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NOTES ON THE PRESENCE OF *EREBIA ALBERGANUS* (DE PRUNNER 1879) (LEPIDOPTERA: NYMPHALIDAE) FOR KOSOVO

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Key words: Mokna Mt., Peklena Mt, survey, univoltine, Balkan Peninsula, Bjeshket e Nemuna.

Abstract

Erebia alberganus (De Prunner 1798) is European endemic and high mountain species of Nymphalidae butterfly family, with scarce and isolated records along the Balkan peninsula. Its presence for Kosovo was recently confirmed, thus the aim of this paper is to present this interesting discovery in the National park Bjeshket e Nemuna and highlight its distribution in the neighboring countries. During our surveys, five specimens of *E. alberganus* are observed in two locations: Mokna Mt. and Peklena Mt. Previous record of this species at the border of Kosovo and North Macedonia was not reliable, although recent data from North Macedonia suggest that the species could also be present in Sharr. Mt., in the southern part of the country. With this record, the number of butterfly species in the Republic of Kosovo riches 174. A population of *E. alberganus* at Peklena Mt. was destroyed by habitat alteration, which highlights the need for better conservation practices on one side and species vulnerability to such habitat changes on the other.

Introduction

Erebia alberganus is high mountain butterfly restricted to European continent. It belongs to the family Nymphalidae, the largest family of butterflies (Papilionoidea, former Rhopalocera), which includes 8 subfamilies. According to the European checklist of Butterflies, it is classified into the subfamily Satyrinae (WIEMERS et al. 2018). Based on this list, 57 species

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of *Erebia* have been recorded in Europe so far. *E. alberganus* is a univoltine butterfly that can be seen from June to late August in grasslands in the forest belt, to subalpine meadows, at an altitude from 900–2500 m. The larvae feed on *Anthoxanthum odoratum*, *Festuca ovine* and *Ochlopoa annua* (Tshikolovets 2011).

Being European endemic, *Erebia alberganus* is distributed in the Alps, in the northern mountains of Spain, in the Dolomites of Italy, in the southern Alps of Switzerland, Austria, central Italy, and France (van Swaay et al. 2010). It also has a fragmented distribution in the Balkan peninsula, in countries: Montenegro (Kudrna 2015, Franeta 2018, Jakšić and King 2021), Bulgaria (Abadjiev 2001, Hristova and Beshkov 2017), Serbia (Popović et al 2013, Popović and Verovnik 2018), in North Macedonia (Jakšić 1988, Melovski 2002, Abdija 2013). A recent publication on butterflies of Albania (Cuvelier et al. 2023) has confirmed the first record of *E. alberganus* near Valbonë in the North Albanian Alps (Bjeshket e Nemuna). As it spreads mainly at high altitudes, the risk of extinction is low, except in low altitude zones where it can be threatened by the human factor due to the destruction of its habitat. At European level, this species is not believed to face major threats (Van Sway et al. 2010).

The only previous mention of *E. alberganus* for Kosovo is from the maps of butterflies of Yugoslavia, with single point located at the border between Northern Macedonia and Kosovo (Jakšić 1988). We have recorded this species in Bjeshket e Nemuna National Park (63,028 ha), also known as Prokletine Mts., and located in the western and north-western territories of Kosovo. With recently confirmed presence of *E. alberganus* for Kosovo, we aim to present this interesting discovery and highlight its distribution in the neighboring countries.

Material and Methods

Study area

The Mountain massif "Bjeshket e Nemuna" (Prokletije) is located in the northwestern part of Kosovo, north of Albania, and the southeast of Montenegro. Bjeshket e Nemuna massif makes up the whole western border of the country. It belongs to Dinaric Alps or Dinarides, a great mountain range of south-eastern Europe. Due to the high natural and geomorphological values, with high biodiversity of flora and fauna and characterized by beautiful mountains, the mountain massif "Bjeshket e Nemuna" is declared a National Park in 2013. Moreover, the mountains are characterized by a large number of butterfly species, being also declared a Prime butterfly area (MESP 2015). Our research area included many locations in these mountains, including the high peaks above 1000 m whereas the localities where the species was recorded are located in the northern part of the Park, named Mokna Mt. and Peklena Mt. (Figure 1). Mokna Mt. is located on the Kosovo border with Serbia and Montenegro, at an altitude of 1640 m above sea level (42°53’14.73”N, 20°33’47.05”E) while Peklena Mt. at an altitude of 1300 m above sea level (42°40’30.56”N, 20°14’26.20” E). The vegetation in the National Park is very rich and diverse. Oak forests lie at an altitude from 300 m to 900 m, with the species *Quercus cerris, Quercus frainetto, Quercus pubescen,* and *Quercus petraea;* beech forest at altitude 800–1200 m, mixed *Picea* sp.*, Abies alba* and *Pinus heildreichii*, with the dominant species *Fagus moesaica*. Coniferous forests lie at an altitude of 2000–2200 m, with characteristic species *Pinus heildreichii, Pinus peuce*. At the lower limit of this zone, *Abies alba* and *Picea* sp. are encountered, while on the upper border lies *Pinus mugo, Juniperus nana,* and *Juniperus intermedia.* At the upper limit of the forest*,* lie Alpine and subalpine pastures (Rexhepi 1994, Millaku 1999).

Fig. 1. The map of Kosovo with the surveyed localities in the National Park Bjeshket e Nemuna *Source*: Valbon Bytyqi (2023).

The last data on the butterflies of Bjeshket e Nemuna date from the nineties (JAKŠIĆ 2006). Our survey is conducted after more than 20 years of gap in butterfly studies in this area and resulted in two new records for National Park Bjeshket e Nemuna and for the country. Apart from the record of *Erebia alberganus* which is the subject of this paper, the species *Heteropterus morpheus* (Pallas, 1771) is recorded for the first time in Kosovo (KABASHI KASTRATI et al. 2022). Regarding the species of the genus *Erebia*, until now 15 species have been recorded in an earlier survey in "Bjeshket e Nemuna" (JAKŠIĆ and ŽIVIĆ 1998, JAKŠIĆ 2006).

Sampling

In the period from 2019 to 2021, several field trips were conducted to the mentioned locations. The research was carried out on warm and sunny days, mainly in the middle of the day during the months June, July, and August. The butterflies were collected with entomological nets, the species were mainly identified in the field and released back into nature, but some specimens were identified in the laboratory of zoology, in the Department of Biology (Faculty of Mathematics and Natural Sciences in Pristina, Kosovo), collected specimens have been spread and stored in the entomological boxes. Species identification was done according to TOLMAN and LEWINGTON (2008) and TSHIKOLOVETS (2011).

Habitats

According to EUNIS classification, the habitat in Mokna Mt. where *E. alberganus* is recorded (20°14'26.74" N, 20°33'50.64" E) is *Abies and Picea* woodland (MILLAKU 1999). Southern European Norway spruce forests, outlying *Picea abies* formations of the Apennines, the southern Dinarides, the Balkan Range, and the Rhodopides, at the southern limit of the range of the species and mostly south of its continuous range (Figure 2*a* and Figure 2*b*).

Habitat and vegetation in Peklena Mt. where *E. alberganus* was collected (42°40'31.25" N, 20°14'12.10" E) is *Fagus* woodland (beech woodland). Forest dominated by beech species *Fagus orientalis* and other *Fagus* species in southeastern Europe and the Pontic region. Many montane formations are mixed beech-fir or beech-fir-spruce forests (EUNIS classification, 2020)

Fig. 2. Surveyed localities: *a*, *b* – Mokna Mt. 15 August 2021; *c*, *d* – Peklena Mt. 20 July 2019
Source: photos by E. Kabashi Kastrati

Results

Erebia alberganus (De Prunner 1798) was observed for the first time in the Peklena peak, on a hot and sunny day on July 20th 2019, on the roadside, near the bushes, where we found two specimens. After this period, the original habitat was destructed due to the work for the construction of a new mountain road, which has caused habitat fragmentation and the disappearance of the species from this location. On, July 21st 2020, *Erebia alberganus* was observed in Mokna at an altitude of 1640 m, where two specimens are registered. Another specimen was found in the same place on August 15th 2021. Thus, from this research, in three years of surveys we have a total of five specimens of *Erebia alberganus*.

Erebia alberganus differ from other *Erebias* in wings pattern and coloration. They have mainly dark brown wings and almond shapes with black spots and white highlights (Figure 3). All markings are prominent and the species is overall easy to identify (Lewington and Tolman, 2008).

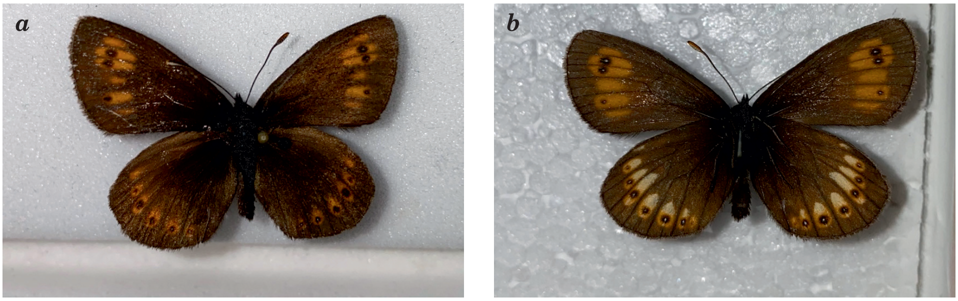

Fig 3. *Erebia alberganus*, male, Peklen, 20 July 2019, spread wings (dorsal view) and spread wings (ventral view)

Discussion

This paper describes the first record of *Erebia alberganus* for the National Park Bjeshket e Nemuna, and first reliable data from Kosovo. There were some dilemmas that *E. alberganus,* registered by JAKŠIĆ (1988) in Sharri Mt., in the border of Kosovo with North Macedonia, was a record from Kosovo's territory, but the same author didn't include *E. alberganus* in his following publications on butterflies in Kosovo and Metohija (JAKŠIĆ and ŽIVIĆ 1998), nor in the Red Data Book of Serbian Butterflies (JAKŠIĆ 2003). For this reasons, the species was not included in previous lists of butterfly species of Kosovo. Recent publication on Butterflies in Sharr Mt. have confirmed the presence of *E. alberganus* in North Macedonia (MELOVSKI 2002, ABDIJA 2013, KRPAČ et al. 2021). Being present at the Ljuboten peak located at the border between the two countries, its presence is also expected on Sharr mountain range in Kosovo and pending to be confirmed.

E. alberganus was recorded in the same mountain range (Bjeshket e Nemuna) in Montenegro (FRANETA 2018), more precisely in the locality "Čakor pass" which is less than 5 kilometers from the border with Kosovo and 15–20 km from the new locality where we have observed the species, therefore its occurrence in our country is expected. In Serbia, *E. alberganus* is regularly reported from Stara planina Mt. (JAKŠIĆ 1996, POPOVIĆ et al. 2013, LANGOUROV, 2019) and has been recently found in Mokra Gora, a small part of Bjeshket e Nemuna Mts. in the southwestern (POPOVIĆ et al. 2020). According to POPOVIĆ et al. (2013), this species has an extremely fragmented distribution in the mountains of the Balkan Peninsula.

During our three-year research period we managed to register only five specimens of *E. alberganus*. In Peklena Mt, the species was observed only in the first year of the survey, when two specimens were collected.

After the habitat destruction the species was not recorded in 2020 and 2021. Even though the surveyed area is in the National Park which is protected by the Law, and also proclaimed as a Prime butterfly area, the threats for this species are evident. On the contrary, in Mokna Mt. the species was recorded during each year of the survey and the risk of habitat alteration here was much lower due to higher altitude. In the European Red List of Butterflies, *Erebia alberganus* (De Prunner, 1798) is considered LC (Least Concern) (Van Sway et al. 2010). Apart from the habitat destruction, possible threats for this species in Kosovo are high number of visitors in National parks and other mountain areas, fires caused by human carelessness, construction of houses and hotel facilities and climate changes.

Conclusion

This research increased the number of *Erebia* species recorded for Kosovo and National Park "Bjeshkët e Nemuna". With this record, the number of butterfly species in the Republic of Kosovo is 174.

During the realization of our research, we were witnesses to the effects of anthropogenic impact on the population of *E. alberganus* and the disappearance of the species from its original habitat. It is evident that habitat destruction has a negative impact on species status, therefore, we consider that much more rigorous management measures are needed to protect not only butterfly species in the National Park, but also its overall biodiversity, which is its most valuable asset.

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CONDITIONING OF NORWAY SPRUCE (*PICEA ABIES* (L.) KARST.) SOWING MATERIAL

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Key words: semiconductor laser, Norway spruce, seeds, seedlings, bio-stimulation.

Abstract

Norway spruce (*Picea abies* (L.) Karst.) is a relatively demanding species (e.g. it does not tolerate drought, is very sensitive to environmental pollution, diseases and pests) compared to other conifers. It is therefore important to provide it with optimal growth conditions in the early stages of development. The laboratory and pot experiments were carried out on seed material of 10 different genotypes of Norway spruce bio-stimulated with semiconductor laser light. The following variants were used: control without irradiation (C) and multiplied dose variants ($0.25 \cdot 10^{-2}$ J/cm^2), three-fold (D3), six-fold (D6), and nine-fold (D9). Morphological features, fresh and dry weight of seedlings and the content of photosynthetically active pigments were assessed. Under the influence of the applied radiation, morphological features of seedlings were stimulated, and dose D6 turned out to be the most effective. It caused the stimulation of the tested features both in the experiment conducted in laboratory conditions and in a tunnel. The application of doses D3 and D9 caused an increase in chlorophyll content.

Introduction

Norway spruce (*Picea abies* (L.) Karst.) is an evergreen, fast-growing, erect tree species with many forms and varieties (MATĚJKA et al. 2014). It naturally grows in European forests from Scandinavia to Greece. In Central and southern Europe, the species naturally occurs mostly only in the mountains. Norway spruce moderately adapts to various climatic conditions. A growing season of at least 60 days and a minimum of 120 days of winter rest with freezing temperatures is necessary for it to develop properly (SVYSTUNA et al. 2021).

It has moderate soil requirements; it grows well on brown, fresh, or

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loam soils which are deep, moderately rich, not too acidic, and have an average depth of groundwater level (MURAT 2002, JAWORSKI 2011, MATĚJKA et al. 2014).

In Polish forests, the share of stands with a predominance of spruce is 5.6%. Spruce acts as an ecological admixture or acts as a second floor in stands in various habitats. Unfavorable changes occur in forests as a result of negative abiotic and biotic factors. In Europe, these changes include disturbances in the physiological processes of trees, such as flowering, fertility and quality of seeds obtained from forest tree seeds. The occurring changes in the genetic material result in a heritable mutation of some genotypes in individuals. Thus, a reduction in the quality of seeds and seedlings may be the effect of these changes (KORCZYK et al. 2010).

It seems that not only environmental contamination, but also long-term storage of seed material, e.g. in gene banks, also causes a quality drop associated with changes in their condition – aging – and therefore the seeds are subjected to various forms of refinement (COPELAND and MC DONALD 2001, KUBALA et al. 2013). In pre-sowing conditioning of seed materials, stratification is used (GRZESIK et al. 2012). Chemical methods are also used, e.g. treating dry, hard seeds with concentrated sulfuric acid (WOJCIECHOWSKA 1983).

In recent years, due to the global desire to reduce the excessive use of chemical compounds in plant production and the increasing acreage of plants cultivated using organic methods, in the improvement of seeds, other factors are used that have a beneficial effect on physiological processes (GRZESIK et al. 2012, HERNANDEZ-AGUILAR et al. 2016).

One of these factors is radiation, for example caused by a semiconductor laser (RYBIŃSKI AND GORCZYŃSKI 2004, KLIMONT 2006, SOLIMAN and HARITH 2010, GRZESIK et al. 2012). It is mainly used to stimulate cultivated plant growth (KLIMONT 2006). Seeds conditioning with laser rays positively influences germination processes, plant growth and development, as well as the size and quality of the obtained crop (DOBROWOLSKI and RÓŻANOWSKI 1998, HERNANDEZ-AGUILAR et al. 2016, SZAJSNER et al. 2017, KOSZELNIK- LESZEK et al. 2019, SZAJSNER et al. 2019).

Due to the lack of reports on the effects of laser radiation on seeds of forest trees (e.g. germination parameters and morphological characteristics of seedlings), an attempt was made to study the response of seed material of ten different genotypes of Norway spruce using semiconductor laser light under laboratory conditions and in a pot experiment.

The characteristics and origin of particular Norway spruce genotypes

Norway spruce seeds were obtained from Forest Gene Bank in Kostrzyca (Forest Reproductive Material 50°81′70″N 15°77′71″E) and were used for both the foil tunnel experiment (pot experiment) and the laboratory experiment. 10 spruce genotypes were selected (including A-11/ZP/12; B-106/ZP00; C-251/ZP04; D-251/ZP/04; E-262/ZP/07; F-470/ZP/04; G-514/ZP/03; H-567/ZP/03; J-986/ZP/06; K-1514ZP/00). Batches of seeds were collected during multiple years (genotypes: A – 2011, B – 2000, C – 2004, D – 2006, E – 1992, F – 2009, G – 2003, H – 2003, J – 2006, K – 2000).

The selected genotypes came from the following sites:

- Forest District Borki (A, D), 54°08′97″N – 21°91′22″E;
- Forest District Rokita (B, C), 53°76′76″N – 14°83′76″E;
- Forest District Nowy Targ (F, J), (49°69′95″N – 20°06′24″E;
- Forest District Śnieżka in Kowary (E), 50°79′06″N – 15°83′81″E;
- Forest District Wałbrzych (G), 50°73′57″N – 16°21′25″E;
- Forest District Ujsoły (K, H), 49°47′35″N – 19°11′78″E.

The propagating material used in the experiment belongs to the selected or qualified category (1999/105EC EU directive seed categories) and was of indigenous origin. The purity of the seed material used in the experiment varies as follows: 96% – H, 96.2% – J, 97% B, 97.2% – G, 97.4% – C, 98% – D, 98.3% – E, 99% – K, 99.9% – A, 100% – G; the average moisture content of all seeds was 5,3% (KORCZYK et al. 2010).

Laboratory experiment. The laboratory experiment was carried out in a SANYO environmental chamber, type MLR-351H. Norway spruce seeds were treated with semiconductor laser irradiation, type CTL 1106MX (with a power of 200 mW and a wavelength of 670 nm), working with a CTL-1202 S scanner. Three-(D3), six-(D6) and nine-fold (D9) doses were applied, with the basic dosage of $0.25 \cdot 10^{-2}$ J/cm^2. The duration of individual exposure was 4.1 min. The control group C(D0) consisted of seeds without any dosages.

Control and treated seeds laser light were placed in cuvettes lined with filter paper soaked in water distilled. The experiment was set up in three repetitions, 30 seeds per repetition. Energy and germination capacity was tested respectively 7 and 14 days after sowing (accordance with the methodology proposed by ISTA (2008). Moreover, the length of the radicle, hypocotyl and aboveground part of seedlings (stem) were measured. The content of fresh and dry weight of seedlings was also assessed.

Pot experiment. The experiment with sowing material of Norway spruce treated with laser radiation (see laboratory experiment) was also conducted also in pots grown under a cover (foil tunnel). The substrate in pots was peatmoss with NPK fertilizer. After four weeks, the following seedling parameters were assessed: the number and length of roots, the height of seedlings and the content of fresh weight of roots and above-ground parts of plants. Moreover, photosynthetically active pigments were tested in plant material obtained after eight weeks – chlorophyll a, b, and total. Chlorophyll was tested using spectrophotometry by measuring the absorbance of the prepared filtrates at a wavelength of 663 (chlorophyll *A*), 645 (chlorophyll *B*), 652 (chlorophyll *A* + *B*) and 765 for polyphenols (LICHTENTHALER 1987).

Statistics. The results of the research were statistically evaluated using variance analysis for a two-factor experiment, with the use STATISTICA 13.1 by Stat Soft Polska. The significance of differences was calculated at the level of $\alpha = 0.05$ using the Duncan test.

Results and Discussion

Laboratory experiment

The statistical analysis of data on energy and germination capacity for all tested ganotypes showed no significant influence of laser radiation on these features.

This is most likely due to the fact that the seed material used in the experiment was of high quality – they belonged to the selected or qualified categories (KALINIEWICZ et al. 2011).

The studied morphological features of the seedlings: the length of radicle and hypocotyl, the length of the stem and the fresh weight of the seedlings showed a significant stimulating effect of the applied semiconductor laser radiation. The length of the embryonic root increased by 14.79% in relation to the roots of control plants after six- and nine-fold seed irradiation (Table 1). The length of hypocotyl showed stimulation under the influence of any applied doses of laser radiation – by 7.8% for dose D6; respectively 5.4% for D3 and 5% for nine-fold exposure (Table 1). In the case of stem length, the value of this feature was increased only through dose D6 – over 7.5%. The fresh weight of the seedlings grown from the irradiated seeds, in relation to the control seedlings, was higher by 16.5% and by 15%, respectively, after the application of the D6 and D9 doses. For the dry matter of seedlings, no effect of pre-sowing treatment of seeds with laser radiation was found (Table 1).

Table 1

Laboratory experiment: Average values and homogenous groups for tested features of Norway spruce

Dose	Length of the radicle [mm]	Length of the hypocotyl [mm]	Length of the stem [mm]	Fresh weight [g]
C(D0)	25.15^{b}	29.76^{b}	39.23^{b}	0.206^{b}
D3	26.46^{b}	31.38^{a}	$40.90^{a,b}$	$0.229^{a,b}$
D6	28.87^{a}	32.08^{a}	42.19^{a}	0.240^{a}
D9	28.87^{a}	31.26^{a}	$40.69^{a,b}$	0.237^{a}

The performed statistical analysis showed an interaction of the studied genotypes with the applied doses of laser radiation for all examined morphological features and for both the fresh and dry weight of seedlings. The length of the embryonic root increased after all doses in genotypes D, J and K. Doses D3 and D9 induced stimulation in genotypes C. Six-fold seed exposure stimulated the root length in four out of ten tested genotypes: A, E, F and G.

The reduction in length was observed twice: in B under the influence of doses D3 and D6, and in H for doses D3 and D9. Very diverse reactions to pre-sowing laser irradiation were observed for the length of hypocotyl. Under the influence of all applied doses, genotype F showed stimulation, while genotype B showed a reduction in the value of this feature. Stimulation was also observed in form A (dose D6), form C (doses D3 and D9), and form E – D9. In genotype H there was a reduction after application of doses D3 and D9.

Four of the studied genotypes (D, G, J and K) showed no significant response to the treatment of seeds with semiconductor laser radiation. The interaction obtained for the length of the stem with the needles showed a significant stimulation in genotype F under the influence of all applied doses, in genotypes C and G under the influence of three-fold irradiation, in genotypes E – dose D9, K – dose D6. Three of the tested forms (genotype A, D, J) did not respond to pre-sowing seed irradiation. Stem length reduction occurred in form B after all doses, and in form H after doses D3 and D9 (Table 2). The results obtained in the studies conducted by SZAJSNER et al. (2017) on sugar beet (*Beta vulgaris*) showed that a magnetic field and laser radiation modify the process of cluster germination. Moreover, they extend the germinal root and stimulate pigment condensation in seedlings

For fresh weight of seedlings, a response to irradiation was observed in six out of ten tested genotypes. Three of them – form C, F and G – were characterized by an increase in the content of fresh weight of seedlings after applying three irradiation dosages, in relation to the control plants. Genotype E reacted to dose D9; genotype K to dose D6. No reaction was observed in forms A, B, D and J. Reduction in fresh weight of seedlings occurred only in genotype H under the influence of three-fold irradiation. The dry matter content of seedlings obtained from irradiated seeds was reduced only in the F genotype under the influence of D3 and D9 doses. The remaining forms did not show any reaction to the applied laser radiation.

Table 2

Laboratory experiment: Average values and homogenous groups for tested features of Norway spruce – interaction between genotype and does

Genotype	Dose	Length of the radicle [mm]	Length of the hypocotyl [mm]	Length of the stem [mm]	Fresh weight [g]	Dry weight [g]
1	2	3	4	5	6	7
A	C(D0)	$25,53^{b}$	34.30^{b}	47.20^{a}	0.25^{a}	0.037^{a}
	D3	25.70^{b}	$38.17^{a,b}$	51.2^{a}	0.22^{a}	0.041^{a}
	D6	31.97^{a}	41.60^{a}	52.93^{a}	0.28^{a}	0.041^{a}
	D9	25.87^{b}	$38.70^{a,b}$	49.37^{a}	0.25^{a}	0.043^{a}
B	C(D0)	28.40^{a}	32.53^{a}	42.97^{a}	0.23^{a}	0.040^{a}
	D3	21.13^{b}	21.2^{b}	28.20^{b}	0.19^{a}	0.050^{a}
	D6	21.37^{b}	25.17^{b}	34.17^{b}	0.21^{a}	0.040^{a}
	D9	$24.30^{a,b}$	23.47^{b}	33.80^{b}	0.22^{a}	0.050^{a}
C	C(D0)	23.27^{c}	29.20^{b}	33.57^{b}	0.19^{b}	$0.040^{a, b}$
	D3	27.93^{b}	37.77^{a}	41.77^{a}	0.28^{a}	0.050^{a}
	D6	$25.73^{b,c}$	31.20^{b}	33.57^{b}	0.24^{b}	0.030^{b}
	D9	34.90^{a}	38.00^{a}	$39.50^{a. b}$	$0.26^{a,b}$	0.030^{b}
D	C(D0)	19.03^{b}	30.93^{a}	41.93^{a}	0.20^{a}	0.030^{a}
	D3	25.47^{a}	33.10^{a}	44.10^{a}	0.21^{a}	0.030^{a}
	D6	24.0^{a}	32.63^{a}	44.70^{a}	0.21^{a}	0.040^{a}
	D9	25.77^{a}	33.37^{a}	44.43^{a}	0.22^{a}	0.040^{a}
E	C(D0)	26.63^{c}	26.27^{b}	36.23^{b}	0.17^{b}	0.040^{a}
	D3	24.23^{c}	$27.57^{b,a}$	35.63^{b}	$0.20^{a,b}$	0.040^{a}
	D6	31.73^{b}	$29.3^{b,a}$	$40.93^{a, b}$	$0.19^{a,b}$	0.040^{a}
	D9	36.37^{a}	31.63^{a}	43.43^{a}	0.22^{a}	0.040^{a}
F	C(D0)	21.50^{b}	17.83^{c}	24.07^{c}	0.16^{b}	0.050^{a}
	D3	28.63^{b}	30.47^{a}	41.77^{a}	0.23^{a}	0.030^{b}
	D6	33.13^{a}	32.2^{a}	42.47^{a}	0.14^{b}	0.040^{a}
	D9	24.53^{b}	25.27^{b}	33.27^{b}	$0.20^{a, b}$	0.030^{b}

cont. Table 2

1	2	3	4	5	6	7
G	C(D0)	27.07^{b}	$36.47^{a,b}$	47.50^{b}	0.28^{b}	0.040^{a}
	D3	$30.77^{a,b}$	40.20^{a}	55.73^{a}	0.33^{a}	0.040^{a}
	D6	32.5^{a}	35.23^{b}	47.00^{b}	0.28^{b}	0.040^{a}
	D9	26.93^{b}	33.10^{b}	44.83^{b}	0.28^{b}	0.050^{a}
H	C(D0)	28.43^{a}	30.43^{a}	38.77^{a}	0.28^{a}	0.040^{a}
	D3	18.83^{c}	20.93^{b}	26.67^{c}	0.18^{b}	0.050^{a}
	D6	$26.00^{a,b}$	28.97^{a}	39.50^{a}	0.26^{a}	0.040^{a}
	D9	$22.83^{b,c}$	23.23^{b}	32.17^{b}	0.24^{a}	0.050^{a}
J	C(D0)	22.13^{c}	29.97^{a}	$40.60^{a,\ b}$	0.22^{a}	0.050^{a}
	D3	27.47^{b}	30.80^{a}		0.21^{a}	0.040^{a}
	D6	$28.17^{a,\ b}$	30.70^{a}	$41.13^{a,\ b}$	0.25^{a}	0.040
	D9	32.60^{a}	34.40^{a}	45.77^{a}	0.24^{a}	0.050^{a}
K	C(D0)	29.53^{b}	29.70^{a}	39.41^{b}	0.22^{b}	0.050^{a}
	D3	34.47^{a}	33.53^{a}	$44.10^{a,\ b}$	$0.25^{a,\ b}$	0.050^{a}
	D6	34.10^{a}	33.70^{a}	45.37^{a}	0.27^{a}	0.050^{a}
	D9	34.63^{a}	31.43^{a}	$40.33^{a,\ b}$	$0.26^{a,\ b}$	0.050^{a}

Pot experiment

In the experiment conducted in foil tunnels, like in the laboratory experiment, the D6 dose turned out to be the most effective. It stimulated four out of five examined morphological features of spruce seedlings. The length of the root increased by 15.9%, the height of the seedling by 29.3%, the weight of the above-ground part of the seedling by 26.7%, and the weight of the roots by 28.6%. The stimulation of seedling height was caused by the D9 dose and the increase in seedling weight by the D3 dose. The only feature reduced under the influence of laser radiation was the number of roots – on average from 3.84 PCs to 2.54 PCs (Table 3).

