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Artificial Waterholes for European Bison as Biodiversity Hotspots in Forest Ecosystems: Ecological Effects of Species Reintroduction Activities

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Abstract: Despite the growing population of European bison (*Bison bonasus*), it is necessary to plan the reintroduction of these animals to new areas. Reintroduction of European bison often requires the improvement of natural conditions. Such preparatory activities allow European bison to more easily adapt to new places, but also impact the functioning of animals from other taxa. The aim of the presented study was to examine the impact of waterholes for European bison on the development of local populations of amphibians and dragonflies (Odonata), as well as the creation of new feeding grounds for bats. We examined 15 reservoirs in the Augustów Forest District located in northeastern Poland, of which five were waterholes for European bison built in 2013–2014, four were semi-natural reservoirs transformed into waterholes for European bison in 2018, and six were natural reservoirs. Dragonflies were studied in 2021–2022; amphibians in 2018 and 2020; and bats in 2018, 2019, and 2020. In total, 24 species of dragonflies (Odonata), 10 species of amphibians, and 13 species of bats were found. The results of the inventory of three taxonomic groups using different comparative variants indicate a significant impact of the construction of waterholes for European bison on the biodiversity of the forest ecosystem. We concluded that the waterholes for European bison present better resistance to drying out than natural reservoirs. In addition, waterholes warm up more quickly, supporting better conditions for amphibians. The surface of the reservoirs and their exposed surroundings are favorable for insects (including dragonflies), and these are a source of food for bats, becoming attractive feeding grounds for them.

Keywords: *Bison bonasus*; conservation; waterholes; dragonflies; amphibians; bats

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1. Introduction

The reintroduction of the European bison (*Bison bonasus*) in recent years has had a clear effect on the growing population [1]. Still, however, the distribution of European bison is not satisfactory and there is a need for the further reintroduction of this species into new areas, especially in view of current threats [2–4]. For this reason, active efforts are still being made to reintroduce European bison [5,6], as well as to assess the habitat suitability and improve the reintroduction methods [7–10].

The impact of European bison on habitats is still poorly known. Although the species is regarded as a grazer, its diet can consist of up to 33% woody plants [11]. Nevertheless, European bison present high foraging plasticity [12]. For this reason, this species can occupy various habitats depending on the availability within its home range [13]. European bison can have an impact on plant species composition and seed bank through endo- and epizoochory [14–16], as well as direct grazing and debarking in the forest habitats [17], which may also lead to significant damage to tree stands [18,19]. The ecological role of the European bison in influencing the biodiversity of open areas [20] and its indirect impact on invertebrates in forest habitats was also indicated [21].

Introducing the European bison to new habitats involves not only transporting animals, but also a number of activities aimed at preparing the environment for easier adaptation of the animals in the new place. One such activity is the construction of waterholes, which is recommended in areas where access to natural water is difficult [22]. Such waterholes are built in numerous projects aimed at the protection of European bison in Poland [23]. Ponds are seen as biodiversity habitats, especially in forest ecosystems [24,25]. The creation and restoration of water reservoirs provide conditions for the development of local amphibian populations [26,27] and improve the quality of feeding grounds for bats [28,29]. Anthropogenic water bodies also provide valuable secondary habitats for some dragonfly (Odonata) species [30,31]. Many insects thriving in water are a rich food source, e.g., for bats. These species also include Trichoptera and Ephemeroptera. Two bats that specialize in these insects are Daubenton's bat and the pond bat (*Myotis daubentonii* and *Myotis dasycneme*); all small bats of the genus *Myotis* and *Pipistrelles* (*Pipistrellus pipistrellus* and *Pipistrellus nathusii*) and *Noctaltule* (*Nyctalus*) can also hunt them [32]. However, the pond bat mainly hunts in large bodies of water, both standing and flowing, such as rivers, wide canals, fishponds, lakes, and other unvegetated areas [33,34].

Taking the above into account, this study aims to assess the impact of wisent ponds (waterholes made for European bison) on biodiversity in the forest ecosystem. The study covers three taxonomic groups: dragonflies, amphibians, and bats, which were analyzed in 15 waterholes in the Augustowska Forest complex (Poland). Two hypotheses were posed:

The artificial reservoirs created for the European bison contribute to the species diversity of dragonflies, amphibians, and bats.

Artificial water reservoirs in the Augustowska Forest contain a greater number of species and more often rare species of dragonflies and amphibians than natural reservoirs.

2. Material and Methods

The study was conducted in Augustów Forest District, located in northeastern Poland. The forest district covers an area of 26,048.78 ha and is a part of one of the largest forest complexes of the Augustowska Forest in Poland. The research area covered 15 water reservoirs in the central part of the forest complex (Figure 1, Table S1).

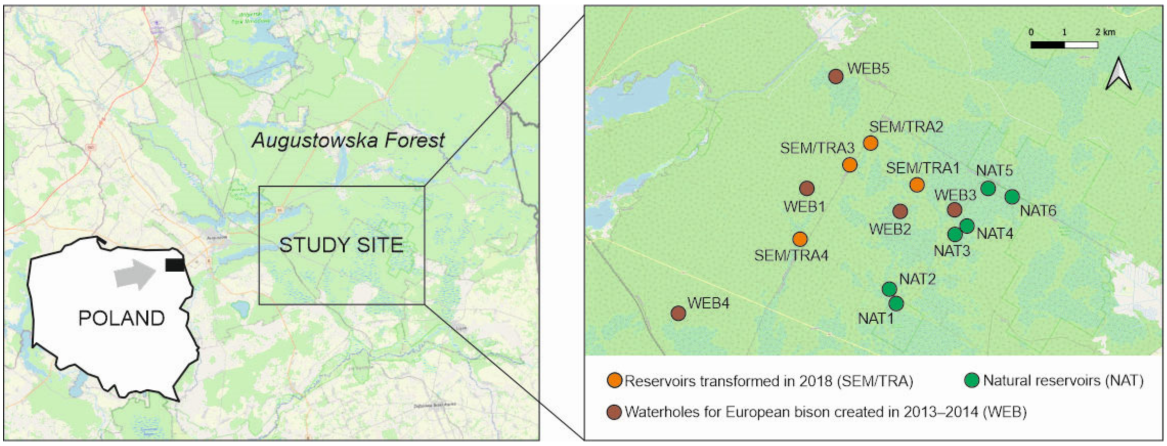

Figure 1. Location of reservoirs studied within the study site.

- Five waterholes for the European bison in mid-forest meadows created in 2013–2014, herein called WEB;
- Four seminatural waterholes, which were transformed into waterholes for the E. bison in 2018. Transformation into waterholes consisted in cleaning the reservoir, enlarging its surface, creating shallow banks, and uncovering the surroundings by

cutting down the tree vegetation near the reservoir. Before transformation, waterholes were called SEM, and after transformation, they were named TRA. The semi-natural reservoir's (SEM) numbers correspond to the same reservoir after transformation (TRA), i.e., TRA1 is the same reservoir as SEM1, but after transformation;

- Six natural water reservoirs detected near artificial/transformed waterholes for European bison. Waterholes for European bison are usually created in places where natural water reservoirs [22] are not available; therefore, natural reservoirs located as close as possible to artificial/transformed waterholes for European bison were selected for comparative studies. The reservoirs were named NAT. Natural reservoirs were either ephemeral or heavily overgrown and significantly shaded.

Due to the work with protected species, approvals from the relevant Directorates for Environmental Protection were obtained prior to undertaking the field research. The research did not require approval from ethics committees in light of Polish law.

2.1. Dragonflies

The study was carried out in all 15 reservoirs in 2021–2022 (Figure 2). Each reservoir was visited twice—the summer aspect of the odonatofauna was examined in the third decade of August 2021, and the spring aspect in the second decade of June 2022. Each visit to individual reservoirs lasted several dozen minutes. The methodology was based on direct observation of imagines and collection of exuviae, which were carried out during a walk along the entire shoreline of the reservoirs, or a section of the shoreline if it would be difficult or impossible to walk around the reservoir. All observed exuviae were collected. Estimated or exact adult numbers were recorded, as well as reproductive behavior: egg-laying, copulations, tandems, and territorial behavior. Information on the presence of juvenile or teneral individuals of a given species was also recorded and/or their photographic documentation was prepared.

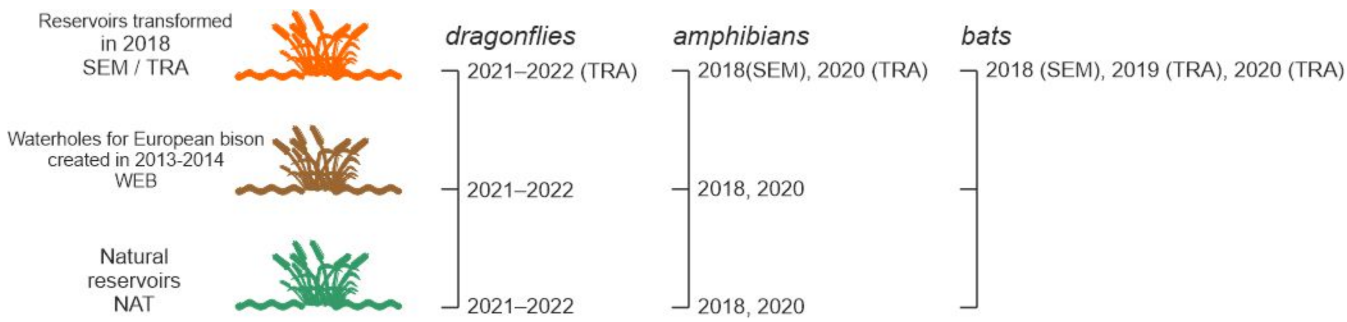

Figure 2. The scheme of the studied taxonomic groups in the given reservoirs (SEM-seminatural reservoirs before transformation, TRA-reservoirs after transformation).

A total of 441 exuviae were collected, of which about 80% of the material was identified to the species level. The rest was identified to the genus or family level, usually due to low-quality material. One dead larva was also photographed and identified to the species level.

The following categories of species occurrence were distinguished [35,36]:

- Autochthonous species: when exuviae were collected, and/or teneral individuals were observed, and/or numerous breeding behaviors were detected;
- Probable autochthonous species: when few or isolated breeding behaviors such as tandems, copulation, or oviposition were recorded and/or at least a few individuals displaying territorial behavior and/or numerous adults in an environment suitable for development were detected;
- Recorded species: in other cases.

2.2. Amphibians

The study was conducted in two years, 2018 and 2020, and also covered all 15 reservoirs (Figure 2). In 2018, the study was conducted on SEM, but in 2020, the study was conducted on transformed reservoirs (TRA). In 2018, four inspections were carried out: on 14 April, 18–19 May, 22–23 June, and 28–29 July. In 2020, three inspections were carried out: on 4–5 May, 1–2 June, and 7–8 August. Due to the COVID-19 pandemic, it was impossible to perform an early spring inspection. To identify the presence of each amphibian species in each reservoir, we used the following methods:

- The listening method—to record the presence of anuran amphibians during the visit, lasting from 10 to 30 min, depending on size of the pond;
- Direct observation of water (including water plants) and banks, where individuals in different stages of development (including eggs) were visually sought. On each reservoir, one passage was made along the banks around the entire reservoir, except for in inaccessible places;
- Capturing individuals with a herpetological net.

Each noticed specimen was described according to the species and age, if possible. Due to the difficulty of determining the taxonomic affiliation of green frogs in the field, each individual we encountered was described as belonging to the group of "green frogs" (*Pelophylax esculentus complex*) [37,38].

