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The reproductive phenology of *Isoetes lacustris* L.: Results of field studies in Scandinavian lakes

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Abstract

The phenologic change of the leaf rosette structure of *Isoetes lacustris* L. was studied in 26 lakes of temperate, boreal, or subarctic Scandinavia between 59° and 70° n.l. The investigations were carried out during six defined seasons: late winter, spring, early summer, late summer, autumn, and early winter. From 640 plants, gained with the aid of SCUBA, six leaf types were distinguished: immature megasporophylls, mature megasporophylls, immature microsporophylls, mature microsporophylls, and sporophylls that had released their spores and leaves with undeveloped sporangia. Mean numbers per rosette of each leaf type were established in each study lake and study season, resulting in a common pattern embracing all lakes studied.

Megasporophylls are developed throughout the year, whenever the water temperature is about 10 °C. Their share was always more than 30%, excepting winter. