# Initial decomposition of floating leaf blades of waterlilies: causes, damage types and their impact (#29085)



First submission

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# Initial decomposition of floating leaf blades of waterlilies: causes, damage types and their impact

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Initial decomposition (i.e. leaf damage and leaf loss) of large-leaved plants such as waterlilies can be studied well: the turnover of the floating leaf blades is low and the leaves exist for a relatively long time. Floating leaf blades of *Nuphar lutea* (L.) Sm., *Nymphaea alba* L. and *Nymphaea candida* Presl, were studied in separate plots in three fresh water bodies differing in environmental conditions such as trophic status, pH and alkalinity. All floating leaves in a plot were numbered and leaf length, percentages and types of leaf damage and decay of each leaf were measured and estimated weekly for all plots during the growing season.

Initial decomposition and its various causes are depicted and described. Also leaf damage with respect to the potential leaf area per species per plot, contributions to leaf damage by external causes, leaf loss in time and succession of damage causes are presented. Only a few damage causes had a significant impact on leaf damage and leaf loss: autolysis, fungi, snails and mechanical damage. The floating leaves offer food for a series of specialized insects consuming leaf area from below the water surface, from the upper surface or by mining the leaf tissue. Waterfowl (e.g. Rallidae) consume leaf parts and walk on the leaves scratching the upper surface. Several forms of succession of damage can be distinguished such as erosion of the wax layer, followed by cellulolytic bacteria, or fungi, followed by snails, or mechanically damaged leaves (by wind and wave action, desiccation and hail stones), followed by biotic causes and decay, or autolysis, followed by microbial decay, followed by tissue removal by snails, followed by breaking up of leaves. In alkaline waters the seasonal patterns of initial decomposition differed between *Nymphaea* and *Nuphar*.

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## Abstract

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followed by snails, or mechanically damaged leaves (by wind and wave action, desiccation and hail stones), followed by biotic causes and decay, or autolysis, followed by microbial decay, followed by tissue removal by snails, followed by breaking up of leaves. In alkaline waters the seasonal patterns of initial decomposition differed between *Nymphaea* and *Nuphar*.

**Keywords** Decomposition causes · Floating leaf blade decomposition · Fresh water body · Nymphaeaceae · Nymphaeid growth form · Seasonal change

## Introduction

Already during their development plant leaves are exposed to abiotic factors (such as weather conditions, causing physical damage, fragmentation and drying out) as well as biotic factors (such as infection by fungi and viruses, herbivores, and animals using these parts of the plant in various ways). This exposure is well-known for crops and ornamental plants as it causes economic damage. Plant resistance depends on age and plant injuries (Kennedy & Barbour, 1992). Initial ~~causes of~~ decomposition (when the leaves are still connected to the plant) precede the process of leaf material entering the decomposition cycle on the soil. In ecological studies much attention is paid to the latter because these soil processes are important for the biogeochemical cycles. However, with the exception of agriculture, horticulture and forestry (for which phytopathology is a main discipline), much less attention is paid to the first process, in particular for aquatic macrophytes.

Decomposition of aquatic macrophyte tissue consists of a complex series of interacting processes (Kok, 1993; Fig. 1). Often various stages of the decomposition process can be found on one plant or even on one leaf. During initial decomposition vital macrophyte tissue can be used by herbivores and microorganisms (phytopathogens). Before the plant material dies away, the plant tissue goes through the senescence phase. During senescence further decomposition and fragmentation (by weak pathogens, facultative herbivores, grazers and scrapers) occur, leading to the production of faecal pellets. The (bio)chemical composition of plant tissue also changes during senescence due to hydrolysis of macromolecules like DNA and proteins and due to resorption of soluble nutrients. This leads to a loss of tissue structure, sometimes to a loss of secondary chemical compounds and to the colonization of the tissue by microorganisms, making senescent tissue more attractive for facultative detritivorous macroinvertebrates than vital tissue (Rogers & Breen, 1983).

These phases of initial decomposition can be studied well in the leaf blades (laminae) of large leaved plants such as waterlilies in which the turnover of floating leaf blades (further indicated as floating leaves or leaves) is low ($P/B_{max}$ 1.35-2.25) and the leaves exist for a relatively long time (on average 38-48 days) (Klok & Van der Velde, 2017). The study of waterlilies has several other advantages since waterlilies occur worldwide (Conard, 1905; Wiersema, 1987; Padgett, 2007). Furthermore, they have a fixed position in the vegetation zonation along water bodies between helophytes and submerged macrophytes. The nymphaeid growth form is shown by the possession of floating leaves and by rooting in the sediment (Luther, 1983; Den Hartog & Van der Velde, 1988). These floating-leaved plants will not float away as other floating-leaved plants which are free floating. Besides floating leaves, waterlilies also produce thin submerged leaves and aerial leaves at crowding at the water surface and at lowered water level (Glück, 1924; Van der Velde, 1980).

When vital, plant organs have defense mechanisms against damage and decay to slow down decomposition processes. Because of their development under water and subsequent occurrence on the water surface, floating leaf blades of waterlilies can be attacked by microorganisms, fungi and herbivorous animals such as folivores, both from the air above and from the surrounding water below (Lammens & Van der Velde, 1978; Van der Velde *et al*., 1982; Van der Velde & Van der Heijden, 1985; Martínez & Franceschini, 2018). Young leaves can already be attacked under water before they unroll. Longterm effects of folivores on plant growth are reported as negative (Marquis, 1992) reducing leaf density (Stenberg & Stenberg, 2012). Defenses of waterlily leaves against attacks include replacing old floating leaves by new ones, hydrophobic epicuticular wax layers (Riederer & Müller, 2006; Aragón *et al*., 2017), spines (Zhang & Yao, 2017), sclereids with calcium oxalate crystals (Brock & Van der Velde, 1983; Franceschi & Nakata, 2005), tough tissue (Mueller & Dearing, 1994; Kok *et al*., 1992), and plant secondary metabolic chemical compounds such as alkaloids and phenolics (Hegnauer, 1969; Goleniewska-Furmanova, 1970; Hutchinson, 1975; Peura & Lounasmaa, 1977; Kok *et al*., 1992; Vergeer & Van der Velde, 1997; Smolders *et al*., 2000; Martínez & Franceschini, 2018). This selects specific species which can break through the defense causing initial decomposition, while other species have to wait for autolysis or other factors weakening the defense system (Kok *et al*., 1992). In the first case the attacking species are more or less specialized and often restricted to plant species, genus or family. Damage of leaves can cause a leach out of soluble carbohydrates such as oligosaccharides and starches, proteinaceous material and phenolic compounds which are metabolized at high rates by microorganisms during the initial decomposition (Brock, Boon & Paffen, 1985).

Fully decayed floating leaf material that sinks to the bottom makes a significant contribution to the detritus food chain by further decomposition processes (Brock *et al*., 1983; Brock 1984; Brock, 1985; Brock, Boon & Paffen, 1985; Brock *et al*., 1985; Van der Velde & Van der Heijden, 1985; Kok, Meesters & Kempers, 1990; Kok & Van der Velde, 1991; Kok, 1993). They reach the bottom as debris, decayed leaves, leaf fragments and faecal pellets which fuel the benthic communities serving as food for detritivores and saprophytes (Kok *et al*., 1992).

A study with laminae of the waterlily *Nymphaea alba* in litter bags in the field and the laboratory showed that weight loss during decomposition was low under acid conditions in a moorland pool (Voorste Goorven) and fast in an eutrophic alkaline oxbow lake (Oude Waal) with similar results under laboratory conditions mimicking a comparable water quality as in the field. During the first 10-30 days a pronounced weight loss and a rapid change in organic matter composition was observed, after that period changes are small and an accumulation of structural carbohydrates such as cellulose, hemicellulose and lignin from the cell wall fraction could be observed. The disappearance of that fraction was dependent on the water quality of the water body (Brock, Boon & Paffen, 1985).

The present study focusses on initial decomposition ~~patterns and causes~~ of floating leaves of three species of waterlilies in three water bodies differing in pH, buffering capacity, nutrient levels and surroundings. Data ~~were~~ collected to answer the following research questions:

- ~~Which causes and patterns of initial decomposition of floating leaves can be identified~~?
- What is the ~~impact~~ of each cause on initial decomposition?
- How ~~succession of~~ decomposition progress~~ed~~ during the season?

## Materials and Methods

### Sites

Field research took place in three different water bodies in The Netherlands: Haarsteegse Wiel (HW), Oude Waal (OW) and Voorste Goorven (VG). In these water bodies, dense, nearly mono-specific waterlily stands occurred. Three plots were laid out in stands of *Nuphar lutea* (HW and OW, 1977; VG, 1988), two plots in stands of *Nymphaea alba* (OW, 1977; VG, 1988) and one plot in a stand of *Nymphaea candida* (HW, 1977).

The Haarsteegse Wiel (Province of Noord-Brabant; 51$^{0}$43'05" N, 5$^{0}$11'07" E) originates from two connected breakthrough ponds created by dike bursts along the river Meuse in the past. It is an isolated eutrophic water body with low alkalinity. The water level depends on precipitation, seepage and evaporation. During the summer period stratification occurs. The bottom consists of sand and a sapropelium layer with increasing thickness towards the littoral border. The waterlily beds are situated in the wind-sheltered part of the lake.

The Oude Waal (Province of Gelderland; 51$^{0}$51'13" N, 5$^{0}$53'35" E) is a shallow highly eutrophic, alkaline oxbow lake in the forelands of the river Waal. The depth during the growth season is shallow, except for three remnants of former breakthrough ponds. The water level is dependent on precipitation, upward seepage, overflow of the River Waal in winter and/or spring (which strongly influences water chemistry and quality), and evaporation. The bottom consists of clay and sand, covered by a sapropelium layer of varying thickness in the nymphaeid beds.

The Voorste Goorven (Province of Noord-Brabant; $51^{0}$33'53" N, $5^{0}$12'26" E) is a shallow, oligotrophic, isolated, culturally acidified moorland pool, showing very low alkalinity values. The hydrology is mainly dependent on precipitation, upward seepage and evaporation. The lake has a poorly buffered sandy soil and is surrounded by forests.

Characteristics of the investigated water bodies are listed in Table 1. Chemical characteristics were derived from Brock, Boon & Paffen (1985) and Kok, Van der Velde & Landsbergen (1990).

In none of these water bodies boating or navigation occurred, which is important to mention as in that case floating leaves can also be damaged by boat propellers, etc. For the present study we used a small zodiac with peddles to gently reach the plots.

**Initial decomposition and causes**

Initial decomposition includes both leaf damage (i.e. damage to leaf tissue) and leaf loss (i.e. lost leaf tissue) (Lammens & Van der Velde, 1978 ;Van der Velde *et al.*, 1982; Van der Velde & Van der Heijden, 1985). Even before a leaf unrolls, initial decomposition occurs. A classification of the various causes of initial decomposition of floating leaves was proposed earlier (Van der Velde *et al.*, 1982). Herein a primary division is made in internal and external causes, the internal due to physiological factors (autolysis), the external due to either abiotic or biotic factors. Roweck (1988) added water level fluctuations as abiotic factor and mass starvation as a result of stress factors under internal factors to this classification. As result of the current research a slightly enhanced version of the original classification is shown in Table 2.

**Potential and actual leaf area**

The various causes of initial decomposition that were identified during this study are described and quantified. To quantify leaf loss, a distinction was made between potential and actual leaf area. The potential area was defined as the area of the entirely intact leaf. The actual area was defined as the potential area minus the area that was missing.

The potential leaf area was calculated by correlation with the leaf length, using a quadratic regression equation (Van der Velde & Peelen-Bexkens, 1983; Klok & Van der

Velde, 2017). Randomly harvested undamaged, fully green leaves sampled outside the plots were taken to the laboratory and both length and area (using a planimeter) were measured in order to determine equation coefficients between leaf length and area. With the aid of these equations the areas of floating leaves in the plots were calculated. Mathematically, the equation is described by:

$$A(L) = c_i L^2 \qquad (1)$$

where:

$A(L)$ = potential leaf area at length $L$ ($cm^2$)

$L$ = leaf length from the leaf tip to a basal lobe tip (cm)

$c_i$ = correlation coefficient of species $i$

$i$ = species (*Nuphar lutea*, *Nymphaea alba*, *Nymphaea candida*)

**Collected plot data**

To collect data on initial decomposition during the growing season, six representative plots of 1 $m^2$ were laid out in the center of mono-specific stands, surveying one rhizome apex per plot. A non-destructive leaf-marking method was used to mark all floating leaves within a plot, which enabled data collection during the complete life-span of the leaves. A square perforated PVC tube frame, held approximately 15 cm below the water surface by cork floaters and anchored by four bricks, bordered a plot. In this way the unrolling of floating leaves in the plot was not hindered and all leaves having their petioles within the frame were counted and measured. A leaf was considered still present as long as, after fragmentation, tissue of the lamina was connected to the petiole in the case of OW and HW. In VG the leaf was considered gone when it was completely decayed and sunk under the water surface or when it disappeared.

Measurements and observations of all leaves within a plot took place weekly during the growing season. It included tagging newly unrolled leaves with uniquely numbered Rotex tapes (fixed around the petiole just under the leaf), counting the actual number of leaves, measuring leaf length in mm (from the leaf tip to a basal lobe tip) and visually estimating the different types of initial decomposition as percentage of the potential leaf area of each leaf. During the whole growing season, undamaged leaves were harvested at random a few meters outside the plots at each location to measure length (mm) and area ($cm^2$) to ~~eventually~~ determine the coefficients of equation (1).