2.3. Bats

The study was conducted in three consecutive years, 2018, 2019, and 2020, and covered four seminatural reservoirs: SEM (study conducted in 2018), and the four reservoirs transformed (TRA, study conducted in 2019 and 2020) (Figure 2). The field study was carried out each year in three seasons: spring, summer, and autumn. Such a work schedule allowed us to record the activity of bats during spring migrations, the creation of breeding colonies, reproduction, colony breakdown, and the beginning of autumn migrations [39]. The following methods were used in the field:

- Capturing bats in chiropterological nets. A net, 6 m long, was placed as close as possible to a given reservoir. Capturing was carried out for three full, consecutive nights (in each reservoir and in each year of the study). The caught individuals were taken out of the net and marked according to species using standard methods [32,34,40].

3. Results

3.1. Dragonflies

A total of 24 species of dragonflies (Odonata) were recorded, but full development, considered certain or probable, was observed in 14 species in at least one reservoir (Table S2, Figure 3). In total, 9–13 taxa were found in reservoirs constructed in 2018 (on average, 11 taxa per reservoir), 4–11 taxa (on average, eight taxa per reservoir) on WEB reservoirs, and zero to nine taxa (on average, three taxa per reservoir) on NAT reservoirs. Within the WEB2 reservoir, 13 taxa were recorded, which is the highest number found in the context of the studied sites. Among the three groups of reservoirs, the fewest taxa were found in natural reservoirs, with no dragonflies found in reservoir NAT2. Only the NAT6 reservoir was distinguished by a relatively high number of nine species, a number comparable to or even higher than some European bison waterholes, while the other natural reservoirs presented much lower values (Figure 3).

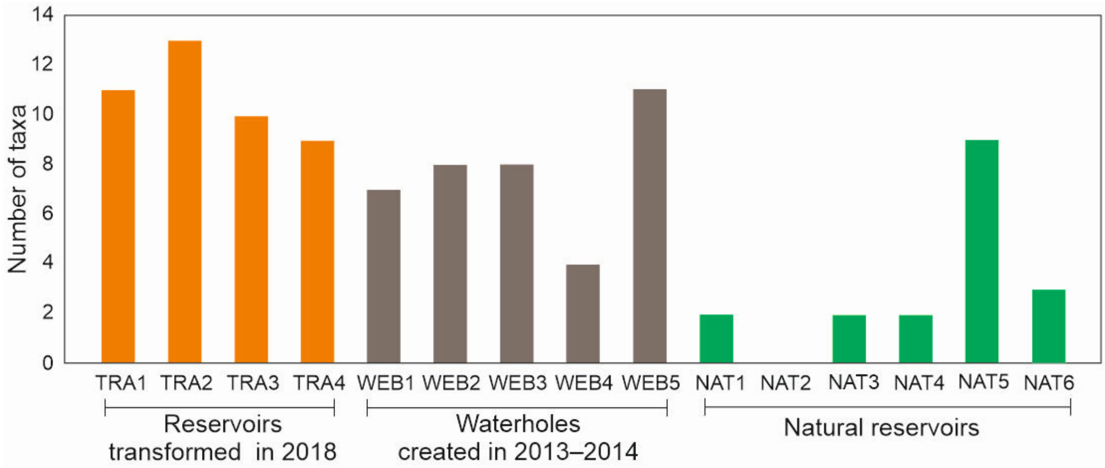

Figure 3. Number of dragonfly taxa found in the examined water reservoirs.

The most common species was *Coenagrion puella*, recorded at all of the waterholes, as well as within almost all natural reservoirs, often in large numbers, in the form of adults and/or exuviae. *Aeshna cyanea*, *Libellula quadrimaculata*, *L. depressa*, and *Leucorrhinia pectoralis* were also common and relatively numerous in some of the studied locations. One species which we found-*Leucorrhinia pectoralis*—is strictly protected in Poland [41], and is included in Annexes II and IV of the Council Directive 92/43/EEC of 21 May 1992 [42]. This species was observed in all TRA reservoirs, in two out of five WEB reservoirs (WEB1, WEB2), and in two out of six natural reservoirs (NAT5, NAT6). Among the listed species, the rarest in Poland was *Coenagrion lunulatum* [36,43], recorded in the TRA2 reservoir and transformed in 2018.

Eurytopes predominated among species found both in WEB reservoirs and in natural reservoirs (NAT), and only in TRA reservoirs did they account for half of all species (Figure 4). In the latter, the most tyrphophiles were found. Interestingly, in the WEB reservoirs, two pioneer species were recorded—*Libellula depressa* and *Ischnura pumilio*. In TRA reservoirs, *Libellula depressa* was also found. Two species of small water bodies, *Lestes dryas* and *Lestes barbarus,* were recorded in WEB reservoirs, while only *Lestes barbarus* was found in the other two types of reservoirs. The TRA reservoirs were distinguished by the number of rheophilic/reobiontic species recorded there, although only one, *Pyrrhosoma nymphula*, was considered likely to develop in one of these reservoirs. The remaining species preferring watercourses, *Calopteryx splendens* and *Gomphus vulgatissimus* (Table S2), and the species obligatorily associated with watercourses, *C. virgo*, were found only singly in few sites in their adult forms. Their records should be considered accidental, especially in the case of *C. virgo*.

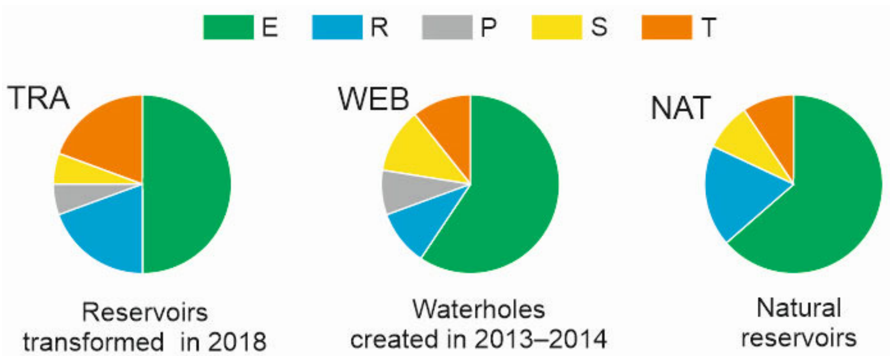

Figure 4. Ecological elements in the dragonfly fauna found in the examined water reservoirs: E—eurytopes; R—rheobionts and rheophiles; P—pioneer species; S—species of small water bodies; T—tyrphophiles.

3.2. Amphibians

In 2018, a total of six amphibian species and one taxon were found: *Triturus cristatus*, *Lissotriton vulgaris*, *Bufo bufo*, *Pseudepidalea viridis*, *Rana temporaria,* and *Rana arvalis*, as well as a complex of green frogs (Table S3.Only in one reservoir (natural reservoir NAT2) were no amphibians found. Amphibians were found in the remaining 14 examined reservoirs, although the number of species varied. Ten reservoirs out of fifteen were used by amphibians for reproduction. Waterholes for European bison (WEB) presented the greatest species diversity and the highest degree of settlement. All the species and the taxon mentioned above were found in these waterholes. From three to five species of amphibians were observed in individual waterholes (on average, four species per reservoir). In the reservoir (WEB2) the presence of five species of amphibians was confirmed, which was the highest species richness among all monitored reservoirs. Representatives of the green frog complex were observed in all of the reservoirs. Brown frogs (*Rana temporaria/Rana arvalis*) and *Lissotriton vulgaris* were present in four out of five reservoirs (Table S3). *Triturus cristatus* and *Bufo bufo* were present in three out of five reservoirs. *Pseudepidalea viridis* was found only in one WEB reservoir (WEB4). All waterholes for European bison were breeding sites for amphibians. However, no eggs or larvae of *Lissotriton vulgaris* could be observed in two waterholes, namely, WEB4 and WEB5, despite finding adult individuals (Table S3). In SEM reservoirs, the amphibian species representation was slightly lower, but all were colonized. Both species of newts, brown frogs, and a complex of green frogs were found in them. In individual reservoirs, one to four species of amphibians were recorded (on average, 2.5 species per reservoir). *Triturus cristatus* was present in three out of four ponds (SEM2, SEM3, and SEM4); *Lissotriton vulgaris* was present in two ponds (SEM3 and SEM4); and brown frogs were observed only in one pond (SEM3) (Table S3). Reproduction was confirmed in three out of four reservoirs (SEM2, SEM3 and SEM4). Eggs/larvae of *Triturus cristatus* (SEM2 and SEM4) and *Lissotriton vulgaris* (SEM3 and SEM4) were also observed.

Natural reservoirs were characterized by the lowest number of individuals, but the species richness was greater than in SEM reservoirs. The presence of *Triturus cristatus* and *Lissotriton vulgaris*, brown frogs, *Bufo bufo*, and a complex of green frogs were confirmed. In individual ponds, from zero to three species were found (1.5 species/reservoir). The presence of amphibians was confirmed in five out of six reservoirs (Table S3). *Bufo bufo*, brown frogs (NAT1), and *Triturus cristatus* and *Lissotriton vulgaris* (NAT5) were found in one reservoir. Breeding of five species was confirmed in only two ponds (NAT1 and NAT5): *Bufo bufo* (NAT1), brown frog (NAT1), *Triturus cristatus* (NAT5), and *Lissotriton vulgaris* (NAT5) (Figure 5, Table S3).

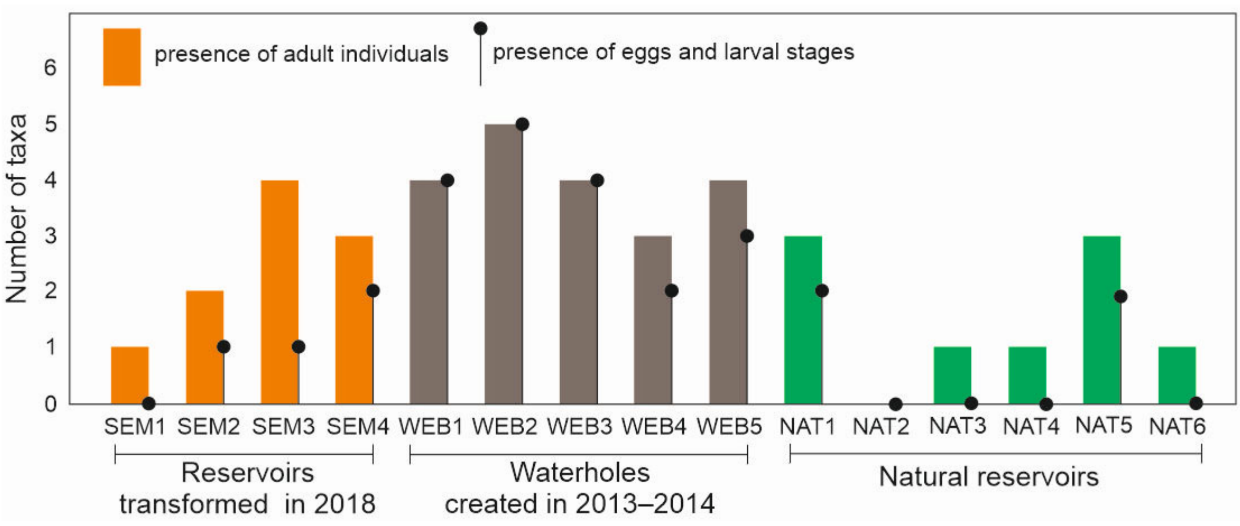

Figure 5. Number of amphibian taxa found in the examined water reservoirs in 2018.

