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A Perspective in the Study of Biodiversity of Order Lepidoptera in Albania: Case Study using the MRR Methodology

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Abstract
The Mark-Release-Recapture (MRR) method is a widely used method to estimate populations of different organisms in a given environment throughout the field of biology. The biological material was collected during the year 2023. This study uses the MRR (Mark – Release – Recapture) method to assess the population, distribution, territoriality and behavior of the study species *Pseudochazara Tisiphone* Brown, 1980 in the area of Drenova. The MRR method involves marking individuals, releasing them into the environment, and then recapturing them again to calculate the number of marked recaptures. Based on the number of marked individuals that are recaptured, we determine the total number of individuals in a population. This method provides a sustainable and effective way to monitor and study natural populations in their environments. During this year we managed to catch 32 species of this type, of which we found 21 females (65.6%) and 11 males (34.3%) based on behavioral differences. The captured individuals are tagged and released, according to the MRR methodology. By analyzing the marked species, it turns out that we have 6 individuals or (18.7%) of the *Pseudochazara Tisiphone* species found again. Of these 6 individuals, 4 individuals are female (12.5%) and 2 are males (6.25%). This recurrence rate is low. From the analysis of the days according to the months, in which we met and re-met more often individuals of *Pseudochazara Tisiphone*, it turns out that the frequency of meeting this type results more at the end of summer.

Keywords: *Pseudochazara Tisiphone*, Mark - Release- Recapture, Conservation, Lepidoptera, Albania

Introduction

In recent years, there have been important developments regarding the recognition of the distribution of Lepidoptera species in Albania. These studies are mainly focused on the taxonomic distribution of butterflies, carried out by foreign entomologists and local entomologists as a contribution to the knowledge of the lepidoptera fauna of Albania (Beshkov, 1995; Beshkov & Misja, 1995; Beshkov *et al*., 1996) [1, 2, 3]. In recent years, interest in Lepidoptera species in Albania has increased, and several publications are referred to, which present updates of the species list of Lepidoptera, as well as a new atlas has been published where 205 species of butterflies have been confirmed (Cuvelier *et al*., 2022) [6].

Our study, unlike the studies so far mainly in the systematic taxonomy of Lepidoptera in Albania, cited above, aims to analyze the biological behavior of the species *Pseudochazara tisiphone* Brown, 1980, in a study station in Drenove in the area of Korça (Albania). Analyzing this biological behavior of this species will provide us with data to design conservation models for Lepidoptera species (Williams *et al*., 2018) [19].

Pseudochazara Tisiphone was discovered in the ophiolite zone of northern Pindos (Greece) by Brown (1976) [4], and since then populations of this species have declined mainly due to human activity (Cuvelier *et al*., 2018) [7]. It is found on stony plateaus and rocky slopes, on ophiolite substrates, with loose gravel parts and scattered grasses from 1000 to 1700 m (Cuvelier *et al*., 2018) [7].

In Albania, this species was referred in 2017 in the area of Bulqiza (Dibër) (Cuvelier *et al*., 2018) [7], and in 2022 in Lurë-Deja National Park (Dibra) (Cuvelier *et al*., 2022) [6]. Albania has a decisive role in preserving these populations of this species with significant genetic variations. This will have to go hand in hand with the protection of these special habitats that are prone to serious threats (Cuvelier *et al*., 2022) [6].

Our study, with the Mark-Release-Recapture (MRR) method, applies an effective method for obtaining demographic information about animals, including insects (Gall, 1984; Pollock *et al*., 1990; Sandercock, 2006) [12, 15, 17]. The marking of animals with different methods dates back to 218 BC, when ornithologists first marked birds with tape (Fisher, 1964) [11]. Marking insects for scientific studies began around 1920, when researchers used dyes, and stains in insect population studies (Dudley, 1923) [10]. Meanwhile, studies of Lepidoptera with the MRR method of butterflies were initiated for the first time in Britain and Europe in the 1930s (Brett, 1936; Dowdeswell *et al*., 1940) [5, 9]. During studies using the MRR method, marking does not affect the later survival of the animal (Manly, 1971; Pollock *et al*., 1990; Pollock, 2005) [13, 15, 16]. Information derived from the number of Lepidoptera captured and recaptured provides important variables such as: Population size, reproduction rate, threat level, etc.

Materials and Method

Our study was developed throughout the year 2023 in the Region of Korça, in the area of Drenova for the species.

Fig 1: Marking of individuals *P.tisiphone* photo by @Xh.Qirinxhi

Pseudochazara Tisiphone developed in a transect of this area with a surface of 5 Ha each, according to Williams (Williams *et al*., 2018) [19]. Monitoring for *Pseudochazara Tisiphone* was carried out between 09^{30} and 12^{30} a.m, during the months of August - September which coincides with the flight time of the Order Lepidoptera (Pollock, 2005) [16].

