biology of the species and the conservation problems. For this reason, during the period 2006-09 a technical study was carried out to overcome these deficiencies. As a result of the conclusions derived from this study, since 2010 a monitoring program of natural populations was started, as well as the creation of new populations through the release of individuals from the incipient breeding program in captivity that had previously begun in 2007. However, the progress in the study of the Montseny newt also highlighted the threats and negative impacts affecting the populations and the conservation of their habitats. The development of these actions (monitoring of the habitat and, the natural and reintroduced populations), the realisation of logistical and budgetary limitations, and the need to integrate all the actions under a unified project, give place to the presentation of a life project. The LifeTritoMontseny (LIFE15 NAT/ES/000757) began to operate in October 2016 and ended in December 2020, although the delay in meeting the deadlines for the completion of some actions made it necessary to extend their development until December 2022. Thus, the LifeTritoMontseny included five lines of work, which aim to a) increase the degree of legal protection of the species and its habitats within the PN-RB Montseny, b) disseminate the knowledge about the species and place it in social value, particularly in the local population, c) implement in-situ and ex-situ conservation actions, especially within the framework of the captive breeding program, d) carry out research in various areas to improve knowledge of the species and monitor *Calotriton arnoldi* populations as well its habitats and e) carry out actions to restore the altered habitats and minimise the impacts that affect them. These lines of work have developed into 49 different actions, integrating a multidisciplinary team of more than 120 people, with a total budget of almost 3 million euros.

## 2.- Action D2: Monitoring of the natural populations of Montseny's newt

Within LIFE15 NAT/ES/000757, action D2 is defined as the monitoring of natural and reintroduced populations of the Montseny newt. Nevertheless, it has been chosen to separate the monitoring of natural populations from reintroduced ones in the final report of the Life project. The reason for this is to connect and integrate monitoring of reintroduced populations with the captive breeding program. For this reason, the results of the monitoring of the reintroduced populations are shown in the C15 action of the LifeTritoMontseny, while in this report we also included the monitoring of natural populations.

The natural populations monitoring program established in action D2, represents the continuation of the started in 2010, which initially consisted of sampling a linear stretch of stream of less than 150m in seven of the eight streams inhabited by species. Given that the samplings were carried out at night and by a single person, for safety reasons these short sections were selected because they were the most accessible and the least difficult to traverse. Since 2015, the participation of the mountain group of the Rural Agents of the Generalitat de Catalunya in the sampling program, allowed the building of a field work team with the aim of covering almost the entire geographical distribution of *Calotriton arnoldi*. This last sampling system incorporated within the species monitoring program was adopted in action D2 of the LifeTritoMontseny as a fieldwork routine. However, from 2018 a new survey has been incorporated to D2 action: the intensive survey of A2 population in order to estimate demographic parameters needed to have a good scientific knowledge of species biology and ecology and needed to build models of long-term survival in new populations. Then, two types of monitoring of the wild populations were carried out. An extensive one that included all the wild populations and an intensive one on the eastern A2 population.

**2.1.-Goals and Methodology**

**2.1.1.- Purpose and objectives**

The monitoring of natural populations of *Calotriton arnoldi* has been an essential tool for the management of the species during the development of LIFE15 NAT/ES/000757. The main purpose of the action is to periodically diagnose the state of conservation of the species and secondarily to provide specific information on the situation of specific populations. In this framework, the main objectives of the D2 action have been:

a) Achieve the most detailed knowledge about the geographical distribution and quantify the spatial extent of the populations and the whole species.

b) Achieve demographic parameters in wild eastern population (A2) in order to estimate ecological and biological parameters of the species by means of capture-recapture.

c) Compute representative indicators of the conservation status of the species at the level of the population, population groups and the species as a whole.

d) Support of complementary scientific and technical activities not directly programmed in the monitoring.

**3.- Study area**

The field work intended to achieve the objectives of action D2 has been carried out within the distribution area of *Calotriton arnoldi*, the Upper Tordera Basin. This water course is arranged over the territory oriented to the southwest, thus generating the Tordera valley and dividing it into several eastern and western second order basins. The species inhabits an eastern and a western basin, forming two groups of populations separated approximately 5 km in a straight line. Nevertheless, following the course of the Tordera – the natural connector-, this distance is more than 30 km. In this technical report, these two groups will henceforth be referred to as the eastern and western groups or basins. The eastern basin is fundamentally oriented to the southwest and experiences a greater human impact. In this basin the species inhabits three streams (A1, A2 and A3) that converge in the eastern river tributary of La Tordera, but because the Montseny Brook newt populations do not occupy the lowest sections despite the fluvial continuity among them, they are not interconnected. In the western basin there are five populations located on four streams (B1, B2, B3 and B4) that flow into the wester brook, but the newts that live there are considered an independent fifth population (B5). In this group of populations, the only population isolated and without continuity with the others is the outermost one in the valley, the B1.

**4.- Tasks done**

The development of the D2 action has led to the development of several tasks involving field actions and analysis of the data obtained. Prior to the field work, the request for logistical support by the mountain group of the Rural Agents of the Generalitat of Catalonia and the planning of the campaigns have been carried out. Field actions have consisted of sampling natural populations and related complementary tasks (see methodology). At the end of these tasks and depending on the LifeTritoMontseny project schedule, the corresponding development reports of action D2 have been carried out as scheduled. In conclusion two types of surveys were done: Extensive survey in all wild populations known and intensive survey in A2 eastern population.

## 5.- Extensive survey

### 5.1.- Field methodology

The monitoring program of the natural populations of *Calotriton arnoldi* consisted of sampling the distribution of the species by means of nocturnal routes carried out by a team made up of a researcher and two rural agents. The campaigns have been developed primarily during the springs, but water flow problems have forced in certain years to continue them during the following autumn. Specifically, the detectability of the Montseny newt is limited by the availability of enough superficial water level to enable the individuals' activity on the stony stream-bed, where they are visible to the eye without having to lift rocks with the consequent alteration of the microhabitats what this entails.

At the same time, after episodes of heavy rain the water flows rise enormously, increasing the depth of the torrent pools and the turbulence of the water. This also makes it difficult to detect the newts, both due to the movement of the water and the fact that the individuals select deeper places or take refuge under stones. Finally, temperatures also limit surface activity, so that during winter and summer the detectability of newts decreases. However, during the summer the main handicap is actually the drop in water levels due to the drought and not so much the increase of temperatures above the physiological tolerance of *Calotriton arnoldi*.

Confirmation of this assumption is found in the fact that individuals can be observed active during the summer in the deepest wells of the creeks and lower sections of the largest stream. These factors act differently according to the characteristics of each stream and even within each of them showing gradual differences between the upper and lower sections. At the same time, the mountain slopes where the streams are placed are very steep, which makes it difficult to walk. For this reason, instead of visiting each stream separately, itineraries have been established combining sections of different streams at the same time to facilitate the team mobility and thus optimise sampling.

The support of the rural agents of the mountain group was very important, not only to carry out the field tasks safely, but also to collaborate in the counting of individuals.

Thus, the following order of sampling itineraries was established by combining sections of different streams: 1st B1+B2b (lower section), 2nd A2+A3, 3rd B3+B2b (upper section), 4th 1 (upper section), 5th B2a, 6th B4 (upper section), 7th B4 (lower section), 8th A1 (lower section) and 9th B5. This prioritisation makes it possible to visit in the first place the streams and sections that usually dry up earlier and to leave late those that have more water flow so that the decrease in water levels during summer allows a better survey.

Due to accentuated pluviometric irregularity and severe droughts that have occurred in recent years, we partially changed this pre-established program and even completed it with samplings carried out during the autumn. In some cases, after heavy episodes of rain, field work has had to be stopped for a week, waiting for the water flow to decrease. During the development of the field tasks, biosecurity rules have been followed in order to avoid the contagion of emerging infectious diseases to the newts.

The sampling consisted of walking on each itinerary twice a year - although this has not always been possible due to poor water-levels- during the night, visually locating newts and therefore without moving the stones that serve as shelter. Two types of data have been obtained during these samplings. The first was the geolocation of individuals using the digital cartography of the PN-RB del Montseny as a reference. Given that in the populations with higher densities nets were sometimes close to each other, when it happened, a single location was taken, due to the fact that the geolocation error was large compared to the distance among them.

The other data we obtained was the count of the number of individuals. In fact, these two types of data were recorded in the field at the same time before transferring them to the digital database. However, whenever possible, each observed individual was categorised as adult (male or female), subadult or larvae. In order to do it, subadults were defined as those individuals with a keratinised and granular skin, but without developing the typical sexual dimorphism of the species (Amat et al., 2015). However, a body size of less than 50 mm has been established as a cut-off point, below which metamorphic individuals are defined as subadults and from which sexual maturity occurs. In the case of adults, sexing was performed based on external dimorphic characteristics (Amat et al., 2015) and when this was not possible -because newts escaped quickly- individuals were recorded as simply adults. Finally, the larvae were those individuals defined by having a non-keratinised skin, developed gills, caudal membranes and, elongated and tubular head. On very rare occasions, post metamorphic have been observed, which are similar to larvae but lack gills.
During the development of the samplings, a series of complementary tasks were developed in the framework of the development of other LifeTritoMontseny actions. At a technical level, negative impacts on the habitats were located and assessed as well as the functionality of the works intended to renaturalise or improve them based on the ecological characteristics of *Calotriton arnoldi*. However, during the development of the field work and taking advantage of the capture of specimens for research purposes, sampling of skin secretions has been carried out for detection of emerging diseases. At a scientific level, we participated in the study of diet, population genetics and the detectability of the species using environmental DNA. In this sense, genetics sampling was used to carry out a mark-recapture sampling in a stretch of stream inhabited by the B5 population, in order to estimate the population size and density.

### 5.2.- Data analysis

The data obtained during the fieldwork have been recorded into a digital database with the monitoring of reintroduced populations and the specific demographic studies carried out in the A2 population.
The analysis of the data allowed the mapping of the geolocations using digital cartography of the PN-

RB Montseny, showing the extent of the geolocation error. Therefore, we corrected the raw locations by placing them on the shortest straight line to the stream and entering the new locations into the initial database in UTM format. This corrected mapping has been used to compute the stream lengths occupied by populations. In order to homogenise the sampling effort, the number of nights (maximum two by year) used to sample the populations has been taken into account. Thus, considering this sampling effort, the counts and geolocations for each population category (males, females, adults, immatures and larvae) and computed distances, the following numerical indicators have been estimated:

**a) Absolute abundance**. Annual maximum values as the highest number of adult individuals on one of the two sampling nights. Since the sampling of some streams has been carried out in sections, and the frequency in which they have been sampled is not the same, in the case of the maximum abundances they have been calculated in a weighted manner

**b) Relative abundance**. It represents the number of adult newts counted with respect to the length of the stream occupied according to the geolocations, using the annual maximum values.

**c) Occupancy.** Length of the stream where newts have been found in relation to the total using the geolocations obtained during the fieldwork and calculating the total length using digital mapping using Q-GIS software.

We also have performed an estimation of the population size of adult newts based on the gathered data during the period 2017-2020. This was done by defining stream sections of high, medium and low abundances based on the concentration of geolocalizations along the watercourses. We used a density of 1 adult newt / m based on the results of a short demographic study of a section of the population B4 for the high abundances stream sections and values of 0.66 and 0.32 adults /m for medium and low abundance according to the results of two long-term demographic studies carried out in the A2 and B2 populations previously to the life project implementation. Connectivities among populations of each basin were also computed using a dendritic index as a product of the length of the sections of the stream inhabited by newts divided by the total length of the watercourses of the basin and multiplied by the inverse distance to each of the other populations.

**5.3.- Results**

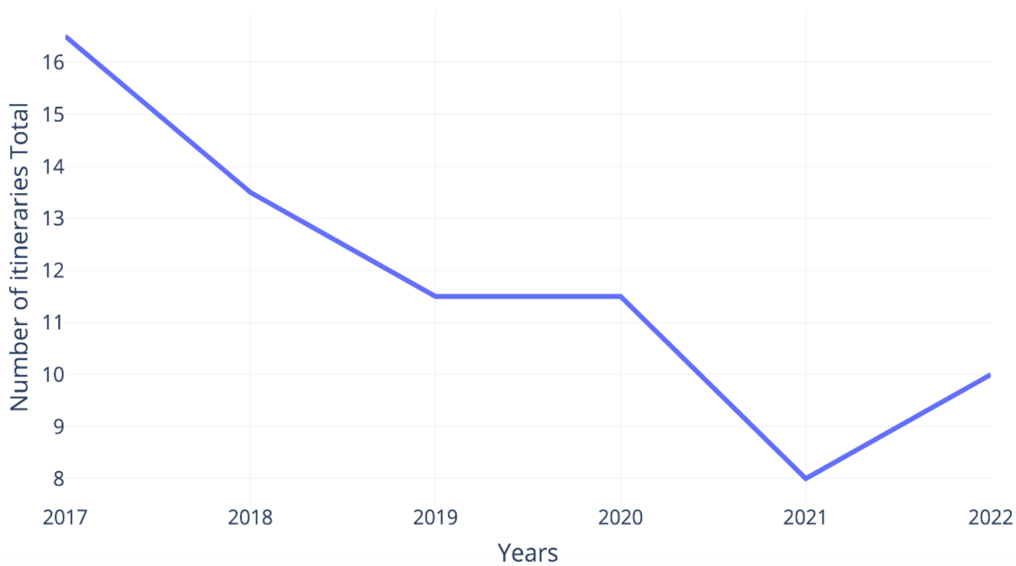
Herein we report the data gathered during the field samplings we performed in the framework of the D2 action of the LIFE15 NAT/ES/000757 and the results derived from their analysis. Previously to the results presentation, we quantify and summarise the main field data collected during the field surveys performed during the period 2017-2022. Results are presented, first analysing the spatial-temporal structure of the distribution and abundance of the Montseny newt. After that, we report the overall and population annual variation of the three main indications of species conservation: occupation of the streams, and abundance and relative density. In addition, we used this primary data to conduct an analysis to understand how each population is interconnected and determine how much isolation there is among the other populations. Finally, we also used data to estimate population size for each population, the two groups of them and the whole species. The dynamics of the three indicators during the period of the LifeTritoMontseny implementation coupled with the information provided by the analysis of connectivity and population size were posteriorly used to diagnose the current state of conservation of the species after the end of the Life project.