In 2020, the presence of amphibians was confirmed in 11 out of 15 surveyed reservoirs; only *Bufo bufo*, which was present in 2018, was not confirmed in 2020. A new species for the study area was *Hyla arborea* (but without confirmed reproduction), which was found in the TRA4 reservoir. Amphibians bred in all 15 water reservoirs. In waterholes for European bison (WEB), only two species and one taxon (*Triturus cristatus*, *Pseudepidalea viridis*, and green frog complex) were found (Table S3). All of the reservoirs were inhabited by amphibians, but no more than two species were found in each (Figure 6). The presence of a green frog complex was confirmed in four out of five reservoirs (WEB1, WEB2, WEB3, and WEB5), *Triturus cristatus* only in two (WEB2 and WEB3), and *Pseudepidaleas viridis* was still present in the same waterhole (WEB4). Each pond was a breeding site for the amphibian species observed in it. *Lissotriton vulgaris*, brown frogs, and *Bufo bufo* were not recorded. In waterholes transformed in 2018 (TRA), the presence of three species and one taxon was recorded: *Triturus cristatus, Lissotriton vulgaris*, *Hyla arborea,* and a complex of green frogs. *Triturus cristatus* was observed only in two reservoirs (TRA1 and TRA2), similarly to the *Lissotriton vulgaris* (TRA1 and TRA4). For the first time, it was possible to confirm the presence of a new species, *Hyla arborea*, which was recorded while calling near the TRA4. This was the only pond of all those examined in the vicinity, which had its presence confirmed. Only two reservoirs were used as breeding sites (*Lissotriton vulgaris* in TRA1, green frog complex in TRA4) (Figure 6, Table S3).

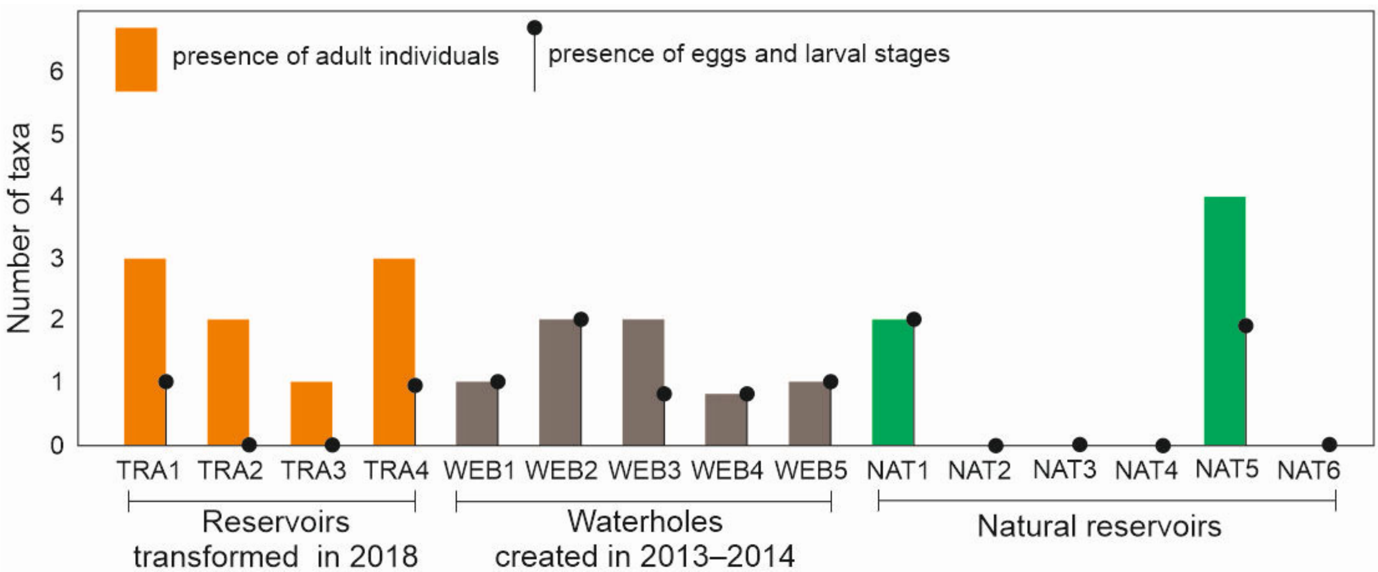

Figure 6. Number of amphibian taxa found in the examined water reservoirs in 2020.

Again, natural reservoirs were characterized by the smallest number of individuals compared to other groups of reservoirs. Amphibians were found in only two out of six monitored reservoirs. However, in terms of species richness, natural reservoirs supported more amphibians species than artificially created waterholes—four species and one taxon of amphibians were observed: *Triturus cristatus* (NAT1 and NAT5), *Lissotriton vulgaris* (NAT5), brown frogs (NAT5), and a complex of green frogs (NAT1 and NAT5 DS10). Amphibian reproduction was confirmed in two out of six controlled reservoirs. The breeding species were *Triturus cristatus* and green frog complex (NAT1) and *Lissotriton vulgaris* and brown frog (NAT5).

3.3. Bats

In 2020, the number of species was similar to 2019 (TRA reservoirs), and higher than in 2018 (SEM reservoirs) before the transformation (Figure 7). More pronounced differences were found in the number of individuals, which showed a two-fold increase in 2020 compared to 2018 (Figure 7).