The transect was observed for almost 2 hours at a slow and steady pace.During monitoring, unmarked individuals were noted, as well as individuals that had been previously marked. (Manly, 1971) [13]. In using the MRR technique, butterflies in the field were collected by aerial entomological netting. Individuals are then removed from the net (Fig 1), holding the butterfly by the wings between the thumb and forefinger of the right hand (Morton, 1982) [14]. Individuals are passed in the left hand (the right hand is free for marking), and it is held on the thorax at the base of the lower wings, which are raised in this position, and at the base of these wings a symbol (number /line/ dot) with a gold metallic ink tip pen, to be recognizable (Williams *et al*., 2002) [18].

Marked insects are released back into the same field/place. Then we return to these same stations, and aim to meet again these same individuals marked by us. In each case, maximum care was taken not to damage the wings and disturb the marked individual (Gall, 1984) [12]. Recaptured butterflies were then checked for the presence of the marker to differentiate from unmarked butterflies.

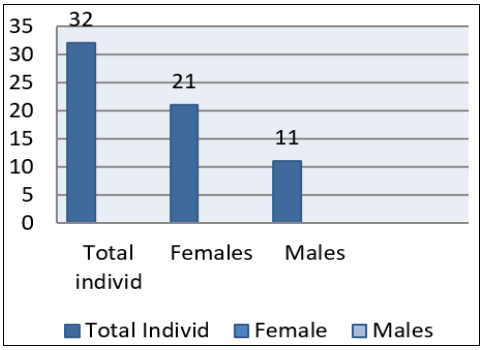
Results and Discussion

To test the marking procedure on Lepidoptera survival, we managed to capture 32 individuals using the MRR (Mark–Release–Recapture) method. This is the standard way of testing for a temporal marker effect on later survival (Gall, 1984; Sandercock, 2006) [12, 17]. The survival rate was evaluated with two parameters: One for the first day after marking and the other for the following days. The butterfly that was studied is *Pseudochazara Tisiphone* Brown, 1980, family Nymphalidae Rafinesque, 1815, subfamily Satyrinae Boisduval, 1833.

Sampling was carried out in the interval for 24 days in the period August 17 - September 10. The transect where the monitoring and marking was carried out was observed for almost 2 hours with slow and steady walking. During the process *Pseudochazara Tisiphone* were caught with entomological nets. After capture the individuals were marked with an identification mark on the lower part of the front wing of the hind wings. 32 individuals were captured of which 21 females (65.6%) and 11 males (34.3%), (Table 1, Graph 1) determination based on behavioral differences (Williams *et al*., 2018) [19].

Table 1: Number of individuals by sex

Sex ♀/♂	**Number of specimens**	**% of specimens**
♂ (Males)	11	34.3%
♀ (Females)	21	65.6%

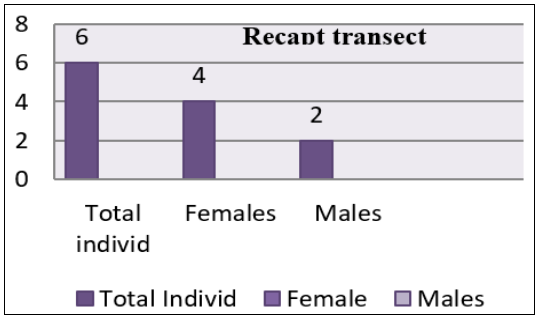


Graph 1: Number of specimens by sex ♀/♂

6 individuals were recaptured, so the rate of recapture is 18.7%, this frequency of re-encounter is low (Williams *et al*., 2018) [19], where 4 individuals are female (12.5%) and 2 individuals are male (6.25%), the determination based on behavioral differences. During the marking process, we also have 1 dead individual (Table 2, Graph 2).

Table 2: Number of re-encounters during marking

Sex ♀/♂	**Number of specimens**	**% of specimens**
♂ (Males)	2	6.25 %
♀ (Females)	4	12.5%



Graph 2: Nr of re-encounters by sex ♀/♂

Based on these preliminary data, we think that in the future we should increase the number of days in the field, to increase the possibility of re-meeting individuals, to have a clearer picture in order to design conservation models for Lepidoptera species.

Conclusion

This study employs the Mark-Release-Recapture (MRR) method to assess the population size, distribution, territorial behavior, and movement patterns of the study species *Pseudochazara tisiphone* during 2023 resulted in 32 captured individuals of this species, of which 21 were females (65.6%) and 11 males (34.3%) based on behavioral differences.

From the analysis of the marked individuals, it turns out that we have 6 individuals or (18.7%) of the *Pseudochazara Tisiphone* re-encountered where 4 individuals are females (12.5%) and 2 males (6.25%). This re-encounter frequency is low, as a result of the impact of the death of male individuals during the period between the capture and recapture of the species by our team, but also the difficulties of applying the methodology.

From the analysis of *Pseudochazara Tisiphone* individuals that we caught and recaptured more often, we conclude that the frequency of this species is higher in summer.

This case study using the MRR method for the species *Pseudochazara Tisiphone*, and the effectiveness of this method that resulted during this work, creates a perspective in the future, for the study of the biodiversity of Lepidoptera in Albania, and helps to design conservation plans for this endangered species

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