Table 3

Pot experiment: Average values and homogenous groups for tested features of Norway spruce

Dose	Height of the seedling [mm]	Root numbers [pcs]	Root length [mm]	Weight of the above-ground part of the seedlings [g]	Root weight [g]
C(D0)	21.26^{b}	3.84^{a}	74.02^{b}	0.15^{b}	0.05^{b}
D3	$21.18^{a,\ b}$	$2.78^{b,\ c}$	71.36^{b}	0.19^{a}	0.04^{b}
D6	27.48^{a}	3.12^{b}	85.8^{a}	0.19^{a}	0.07^{a}
D9	26.12^{a}	2.54^{c}	72.64^{b}	0.15^{b}	0.04^{b}

The performed statistical analysis showed a diversified reaction of the studied spruce genotypes to the applied doses of laser radiation (Table 4). Only dose D6 stimulated the height of the seedling in 4 out of 10 tested spruce genotypes. They were as follows: genotype C, F, H and K (by 38.90%; 53.79%; 115.38% and 53.33%, respectively). Genotype H deserves special attention – its elongation was over 115%. In genotype G, a reduction in the value of this feature was observed after the use of any of the three doses of laser radiation, and in genotype B under the influence of the strongest dose – nine-fold irradiation. The remaining genotypes (A, D, J) did not show any reaction to the applied doses of radiation.

The other applied doses of laser radiation did not produce any effect. Genotype G again turned out to be a form sensitive to any dose of laser radiation used in the experiment. This time, the number of roots was stimulated. The greatest effect was obtained with the dose D9 (from 2.0 to 4.0). The number of roots also increased in genotype A after irradiation with the D6 dose (from 3.6 to 7.0); while in genotype F, under the influence of six- and nine-fold irradiation, from 1.8 to 3.6 for D6 and 2.6 for D9. Genotype B responded by reducing the number of roots to irradiation with any dose. The lowest dose (D3) reduced the number of roots in forms E and F while the highest dose (D9) – in forms A, D and K. The remaining genotypes – C and J – did not show any reaction to pre-sowing irradiation.

The increase in the fresh weight of the above-ground part of the seedlings was achieved in the C form – by 35.30%, the F form – by 42.9%. and the H form – by 50%; in all cases after the D6 dose. The G and K genotypes responded with a reduction in seedling fresh weight to the application of any of the three doses of laser light. The other genotypes showed no significant effect of seed irradiation. An increase in root weight by 57% was obtained with the D6 dose only in genotype A. Genotypes B, D and K showed a reduction in root mass. while genotypes C, E, G, H and J did not respond to pre-sowing irradiation.

Table 4

Pot experiment: Average values and homogenous groups for tested features of Norway spruce – interactions

Genotype	Dose	Height of the seedling [mm]	Root numbers [pcs]	Weight of the above-ground part of the seedlings [g]	Root weight [g]
1	2	3	4	5	6
A	C(D0)	24.0^{a}	3.6^{b}	$0.20^{a,b}$	0.07^{b}
	D3	16.0^{a}	2.8^{b}	0.11^{b}	$0.05^{b,c}$
	D6	23.0^{a}	7.0^{a}	0.21^{a}	0.11^{a}
	D9	21.0^{a}	3.0^{b}	0.15^{b}	0.03^{c}

cont. Table 4

1	2	3	4	5	6
B	C(D0)	23.4[a]	7.4[a]	0.20[a]	0.12[a]
	D3	21.0[a,b]	3.8[b]	0.15[a,b]	0.04[b]
	D6	28.0[a]	2.8[b,c]	0.20[a]	0.04[b]
	D9	13.0[b]	1.6[c]	0.10[b]	0.03[b]
C	C(D0)	26.2[b]	3.0[a]	0.17[b]	0.04[a]
	D3	26.8[b]	2.2[a]	0.16[b]	0.04[a]
	D6	36.4[a]	2.2[a]	0.23[a]	0.04[a]
	D9	20.6[b]	2.6[a]	0.12[b]	0.03[a]
D	C(D0)	20.0[a]	3.8[a]	0.13[a]	0.10[a]
	D3	19.0[a]	4.2[a]	0.16[a]	0.04[b]
	D6	19.0[a]	3.4[a]	0.13[a]	0.05[b]
	D9	25.2[a]	1.4[b]	0.13[a]	0.03[b]
E	C(D0)	18.6[a]	2.8[a]	0.14[a]	0.04[a]
	D3	24.6[a]	1.6[b]	0.15[a]	0.04[a]
	D6	23.0[a]	2.8[a]	0.14[a]	0.03[a]
	D9	21.8[a]	3.6[a]	0.14[a]	0.04[a]
F	C(D0)	26.4[b]	1.8[b]	0.14[b]	0.05[a]
	D3	17.6[b]	2.0[b]	0.18[a,b]	0.02[b]
	D6	40.6[a]	3.6[a]	0.20[a]	0.03[a,b]
	D9	20.8[b]	2.6[a]	0.16[a,b]	0.03[a,b]
G	C(D0)	44.8[a]	2.0[b]	0.311[a]	0.07[a]
	D3	30.0	3.2[a]	0.20[b]	0.05[a]
	D6	22.2[b]	3.0[a]	0.20[b]	0.07[a]
	D9	25.0[b]	4.0[a]	0.19[b]	0.06[a]
H	C(D0)	15.6[b]	3.2[a,b]	0.16[b]	0.05[a]
	D3	22.0[b]	3.8[a]	0.17[b]	0.06[a]
	D6	33.6[a]	2.6[a,b]	0.24[a]	0.05[a]
	D9	24.8[a,b]	2.0[b]	0.17[b]	0.04[a]
J	C(D0)	30.0[a]	2.8[a]	0.18[a]	0.05[a]
	D3	2 0.8[a]	2.6[a]	0.15[a]	0.03[a]
	D6	28.0[a]	3.4[a]	0.19[a]	0.03[a]
	D9	24.4[a]	3.2[a]	0.18[a]	0.06[a]
K	C(D0)	21.0[b]	3.0[a]	0.25[a]	0.07[a]
	D3	14.0[b]	1.6[a,b]	0.13[b,c]	0.04[b]
	D6	32.2[a]	2.4[a]	0.17[b]	0.03[b]
	D9	16.0[b]	1.4[b]	0.11[c]	0.02[b]

Chlorophyll A and chlorophyll B are the main photosynthetic pigments. Both absorb light, but chlorophyll A plays a unique and key role in the conversion of light energy into chemical energy (BICZAK et al. 2016). When examining the effect of the applied doses of laser radiation on spruce seeds, a significant stimulating effect on the content of chlorophyll in the obtained seedlings was found. Both three- and nine-fold irradiation increased the content of chlorophyll A, B and total (dose D3 by 26.7%. 13.33% and 22.80%, respectively; and dose D9 by 24.47%. 15.86% and 21.94%, respectively). Six-fold irradiation caused an increase only in the content of chlorophyll A (by 10.13%) see Table 5. Statistical analysis of the obtained results showed the interaction of genotype x applied laser light dose for chlorophyll A, B, and total. Under the influence of pre-sowing irradiation. five of the studied spruce genotypes produced seedlings with significantly increased chlorophyll content and under the influence of any of the three applied doses. These were genotypes B, C, G, H and K. Forms B and C showed the highest content under the influence of dose D9 (by 40.5% and 44.80%, respectively), while the lowest dose (D3) increased the content of chlorophyll A in genotypes G (by 33.40%), H (by 20.60%), and K (by 45%).

Table 5

Pot experiment: Contents of chlorophyll A, chlorophyll B and total chlorophyll in Norway spruce seedlings [mg g^{-1} F.W.]

Dose	Chlorophyll A	Chlorophyll B	Total chlorophyll
C(D0)	1.046^{c}	0.435^{b}	1.481^{b}
D3	1.326^{a}	0.493^{a}	1.819^{a}
D6	1.152^{b}	0.440^{b}	1.592^{b}
D9	1.302^{a}	0.504^{a}	1.806^{a}

Genotypes A, F reacted to doses D3 and D9 with stimulation, while dose D6 caused a reduction in chlorophyll A content in three tested genotypes – A, D, and E. The content of chlorophyll B was stimulated in genotypes A, C, H after three- and nine-fold irradiation. dose D3 caused an increase in the content of this pigment in forms D and K, while D9 – in F, G and J. Genotype B turned out to be the most sensitive and reacted to all applied doses of laser light with a significant stimulation of chlorophyll B content. The reduction was observed in genotype E (doses D6 and D9). For total chlorophyll, the best effects were achieved in genotypes B, C, G, H – stimulation after all applied doses. D3 increased the content of this pigment in forms A, D, E and K, while dose D9 in forms A, F and J. The reduction occurred only in two cases – in forms A and E under the influence of dose D6 (Table 6). In studies conducted on other species. it was found that short-term exposure to pre-sowing electromagnetic radiation of amaranth seeds affects the germination energy, but not the content of photosynthetic pigment (DZIWULSKA-HUNEK et al. 2013). Higher chlorophyll content and leaf surface area under the influence of a magnetic field were observed by EŞITKEN and TURAN (2004), whereas SZAJSNER et al. (2017). in their research on sugar beet pre-sowing stimulation, found that a magnetic field and laser radiation modify the sprouting process and stimulate pigment condensation in seedlings. SACAŁA et al. (2012) also observed a significant stimulating effect of laser radiation on the content of chlorophyll and carotenoids in the study on sugar beet as a result of five- and seven-fold irradiation of the clusters with semiconductor laser rays.

Table 6

Pot experiment: Contents of chlorophyll A, chlorophyll B and total chlorophyll in Norway spruce seedlings for interaction genotype x dose – average values and homogenous group

Genotype	Dose	Chlorophyll A	Chlorophyll B	Total chlorophyll
1	2	3	4	5
A	C(D0)	0.879^{c}	0.324^{b}	1.203^{b}
	D3	1.015^{b}	0.406^{a}	1.421^{a}
	D6	0.721^{d}	0.284^{b}	1.005^{c}
	D9	1.046^{a}	0.407^{a}	1.453^{a}
B	C(D0)	0.945^{d}	0.351^{c}	1.296^{c}
	D3	1.094^{c}	$0.455^{a,b}$	1.549^{b}
	D6	1.144^{b}	0.436^{b}	1.580^{b}
	D9	1.328^{a}	0.501^{a}	1.829^{a}
C	C(D0)	0.872^{c}	0.327^{c}	1.199^{c}
	D3	1.117^{b}	0.416^{b}	1.533^{b}
	D6	1.043^{b}	$0.384^{b,c}$	1.427^{b}
	D9	1.263^{a}	0.503^{a}	1.766^{a}
D	C(D0)	$1.365^{a,b}$	0.491^{b}	1.856^{b}
	D3	1.427^{a}	0.581^{a}	2.008^{a}
	D6	1.254^{c}	0.508^{b}	1.762^{b}
	D9	1.344^{b}	$0.526^{a,b}$	1.870^{b}
E	C(D0)	1.246^{b}	0.494^{a}	1.740^{b}
	D3	1.363^{a}	0.532^{a}	1.895^{a}
	D6	1.098^{c}	0.407^{b}	1.505^{c}
	D9	1.212^{b}	0.462^{b}	1.674^{b}
F	C(D0)	1.122^{c}	0.434^{b}	1.556^{b}
	D3	1.207^{b}	$0.447^{a,b}$	1.654^{b}
	D6	$1.145^{b,c}$	0.432^{b}	1.577^{b}
	D9	1.316^{a}	0.502^{a}	1.818^{a}

cont. Table 6

1	2	3	4	5
G	C(D0)	1.112^{d}	0.441^{b}	1.553^{c}
	D3	1.483^{a}	0.441^{b}	1.924^{a}
	D6	1.225^{c}	0.497^{a}	1.722^{b}
	D9	1.370^{b}	0.529^{a}	1.899^{a}
H	C(D0)	1.114^{c}	0.456^{b}	1.570^{c}
	D3	1.344^{a}	0.519^{a}	1.863^{a}
	D6	1.213^{b}	0.493^{a,b}	1.706^{b}
	D9	1.313^{a}	0.494^{a}	1.807^{a,b}
J	C(D0)	1.331^{b}	0.504^{b}	1.835^{b}
	D3	1.373^{b}	0.531^{a,b}	1.904^{b}
	D6	1.320^{b}	0.538^{a,b}	1.858^{b}
	D9	1.477^{a}	0.581^{a}	2.058^{a}
K	C(D0)	1.267^{c}	0.531^{b}	1.798^{b}
	D3	1.837^{a}	0.603^{a}	2.440^{a}
	D6	1.354^{b}	0.421^{c}	1.775^{b}
	D9	1.353^{b}	0.530^{b}	1.883^{b}

Conclusions

1. The response of the studied genotypes of Norway spruce to pre-sowing seed irradiation was varied and depended on both the genotype and the applied doses of laser radiation.

2. In laboratory conditions, all examined morphological features of the seedlings showed a positive effect of stimulation.

3. The genotypes C, E and F turned out to be the most susceptible to laser irradiation in laboratory conditions and showed stimulation of all examined characteristics.

4. In the pot experiment, the examined features such as seedling height, root length or seedling and root weight showed a significant stimulation effect of laser radiation. Only the number of roots was reduced.

5. The D6 dose turned out to be the most effective, inducing the stimulation of the examined features both in the laboratory and in a pot experiment.

6. The application of doses D3 and D9 of laser radiation for pre-sowing spruce seed bio-stimulation increased the content of chlorophyll in seedlings, which may increase the intensity of photosynthesis and thus plant biomass.

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THE DIVERSITY OF SPHINGIDAE (INSECTA: LEPIDOPTERA) IN KORITNIKU MOUNTAIN, THE REPUBLIC OF KOSOVA

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Abstract

Moths of the family Sphingidae are large and usually easily recognizable moths, that remain not sufficiently surveyed in some parts of Europe, including the Republic of Kosovo. From 2020 to 2022, Sphingidae diversity was surveyed in Kosovo's part of mountain Koritniku, near the border with Albania. The moths were collected with light traps in the period April-October in each year of the survey. In total 12 Sphingidae species were recorded, which compose about 30% of European fauna. Of those, 7 have been recorded in Kosovo for the first time: *Daphnis nerii* (Linnaeus, 1758), *Deilephila elpenor* (Linnaeus, 1758), *Hyles livornica* (Esper, 1780), *Acherontia atropos* (Linnaeus, 1758), *Laothoe populi* (Linnaeus, 1758), *Mimas tiliae* (Linnaeus, 1758) and *Smerinthus ocellata* (Linnaeus, 1758). The recorded number of Sphingidae species in our research is only a modest contribution to a better knowledge of the moth fauna of Kosovo, but still represents an update in comparison with historical records. Considering the number of species of this family in neighbouring countries, we can suppose that the number of recorded species is not a final one and further research is needed.

Introduction

Family Sphingidae Latreille, 1802 (hawk moths) is a moth family, represented by more than 1450 species in all continents, except Antarctica (VAN NIEUKERKEN et al. 2011). The number of species in Europe registered so far is 40 (KRPAČ et al. 2019, DE JONG et al. 2014).

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They are among the largest Lepidoptera, with a strong robust bodies, fast fliers, and with a long proboscis. A lot of species of hawk moths are very important pollinators. Long proboscis in many species enables them to feed with nectar in plants with long flowers, whereas the moths with shorter proboscises can't access the flowers' nectar (CALDAS and ROBINS 2003).

Most hawk moth species fly at night, with exception of a few species, like the genus *Hemaris,* that fly during the day (POWEL 2009).

The caterpillars of hawk moths are usually easily recognizable due to their large size and the curved horn on the eighth abdominal segment (LERAUT 2006). Many Sphingidae species are migratory and may occur far from their habitats, and can fly distances between the continents (LERAUT 2006).

This family has never been systematically studied in the Republic of Kosovo, and the existing records are mostly historical and few (REBEL and ZERNY 1931, VASIĆ et al. 1978, DOROVIĆ 1979, JAKŠIĆ 1986). Even some of the most common species have not been recorded in the country, indicating the need for additional surveys. Therefore, this research aimed to gain insight into the diversity of Sphingidae of Koritnik Mountain and Kosovo itself.

Material and Methods

Koritniku Mt. is located in the south of the Republic of Kosovo and stretches along the state border with Albania. The highest part of the mountain, above 1470 m, with an area of 818 ha, is designated as a strict nature-protected area. The reserve represents the area of high limestone mountains, characterized by special geomorphological and biodiversity values. The special feature of this mountain is the presence of the largest area (nearly 2000 ha) of Heldreich's pine forest (*Pinus heldreichii*) in the Balkans (REXHEPI 1994).

Moths were collected with a 6 W 12 V Portable Heath Moth Trap and a portable trap consisting of a single 20 W black light bulb, in the period April-October in each year of the survey 2020 and 2022. All the collected specimens were transported to the Laboratory of Zoology at the Department of Biology, FMNS, University of Prishtina. In the lab, specimens were fixed in entomological pins, labelled, and preserved. The taxonomy is based on PITTAWAY (1993).

This survey was done on two main localities. The given habitat types according to EUNIS, with geographic coordinates and altitude:

– Koritniku: Koritniku Mt., 835 m, Agriculturally improved, re-seeded, and heavily fertilized grassland, in an area of oak forest (*Quercus frainetto* woods 9280), 42°09'24" N, 20°39'21" E, light trap, obs. PB, Figure 1 and Figure 2;

– Koritniku: Koritniku Mt., 1245 m, Fagu's woodland (41.1 Beech forests), 42°04'52" N, 20°36'20" E, light trap, obs. PB, Figure 1 and Figure 3.

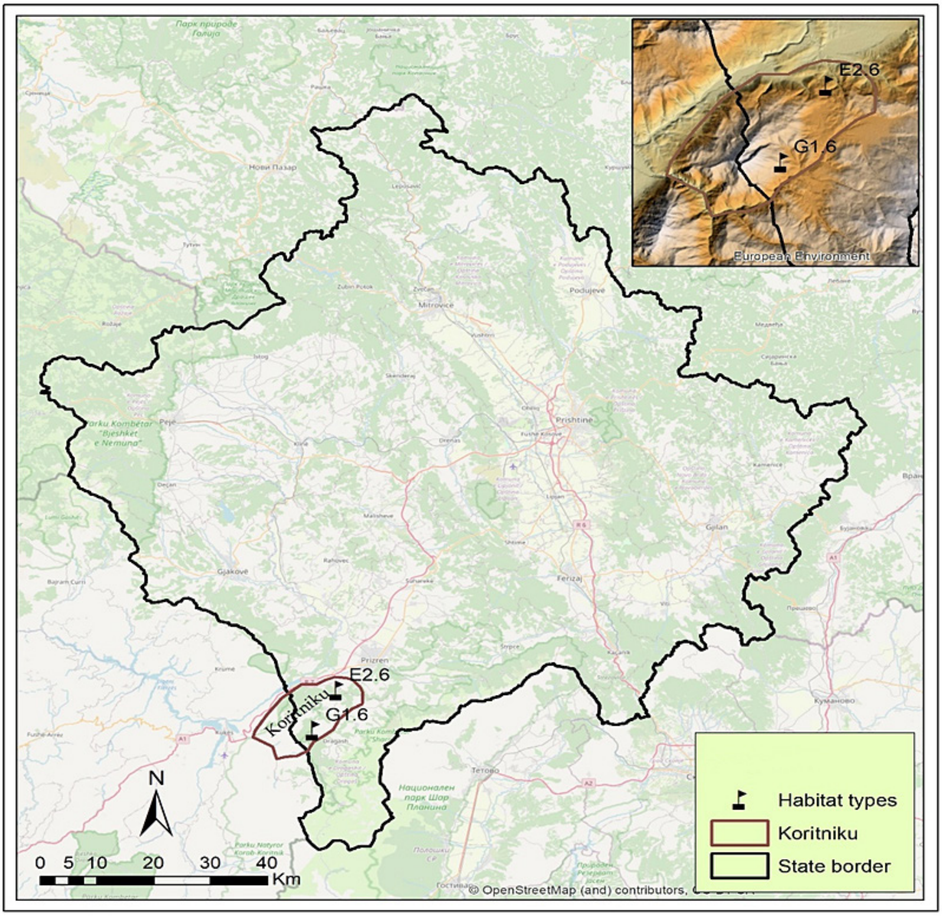

Fig. 1. The Map of Kosovo with the two surveyed localities on Mt. Koritniku

Fig. 2. The first habitat of the hawk-moths survey in Koritniku Mt.
Source: photo by Pajtim Bytyçi in Zhur, 10. June 2022

Fig. 3. The second habitat of the hawk-moths survey in Koritniku Mt.
Source: photo by Pajtim Bytyçi in Rapçë, 10. May 2022

Results and Discussion

Family Sphingidae

During this survey, a total of 76 Sphingidae specimens were collected at Mt. Koritniku. From them, 12 species were identified, six belonging to the subfamily Macroglossinae, four to the subfamily Smerinthinae and two species to Sphinginae. For each species, all the available literature records for Kosovo are presented, as well as their global distribution and comments.

The subfamily Macroglossinae

1. *Macroglossum stellatarum* (Linnaeus, 1758).

Global distribution. *Macroglossum stellatarum* (Linnaeus, 1758) is a common moth species found across the Holarctic region (Cuadrado 2017).

Distribution in the region. It is known from Albania (Rebel and Zerny 1931), North Macedonia (Rebel and Zerny 1931, Daniel 1964, Krpač et al. 2019), Montenegro (Rebel and Zerny 1931), Serbia (Vasić et al. 1978, Habiprot 2022), Bulgaria (Hristova and Beshkov 2017), Bosnia and Herzegovina (Lelo 2004), Croatia (Koren 2018), Slovenia (Carnelutti 1992), Greece (Fritsch et al. 2014), Romania (Székely 2010).

Literature records: Zhleb (Rebel & Zerny 1931).

New records: Koritnik, 26. July 2021.

2. *Daphnis nerii* (Linnaeus, 1758).

Global distribution. *Daphnis nerii* is a large hawk-moth found in wide areas of Africa and eastwards as far as Middle-East, and southern Europe where it ventures north, sometimes as far as Scandinavia and Finland, and also Ireland and England (Goater 1974, Leraut 2006). Extra-limital range – to south-east Asia and the Philippines; Hawaii, the islands of Saipan and Guam as well as Chichijima, Japan (*Daphnis nerii.* 2023).

Distribution in the region. It is known from Albania (Rebel and Zerny 1931, Beshkov and Nahirnić-Beshkova 2021), North Macedonia (Daniel 1964, Krpač et al. 2019), Serbia (Vasić et al. 1978, Habiprot 2022), Croatia (Koren 2020), Montenegro (Rebel and Zerny 1931), Bulgaria (Hristova and Beshkov 2017), Bosnia and Herzegovina (Lelo 2004), Slovenia (Carnelutti 1992), Greece (Fritsch et al. 2014), Romania (Székely 2010).

New records. Koritnik, 19. June. 2021. No published records of this species in Kosovo were found, but some of the records from Krpač et al. (2019) may refer also to Kosovo.

3. *Hyles euphorbiae* (Linnaeus, 1758).

Global distribution. it is distributed from Europe to China, in Europe it is distributed from Spain to the Balkans (Leraut 2006).

Distribution in the region. It is reported from Albania (Rebel and Zerny 1931, Beshkov and Abadjiev 1996), Montenegro (Rebel and Zerny 1931), North Macedonia (Daniel 1964, Krpač et al. 2019), Serbia (Vasić et al. 1978, Habiprot 2022), Bulgaria (Hristova and Beshkov 2017), Bosnia and Herzegovina (Lelo 2004), Croatia (Koren and Ladavac 2013), Slovenia (Carnelutti 1992), Greece (Weídlich 2016), Romania (Székely 2010).

New records: Koritnik, 27. May 2021.

Literature records: Novosellë (Rebel and Zerny 1931).

4. *Hyles livornica* (Esper, 1780).