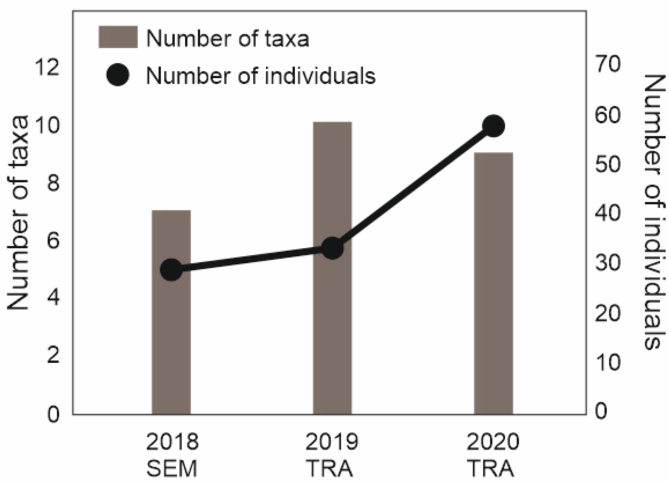

Figure 7. Number of bats species and individuals in given years of the study: 2018—before transformation to waterholes for European bison (SEM); 2019–2020—after transformation (TRA).

In 2018, 29 individuals belonging to 7 species of bats were caught near the SEM reservoirs (*Myotis daubentonii*, *Pipistrellus pispistrellus*, *Pipistrellus nathusii*, *Barbastella barbastellus*, *Plecotus auritus*, *Nyctalus noctula*, and *Nyctalus leisleri*). In 2019, after transformation into waterholes for European bison (TRA), 33 bats of 10 species were caught (*Eptesicus nilssonii*, *Vespertilio murinus*, *Myotis daubentonii*, *Pipistrellus pygmaeus*, *Pipistrellus pispistrellus*, *Pipistrellus nathusii*, *Barbastella barbastellus*, *Plecotus auritus*, *Nyctalus noctula*, *Nyctalus leisleri*, and 1 individual classified to the genus *Pipistrellus* spp.). In 2020, 58 individuals belonging to 9 species were caught (*Eptesicus serotinus*, *Myotis nattereri*, *Myotis daubentonii*, *Pipistrellus pygmaeus*, *Pipistrellus pispistrellus*, *Pipistrellus nathusii*, *Barbastella barbastellus*, *Nyctalus noctula*, *Nyctalus leisleri*, and one individual classified to the genus *Pipistrellus* spp.) (Figure 8).

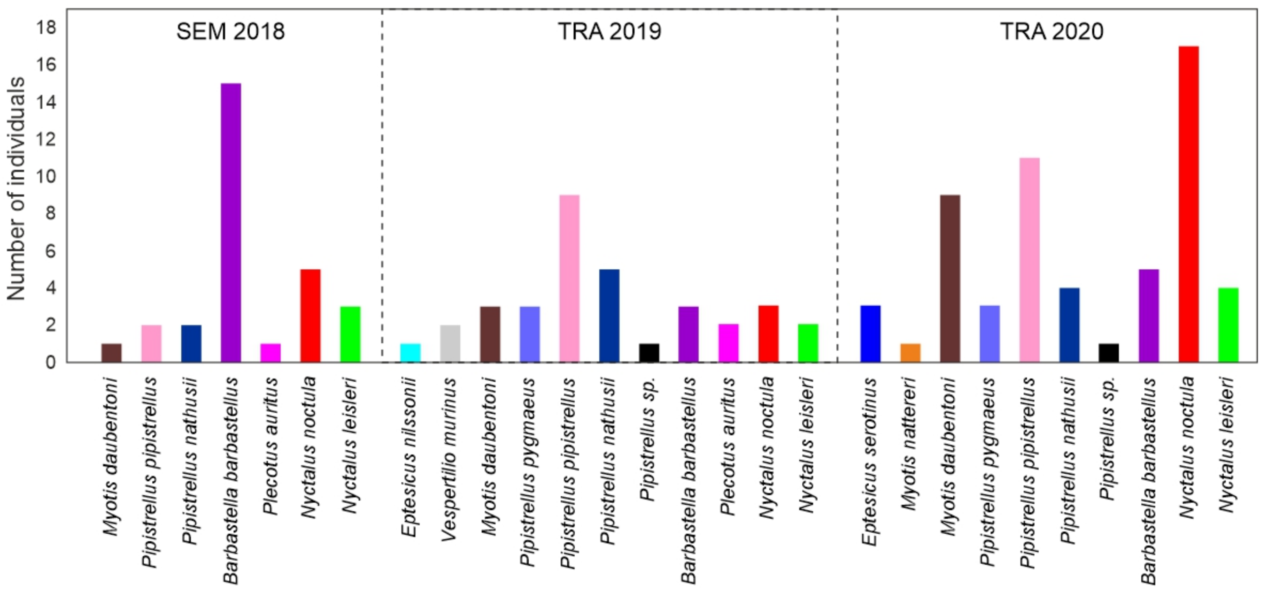

Figure 8. Catch results in 2018 (before transformation) (SEM), and in 2019 and 2020 (TRA) after transformation to waterholes for European bison.

In 2018, the bats found in SEM reservoirs presented lower species richness compared to that found in the following years after the transformation (TRA reservoirs). In 2018, out of the 7 species found, over 50% (15 individuals) represented the typical forest species *Barabastella barbastellus*. The second relatively common species was *Nyctalus noctula*, of which five specimens were collected. After the transformation of the reservoirs, an increase in the number of bat species in the first growing season was noted. In addition, the proportions of representatives of individual species changed. In 2019, in the TRA reservoirs, the number of recorded species was found to increase from 7 to 10. With the increase in the number of species, a significant decrease, to 9%, in the share of *Babastella barbastellus* (dominating 2018) was observed. The opposite trend was observed in the case of *Pipistrellus pipistrellus*, whose share in the captured bat species increased from 7% to 28%. In addition, three species that were not found in 2018 (SEM reservoirs) appeared: *Eptesicus nilssonii*, *Vespertilio murinus*, and *Pipistrellus pygmaeus*. In 2020 (TRA reservoirs), the number of bats caught increased significantly, to 58 individuals. The number of species decreased from 10 to 9. No individuals belonging to the *Eptesicus nilssonii*, *Vespertilio murinus*, and *Plecotus auritus* species were caught, although they were present in 2019 (TRA reservoirs). The new species caught in the study area were *Eptesicus serotinus* and *Myotis nattereri*. In 2020 (reservoirs), three bat species dominated: *Nyctalus noctula*, *Pipistrellus pispistrellus*, and *Myotis daubentoniid.*

4. Discussion

The results of the inventory of three taxonomic groups of water reservoirs, using various comparative variants, indicate an impact of the construction of waterholes for European bison on the biodiversity of the forest ecosystem. Ecological effects are not always unambiguous, and often require explanation; this is presented below, broken down into individual taxonomic groups.

