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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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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).

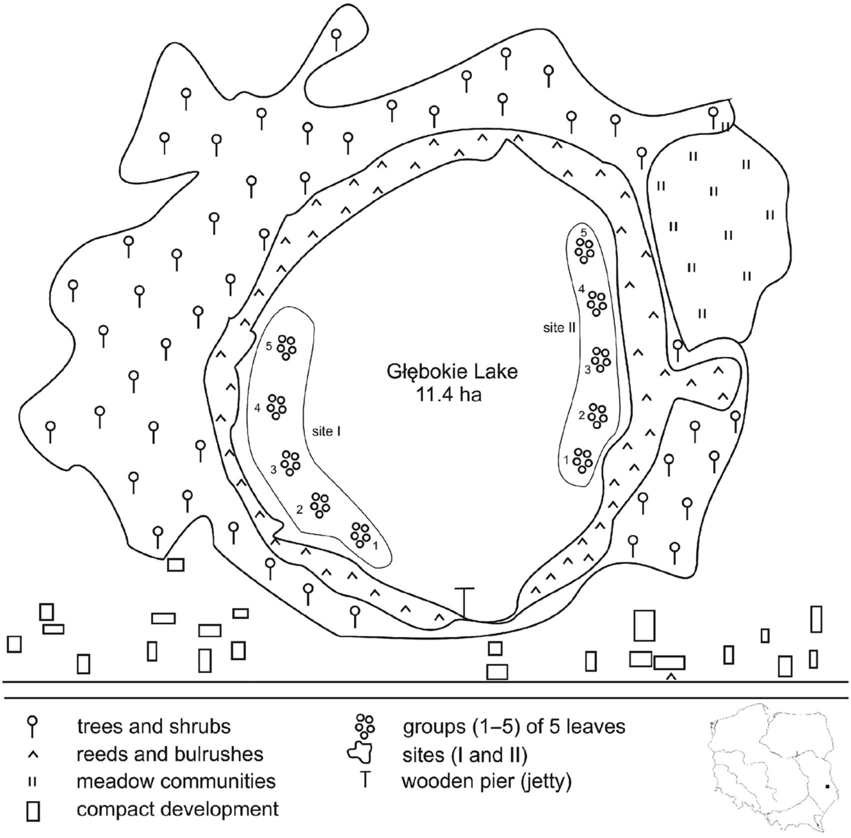

Fig. 1. Precise locations of nympheids in the study area

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.

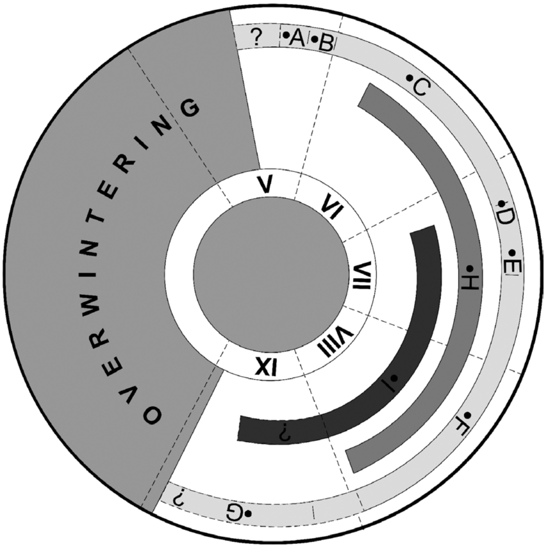

Fig. 3. Life cycle of *Galerucella nymphaeae* in Głębokie Lake. Development stages: light grey strip – adult, medium-grey strip – larva, dark grey strip – pupa, V–IX – months May – September; A – leaving of winter hideouts and complementary feeding of adults; B – first copulating pair; C – egg laying; 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 ($Z = 0.58$; $p = 0.561$).

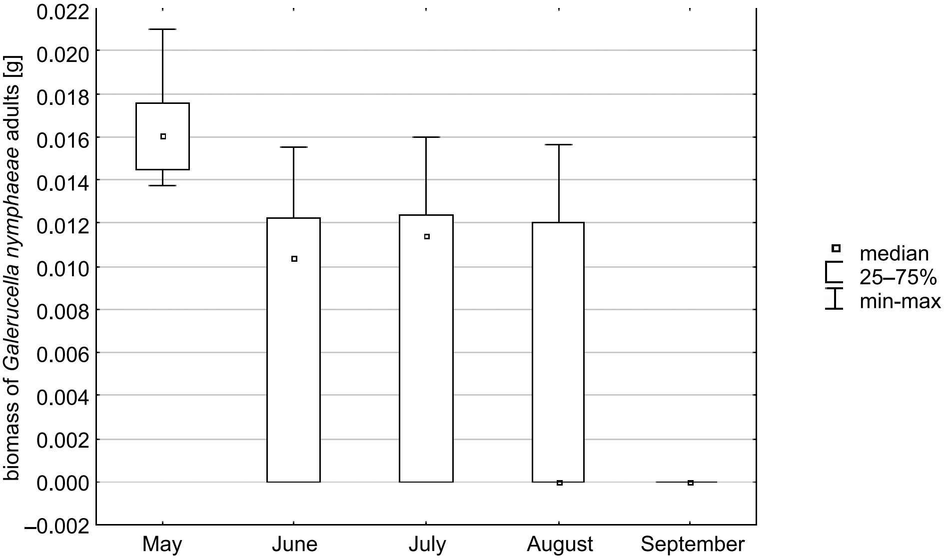

Fig. 4. Biomass of *Galerucella nymphaeae* adults in each month of the study

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.