## Results

Vegetation period, total number of leaves produced and total potential area of leaves of the species in the plots are presented in Table 3. Data on damage to leaves are presented per damage cause for all plots in Table 4. This table shows the percentage affected leaves of the total number of produced leaves, the average and the maximum percentage affected area of the potential leaf area and the area of lost surface tissue of the total potential area.

Leaf loss by external causes per plot in time is given in Fig. 2 and percentual contributions to leaf damage by external causes per plot per cause in time are given in Fig. 3.

**Causes of initial decomposition and their impact**

Parts of a floating leaf can be damaged or lost by various causes. A description of all damage causes found follows below.

**Autolysis**. The newly enrolled floating leaves are green and hydrophobic by an epicuticular wax layer (Fig. 4). During senescence this wax layer erodes by colonization of bacteria and fungi. In this stage the leaf tissue can be attacked by cellulolytic bacteria (Howard-Williams *et al*., 1978; Robb *et al*., 1979; Rogers & Breen, 1981; Barnabas, 1992). Autolysis starts shortly after the first leaves are fully grown and continues the whole floating leaf vegetation period. The leaf turns from green to yellow, which leads at the end of the existence of the floating leaf to total microbial decay, the leaf turning brown. Autolysis is controlled by the plant itself by hormones (e.g. Osborne, 1963). As expected, the influence of autolysis reached its maximum towards the end of the growing season. In October the percentage of affected leaves rose to 100%, however, the surface area affected was quite stable and generally around around 10%. For separate leaves the area affected by autolysis may decrease in time, since microbial decay will take over part of the area (Fig. 5).

**Frost**. Frost in early spring may damage the tips of young leaves sticking out of the water. Frost does not occur frequently, but because of frost individual leaves may lose up to one third of their area (Fig. 6). The effect on surface area was less than 5%.

**Hail stones**. Occasional hail stone showers damage the floating leaves by penetrating the leaf and making typical scars on the leaves (Fig. 7). Leaf damage was minimal.

**Dehydration**. Due to hard wind floating leaves are lifted from the water, flip over and subsequently air exposed parts (in particular the leaf margin) dry out (Fig. 8). The effect on leaf surface area was generally less than 5%.

**Mechanical damage**. This damage is caused by wind and wave action, and consists of cracks in the leaves or lost leaves by breaking of the petiole (Fig. 8). The percentage of affected leaves was quite high during the whole data taking period for plots (*Nuphar lutea,* HW), (*Nymphaea alba*, OW) and (*Nymphaea candida*, HW), ranging about 60-80%. Plot (*Nuphar lutea*, OW) showed peaks of 90% in spring and 70% in autumn with a dip of

10% in summer. Plots (*Nuphar lutea*, VG) and (*Nymphaea alba*, VG) showed no damage at all for this cause.

**Scratches**. Damage by scratches is caused by the fingernails of birds, mostly Coot (*Fulica atra* L.) and also Moorhen (*Gallinula chloropus* L.), as they are walking or running over the leaves (Fig. 9). In general the scratches are straight and effect only the epidermis of the leaf, but angle-shaped cuts due to nails penetrating the leaf tissue also occur (Lammens & Van der Velde, 1978). The impact on leaf surface was low, generally below 5%, despite the high percentage of affected leaves, sometimes up to 100% for plots (*Nuphar lutea,* HW), (*Nuphar lutea*, OW), (*Nymphaea alba*, OW) and (*Nymphaea candida*, HW). Plots (*Nuphar lutea*, VG) and (*Nymphaea alba*, VG) showed no damage at all.

**Damage by *Elophila nymphaeata* (L.) (Crambidae)**. The caterpillar of the moth *Elophila nymphaeata* damages the leaf in two ways. The larva consumes leaf tissue and cuts out oval patches from the floating leaf. It can attach a patch to the underside of a floating leaf to make a shelter below the leaf or it spins two patches together to make a floating shelter (Fig. 10). Life cycle and behavior of *E. nymphaeata* are described by Reichholf (1970). The effect on leaf surface was low, at most 5%, while leaves in plots (*Nymphaea candida*, HW), (*Nuphar lutea*, VG) and (*Nymphaea alba*, VG) showed no damage.

**Consumption by *Fulica atra* L. (Rallidae)**. Damage by consumption of leaf tissue by the Coot (*Fulica atra*) can be recognized by omissions in the form of triangular areas at the edge of a leaf. Sometimes a major part of the leaf has been consumed. Generally prints from the beak are visible around the consumed areas (Fig. 11). The total effect on leaf surface area was minimal, while plots (*Nuphar lutea*, VG) and (*Nymphaea alba*, VG) showed no damage at all.

**Consumption by pond snails (Lymnaeidae)**. Damage on fresh leaves is caused mainly by *Lymnaea stagnalis* L. and to a lower extent by other lymnaeids. Since snails grow best and become larger by eating soft fresh leaf material, unfolded leaves still under water are the victim of consumption, which can be seen from rows of holes, large near the edge and becoming smaller towards the center of the leaf (Fig. 12). In general snails have a preference for decaying leaf material, e.g. consuming areas that were infected by fungi. Van der Aa (1978) notice small holes in the center of many spots and suggested that an arthropod has been active. Possible he observed the result of grazing by snails on the spots. Damage generally is an important cause during the whole period of data taking for both *Nuphar lutea* and *Nymphaea alba*, with a contribution of 20-40%.

**Consumption by *Donacia crassipes* F. (Chrysomelidae)**. Host plants of the beetle *Donacia crassipes* are waterlilies (*Nuphar* spp. and *Nymphaea* spp.). The imagines live on the floating leaf upper side where they feed on leaf tissue (upper epidermis, parenchym till the under epidermis). The spots consumed are round to oval. Around these spots decay starts after some time. Eggs are deposited on the underside of leaves. For that purpose the beetle gnaws a round to oval hole in the leaf by which it can stick the abdomen for oviposition at the underside of a leaf (Figs. 13 and 14). Hatched larvae sink to the bottom and feed on roots. After a three-year life-cycle, they overwinter as pupae in cocoons attached to roots (Bienkowski, 1996). The percentage of damaged leaf surface is minimal.

**Consumption by *Bagous rotundicollis* Bohemann (Curculionidae)**. The beetle *Bagous rotundicollis* feeds on waterlily leaves (Van der Velde *et al.*, 1989). The adult scrapes off spots with a diameter of ca. one cm from the underside of the floating leaf near its margin in which way the lower epidermis and sponge parenchyma are consumed, leaving the palisade parenchyma and upper epidermis intact (Fig. 15). Damage was found in plot (*Nymphaea alba*, VG) only with up to 30% infected leaves.

**Consumption by *Galerucella nymphaeae* L. (Chrysomelidae)**. The Waterlily Beetle (*Galerucella nymphaeae*) completes its full life cycle on the upper surface of floating leaves. In winter the adults hide in remains of dead helophytes, under the bark of trees or in ground litter. Simultaneous with the development of floating leaves the beetles appear. Eggs are attached to the upper surface of floating leaves. Hatching of eggs is followed by three larval stages and pupation, taking 15-29 days. Both imagines and larvae feed on the upper surface of floating leaves by grazing epidermis and palisade and sponge parenchyma. The larvae, which can be considered halfminers create irregular trenches on the surface leaving the under epidermis of the leaf intact. In the trench they deposit their faeces which leads to decay. The under epidermis decays and disappears, which makes the leaves vulnerable to fungal and microbial attacks (Wesenberg-Lund, 1943; Roweck, 1988). So leaf disappearance is finally caused by fungi and bacteria, but the process is initiated by the beetle (Wallace & O'Hop, 1985). The pattern of damage to the leaves is easily recognized. Imagines make smaller eating spots in contrast to the larvae (Fig. 16). These spots with regular margins made by *Galerucella nymphaeae* imagines can be distinguished from those made by *Donacia crassipes* of which the margins are more ragged (Roweck, 1988). Damage was found in plot (*Nymphaea alba*, VG) only with infected leaves starting half June going up to 30-40% in August until October and a sharp peak of 60% half October.

**Mining by *Hydromyza livens* (Fabricius) (Scatophagidae)**. The larvae of the fly *Hydromyza livens* only occur in *Nuphar* leaves. The autecology of this fly species is extensively described in Brock & Van der Velde (1983). The larvae of *Hydromyza livens* mine in the leaf tissue which they consume. The eggs of this fly are laid at the underside of the leaves. For that purpose the fly goes via the margin under water and follows the dichotomous nerves till it reaches the midrib of the leaf, where it lays an egg. From the egg the larva immediately starts to mine in the leaf tissue. The mine track shows a very characteristic shape as the larvae first mine towards the margins of the leaf, then bend and mine parallel to the leaf margin, bend again towards the midrib and mine further into the petiole where they pupate (Fig. 17). Since they also mine the petiole, they create a weak breaking point where the leaf can break off and float away. The total effect on decomposition of floating leaves was less than 8%. With translucent light it appeared that the real damage was higher due to leakage, etc.

**Mining by Chironomidae**. Larvae of some Chironomidae mine in the leaf tissue and dig/eat their way through the leaf tissue. Typical damage on *Nuphar* leaves is caused by the chironomid larvae of *Tribelos intextus*. The larvae mine the leaves when they are still folded and below the water surface and thus damage rolled leaf. So when the floating leaves enroll at the water surface rows of small holes become visible (Van der Velde & Hiddink, 1987) (Fig. 18). Other miners observed are larvae of *Cricotopus trifasciatus* (Meigen in Panzer, 1813) (Fig. 19), which is a half miner intensively damaging the floating leaves of *Nymphoides peltata* (Gmel.) O. Kuntze (Lammens & Van der Velde, 1978). It is observed to cause some damage at the leaf margins on floating leaves of *Nuphar lutea* in the neighbourhood of *Nymphoide*s in OW (Van der Velde & Hiddink, 1987). The total effect on the decomposition of floating leaves was minimal.

**Mining by *Endochironomus* spec. (Chironomidae)**. The larvae of these midges mine in floating leaves. In 1977 there may have been two not so well separated generations. Could clearly be distinguished from other Chironomidae (previous cause), since the mines are visible on the floating leaf upper side as straight dark stripes (Roweck, 1988) (Fig. 20). The total effect on the decomposition of floating leaves was minimal.

**Fungi**. The leaves of *Nuphar lutea* were infected by *Pythium* “type F” (Jacobs, 1982) (Fig. 21) and the leaves of *Nymphaea alba* and *Nymphaea candida* by *Colletotrichum nymphaeae* (Van der Aa, 1978) (Fig. 22). The percentage of damage for the surface area was around 15% for *Pythium* “type F” and up to 55% for *Colletotrichum nymphaeae*.

**Microbial decay**. Due to autolysis the resistance of a leaf against microbial infection disappears quickly, which gives rise to microbial decay. The effect on the affected surface area ranges from about 15-25%, with an exceptional peak of 60% in plot (*Nymphaea candida*, HW) at the very end of the growing season.

**Unknown causes**. Missing (parts of) leaves can be caused by consumption or damage by aquatic animals, however in many occasions the real cause of lacking leaf parts could not be determined. Leaves disconnected from their petioles are scattered by wind and wave action and could not be followed anymore. Damage occasionally went up to 60% for *Nuphar lutea* in HW, *Nymphaea alba* in OW and *Nymphaea candida* in HW, however, for the other plots (*Nuphar lutea*, OW), (*Nuphar lutea*, VG) and (*Nymphaea alba*, VG), this type of damage was hardly found.

## Discussion

Initial decomposition tends to increase in time during the vegetation season with the exception of the acid Voorste Goorven (with *Nuphar lutea* and *Nymphaea alba*). In the alkaline waters (OW, HW) leaf damage for *Nuphar lutea* was less than 20% related to the total potential leaf area in the plot until half September, but afterwards increased to more than 50%. For *Nymphaea alba* and *Nymphaea candida* it was less than 10% with an increase to almost 20% in October.

It seems that the overall patterns of decomposition of floating leaves in the plots differ for waterlily species, water quality (alkaline vs. acid) and wind exposure. The acid water is also most sheltered against wind and wave action which allowed *Galerucella nymphaeae* to become an important herbivore, which is lacking in the other plots in water bodies with often a strong wind exposure. In the acid water plots no consumption by snails was observed in contrast to the alkaline plots. Consumption of leaves was occurring by specialized insect species only and their impact was low. Leaf fragmentation was hardly observed in the acid plots.

In the two alkaline waters *Nuphar lutea* showed a similar seasonal decomposition pattern, that differed from that of *Nymphaea alba* and *N. candida*, which were similar to each other. In *Nuphar* there is an increase in share of microbial decay followed by fungi

(*Pythium* spec.), which both have an important role in the decomposition from the beginning of the season. In *Nymphaea* decay by a fungus (*Colletotrichum nymphaeae*) started and increased in importance towards the end of the season.

In general microbial and fungal decay increased in relative importance during the season, while unknown causes diminished just as all other damages causes together.

From the list of causes of initial decomposition and their impact it is clear that autolysis was the most important for the decomposition of the floating leaves. Also microbial decay and unknown causes have high impact, except for Voorste Goorven, where the floating leaves showed no damage for these causes. Minor causes occurring incidentally at once during the vegetation period were frost that can cause serious leaf loss and hail stones that hardly have impact with respect to disappeared area, but can contribute to further fragmentation of the leaves. Dehydration and mechanical damage are dependent of wind and wave action. High solar radiation and air temperatures cause the dehydration of the flipped over leaves with a high impact in Haarsteegse Wiel and Oude Waal in contrast with the wind protected Voorste Goorven where the leaves hardly show that type of damage. In the Voorste Goorven damage was mainly caused by specialized consumers of floating leaf tissue in particular herbivorous beetles, fly larvae, chironomid larvae and the omnivorous Coot. From the start of the growing season the mining by *Endochironomus* spec. was dominant at plots (*Nuphar lutea*, VG) and (*Nymphaea alba*, VG).

The difference in leaf damage and leaf loss between acid and alkaline waters was clear. In the acid VG (with *Nuphar* lutea and *Nymphaea alba*) the effect of leaf damage and leaf loss was minimal. Low pH of the water caused a low rate of decomposition of the leaves by several interacting factors such as low $HCO_3$, high Al concentrations, low pH in the plant tissue, high phenolics stored in the tissue as cell wall degradation is inhibited. Al and low pH cause also a lower number of detritivores leading to low feeding and low leaf fragmentation. Inhibition of cell wall degradation leads to low fragmentation and prevents softening of microbial enriched plant tissue which means that also by high phenolics stored in the tissue the plant tissue has a low resource quality for detritivores. Snails are absent under acid conditions because of lack of calcium for their shells. Snails prefer to consume decaying parts of leaves under high pH and alkaline conditions (Kok,1993). *Nymphaea candida* (HW) showed an increase for nail scratches towards the end of June, which may be the influence of young coots.

## Conclusions

Of the causes of initial decomposition of floating leaves that have been found, only a few have significant impact on leaf damage and leaf loss. The floating leaves offer food for a series of specialized insects consuming leaf area from below the water surface as well as from the upper surface or are mining in the tissue and birds (Rallidae) which swim around in the neighborhood consuming leaf parts and walk on the leaves scratching the upper surface. High impact causes are autolysis, fungi, snails, mechanical damage and unknown causes. As a consequence of  microbial decay, tissue removal is very prominent in some cases.

During the vegetation growth period the development of new floating leaves and the dying off of old leaves continues during a long period. Also the growing period of leaves stops earlier than the dying off of the older leaves and comprises 53 to 73 % of the vegetation period (Klok & Van der Velde, 2017).

Other aspects of influence are abiotic conditions and physico-chemical characteristics of the water bodies. Wind-sheltered plots showed different insects species with different impact and no mechanical damage by wind and wave action. The surrounding biotopes are also important as meadows are important for Coots to survive winter time by grazing grass in groups. High densities of waterfowl leads to higher damage of the leaves. Acid and alkaline also show different impact of damage causes. Typically, this was the case for the acid Voorste Goorven (*Nuphar lutea* and *Nymphaea alba*), which is sheltered against wind action by trees, in contrast to Haarsteegse Wiel (*Nuphar lutea* and *Nymphaea candida*) and Oude Waal (*Nuphar lutea* and *Nymphaea alba*).

Prolonged dark, cloudy and wet weather conditions by rain and/or shadowing are a stress factor weakening the defense of waterlily leaves due to reduced availability of sunlight and stimulate heavy infection and decay by fungi. Shading as a stress factor reduces the phenolic content making the mature leaves vulnerable to infection by fungi as phenolics have fungistatic properties (Vergeer & Van der Velde, 1997).

In summary several forms of succession of damage can be distinguished such as eroded wax layer, followed by cellulolytic bacteria, fungi, followed by snails, abiotically damaged leaves, followed by biotic causes and decay, autolysis, followed by microbial decay, followed by tissue removal by snails, followed by breaking up of leaves.

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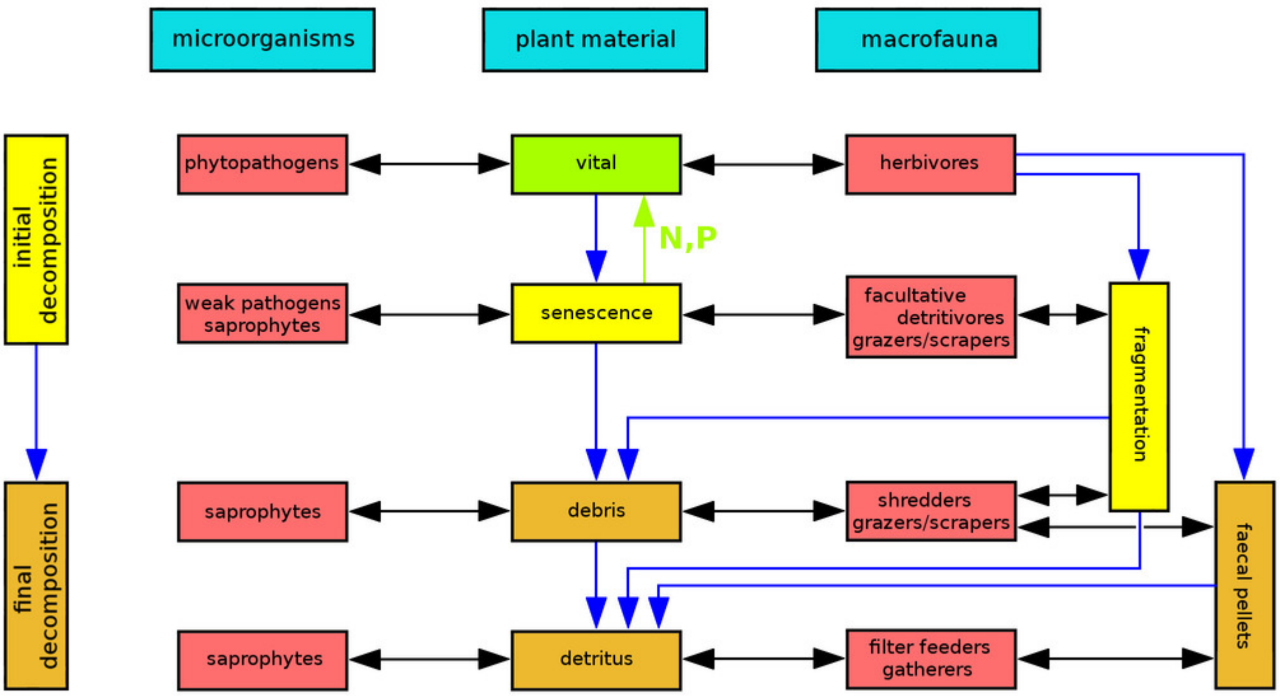
# Figure 1

[p] Relations between decomposition stages and the organisms involved in various stages (modified after Kok, 1993).

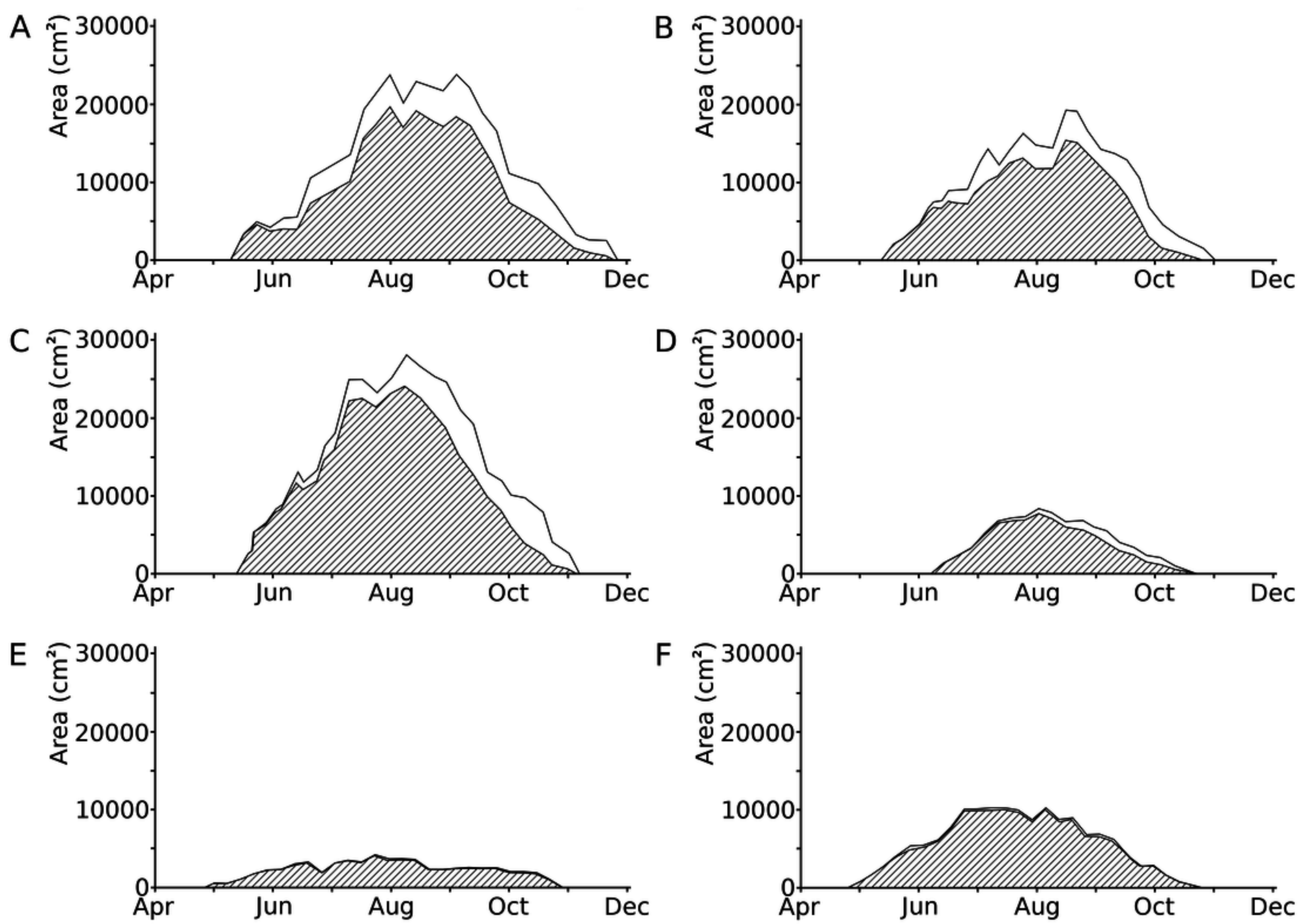
Relations between decomposition stages and the organisms involved in various stages (modified after Kok, 1993). Where double (black, horizontal) arrows indicate interaction and single (blue, vertical) arrows indicate succession or result. During senescence resorption of N and P is indicated by an up-arrow (green).



# Figure 2

Leaf loss by external causes in time per plot.

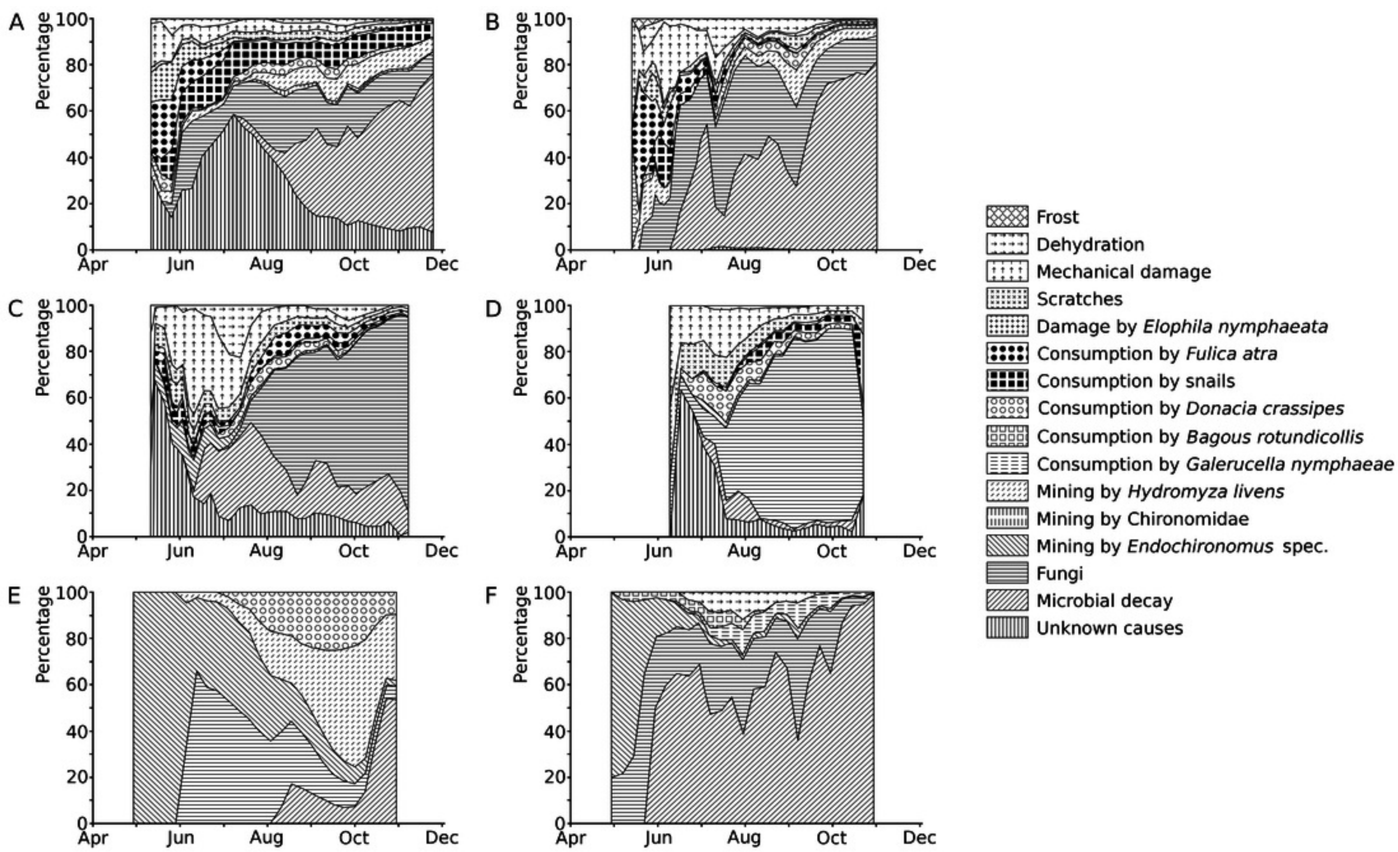
Leaf loss by external causes in time per plot (white), shown by the actual surface (hatched) and the potential surface (white + hatched). (A) *Nuphar lutea*, Haarsteegse Wiel, 1977, (B) *Nuphar lutea*, Oude Waal, 1977, (C) *Nuphar lutea*, Voorste Goorven, 1988, (D) *Nymphaea candida*, Haarsteegse Wiel, 1977, (E) *Nymphaea alba*, Oude Waal, 1977, (F) *Nymphaea alba*, Voorste Goorven, 1988.