**5.3.1.- Sampling data**

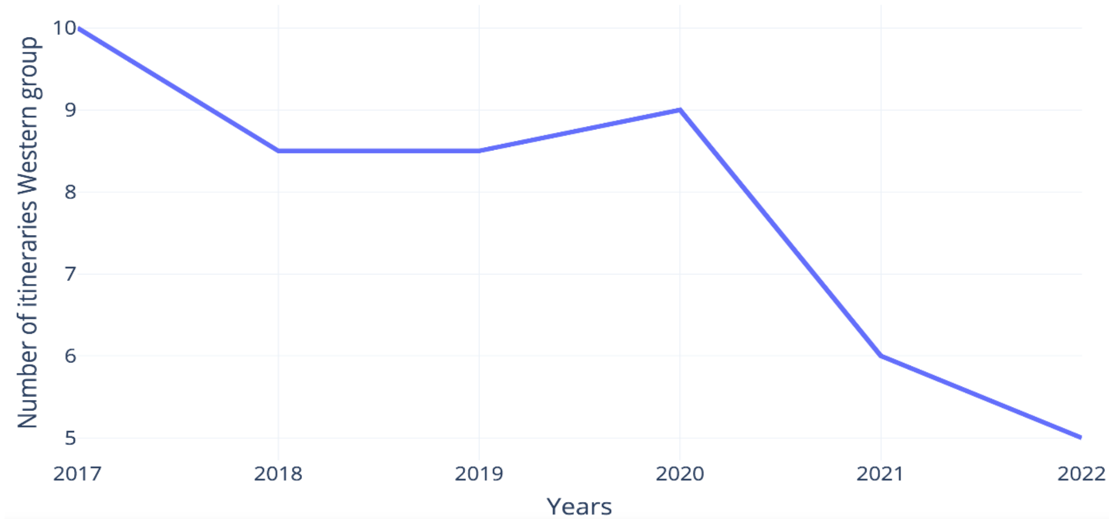
Field survey expended a total of 72-night itineraries (mean by year =12) during the period 2017-2022, allowing to sample several sections from different streams in the same itinerary.

The fluctuation of the sampling effort (number of nights per year and population) was very informative about how the droughts have affected the water availability in the aquatic habitats of *Calotriton arnoldi*. The total number of night itineraries performed by year showed a clear tendency to decrease during the two last years for the whole species range, although this pattern was different between the two groups of populations. In the eastern group of populations, the number of night itineraries declined during the halftime of project execution, increasing in 2020 and became posteriorly stable whereas in the case of the western group the number of itineraries invested remained stable until the two last years when declined. Thus, the overall tendency was caused by the dynamics of the sampling in this latter group of populations revealing a strong negative effect of the severe drought experienced during the last two years. Comparing the sampling effort between basins, the western one involved more nights, because of the larger number of streams, but nevertheless the trend towards a decrease is more marked in comparison with the dynamics of the eastern basin.

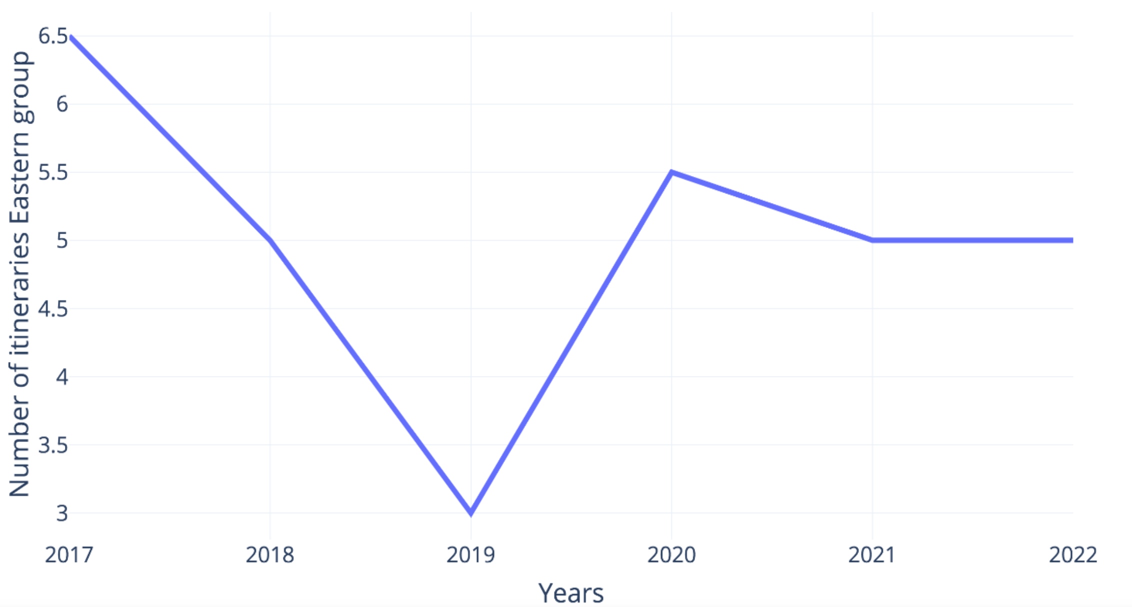
Pooling all the populations in one unique sample, the maximum number of observed adult newts by year was unrelated to the number of standardised nights devoted to each annual survey (Spearman correlation = 0.521, P=0.305). After the completion of the Life project, we obtained 1211 geolocalized observations of Montseny Brook Newts – comprising adults, subadults and larvae- and varying among years from 373 in 2019 from a minimum of 84 in 2021 (annual mean 202). Strikingly, the two lowest number of observations were gathered in the two last years. By population classes, larvae were exceptionally encountered - they were the 0.9% of the total observations-, whereas subadults were rarely found (15.2%) and therefore most part of the geolocations corresponds to adult newts (83.9%).



Dynamics of the number of night itineraries invested on sampling the Montseny Brook newt by year for the whole species range.

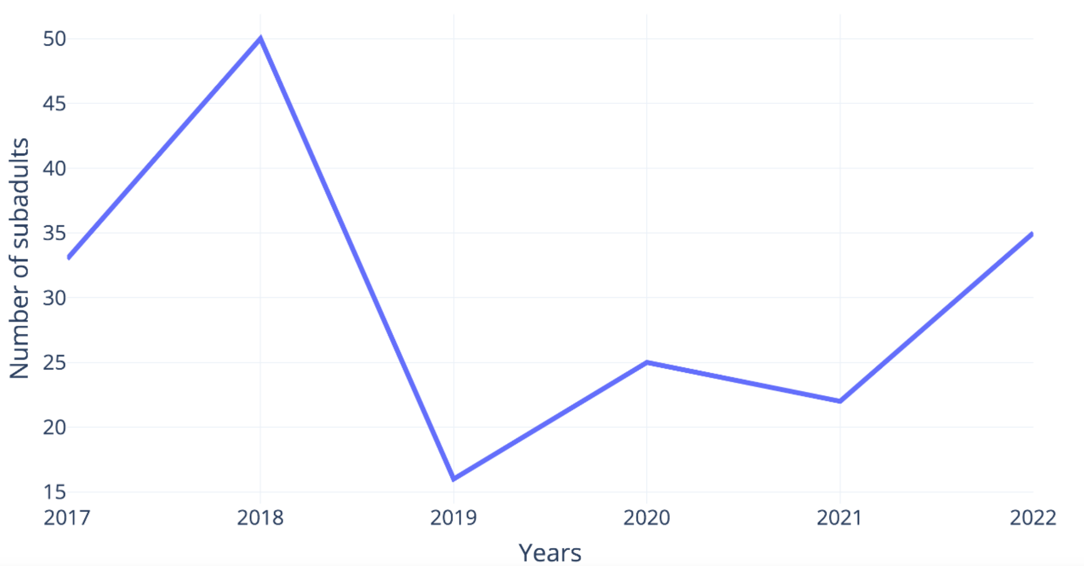


Dynamics of the number of night itineraries invested on sampling the Montseny Brook newt by year for the western group of populations.

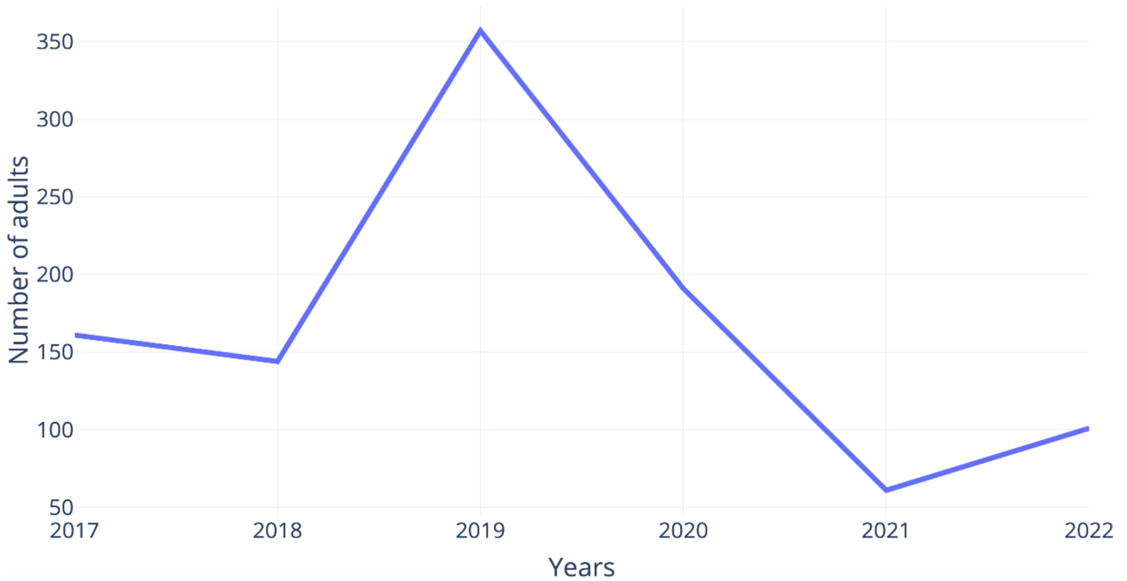


Dynamics of the number of night itineraries invested on sampling the Montseny Brook newt by year for the eastern group of populations.

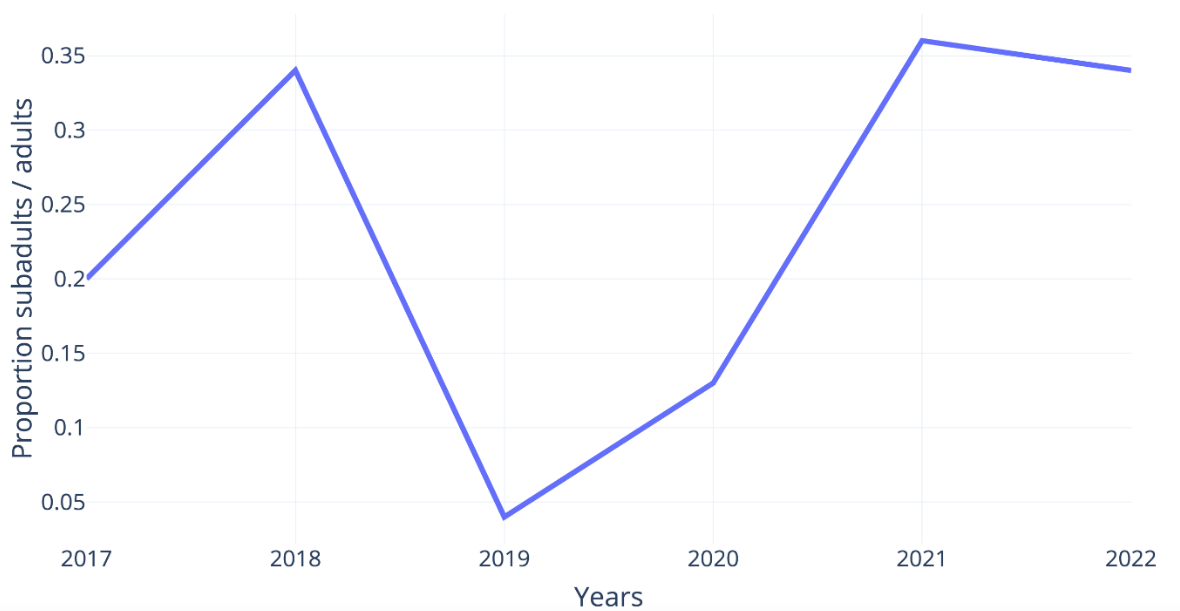
By population classes, larvae were exceptionally encountered - they were the 0.9% of the total observations-, whereas subadults were rarely found (15.2%) and therefore most part of the geolocations corresponds to adult newts (83.9%). Excluding larvae from the analysis and comparing the number of subadults and adults observed through the six years of sampling, we found significant differences among years (Chi-square test = 74.1, d.f.=5, P<0.001). The number of observed adults experienced a marked drop in 2019 in comparison with previous years slowly recovering during the rest of the study period, whereas subadults showed an opposite pattern characterised by a striking maximum in this year. As a consequence to that, the proportion between these two population classes, if well favourable to the adults during all the period, was strongly reduced in 2019. Considering only the adults that were confidently sexed (325 females and 262 males), we also found annual differences between the proportion of males versus females (Chi-square test=26.0, d.f.=5, P<0.0001). The number of females and males clearly increased in the sampling of 2019 and decreased posteriorly, whereas the proportion between sexes oscillated between 0.4 and 1.2 with the exception of 2021, when males extremely outnumbered females.



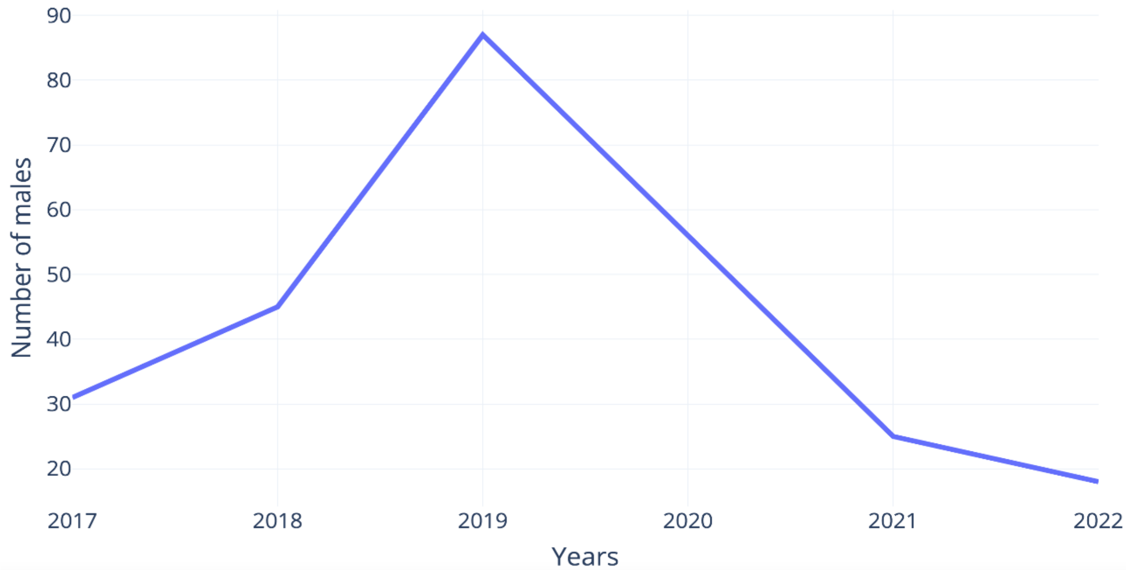

Dynamics of the number of subadult Montseny Brook newts observed by year.