Global distribution. This species is found in Africa, and from Europe to India and Japan (Leraut 2006).

Distribution in the region. It is reported from Albania (Beshkov and Abadjiev 1996), North Macedonia (Daniel 1964, Krpač et al. 2019), Bulgaria Hristova and Beshkov 2017), Bosnia and Herzegovina (Lelo 2004), Croatia (Koren 2020), Greece (Hassler et al. 1988, Fritsch et al. 2014), Romania (Székely 2010).

New records: Koritnik, 05. August 2022.

Comments. New species for Kosovo. No published records of this species in Kosovo were found, but some of the data in research by Krpač et al. (2019) may refer also to Kosovo.

5. *Deilephila elpenor* (Linnaeus, 1758).

Global distribution. It is a species distributed throughout the Euroasiatic distribution, not presented in N Africa, from Europe to Japan (Bestmann et al. 1992, Leraut 2006), it has also been found in Canada and the USA (northern Washington State (Waring 2017).

Distribution in the region. It is reported from Albania (Beshkov et al. 1996), North Macedonia (Daniel 1964, Krpač et al. 2019), Serbia (Vasić et al. 1978, Habiprot 2022), Bulgaria (Hristova and Beshkov 2017), Bosnia and Herzegovina (Lelo 2004), Croatia (Koren 2018), Slovenia (Carnelutti 1992), Romania (Székely 2010).

New records: Koritnik, 26. April 2022, 18. May 2021.

Comments. New species for Kosovo. No published records of this species in Kosovo were found, but some of the data in research by KRPAČ et al. (2019) may refer also to Kosovo.

6. *Deilephila porcellus* (Linnaeus, 1758):

Global distribution. It is distributed from Europe to Central and Eastern Asia (Amurland) as well as North Africa (LERAUT 2006).

Distribution in the region. It is reported from Albania (EICHLER and FRIESE 1965, BESHKOV et al. 2020b), Serbia (VASIĆ et al. 1978, HABIPROT 2022), Bulgaria (HRISTOVA and BESHKOV 2017), Bosnia and Herzegovina (LELO 2004), Croatia (VIGNJEVIĆ et al. 2010, KOREN 2018), Slovenia (CARNELUTTI 1992), Romania (SZÉKELY 2010).

Literature records: Pejë (REBEL and ZERNY 1931).

New records: Koritniku, 20. May 2021.

The subfamily Sphinginae

1. *Agrius convolvuli* (Linnaeus, 1758).

Global distribution. It is distributed throughout Europe, Asia, Africa, Australi, Indonezia and New Zealand (LERAUT 2006, BUTLER 1879).

Distribution in the region. It is reported from Albania (REBEL and ZERNY 1931), North Macedonia (DANIEL 1964, KRPAČ et al. 2019), Serbia (VASIĆ et al. 1978, HABIPROT 2022), Bulgaria (HRISTOVA and BESHKOV 2017), Bosnia and Herzegovina (LELO 2004), Croatia (VIGNJEVIĆ et al. 2010), Slovenia (CARNELUTTI 1992), Greece (HASSLER et al. 1988), Romania (SZÉKELY 2010).

Literature records: Novosellë (REBEL and ZERNY 1931).

New records: Koritnik, 20. June 2020, Koritnik, 23. August 2021.

2. *Acherontia atropos* (Linnaeus, 1758).

Global distribution. This is an Afrotropical species distributed from Northern Africa, the Mediterranean regions of Europe (with migrating specimens found all across Europe) to the Middle East (to Iran, Turkmenistan and Kazakhstan) (PITTAWAY 1993).

Distribution in the region. It is reported from Albania (BESHKOV et al. 2020), North Macedonia (DANIEL 1964, KRPAČ et al. 2019), Serbia (VASIĆ et al. 1978, HABIPROT 2022), Bulgaria (HRISTOVA and BESHKOV 2017), Bosnia and Herzegovina (LELO 2004), Croatia (KOREN and LADAVAC 2013), Slovenia (CARNELUTTI 1992), Greece (HASSLER et al. 1988), Romania (SZÉKELY 2010).

New records: Koritnik, 28. May 2021.

Comments. New species for Kosovo. No published records of this species in Kosovo were found, but some of the data in research by Krpač et al. (2019) may refer also to Kosovo.

The subfamily Smerinthinae

1. *Marumba quercus* (Denis & Schiffermüller, 1775).

Global distribution. It is widespread and is found in southern Europe, the Middle East, North Africa, and Mesopotamia (Pittaway 2018).

Distribution in the region. It is reported from Albania (Rebel and Zerny 1931), Montenegro (Rebel and Zerny 1931), North Macedonia (Daniel 1964, Krpač et al. 2019), Serbia (Vasić et al. 1978, Habiprot 2022), Bulgaria (Hristova and Beshkov 2017), Bosnia and Herzegovina (Lelo 2004), Croatia (Koren 2020), Slovenia (Carnelutti 1992), Greece (Hassler et al. 1988), Romania (Székely 2010).

New records: Koritnik, 10. May 2021.

Literature records: Biraç (Dorović 1979).

2. *Mimas tiliae* (Linnaeus, 1758).

Global distribution. This is a Euroasiatic species, distributed from Spain to Finland, Asia Minor, Iran and Mongolia and China to the East (Danner et al. 1998, Pérez et al. 2009).

Distribution in the region. It is reported from Albania (Beshkov et al. 2020), North Macedonia (Daniel 1964, Krpač et al. 2019), Serbia (Vasić et al. 1978, Habiprot 2022), Bulgaria (Hristova and Beshkov 2017), Bosnia and Herzegovina (Lelo 2004), Croatia (Vignjević et al. 2010, Koren 2018), Slovenia (Carnelutti 1992), Greece (Koutsaftikis 1970), Romania (Székely 2010).

New records: Koritnik, 26. June 2022.

Comments. New species for Kosovo. No published records of this species in Kosovo were found, but some of the data in research by Krpač et al. (2019) may refer also to Kosovo.

3. *Smerinthus ocellata* (Linnaeus, 1758).

Global distribution. Has spread throughout Europe (with the exception of much of Scandinavia and the Baltic Republics) (Leraut 2006), it is also found in the east through Russia, and to eastern Kazakhstan and the Altai (Danner et al. 1998), North Africa and Middle-East (Leraut 2006).

Distribution in the region. Albania (Beshkov et al. 2020a), North Macedonia (Daniel 1964, Krpač et al. 2019), Serbia (Vasić et al. 1978, HABIPROT 2022), Bulgaria (HRISTOVA and BESHKOV 2017), Bosnia and Herzegovina (LELO 2004), Slovenia (CARNELUTTI 1992), Croatia (VIGNJEVIĆ et al. 2010, KOREN 2018), Greece (HASSLER et al. 1988), Romania (SŹEKELY 2010).

New records: Koritnik, 26. June 2022.

Comments. New species for Kosovo. No published records of this species in Kosovo were found, but some of the data in research by KRPAČ et al. (2019) may refer also to Kosovo.

4. *Laothoe populi* (Linnaeus, 1758):

Global distribution. It occurs throughout Europe to Central Asia (PITTAWAY *1993).*

Distribution in the region. It is reported from Albania (EICHLER and FRIESE 1965), North Macedonia (DANIEL 1964, KRPAČ et al. 2019), Serbia (VASIĆ et al. 1978, HABIPROT 2022), Bulgaria (HRISTOVA and BESHKOV 2017), Bosnia and Herzegovina (LELO 2004), Croatia (VIGNJEVIĆ et al. 2010, KOREN 2018), Slovenia (CARNELUTTI 1992), Greece (FRITSCH et al. 2014), Romania (SZÉKELY 2010).

New records: Koritnik, 26. June 2022.

Comments. New species for Kosovo. No published records of this species in Kosovo were found, but some of the data in research by KRPAČ et al. (2019) may refer also to Kosovo.

During our survey, 12 Sphingidae species have been recorded on Mt. Koritnik, and of those seven have been recorded in Kosovo for the first time. Before our survey, only seventh species were reported: *Macroglossum stellatarum* (Linnaeus, 1758), *Hyles euphorbiae* (Linnaeus, 1758), *Deilephila porcellus* (Linnaeus, 1758), *Agrius convolvuli* (Linnaeus, 1758), *Marumba quercus* (Denis & Schiffermüller, 1775), *Hemaris tityus* (Linnaeus, 1758), and *Hyles vespertilio* (Esper, 1780) (REBEL 1913, REBEL and ZERNY 1931, DOROVIĆ 1979, JAKŠIĆ 1986). Based on our results and literature data, 14 species of Sphingidae moths are recorded in Kosovo so far, however considering the data from the neighbouring countries, this number will for sure increase with additional surveys in the region.

Regarding the number of Sphingidae species in other countries in the Balkans and region, 20 species are known from Albania (REBEL 1913, REBEL and ZERNY 1931, BESHKOV and ABADJIEV 1996, BESHKOV et al. 1996, BESHKOV 1998, BESHKOV and NAHIRNIĆ 2019a, 2019b, BESHKOV et al. 2020a, 2020b), 25 species are registered in North Macedonia (KRPAČ et al. 2019), 20 species in Serbia (VASIĆ 1978, KOREN 2022), 20 in Bosnia and Hercegovina (LELO 2004, KOREN 2022), 21 species are recorded in Slovenia (Carnelutti 1992), 22 in Croatia (Koren 2018, 2020, Koren et al. 2022), and 26 species in Bulgaria (Hristova and Beshkov 2017).

With additional surveys, 9 species can be expected in Kosovo: *Sphinx ligustri* (L.), *Hyloicus pinastri* (Linnaeus, 1758), *Hemaris fuciformis* (Linnaeus, 1758), *Hemaris croatica* (Esper, 1800), *Proserpinus proserpina* (Pallas, 1772), *Rethera komarovi* (Christoph, 1885), *Hyles nicaea* (de Prunner 1798), *Hyles gallii* (Rottemburg, 1780), *Hippotion celerio* (Linnaeus, 1758).

We can conclude that our research contributed to the expansion of the list of moth species in Kosovo with additional seven species from this research. It should be noted that though most of these species are common in the Balkans, due to the lack of surveys they were not recorded earlier.

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VALUATION OF CARBON STOCK IN TREE FOR SMALL COMMUNITY FOREST: CASE STUDY BAN KHUM COMMUNITY FOREST, UTTARADIT PROVINCE, THAILAND

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Key words: carbon offset, carbon stock, dry dipterocarp forest, voluntary market, community forest.

Abstract

This study aimed to assess the tree species and density, biomass, carbon stocks, and the value of carbon stocks in a small community forest in Thailand. The research estimated the biomass above and below ground, carbon stocks, carbon dioxide absorption, and the value of carbon stocks using the European Union Allowance (EUA) and US California Carbon Market exchange rates. The conserved forest zone had 14 different tree species and a density of 618 trees per ha, with a total biomass of 68.130 tonnes, 3.204 tonnes of carbon stored, and 91.74 tonnes of carbon dioxide absorbed. The carbon stock value was calculated at € 1,478.08 and $ 965.66, respectively. In contrast, the utilized forest zone had 7 tree species and a density of 46 trees per ha, with a total biomass of 1.584 tonnes, which was equivalent to 1.584 tonnes of carbon stored or 4.50 tonnes of carbon dioxide absorbed. The value of carbon stock in this zone was € 39.78 and $ 60.90, respectively. The total value of carbon stock at the Ban Khum community forest was $ 2,853.14, assuming a 6% annual interest rate and a carbon trading price of $ 50/tonne CO_2.

Introduction

The impact of population growth on natural resources and the environment, particularly forest resources, is immense. As the population increases, forest areas are being encroached upon for agriculture, and illegal logging is also rampant in Thailand and other places. The situation is exacerbated by the rising carbon dioxide emissions from the industrial and transportation sectors, which further deplete forest resources. According to data from the Royal Forest Department of Thailand, the forested area of the country was 31.58% in 2016, and it has remained close to 31% in

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recent years, despite the shrinking forest area, which is opposite to the trend of increasing population (Ounkerd et al. 2015). As the population grows, the demand for resources increases in many areas, such as housing and subsistence agriculture, leading to greater forest encroachment and the need for wood, paper, furniture, energy, and other products and services. This is particularly challenging in light of climate change.

The accumulation of greenhouse gases is the primary cause of global climate change, which results in increased global temperatures. Greenhouse gases are usually responsible for regulating temperatures since the world would be too cold without them, and living things would not be able to survive (Surin 2008). However, greenhouse gases are increasing in the atmosphere, partially due to deforestation. Forests play a crucial role in moderating the amount of carbon dioxide in the atmosphere since trees use carbon dioxide to create oxygen. Carbon dioxide is taken out of the atmosphere through photosynthesis and stored in the form of biomass (Timilsina et al. 2014). However, when forest resources are depleted, the storage of carbon dioxide is also reduced, leading to an increase in severe natural disasters. According to predictions by the IPCC (2007), the average temperature of the earth's surface will rise by approximately 1.1–6.4°C between 1990 and 2100, resulting in a sea level rise of 1.5–95 centimeters (with moderate predictions of 50 centimeters) and an increase in severe storms. Trees contain 47% carbon by dry weight (IPCC 2006), making it necessary to reduce deforestation and conserve existing forested areas, especially through community-based forest management and other forms of forestry, to reduce the amount of carbon dioxide in the atmosphere.

The voluntary market in particular has had an important role in forestry sector that allows carbon to be voluntarily trades as carbon credits called Verified Emission Reduction (VER) or Carbon Offset in order to trade (Issarapap and Jaruntorn, 2019) in key markets such as US California Carbon Market (CCA), European Union Allowance (EUA), Climate Registry (CR) and bilateral trading between buyers and development project (Over-the-Counter: OTC). Sales contracts can be made between carbon trading organizations and farmers or departments during any participating period by calculating the carbon in soil (soil offset projects) in agricultural lands (Ignosh et al. 2009).

Community forestry in Thailand is not formally recognized by legislation, yet over 8,300 community forests covering an area of approximately 500,000 hectares have been registered with the Royal Forest Department (RFD) of the Ministry of Natural Resources and Environment (MONRE), with another 3,500 community forests in the process of registration. However, the issue of the illegality of community forestry in national parks, reserves, and sanctuaries is a matter of debate in Thailand and affects approximately 2 million people who rely on forest resources in those areas. Forests in Thailand have become an important source of carbon dioxide absorption and a potential carbon stock.

The report presented the advantages of selling carbon credits for agricultural operators and landowners. The public sector has already implemented credit programs in Thailand's community forests in 2019. To showcase the income potential through carbon trading using the US California Carbon Market (CCA) and European Union Allowance (EUA), the study evaluated the revenue generated from the carbon stock in community forests and soil organic carbon throughout the project's duration.

Materials and Methods

The study area

The study area is located at Uttaradit province, northern Thailand (Figure 1), covering approximately 300 hectares. The area is flat, hills, and the soil is sandy loam with laterite. The local climates were tropical and subtropical with three distinctive seasons – summer, rainy and winter, with an average annual rainfall of 1,400 mm. The different ecotypes surrounding the study area rage from mixed deciduous forest and dry dipterocarp forest. Geographic coordinates at 100.027 E, 17.641 N.

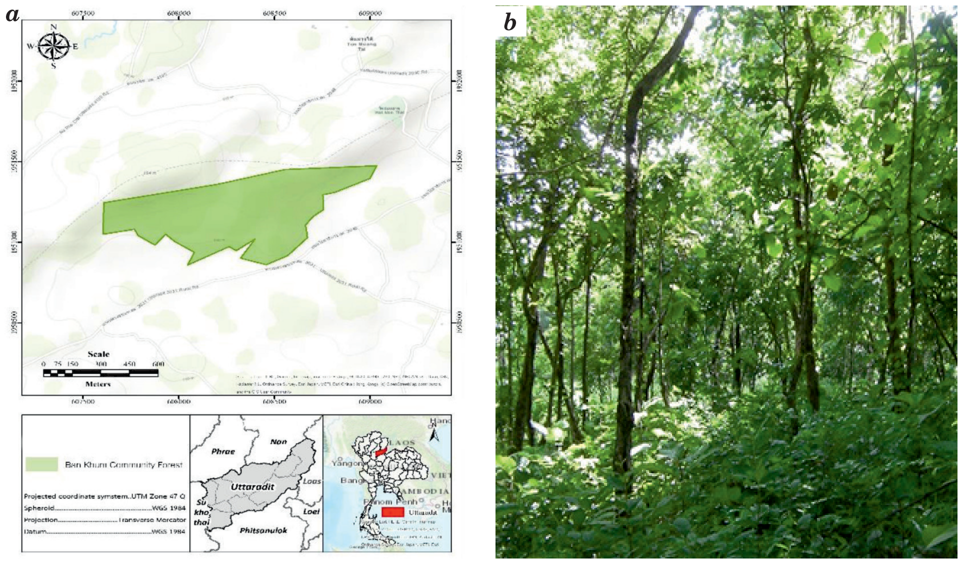


Fig. 1. Study area Ban khum community forest located at Chai Chumphon Subdistrict, Laplae district, Uttaradit province, Thailand: *a* – map of study area; *b* – characteristics of the community forest area used in the research

Study design and methods

Stratified random Sampling in two zone were conserved forest zone and utilized zone. The conserved forest zone mentioned in the previous text refers to an area of the forest that is strictly protected and where the use of forest products is prohibited. On the other hand, the utilized zone mentioned in the text is an area of the forest where some level of utilization of forest products is allowed, such as for subsistence agriculture or small-scale timber extraction. It is important to note that the degree of utilization allowed in the utilized zone may vary depending on the regulations and management practices in place in each specific community forest.

The data was collected from permanent plots in 40 × 40 m^2 quadrate that divided into 25 sub plots of 10 × 10 m^2. A vegetation census was used to collect data on forest structure and species composition. Trees in these plots were recorded all trees DBH > 4.5 cm in each plot of 10 × 10 m^2, with five random subplots of 4 × 4 m^2 within this plot selected for the recording of all tree DBH ≤ 4.5 cm (sapling). Finally, all tree heights ≥ 1.30 m was measured in five randomly located plots of 1 × 1 m, while all trees in five plots of 4 × 4 m was recorded for trees DBH ≤ 4.5 cm and height < 1.30 m (seedling). Analysis gives precise measures of the floristic composition, species density, basal area and ecological characteristics.

1. Each plot's tree stems, branches, and leaves were measured for their above-ground biomass. The aboveground biomass of dipterocarp forest was estimated by equations (1–4) (OGINO et al. 1964):

$$\mathrm{Logw_S} = 2.50913 \log D - 0.94402 \tag{1}$$

$$\mathrm{Logw_B} = 1.81022 \log D - 1.98034 \tag{2}$$

$$\mathrm{Logw_L} = 1.81022 \log D - 1.41128 \tag{3}$$

$$\text{and } W_T = W_S + W_B + W_L \tag{4}$$

where:

D – the diameter at breast height [cm]
W_S – the dry weight mass of stem [kg]
W_B – the dry weight mass of branch [kg]
W_L – the dry weight mass of leaf [kg]
W_T – the total dry weight mass of stem and branch [kg].

2. The below ground biomass was estimated by root/shoot ratio biomass, IPCC (2006) have determined the ratio between below ground biomass to above ground biomass equal to 0.28, can be found as in equation (5):

$$B_b = 0.28\ W_t \tag{5}$$

where:

B_b – below ground biomass [kg]
W_t – above ground biomass [kg].

3. C-stock in aboveground biomass was calculated based on IPCC 2006 by multiplying the 0.47 conversion factor to the biomass (McGroddy et al. 2004).

4. Equation (6) is used to determine the amount of carbon dioxide absorbed from the atmosphere by trees during photosynthesis. This is done by calculating the amount of carbon accumulated in the trees, as they absorb carbon dioxide from the atmosphere for photosynthesis. The carbon content of the trees is then multiplied by a factor of 3.667, as per the guidelines provided by the Intergovernmental Panel on Climate Change (IPCC 2006).

$$\text{Amount of carbon dioxide absorbed in tree} = \text{C-stock} \cdot 3.667 \tag{6}$$

5. The carbon sequestration valuation is calculated from the annual incremental rate of carbon sequestration multiplied by the price of carbon in each market traded. The annual increment of carbon sequestration can be calculated from the annual increase of biomass of Ban Khum community forest by using the rate according to the study in the dipterocarp community forest (Sunthornhao et al. 2013, Ounkerd et al. 2015) at 4.52 percent per rai per year. This study, with a study duration of 5 years, assuming that Khao Wong community forests are growing at an accelerated rate, as can be seen in Equation (7):

$$B_t = 1.0452\ t\ B_0 \tag{7}$$

where:

B_t = biomass carbon at t year [kg ha^{-1}]

B_0 – biomass carbon at current year [kg ha^{-1}]

t – time (year) is 1, 2, 3… 5.

Calculate carbon stock values over the next 5 years using multiple market prices from the Thailand Greenhouse Gas Management Organization Public Organization's weekly carbon trading report (2019), including voluntary carbon markets such as the US California Carbon Market; CCAs price. Buying and selling carbon as of 18 September 2019 is equal to 17.62 US dollars/ton of Carbon Dioxide or 538.12 baht/ton of Carbon dioxide. The average exchange rate used during Round 3 at the end of 2019, with an exchange rate of 1 US dollar at an average of 30.54 baht. Official EU market, European Union Allowance (EUA) market, as of September 18, 2019 equals 26.97. Euro/ton carbon dioxide or 911.05 baht/ton carbon dioxide using the average exchange rate during the 3rd quarter of the year 2019, which the exchange rate of 1 Euro is averaged 33.78 baht (Bank of Thailand 2019), as well as compared with the assumptions in order to study the sensitivity of the trading in 3 different levels, 500, 750 and 1,000 baht/ton of carbon dioxide, and compared at the interest rates of 4, 6, 8 and 10%, can be found as in Equation (8) Sunthornhao et al. (2013), Ounkerd et al. (2015):

$$V_t = V_0 \cdot (1.0r)\ t \tag{8}$$

where:

V_t – valuation of carbon stock at t year [baht/ha]
V_0 – valuation of carbon stock at current year [baht/ha]
r – interest rate equal to 4,6,8 and 10
t – times (year) is 1, 2, 3... 5.

Results and Discussion

Community composition and ecological status of Ban Khum community forest

The selected Ban Khum community forest showed variability in various forest structural attributes such as density, diversity, species riches, and total basal cover.

Table 1

Geographical coordination of the study area of Ban Khum community forest

Name	Location	Altitudes [m asl]	Forest types	Dominant species
Ban-Khum Lab-Lare district, Uttaradit province, Thailand	100.027 E 17.641 N	320	dry dipterocarp forest	Dipterocarpus obtusifolius

* MDF is mixed deciduous forest

The findings demonstrate that all research plots have approximately as many species as other forest areas (Table 2). The MEF and DEF's soils had more moisture than the other woods at the other site, most likely. Moisture levels are an important factor controlling the species composition of each forest (PONGUMPAI 1976, GLUMPHABUTR et al. 2006). The number of species depends on soil moisture in the forest, and it will increase as soil moisture content increases from dry dipterocarp forest to mixed deciduous forest, and the dry evergreen forest and hill evergreen forest to the moist evergreen forest, respectively (OGAWA et al. 1965). Compared to other forests in Thailand, for example Khao Kaset Forest area (KHOPAI 2006), Khun Korn Waterfall Forest Park, Thailand (NUKOOL 2002) and Thung Salaeng Luang National Park (CHATTANONG 2013).

Tree density

The community forest had a lower density of trees with DBH ≥ 4.5 cm compared to other forests such as the dry dipterocarp forest, mainly due to the presence of numerous small trees. Table 2 provides a comparison of the tree density in the study plots with that of other forest types located in different parts of Thailand. The tree density in all study plots was relatively high, particularly in the Ban Khum community forest, which had a comparable density to that of other mixed deciduous forests.

Table 2

Number of species, tree density and basal area of community forest and other mixed deciduous forests in Thailand, only trees with DBH ≥ 4.5 cm

Land use	Forest types	Area	Number of species [sp. ha^{-1}]	Tree density [tree ha^{-1}]	Basel area [%]	Source
Community forest	MDF	Ban-Khum Community Forest	21	664	1.351	present study
Natural forest	MDF	Thung Salaeng Luang National Park	35	2,205	–	PODONG et al. (2013)
Natural forest	MDF	Khao Kaset Forest area	33	959	–	KHOPHAI (2006)
Natural forest	MDF	Khun Korn Waterfall Forest park	62	358	0.358	NUKOOL (2002)

Tree biomass

The study conducted a biomass assessment of trees, which was divided into two categories: above ground biomass, comprising the stem, branches, and leaves, and below ground biomass, comprising the roots. The results revealed that the Ban Khum community forest area had an average biomass content of 1.577±0.498 tonne ha^{-1} divide into stem, branches leaves and root sections of 0.401±0.127 tonne ha^{-1}, 0.400±0.127 tonne ha^{-1}, 0.401±0.127 tonne ha^{-1} and 0.345±0.109 tonne ha^{-1}, respectively. Total biomass was 71.496 tonne, with conserved forest zone having the highest biomass content average 2.271±0.678 tonne ha^{-1} divide into stem, branches leaves and root sections of 0.430±0.134 tonne ha^{-1}, 0.400±0.127 tonne ha^{-1}, 0.401±0.127 tonne ha^{-1} and 0.345±0.109 tonne ha^{-1}.The forest utilized zone had the least amount of biomass average 0.187±0.077 tonne ha^{-1} divide into stem, branches leaves and root sections of 0.540±0.021 tonne ha^{-1}, 0.400±0.127 tonne ha^{-1}, 0.401±0.127 tonne ha^{-1} and 0.345±0.109 tonne ha^{-1}, respectively (Table 2). However, there is a difference in biomass between conserved forest zone and utilized forests zone statistically significant ($p \leq 0.05$). This tree biomass distribution was found in the study showed that the biomass distribution was in the 0.003–1.492 tonne ha^{-1} (Figure 2).