4.1. Dragonflies

The higher-than-average number of dragonfly species in European bison waterholes compared to natural reservoirs suggests that waterholes play a role in increasing local biodiversity in forest ecosystems. The differences in the number of recorded species and the diversity of ecological elements of the dragonfly fauna in the three groups of reservoirs are related to the habitat features of these reservoirs. The number of species found in the studied reservoirs seems to be relatively small, although further research would most likely allow for the detection of more species. Capturing a representative spectrum of adult odonate richness at a single site is dependent on the number of surveys [44]. The relatively small number of species may also be related to the small area of individual reservoirs, as the diversity of odonatofauna is positively correlated with the size of the habitat [45,46]. A higher average number of species in TRA reservoirs (transformed in 2018), compared to the waterholes for European bison (WEB), may indicate that their usefulness for the odonatofauna is decreasing over time.

The natural reservoirs with the lowest number of species, i.e., NAT1, NAT2, NAT3, and NAT4, were ephemeral or heavily overgrown, and also significantly shaded, which is associated with a cooler microclimate. Shading may directly limit the number of adult dragonflies [47], and also indirectly impacts the species richness of dragonflies [48]. The only species found in these four reservoirs were eurytopes, which are very common in Poland, e.g., *Coenagrion puella* [36]. Among the natural reservoirs, the NAT5 reservoir was distinguished by having the largest number of species and the greatest diversity of ecological elements of the odonatofauna; it was, of course, the most diverse habitat. Unlike most other natural reservoirs, it was exposed on one side, and at the same time, it was not overgrown to such an extent as, for example, reservoir NAT1. The nature of the NAT6 reservoir, including the presence of *Sphagnum* sp. and very numerous *Eriophorum* sp., allows us to assume that it may be a habitat for more thyrphophilic species than just the recorded *Leucorrhinia pectoralis*.

The waterholes for European bison (WEB) differ from -natural reservoirs, as there is less shading and/or a lower degree of overgrowth. The fewest species were recorded in the WEB4 reservoir, which may be related to its small area in comparison to the other reservoirs in this group. Most species were found in the well-insulated WEB5 reservoir, characterized by very little overgrowth by macrophytes.

Waterholes transformed for European bison in 2018 (TRA) do not seem to be significantly different from WEB reservoirs in terms of insulation and the degree of overgrowth. The most overgrown of this group of reservoirs, TRA4, shows even greater intensity of this feature than, for example, the WEB5 reservoir; fewer species were found in the former than in the latter. The TRA reservoirs constitute a more attractive habitat for pioneer species than the other two types of reservoirs, as for all of them, *Libellula depressa* was classified as an autochthonous or probably autochthonous species, with 54 exuviae of this species collected in one visit within the TRA1 reservoir. This is a large number, especially considering the small area of the reservoir. Although two pioneer species were found together in the WEB reservoirs, none were classified as autochthonous or probably autochthonous.

Leucorrhinia pectoralis was found in all TRA reservoirs, but only in two out of five WEB reservoirs. This result is comparable to natural reservoirs (NAT), where *Leucorrhinia pectoralis* was found in two of six surveyed reservoirs. The presented results confirm that anthropogenic waters are of great importance for this species [30], and waterholes are relatively valuable secondary habitats for tyrphiles. *Leucorrhinia pectoralis* is not threatened with extinction in Poland, but, rather, widely distributed and widespread [36]. However, it is considered an umbrella species, found in *Sphagnum* bogs and dystrophic reservoirs, for example, which are natural waterbodies bordered by *Sphagnum* and peat pools [49]. Therefore, the most valuable of the examined reservoirs should be considered as those within which the development of *Leucorrhinia pectoralis* was confirmed, i.e., TRA4, TRA3, TRA1, and WEB2. At the same time, these are the sites with the highest number of autochthonous and probably autochthonous species; they also have a relatively high number of all recorded species.