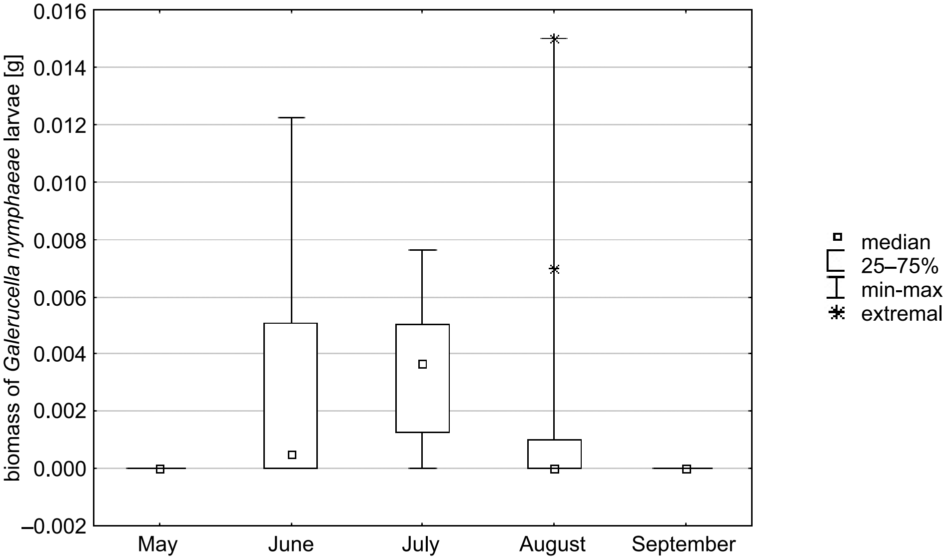

Fig. 5. Biomass of *Galerucella nymphaeae* larvae in each month of the study

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.

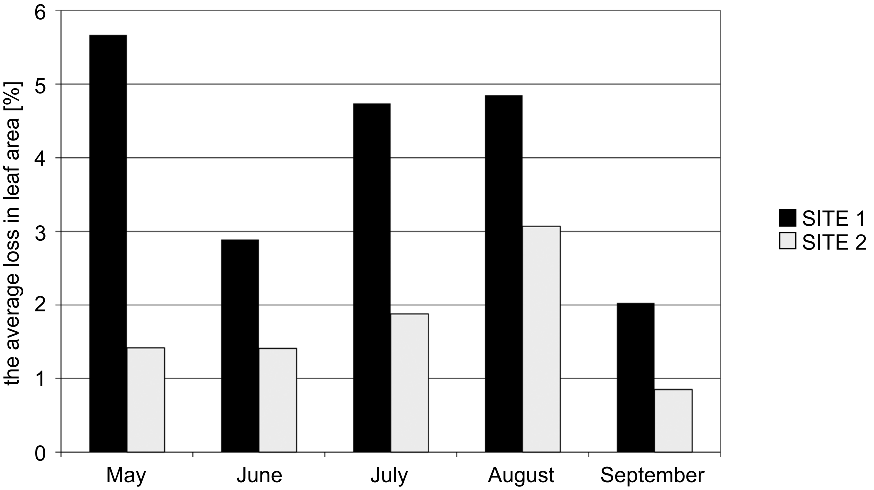

Fig. 6. The average loss in leaf area of yellow water lily [%] at sites I and II in each month of the study due to feeding by *Galerucella nymphaeae* adults and larvae (GN)