# Figure 3

Relative contributions to leaf damage by external causes per plot in time.

Relative contributions to leaf damage by external causes per plot in time. (A) *Nuphar lutea*, Haarsteegse Wiel, 1977, (B) *Nuphar lutea*, Oude Waal, 1977, (C) *Nuphar lutea*, Voorste Goorven, 1988, (D) *Nymphaea candida*, Haarsteegse Wiel, 1977, (E) *Nymphaea alba*, Oude Waal, 1977, (F) *Nymphaea alba*, Voorste Goorven, 1988.

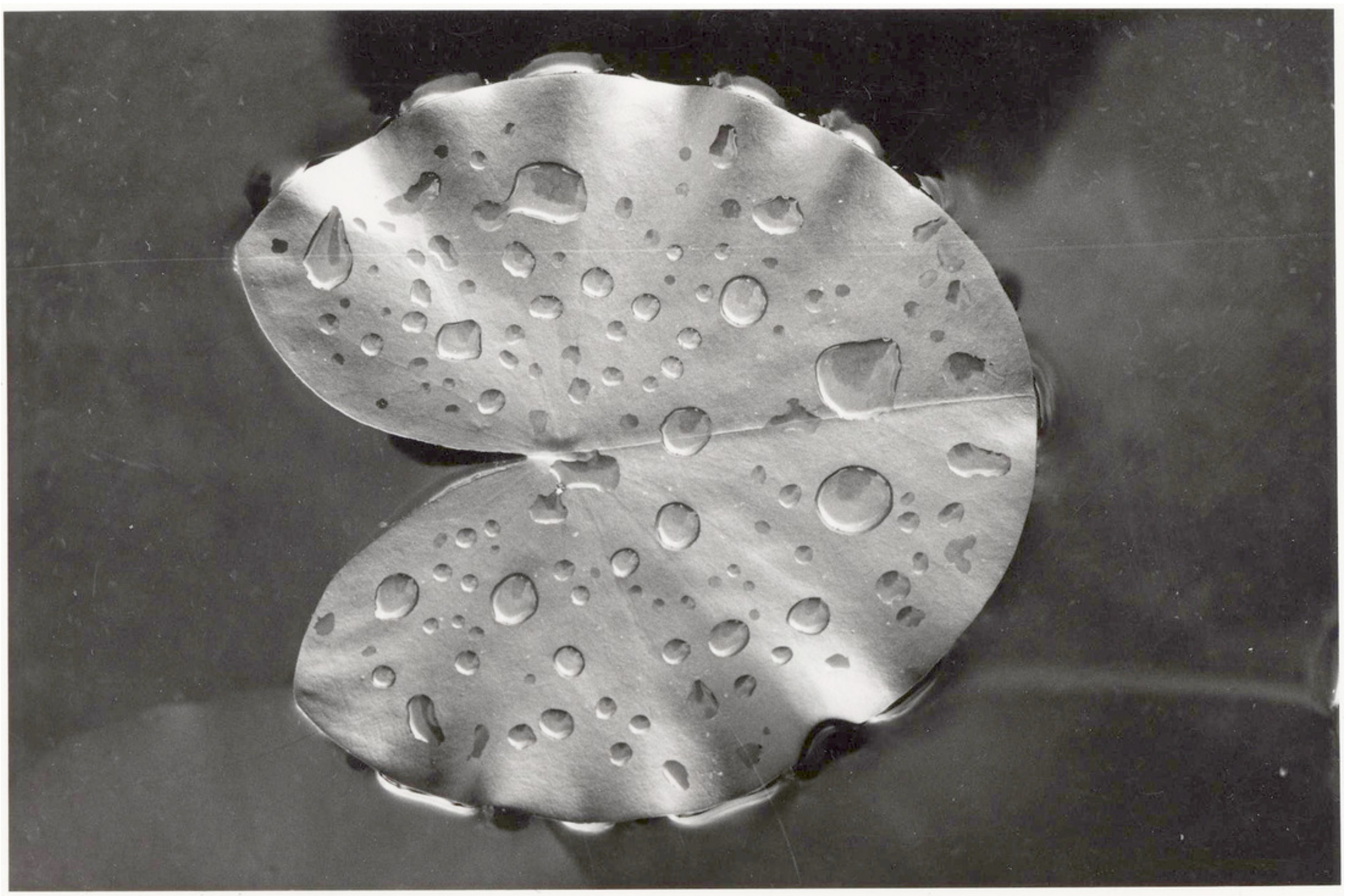


# Figure 4

Defense system.

Defense system. A just enrolled new floating leaf of *Nymphaea alba* showing the hydrophobic wax layer as indicated by the water droplets.

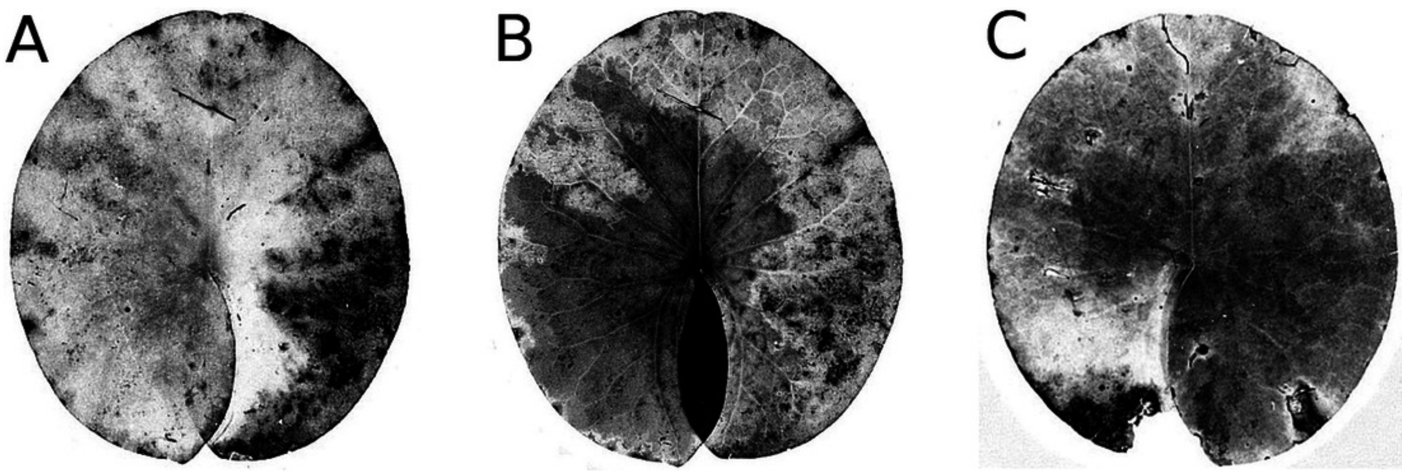
**Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.*



# Figure 5

Autolysis and microbial decay.

Autolysis and microbial decay. (A, B, C) show damage by autolysis and by microbial decay on *Nymphaea candida*, photographed by translucent light. Autolysis is indicated by the lighter areas and microbial decay by the blackish areas. Darker areas are green living tissue.

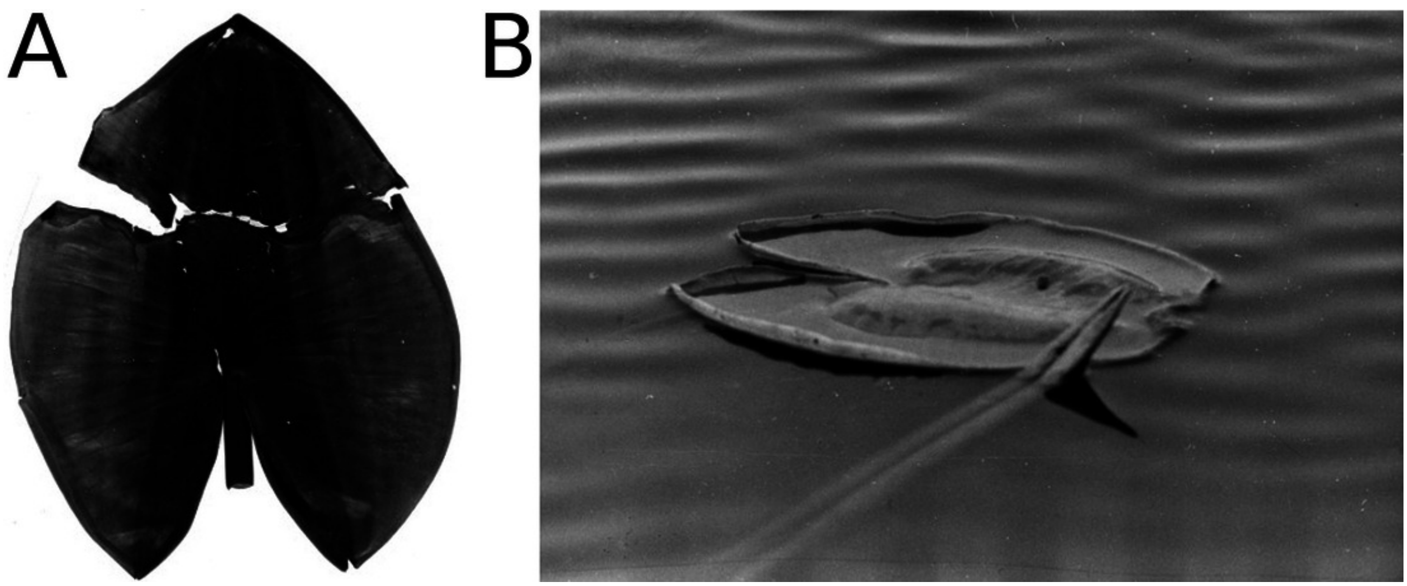


# Figure 6

Frost.

Frost. (A) shows damage by frost on *Nuphar lutea*, (B) shows the tip of a leaf above the water which might be frozen off and detached.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.

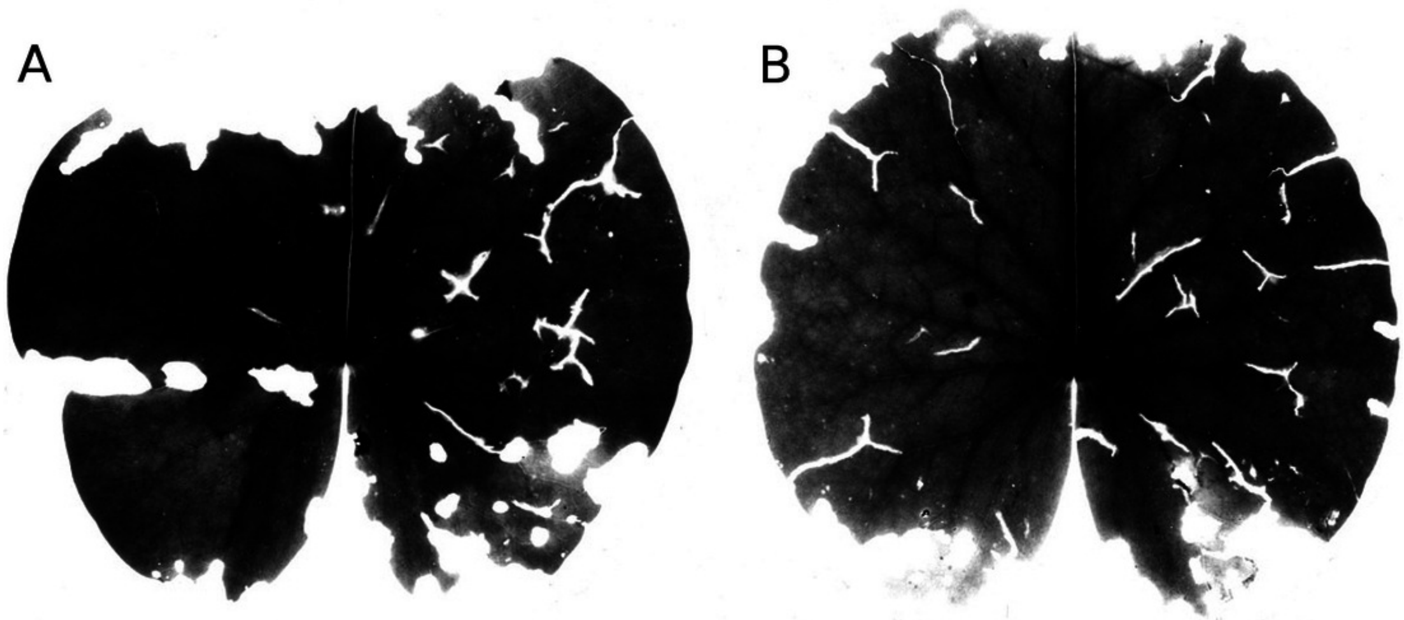


# Figure 7

Hail stones.