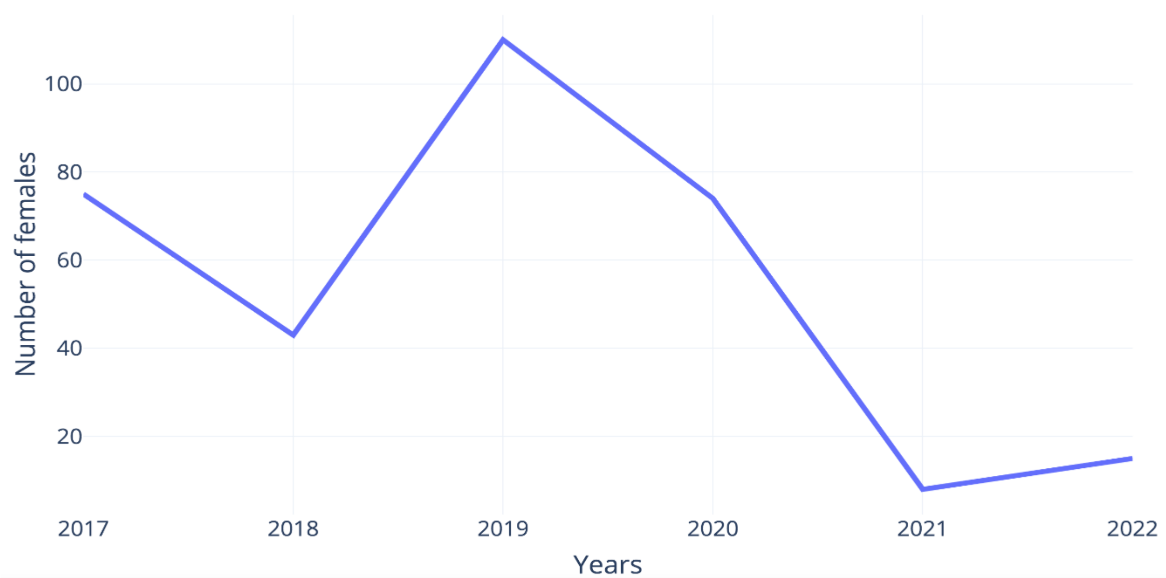

Dynamics of the number of adult Montseny Brook newts observed by year.




Dynamics of the proportion of subadult versus adult Montseny Brook newts observed by year.



Dynamics of the number of male Montseny Brook newts observed by year.



Dynamics of the number of female Montseny Brook newts observed by year.

### 5.3.2.- Geographic distribution

The range of the Montseny Brook Newt is just seven streams located in two separated second-order basins of the Tordera River. The western group of populations (four) were not homogeneously distributed along the watercourses of the basin and some stream sections were not inhabited by the newts leading to between and into population isolation. In general, geolocations were very spatially concentrated giving place to streams sections of high abundance whereas in the margins of those sections rarely there were geolocalizations. The eastern group of populations (three) were more extremely fragmented showing a bigger degree of isolation. Unlike the western group, in the eastern basin mapping revealed stream sections of low abundance in each of the three streams and there was only sections of high abundance just in two of them. During the night samplings, larvae were only found in two population (B4 and B1, although in this latter, only one was detected) and all of them only in the western group of populations. Subadults however, were more frequently found and geographically more widespread in both population groups and overlapping its distribution with the adults.

Nonetheless, we failed to find them during the six years of project development in two populations (B2 and B5) of the western group and one of the eastern (A3) populations. Males and females were found in the same stream sections with the only exception of the A3 population where the low number of observed adults were in all the cases, females.

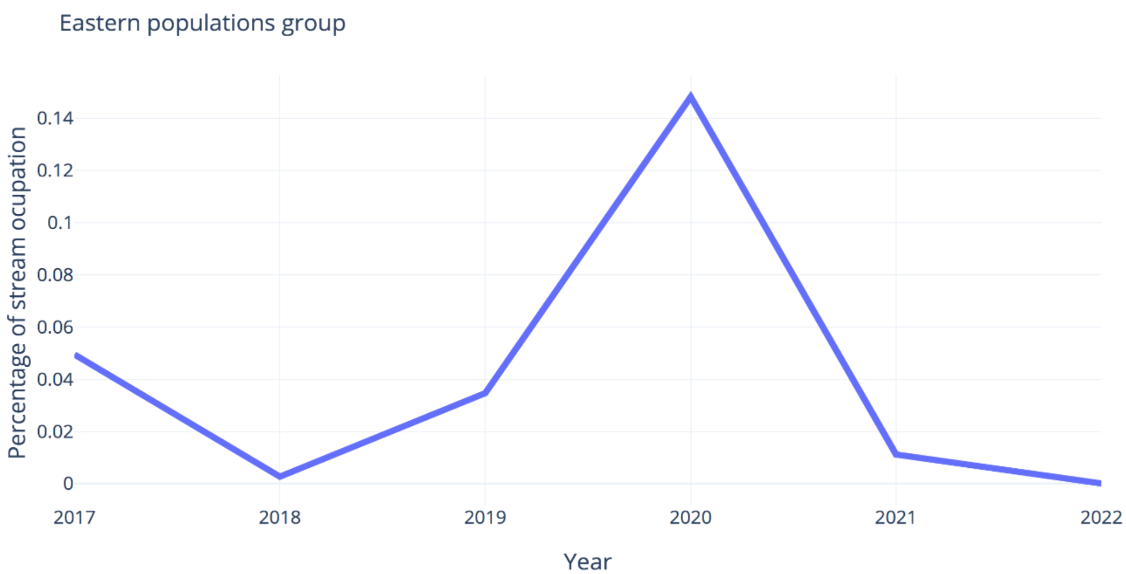
### 5.3.3.- Stream occupancies and abundances

The percentage of water courses occupied by the overall newt population (pooling the two groups of populations) had a mean yearly value of 0.113 ± 0.021 (standard error), oscillating between 0.188 and 0.050 during the project Life implementation. Nevertheless, there were marked differences between the two groups of populations (mean values 0.041 ± 0.022 and 0.162 ± 0.040 for eastern and western groups). For this, reason the dynamic of change on the percentage occupied by year was shown separately for each group of populations.

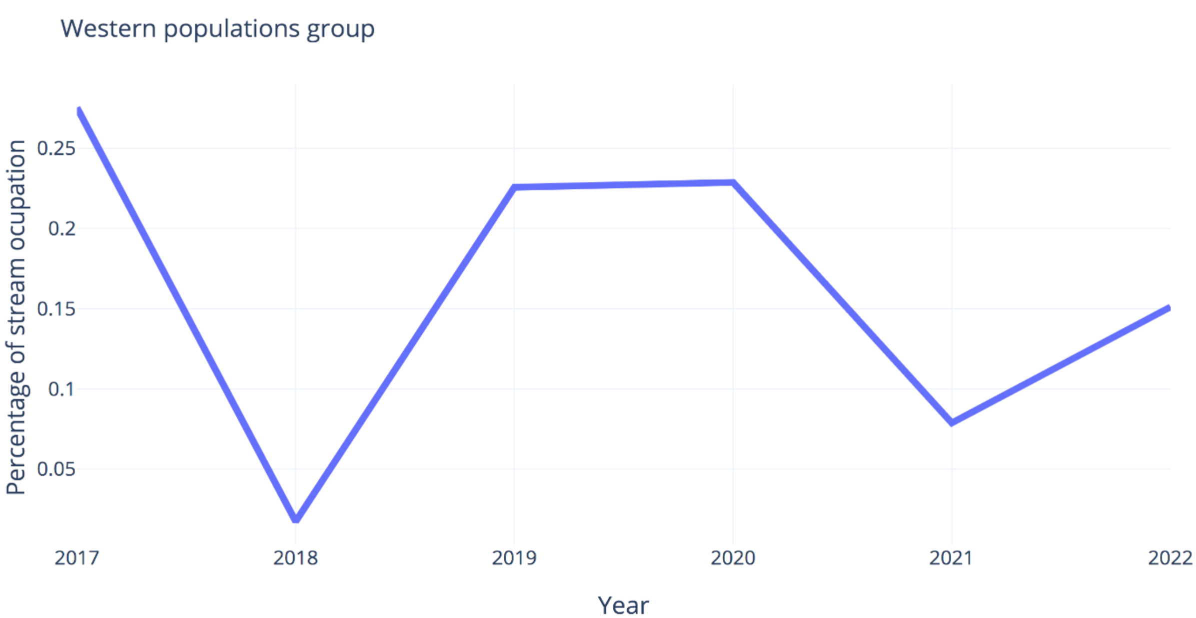
Mean occupation in the overall populations of the eastern group during the period 2017-2022 was clearly lower than the western one, due to the lower values of all the three populations. This was dramatically true in the population A3, where were unable to find newts since 2019. The evolution of the percentage of stream occupation for the whole group of eastern populations only experienced comparable high levels to the western group in 2020 and severely decreased in the last two years.

In contrast, only two populations of the western group (B1 and B3) had similar lower percentages of occupancy than found in the eastern group whereas the overall mean occupancy was clearly higher. The temporal dynamic of the occupancy during the Life project implementation fluctuated showing minimum mean values in 2018 and 2021 and an increase in the last year. In the western group of populations the variation in occupancy among years was higher than in the eastern. Besides these two populations which had the lowest percentages, three populations B2, B4 and B5 had contrastingly higher mean occupancies during the period.

To summarise the results of the absolute abundances of newts, we report those using the maximum number of adults detected in one night survey by population and summed over population groups and the whole species range. Correspondingly as found in the case of stream occupancies, there was a clear difference between groups of populations on newts' abundances and concurrently they were generally larger in the western group in comparison with the eastern group. Thus, the overall mean adult abundance during the period 2017-2022 was 122 ± 26.7 for the populations of the western basin and 11.8 ± 3.4 for the eastern one. The dynamics of newt abundance through time were contrastingly different between the two groups of populations.

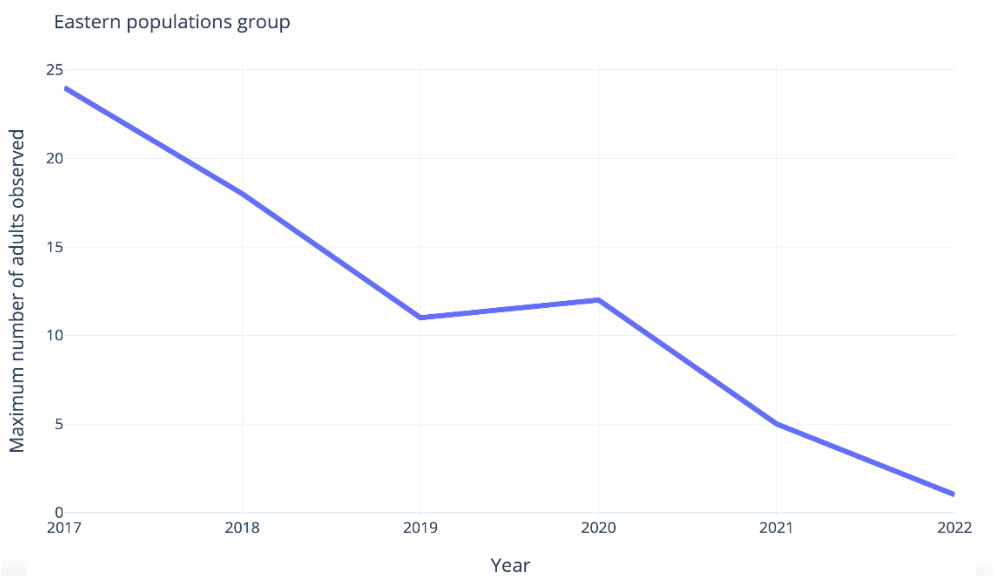



Dynamics of the percentage of stream occupation in the eastern basin during the period 2017-2022.

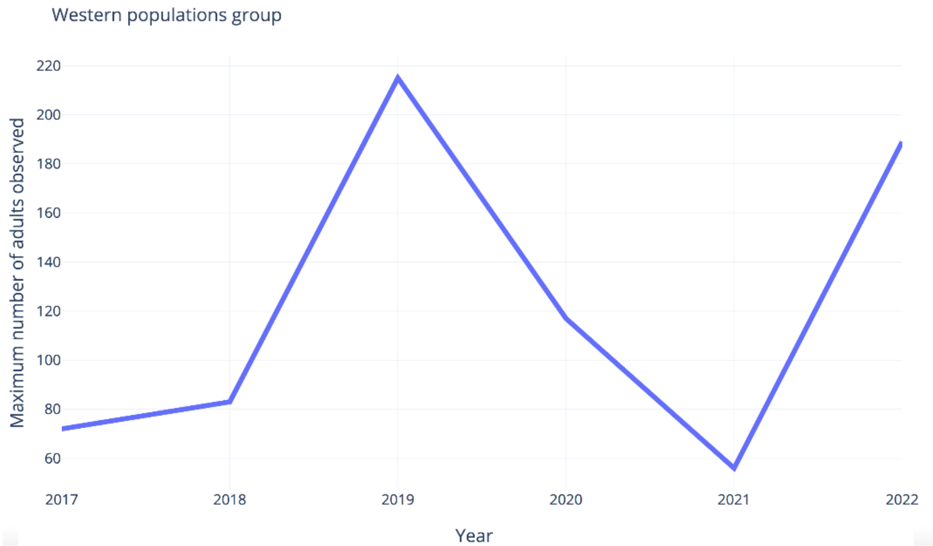



Dynamics of the percentage of stream occupation in the western basin during the period 2017-2022.
newt abundances

Populations in the eastern basin showed evidences of decline along the period, with the only exception of a slight recovery detected in 2020, whereas in the western basin the trend fluctuated over time. However, besides the temporal pattern observed in each group population, we found differences among populations within those placed in the same group. In the eastern basin, the population A3 had experienced a clear decline (no newts observed during the last years) and had a dramatically low mean adult abundance of 0.6 ± 0.4. In contrast, the populations A1 and A2 had larger means of maximum adult abundances during the period 2017-2020 (6 ± 1.6 and 5 ± 1.8, respectively), although clearly lower in comparison with most of the populations of the western basin. In this basin, however, the A1 population developed a trend to decrease though 2017-2022 whereas the A2 experienced fluctuation through the same period.




Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the eastern group of populations.

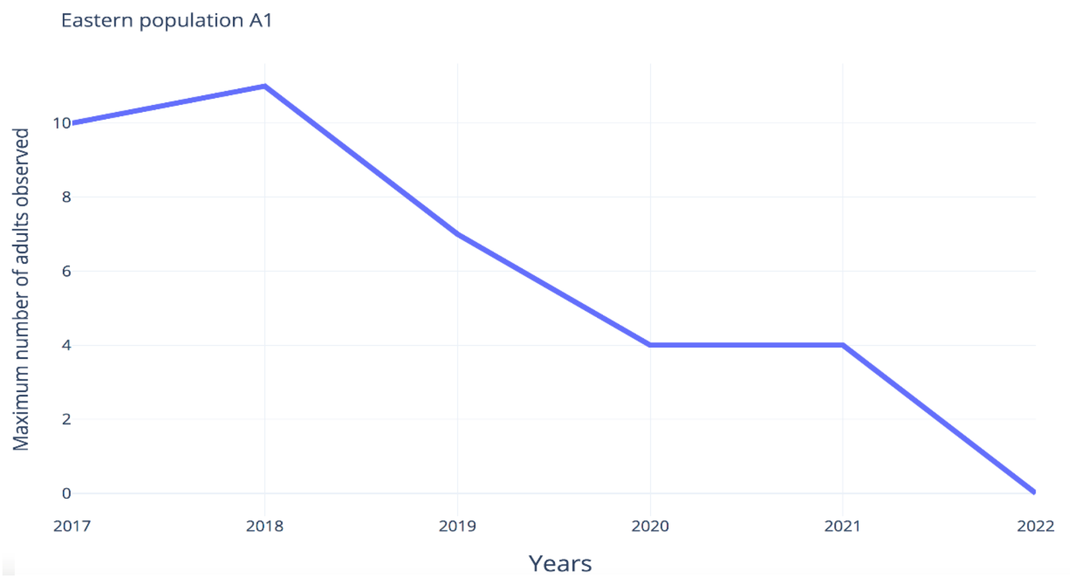



Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the western group of populations.

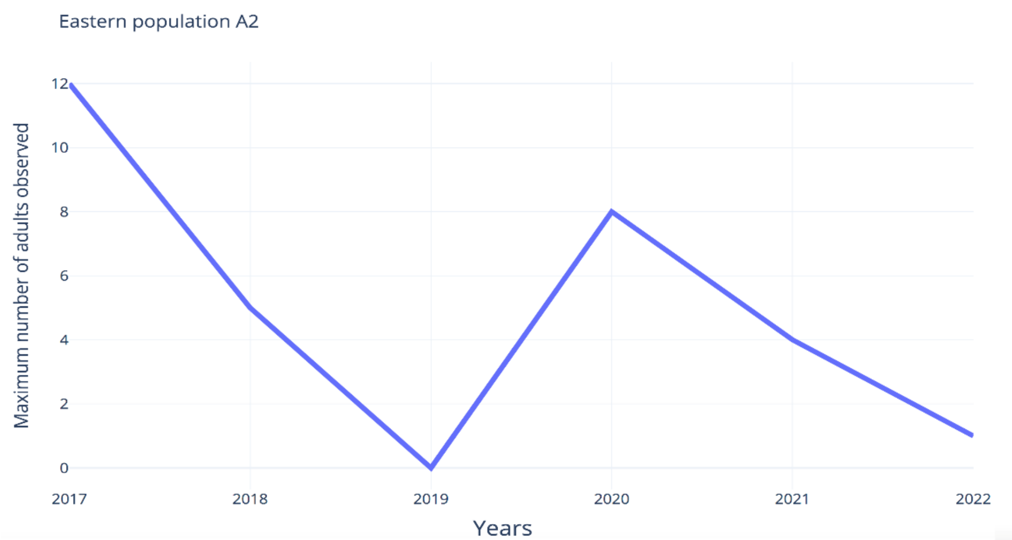
In the western group of populations, only the B3 population showed comparable lower abundances (mean = 6 ± 1.6). to the eastern group. Thus, the adult newt abundance seems to be related to the length of the stream inhabited by newts. For example the two bigger abundances were found in the streams with larger sections occupied by newts: B5, 21 ± 7.9 and B4, 78 ± 36.0. In contrast and in concordance with the B3 population, the shortest streams B2 (14.8 ± 3.2) and B1 (8.8 ± 3.5) had lower adult mean abundances. The B3 population showed a decrease in the last years to the contrary to the other western populations, which fluctuated over the period, B1 and B2 achieving lower abundances than the first years of the study and, B4 and B5 experiencing maximum values in 2019.

### 5.3.4.- Relative abundance

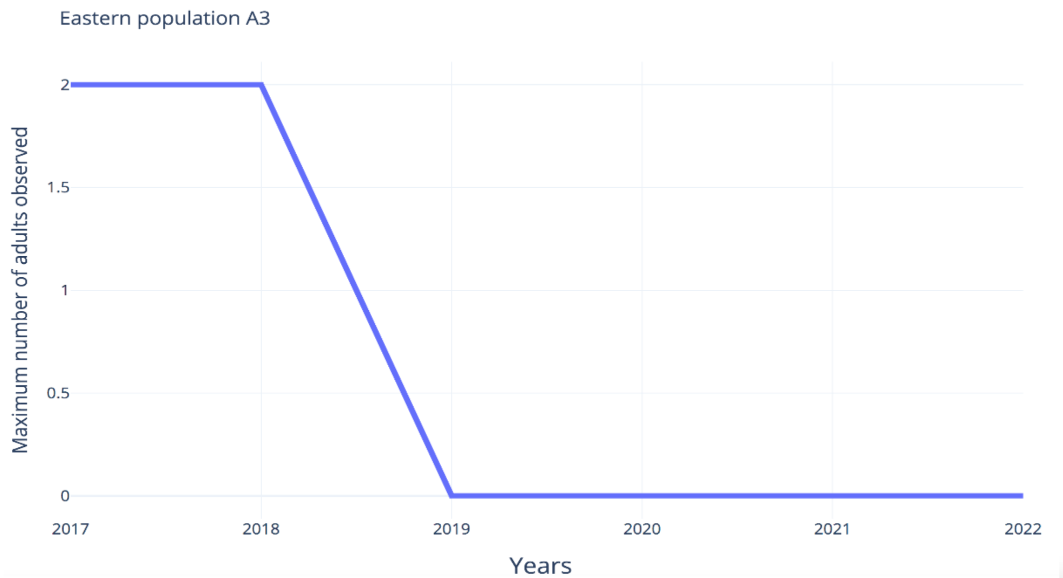
Likely the newt abundance was related to the length of the stream occupied. We found a Spearman correlation between the mean values of these variables for the whole period was 0.718, although marginally significant, P=0.051.



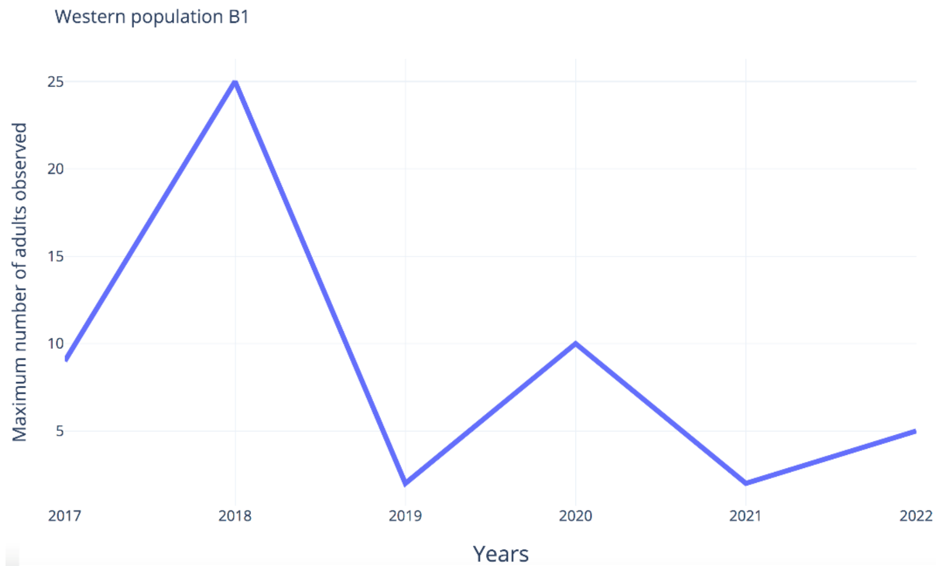

Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the eastern population A1.



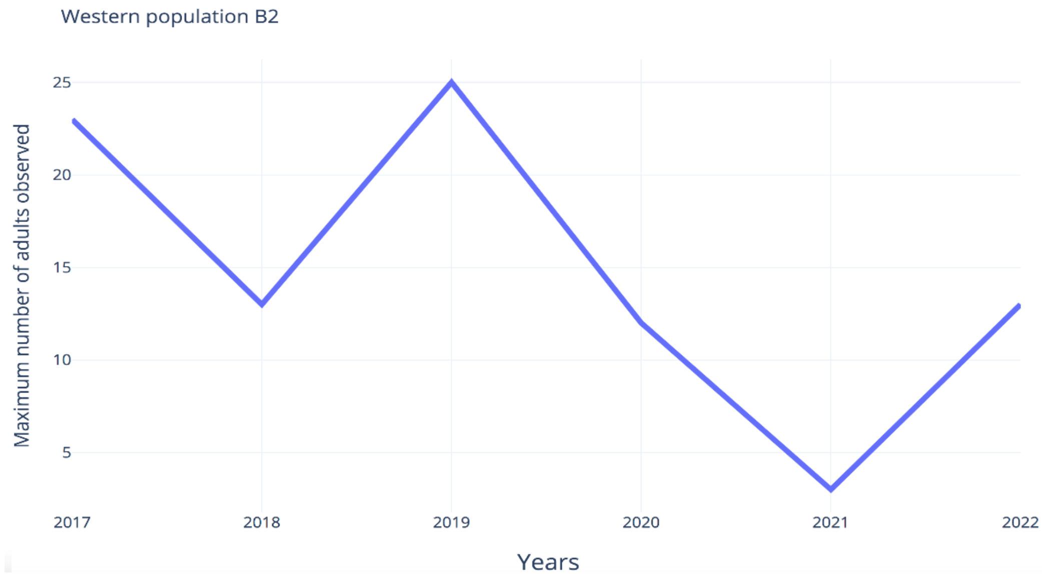

Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the eastern population A2.



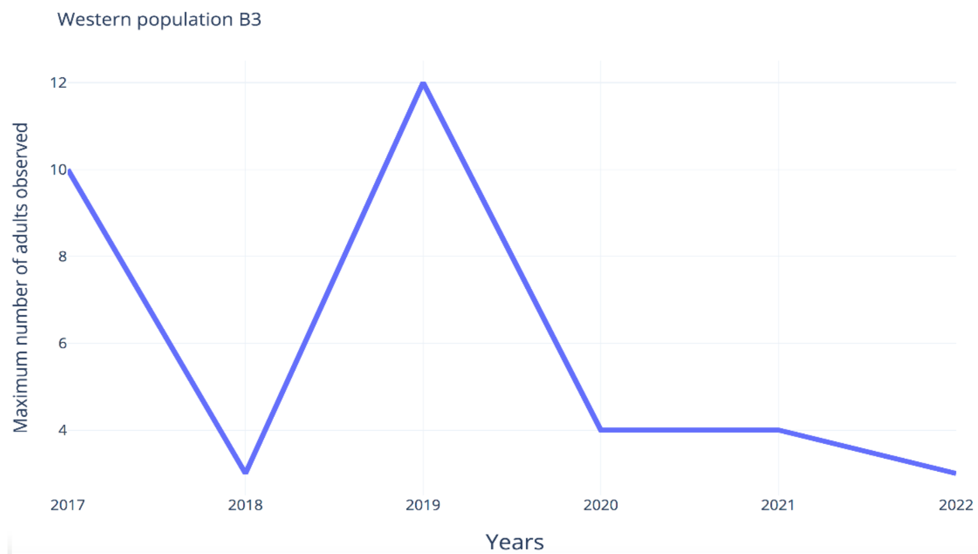

Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the eastern population A3.



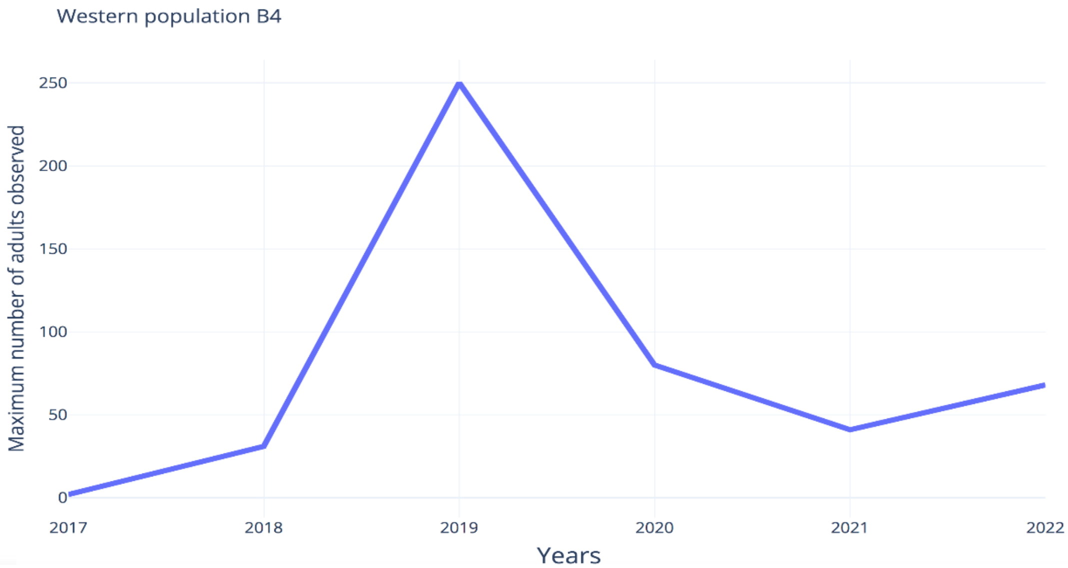

Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the western population B1.