Table 2

Tree biomass of Ban Khum community forest, Uttaradit province, Thailand

Types of forest zone	Area [ha]	Biomass [tonne ha^{-1}]					Total Biomass [ton]
		stems	branches	leaves	roots	total	
Conserved forest zone	30	0.430±0.134	0.400±0.127	0.401±0.127	0.345±0.109	2.271±0.678	68.130
Utilized forest zone	18	0.540±0.021	0.047±0.019	0.046±0.019	0.041±0.017	0.187±0.077	3.366
Mean	–	0.401±0.127	0.400±0.127	0.401±0.127	0.345±0.109	1.577±0.498	–
Total	48	–	–	–	–	–	71.496

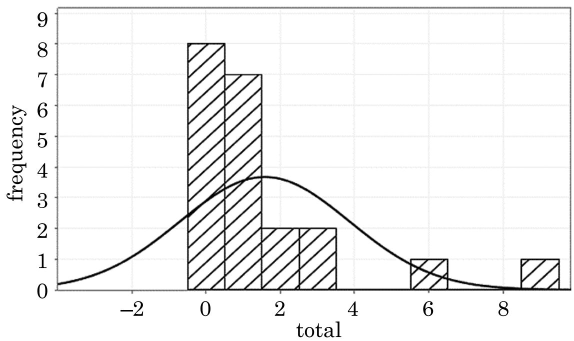


Fig. 2. Histogram with normal curve of total biomass Ban Khum community forest, Uttaradit province, Thailand

Carbon stock

Carbon stock in tree biomass showed that the Ban Khum community forest area had an average carbon stock of 0.741±0.234 tonne C ha^{-1}, divide into stem, branches leaves and root sections of 0.202±0.060 tonne C ha^{-1}, 0.198±0.060 tonne C ha^{-1}, 0.401±0.127 tonne C ha^{-1}, respectively. Total carbon stock was 4.788 tonne C ha^{-1}, with conserved forest zone having the highest biomass content average 1.068±0.319 tonne C ha^{-1} divide into stem, branches leaves and root sections of 0.291±0.086 tonne C ha^{-1}, 0.272±0.082 tonne C ha^{-1}, 0.174±0.649 tonne C ha^{-1} and 0.230±0.070 tonne C ha^{-1}, respectively. The forest utilized zone had the least amount of biomass average 0.088±0.036 tonne C ha^{-1}divide into stem, branches leaves and root sections of 0.025±0.009 tonne C ha^{-1}, 0.047±0.019 tons C ha^{-1}, 0.047±0.020 tonne C ha^{-1} and 0.019±0.010 tonne C ha^{-1}, respectively (Table 3). The Ban Khum community forest slightly higher biomass (1.577 tonne ha^{-1}) and carbon stock (0.741 tonne C ha^{-1}) than other deciduous forests in Thailand, such as Thong Pha Phum National Forest (TERAKUNPISUT et al. 2007), western Thailand (CHIYO et al. 2011) and lower northern Thailand (KAEWKROM et al. 2011). Net primary production in a tropical forest is ~11–21 tonne ha^{-1} with 25–65% contributed from leaf litter (BROWN and LUGO 1982). Though the turnover time of biomass is approximately 34 years, the turnover time of litter is much shorter, < 1 year (BROWN and LUGO 1982) – Table 4.

Table 3

Total carbon stock of Ban Khum community forest, Uttaradit province, Thailand

Types of forest zone	Area [ha]	Carbon stock [tonne C ha^{-1}]					Total biomass [tonne C]
		stems	branches	leaves	roots	total	
Conserved forest zone	30	0.291±0.086	0.272±0.082	0.174±0.649	0.230±0.070	1.068±0.319	3.204
Utilized forest zone	18	0.025±0.009	0.047±0.019	0.047±0.020	0.019±0.010	0.088±0.036	1.584
Mean	–	0.202±0.060	0.198±0.060	0.401±0.127	0.162±0.235	0.741±0.234	–
Total	48	–	–	–	–	–	4.788

Table 4

Summary of carbon stock and above-ground biomass in tropical forest

Locations	Aboveground biomass [tonne ha^{-1}]	Biomass carbon stock [tonne C ha^{-1}]	Source
South East Asia	–	0.078–0.18 (continental), 0.0096–0.225 (insular)	IPCC (2006)
Mixed deciduous forest, Lower Northern Thailand	0.0509 (secondary forest) 0.1045 (primary forest)	0.0307 (secondary forest) 0.0519 (primary forest)	KAEWKROM et al. (2011)
Ratchaburi province, West	0.0309 (dry dipterocarp forest) 0.054 (mixed deciduous forest)	0.0145 (dry dipterocarp forest) 0.0279 (mixed deciduous forest)	CHAIYO et al. (2011)
Kanchanuburi province, West	0.0962 (mixed deciduous forest)	0.0481 (mixed deciduous forest)	TERAKUNPISUT et al. (2007)

Figure 3 shows an obvious linear relationship between carbon stock and number of species (n = 21, R = 0.98, a = 0.1111) and carbon stock and tree density (n = 21, R = 0.98, a = 0.5247). The carbon stock with number of species and tree density in Ban Khum community forest showed a stronger positive correlation

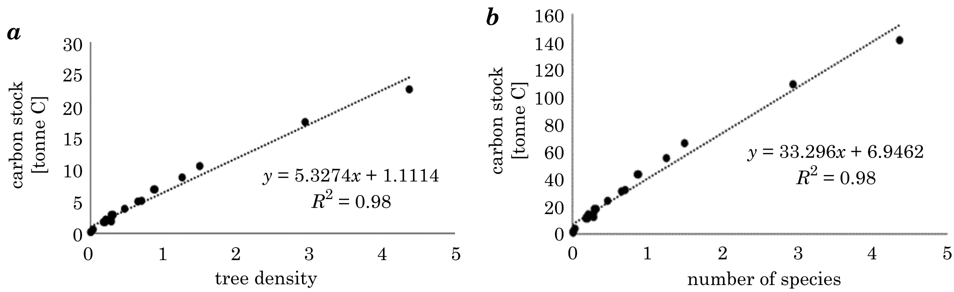
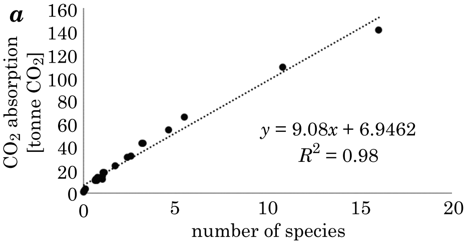


Fig. 3. Relation between carbon stock and number of species, carbon stock and tree density of Ban Khum community forest

Carbon dioxide absorption

When determining the carbon stock in tree biomass to assess the carbon dioxide absorption, it was found that Ban Khum community forest area had an average 0.594±0.188 tonne CO_2 ha^{-1}, divide into stem, branches leaves and root sections of 2.123±0.671 tonne CO_2 ha^{-1}, 0.742±0.231 tonne CO_2 ha^{-1}, 0.690±0.220 tonne CO_2 ha^{-1}and 0.594±0.188, respectively. Total carbon dioxide absorption was 91.74 tonne CO_2, with conserved forest zone having the highest carbon dioxide absorption average 3.058±0.913 tonne CO_2 ha^{-1} divide into stem, branches leaves and root sections of 1.066±0.315 tonne CO_2 ha^{-1}, 0.995±0.299 tonne CO_2 ha^{-1}, 0.997±0.299 tonne CO_2 ha^{-1} and 0.856±0.256 tonne CO_2 ha^{-1}, respectively. Total carbon dioxide absorption was 4.50 tonne CO_2, with utilized zone having the highest carbon dioxide absorption average 0.250±0.104 tonne CO_2 ha^{-1} divide into stem, branches leaves and root sections of 2.123±0.671 tonne CO_2 ha^{-1}, 0.742±0.231 tonne CO_2 ha^{-1}, 0.690±0.220 tonne CO_2 ha^{-1} and 0.070±0.029 tonne CO_2 ha^{-1}, respectively (Table 5). Conserved forests zone has higher amounts of carbon dioxide absorption than forests utilized zone for. Which tends in the direction of biomass and carbon stock.

Figure 4 shows an obvious linear relationship between CO_2 absorption and number of species (n = 21, R = 0.98, a = 0.3516) and CO_2 absorption and tree density (n = 21, R = 0.98, a = 0.9591). The CO_2 absorption with number of species and tree density in Ban Khum community forest showed a stronger positive correlation.



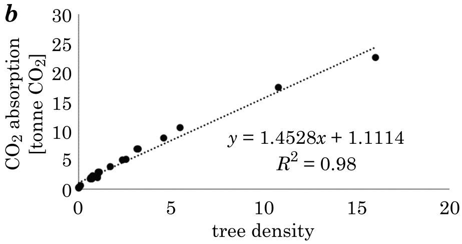


Fig. 4. Relation between CO_2 absorption and number of species, carbon stock and tree density of Ban Khum community forest

Table 5

Carbon dioxide absorption of Ban Khum community forest, Uttaradit province, Thailand

Types of forest zone	Area [ha]	Biomass [tonne CO_2 ha^{-1}]					Total Biomass [tonne CO_2]
		stems	branches	leaves	roots	total	
Conserved forest zone	30	1.066±0.315	0.995±0.299	0.997±0.299	0.856±0.256	3.058±0.913	91.74
Utilized forest zone	18	0.093±0.037	0.078±0.034	0.080±0.033	0.070±0.029	0.250±0.104	4.50
Mean	–	2.123±0.671	0.742±0.231	0.690±0.220	0.691±0.220	0.594±0.188	–
Total	48	–	–	–	–	–	96.24

Carbon stock value

Estimation of carbon stock in the last five years (2015–2019) in Ban Khum community forests was 120.05 tonne CO_2 ha^{-1}. Assessment of carbon stock at an interest rate of 6 percent per year at each level of carbon trading prices (as of 18 September 2019) revealed a total carbon stock value of € 1,538.98 at the price level of the EUA market, equal to € 26.97 per ton of carbon stock. For the CCA market, the purchase price of carbon was $ 17.62 per tonne of carbon stock, providing a total carbon stock value of $ 1,005.44. If the purchase price increased up to $ 20, $ 30 and $ 50 per tonne of carbon stock total value would be $ 1,141.26, $ 1,711.88 and $ 2,853.14. Forest ecosystems are quite variable in the determination of prices. Whether the forest in question is a community or natural forest, pricing depends on the potential and present ecological management of the area. A company's main area will depend on the number of trees if not handled properly by the major academic inevitably result in the environmental sustainability of the system easily, as shown in Table 6.

Table 6

Carbon stock value of Ban Khum community forest, Uttaradit province, Thailand year 2015. The given interest rate of 6 percent per year

Types of forest zone	Area [ha]	Carbon dioxide absorption [tonne CO_2]	Carbon stock value by given carbon price level [tonne CO_2]				
			EUA € 26.97	CCAR $ 17.62	Carbon price [$]		
					20	30	50
Conserved forest zone	30	91.74	1,478.08	965.66	1,096.10	1,644.14	2,740.24
Utilized forest zone	18	4.50	60.90	39.78	45.16	67.74	112.90
	48	96.24	1,538.98	1,005.44	1,141.26	1,711.88	2,853.14

In this study, we explicitly set a fixed volume of carbon from 2014–2020 to be utilized in income estimation for the initial five years. Exchanging and appraisal of carbon rely upon purchasers and vendors who may wish to continue to exchange after the period ends. In addition, carbon stock is to be checked at regular intervals, pretty much as settled upon. The agreement should be reasonable and dependent on academic standards. For example, if the amount of carbon stock is confirmed at regular intervals, and the agreement is made for 25 years, confirmation should be performed multiple times throughout the period. Incomes are, therefore, liable to change, depending on the carbon stock determination (IGNOSH et al. 2009). Carbon credit contracts for zones with forestation may fluctuate rapidly, and strategies are required to determine charges, obligations and income. All of these factors can produce a variety of results following an alternate check charge or expense exception on the 20% carbon stock (FARLEE and STELZER 2008). Some organizations may apply a higher compensation for the same stock (CURRENT et al. 2010) due to the market's diverse referential valuing or if the value is below USD 4 per weight, among other reasons. Therefore, the total income of carbon credits can be changed from the study of other trees such as rubber, it was found that the average income compared to land tenure for rubber plantation in Thailand was approximately 1.6 hectares per household (ISSARAPAP and JARUNTORN 2019). The results of this study indicate that forest community can be developed and placed in voluntary access programs, as well as other forest sectors. Stock or large numbers, both on the aboveground and belowground with good management to reduce train emissions (ISSARAPAP and JARUNTORN 2019) throughout the use of forest community will be able to stretch the deal in accordance with US California Carbon Market and European Union Allowance (EUA) market, however, contract or should not be carefully scrutinized to determine the appropriate value with an effective methodology program for forest community.

Conclusion

In conclusion, the study found that the conserved forest zone had a higher tree species diversity and density compared to the utilized forest zone. The conserved forest zone had a total biomass of 68.130 tonne and a carbon stock of 3.204 tonne C, which resulted in the absorption of 91.74 tonne CO_2. The value of carbon stock in the European Union Allowance (EUA) market was € 1,478.08 and in the US California Carbon Market was $ 965.66. On the other hand, the utilized forest zone had a lower tree species diversity and density with a total biomass of 1.584 tonne, which was converted to a carbon stock of 1.584 tonne C and absorbed 4.50 tonne CO_2. The value of carbon stock in the European Union Allowance (EUA) market was € 39.78 and in the US California Carbon Market was $ 60.90. These findings highlight the importance of conserving forests for carbon sequestration and the potential economic benefits of carbon trading in markets such as the EUA and US California Carbon Market. Previous studies have also shown the importance of forest conservation for carbon sequestration and the potential for carbon trading as a means of promoting conservation efforts. For example, a study by Houghton et al. (2000) found that tropical forests are responsible for absorbing approximately 1.4 billion tonnes of carbon per year, highlighting the potential impact of forest conservation on mitigating climate change. Another study by Börner et al. (2010) investigated the potential for carbon trading in the Brazilian Amazon and found that a system of payments for environmental services could be a viable mechanism for promoting forest conservation and mitigating greenhouse gas emissions. These findings support the importance of continued research and implementation of policies and practices that promote forest conservation and carbon sequestration.

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COMPARISON OF TWO RIVER SEGMENTATION METHODS IN DETERMINING THEIR RESTORATION POTENTIAL

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Key words: watercourse, river, need and possibility of rehabilitation, evaluation method.

Abstract

The aim of the study was to determine the restoration potential of urban river reaches with two different river segmentation methods. The first method was the evaluation of sections with similar characteristics, delineated before assessment. The second method was the assessment of 100 meter [m] long sections. The method of preliminary sections based on the results can be used if it is possible to delimit sections with similar characteristics from most evaluation criteria. While in cases where several different evaluation criteria are applied, the delimitation of shorter sections with the same length can give good results. From the point of view of the plan to be developed, the method of identifying sections with similar characteristics can play a greater role in delineating the most important restoration sub-goals and their target areas. While the method of shorter sections of the same length can be well-used in the development of more detailed concepts or plans. In addition, the territorial scale is also important. In small areas, on shorter river reaches, the method of short, 100 m long sections can also be used. However, it is better to delimit sections with similar characteristics on a large scale or if field surveys cannot be carried out along the entire section length.

Introduction

Watercourses and landscapes along watercourses have been and are currently being shaped by many natural processes and human activities, the adverse effects of which make their protection and restoration one of

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the most important challenges today in Hungary, as well as throughout Europe. A significant part of European watercourses has been changed by human alteration and nearly 95% of the floodplains have been lost (Pedersen et al. 2014 and citations therein). The importance of the implementation of watercourse restoration tasks is emphasized by numerous international directives (EU Water Framework Directive – WFD, EU Biodiversity Strategy until 2030, The European Green Deal, Proposal for a Nature Restoration Law), and Hungarian plans and strategies (5th National Nature Conservation Basic Plan – NTA-V, National Landscape Strategy – NLS). The WFD's list of measures includes rehabilitation projects aimed at restoring the beds, banks, and shorelines of watercourses, as well as mitigating the adverse effects of artificial structures. The planned measures of the second revision of Hungary's river basin management plan (Danube River Basin Management Plan 2021) include the restoration of longitudinal continuity, improvement of hydromorphological conditions, enforcement of ecological aspects (e.g. protection of damaged water and wetland habitats because of the modified water flow, inadequate water supply, improper management, poor water quality or invasive species), and promotion of natural water retention.

Rivers are one of the categories of surface watercourses that can be divided into groups according to size. Rivers have a large catchment area, are considerable in length, and have a significant average water flow (Dévai et al. 1998). Small rivers are 50–250 km long, have 500–10,000 km^2 catchment area, and 5–50 m^3/sec annual average water flow based on the categories of Dévai et al. (1998). Among the 28 watercourses, which are listed in the Act CXCVI of 2011 on national assets as rivers, and in the second revision of Hungary's river basin management plan, they have a "river" water management classification, 64% belong to the small river category. The subject of this research is the small rivers in Hungary.

Different natural and social features and resulting rehabilitation needs require different solutions when planning the rehabilitation of rivers. These differences must be explored and taken into account. It is important to properly establish the rehabilitation projects and to develop the methods of evaluation of rivers, one of the tools of which can be the determination of their rehabilitation potential.

In addition to the more widespread research on determining the restoration potential of river reaches in rural areas, it is also important to place greater emphasis on the rehabilitation of urban river reaches. Previous research on the topic has mainly focused on river sections in rural areas (Erdei 2020a). Watercourses are an important pillar of blue infrastructure and are receiving increasing attention in developing green infrastructure in urban areas (Vaszócsik et al. 2014, MTA-OIA 2017). They represent an outstanding value, and their rehabilitation also contributes to the increase of many ecosystem services. Settlements along rivers can also be important destinations from a tourist point of view, for example, Starczewski et al. (2018) analyzed the tourism development potential of towns along the Krzna river. Due to existing limiting factors, urban river sections are mainly rehabilitated rather than fully restored.

Hulse and Gregory (2004), Boitsidis et al. (2006), Francis et al. (2008), Norton et al. (2009), Gurnell et al. (2014), Guida-Johnson and Zuleta (2019), and Zuo et al. (2020) are examples of international research that, due to their scale or criteria, used methods applicable in urban areas. In the Hungarian literature, publications on the management principles of the rehabilitation of small watercourses (Báthoryné Nagy 2007), the hydromorphological and landscape ecological evaluation of floodplains (Lóczy 2011), or the improvement of continuity and the prioritization of barrier removal (Erős and Czeglédi 2019) can also be mentioned. In these studies, various segmentation methods were used. As a larger-scale study, Norton et al. (2009) dealt with the assessment of water bodies; Zuo et al. (2020) evaluated the studied river by dividing it into upper, middle, and lower sections. Lóczy 2011 divided the investigated river into sections with similar morphological characteristics based on preliminary GIS analyses. Báthoryné Nagy (2007) evaluated the studied stream by segmenting it based on landscape types and landscape management zones. In contrast to these, Hulse and Gregory (2004) evaluated sections of the same length while examining a longer river section and divided it into 1 km long sections. Boitsidis et al. (2006) examined urban river sections, during which they evaluated river sections with the same length of 500 m.

Taking into account the definitions found in the literature, in this research, we call rehabilitation the improvement of the landscape's physical, chemical, ecological, and aesthetic condition along the river, as well as its integration into the urban environment by increasing its multifunctional role. The present research aims to facilitate the realization of urban river rehabilitations, as its goal is to develop an evaluation methodology suitable for determining the restoration potential of urban river reaches and the restoration sub-goals for a given river reach. The restoration potential was determined by applying two types of watercourse segmentation methods and comparing their results. Our aim is to evaluate the need and possibility of restoration with the established methodology and compare them territorially. In this way, areas that can be included in rehabilitation planning with a better chance can be mapped.

Study area

The study area of the research was the urban river reach of Zagyva, in Szolnok. Szolnok is a Hungarian city in the Northern Great Plain region, in the county of Jász-Nagykun-Szolnok, with a population of nearly 70,000. The city is located at the mouth of the Zagyva, which flows into the Tisza, the largest river in the Great Hungarian Plain. The Zagyva River reach included in the evaluation was delineated with the help of the 300 m buffer area of the land use units of Szolnok with residential, holiday, and recreational functions. The study area contained the active floodplain of the river reach (bed, banks, and floodplain). According to the type of water body, the Zagyva is a lowland river with a small slope and a large catchment. The examined Zagyva reach was affected by river regulation works, the cut-off meander called 'Holt-Zagyva' is located north of the settlement. The floodplain accessible for flooding has been significantly reduced to secure more space for the city and eliminate flood risk on agricultural land. There are significant elements of the green infrastructure north of the city, on the former floodplain (mainly due to public welfare forests). The main human interventions affecting the river reach in Szolnok are three transversal structures (weirs) in the channel and maintenance practices (afforestation) on the active floodplain.

Materials and Method

In the Szolnok study area, we evaluated the river reach connected to the urban areas to determine the rehabilitation potential (Figure 1). The steps for this are explained in detail in the subsections below. As a first step, we defined the examined river reach units and the possible sub-goals related to their rehabilitation. The determination of the restoration potential was made regarding the sub-goals. A separate evaluation system was compiled from the examination aspects for each sub-goal. This publication presents evaluations related to the sub-goals related to the ecological and hydromorphological state of the riverbed, the bank, and the active floodplain. During the evaluation, the need for restoration (from the condition and characteristics of the river) and the possibilities of restoration (from the presence of factors limiting its implementation) were determined using a scoring method. By comparing the need and possibilities of restoration, we obtained the restoration potential of the given section for the sub-goal. With the help of this, the territorial differences within the examined river reach were revealed, and the areas with good potential in terms of the various restoration sub-goals could be delineated. By comparing the results of the restoration potential per sub-goal, it was also possible to base the planning of the restoration.

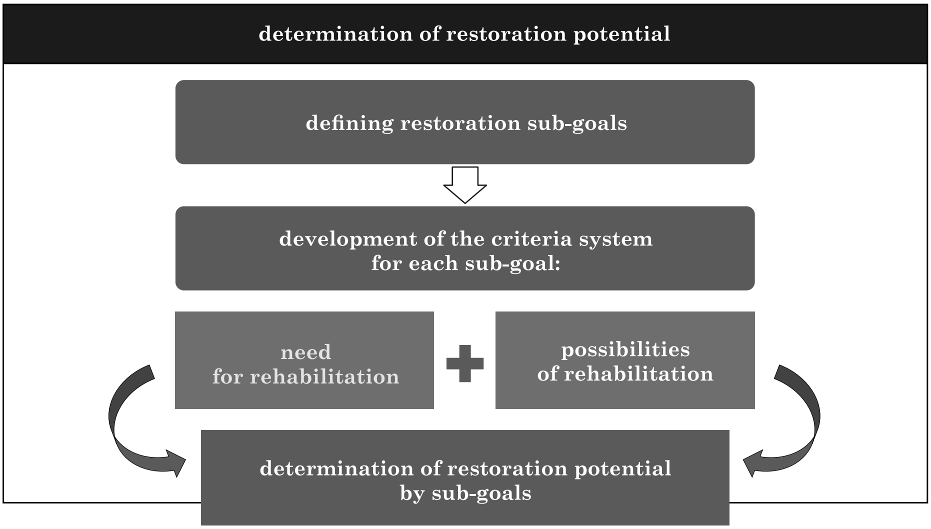

Fig. 1. Method of determining restoration potential

The evaluation was prepared using two types of segmentation methods, which are presented in detail below:

1. Pre-delineated sections with similar characteristics.
2. Sections with the same length of 100 m.

Defining restoration sub-goals

The restoration potential of the examined river reach was determined by establishing restoration sub-goals. The most typical restoration sub-goals related to urban rivers were determined based on the restoration objectives summarized by NAGY and NOVÁK (2004), the guidance standard for assessing the hydromorphological features of rivers (14614:2020) and the analysis of Hungarian projects carried out as a prelude to the present research (ERDEI 2020b). In connection with the most common problems, we determined the restoration sub-goals for which we prepared the evaluation of the restoration potential. The sub-goals of this research are those that primarily affect the area of the riverbed, the bank, and the active floodplain, during which the hydromorphological aspects are an essential part of determining the restoration potential.

The sub-goals evaluated in the current research were the following:

1. Improving the longitudinal connectivity, where artificial structures limit fish migration.

2. Improving the ecological and hydromorphological condition of the active channel.
3. Achieving a more natural channel planform.
4. Improving the naturalness of floodplain vegetation.
5. Improving water quality.

Development of the criteria system for each sub-goal and the source of the data

During the evaluation, the need for restoration (which follows from the condition of the river) and the possibilities of restoration (which follows from the presence of factors limiting the implementation) were determined using a scoring system. The collection of assessment criteria was based on a review of the literature on restoration potential (Erdei 2020a), the literature related to small watercourses (Báthoryné Nagy 2007), the guidance standard CEN 14614:2020, and the methodological manual on the generation and evaluation of hydromorphological data (Viziterv 2019). In addition, aspects related to the bed, banks, and floodplain were taken into account in the research and summarized in Table 1.

Table 1

Evaluation criteria related to the need and possibility of restoration

Zone*	Evaluation criteria	Sub-goal
Need of restoration		
1	2	3
Be	water quality based on the informative environmental rating index	5
Be	ecological continuity of artificial structures in the channel	1
Be	ecological and hydromorphological impact of artificial structures in the channel	2
Be	naturalness of the channel form	2
Be	average proportion of aquatic or wetland vegetation cover in open water	2
Be, F	frequency and type of specific morphological features	2
Be	degree of modification of the planform	3
Be	degree of change in the sinuosity index	3
Be	distance of arable land from the bed	5
Be	presence and effect of polluting sources	5
Ba	bank slope degree modifications	2
Ba	bank erosion depending on the influence of the river movement	3
Ba	proportion of river sections affected by bank reinforcement	2, 3
Ba	shading effect of riparian woody vegetation	2
Ba	continuity of buffer vegetation on the river bank	2

cont. Table 1

1	2	3
Ba	width of buffer vegetation on the river bank	2
Be, Ba, F	naturalness of lateral vegetation zonation	4
F	naturalness of floodplain woody vegetation	4
F	proportion of habitat patches infested with invasive species	4
F	proportion of areas affected by human activity	4
F	proportion of areas with nature conservation importance	4
Possibility of restoration		
Be	reduction options for the impacts of artificial structures	1, 2, 3
Be	naturalness of the channel form	4, 5
Be, Ba	degree of influence by sedimentation	2
F	width of the floodplain	4, 5
F	width of the floodplain potentially suitable for the movement of the river	1, 3
F	proportion and naturalness of woody vegetation	1, 3
Ba, F	occurrence of protected species	1, 3
Be, Ba, F	proportion of areas with environmental importance	1, 3, 4, 5
Be, Ba, F	occurrence of areas and values with heritage protection	1, 3, 4, 5

*Be – river bed; Ba – river bank; F – floodplain

The sources of the data included the data provided by the General Directorate of Water Management (artificial structures – characteristics, continuity, possible measures; bank protection, map of the related flood defence system; water bases); data provided by the Middle Tisza District Water Directorate (map of the channel at low flow; bank reinforcement), data provided by the Hortobágy National Park Directorate (occurrence of invasive species and protected species; nature conservation areas). For the remaining aspects of the study, analysis of field surveys, satellite imagery, settlement plans, and historical maps were the sources of the data.