Hail stones. (A, B) show damage by hail stones and snails on *Nymphaea alba*.

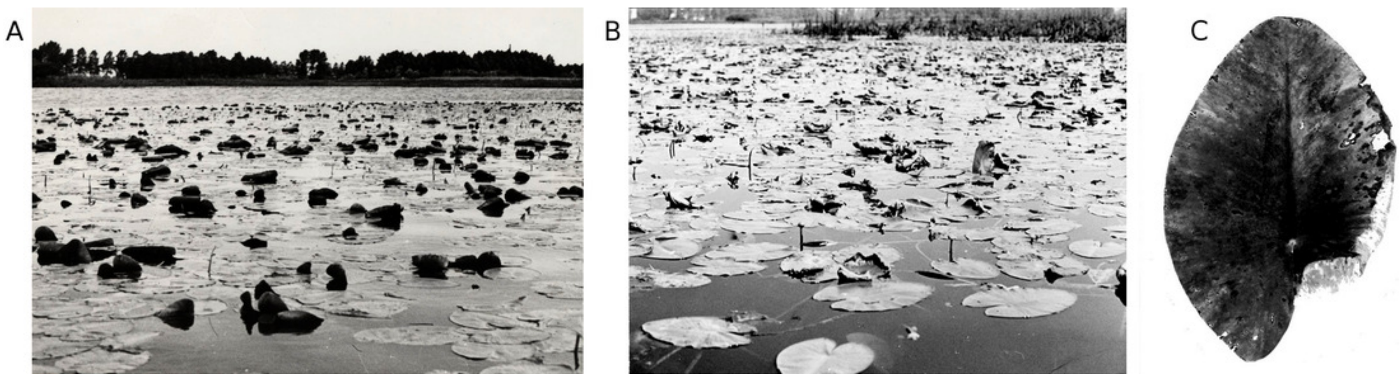
*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.



# Figure 8

Wind and wave action.

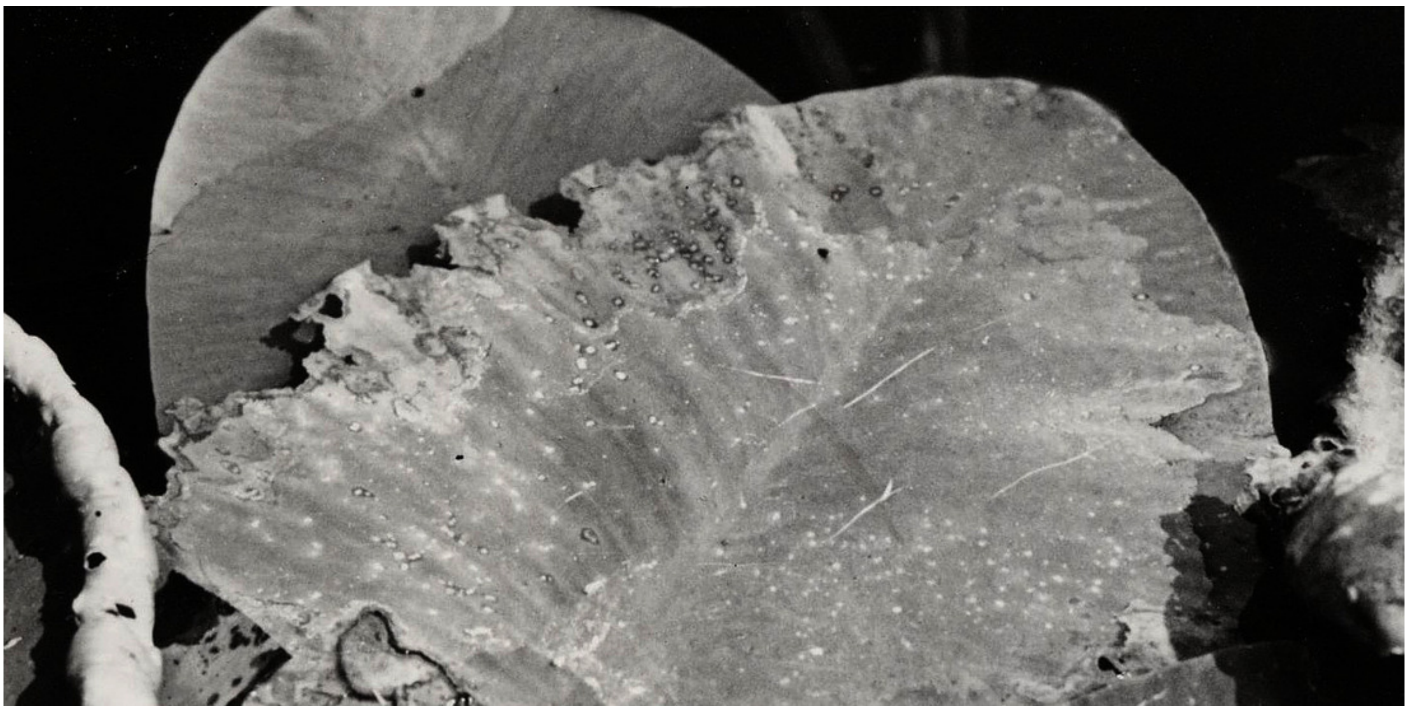
Wind and wave action. (A) and (B) show uplifted leaves as result of wind and wave action, leading to dehydration and mechanical damage.(C) shows dehydration of the leaf margin of *Nuphar lutea* by additional air and sun exposure.



# Figure 9

Damage by scratches.

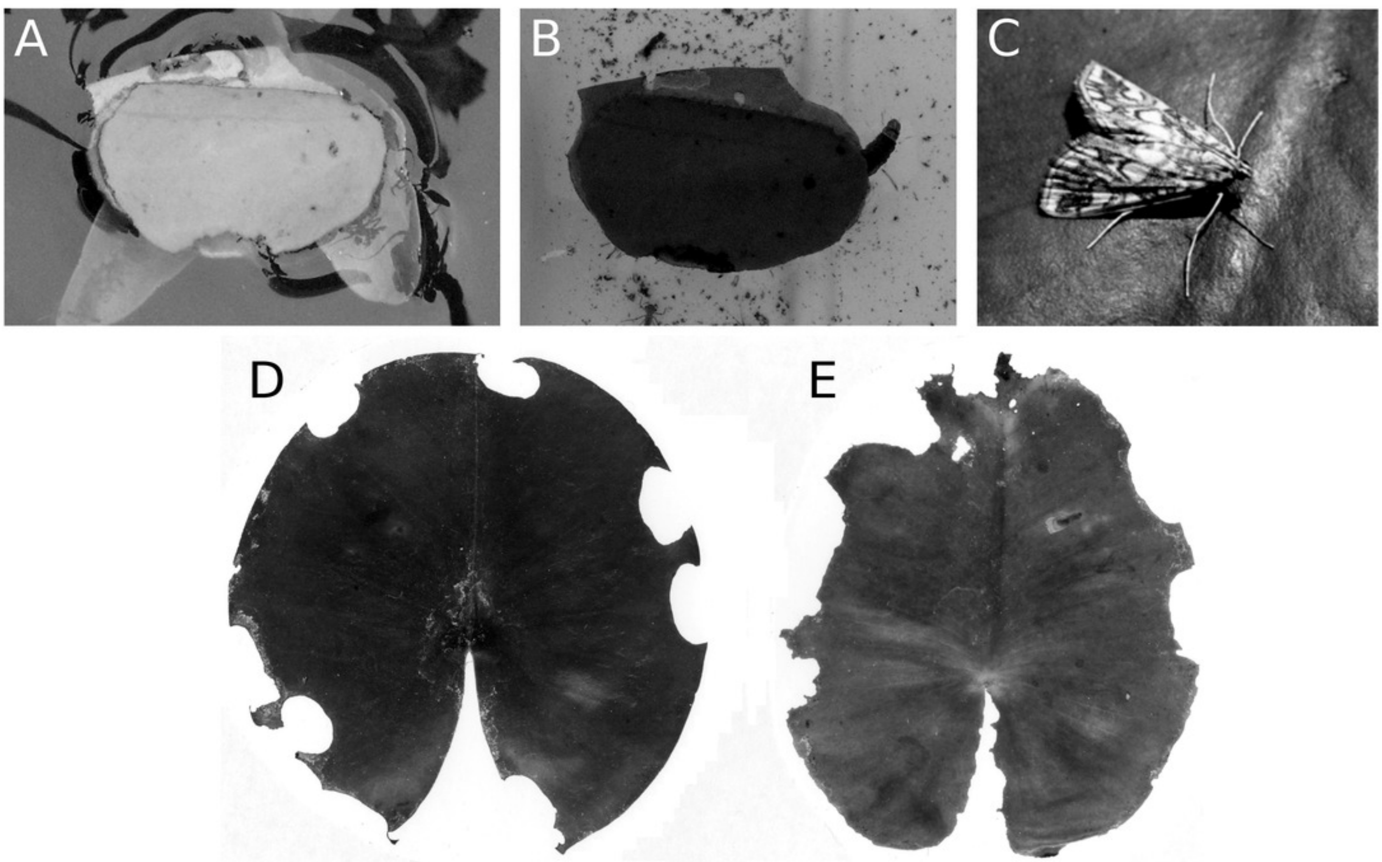
Damage by scratches caused by the nails of *Fulica atra* or *Gallinula chloropus*. Also visible are damage by *Pythium* “type F” and dehydration of the leaf margin.



# Figure 10

Damage by caterpillars.

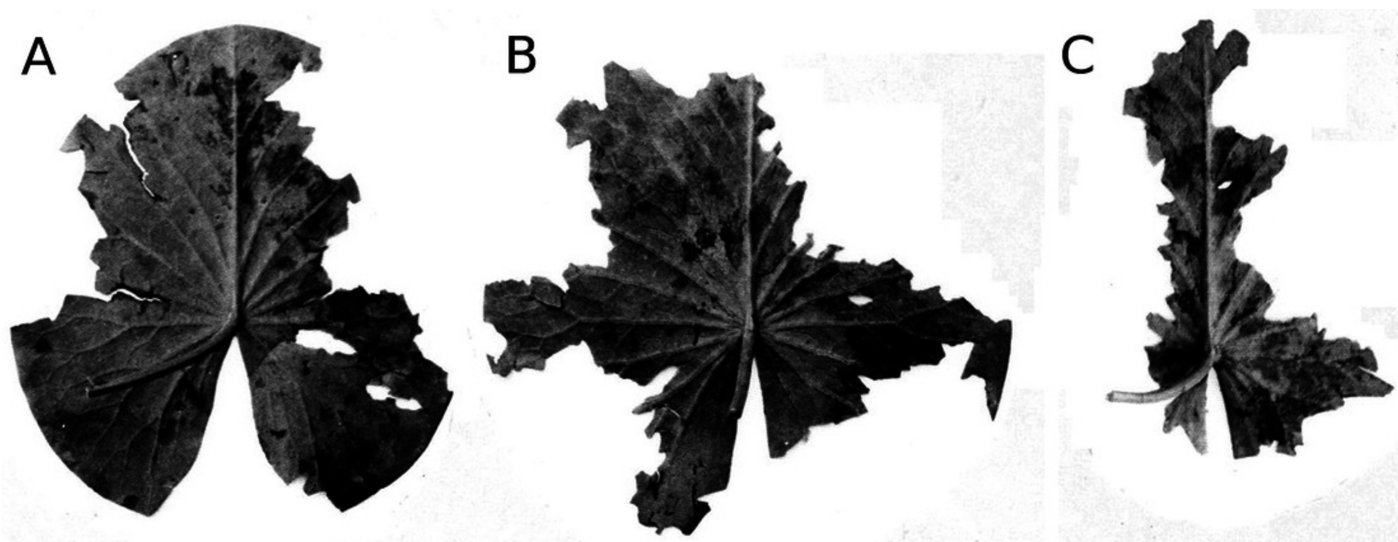
Damage by caterpillars of the moth *Elophila nymphaeata* on *Nymphaea alba* . Where (A, B) show a caterpillar in a free floating shelter composed of two pieces of floating leaf, (C) shows a moth on a leaf, (D, E) show damage on floating leaves of *Nymphaea alba*.



# Figure 11

Consumption by water birds.