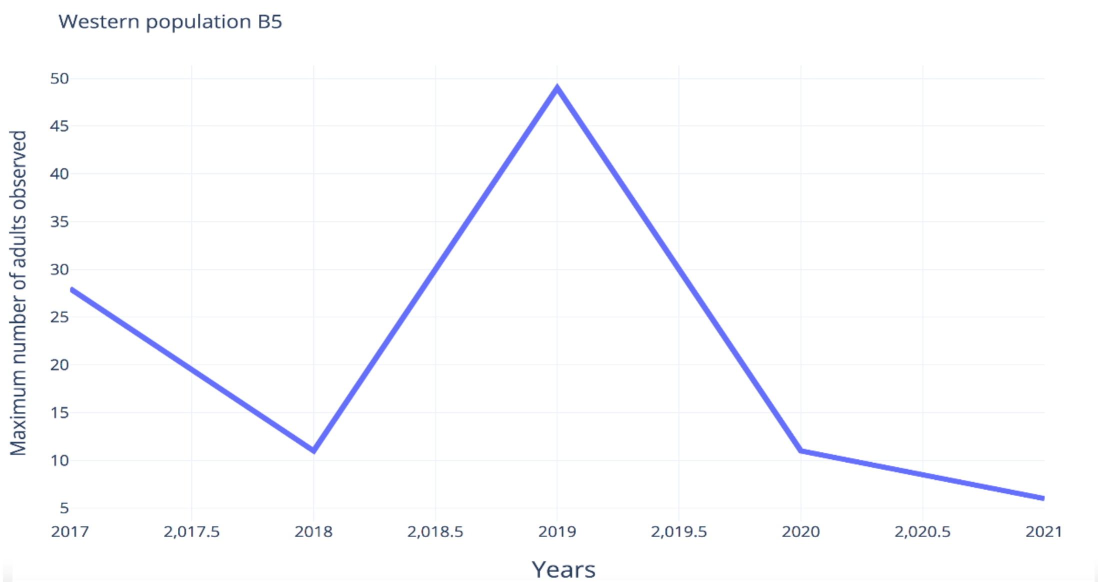
Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the western population B2.



Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the western population B3.




Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the western population B4.

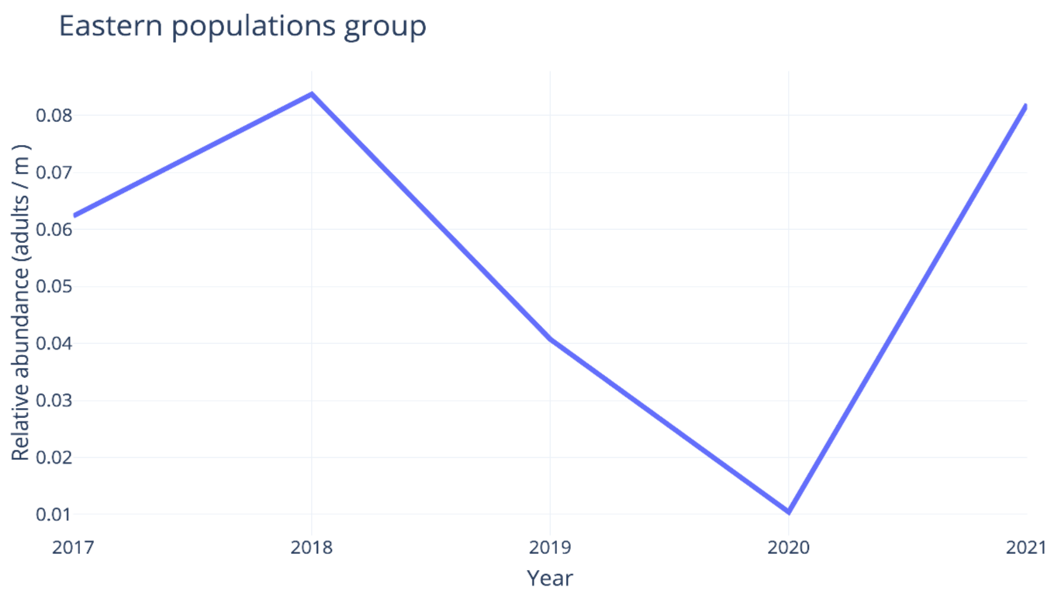



Dynamics of the maximum number of adult newts observed in one of the night surveys during the period 2017-2022 in the western population B5.

For this reason it is interesting to elucidate whether some populations had larger or smaller abundances than expected based on the lengths of the sections inhabited by *Calotriton arnoldi*. The two groups of populations of *Calotriton arnoldi* inhabiting each basin had very different relative abundances, the eastern group had a mean value estimated through the study period two times lower than the western group: 0.055 ± 0.013 and 0.122 ± 0.043. The dynamics of each population group was also distinctive. In the western basin the overall trend was towards the increase of the relative abundance through the time, although the pattern was irregular and inconclusive about a tendency. In the case of the eastern group of populations, relative abundance declined from 2018 until 2020 and subsequently increased. Among populations, relative mean abundances through the period 2017-2022 were higher in the shortest inhabited streams (A3, B3 and B1) ranging from 0.25 adults / m to 0.168. In the case of the eastern populations A2 and A3, the observation of one or none individuals precluded estimate the relative abundance through the study period. In the case of the other populations after fluctuation during most part of the period 2017-2022, there was an increase in relative abundance in the populations A1, B1, B2 and B5. The exceptions in this trend were the B3 and B4 that experienced oscillations on relative abundance through the period.

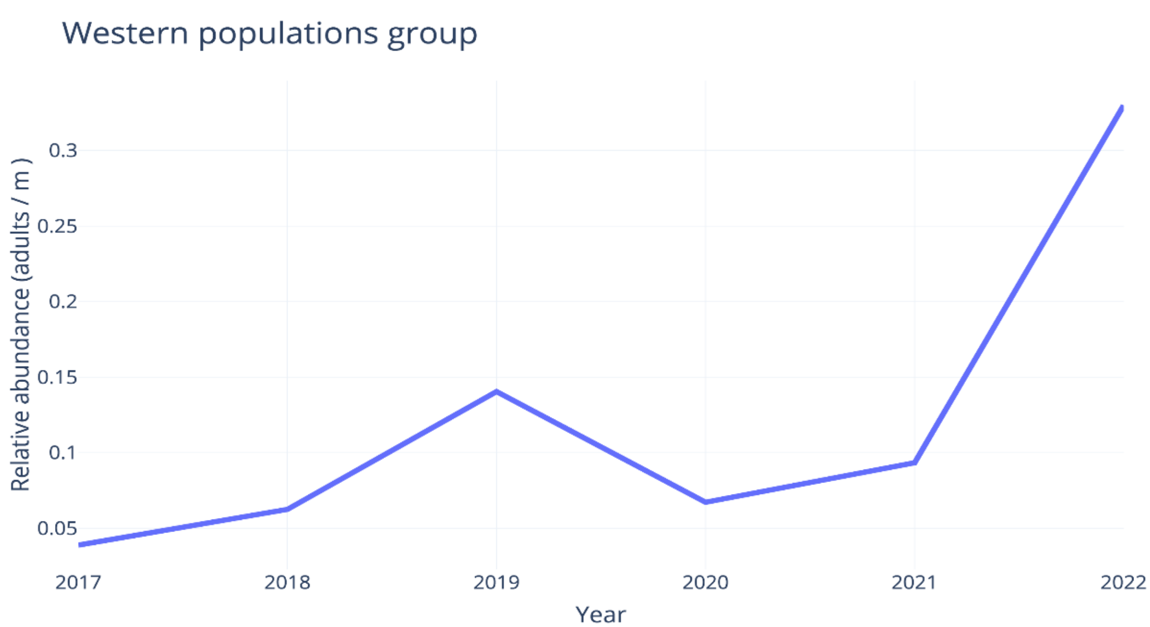
### 5.3.5.- Structure and connectivity

The computed inter-population connectivities for each of the two ranges inhabited by the species offered a clear picture of the species geographic structure concordant with the results achieved by the examination of the range maps. Connectivities in the eastern group of populations were lower (mean ± SE = 0.0000006 ± 0.0000001) than in the western (mean ± SE = 0.000615 ± 0.000009) and barely zero between the two basins. Into the eastern basin the lowest connectivity were found involving the A3 population with the others. In the case of the western group of populations, differences were not so apparent, but the B2 population were placed more equidistantly to the others and thus was the best connected.




Evolution of the relative abundance of *Calotriton arnoldi* during the period 2017-2020, for the whole populations of the western basin.

Evolution of the relative abundance of *Calotriton arnoldi* during the period 2017-2020, for the whole




populations of the eastern basin.

| | A1 | A2 | A3 |
|---|---|---|---|
| A1 | | 0.00000992 | 0.0000065 |
| A2 | 0.00000350 | | 0.0000018 |
| A3 | 0.00000058 | 0.00000046 | |

Matrix of connectivity among the three populations of the eastern basin.

| | B1 | B2 | B3 | B4 | B5 |
|---|---|---|---|---|---|
| B1 | | 0.00092 | 0.00060 | 0.00136 | 0.00007 |
| B2 | 0.00092 | | 0.00099 | 0.00110 | 0.00099 |
| B3 | 0.00048 | 0.00099 | | 0.00090 | 0.00123 |
| B4 | 0.00010 | 0.00023 | 0.00018 | | 0.00031 |
| B5 | 0.00014 | 0.00050 | 0.00013 | 0.00016 | |

Matrix of connectivity among the five populations of the western basin.

### 5.3.6.- Population size

Population size is a very relevant parameter to evaluate the state of conservation of a species, as it is one of the most valuable criteria for the IUCN evaluation. Thus, we estimated population size for each population, basin and the whole species given that the distribution range of *Calotriton arnoldi* is highly fragmented. Based on the scheme of specific densities estimated by demographic studies associated with different abundances by stream sectors (see methodology), we estimated the overall population size of *Calotriton arnoldi* through the species range in 2402 adults. Populations in the eastern basin were less numerous, 605 estimated adults than in the western, 1770 adults. Although demographic studies allow us to estimate the 95% of confidence intervals of the density estimations, we choose not to use them to estimate the confidence in population size in order to not complicate the interpretation of our results.

Estimates for adult populations ranged from 28 (population A3) to 806 (population B4). The population A3 had severely declined and likely could have now disappeared. If this is true, the overall eastern population could be reduced to 596 adults and for the whole population, 2366 adults.

**6.- Intensive survey A2 population**

**6.1.- Field methodology**

In the A2 stream we chosen for population study, a 150m stretch was selected and divided into sections of 10m each for quantifying the nets displacement. The surveys were performed from march, 20 of 2018 to June 26 of 2022. After this date extreme drought occurred and surface water-flow disappeared until December.

This stream belongs to the eastern basin and is located between 1110 and 1420 m, although from 1200 m the superficial circulation of the water disappears. It is oriented towards the south-southwest and the sampled section was located at the limit of the domain of the beech forest. It was a stream that has a population of wild *Calotriton arnoldi* in apparently good condition. The A2 stream was selected for sampling given that it is one of the most representative populations of the eastern core, its ease of access, and some preliminary studies had already been done (Montori and Pascual, 1981; Montori and Campeny, 1991; Amat and Carranza 2005). According to these studies, the torrent had a priori stable population with a sufficient number of individuals to be able to carry out a robust demographic study. Despite this, it was a population that can be subjected to strong anthropic pressure given the aforementioned accessibility.

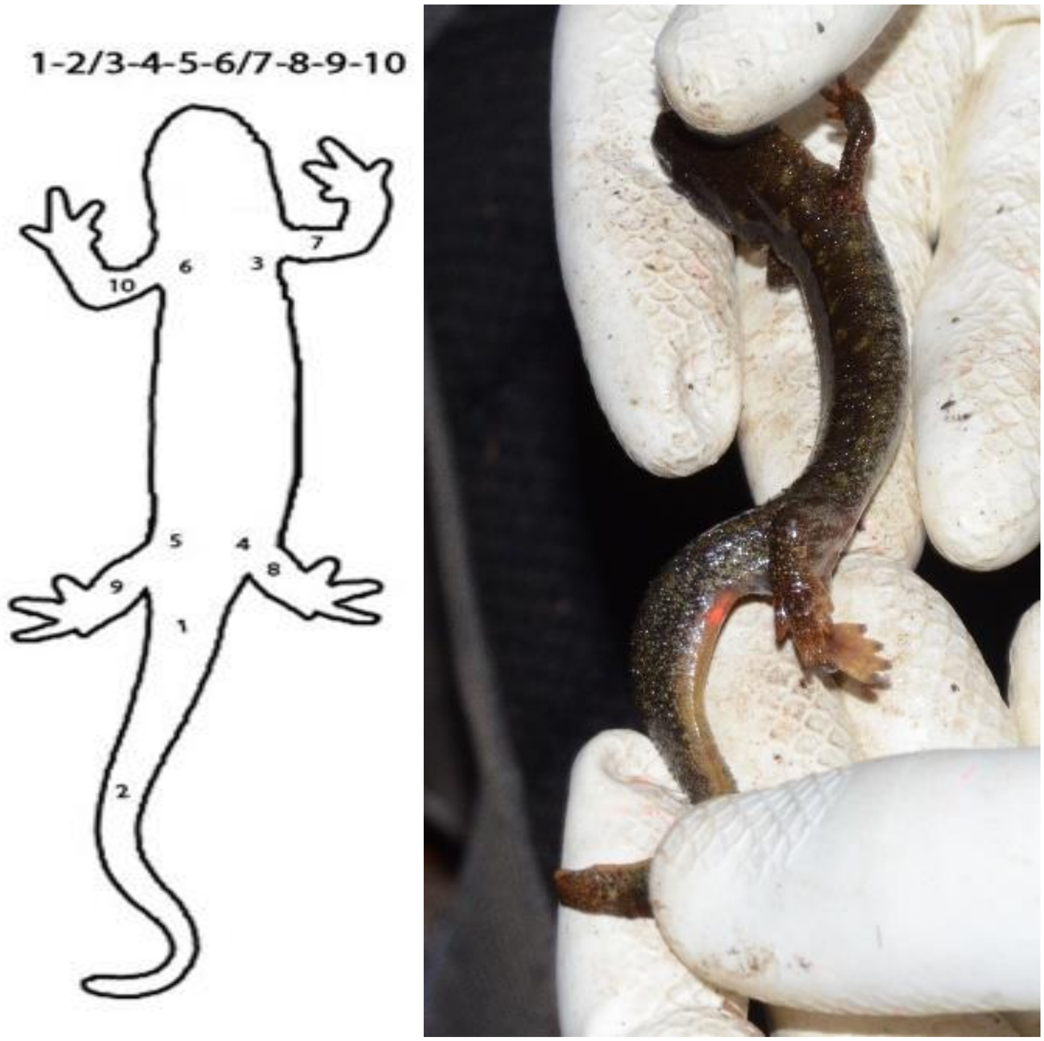
Each survey was carried out at night in an upward direction and with an active search, trying to avoid stepping on the bed of the stream. When a specimen was located, it was captured and the place of capture was marked with a flag in order to release it in the same place. The captured and recaptured individuals were either georeferenced, measured and sexed, or assigned to one of the four immature age-classes that were taken into consideration (larvae, metamorphic, juvenile and subadult). In the study, immature individuals were considered larvae, metamorphic, juveniles and subadults. Larvae were all those individuals that maintain gills and had no signs of metamorphosis. Metamorphic were those individuals who have just metamorphosed. Juveniles were individuals that, having completed metamorphosis, had a larger size and not yet spent a winter in the water after metamorphosis. Subadults were all those specimens that have spent a winter in the aquatic environment after metamorphosis but have not yet reached sexual maturity. The variables that have been taken for each specimen captured were:

- Code: CA18SO00. (species code -2 digits-, year -2 digits-, Stream -2 digits-, order number -2 digits-).
- Date (day/month/year)
- Capture time (hh:mm)
- Genetic sample code (CN00000 or AV00000).
- Capture section. When indicating the section, three possible locations were also indicated. For example, in the section 12-13 it may be 12· - -13 if the newt is closer to indicator 12, 12- · -13 if it is equidistant from both indicators or 12- - ·13 if it is located closer in indicator 13.
- GPS coordinates (x,y) with an accuracy of 1m and indicating the location error when the GPS coverage allowed it.
- Sex (♀,♂, Juvenile, Metamorphic, Larva).

- Weight (in g).
- Body length (in mm). Head-cloaca length including this last organ (SVL).
- Tail length (in mm) (LCua).
- Marking code (elastomer colour code)
- Photo code (numeric)
- Observations.

### 6.1.1.- Marking methodology

Newts were marked (Amat et al. 2021) by injecting a visible implant elastomer (VIE, Northwest Marine Technology, Inc.) and using a visual code based on ten injection points: four in the abdomen, four in the legs and two in caudal region.




Marked individual with elastomer in caudal area. Code: O0/0000/0000

To be able to recognise the specimens individually and to study different aspects of their biology such the population size, the displacements of the specimens or even their growth or biological state, the captured specimens must be marked.

In the A2 population, it was decided to make a marking using the elastomer implant, which is a material that does not interact with the animal's tissues. This technique involves making a mark under the skin with a hypodermic needle using a material based on liquid silicone. This material is composed

of two parts: a pigment and a catalyst, which are mixed at a ratio of 10:1 just before use. When inserted under the skin, the material becomes solid, flexible and stays in place. The result is an easily visible mark under an amphibian's transparent or translucent skin. In addition, if we use a fluorescent pigment, as is our case, when illuminated with a UV LED lamp they become very visible.
This technique involves minimal impact on the animal, especially when compared to other techniques such as amputation of phalanges or cuts to the tail. Elastomer implants do not bother or irritate the animal and the needle used is very thin. In addition, it is a relatively inexpensive method that allows the specimens to be marked quickly and therefore handle the newt in a short time. The possibility of using different colours to make various marks generates a wide variety of combinations and designs that allow individuals to be distinguished. All this makes elastomer marking a recommended marking technique for amphibians, especially small-sized ones.
Newts were marked using different colours elastomers (blue, yellow, pink, red and green) in different parts of the body (tail and lower part of the limbs). In this way, we managed to individualise the markings. Once the variables indicated in the previous section were marked and taken, the specimens were released at the same place of capture.

**6.1.2.- Analysis of the longitudinal displacement of the specimens**

Dispersal activity were estimated by means of meters displaced from capture to each recapture for males and females. The sex ratio was calculated as the proportion of mature males in relation to the total number of adults. Immature individuals were not included in estimated population size models. To estimate population size, we assumed that populations were closed. This assumption is based on the fact that *Calotriton arnoldi* newts are not particularly mobile organisms and the selected stretch includes the upper limit for the presence of water and the lower limit for the presence of the species in this stream.
The methodology described by Daugherty and Sheldon (1982) was applied. When an individual was recaptured in an upper or lower section of the stream, a rank or meters value was assigned, where (n) was the number of sectors between the sector of capture and recapture, and the positive or negative sign

indicated the direction of displacement (upstream: [+] or downstream: [-]) (Montori et al., 2008). Subsequently, the dispersion relative to the area in which an individual had been marked was established for each month. Site fidelity versus dispersal tendency was tested by comparing the monthly distributions relative to each individual's first capture and the standard deviation of these distributions over time using ANOVA, using the number of segments displaced from the first capture as the dependent variable and time (months) as a categorical predictor. According to Daugherty and Sheldon (1982), we would expect the deviation of the monthly distribution of newts from the first capture to increase if migration occurs.

In addition to determining whether individuals moved significantly in one direction or another of the stream, the dispersion by gender and the sections and meters moved, both in terms of total and absolute displacement, was analysed. The analysis of displacements has been carried out based on recaptures and has considered both the displacement between two consecutive captures, as well as the displacement between the first capture and the last recapture. The results are presented both in absolute value of displaced segments and meters with an accuracy of 3.3m. When a specimen was located, it was georeferenced and the segment or section where it was found, indicating whether within the section it was located in the first third, second or third. Thus, as the sections measure 10m we can reach a precision of 3.3m. This precision was in most cases greater than what the GPS gives us, which was why this location was used in the torrent. As an example, we can assume an individual who has been located for the first time in section 10 in the first third. In this case the field sheet was referred to as 10-·-11. If it is subsequently recaptured in the middle third of section 11 (11-·-12), it means that it has moved 13.3m upstream. If the recapture had been made in the upper third of section 9 (9--·) we would consider that it has moved 3.3m downstream and it would be indicated as -3.3m.

### 6.1.3.- Population size

The POPAN model estimator (adaptation of the Jolly-Seber model) was used to estimate population parameters using the MARK$^{TM}$ software. The selected model with lower Akaike value was ϕ (.) p (t) pent (t) N, where apparent survival (ϕ) was considered to be constant over time, while the probability of capture (p) variable over time, and the probability of entry into the population by chance (pent) varied during the period in which they were studied. Sex-ratio were estimated (♂♂/(♂♂+♀♀)) following Wilson (2002).

Measuring SVL and recording the weight of *Calotriton arnoldi* adult.

## 6.2.- Results

### 6.2.1.- Intensive surveys in A2 population.

The first survey in A2 stream was carried out in April 2018 and the last in June 20, 2022. From this date no survey could be carried out due to a drought that persisted even in autumn, the rainy season in Montseny N.P. The lack of rain causes the disappear of superficial flow water to, making it impossible to locate the individuals. A monthly survey was carried out from July to December 2022 to determine if there was surface flow of water or not.

In the A2 stream 81 adult newts (32 ♂♂ and 49 ♀♀) were captured, marked and released from 2018 to 2022 and the adult recapture rate reached 29.3%. Twenty-four immatures, metamorphic and larvae were captured during this period, but they were not marked due to their small size. In total, from 2018 to 2022 157 newts were captured, with a recapture rate around 30%. The sex-ratio average obtained

(♂♂/(♂♂+♀♀)), was favourable to females (0.42), even throughout the year. These observations were according with the results obtained in extensive survey from other wild populations.

| Month | Males | Females | Immatures | Total | Total /Surveys | Males /Surveys | Female /Survey | Immature /Surveys | Mean water temperature |
|---|---|---|---|---|---|---|---|---|---|
| January | 0 | 0 | 0 | 0 | 0 | | | | 7.20 |
| February | 0 | 0 | 0 | 0 | 0 | | | | 6.70 |
| March | 0 | 0 | 0 | 0 | 0 | | | | 7.60 |
| April | 7 | 11 | 1 | 19 | 4.75 | 1.75 | 2.75 | 0.25 | 7.70 |
| May | 7 | 21 | 7 | 35 | 8.75 | 1.75 | 5.25 | 1.75 | 8.90 |
| June | 18 | 21 | 7 | 46 | 7.67 | 3 | 3.50 | 1.17 | 11.70 |
| July | 13 | 18 | 6 | 37 | 4.63 | 1.63 | 2.25 | 0.75 | 12.32 |
| August | 0 | 0 | 0 | 0 | 0 | | | | 13.50 |
| September | 0 | 0 | 4 | 4 | 4 | 0 | 0 | 3 | 13.00 |
| October | 1 | 0 | 1 | 2 | 2 | 1 | 0 | 1 | 11.70 |
| November | 2 | 1 | 0 | 3 | 3 | 2 | 1 | | 8.65 |
| December | 4 | 7 | 0 | 11 | 5.50 | 2 | 3.50 | | 8.14 |
| | 52 | 79 | 26 | 157 | 3.36 | 1.64 | 2.28 | 1.32 | 9.70 |

Captured newts (including recaptures), from 2018 to 2022 in absolute values and related to number of surveys.

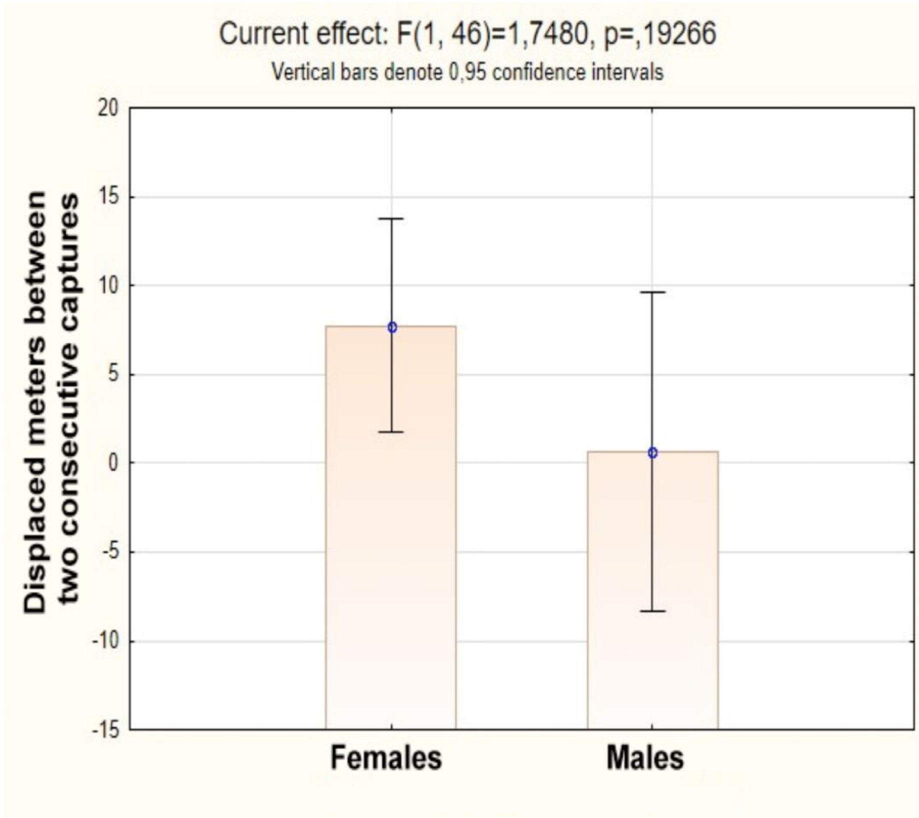
In the surveyed stream (A2), the largest number of specimens were caught in spring, mainly May-June. Stream surface groundwater activity decreases during the cold period from January to March and quickly during the summer (second half of July and August), coinciding with the torrent's dry period. These data was in accordance with those provided by different studies. There was also a significant increase in activity in autumn and early winter. The species has the tendency of inhabiting underground aquifers, between the cracked rocks in the stream, and this influences the timing of surveys because during dry periods, surface activity is almost non-existent.

**6.2.2.- Dispersal activity.**

After five years, the longitudinal average displacement is 5.53 m. This mostly consists of females, whose preference is to go upstream. Males show poorly defined displacement. However, it can be considered that the species remains faithful to the same area, despite not being territorial. There were newts that after more than 1000 days have only moved 3.3 meters and others that after 744 days have not moved at all. The largest displacements observed are 56.6 m upstream and 20 m downstream. Most of the specimens, however, did not show significant displacements. Although females tend to move mostly upstream and males were more wanderers, no significant differences between the two sexes have been obtained (ANOVA $F_{1\ 46}$ =1,.750, P=0.193).

**6.2.3.- Population size.**

In the A2 stream 81 adult newts (32 ♂♂ and 49 ♀♀) were captured, marked and released from 2018 to 2022 and the adult recapture rate reached 29.3%. Over time, population size was not constant because the estimations correspond only to surface active newts. The estimated population size in the 150 meters surveyed was 93.07 newts with a confidence interval of 95% from 80.94 to 119.41. Their estimated survival was very high ($\phi$ = 0.9984 95% confidence interval: 0.9965 - 0.9994) and the average density of newts was 0.63 newts/m (0.54 - 0.79). The number of captured newts decreased throughout the study, most likely due to the drought of the last two years. It was observed both in the absolute number of captures and relative to the number of surveys.




Displaced meters between two consecutive captures. Average and 95% confidence intervals for males and females by segments of 10 meters. Differences between sexes were not significative.

Only in 2022 does the population of the surface aquifer seem to recover due to the intense rains of that year. In this year, the population could not be sampled from the end of June since the surface aquifer has dried up. The expected rains will allow us to see the importance of the underground aquifer for the survival of populations.