Determining restoration potential

To determine the restoration potential, the presented evaluation criteria were assigned to the restoration sub-goals, so the restoration potential was determined for each sub-goal. To determine the restoration potential, we evaluated each aspect on a 5-point scale. In the case of aspects relating to the river bank, the characteristics of the right and left banks were also taken into account, however, the characteristics of the two sides were summed up, and the river section was evaluated together based on them. In that way we could describe the given section with one evaluation result according to the characteristics of the bank. In the case of the floodplain, this is less possible, there can appear much different characteristics, and they are geographically far from each other, so we found it more worthwhile to evaluate them separately, which resulted in two separate evaluations result. Aspects were weighted in terms of importance. The scores for each section were summed and averaged using weighting. We separately aggregated the evaluation results of the aspects related to the need for restoration (current condition of the river: higher score = greater need) and separately the evaluation results of the aspects related to the possibilities of restoration (limiting factors: higher score = better implementation possibilities) per subgoal.

To determine the restoration potential of the given section, the need and the possibilities of restoration were compared (Table 2). Based on the results of the separate evaluation of the necessity and the possibility,it can be seen which makes the restoration potential of the given section worse, so the two partial results and the final result can be interpreted together.

Table 2

Determining restoration potential based on the need and possibility of restoration

Specification		Possibility of restoration		
		high	medium	small
Need of restoration	high	5	4	3
	medium	4	3	2
	small	3	2	1

During the evaluation, we considered the need for restoration to be greater, the more the given section is in a modified/unfavourable condition. On the other hand, we considered restoration possibilities to be better, the fewer the limiting factors. During the restoration potential determination, the sections with the most modification or worse condition and the fewest limiting factors became those with the best restoration potential. On the other hand, the less modified sections with many limiting factors were the ones with the worst restoration potential.

Segmentation methods

Sections with similar characteristics. During the evaluation, pre-delineated sections (including the river bed, river bank, and floodplain) were evaluated in accordance with the recommendation of the CEN 14614:2020 standard. The floodplain sections were delineated in connection with the river sections, which in some cases were further divided due to the different land uses along the floodplain. In the case of the method, the purpose of segmentation is to determine river and floodplain sections with similar properties, even before more detailed investigations, based mainly on aspects that can be clearly delineated from maps and databases. In the present research, sections were delineated, taking into account the following aspects:

– location (urban/rural),

– main land use next to the active floodplain (forest/grassland/agricultural area/horticultural area/*residential area/holiday-weekend house area/industrial area/mixed area* – last four together built-up areas),

– width of the active floodplain (narrow/medium or variable/wide),

– presence of significant cut-off meander and

– presence of artificial structures.

The location (rural/urban area) and the land use characteristics of the areas adjacent to the floodplain have an impact on, among other things, the loads on the river section, the natural condition, and the use of the section. The main land use was determined based on settlement plans and satellite images. The width of the floodplain has a fundamental effect on restoration options, and major cut-off meanders indicate sections affected by previous significant river regulations. Based on these, seven river sections in the Szolnok study area were delineated. During the evaluation, the floodplains on the right and left banks were treated separately.

By delineating the sections with similar characteristics, seven sections were defined in the examined river reach (Figure 2). The first three sections are all rural areas outside the city and have a wide active floodplain. Along Sections 1 and 2, there are forest and agricultural areas. Meander cutting took place in the case of Section 2, where a cut-off meander called 'Holt-Zagyva' is located. On the eastern side of Section 3, there are areas with holiday houses. On the western side of Section 4 lie built-up areas, where mainly residential and industrial areas are located. This section typically has a wide (>300 m) active floodplain. Sections 5 and 6 cross the built-up areas of the city and have a medium-width (100–300 m) active floodplain. Along Section 5, mainly residential areas are typical, and along Section 6, there are both residential and mixed areas adjacent to the floodplain. Section 7 is located at the mouth of the Zagyva, which crosses the city centre. The floodplain is narrow here (<100 m width), and mixed land use is typical along the floodplain.

Sections with the same length of 100 m. According to the second method, the examined river reach was divided into equal sections of 100 m in length. The method's purpose is to reveal the differences in the area of the river and floodplain during the evaluation with sections of the same length instead of preliminary delimitation based on characteristics. For this purpose, the centerline of the river was uniformly divided into sections of 100 m in length. We defined the floodplain sections in connection with river sections of the same length. Due to the meandering nature of the river, the resulting floodplain sections cannot form completely uniform, parallel boundaries. Because of this and due to changes in the width of the floodplain, their territorial extent is not the same. In the Szolnok study area, 75 sections of 100 m were demarcated (Figure 2).

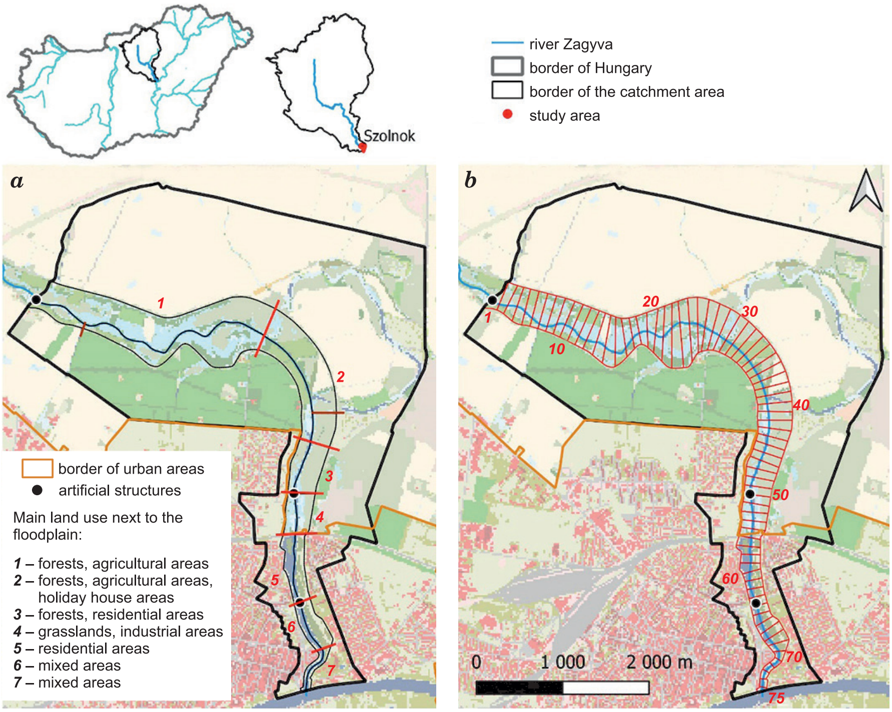

Fig. 2. River sections with similar characteristics, delineated before assessment (*a*) and 100 m long river sections (*b*) in the Szolnok study area

Results

During the research, we determined the restoration potential for the following restoration sub-goals: (1) improving the longitudinal connectivity where artificial structures limit fish migration, (2) improving the ecological and hydromorphological condition of the active channel, (3) achieving a more natural channel planform, (4) improving the naturalness of floodplain vegetation, and (5) improving water quality. Figure 3 shows the results of the two types of segmentation methods for sub-goals 1 and 2; Figure 4 shows the results for sub-goals 3 and 4; and figure 5 shows the results for sub-objective 5.

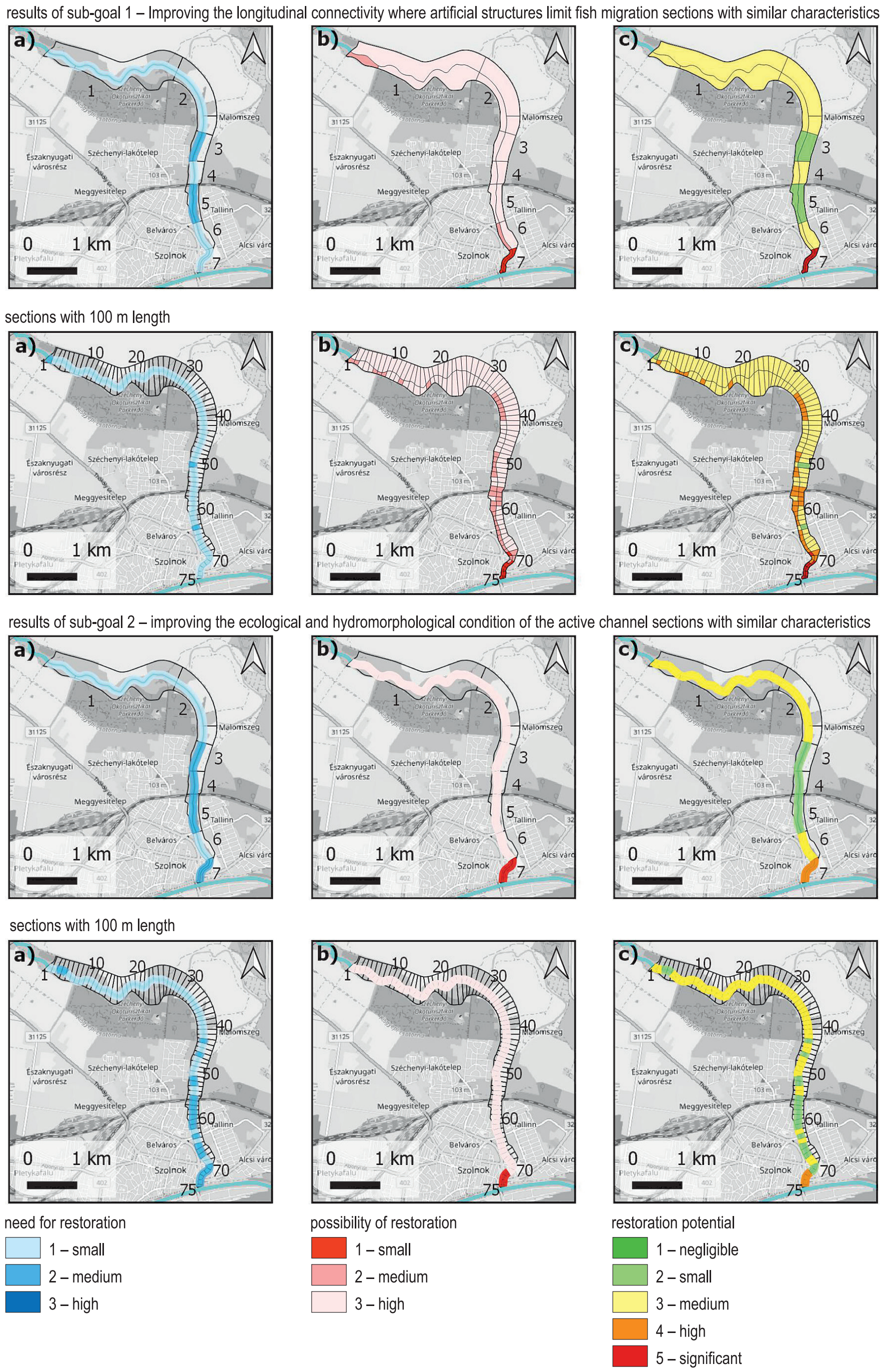

Fig. 3. Evaluation results for sub-goal 1 and 2: *a* – need of restoration; *b* – possibility of restoration; *c* – restoration potential

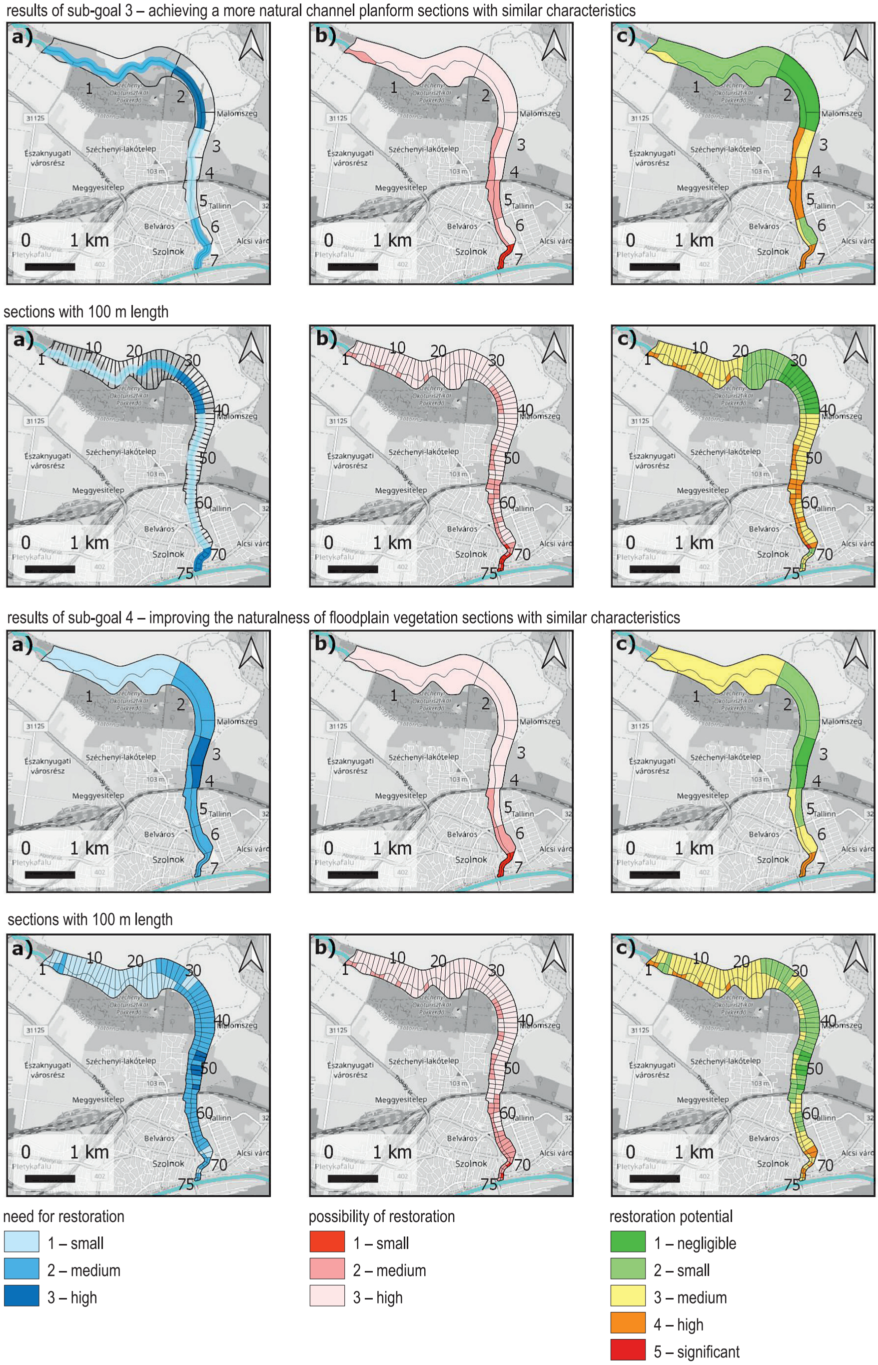

Fig. 4. Evaluation results for sub-goal 3 and 4: *a* – need of restoration; *b* – possibility of restoration; *c* – restoration potentia

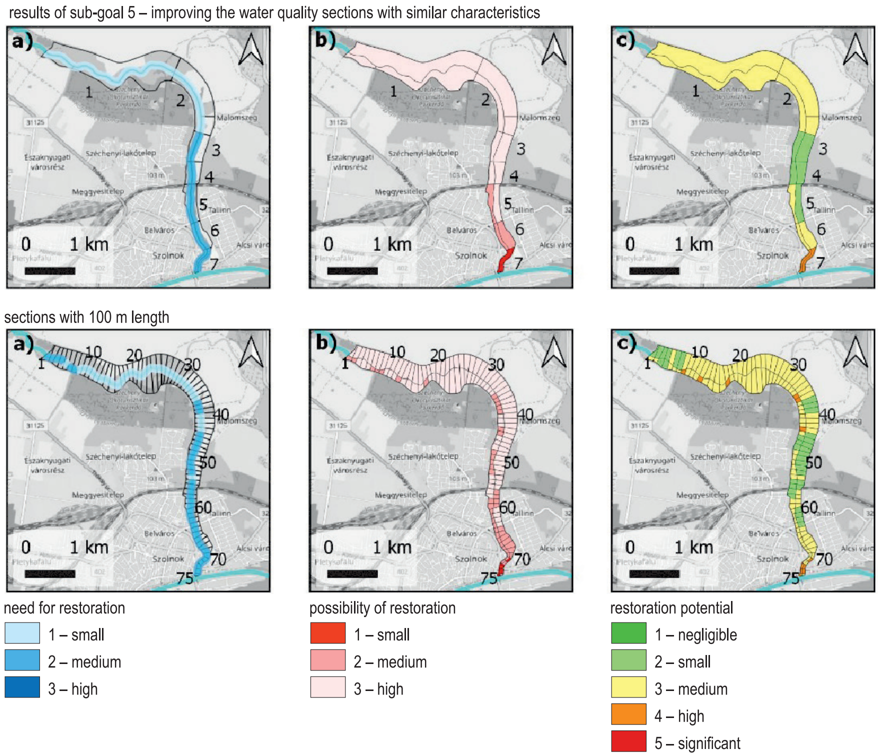

Fig. 5. Evaluation results for sub-goal 5: *a* – need of restoration; *b* – possibility of restoration; *c* – restoration potential

In the case of **sub-goal 1 – improving the longitudinal connectivity where artificial structures limit fish migration** – based on the evaluation results of sections with similar properties, it can be seen that Sections 3 and 5 have good restoration potential. These sections are affected by the presence of artificial structures at their downstream boundaries. Based on the results, achieving the sub-goal is a realistic goal for all artificial structures located on the river reach. The reason for this is that each of the artificial structures present only provides connectivity periodically, and they could all be rebuilt with a more natural solution. In addition, it is possible in the case of artificial structures created to reduce channel slope, e.g. to make the river bed pattern more meandering, as a significant limiting factor does not affect the given floodplain sections. A limiting factor is mainly the width of the floodplain potentially suitable for the movement of the river in section 7, but this is not affected by the presence of an artificial structure.

In the evaluation results of the 100 m sections, the location of the sections affected by the artificial structures (sections 50 and 63) is shown more precisely due to the shortness of the evaluated sections. Based on the results, it can be seen that in the neighbourhood of the sections affected by the artificial structures, there are always sections on at least one side of the river that have a floodplain of adequate width so that they can be included in the restoration.

In the case of **sub-goal 2 – improving the ecological and hydromorphological condition of the active channel** – based on the evaluation results of the sections with similar properties, the urban and adjacent sections 3, 4 and 5 have a high restoration potential. The need to achieve the sub-goal in these sections arises mainly due to the lack of woody vegetation along the bank, the unfavourable ratio of the bed being covered with aquatic vegetation, and the lack of special morphological elements in the riverbed. In the 7th section, it would be necessary to improve the condition of the small water bed (a trapezoidal bed and an unfavourable bank slope are typical), but the condition of the estuary section is strongly influenced by the Tisza floods. The section is characterized by sedimentation. The main possible interventions on the sections with high restoration potential affect the riverbed and the river bank, so they do not involve areas of the floodplain. Such e.g. increasing the variety of bed depth and width; placement of the bank reinforcement "backwards", thereby providing more space for the river; increasing the variety of flow dynamics, and placing elements in the river bed that match the type of river (e.g. dead wood); restoring the near-natural shape of the riverbed and bank or planting woody vegetation along the river.

During the evaluation of the 100 m long sections, sections 51–63, which have high rehabilitation potential based on the results, are similarly visible. In addition, among sections 65–71, it can also be a realistic goal to improve the ecological and hydromorphological condition of the small water bed and bank, as they have a high restoration potential.

In the case of **sub-goal 3 – achieving a more natural channel planform** – based on the evaluation results of the sections with similar properties, based on the results of section 2, which is affected by the presence of the cut-off meander called 'Holt-Zagyva', has significant restoration potential, as this is where the biggest changes took place during the regulation, in addition, the potential for restoration is high thanks to a wide floodplain. Section 1 also has great potential for restoration since the alignment of the bed has been changed here as well (although only within the area of the floodplain), and it also has a few limiting factors. The position of the river bed was also changed in section 7, where the mouth was once located on the eastern side of the Szolnok castle. However, the possibility of restoration in this section is small, mainly due to the narrow floodplain, so the restoration potential is small.

Based on the evaluation results of the 100 m long sections, the 30–40 sections affected by the 'Holt-Zagyva' cut-off also have significant restoration potential. In the case of the preceding sections, there is a visible difference in the results. Here, sections 21–29, in which the alignment of the bed changed within the area of the floodplain, can be seen more precisely. The possibility of restoration is typically high in these sections as well, so their restoration potential is also high.

In the case of the **sub-goal 4 – improving the naturalness of floodplain vegetation** – based on the results of the sections with similar characteristics, the restoration potential is significant in the left floodplain of sections 3 and 4. The main reasons are that there is a high level of invasive species coverage, there are typically no limiting factors in the area, and there are areas of nature conservation importance, which makes implementing the sub-goal even more important. In addition, the restoration potential for the realization of the sub-goal is also high on the 2^{nd} section and the left floodplain of the 5^{th} section. In the 1^{st} section, the vegetation is semi-natural, which means that the need for restoration and the restoration potential is only moderate. In the urban sections, the restoration potential is low or moderate due to limiting factors (e.g. narrow floodplain, the presence of areas of environmental or heritage protection importance). From the point of view of recreation and aesthetic value, the improvement of vegetation on the floodplain may also be important in these urban sections, but these will be evaluated as a separate restoration sub-goal in the later phase of the research.

This result can also be seen by evaluating the 100 m sections. Sections 47–54 have significant restoration potential due to the reasons described above. The restoration potential is typically high in the 25–63. Sections, at least on one side of the floodplain, since the need for restoration on these sections is moderate (e.g. lack of woody vegetation on the river bank, lateral vegetation zonation is not close to nature, presence of plantation forests), and the possibility of rehabilitation is high (floodplain width is adequate).

From the point of view of **sub-goal 5 – improving the water quality** – the restoration potential is high in sections 3, 4 and 5. In the case of section 5, there is a better opportunity for the application of restoration solutions on the left side of the floodplain. The need for restoration in these sections is moderate due to the water quality characteristics and lack of woody vegetation/wetlands near the river that would have a buffer capacity. The water quality in the entire river reach is unfavourable. The other urban sections are also characterized by the lack of buffer vegetation on the riverbank. However, due to the limiting factors (e.g. narrow floodplain, the presence of areas of environmental or heritage protection importance) in these sections, it may be more difficult to create vegetation of significant impact.

Based on the evaluation results of the 100 m sections, there are also some sections (1–7 and 37–39) in the rural areas that are in great need of restoration, mainly due to the lack of woody vegetation or wetlands capable of buffering on the river bank. In accordance with the evaluation of sections with similar characteristics, on sections 71–75, the possibility of restoration is small (narrow floodplain, the naturalness of the channel form is not suitable). Therefore, the restoration potential is also small.

Discussion

Based on the results of the study area evaluations, the method of sections with similar characteristics can be used well in case of aspects that characterize the occurrence frequency of point-like elements (such as the occurrence of special morphological elements in the riverbed or protected species). By evaluating longer sections, sections with a different frequency of occurrence are more clearly defined. When applying the method, it is particularly important to properly define the section boundaries because if the properties of some aspects do not change at the boundary of the pre-delineated sections, then certain information loss must be expected. Therefore, it is important that the criteria selected for preliminary delimitation enable the appropriate delimitation of the sections, i.e. the most significant properties are taken into account (or it is also possible to delimit the section boundaries after the examination has been carried out, but this may cause difficulties during the examination and evaluation of a large number of criteria).

The difficulty of applying the method of the same 100 m long sections based on the results of the study area evaluations is that the frequency of occurrence of point-like elements can be evaluated less well with it. The more precise location of their occurrence can be clearly detected by evaluating short sections, but the sections with a similar frequency of occurrence can be defined only by analyzing the pattern of the sections. Suppose we apply many evaluation aspects during the evaluation (and during the preliminary delimitation we would not be able to take them all into account - with the same weight), then by evaluating sections of appropriate length, even in the case of less significant aspects. In that case, local differences can be better distinguished since within longer sections, there is a greater chance that several characteristics are typical for some aspects in the same section (e.g. how the vegetation cover of the riverbed changes), so some local differences can be less distinct.

In order to choose the segmentation method used in evaluating a river reach, it is necessary to define the purpose of the given assessment and at what scale we want to explore the characteristics of the given river section. By evaluating longer sections with similar characteristics, the results may become homogenized if inappropriate section boundaries are chosen; or in the case of using a large number of evaluation criteria, when not all of them can be taken into account for the preliminary delimitation so that some local characteristics may disappear in the evaluation.

The application of pre-delineated sections works well when preparing for homogeneous sections, or the effect of point-like elements can also be properly evaluated on the entire section (e.g. by introducing an index). However, if the evaluated sections are too short, it can be difficult to interpret the results and their applicability in certain cases (e.g. in the case of the frequency of appearance of point-like elements). In addition, section boundaries that do not adapt to local conditions, but are delineated mechanically, do not adapt to changes in the properties of the aspects. Therefore it is important to create indexes during the evaluation in order to more accurately show the appearance rate of each characteristic.

If, among the aspects to be evaluated, it can be well determined in advance which ones influence the main properties of the river, and thus homogeneous sections can be delineated for the majority of the aspects to be evaluated, then clearer results can be obtained by applying this method. Suppose we have a large number of aspects to be evaluated during the evaluation, and due to their differences, it is impossible to create homogeneous sections by prior delimitation. In that case, the evaluation of shorter, same-length sections can also be used, but it is important to choose the appropriate length of the sections.

During the research, field surveys played an important role in most of the evaluated aspects. Pre-delineated sections with similar characteristics can also be used with less precise surveys. In contrast, in the case of evaluating shorter sections of the same length, it is important to assess the exact territorial location of the given properties. Also, based on the guidance standard CEN 14614:2020, the level of detail depends on the purpose but emphasizes the importance of consistent internal properties for pre-delineated sections. The pre-delineated sections with similar characteristics can therefore be well used for the conceptual delineation of the most important restoration sub-goals and their main target areas in the river reach. Shorter sections with the same length can play a role in establishing a more detailed concept.

In small areas, on shorter river reaches, such as in urban areas, the method of short sections with the same length can also be used, as long as the river reach is easily accessible. On a large scale of investigation or if the river reach cannot be easily accessed, which means that surveys cannot be carried out along the entire length of the river reach, it is better to delimit sections with similar characteristics. In this case, it is possible to select smaller, representative locations where the characteristics of the given aspect can be assessed, and the result can be extrapolated to longer sections.