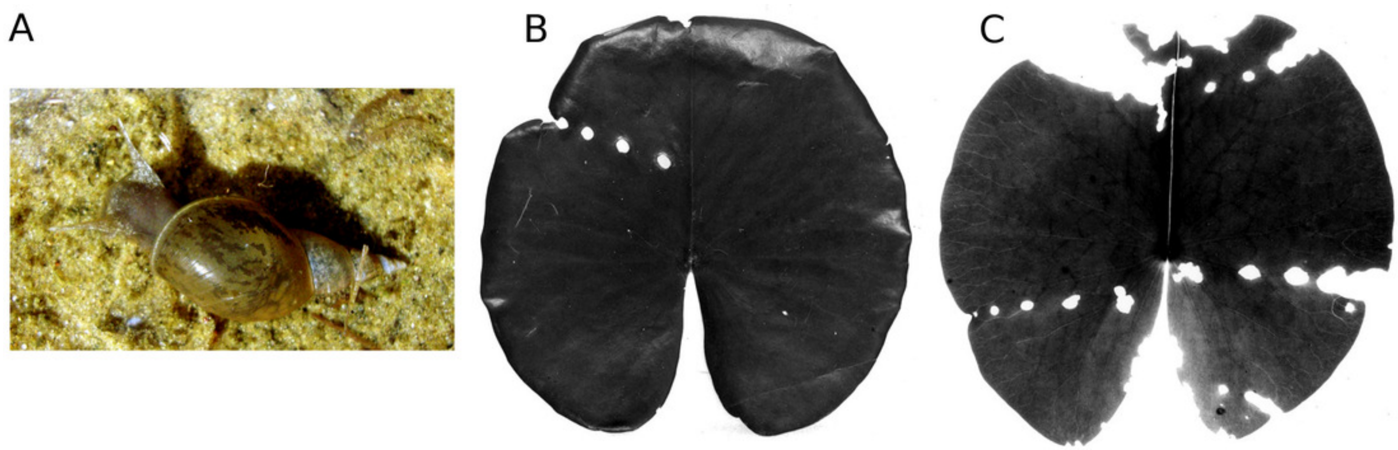
Consumption by water birds. (A, B, C) show damage by consumption of leaf tissue by *Fulica atra* on *Nymphaea alba*.



# Figure 12

Damage by snails.

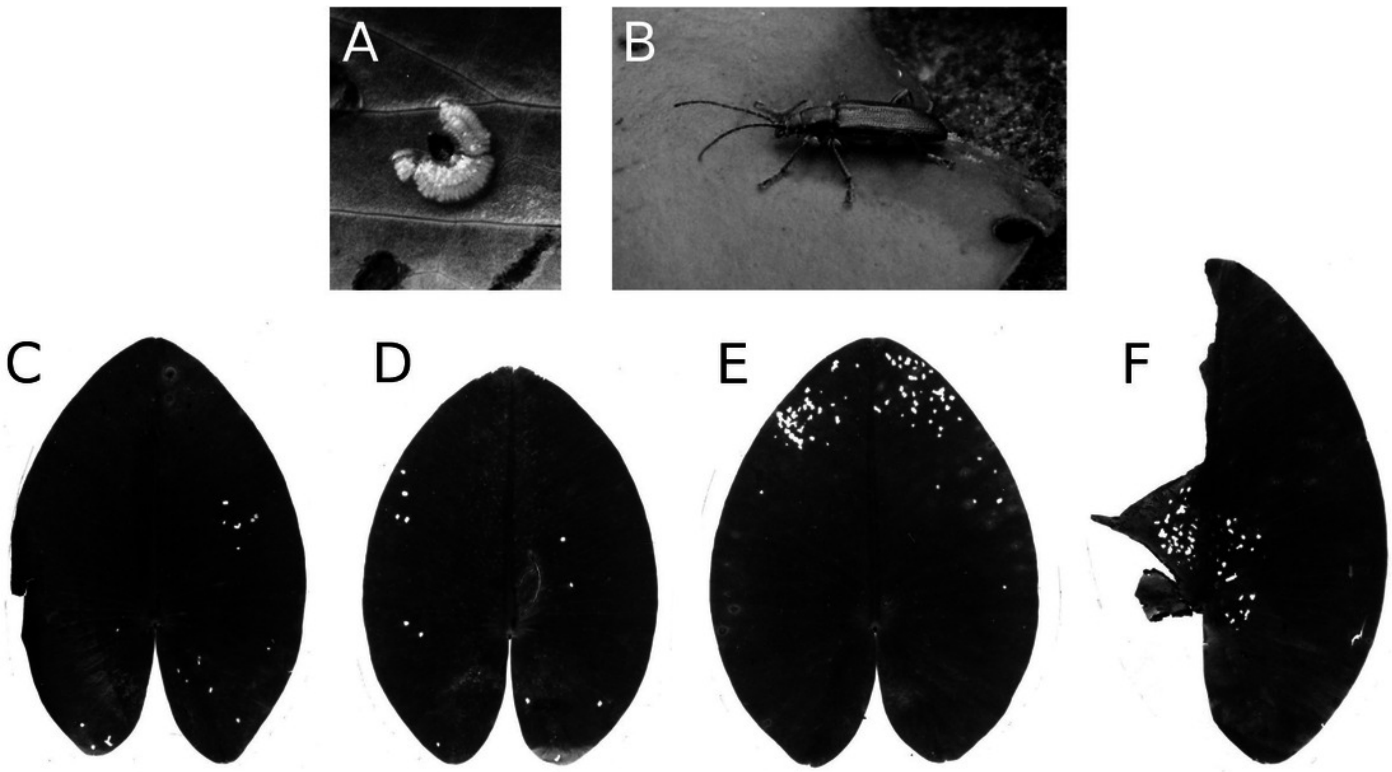
Damage by snails. (A) shows the snail *Lymnaea stagnalis* , (B, C) show damage by *Lymnaea stagnalis* on *Nymphaea alba* (row of holes in leaf created before unrolling of the leaf).



# Figure 13

Damage by imagines of the beetle *Donacia crassipes*.

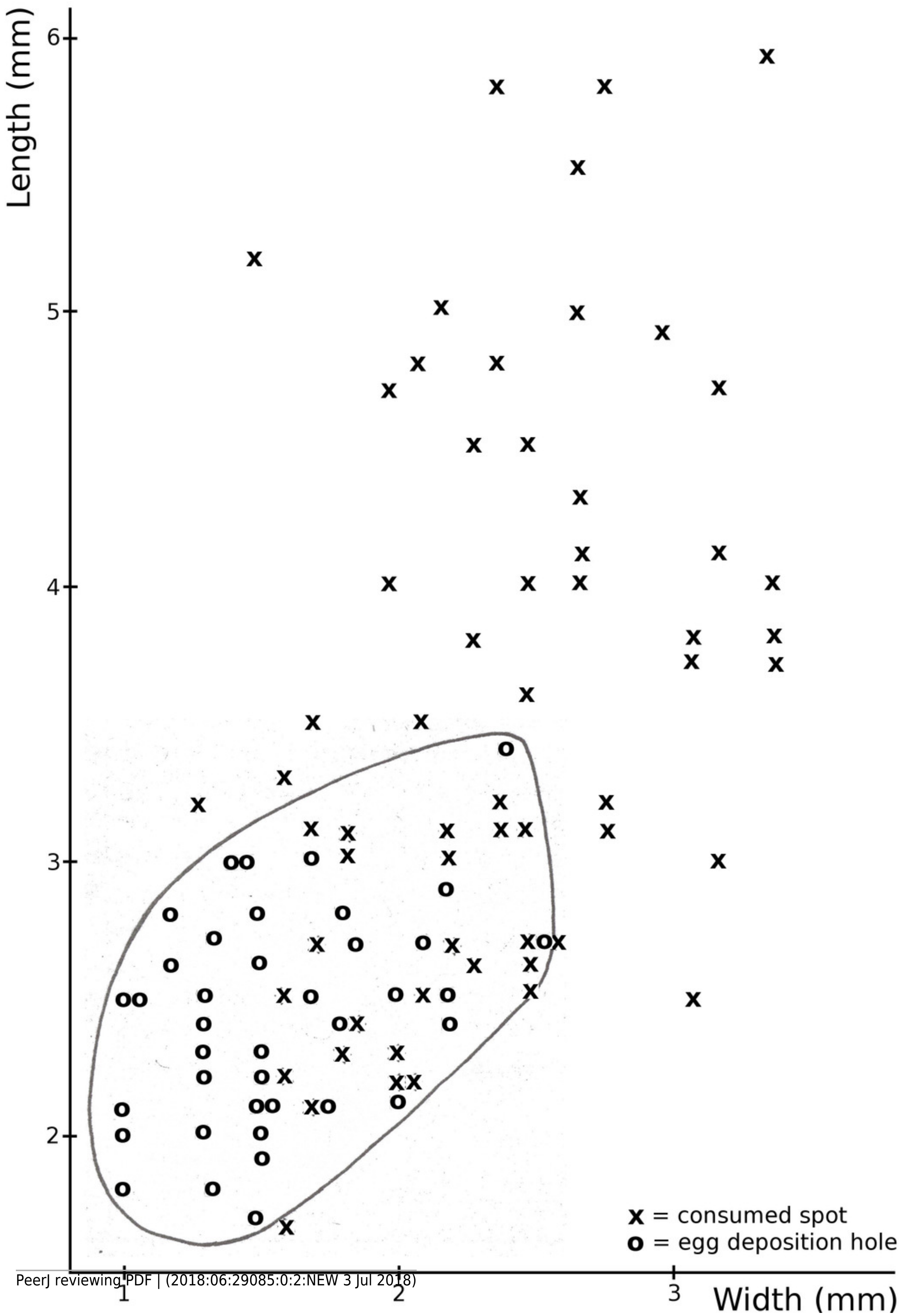
Damage by imagines of the beetle *Donacia crassipes* on floating leaves of *Nuphar lutea* . (A) shows eggs of *Donacia crassipes* at the underside of a floating leaf of *Nuphar lutea* , (B) imago of *Donacia crassipes* on *Nymphaea alba* , (C, D, E, F) leaves of *Nuphar lutea* damaged by consumption of *Donacia crassipes*.



# Figure 14

Damage by imagines of the beetle *Donacia crassipes.*

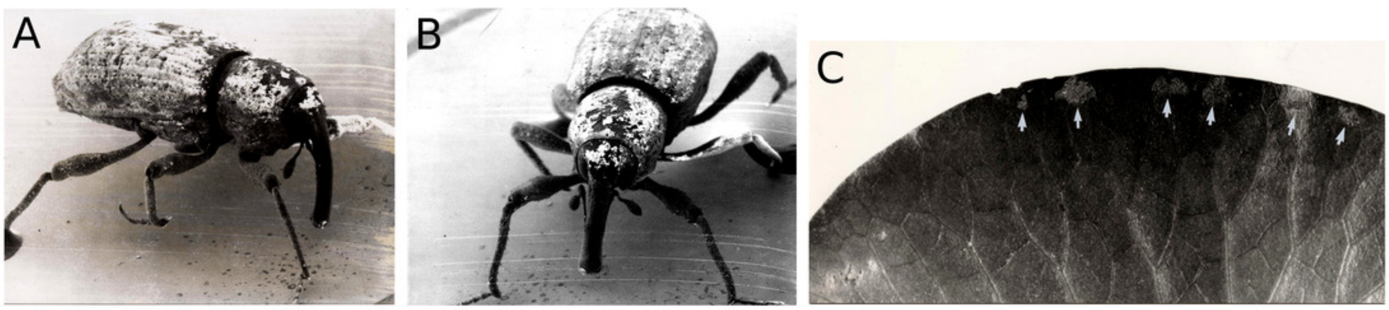
Damage by imagines of the beetle *Donacia crassipes* on floating leaves of *Nuphar lutea*. (A) shows eggs of *Donacia crassipes* at the underside of a floating leaf of *Nuphar lutea*, (B) imago of *Donacia crassipes* on *Nymphaea alba*, (C, D, E, F) leaves of *Nuphar lutea* damaged by consumption of *Donacia crassipes*.


Length (mm)
6
5
4
3
2
1
2
3
Width (mm)
x = consumed spot
o = egg deposition hole

# Figure 15

*Bagous rotundicollis*.

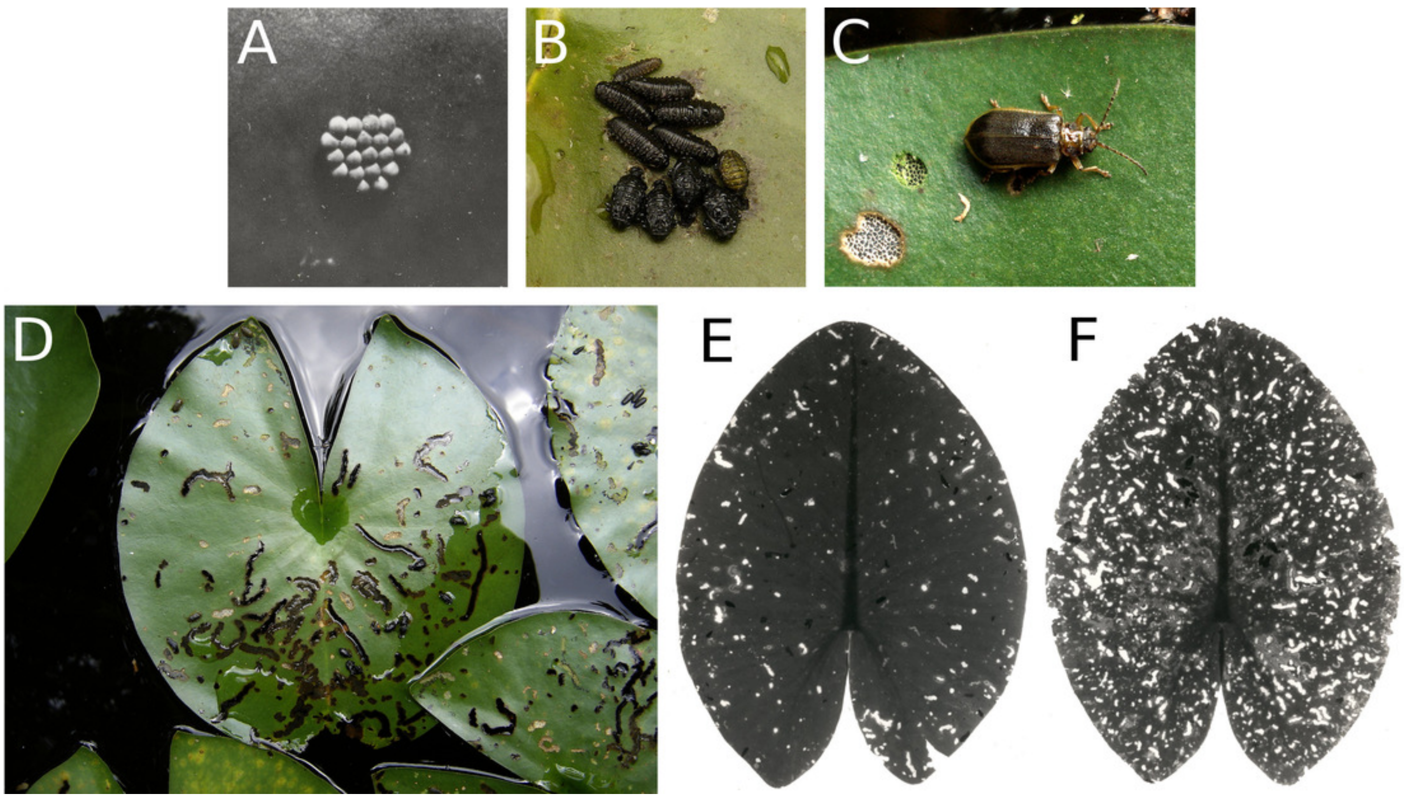
*Bagous rotundicollis*. (A, B) show an imago and (C) shows the damaged spots indicated by white arrows along the margin on the underside of a leaf.