### 6.2.4.- Biometrical data

Our results confirm the data obtained in other studies (Montori and Pascual, 1981; Campeny and Montori, 1986; Amat and Carranza, 2005). Males and females have similar body size and differ fundamentally in tail length which undergoes negative allometry in males from maturity.

| Month | Males | Mean | SD | Maximum | Minimum | n |
|---|---|---|---|---|---|---|
| Weight (g) | Males | 5.29 | 0.77 | 6.57 | 3.80 | 47 |
| | Females | 4.31 | 0.72 | 6.20 | 2.50 | 61 |
| | SA-Juveniles | 1.98 | 0.72 | 3.50 | 1.40 | 6 |
| | Metamorphic | 0.97 | 0.07 | 1.00 | 0.90 | 11 |
| SVL (mm) | Males | 59.86 | 3.45 | 65.00 | 45.00 | 47 |
| | Females | 59.71 | 4.05 | 69.00 | 44.50 | 61 |
| | SA-Juveniles | 43.10 | 3.92 | 50.00 | 40.00 | 6 |
| | Metamorphic | 32.50 | 0.71 | 33.00 | 32.00 | 11 |
| Tail length (mm) | Males | 43.49 | 3.54 | 50.50 | 35.00 | 47 |
| | Females | 45.32 | 3.66 | 53.00 | 33.00 | 61 |
| | SA-Juveniles | 33.13 | 3.30 | 39.00 | 30.00 | 6 |
| | Metamorphic | 24.67 | 4.60 | 27.50 | 20.00 | 11 |
| Total length (mm) | Males | 103.35 | 5.88 | 115.50 | 81.00 | 47 |
| | Females | 105.03 | 6.82 | 121.00 | 83.50 | 61 |
| | SA-Juveniles | 76.23 | 6.85 | 87.50 | 71.00 | 11 |
| | Metamorphic | 57.17 | 5.30 | 60.00 | 52.00 | 6 |
| Tail / SVL | Males | 0.73 | 0.06 | 0.83 | 0.56 | 47 |
| | Females | 0.76 | 0.06 | 0.88 | 0.61 | 61 |
| | SA-Juveniles | 0.77 | 0.05 | 0.87 | 0.70 | 11 |
| | Metamorphic | 0.76 | 0.13 | 0.85 | 0.63 | 6 |

Descriptive biometrics of newts caught for the first time and that did not present malformations or tail amputations.

Metamorphic individuals, juveniles, subadults and females have a similar tail length proportional to SVL and higher than that of males. The specialisation of the amplexus in this genus, where the male captures and holds the female with the tail to transmit the spermatophore, is most likely the cause of this negative male allometry in tail length. On the other hand, males are more robust as shown by the fact that despite being smaller than females, they are more weighted. The smallest specimen captured and which had completed metamorphosis measured 52 mm in total length and this can be considered, for now, the minimum metamorphosis size. The smallest male measured 81mm in total length and the smallest female 83.5 mm.

## 6.3.- Discussion and Conclusions

### 6.3.1.- Methodological considerations.

The Montseny massif is currently experiencing the effects of climate change on the ecosystems and the species. For example, the other most interesting amphibian of the massif, a cryophilus species - the common frog (*Rana temporaria*) - tends to breed early in comparison with previous decades and its southernmost populations are in regression in the Montseny (Montori & Amat 2023). Because of its ecological requirements, *Calotriton arnoldi*, our results indicate that the climatic changes put the species in higher risk of extinction. Critically this species depends not only on the maintenance of a minimum subterranean water-flux, but also in the existence of periods - particularly in spring and autumn - of overflow that allows individuals to develop activity on the stream-bed where likely the availability of food resources is higher. In this context the monitoring of the natural populations is crucial to periodically evaluate the situation of the species. Nevertheless, Montseny Brook newts are not easy to survey because during unfavourable periods they remained unobservable to the researchers. Thus, during the last years due to the severe droughts the window of time where it is feasible to sample during good environmental conditions has been substantially reduced. In order to overcome it, during the samplings of 2023 the guards of the natural park will participate in some samplings even though the most of them will continue to be realised with the rural agents of the Generalitat de Catalunya. This small change in the methodology will provide more personnel to optimise the number of days for population survey. The collaboration among the main researcher and the rural agents was very positive as revealed by the high implication of them in the field samplings, the good coordination and the consolidation of a stable team. These and the incorporation of the guards of the natural park is essential for the continuity of the population monitoring after the life project.

**6.3.2.- Ecological population parameters and implications on species conservation**

Our results evidence strong differences among the numbers of adults, subadults and larvae we observed, raising the question about why it happens. The low number of larvae observations cannot be considered as evidence of population decline because they usually avoid the activity on the stream-bed to elude predation or be exposed to higher temperatures. One of the limitations of the study of this endangered species is that we cannot use invasive methodologies to study the fecundity of the species and the larvae survivorship and unfortunately data gathered from captive individuals is not representative of that happens in natural conditions. Demographic monitoring of the A2 and occasional observations in the A3 populations of larvae indicate that its rarity in the natural population surveys does not indicate a real decline of this population class. In contrast, although adults are more numerous than subadults in our surveys, we have detected an increase in the observations of individuals belonging to this latter population class during the last years that has been climatically clearly unfavourable for the species. It is unclear what this observation could indicate. One possibility is that subadults need to develop more activity than adults even during unfavourable periods because they need to sustain a high rate of growth and hence devote more time in feeding. This hypothesis assumes that likely in the stream bed food availability is higher than in the rocky matrix. Thus, during unfavourable periods more adults tend to seek refuge in the deepest rock areas to avoid physiological stress, whilst subadults take advantage of the retreat of adults trying to use the trophic resources in the stream-bed. Alternatively, the reduction on the number of adult observations could be the effect of a rise of the mortality in this population class, allowing subadults to increase their superficial activity, implying differential mortality rates among these to population classes. These two hypotheses are difficult to test due to the particular species ecology, but the demographic monitoring of the A2 population should offer answers to this question. The minor number of observations of subadults and larvae among populations should be related with the population sizes (in larger populations more these two classes are more probable to be found), but also to the structural characteristics of the streams.

Larvae elude strong water fluxes and maybe high densities of adults to avoid predation. Nevertheless, the two streams where larvae were found (B1 and B4) have very different structure, so it is not clear why they are found in some streams and not in others. The dynamics on sex proportion should be a consequence of the different requirements on reproductive investment between males and females affecting the time they are active in the stream-bed. It is expected that as in most amphibians the sex proportion in the population is close to one (Fernandez-Guiberteau et al., 2020), and the estimated value should be a consequence of a discordance of the periods of superficial activity. Nevertheless, the results achieved by the demographic monitoring of the A2 are inconclusive given the short period of accumulated sampling.

The analysis of the spatial distribution of the newt observations along the streams highlights the consequences of the negative impacts produced by the anthropogenic activities on the geographic range of *Calotriton arnoldi*. Specifically in the western populations B1, B2, B3 and B4 there are a gap of observations several metres upwards and downwards in the point where forest and clearing tracks intercept the streams. This evidence shows how sensible is the Montseny Brook newt to alterations on stream structure and loss of forest cover and the necessity of suppressing the forest tracks not currently in use and predictable in the future, and build animal crossings when this is not possible.

The only population not affected by this impact is the B5, because the stream is placed in the depth of the main valley far away from human settlements and activity. In the case of the eastern basin, the population A1 is intercepted by a road but the lack of newts below and above this point seems, on the basis of genetic results, not only as a result of this impact, but also by natural habitat limitations. This same road crosses the stream inhabited by the A2 population and despite newts being found in both sides of the stream and very close to the interception point, demographic monitoring of this population revealed that rarely newts use the primary stream canalisation placed under the road. In this case the alteration of the road does not seem to have so severely impacted on the population in comparison to other cases due to the large forest cover provided by a well developed forest of beeches, although a negative impact on the ability of displacement has been observed (Fernandez-Guiberteau et al., 2020). The conservation of the habitat of the A3 population is actually very bad by combination of severe alteration of the native forest, plantation of allochthonous trees and water catchment. It is likely that the dense net of forest tracks affected the species negatively in the past, but is difficult to demonstrate because when the monitoring started the situation of this population was already disastrous.

**6.3.3.- Population dynamics before and during the Life project implementation.**

Comparing the results achieved by the monitoring of the natural populations before the starting of the LifeTritoMontseny and at the end of the project we concluded that the species is declining due to the because of the harsh climatic conditions occurred during the last years that affects water availability combined with the extreme fragmentation of the populations. The first evidence of this conclusion is the reduction of the extent of the species range as evidenced by the comparison of the maps of the newts locations since the discovery of the species. The low ability of the species for dispersal and the low number of individuals in most populations coupled with this inter and, in some cases, intra population fragmentation of the habitat have avoided the recolonisation of places where the species disappeared or the rise of the population size where it became rare. Concretely, the species were found in the middle altitude stream sections of the A3 and A2 populations in samplings performed during the period 2006-2009. In the case of the A3, we failed to find the species in this section since 2009 and during the three last years of the implementation of the Life project we have not observed any newt in the upper stream section. Thus, our data indicates a serious decline of this population and dramatically it is likely that it could be now extinct or the number of individuals is too low to naturally recover. Observations of newts in the lower section of the A2 population were rarest since 2009 suggesting a decline of this population. This conclusion is reinforced by the fact that in the upper sector of this stream newts were only found in the superior half close to the water source during the samplings of the D2 actions. In contrast, during the demographic study carried out during 2006-2009, newts were found along the entire upper section of the stream.

The demographic study of the same section that is currently being undertaken also failed to find newts in the lower part (Guiberteau et al., 2020) independently supporting this evidence. Thus, populations A2 and A3 are likely experiencing the same phenomena, a decline that starts with the ratification of Montseny Brook newts in the lower stream sections where temperatures are higher and the habitat is affected by the alteration of the riparian forest and lead finally to the loss of the population. Probably this has already occurred in the A3 population and now could be happening in the A2.
In fact, the number of adult newts observed have declined in the last years of monitoring. The specific demographic study of this latter population currently in progress must evaluate if this is true. In both cases populations retreat to the highest parts of the stream upper section close to the source, where, because of the subterranean supply, water temperatures could be lower and more constant through the time. It could be related with the rise of environmental temperatures due to the climate change that also could explain the species extirpation in the lower stream sections.

The most dramatic evidence of the effects of climate change are the severe droughts experienced during the last two years in the Montseny massif. Our surveys indicated that this effect seems to be more important in the western than in the eastern basin. The reason why this happens could be the reduction of water provided by the rains, whereas the delivery by the subterranean sources remain less affected. It is possible that in the eastern streams most part of the water-flux is dependent on the subterranean sources and the water provided by rains is occasional, which reduces the impact of the droughts. In the western basin, we inferred the decline of the population B3, which occupies a short section in the upper part of the stream where the source feeds the watercourse. Although there are more meters of stream above this water source, usually this upper section is completely dry and we don't find evidence of subterranean water. The main evidence of this decline is the decrease in the number of observed adults during the last years of monitoring and as observed in the two eastern populations, and a significant reduction of the stream occupancy produced by concentration of newts close to the subterranean source. Apart from the drop of water flow during the last two years produced by the severe drought, the only apparent change is the algal growth occurred in the lowest part of the stream

section that could indicate an increase in organic matter coupled with the rise of the temperatures, although the reason that could have cause it is completely unknown.
On the other hand there are sections of the A1, B3, B4 and B5 where the habitat is formed by high cascades and there is little forest cover because of the stepped terrain and the lack of enough soil for tree implantation. In this case the lack of the species is merely attributable to natural habitat limitations that seems to be very important from an historical perspective as indicated by genetic analysis.

d) Stream connectivity and isolation among populations.
The dendritic index of connectivity portrays a highly fragmented species range, more dramatically in the case of the populations of the eastern basin than in the western. However, the index we used has some limitations because it only considers the distance separating the populations by inhabited stream sections, without taking into account the direction of the potential migration of the individuals (up or down) and what causes the isolation. Practical connectivity among populations can be inferred from the ability of displacement of individuals and in a historical perspective from the analysis of genetic data. In the first case, demographic monitoring of the population A2, showed that displacement upwards the stream was rarer than downwards, most of the displacements were less than 10 m with a maximum up to 60m (Fernandez Guiberteau et al., 2020). These results were achieved by monitoring the adults and subadults. Because larvae are smaller and inhabit the inner rocky matrix, it is likely that they are able to displace even less than the other two population classes. Thus, stream sections where there are anthropogenic barriers and/or extensive habitat alteration or lacks of a minimum subterranean water-flow that are more than 100m long, can be considered as a strong impediment for the migration of individuals and thus, negatively affecting the inter-population connectivity.
However, extreme episodes of water-flow after exceptionally strong rains could produce dragging of newts overcoming this limitation. This kind of migration is involuntary and should involve a few individuals, because *Calotriton arnoldi* is well adapted to these situations by individuals seeking refuge in the deepest parts of the stream-bed into crevices and holes when it happens. Obviously the dragging of newts provides an exceptional rare and unidirectional flow of individuals from the high sections of

the streams to the lowest not in the opposite direction. Unpublished genetic analysis of SNPs polymorphism offers a picture of the connectivity among populations from an historical point of view. Migration upwards is unlikely based on the observation that between the isolated two sections of the eastern A1 populations and was very low and occurred always downwards. This is also the case of the migration between the western B3 and the B4 streams based on genetic analysis.