4.2. Amphibians

Despite the comparable number of amphibian species found in 2018 and 2020, in individual reservoirs, species diversity was lower in 2020. However, in the waterholes created/transformed for European bison, a larger number of individuals was found compared to in natural reservoirs. The lower number of amphibian species in 2020 may be due to at least two reasons. The first is the lack of inspection in early spring, a period during which there was the greatest chance of detecting species such as common frogs, moor frogs, and common toads. These species were found in large numbers in 2018 during the April visit in most of the monitored ponds. The second reason for the smaller number of amphibians were weather conditions, as the 2020 season was characterized by a relatively dry spring and summer, which could have impacted the durability of the water reservoirs (Figures S1–S10). Most of the natural reservoirs had a very low water level, and some were completely dry (NAT2, NAT3, and NAT4). Extremely dry and hot summers force amphibians to reduce their activity, which can have consequences on their food intake, resulting in lower levels of fat deposition than are needed for winter, as well as decreased reproduction and survival potential [50]. However, large fluctuations in amphibian populations occur naturally, and may depend on competition, predators, and hydrological and temperature conditions [51,52]. Amphibian populations can survive periods of drought when their breeding ponds dry up. However, this is only possible if there are other permanent ponds in the area. Therefore, it is very important that water reservoirs are not isolated and that they form a network of ponds. The persistence of high temperatures and low rainfall in the spring and summer period in subsequent years may contribute to the complete drying of ponds, and, consequently, to the disappearance of local amphibian populations [53]. This suggests the importance of waterholes in periods of low rainfall, as all of the studied waterholes held water and supported amphibian populations. This finding was contrary to the data from the natural reservoirs, which showed that only two out of six were inhabited by amphibians in 2020. The abundance of amphibian species in waterholes originally created for European bison may also be a result of the open habitats around them. Access to light increases water temperature more quickly; thus, the development of larvae is accelerated [54]. In addition, the gentle slopes of the waterholes provide better resting places for juvenile individuals, as they can easily leave the water habitat [55]. *Triturus cristatus* is protected under Annex II of the Habitats Directive, i.e., it is subject to a special form of protection in the EU Member States, as it is the fastest declining amphibian species in Europe [56,57]. It was found in seven reservoirs in 2018, of which six were waterholes for European bison, and in six reservoirs in 2020, of which four were waterholes. The great crested newt is a species with high environmental requirements [58], preferring deeper and larger (500–750 m^2) ponds with emergent and submergent aquatic vegetation, low numbers of breeding waterfowl, suitable terrestrial habitat within 0.5 km, and a network of ponds within a 1 km radius [59]. Its presence proves the importance of water reservoirs for European bison in maintaining the metapopulation of this species.

4.3. Bats

The obtained results confirm the impact, indicated above, of the reconstruction of reservoirs on biodiversity. Specifically, after the reservoirs were transformed into waterholes (SEM to TRA), the number of bat species and the number of individuals increased. For many bat species, water bodies are of primary importance [60]. They provide bats with both food and drinking water [61]. A general increase in the number of individuals and bat species does not mean that all species react in a similar way to the changes, which can clearly be seen in the example of *Babastella barbastellus.* The dominance of this species in SEM reservoirs indicates the proximity of its daytime roosts. It is a typical forest species that travels short distances in search of food [62,63] and usually chooses riparian vegetation and deciduous forests as foraging sites, where it preys on moths, which make up the bulk of its diet [62,64–66]. After the reservoirs were transformed into waterholes (TRA), the vegetation around the ponds was significantly reduced and the water surface, previously covered with duckweed, was cleaned. Such water reservoirs are often places where water insects appear, and *Barbastella barbastellus* does not hunt these species. The smooth water surface, which was created after the conversion of tanks and the related appearance of aquatic Diptera in 2019 and 2020, was probably the reason for the significant increase in the numbers of *Myotis daubentonii* and *Pipistrellus pispistrellus,* as they form the basis of these bats' diet. A slight increase in abundance was revealed in 2020 for *Nyctalus noctula*, which often hunts above the water surface [32,67–69].

Unfortunately, we do not know to what extent European bison use waterholes. The herd was observed in the vicinity of some reservoirs (Kolator, personal communication), but it is not known whether all of them are in use. It can be assumed that natural reservoirs are not often visited by European bison due to the often swampy ground around them and the dense cover with woody plants.

It is also difficult to assess the potential impact of European bison on the studied species. Previous studies have shown the negative effects of ungulates on amphibians or dragonflies, which were associated with trampling and consuming ground flora around reservoirs and changing water quality in reservoirs [70–73]. However, a negative effect was not observed for all species, and was usually due to the intensive use of reservoirs by ungulates. European bison in Augustowska Forest are characterized by a low-density population [21], which excludes the intensive use of water reservoirs. Moreover, the study outlined in [21] showed a positive effect of European bison on carabid beetles, which constitute food resources for some bat species [32,34]. In addition, a potential effect resulting from the impact of European bison on woody plants (inhibiting their development) can be observed, which may result in slowing of the succession and gradual shading of reservoirs.

5. Conclusions

Our study, based on three taxonomic groups, indicates that the construction of waterholes for European bison results in an increase in biodiversity. This was demonstrated in both the static comparison of built waterholes to natural and semi-natural reservoirs and the effect of transforming semi-natural waterholes into waterholes for European bison. The main advantage is the resistance of waterholes to drying out, which was demonstrated in dry years when even insufficient rainfall did not cause the waterhole to dry out. Frequent desiccation was found in natural reservoirs. In addition, the structure of the shores, which were shallower on at least one side, caused faster warming in the summer and growth of vegetation favorable for the development of amphibians. The surfaces of the reservoirs and their exposed surroundings were favorable for the development of insects (including dragonflies), which are a source of food for bats. For this reason, the vicinities around bison waterholes have become important feeding grounds for bats. The short period of research in this study did not allow for the assessment of the durability of these reservoirs; however, as has been shown in studies on amphibians, such waterholes present much greater species diversity than natural reservoirs a few years after construction.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15030446/s1, Figure S1. The chart of precipitation in Suwalki, 10.03.2018–30.06.2018 [74]; Figure S2. The chart of precipitation in Suwalki, 10.03.2019–30.06.2019; Figure S3. The chart of precipitation in Suwalki, 10.03.2020–30.06.2020; Figure S4. The chart of precipitation in Suwalki, 10.03.2020–30.06.2021; Figure S5. The chart of precipitation in Suwalki, 10.03.2022–30.06.2022; Figure S6. The chart of minimum and maximum temperature in Suwalki, 10.03.2022–30.06.2018; Figure S7. The chart of minimum and maximum temperature in Suwalki, 10.03.2022–30.06.2019; Figure S8. The chart of minimum and maximum temperature in Suwalki, 10.03.2020–30.06.2020; Figure S9. The chart of minimum and maximum temperature in Suwalki, 10.03.2021–30.06.2021; Figure S10. The chart of minimum and maximum temperature in Suwalki, 10.03.2022–30.06.2022; Table S1: Basic information on studied reservoirs; Table S2: List of Odonata taxa recorded; Table S3: List of Amphibians taxa recorded in 2018 and in 2020.

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