# Figure 16

Damage by larvae and imagines of *Galerucella nymphaeae*.

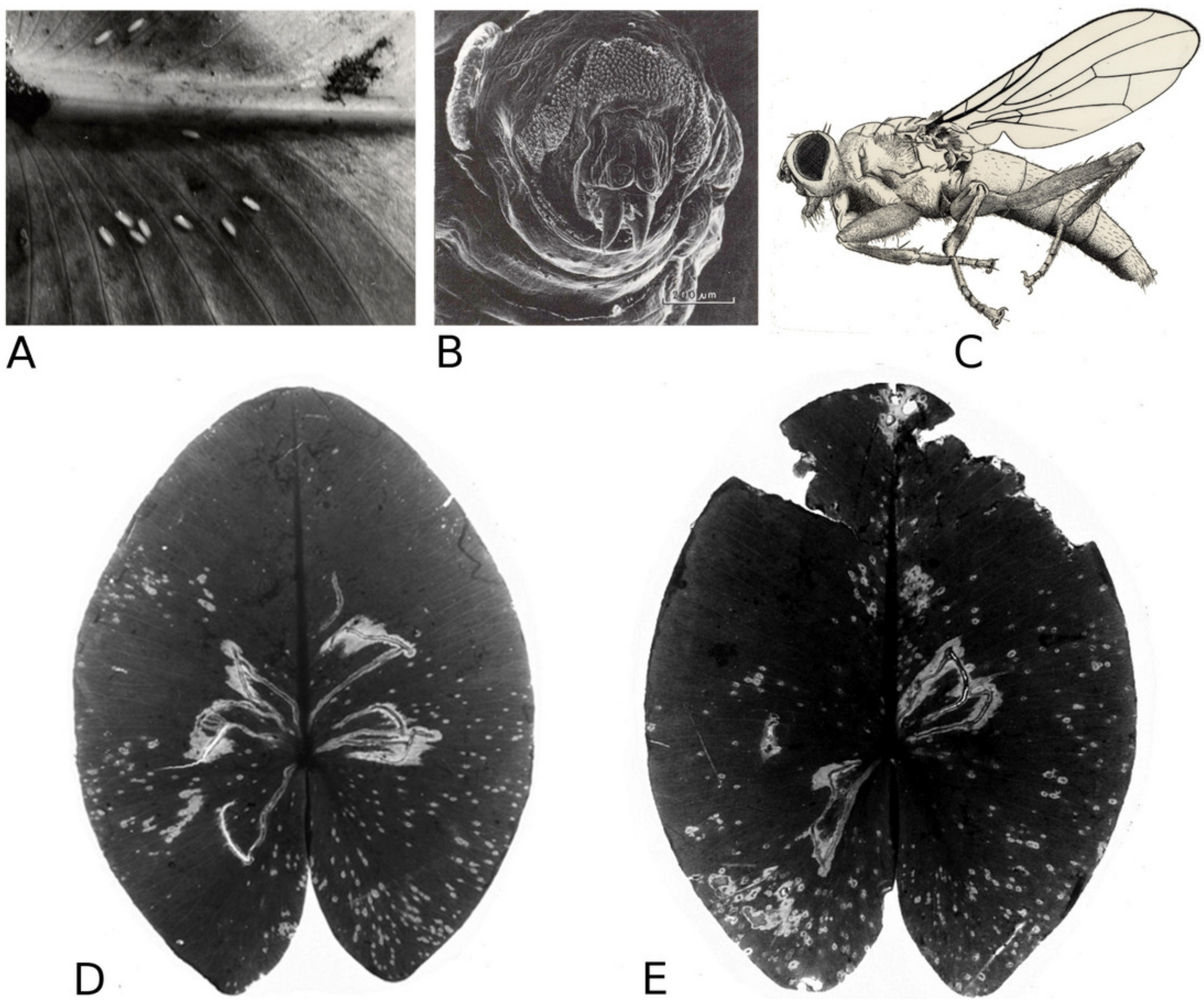
Damage by larvae and imagines of *Galerucella nymphaeae* by consumption of floating leaves. (A) shows eggs, (B) shows larvae and pupae, (C) shows an imago with consumption spots, (D) shows typical damage patterns by larvae on *Nymphaea alba* and (E, F) show damage patterns by larvae and imagines on *Nuphar lutea*.



# Figure 17

Damage by *Hydromyza livens* larvae.

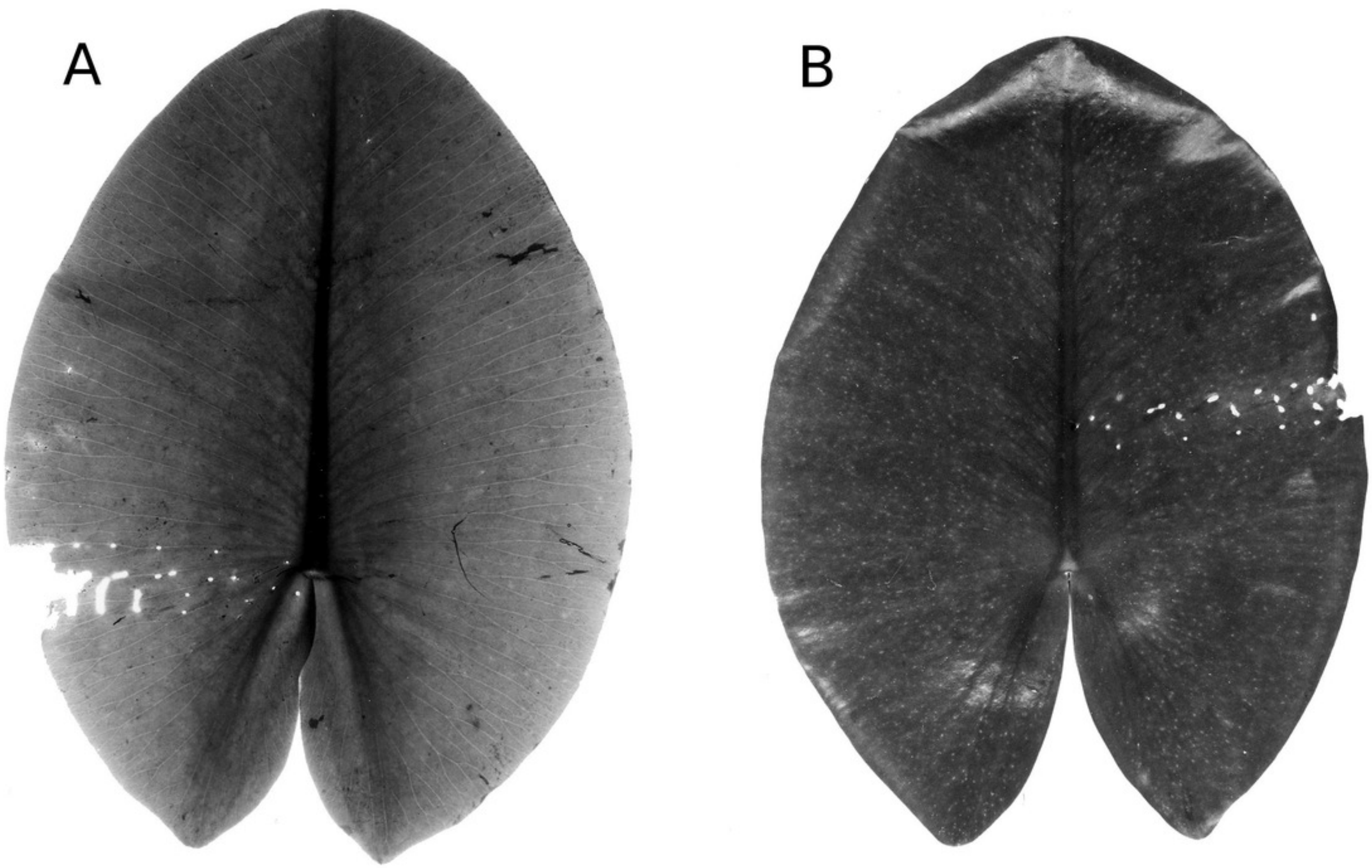
Damage by *Hydromyza livens* larvae. (A) shows eggs of *Hydromyza livens* on the underside of a *Nuphar lutea* leaf, (B) shows a scanning electron microscope image of the head of a larva, (C) shows an imago, (D, E) show mine tracks of larvae on *Nuphar lutea* (D, E). The photos (D, E) also show infection by *Pythium* spec. (scattered small spots). Photos of leaves made with translucent light.



# Figure 18

Damage by larvae of the chironomid *Tribelos intextus.*

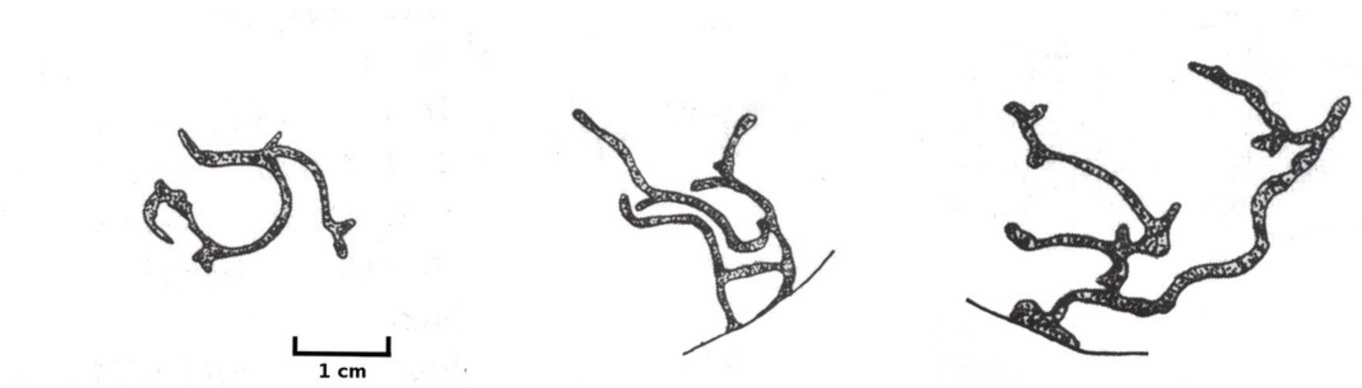
Damage by larvae of the chironomid *Tribelos intextus* on *Nuphar lutea* (A, B).



# Figure 19

Typical mining patterns by larvae of *Cricotopus trifasciatus.*

Typical mining patterns by larvae of *Cricotopus trifasciatus* (Chironomidae) on floating leaves. Patterns on the leaf (left) and near the leaf margin (middle and right).