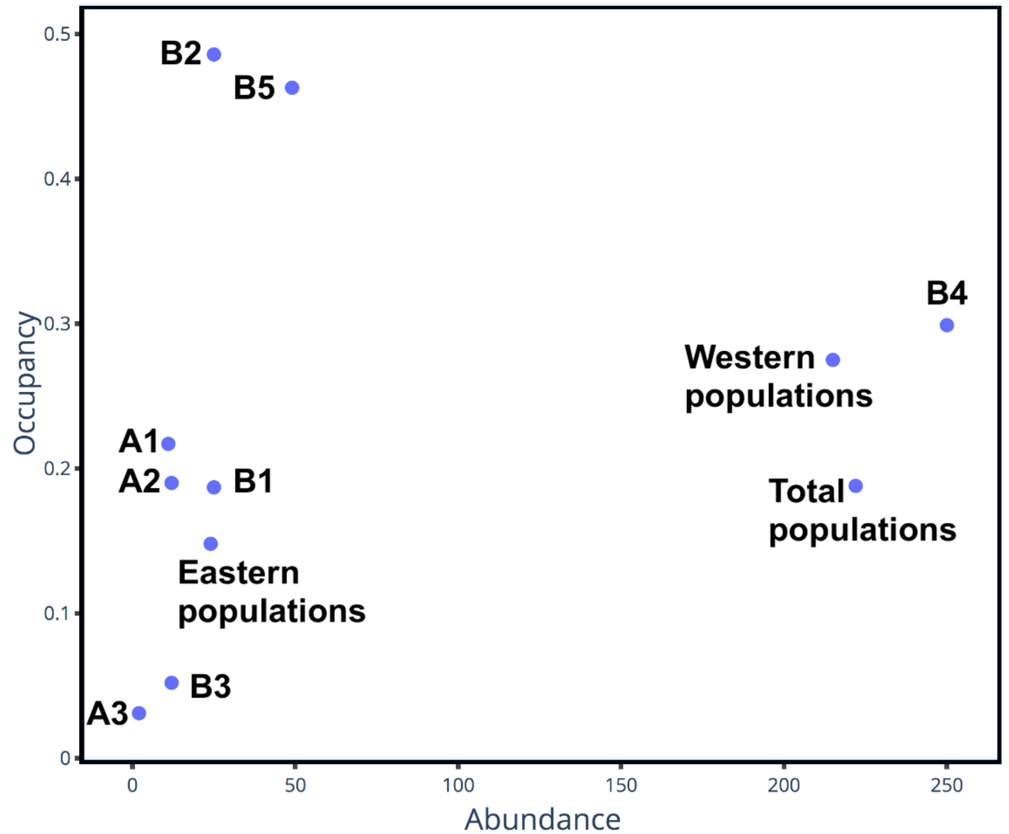
In the case of the A1 population, the cause of the intrapopulation isolation is the existence of a section of stream that is usually shallowly dry and if a subterranean water flow exists it could be very small, intermittent and be placed very deeply. In the case of the B3 population, the lower stream section is formed by a concatenation of large wall rocks generating high cascades with a few ponds placed among them that have few rocks. This habitat is very hostile to the newts becoming a natural barrier between this population and the closest B4. The most likely explanation of the observed pattern is that migration occurred in these cases by involuntary displacement, very rare and stream downwards probably by newts dragging after strong water fluxes produced by heavy rains. In the case of the best connected populations, the genetic homogeneity observed in the other western populations suggest a more frequent migration of newts and in both directions by voluntary movements of newts. Populations of the western and eastern basins were completely isolated as genetic data clearly indicated since several thousands years ago even leading to the development of different morphologic characteristics. The connection between both basins is only possible when they join in the Tordera river at very low altitude in an environment where the species cannot survive. This and the lack of ability for terrestrial dispersal are the causes of the complete isolation between the two groups of populations. As a consequence, the management of the two population groups was being done independently for each of them.

### 6.3.4.- Relevant population parameters for species conservation

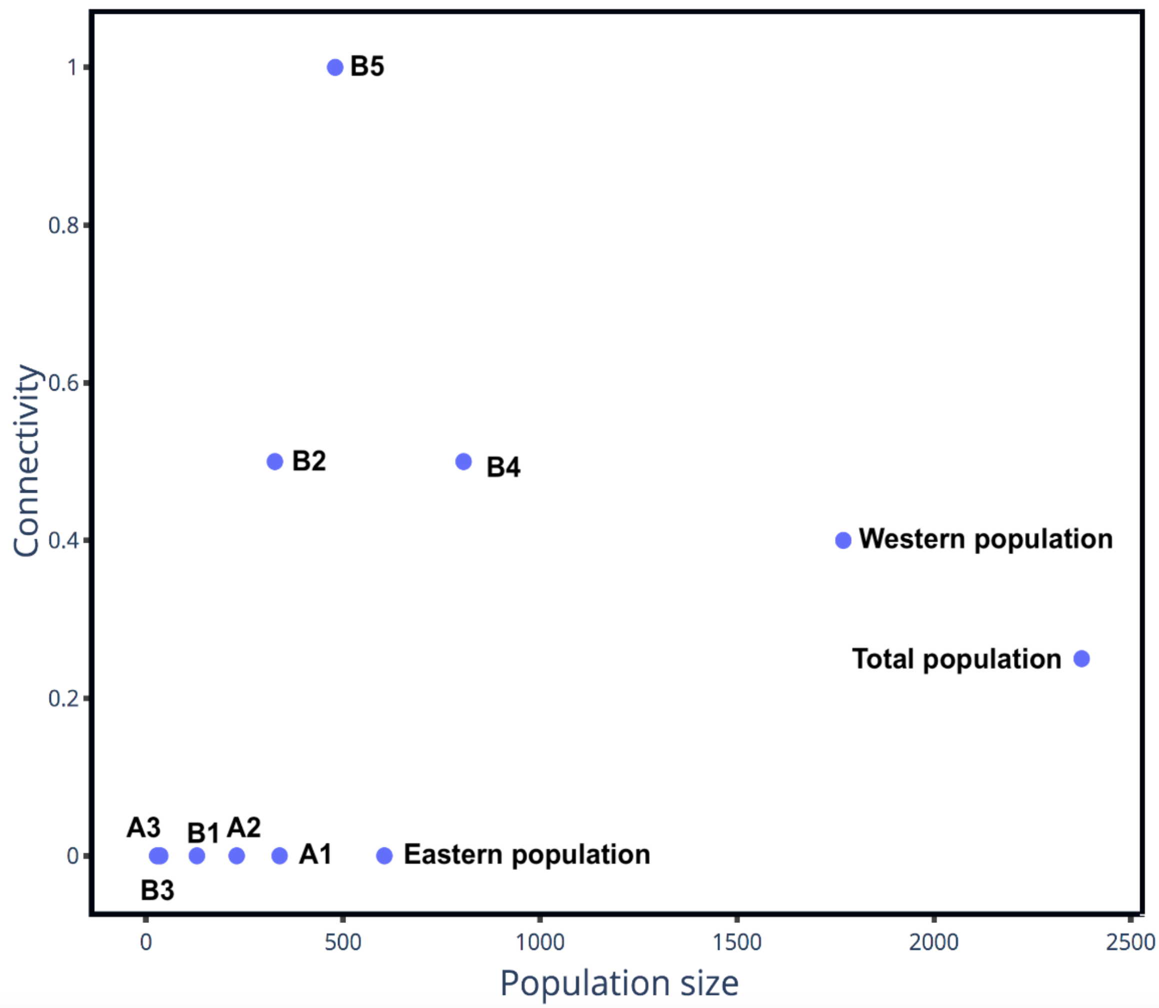
Our study allowed us to estimate two parameters that are of special concern to assess the state of conservation of a species based on the criteria of the IUCN (2001). Population size - in our case referring to the number of adults - is likely the most important because it is directly conversely related with the risk of extinction. The Montseny newt populations dramatically differ on population size, probability due to marked differences on the habitat characteristics, which shapes the carrying capacity. Stream size is the most important variable affecting population size. In the case of Calotriton arnoldi it is not only the linear dimension, but also the volume because the species use the inner rocky matrix as a shelter when environmental conditions in the stream-bed are unfavourable. Nevertheless, because it is impossible to quantify the rocky volume of the stream we calculated population densities and habitat occupancies using the linear length of the stream. Population sizes were calculated using densities and the length of the stream occupied by newts, thus entailing a logical positive relationship between population size and stream length. Nevertheless, we did not use the estimations of relative abundances, but estimations of population density derived from demographic studies independent of the monitoring of natural populations. In this way, we avoided the circularity in our procedure, thus assuring that the connection between habitat and population sizes is truly realistic and not in artefact. Another problem of the indicator of relative abundance is that its dynamics through the time can be caused by changes in the number of individuals, stream occupancy or both. Then an increase of relative abundance may indicate that roughly the same number of newts are concentrated in a short section of stream in relation to previous years instead of an increases on the quantity of individuals. The other parameter is the extent of habitat that the species uses. Preliminary results of demographic and genetic studies in progress indicate that Calotriton arnoldi is a very philopatric species. For this reason, it is very interesting to analyse the percentage of stream length that is inhabited by newts instead of the absolute length. Thus, the sustained reduction of the stream occupancy through time we observe in populations of Calotriton arnoldi could be represent a truly decline if a limited ability of dispersal is assumed.

**6.3.5.- Diagnose of the conservation status**

The finality of the D2 action is the continuous evaluation of the state of conservation of *Calotriton arnoldi* in relation to the IUCN previous cataloguing as "critically endangered". Thus, at the end of the implementation of the LifeTritoMontseny we herein analyse the current state of conservation of the species both globally and individually for each of the eight natural populations, using the information gathered during the period 2017-2022. Thus, we selected two population indicators - abundance and stream occupancy - and two derived estimators -population size and connectivity - derived from the compiled field data. We don't use the indicator of relative abundance because it is a combination of two indicators - population abundance and stream occupancy- already used in these analyses. In the case of these two indicators, we used mean values computed through the years of implementation of the Life project, for each population of the species. Mean abundances sizes were calculated using the maximum number of adults observed by night and year and stream occupancies were computed as the mean percentage of the length of the stream where newts were found. However, we also repeated the computations using the minimum values through the years of sampling to represent the situation of the populations in the worst environmental context. We used the population sizes estimated in this report, but modified the connectivities among populations based on the results of our analysis using the indirect evidence of potential rates of the migrations and its directions. Thus, we assumed total isolation among the two basins that constitute the species range as well among the three eastern populations and for the B1 and B3 populations of the western basin, assigning a value of 0 connectivity. Connectivities of the B2 and B4 western populations are also possible with the B5 by dragging of newts after heavy water-flux, whereas because of its geographic placement the B5 population can receive individuals from all these two populations. Thus, connectivities of the B2 and B4 are assigned to 0.5 and the B4 to 1. In order to easily visualise how differently the eight populations of the species are in relation to these variables, we performed simply biplot graphics of the indicators and derived parameters.




Spatial ordination of the populations, groups of populations (basins) and the whole species based on the combination of absolute abundance (maximum number of adults) and percentage of occupancy averaged through the period 2017-2022.
Spatial ordination of the populations, groups of populations (basins) and the whole species based on the combination of estimated population size and connectivity through the period 2017-2022.




At population level four types of populations can be distinguished on the basis of the covariation of the maximum abundance and percentage of stream occupancy. The western B4 population is in a very good situation from a conservation point of view because of its high newt abundance and occupancy. Other two western populations, B2 and B5 are characterised by even larger occupancies, but their abundances are lower. The two other groups tend to have similar or slightly lower abundances than the previous group, but in contrast occupancies are moderate (A1, A2 and B1) or very low. This latter is the case of the A3 and B3 populations, which have a higher risk of extinction and it is possible than in the case of the eastern A3 could have already vanished. The same analysis performed using the minimum mean abundances did not substantially change the image of the population's conservation and therefore is not commented. Clearly the western group of populations is in a better situation by sustaining three populations moderately well conserved, whereas in the eastern basin *Calotriton arnoldi* is in a higher risk of extinction because the lack of populations sustaining large abundances in large occupied sections of streams.

Relationships of the populations based on the covariation between the estimated population size and the connectivity of the population with the others gave similar results than when using the indicators. The B5, B4 and B2 populations have moderate-higher connectivities and larger population sizes, indicating a good state of conservation, in contrast with the high degree of isolation among the other populations. Among these latest, the worst situation -low population size and at the same time low connectivity- is found in the B1, and extremely in the B3 and A3, whereas the other two eastern populations A2 and A1, have despite its isolation, not so small population sizes. Again, this analysis revealed that the situation of the species in the eastern basin is clearly worse than in the western. We summarise the situation from a conservation perspective of each population and group population as follows:

| | Population size | Population density | Stream occupancy | Population fragmentation | Population isolation | Negative impacts |
|---|---|---|---|---|---|---|
| B1 | Low | Low | Medium | High | Complete | Forest and water exploitation and forests tracks |
| B2 | Medium | Medium | High | Very low | Medium | Water exploitation |
| B3 | Very low | Low | Very low | Low by restricted extension | Medium | No |
| B4 | High | High | High | High | Medium | Forests tracks |
| B5 | High | High | High | Very low | Low | No |
| Western | Medium | Medium | Medium | Medium | Medium | |
| A1 | Low | Medium | Medium | High | Complete | Forest and water exploitation, alteration of riparian forest and forests tracks |
| A2 | Medium | High | Low | High | Complete | Forest and water exploitation, alteration of riparian forest and forests tracks |
| A3 | Very low | Low | Very low | Low by restricted extension | Complete | Forest and water exploitation, alteration of riparian forest and forests tracks |
| Eastern | Low | Low | Low | High | Complete | |

Categorisation of the populations and groups of populations of *Calotriton arnoldi* based on parameters analysed and listing the negative impacts.

Integrating the results of the analyses performed, we consider the A3 and B3 as the most endangered populations of the species and now experiencing the highest risk of extinction. The A1, A2 and B1 are in a worrying situation that could evolve in the future to the level of the A3 and B3. All these populations may deserve actions of population reinforcement from the captive breeding groups although if the reason causing this dynamics is climate change or there are other unknown factors involved, it is not clear that it will be effective to reverse the situation. The B2, B4 and B5 populations are the best preserved and until now only periodic surveillance is needed.

Based on the main conclusion of the natural populations monitoring, that is the decline of the species has slightly increased since 2010, the preservation of the species will be effective if the implantation of new populations by the reintroduction program is successful. If the worst predictions of climate change become real in the next few years, we believe that only three populations -all in the western basin- could persist through time. It also implies that the stream occupancy of these populations and the number of newts will predictably be reduced. It would be interesting to profit from the few years of exceptionally large precipitations and water availability to evaluate the ability of the species to persist to the extremely hard droughts. Nevertheless, the decline we observed in two populations is not cause of optimism. In this context, the program of reintroduction is releasing newts in streams that based on our observations maintain in most cases a minimum ecologic water flow, thus ensuring the survivorship of the newts in the most critical periods of the year. Our results highlighted the worrying situation of the species in the eastern basin which unfortunately have less potential habitat in the Tordera basin. Additionally, captive breeding of eastern newts is less effective in producing subadults for release in nature, posing more difficulties to the recovery of the species in this part of its range. Anyway and in spite of all these problems we are forced to overcome all this and achieve the success of the reintroduction program because unfortunately and in light of our conclusions this is the only conservation strategy capable of preserving the species in Montseny.

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