Conclusions

During the research, we evaluated the restoration potential of an urban river reach of Zagyva in the city of Szolnok by comparing two types of river segmentation methods. We obtained this by evaluating the need and possibility of restoration for the following restoration sub-goals : (1) improving the longitudinal connectivity where artificial structures limit fish migration, (2) improving the ecological and hydromorphological condition of the active channel, (3) achieving a more natural channel planform, (4) improving the naturalness of floodplain vegetation, and (5) improving water quality. One of the segmentation methods was done by delimiting sections with similar characteristics, and the other by evaluating river sections with the same length of 100 m. Based on the results, the main differences in the river reach in the case of the evaluated sub-goals emerged with both segmentation methods. In the case of a section with similar characteristics, it is very important to choose the appropriate section boundaries. In the case of shorter, 100 m long sections, the territorial extent of the effects of point-like elements (for example occurrence of special morphological elements in the riverbed or protected species, which were located at a given point of the area) should be taken into account. Regarding the examined sub-goals, we determined which river sections have a high restoration potential within the Zagyva river reach of Szolnok, thereby defining which river sections and which restoration sub-goals are more important to achieve.

The method is suitable for evaluating and spatially comparing the need and possibilities of restoration before planning the restoration of urban river reaches. The method helps in the territorial delineation of restoration sub-goals, but further, more detailed analyses are necessary to plan precise interventions. The criteria system of the method can be used on other rivers, even in other countries. Still, the assessment should take into account the reference characteristics/target condition of the given river reach, which may differ. As a continuation of the research, the restoration sub-goals included in the assessment can be expanded, such as improving recreational opportunities or improving urban landscape aesthetic value. As a further continuation, it is possible to experiment with a method where the entire study area is divided by raster, thus resulting in a mosaic area unit assessment. After the evaluations in the study areas, country-scale analyzes are planned as a continuation of the research, with the aim of exploring the possibilities of a national analysis of the restoration potential based on the country-scale available databases.

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THE FACTORS THAT MODIFY THE RATE OF DAMAGE AND DECOMPOSITION OF *NUPHAR LUTEA* LEAVES: AN EXAMPLE FROM A SHALLOW EUTROPHIC LAKE

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Key words: yellow water lily, *Galerucella nymphaeae*, Chrysomelidae, leaf damage, wind and waves.

Abstract

The rate and degree of damage to the leaves of yellow water lily *Nuphar lutea* in the shallow eutrophic lake was studied. Three factors were analysed: gnawing by *Galerucella nymphaeae*, the activity of microorganisms, and physical damage. Differences in the abundance, density and biomass of larvae and adult forms of *G. nymphaeae* were found between the two study sites. Site I was more favourable to the development of leaf beetles. The larger *G. nymphaeae* populations at site I resulted in greater leaf damage by this beetle. In turn, greater leaf damage due to feeding by *G. nymphaeae* resulted in greater damage resulting from microbial activity. The most likely reason for the smaller degree of damage to leaves at site II were their greater exposure to wind and waves. These two environmental factors impeded the development of the *G. nymphaeae* population and thus resulted in less damage to *N. lutea* leaves and a slower rate of decomposition.

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Introduction

In shallow, eutrophic European lakes the yellow water lily *Nuphar lutea* (L.) Sibth. & Sm. and white water lily *Nymphaea alba* L. form one of the more widespread plant association, *Nupharo-Nymphaeetum albae*, which plays the primary role in the shallowing process in the lakes of this type of trophy (MATUSZKIEWICZ 2001). In the typical eutrophic lakes, as the Głębokie Lake in Poland (see study area), this association is a transition zone between associations of completely submerged plants of the Charetea class and rushes of the Phragmitetea class, mainly from the Phragmition alliance.

Plant species in this association are characterized by high biomass production and are an important link in nutrient and energy dynamics (WETZEL 1983). They form communities with a characteristic physiognomy – macrohydrophytes rooted in the bottom with leaves floating on the water surface and flowers projecting above the water table (PADGETT 2007). Also typical of members of the Nymphaeacae family is the production of various alkaloid compounds with allelopathic effects that protect them against many invertebrates and microorganisms (HUTCHINSON 1981, ELAKOVICH and YANG 1996, PADGETT 2007). Hence, only a few taxa, such as *Galerucella nymphaeae* (Linnaeus, 1758), consume the leaves of these plants, often being even monophagous (WARCHAŁOWSKI 1994). Nevertheless, due to the location of the leaf surface at the interface between aquatic and terrestrial ecosystems, leaves may be additionally exposed to damage from other, specialized herbivores, occurring in small numbers, such as the larvae of *Nymphula nitidulata* (Hufnagel, 1767) mining in the leaves (VALLENDUUK and CUPPEN 2004), as well as terrestrial herbivores (SETÄLÄ and MÄKELÄ 1991, KORNIJÓW and ŚCIBIOR 1999b). Throughout the growing season, both sides of the yellow water lily leaf are additionally a site of concealment and breeding for many phytophagous invertebrates, a hunting area for predators (KORNIJÓW 1989, KORNIJÓW and ŚCIBIOR 1999a) and a place of development for several taxa of fungal and bacterial microorganisms (DONDERSKI and KALWASIŃSKA 2002, VORONIN 2014).

In Polish chrysomelid fauna, the genus *Galerucella* Crotch, 1873 is represented by 7 species (BOROWIEC et al. 2011), while the *Catalogue of Palearctic Coleoptera* lists only 6 species from Europe, with *G. aquatica*, *G. sagittariae* and *G. kersteni* synonymized with *G. nymphaeae* (SILFVERBERG 1974, BEENEN 2010). Various publications on morphology and genetics also show that *G. aquatica* is a sibling-species to *G. nymphaeae* and should be excluded from the species complex mentioned above (LOHSE 1989, NESTEROVA 2008, HENDRICH et al. 2015). The distinct status of certain species of the *G. nymphaeae* complex has been intensively researched for northern European and North American populations, mainly by Finnish researchers (NOKKALA and NOKKALA 1989a, b, 1998, NOKKALA et al. 1998). Among all species of this genus, only *G. nymphaeae* is strictly hydrophilous, living and feeding on floating leaves of some Nymphaeaceae (such as *Nuphar* or *Nymphaea*), whereas other species primarily prefer hygrophilous plants, feeding on various species mainly of the Polygonaceae, Rosaceae, Primulaceae, Hydrocharitaceae and Salicaceae families (BROVDIJ 1973, WARCHAŁOWSKI 1994). *Galerucella nymphaeae* is widely distributed in the Euro-Siberian region as well as in North America (BROVDIJ 1973, SILFVERBERG 1974, WARCHAŁOWSKI 1994).

Galerucella nymphaeae causes the most damage to the leaves of *N. lutea* of all the invertebrate taxa that live and feed on it (SETÄLÄ and MÄKELÄ 1991). The insect's habitat is the floating leaves of plant, which both adults and larvae feed on (BROVDIJ 1973, WARCHAŁOWSKI 1994), leading to their rapid degradation (consumption from 0.4–15% of annual net production according to various authors) and the release of considerable amounts of carbon and nitrogen bound in organic compounds into the aquatic environment (SETÄLÄ and MÄKELÄ 1991). The leaf is destroyed within a short time, initially by feeding beetles, which in turn induces numerous processes of decay caused by various species of fungi, fungus-like organisms and heterotrophic bacteria (DONDERSKI and KALWASIŃSKA 2002, VORONIN 2014, MAZURKIEWICZ-ZAPAŁOWICZ et al. 2016), which decompose the collenchyma previously damaged by the beetles (SETÄLÄ and MÄKELÄ 1991). Floating leaves can also be damaged by some species of water birds (PAILLISSON and MARION 2001).

In the present study, observations of the rate of *N. lutea* leaf damage and decomposition caused by biological, microbiological and physical factors were conducted at two sites. Physical factors clearly distinguishing the two sites were wind and waves. For the purposes of the study, a working hypothesis was put forward: physical conditions, namely wind and waves, affect the population parameters (abundance, density and biomass) of *Galerucella nymphaeae* inhabiting those two sites. As *Galerucella nymphaeae* is a trigger inducing decomposition of the leaves of yellow water lily, the total rate of damage and decomposition of the leaves will be different at those two sites. The aims of the study were: 1) to examine in detail the development cycle of the leaf beetle, specifying the period when adults and larvae appear on the leaves; 2) to assess population parameters (abundance, density and biomass) of *G. nymphaeae*, including both adults and larvae; 3) to assess the course of biological, microbiological and physical damage in a monthly cycle; and 4) to assess abiotic conditions affecting *N. lutea* leaves and the *G. nymphaeae* populations colonizing them at each of the study sites.

Methods

Study area

The study was carried out in Lake Głębokie (51°17’28”N 23°05’56”, 168 m a.s.l., situated in the Łęczna-Włodawa Lake Lakeland (eastern Poland) – Figure 1. It is a typical eutrophic lake with a surface area of 11.4 ha and a maximum depth of 5.7 m (RADWAN and KORNIJÓW 1998).

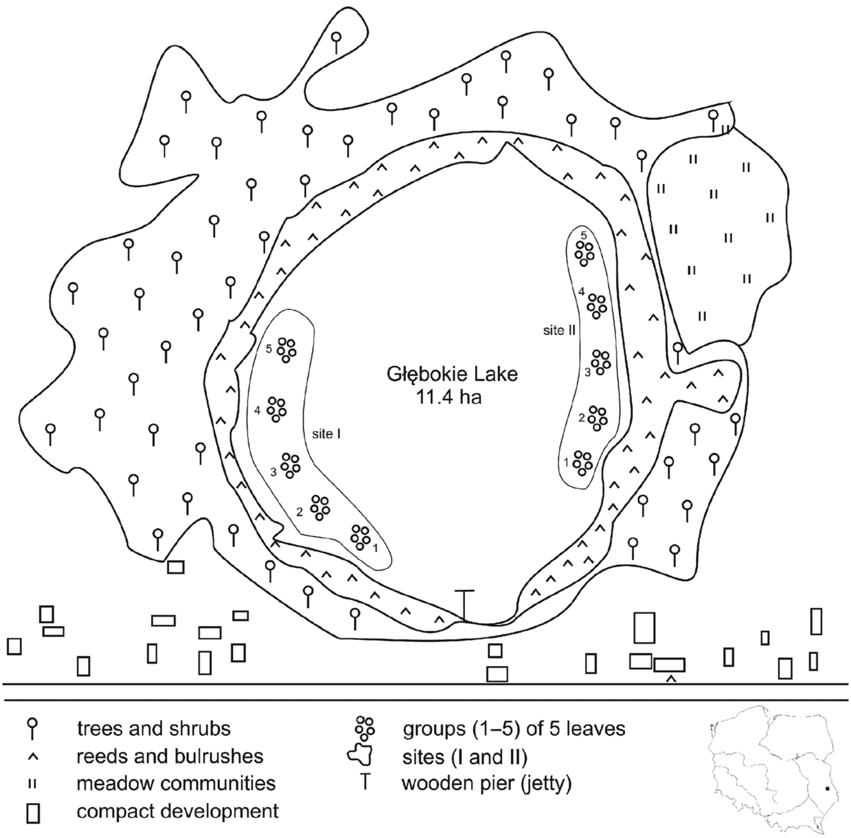

Fig. 1. Precise locations of nympheids in the study area

The two sites were located on the opposite sides of the lake and consisted of belts measuring several dozen metres in which *N. lutea* occurred. Yellow water lily was present at depths no further than 20 m from the edges of the rushes surrounding the lake (Figure 1). The two sites (site I and site II), located 200 m apart, differed in sunlight exposure, wave strength, wind exposure, substrate type, physicochemical conditions, water depth, and the depth at which the leaves were rooted to the bottom (Figure 2, Table 1).

Site II was exposed to the wind in much greater way than site I. As the consequence, the waves were bigger than at site I. Open space, stronger exposure the wind and bigger wave strength caused water to flow over leaf surfaces or flood them for longer periods. Lower density of *N. lutea* at site II indicates that it was less favourable habitat for the plants than site I.

Fig. 2. Microhabitat differences at the two sites in Głębokie Lake. Light conditions and wave strength: *a* – site I; *b* – site II. Leaf damage caused by *Galerucella nymphaeae* and decay factors (in July): *c* – site I; *d* – site II. All photos were taken on the same day

Table 1

Differences in abiotic and biotic factors between sites

Factor	Site I	Site II
Sunlight exposure	sunlit until early afternoon, then strongly shaded by tall trees and bushes	sunlit all day
Wave strength (during analogous, moderate wind force)	usually low or none (places shielded from the wind, shorter petioles)	pronounced, frequently causing water to flow over leaf surfaces or flood them for longer periods (open space, longer petioles)
Plant density	large, densely arranged leaves, frequently overlapping leaf blades; average distance between leaf blades 0–30 cm	moderate or low, leaves rarely overlap, often float separately; average distance between leaf blades 0–100 cm
Width of plant belt	10–15 m	3–8 m
Leaf rooting depth	0.6–1.4 m	1.0–1.8 m
Bottom	sandy, covered with a layer of decomposing leaf material (sediment) several cm thick	sandy, with calcium carbonate, almost no rotting plant material (sediment)

Field research

The present study consists of observations conducted in Lake Głębokie in 2012, and in the case of details of the development cycle of the leaf beetle, also observations from 2013.

At each site and in each month of the study, five leaf blades were collected from five locations (within each site), spaced about 10 metres apart. Thus the total sample consisted of 25 leaf blades. The research was carried out once a month from May to September. There were 125 laminae collected from each of the two sites during the entire study, so that the total material consisted of 250 *N. lutea* leaves.

Leaves were collected according to the methodology described by KORNIJÓW (1998) and KORNIJÓW and ŚCIBIOR (1999a). A hand net was placed under each leaf to collect the hydrobionts inhabiting the underside of the blade, and then the petiole was cut with scissors under water. After draining off the water, the leaves were transferred together with the invertebrates into sealed plastic bags (5 leaves per bag); in addition, organisms visible to the naked eye that remained on the edges of the net were hand-picked with tweezers. The leaves were cooled in a cooler, taken to the laboratory.

Laboratory work

In laboratory, the water lily leaves were transferred to white cuvettes, where they were thoroughly rinsed and all organisms were removed from them. Then the leaves were blotted with filter paper and scanned whole or in parts (together with a millimetre scale), depending on the size of the leaf surface, and saved to a graphic file. When a leaf was scanned in parts, the image was reassembled into a whole using CorelDraw X8 software. On the graphic image of each leaf, the area of all damage was measured in ImageJ software, distinguishing three types of damage: GN, MO, and PH. GN damage consists of holes perforating the leaves or gnawed into the parenchyma, caused by feeding adults and larvae of *G. nymphaeae*. MO designates damage to the leaves beginning with the appearance of dark spots on their surface, caused mainly by fungal microorganisms (moulding and then rotting), and less frequently by bacteria outside the gnawed holes and on the edges of the lamina, and with time, also on the edges of holes previously gnawed by beetles, as well as leaf fragments torn or fallen off due to the activity of microorganisms. PH is physical damage, usually appearing on the edges of the leaves or penetrating the leaf in a triangular shape, natural cracks of the leaf surface, damage arising during the growth of the leaves, damage caused by water birds, weakened fragments broken off by waves, and other damage not caused by decay.

The areas of all types of damages were given in cm^2.

Statistical analysis

The statistical analysis was aimed at determining the impact of two factors, i.e. location (site I, site II) and date (May, June, July, August, September) on five parameters: biomass and density of *G. nymphaeae*, and GN, MO and PH damage.

In the case of *G. nymphaeae* biomass, *G. nymphaeae* density, and PH damage, the data transformations applied failed to achieve compliance with the normal distribution (Shapiro–Wilk test), so the nonparametric Mann–Whitney U test (Z) was used to compare those parameters between sites, and the nonparametric Kruskal–Wallis test (H) was used to verify the supposition that *G. nymphaeae* biomass, *G. nymphaeae* density, and PH damage to the leaves differed significantly between months. Multiple comparison tests were performed to determine the months between which significant differences in the features occurred. The results are presented in bar charts or profile plots.

Two-way ANOVA was performed to verify whether the mean level of GN and MO damage to water lily leaves differed between the two study sites during the leaf beetle season. Before the test was performed, the data were decimal log-transformed to achieve normal distribution. In addition, box charts were used to remove two outliers that disrupted the homogeneity of variance (Levene's test). If significant differences were detected between the mean areas of damage, post-hoc Tukey tests were used.

All tests were performed using Statistica 13.1 software, at a significance level of 0.05.

Results

Seasonality of the occurrence of *Galerucella nymphaeae*

The individual stages of the developmental cycle of *G. nymphaeae* in Lake Głębokie are presented in Figure 3. In the second third of May, numerous adults were already observed, having left their winter hiding places, and were feeding intensively on numerous, fully developed leaves. After copulation (it should be noted that the last copulating pairs were observed in the first 10 days of August), eggs were laid on the leaves. They were laid in masses, with an average of 12 eggs per mass in May, 15 in June, and 9 in July. Larvae were most numerous from June to July, with only isolated individuals found in August. The first beetles of the summer generation appeared in mid-June, and a few adult individuals were found at the end of August. It is very likely that they began to leave the aquatic habitat for their winter hiding places on land at the beginning of September.

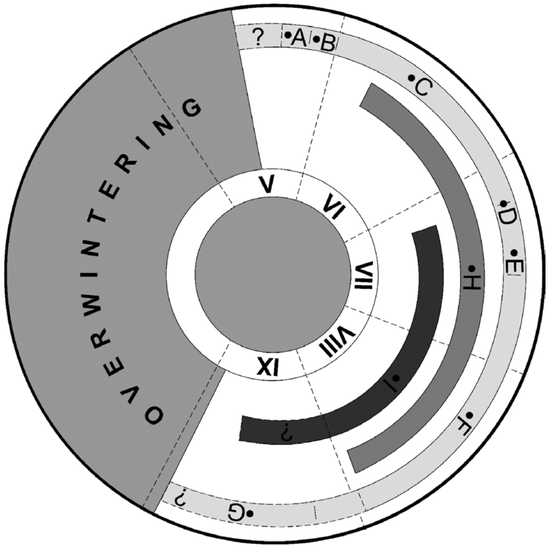

Fig. 3. Life cycle of *Galerucella nymphaeae* in Głębokie Lake. Development stages: light grey strip – adult, medium-grey strip – larva, dark grey strip – pupa, V–IX – months May – September; A – leaving of winter hideouts and complementary feeding of adults; B – first copulating pair; C – egg laying; D – end of egg laying; E – disappearance of beetles of wintering generation; F – appearance of beetles of the summer generation; G – preparing for overwintering; H – period of appearance and occurrence of all larval stages; I – period of appearance and occurrence of pupa stage

Table 2

Numbers of *Galerucella nymphaeae* adults and larvae found at each site in each month

Months	Adults		Larvae	
	site I	site II	site I	site II
May	19	33	0	0
June	25	1	30	2
July	15	3	36	11
August	2	4	2	2
September	0	0	0	0
Total	61	41	68	15

Galerucella nymphaeae adults were caught from May to August, with peak abundance in May (at site II) and June (at site I) – Table 2. More adults were caught in total at site I (61) than at site II (41). *Galerucella nymphaeae* larvae were found in the habitat from June to the end of August (they were undoubtedly also present on the leaves at the beginning of September), with peak abundance in July at both sites (Table 2). Many more larvae (68) were caught at site I than at site II (15). The adult/larvae ratio was 47/53 at site I and 73/27 at site II. The small number of larvae relative to adults at site II may indicate increased mortality of these stages at this site.

Density of *Galerucella nymphaeae*

The two sites differed in terms of sun exposure, wave strength, and wind exposure. In the case of adults, the site was shown to have a significant effect on the mean density of leaf beetles ($Z = 2.24$; $p = 0.025$). The average density of *G. nymphaeae* was higher at site I (0.103 individuals/100 cm^2 leaf area) than at site II (0.072 individuals/100 cm^2) – Table 3. The mean density of *G. nymphaeae* larvae was also higher at site I (0.145 individuals/100 cm^2 leaf area), but there was no significant effect of the location (site) on the density of larvae on the water lily leaves ($Z = 1.11$; $p = 0.240$).

Table 3

Mean densities (ind. per 100 cm^2 of *Nuphar lutea* leaf area ± SE) of *Galerucella nymphaeae* adults and larvae found at each site in each month

Months	Adults		Larvae	
	site I	site II	site I	site II
May	0.129±0.04	0.229±0.14	0	0
June	0.170±0.08	0.006±0.006	0.186±0.10	0.019±0.01
July	0.097±0.009	0.023±0.01	0.235±0.04	0.090±0.04
August	0.015±0.009	0.030±0.02	0,015±0.009	0.012±0.012
September	0	0	0	0
For site	0.103±0.02	0.072±0.04	0.145±0.04	0.040±0.01

Observations were made over several months, which corresponded to the season of occurrence of *G. nymphaeae* on yellow water lily leaves. The time factor (month) was found to have a significant effect on the density of adults ($H(4, 50) = 17.71$; $p = 0.001$). The highest mean density of *G. nymphaeae* adults was recorded in May, and the smallest in June at site II (Table 3). Post-hoc tests showed statistically significant differences between densities of adults in May and September. The difference in density between May and August was smaller. It can therefore be assumed that May is the period in which *G. nymphaeae* adults cause the greatest damage to the lamina of the host plant.

The situation was similar for larvae. For this stage of development as well, the month of the study proved to be statistically significant for the average density on water lily leaves (H (4, 50) = 22.97; $p < 0.001$). However, the highest average density of *Galerucella nymphaeae* larvae was recorded in July (Table 3), which undoubtedly intensified damage to the water lily leaves during this period.

Biomass of *Galerucella nymphaeae*

The box charts (Figure 4 and Figure 5) show the distribution of *G. nymphaeae* biomass in the study period for adults and larvae, respectively. Analysis of Figure 4 shows that adult biomass in May was significantly higher than during the rest of the period of occurrence of leaf beetles. In the second third of September, adult beetles were no longer seen on the water lily leaves. The significant effect of time on the biomass of adults was confirmed (H (4, 50) = 23.49; $p < 0.001$). The mean biomass of adults was greater at site II (0.019 g). Unlike density, however, adult biomass was not found to differ significantly between sites ($Z = 0.58$; $p = 0.561$).

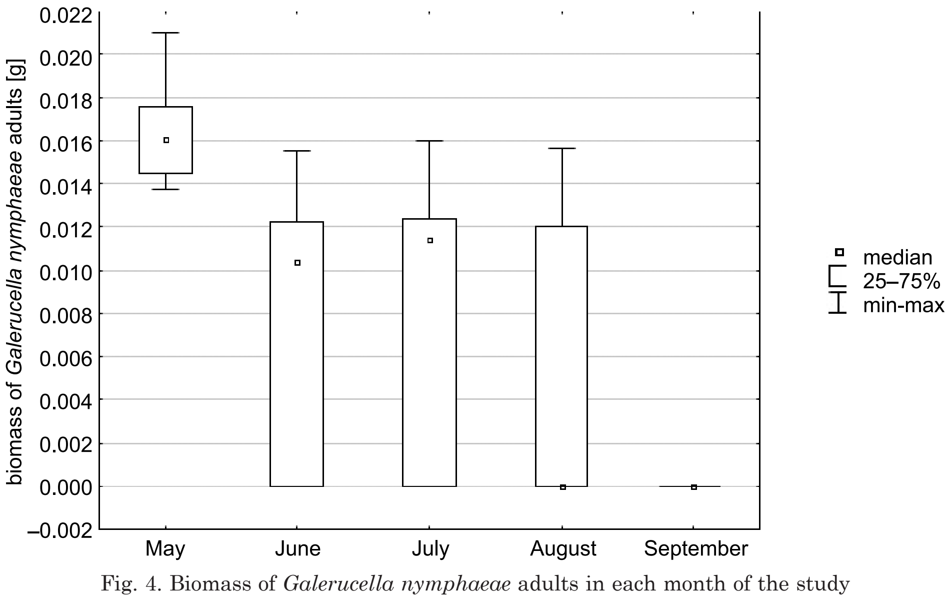

Fig. 4. Biomass of *Galerucella nymphaeae* adults in each month of the study

In the case of larvae, average biomass was higher at site I (0.007 g) than at site II (0.004 g). As in the case of adults, the study site had no significant effect on the average variation in larval biomass ($Z = 1.22$; $p = 0.221$). The average biomass of larvae, however, as in the case of density, differed significantly between months of research (H (4, 50) =19.30; p = 0.0007). Analysis of Figure 5 and the post-hoc multiple comparison tests indicate that the greatest differences in larval biomass occurred between May and July and between July and September. July was the month with the highest median biomass of larvae.

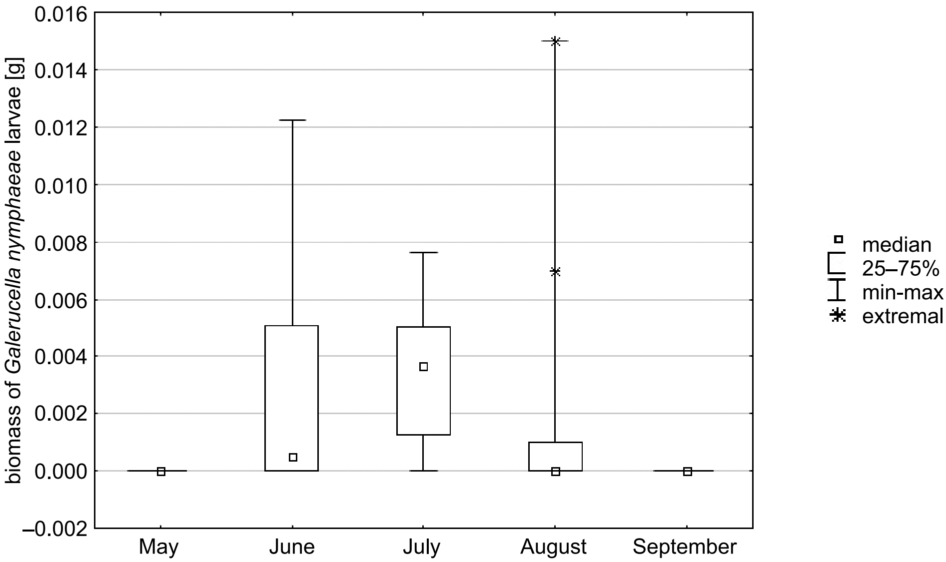

Fig. 5. Biomass of *Galerucella nymphaeae* larvae in each month of the study

Damage caused by *Galerucella nymphaeae* (GN)

As it is not possible to distinguish which holes in the yellow water lily leaves are the result of activity by adults and which are caused by larvae, for further analysis the damage was treated jointly for both stages. There were statistically significant differences in the degree of damage to the leaf blade by *G. nymphaeae* between sites I and II (two-way ANOVA; F (1, 38) = 38.69; $p < 0.001$). Greater damage was found at site I (Figure 6), where the mean area of damage caused by leaf beetles was 4.03% of the leaf blade area (range: 0.04–33.44, SD = 4.62). At site II, the average area of damage caused by *G. nymphaeae* was 1.73% of the leaf blade area (range: 0.00–16.95, SD = 2.42). A significant effect of the time factor on damage to the leaf blade caused by GN feeding was found as well (two-way ANOVA; F (4, 38) = 7.83; $p < 0.001$). The greatest mean damage to the leaf blade was recorded in May at site I. Significant damage to the leaves was also noted in August (sites I and II) – Figure 7.