# Figure 20

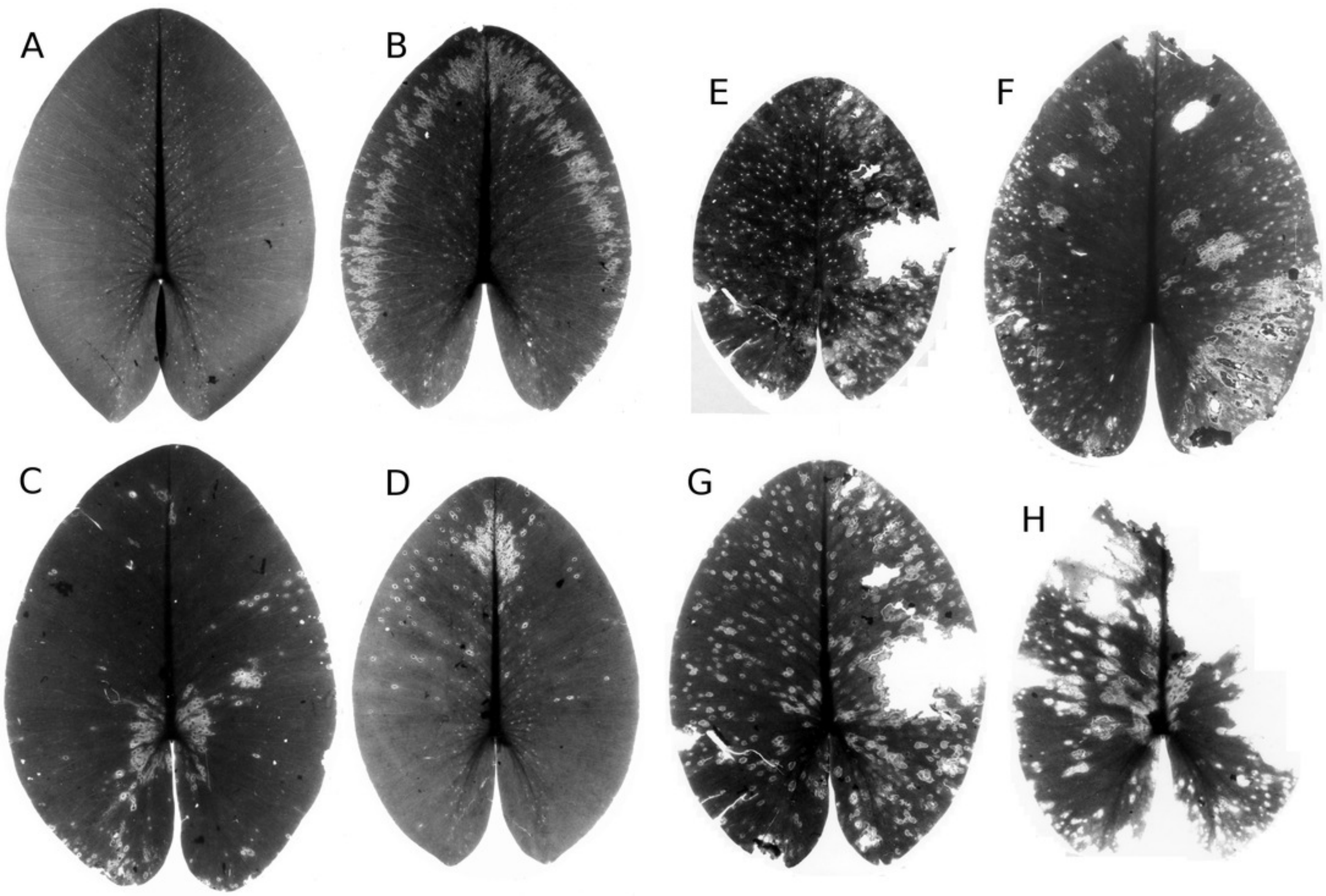
Typical mining patterns by larvae of *Endochironomus* spec.

Typical mining patterns by larvae of *Endochironomus* spec. (Chironomidae). Patterns on the leaf (left and middle) and near the leaf margin (right).

# Figure 21

Damage by *Pythium* “type F”.

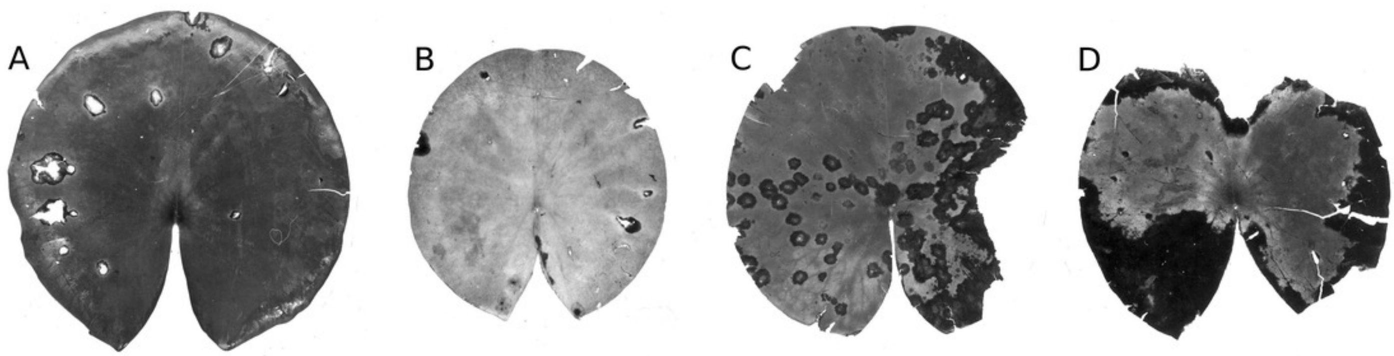
Damage by *Pythium* “type F” on *Nuphar lutea* (A-H). Photos made by translucent light.



# Figure 22

Damage by *Colletotrichum nymphaeae* .

Damage by *Colletotrichum nymphaeae* (A, B, C, D) on *Nymphaea alba.* A shows infection spots that are consumed by snails.



## Table 1 (on next page)

Physico-chemical characteristics of the three investigated water bodies.

Physico-chemical characteristics of the three investigated water bodies. Chemical characteristics according to Brock, Boon & Paffen (1985) and Kok, Van der Velde & Landsbergen (1990).

| | Haarsteegse Wiel (HW) | Oude Waal (OW) | Voorste Goorven (VG) |
|---|---|---|---|
| Area (ha) | 18 | 25 | 5 |
| Depth (m) | 17 | 1.5 | 2 |
| Water level fluctuations | Low | High | Low |
| Stratification | Yes (summer,<br>thermocline at 4-6 m) | No | No |
| Hydrology | Precipitation/evaporation<br>Seepage | Precipitation/evaporation<br>Upward seepage<br>River water overflow | Precipitation/evaporation<br>Upward seepage |
| Direct environment | Trees, bushes, reeds | Meadows | Forest |
| Wind and wave action | Low | Moderate | Moderate |
| Bottom | Sand / sapropelium | Sand / clay / sapropelium | Sand / sapropelium |
| Trophic status | Eutrophic | Highly eutrophic | Oligotrophic |
| Chemical characteristics:<br>Alkalinity (meq.L$^{-1}$)<br>pH | <br>1.5<br>7.1-8.5 | <br>5.2<br>6.7-8.3 | <br>0.0-0.07<br>4.7-5.5 |
| Sampling year | 1977 | 1977 | 1988 |
| Plots, depth (m) | *Nuphar lutea*, 1.5<br>*Nymphaea candida*, 2.5 | *Nuphar lutea*, 1.5<br>*Nymphaea alba*, 1.5 | *Nuphar lutea*, 2<br>*Nymphaea alba*, 2 |

# Table 2 (on next page)

Classification of causes of initial decomposition.

Classification of causes of initial decomposition of floating leaves (after Van der Velde *et al*., 1982).

<table>
<tr><td>internal</td><td colspan="3">autolysis</td></tr>
<tr><td rowspan="7">external</td><td rowspan="3">abiotic</td><td colspan="2">frost</td></tr>
<tr><td colspan="2">hail stones</td></tr>
<tr><td colspan="2">wind and wave action</td></tr>
<tr><td rowspan="4">biotic</td><td rowspan="2">animals</td><td>damage</td></tr>
<tr><td>consumption</td></tr>
<tr><td colspan="2">fungal decay</td></tr>
<tr><td colspan="2">microbial decay</td></tr>
</table>

## Table 3(on next page)

Information about the plots in the sites.

Information about the plots in the sites. HW = Haarsteegse Wiel, OW = Oude Waal, VG = Voorste Goorven.

| Species | Site | Year | Vegetation period | Total number of leaves.m$^{-2}$ | Total potential area of leaves (cm$^2$) |
|---|---|---|---|---|---|
| *Nuphar lutea* | HW | 1977 | May 10 – November 24 | 77 | 49674 |
| *Nuphar lutea* | OW | 1977 | May 11 – November 1 | 59 | 39898 |
| *Nuphar lutea* | VG | 1988 | April 28 – October 27 | 22 | 8440 |
| *Nymphaea candida* | HW | 1977 | June 7 – October 19 | 43 | 11185 |
| *Nymphaea alba* | OW | 1977 | May 11 – November 6 | 108 | 53035 |
| *Nymphaea alba* | VG | 1988 | April 28 – October 27 | 80 | 23053 |

## Table 4(on next page)

Damage to leaves.

Damage to leaves. Per damage cause the percentage of leaves affected, the average (av.) and maximum (max.) percentage of the potential area affected and the area of lost surface tissue for all leaves produced per plot are shown. The total number of leaves and the total potential area of leaves per plot are listed in Table 3. The plots with *Nuphar lutea* are indicated by (1) = HW, 1977; (2) = OW, 1977; (3) = VG, 1988; the plot with *Nymphaea candida* by (4) = HW, 1977; the plots with *Nymphaea alba* with (5) = OW, 1977; (6) = VG, 1988.

| Damage cause | Percentage of leaves affected | | | | | | Percentage of potential area affected | | | | | | | | | | | | Area lost (cm$^2$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) | (1) | | (2) | | (3) | | (4) | | (5) | | (6) | | (1) | (2) | (5) | (4) | (3) | (6) |
| | | | | | | | av | max. | av. | max. | av. | max. | av. | max. | av. | max. | av. | max. | | | | | | |
| Autolysis | 79 | 92 | 91 | 84 | 78 | 64 | 6.32 | 40.00 | 6.19 | 19.00 | 4.84 | 23.50 | 10.92 | 39.00 | 5.39 | 35.00 | 2.94 | 15.71 | 4278 | 2508 | 4727 | 2181 | 1868 | 2748 |
| Frost | - | 2 | - | - | - | - | - | - | 0.01 | 0.83 | - | - | - | - | - | - | - | - | - | 5 | - | - | - | - |
| Hail stones | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Dehydration | 23 | 37 | - | 9 | 28 | 6 | 0.45 | 5.00 | 0.97 | 6.86 | - | - | 0.05 | 0.63 | 0.64 | 7.78 | 0.17 | 8.00 | 384 | 603 | 854 | 9 | - | 48 |
| Mechanical damage | 78 | 47 | - | 74 | 80 | - | 1.05 | 8.75 | 1.15 | 10.00 | - | - | 0.79 | 3.29 | 1.51 | 10.91 | - | - | 546 | 577 | 1118 | 95 | - | - |
| Scratches | 83 | 59 | - | 84 | 77 | - | 0.67 | 1.00 | 0.49 | 1.00 | - | - | 0.64 | 1.00 | 0.61 | 1.00 | - | - | 382 | 223 | 386 | 83 | - | - |
| Damage by *Elophila nymphaeata* | 10 | 3 | - | - | 6 | - | 0.36 | 5.00 | 0.11 | 3.57 | - | - | - | - | 0.12 | 3.89 | - | - | 144 | 43 | 66 | - | - | - |
| Consumption by *Fulica atra* | 36 | 14 | - | 12 | 50 | - | 0.78 | 10.00 | 0.56 | 17.50 | - | - | 0.08 | 0.92 | 0.58 | 3.00 | - | - | 385 | 204 | 442 | 14 | - | - |
| Consumption by snails | 56 | 12 | - | 12 | 13 | - | 2.47 | 10.00 | 0.41 | 5.43 | - | - | 0.34 | 5.00 | 0.25 | 8.00 | - | - | 1113 | 203 | 120 | 26 | - | - |
| Consumption by *Donacia crassipes* | 65 | 63 | 73 | 70 | 54 | - | 0.62 | 2.00 | 0.60 | 1.75 | 0.78 | 2.00 | 0.57 | 1.17 | 0.41 | 1.56 | - | - | 375 | 285 | 324 | 74 | 64 | - |
| Consumption by *Bagous rotundicollis* | - | - | - | - | - | 29 | - | - | - | - | - | - | - | - | - | - | 0.20 | 1.00 | - | - | - | - | - | 63 |
| Consumption by *Galerucella nymphaeae* | - | - | - | - | - | 24 | - | - | - | - | - | - | - | - | - | - | 0.28 | 2.73 | - | - | - | - | - | 85 |
| Mining by *Hydromyza livens* | 65 | 69 | 73 | - | - | - | 1.31 | 6.45 | 1.10 | 4.00 | 1.34 | 3.50 | - | - | - | - | - | - | 786 | 516 | - | - | 119 | - |
| Mining by Chironomidae | 14 | 2 | - | 2 | 6 | - | 0.18 | 5.00 | 0.02 | 1.00 | - | - | 0.01 | 0.38 | 0.05 | 1.00 | - | - | 99 | 7 | 33 | 3 | - | - |
| Mining by *Endochironomus* spec. | 5 | - | 50 | 12 | 25 | 23 | 0.04 | 1.20 | - | - | 1.08 | 5.00 | 0.09 | 1.00 | 0.29 | 1.80 | 0.52 | 5.40 | 34 | - | 181 | 13 | 99 | 110 |
| Fungi<br>*Pythium* "type F"<br>*Colletotrichum nymphaeae* | <br>86<br>- | <br>92<br>- | <br>77<br>- | <br>-<br>79 | <br>-<br>53 | <br>-<br>94 | <br>4.21<br>- | <br>11.75 | <br>6.07<br>- | <br>12.86<br>- | <br>1.02<br>- | <br>4.86<br>- | <br>-<br>6.68 | <br>-<br>17.86 | <br>-<br>6.10 | <br>-<br>21.67 | <br>-<br>2.08 | <br>-<br>8.80 | <br>2879<br>- | <br>3153<br>- | <br>-<br>11464 | <br>-<br>3274 | <br>277<br>- | <br>-<br>767 |
| Microbial decay | 56 | 86 | - | 56 | 72 | - | 4.87 | 26.25 | 9.67 | 26.11 | - | - | 0.39 | 5.25 | 2.84 | 26.78 | - | - | 8803 | 11844 | 5634 | 182 | - | - |
| Unknown causes | 65 | 5 | - | 19 | 34 | - | 7.19 | 33.33 | 0.05 | 1.00 | - | - | 1.04 | 26.67 | 1.59 | 40.00 | - | - | 3888 | 20 | 1235 | 115 | - | - |