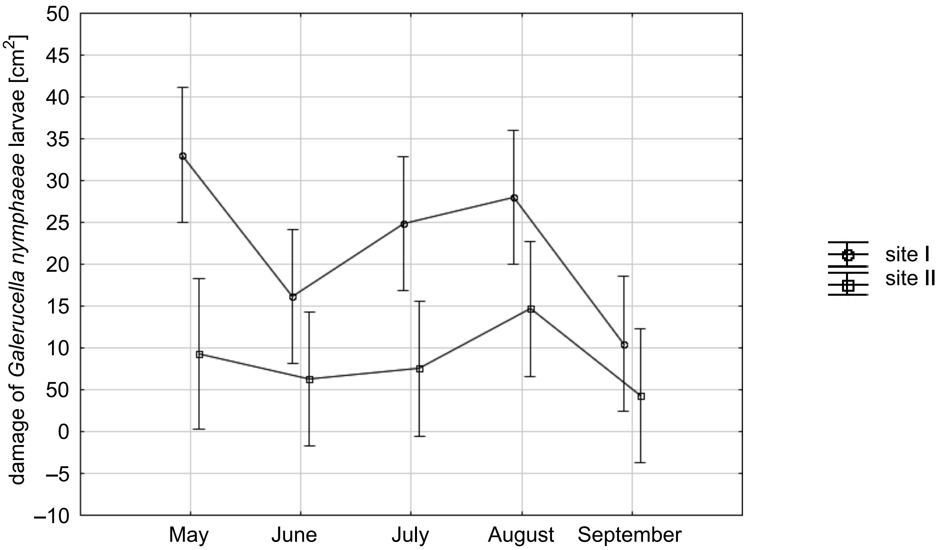

Fig. 7. Profile plot of average loss in leaf area of yellow water lily at sites I and II in each month of the study due to feeding by *Galerucella nymphaeae* adults and larvae (GN)

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: $\omega^2 = 38.9\%$ for sites and $\omega^2 = 18.8\%$ 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*.

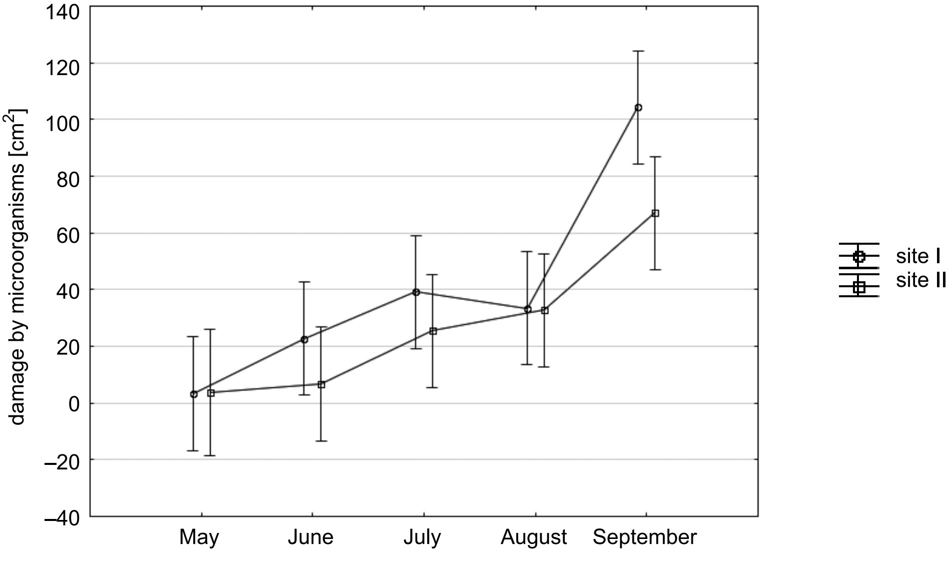

Fig. 8. Profile plot of mean damage to yellow water lily leaves caused by microorganisms (MO) at sites I and II in each month of the study

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).

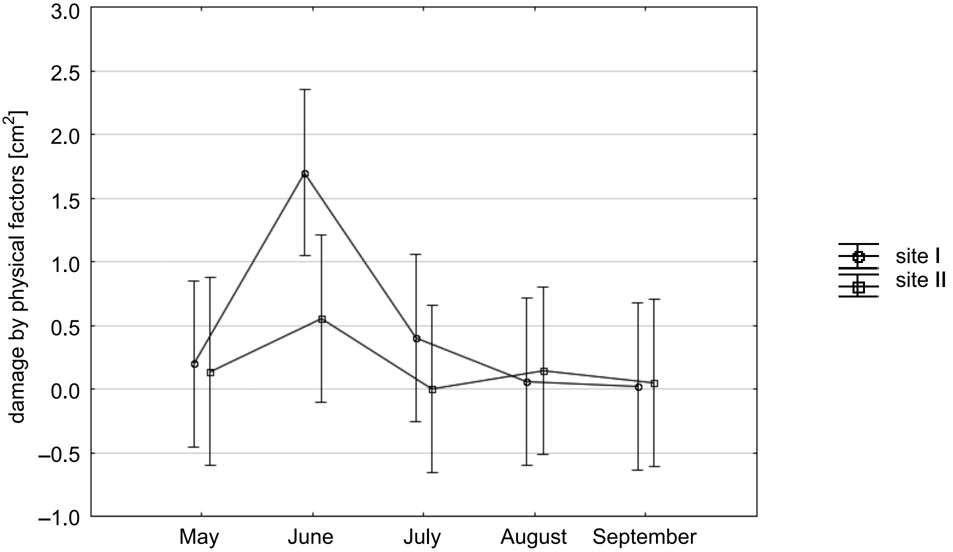

Fig. 9. Profile plot of mean damage to yellow water lily leaves caused by physical factors (PH) at sites I and II in each month of the study

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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