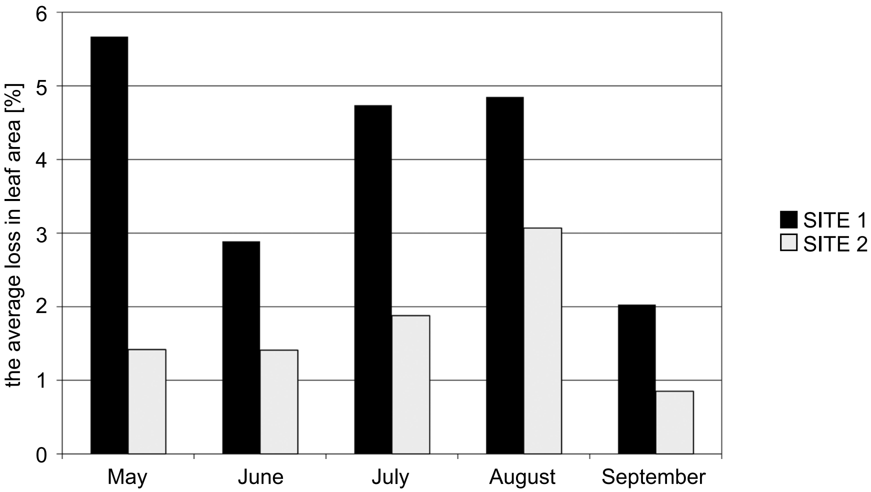

Fig. 6. The average loss in leaf area of yellow water lily [%] at sites I and II in each month of the study due to feeding by *Galerucella nymphaeae* adults and larvae (GN)

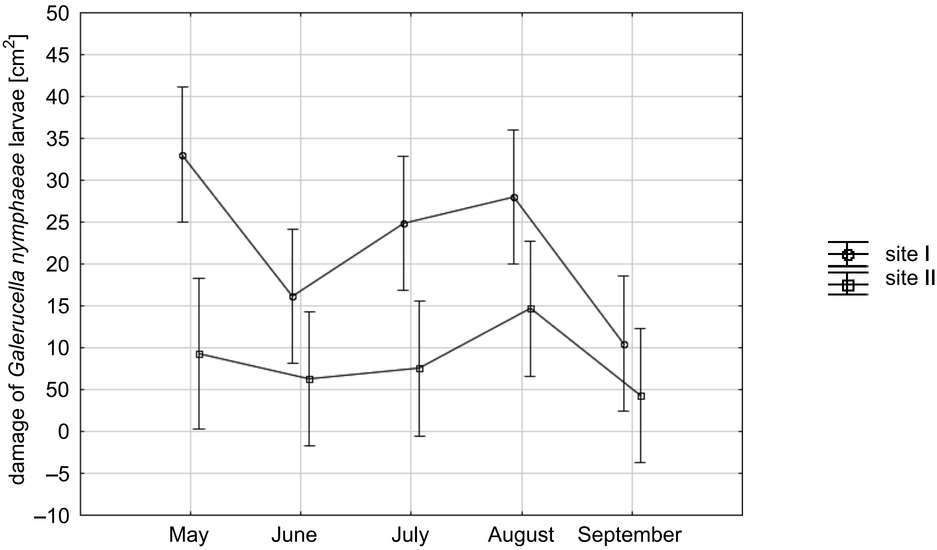

Fig. 7. Profile plot of average loss in leaf area of yellow water lily at sites I and II in each month of the study due to feeding by *Galerucella nymphaeae* adults and larvae (GN)

To assess the influence of the study site and month of research on damage caused by GN feeding, the following coefficients were determined based on the analysis of variance: ω^2 = 38.9% for sites and ω^2 = 18.8% for months. They show that the nature of the habitats (study sites) and the month of research explain 58% of the variation in damage to the lily leaves generated by GN feeding. The rest, i.e. about 42% of the variation in this feature, was associated with sources of variation that were not tested in the research.

Damage caused by microorganisms (MO)

The second and equally important factor influencing the processes of decomposition of *N. lutea* leaves, after *G. nymphaeae*, was microbes appearing at the sites of damage caused by feeding by *G. nymphaeae*. Figure 8 shows the average losses of water lily leaf area caused by microbes. Greater damage was found at site I, where the average damage to the leaf blade was 7.34% of the leaf area (range: 0.00–77.07, SD = 11.62). At site II, the average damage caused by MO accounted for 4.88% of the leaf blade area (range: 0.0–53.98, SD = 8.93). The ANOVA test, however, showed no statistically significant differences in the mean loss of leaf area between sites (two-way ANOVA; $F (1, 38) = 3.65$; $p = 0.064$). On the other hand, the variation in individual months was pronounced (two-way ANOVA; $F (4, 38) = 20.79$; $p < 0.001$). Tukey's post-hoc tests showed that the months of June, July and August formed a homogeneous group in terms of the magnitude of damage caused by MO ($p > 0.05$). The variation in this damage in the remaining months was statistically significant ($p < 0.05$). The average area of leaf damage caused by MO was the smallest in May and by far the largest in September, which undoubtedly results from prior damage generated by *G. nymphaeae*.

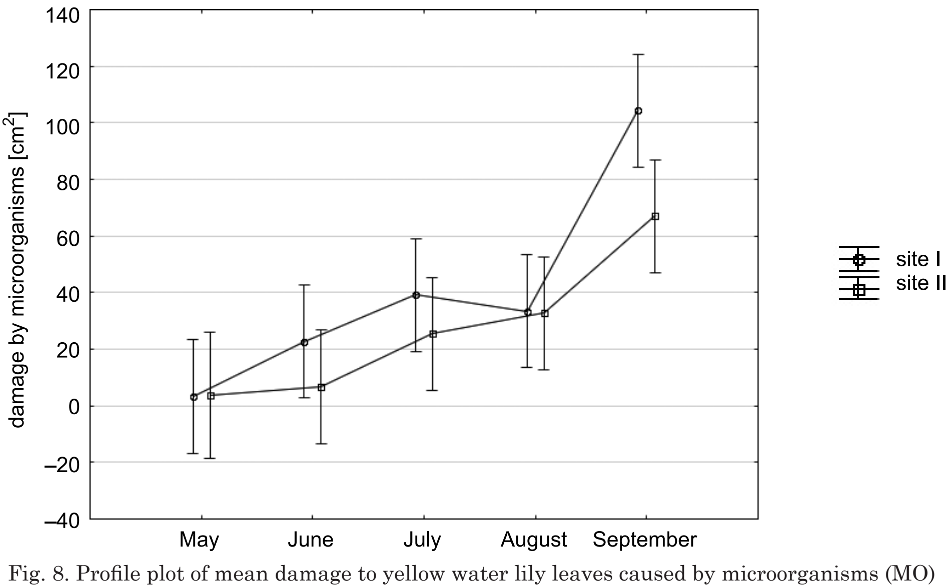

Fig. 8. Profile plot of mean damage to yellow water lily leaves caused by microorganisms (MO) at sites I and II in each month of the study

Damage caused by physical factors (PH)

Physical damage to yellow water lily leaves was not statistically significantly influenced by variation in habitat conditions ($Z = 0.58$; $p = 0.550$) or by the time factor (H (4, 46) = 8.64; $p = 0.072$). At the same time, it was determined that at site I the average damage to the leaf blade resulting from PH was smaller than damage by GN or MO and accounted for 0.07% of the leaf area (range: 0.00–2.52, SD = 0.295), while at site II the mean damage caused by PH was 0.03% of the leaf blade area (range: 0.0–0.87, SD = 0.113). The greatest PH was recorded in June (Figure 9).

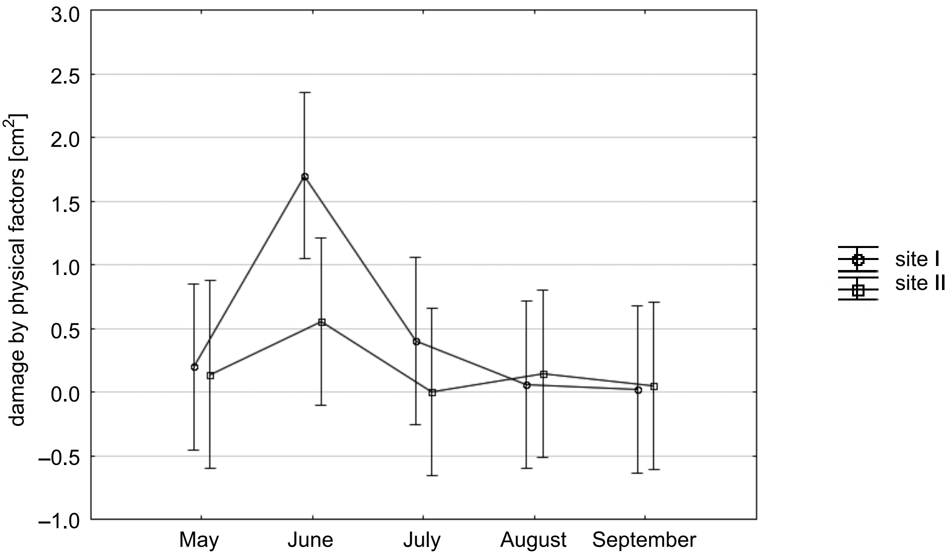

Fig. 9. Profile plot of mean damage to yellow water lily leaves caused by physical factors (PH) at sites I and II in each month of the study

Discussion

The degree of damage to *N. lutea* leaves is directly dependent on the size of the leaf beetle population. The level of damage initiated by the leaf beetles at the start of the growing season in turn affects the rate and scale of damage by microorganisms, and also increases the level of physical damage. Therefore, the degree of development of the *G. nymphaeae* population is crucial to the scale and rate of damage to and decomposition of *N. lutea* leaves during the growing season.

The conducted research indicate that the developmental cycle of *G. nymphaeae* in Lake Głębokie is similar to that described in a study by Brovdij (1973) for Ukraine. In Lake Głębokie, adults of the summer generation were observed during copulation as late as 12 August 2013, but it is not known whether they are able to complete the full development cycle by the end of the plant growing season. Also, the average number of eggs laid by beetles was lower than in BROVDIJ'S (1973) observations. The beetles of the overwintering and summer generations (before overwintering) are present in the habitat at the same time in part of their cycle; from the end of July and beginning of August a significant decrease is observed in the numbers of both adults and larvae (Table 2, Table 3). At the beginning of the development cycle, the greatest threat to the leaves is adult beetles, and after leaving the eggs laid by them, the larvae feeding together with them as well (Figure 3).

The most voracious of these is the third stage, which in a small eutrophic reservoir in central Finland was found to be responsible for 77% of consumption by all three developmental stages (SETÄLÄ and MÄKELÄ 1991). Feeding by beetles of the summer generation is less intensive, in part because of their smaller numbers and in part due to the smaller number of undamaged leaves, which are most rapidly damaged in eutrophic water bodies and last just over 20 days in Finland. However, they are replenished in the habitat during most of the growing season, which is crucial for subsequent developmental forms of *G. nymphaeae* (SETÄLÄ and MÄKELÄ 1991), whose full developmental cycle lasts much longer (Figure 3). Intensive feeding by the beetle on leaves, however, may shift the production of floating leaves towards production of submerged leaves (KOUKI 1993).

The average damage to the leaves of *N. lutea* caused by *G. nymphaeae* ranged in the Głębokie Lake from 1.73% of the leaf blade area at site II (max 16.95) to 4.03% at site I (max 33.44), which is quite low. The results of research by other authors indicate that damage to various macrophytes, including the yellow water lily, by herbivorous species can often exceed 10%, sometimes reaching up to 30.6% (WALLACE and O'HOP 1985, KOUKI 1991b, NEWMAN 1991, KORNIJÓW 1996, BOLSER and HAY 1998, LODGE et al. 1998). BROVDIJ (1973), based on the results of other researchers from the beginning of the last century, reports that in Canada (British Columbia) these beetles appeared on the leaves in such large numbers that their surface was completely destroyed. The feeding beetle utilizes mainly proteins from the leaves for its energy needs, leading to a rapid loss primarily of phosphorus and other nutrients from the leaves, while its droppings and the leaves decomposed by it form microbiologically rich, high-quality detritus which is added to the system throughout the growing season (WALLACE and O'HOP 1985). The minor damage caused by *G. nymphaeae*, as compared to the literature data, resulted from low densities of the beetle: the average density of adults was 0.072 individuals per 100 cm^2 leaf blade at site II and 0.103 individuals per 100 cm^2 leaf blade at site I, while larval density was 0.040 and 0.145, respectively. WALLACE and O'HOP (1985) reported a density of larvae alone of 4.6 ind./100 cm^2 of leaf blade on *N. lutea* leaves in the Ogeechee River. A few years earlier, the average density of adult forms of this beetle on *N. lutea* leaves in Lake Głębokie was 44.8 ind./100 cm^2 at site I and 25.6 ind./100 cm^2 at site II, i.e. several hundred times higher (KORNIJÓW and ŚCIBIOR 1999b). Fluctuations in the population size of this beetle are irregular and occur every two or three generations (NOKKALA and NOKKALA 1989a), so they are not necessarily the result of the emergence of a new factor limiting the abundance of the species in the biocoenosis of the lake.

Biological factors affecting the rate and sequence of leaf decay varied depending on the month. In late spring and summer, *G. nymphaeae* (GN) beetles have the primary role in damaging the leaf blade, with a small contribution of physical factors (PH) in the summer. According to observations by WALLACE and O'HOP (1985), actual leaf consumption by this herbivore is much smaller than the damage resulting from its feeding. In summer and early autumn, damage caused by *G. nymphaeae* was conducive to the rapid development of Ascomycetes fungal microorganisms and chromistan fungal analogues of the class Oomycota, which completed the decomposition of the leaves in the Głębokie Lake. Research conducted by MAZURKIEWICZ-ZAPAŁOWICZ et al. (2016) showed that on the leaves of *N. lutea* this is usually a group of anamorphic, specialized (or unspecialized) pathogens common in Europe, belonging to several species of the genera *Alternaria*, *Chaetomium*, *Cladosporium*, *Colleotrichum*, *Elongisporangium*, *Fusarium*, *Gibberella* and *Septoria*. In addition, phylloplane fungi inhabiting both surfaces of the leaf utilize plant metabolites (amino acids, carbohydrates, auxins and other compounds) as a food source and colonize the leaves of *N. lutea* before they develop, with maximum abundance from June to the end of summer (MAZURKIEWICZ-ZAPAŁOWICZ et al. 2016), which was also confirmed in the present study (Figure 8). The development of fungal species also occurring in bottom sediments under plants increases with increasing concentrations of nitrates and phosphates in the water. Species of fungal microorganisms have also been shown to more readily inhabit the upper surface of the leaf than the underside, where there is a greater risk of being washed away by waves (VORONIN 2014). Such a situation in Lake Głębokie was observed at site II (where the waves were stronger), which was confirmed and reflected in the results obtained.

Damage to the leaf blade by microorganisms in the lake remained at a low level for most of the season, with a sharp increase in September, when it was clearly the predominant type of damage, reaching values more than three times higher than the maximum leaf damage previously caused by *G. nymphaeae*. The sharp increase in damage caused by microorganisms in September is associated with the natural process of decomposition of leaves in autumn, but a relationship with damage caused by *G. nymphaeae* in previous months can be found here. In addition to fungi, proteolytic bacteria may account for a significant proportion (up to 100%) of spring and also autumn leaf decay. On water lilies in the conditions of Poland, these are mainly taxa belonging to the Enterobacteriaceae, to the groups *Flavobecterium-Cytophaga* and *Arthrobacterium-Corynebacterium* (DONDERSKI and KALWASIŃSKA 2002). Damage caused by microorganisms was much greater at site I, where higher abundance, density and biomass of beetles were noted, as well as greater leaf damage induced by them. This indicates that the damage caused by feeding beetles during the summer contributes significantly to the increase in damage caused by microorganisms in later months. However, the optimal temperature for bacterial proteases is 18°C (DONDERSKI and KALWASIŃSKA 2002). Leaves damaged by gnawing of the upper epidermis and consumption of the parenchyma by beetles (many star-shaped cells in the gut) are much more susceptible to the activity of microorganisms, which have easier access to leaf tissues (SETÄLÄ and MÄKELÄ 1991). This results in accelerated leaf decomposition. A severe reduction in the lifespan of individual *Nuphar* leaves due to the activity of *G. nymphaeae* has been found by many authors (WALLACE and O'HOP 1985, JULIANO 1988, KOUKI 1991a). Apart from the obligatory herbivores inhabiting the leaf blade, the vast majority of the epifauna of *N. lutea* prefers the material of ageing leaves (VAN DER VELDE 1980).

Physical damage did not play a significant role for leaves in the lake, but the limiting effect of the presence and feeding of coots biting off the leaf blades of nympheids on the density of *G. nymphaeae* in the summer has been described (PAILLISSON and MARION 2001).

Water lily leaf beetles are relatively sedentary herbivores; adults do fly between neighbouring plants, but typically only for a few seconds at a time (R. BOLSER, cited after BOSLER and HAY 1998). There are locations where *Nuphar* is common but *Galerucella* is conspicuously absent, suggesting that the beetles' dispersal ability may be limited at larger scales (BOSLER and HAY 1998). Our results indicate that *G. nymphaeae* dispersion may be limited not only at a large spatial scale, but also at a much smaller scale. Despite the small distance between sites in the present study (about 300 m), there were differences in the number of individuals caught, density, and biomass of larvae and adults between the two sites. Site I was considerably more favourable to the development and life of the beetles, as more individuals were caught and their density and biomass were higher. More adults were caught at site I (61) than at site II (41), as well as many more larvae (68) than at site II (15). The ratio of adults to larvae was 47/53 at site I and 73/27 at site II. The small number of larvae in relation to adults at site II may indicate higher mortality of these stages at this site. The larvae died sooner, without reaching their maximum size. The biomass of adults was also lower than at site I, which indicates a lower rate of leaf consumption. Physical factors clearly distinguishing the two sites were wind and waves: site I was located near a forested shore, shielded from the wind, where waves were minimal (Figure 1 and Figure 2). Site II was in open water and was exposed to stronger wind and strong waves. These two environmental factors (wind and waves) most likely resulted in the lower numbers, densities and biomass of both adults and larvae of *G. nymphaeae* at site II. The influence of wind and waves at site II in Lake Głębokie probably accelerated the sinking of damaged leaves, forcing the beetles to migrate and colonize new ones more quickly. The less stable environmental conditions and, of necessity, more intensive migration of beetles at site II was most likely the cause of their increased mortality and consequently the lower abundance, biomass and density of *G. nymphaeae* at site II. Similar observations can be found in research by other authors. The mortality of the larvae can be very high, reaching up to 98% (WALLACE and O'HOP 1985), and most of them die during colonization of subsequent leaves (KOUKI 1991b). HARRINGTON and TAYLOR (1990) found that even fine-scale (within the plant) spatial changes in the environment of invertebrate herbivores may cause high mortality in these herbivores if they are not able to efficiently migrate to new available resources. When a resource patch (e.g. a leaf within a plant) is depleted, mortality during dispersal may be a key factor for the overall mortality rate (STILING 1988, HARRINGTON and TAYLOR 1990). It is also evident that the spatial and temporal scale (ecological neighbourhood of an animal) of interest may vary between an animal's individual developmental stages (ADDICOTT et al. 1987), i.e. can be different for larvae and adult insects. Faster sinking of leaves due to wind was the main cause of such high larval mortality at site II. *Galerucella nymphaeae* larvae in Lake Głebokie, as stages with much lower dispersion capacity than adults, were not able to colonize new *N. lutea* leaves fast enough. This thesis is confirmed by results obtained by other authors. According to KOUKI (1991a), *N. lutea* leaves disappear quite rapidly due to herbivory, and the developing larvae must emigrate from the sinking leaves. According to this author, no beetle was able to survive from egg to adult on a single leaf, as it took the beetles only about 3 weeks to cause severe damage, while the developmental time of the beetle is much longer (5–6 weeks). Hence, they have to migrate and colonize other leaves. Such migration may cause severe mortality in the herbivore population, as the environment between the leaves is hostile. Mortality between successive larval instars can be up to two thirds (KOUKI 1991a).

Conclusions

The observations and results obtained in the present study indicate that besides the biotic factors affecting the yellow water lily leaves and causing their decomposition, abiotic factors acting on the scale of the lake (primarily wind and waves) are important as well. In the case of Lake Głębokie, site II, which was more exposed to the effect of wind, causing stronger waves, was clearly less favourable to colonization by *G. nymphaeae* as well as by microbes carrying out the process of leaf decay at the end of the season. As a result of these habitat conditions, the *N. lutea* population at site II is more stable, although smaller. At the same time, it is less susceptible to decomposition, which takes place here on a much longer time scale.

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ALGINATE CHARACTERISTICS AND FUCOXANTHIN CONTENT FROM *SARGASSUM POLYCYSTUM* THROUGH ULTRASOUND-ASSISTED EXTRACTION AND THE RELATIONSHIP WITH WATER QUALITY OF HABITAT

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Key words: macroalgae, alginate, fucoxanthin, ultrasound-assisted extraction (UAE), water quality.

Abstract

The study attempted to obtain alginate and fucoxanthin from *Sargassum polycystum* C. Agardh using ultrasound-assisted extraction (UAE) and to correlate these with the water's environmental condition. The alginate was extracted using an acid pathway, the fucoxanthin was quantified using high-performance liquid chromatography, and the trace element was obtained using an atomic absorption spectrophotometer (AAS). The nutrients and micronutrient composition of *S. polycystum* were dominated by carbohydrates (32.8; 1.6%), with the main trace elements being selenium, iron, copper, and zinc. The alginate was attributed to 8.98±0.1 pH, 12.55±0.5% of water content, 22.63±0.9% of ash content, 70.28±0.5 Cps. of viscosity, and 37.24±0.8% of yield. The extract was also containing fucoxanthin, which was relatively high (0.41–0.59 mg g^{-1}). There was a favourable association between water quality and the content of alginate, fucoxanthin, and nutrition. According to the ultrasonication extraction process, *S. polycystum* could produce high-quality alginate with a high fucoxanthin content.

Introduction

Seaweed as macroalgae is divided into three phyla based on the pigment colours, which are brown seaweed (Ochrophyta, class Phaeophyta),

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red (Rhodophyta), and green (Chlorophyta). More than 4400 species spread throughout the world, among which red species are the most abundant (DAWES 2016). Seaweed in Indonesia is an essential commodity, with 9.3 million tons of cultivated products in 2018 (FAO 2020). The study conducted by PUSPITA et al. (2020) stated the national production was still dominated by red seaweed species, specifically *Kappaphycus alvarezii*, *Eucheuma spinosum*, and *Gracilaria* sp., while *Gelidium* spp. and *Sargassum* spp., are still based on the natural harvested. *Sargassum* is the most diverse brown seaweed and has been reported to dominate Indonesian waters in terms of biomass and abundance, especially in the intertidal zone (WOUTHUYZEN et al. 2016, SETYAWIDATI et al. 2018, SUMANDIARSA et al. 2020b).

One types of *Sargassum* that is most often found in Indonesian waters is *Sargassum polycystum*. The study results by SUMANDIARSA et al. (2021a), found the distribution of this species varied between locations and seasons in Indonesian waters. The nutrients contained was also rich with carbohydrate is the highest, between 40–50% dry weight (KUMAR et al. 2015, PRAIBOON et al. 2018, SALOSSO 2019). In addition, the micronutrients contents such as iron (Fe), manganese (Mn), and iodine (I), were relatively high, which has potential to be used as additives in seaweed-based food and non-food products (FLORES et al. 2015, CIRCUNCISÃO et al. 2018, SUMANDIARSA et al. 2020a).

Alginate, as a polysaccharide, has been extracted from *Sargassum* seaweed, with the characteristics were comparable to those main resources. Currently, the largest alginate producers were *Laminaria* spp., *Lessonia* spp., and *Macrocystis*, accounting for 89% of world production (PORSE and RUDOLPH 2017). Several studies showed *Sargassum* produced a variation of alginate yields, such as about 8.5–45.54% from *S. cristaefolium* (SUGIONO et al. 2019), 23% from *S. natans,* and 17% from *S. vulgare* (RHEIN-KNUDSEN et al. 2017), 12.09–25.77% and 28.22% from *S. polycystum* (DHARMAYANTI et al. 2019, SUMANDIARSA et al. 2020a), and about 24% from *Sargassum* sp. (MOHAMMED et al. 2020).

Meanwhile, fucoxanthin as bioactive compound was also reported as valuable composited and found quite high in *Sargassum*, essentially in *S. horneri* (3.7 mg g^{-1}) and *S. cinereum* (0.179 mg g^{-1}), as well as *Sargassum* sp. (0.47 mg g^{-1}) (KUMAR et al. 2013, NURSID et al. 2015, RENHORAN et al. 2017, MIYASHITA et al. 2020). Thus, bioactive contents found varied depending on species, habitat temperature, depth, season, and the use of extraction method (PÁDUA et al. 2015, TERASAKI et al. 2017).

In response to the information stated above, the purposed of this study is to determine the effect of employing ultrasound-assisted extraction

(UAE) in the production of alginate and fucoxanthin from *S. polycystum*, as well as the influence of water quality on the habitat.

Materials and Methods

Sampling location

Brown *seaweed Sargassum polycystum* was taken from Tidung island waters in October 2019 (Figure 1). The location characteristics were flat and coral formed islands with classic sandy, muddy, and dead coral sediment. Anthropogenic pressure seems to be a big problem on Tidung Island, caused by the dense population (4,651 people km^{-2}) and tourism activities. The limitation of rainfall has also reduced the flow of organic matter to the coastline, in consequence, it was less fertile. However, *Sargassum* were still grow well and dominated as attached to the dead corals or any concrete substrate.

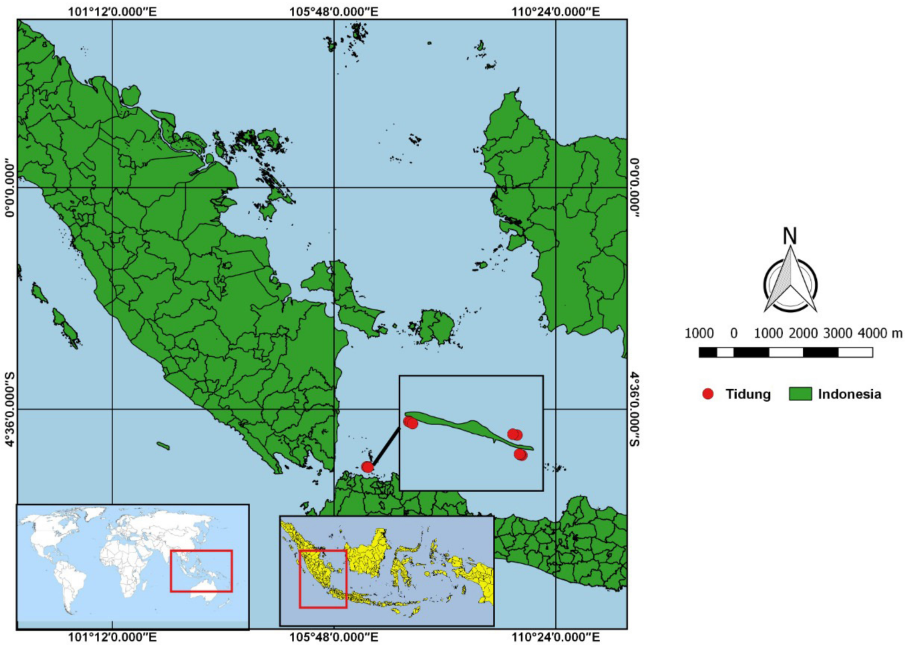

Fig. 1. Research location at Tidung Island, Seribu Island District, Jakarta

Research procedures

Sample collection and preparation

Seaweed samples were taken at optimal low tide with a depth between 15–30 cm. The distance between sampling points was approximately 50 meters parallel to the shoreline. Sample preparation was divide based on nutrition and alginate extraction purposes which were using dried samples, while fucoxanthin extraction from fresh pieces. Drying is done by dried the sample directly under the sunlight for two days, then stored dry until the next test. Fresh samples were prepared through a cleaning process with seawater, then stored in Ziplock plastic that had been given 90% ethanol. The samples were then stored in Styrofoam boxes provided ice to keep them cool and protected from direct sunlight. Furthermore, the samples were stored in cold storage with a temperature of -23^{o}C before further analysis.

Water quality

Parameters measured were dissolved oxygen (DO), pH, temperature, salinity, brightness, nitrate, phosphate, and ammonia. Physical chemistry parameters were measured directly at the research site (in situ) using the multi-parameter testing tool 1P67 Combo 8630. The nitrate, phosphate, and ammonia parameters of seawater were tested using a UV–VIS spectrophotometer using the APHA method (2017).

Nutritional composition analysis

Proximate composition

The Determination includes water, ash, protein, fat, crude fibre, and carbohydrate content. Water content (%) analysis conducted by 2 grams sample and placed in an oven at 105^{o}C for 3 hours. The oven-dried sample was placed in a desiccator until it cooled and then weighed. Ash content was tested through an ashing process using a muffle furnace at a temperature of 550^{o}C until it was white and free of carbon. Samples were weighed after cooling from the desiccator. Both tests refer to the AOAC method. The protein content was analysed using the Kjeldahl method with a conversion factor of 6.25, while the fat was tested by the gravimetric method, which refers to the AOAC standard. The crude fibre composition was tested using the H_2SO_4 destruction method followed by NaOH; then, the measurements were based on the weighing results after the heating process in the Furnace (AOAC 2005). The carbohydrate content of the sample is calculated based on the by difference, which is 100% – (moisture + ash + protein + fat + crude fibre).

Trace elements content

The content of micronutrients in the form of Barium (Ba), Selenium (Se), Iron (Fe), Manganese (Mn), Copper (Cu), Zinc (Zn), and Molybdenum (Mo). 5 g of crushed dry samples were acidified using 5 ml of HNO_3, then stirred. The stirred sample was put in a 100 ml measuring cup, then added 5 ml of HCl and heated in a steam bath for 15 minutes. Then the sample was filtered using filter paper (polycarbonate) with a size of 0.40–0.45 m and added 100 ml of distilled water, stirred again, and analysed with AAS Pin Aacle 900 H with a detection limit of 0.001 mg kg^{-1} with the APHA method, *23rd Edition* in the Proling laboratory of IPB.

Alginate extraction and characterization

Alginate extraction using ultrasonication method (Ultrasound-assisted extraction) based on research by YOUSSOUF et al. (2017). A total of 25 grams of sample was immersed in 250 ml of 80% ethanol then filtered, then extracted on an ultrasonic device in Na_2CO_3 solvent. The Ultrasound was run at 35 kHz for 30 minutes. Furthermore, the precipitation was using a 99% technical isopropyl alcohol solution. All chemicals were purchase from Mallinckrodt chemical, USA.

Alginate yield. Alginate yield was calculated based on the extraction result after the drying process (sodium alginate flour) against the dry weight of the raw material, as figured out below.

$$\text{Yields [\%]} = \frac{\text{weight of alginate acid flour [g]}}{\text{weight of dry seaweed [g]}} \cdot 100\%$$

Water content

The moisture content was obtained from the oven method, namely a 2 g sample was heated in an oven at 105°C for 4 hours, then the weighing was carried out after the sample in the desiccator had cooled. The percentage difference in weight produced is recorded as water content.

Ash content

The ashing method done under a muffle furnace at a temperature of 550^{o}C. A percentage difference between the sample that has become ash and the initial sample is recorded as ash content of alginate.

Viscosity

Determination of alginate viscosity was carried out using a viscometer spindle (TV-10). alginate sample about 2.5 g dissolved in 50 mL of 20% Na_2CO_3 at a temperature of 80^{o}C, then poured into a viscometer tube and set at a speed of 60 rpm. The resulting viscosity value is expressed in centiPoise (cP).

Alginate pH

About 3 g of the samples was dissolved into 197 ml of distilled water. Then, the solution was heated at 80^{o}C for 10 minutes until the alginate was completely dissolved. After the temperature dropped to 250^{o}C, pH measurements were carried out using the IONIX pH5S Spear pH Tester.

Extraction and determination of fucoxanthin content

Extraction using Ultrasound Assisted Extraction (UAE) LC60H made in Germany with 90% Acetone solvent from Mallinckrodt chemical, USA (Pro analyst) with a ratio of 1:6 weight/volume. The frequency of the Ultrasound water bath was 35 kHz with an extraction temperature of <30^{o}C within 120 minutes, where the method was a modification from Kawee Ai et al. (2013). The resulting filtrate was then centrifuged at 4^{o}C and a speed of 4,000 rpm for 20 minutes, then filtered with Whatman 2 paper and evaporated by using a Heidolph HB control rotary evaporator made in Germany. The evaporated solid was then diluted with 10 ml of ethanol and followed by centrifugation at a speed of 3000 rpm for 20 minutes at 4^{o}C. Then the samples were freeze and stored at freezing temperatures (-20^{o}C) before being quantified by HPLC. The fucoxanthin content was analysed using a Shimadzu LC-20A High-Performance Liquid chromatography (HPLC) equipped with a UV–Vis detector and separated using a C18 (20 mm x 250 mm) column (Luna Phenomenex). The mobile phase is HPLC grade water (solvent A) and acetonitrile (solvent B) with gradient elution system: 0 minutes, 80:20 (v/v) A: B, flow rate 15 mL/minute, for 30 minutes, and injection volume 2 mL. The detection wavelength was 254 nm, and the column temperature was 30^{o}C. The amount of fucoxanthin in mg/g was obtained from converting the area of the active fraction of the sample to the standard fucoxanthin curve. The fucoxanthin standard was obtained from Sigma-Aldrich, Germany.

Statistical analysis

All data are displayed with the mean and standard deviation. The response of the habitat environment to the content of nutrients, alginate, and fucoxanthin was analysed using Canonical Correspondence Analysis (CCA) multivariate statistics. Statistical analysis performed by Past Statistical Software V4.02 (HAMMER 2020).

Results

Proximate and trace element contents

The study found that carbohydrate content of *S. polycystum* was the most dominant, reaching 32.8%, followed by ash content (18.2%). The fat and protein contents showed the opposite, which was only 0.6 and 1.45%, respectively. Besides, the highest micronutrient was iron content of 0.219 mg kg^{-1}, followed by zinc, copper, and selenium. In contrast, three trace elements were not detected, which were barium, manganese, and molybdenum. Proximate composition and micronutrient contents are presented in Table 1.

Table 1

Proximate and micronutrient composition of *S. polycystum* (n = 3; mean ±SD)

Test parameters	Amount
Water [%]	18.2 ±0.5
Ash [%]	26.9±0.9
Proteins [%]	1.45±0.1
Fat [%]	0.61±0.08
Crude fibre [%]	20.02±1.5
Carbohydrates [%]	32.8±1.6
Barium [mg kg^{-1}]	0
Selenium [mg kg^{-1}]	0.0011±0.0001
Iron [mg kg^{-1}]	0.219±0.07
Manganese [mg kg^{-1}]	0
Copper [mg kg^{-1}]	0.0083±0.0006
Zinc [mg kg^{-1}]	0.0.0143±0.003
Molybdenum [mg kg^{-1}]	0

Characteristics of ultrasonication extracted alginate

The results obtained from alginate characterization is presented in Table 2, which showed a high yield that more than 37%. The ash and water content levels indicated that the alginate was still in compliance with commercial standards, as they only reached 12.55 and 22.63 percent, respectively. The viscosity value, which was 70 Cps in this study, is one of the most important parameters in determining alginate quality. It is indicated that *S. polycystum* has the potential to be a resource for alginate. This is also supported by a reasonably good pH value of 8.98.

Table 2

Characteristics of alginate (n = 6; mean±SD)

Parameter	Value
Yield [%]	37.24±0.8
Water content [%]	12.55±0.5
Ash Content [%]	22.63±0.9
Viscosity [Cps]	70.28±0.5
pH	8.98±0.1

Fucoxanthin content of *S. polycystum*

The total fucoxanthin extracted from *S. polycystum* is presented in Table 3. Extraction of bioactive from macroalgae using Ultrasound is an environmentally friendly method (green extraction method/technology). The amount of fucoxanthin obtained was relatively high, and there was no statistically significant difference between extractions, with the highest being about 0.59 mg g^{-1} and the lowest being about 0.41 mg g^{-1}.

Table 3

Fucoxanthin from *S. polycystum* seaweed. (n = 6; mean ±SD)

Extraction	Total content [mg g^{-1}]
1	0.436±0.006
2	0.413±0.02
3	0.59±0.02

Sea-water quality

Table 4 shows the physical and chemical conditions of the waters at the time of sampling and the environment of sampling site showed in Figure 2. DO levels indicate a fairly good requirement of seawater quality, which is also supported by pH conditions that are favourable. Furthermore, the salinity is quite high (35.5‰), which corresponds to the average temperature of 27.5°C. Besides, the waters of the study site demonstrated good water fertility, with nitrate, phosphate, and ammonia levels of 0.34, 0.02, and 0.86 mg L^{-1}, respectively.

Table 4

Water conditions in Tidung Island (n = 9; mean ± SD)

Water quality parameters	Value
DO [mg L^{-1}]	6.53±0.8
pH	7.35±0.3
Temperature [°C]	27.50±0.1
Salinity [‰]	35.45±0.5
Brightness <1 meter [%]	100±0.0
Nitrate [mg L^{-1}]	0.34±0.2
Phosphate [mg L^{-1}]	0.02±0.001
Ammonia [mg L^{-1}]	0.86±0.04

Fig. 2. Condition of sampling locations and samples of *S. polycystum* seaweed

Environmental response to nutrient content, alginate, and fucoxanthin

Figure 3 depicts a biplot of canonical correspondence analysis results. According to the statistical analysis, there were variations in the data, with 70.1 percent and 29.9 percent contributions on axes 1 and 2, respectively. It demonstrates that environmental variables have a strong influence on the characteristics studied. Environmental variables such as phosphate, DO, salinity, pH, and temperature all had different effects on alginate yield and carbohydrate percentage. The ammonia content of seawater, on the other hand, influences the majority of the proximate composition of seaweed, including fat, protein, ash, and water content. Furthermore, the iron microminerals found in *S. polycystum* were linked to pH, salinity, DO, and temperature. Zinc, copper, and selenium, on the other hand, had a negative response to these variables. The content of fucoxanthin was only associated with phosphate and was negatively correlated with nitrate and ammonia variables.

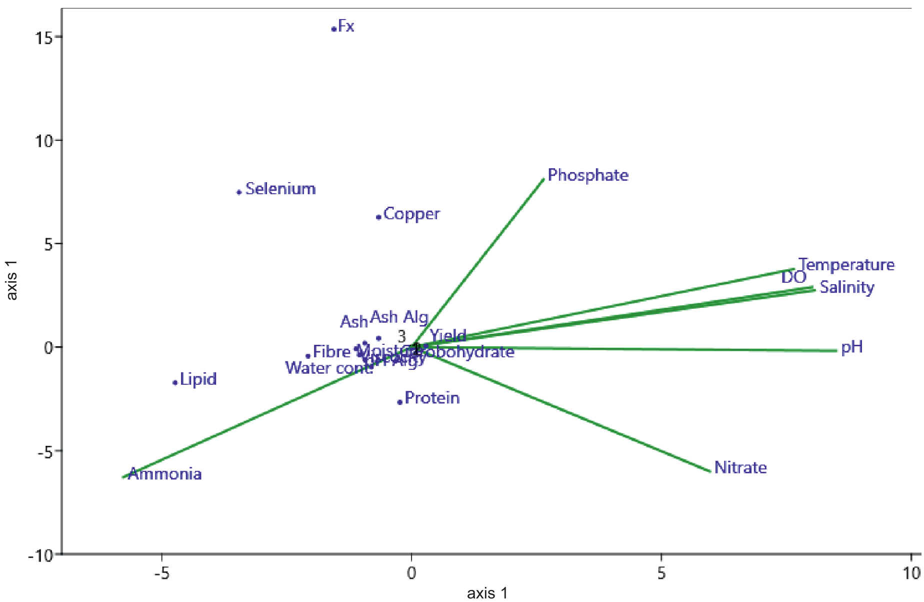

Fig. 3. Results of the canonical environmental correspondence analysis and nutritional characteristics, alginate, and fucoxanthin content of *S. polycystum*

Discussion

Proximate and trace element contents

Carbohydrate's content measured as general that were not differentiated based on their constituent monomers. The abundance of carbohydrates was related to photosynthesis process and food storage function in seaweed thallus. The high percentage was relatively similar with previous studies, such as 33.49% in *S. polycystum* from Malaysian waters (MATANJUN et al. 2009) and 25% from Tamil Nadu, India (PERUMAL et al. 2019). The following essential nutrient was protein content with a lower concentration compared to other types of *Sargassum* such as *S. wightii* reaching 8–12% (PENG et al. 2015), while crude fibre is quite high at 20% when compared with *S. wightii* according to SYAD et al. (2013) which reached 17%. On the other hand, the ash content showed a fairly high concentration and was comparable to studies on the types of *S. natans* and *S. vulgare* of 16 and 34%, respectively (RHEIN-KNUDSEN et al. 2017).

The content of micronutrients shows promising potential, with a trend from the highest to the lowest, namely iron > copper > selenium > zinc > manganese > barium > molybdenum. The last three elements were not detected or below the detection limit of the AAS tool. The concentration of this nutrient varies according to species and growing location, so it is widely used as a bioindicator of the aquatic environment, such as the presence of Cu, Mn, and Zn (BRITO et al. 2012, MALEA and KEVREKIDIS 2014, SIREGAR et al. 2016). On the other hand, these micronutrients are useful for the human diet because they are needed in protein metabolism and enzyme formation (TSUJI et al. 2016). Adequate concentration and regular intake of seaweed products that contain trace elements can help fulfil the diet.

Characteristics of ultrasonication extracted alginate

The yield of alginate extraction in yield characteristics is quite high compared to other *Sargassum* types, namely 13.7% of *S. muticum* (MAZUMDER et al. 2016) and *S. aquifolium* of 39.01% (SETYAWIDATI et al. 2018). The yield of the same species but with different methods was recorded at 12.09–25.77% from Banten waters, Indonesia (DHARMAYANTI et al. 2019) and 28.22% (SUMANDIARSA et al. 2020a) from the waters of Lampung, Indonesia. The amount of alginate in *Sargassum seaweed* is influenced by various factors, including age, fertility phase, and water depth, affecting adaptation to nutrient availability in seawater (PAUL et al. 2020). Alginate from *S. polycystum* is promising due to yield attributes; the utilisation of alginate also depends on the characteristics of viscosity, molecular weight, and monomer composition of mannuronic acid and guluronate (M/G). These rheological characteristics may vary due to biota conditions and habitat environment, such as the research results (SUMANDIARSA et al. 2021b), which found variations in viscosity and M/G ratio in alginate from *S. polycystum* that grows in various small islands in Indonesia. The viscosity obtained was not high, 70.22 Cps, but it was comparable to the results of previous studies. It found that *S. polycystum,* which grew in Banten, Indonesia, reached a viscosity of 35.00–81.33 Cps (DHARMAYANTI et al. 2019) but lower than the type found in the same species grew in Lampung waters was 195.7 Cps (SUMANDIARSA et al. 2020a).

The following characteristics are water content and alginate ash, which were detected at 12.55% and 22.62%. The composition of these two variables plays a role in determining the solubility of alginate required for its utilization. The water and ash content still followed the commercial alginate standard, namely 12% water content and 18–27% ash content (HERNÁNDEZ-CARMONA et al. 2013). On the other hand, the pH value of the alginate produced was relatively high. It was reaching 8.98, which is far above the commercial standard as found in research (KOK and WONG 2018) on the type of *S. polycystum* in Malaysian waters, which is 7.42 and higher than the research results by MAHARANI et al. (2017) of 7.2–7.77 in *S. fluitans* seaweed.

We found that the extraction of alginate from *S. polycystum* by ultrasonication was able to produce alginate characteristics comparable to those produced by conventional extraction. According to research (YOUSSOUF et al. 2017), the Ultrasound-assisted extraction (UAE) method can increase the yield and does not damage the rheological characteristics of the resulting alginate. In addition, this method can minimize the use of chemicals, temperature, and extraction time (FLÓREZ-FERNÁNDEZ et al. 2019). Based on these results, extraction using this method has promising potential. Thus, alginate utilization in the pharmaceutical and food world can be expanded due to supporting factors in physicochemical characteristics such as viscosity, gel properties, strong water solubility, high stability, non-toxicity, and high biocompatibility (ZHANG et al. 2020).

Fucoxanthin content

Extraction of bioactive from macroalgae using Ultrasound is an environmentally friendly method (green extraction method/technology). It refers to various advantages, namely producing good yields, using fewer chemicals, environmentally friendly but capable solvent performance, and being more economical (CHEMAT et al. 2017, OKOLIE et al. 2019, TIWARI 2015). The total fucoxanthin extracted from *S. polycystum* is presented in Table 3. The fucoxanthin obtained was relatively high between 0.41–0.59 mg g^{-1}. These results are comparable with previous studies, including 0.47 mg g^{-1} of *Sargassum* sp. taken from the waters of Lampung, Indonesia (RENHORAN et al. 2017) and around 0.155–0.587 mg/g of *S. polycystum,* which grows in western waters of Indonesia (RENHORAN et al. 2017, SUMANDIARSA et al. 2021b).

The amount of fucoxanthin contained in macroalgae generally varies and is influenced by various factors such as natural conditions, type of species, age, and part of the thallus. The study results (HEFFERNAN et al. 2016, TERASAKI et al. 2017) showed that habitat conditions and extraction methods resulted in varying amounts of fucoxanthin bioactive between species. This compound has excellent potential as a pharmaceutical ingredient because it can be used as an antioxidant, anticancer, anti-obesity, and anti-inflammatory (HEO et al. 2012, MOGHADAMTOUSI et al. 2014, GAMMONE and ORAZIO 2015, JOHNSON et al. 2019).

Fucoxanthin produced from seaweed *S. polycystum* in this study showed great potential. Utilisation as an ingredient in medicines, cosmetics, and other uses can be well explored due to the abundant biodiversity in Indonesian waters. One of the available potentials is the seaweed that grows on small islands such as Tidung Island, which has a large enough biomass and maintained habitat conditions. *Sargassum polycystum* is a brown seaweed that can grow in almost all parts of Indonesia which generally has hard substrates such as dead coral, coral reefs, and coral debris (SUMANDIARSA et al. 2021a). This biota can grow well and dominate the waters because of its adaptability and high tolerance to drastic environmental changes (MAY-LIN and CHING-LEE 2013, MUTA HARAH et al. 2014, HOANG et al. 2016).

Environmental condition

Tidung Island is a small island located in the Thousand Islands, Jakarta Bay, Indonesia, and is famous as a tourism destination. Anthropogenic pressure on this island is large enough to affect the condition of the waters on this island. The physical and chemical conditions of the waters at the time of sampling were determined. As an area with a dense population and the dominance of tourism activities, the waters around Tidung Island are under relatively high pressure, as happened in various other islands with high tourism activities (KURNIAWAN et al. 2016). The measurement results of environmental conditions show that the waters around the island are still quite good, characterised by high DO, good ratio of nitrate and phosphate, and controlled ammonia content. However, there are differences with other studies in the waters of the Thousand Islands with higher DO (13.4 mg L^{-1}) and lower salinity (30 permil) (WIDIARTI et al. 2021).

Anthropogenic factors significantly affect the waters' characteristics because organic materials can quickly enter the waters in large quantities. In addition to anthropogenic, climate change also significantly impacts coastal habitats, including macroalgae based on growth, distribution, and resilience (HARLEY et al. 2012). *Sargassum* can grow in extreme environmental conditions in the intertidal region, especially in limited nutritional conditions due to dynamic hydrodynamic processes (LALEGERIE et al. 2020). The amount of biomass of this biota depends on the requirements of water fertility, namely the concentration of nitrate, phosphate, and ammonia (HOANG et al. 2016).

In general, the nutrients in the waters of Tidung Island are quite fertile, as can be seen from the water quality parameters in Table 4. High water quality can support the growth of various biota well, thus spurring intense competition. These conditions also encourage these biotas to produce specific chemical defences that can threaten between biotas. Species with high adaptive capacity will survive and be characterized by dominant biomass (PUGLISI et al. 2014, BUI et al. 2018). The quality of the habitat may also be seen as an overview of the coastal conditions at the sample location shown in Figure 3.

Environmental response to nutrient content, alginate, and fucoxanthin

Based on the canonical correspondence analysis results, there are variations in the data with contributions on axes 1 and 2 of 70.1% and 29.9%, respectively. It proves that there is a strong response from environmental variables to the characteristics studied. Environmental variables gave various responses, mostly in phosphate, DO, salinity, pH, and temperature, which affected the alginate yield and carbohydrate percentage. On the other hand, the ammonia content of seawater affects most of the proximate composition of seaweed, namely fat, protein, ash, and water content.

Furthermore, the micro minerals of iron contained in *S. polycystum* were associated with pH, salinity, DO, and temperature. On the other hand, zinc, copper, and selenium gave a negative response to these variables. Fucoxanthin content showed remarkable results, which was only associated with phosphate and negatively correlated with nitrate and ammonia variables. The quality of seawater influences the nutrient content of *Sargassum seaweed* as wild macroalgae in its habitat. The study results (PERUMAL et al. 2019, PRAIBOON et al. 2018) stated that the season affected the aquatic conditions of the *Sargassum* habitat and significantly affected its nutritional composition, including the proximate composition. Seasonal variations that have an impact on water quality also affect the metabolic processes of macroalgae. So, the accumulation of macronutrients can vary between species and between species that grow (BALBOA et al. 2016, D'aRMAS et al. 2019).

Metabolites as critical chemical compounds produced by macroalgae also vary among the same species, such as in the production of alginate and fucoxanthin. Alginate characteristics of *Sargassum* are positively correlated with environmental conditions such as water depths that have variations in seawater nutrients. The depth of 10 meters resulted in the highest alginate yield due to low nutrition, which forced this biota to adapt molecularly to produce more food reserves through the production of polysaccharides (PAUL et al. 2020). On the other hand, fucoxanthin as a secondary metabolite is more influenced by the availability of light and the period of exposure to direct sunlight. Fucoxanthin content in *S. polycystum* is strongly correlated with water fertility in nitrate, phosphate, and ammonia in the waters (SUMANDIARSA et al. 2021b).

Based on the results obtained from this study, it was found that *Sargassum polycystum* seaweed has the potential as a source of nutrients, both trace elements and macronutrients. Furthermore, ultrasonication assisted extraction was able to produce better alginate and fucoxanthin than conventional extraction. Thus, extraction by ultrasonication can be suggested to have more prominent metabolites to be one of the best alternatives. This is supported by the results of previous studies by (BORAZJANI et al. 2017, YOUSSOUF et al. 2017, FLÓREZ-FERNÁNDEZ et al. 2019, YUAN et al. 2020), in which ultrasonication was able to produce alginate as a polysaccharide and fucoxanthin as part of bioactive carotenoids with high effectiveness.

Conclusion

Ultrasound-assisted extraction method to obtain metabolites from *S. polycystum* seaweed, namely alginate and fucoxanthin, is encouraging. It is evident from the quantitatively high number of compounds compared to conventional methods. The proximate composition of this species is dominated by carbohydrates and fibre and small amounts of protein and fat. However, the composition of trace elements is quite high in the form of iron, zinc, copper, and selenium, which humans need in the diet process. A total of 37.24% alginate with a viscosity of 70 Cps and 0.41–0.56 mg g^{-1} fucoxanthin was produced from ultrasonication extraction, proving that this method is effective and efficient. It also found that environmental conditions gave a strong response to the content of nutrients, alginate, and fucoxanthin, especially by variations in nitrate, phosphate, and ammonia. Thus, the extraction method and aquatic habitat conditions on Tidung Island affect the nutritional and metabolite characteristics of *S. plycystum*.

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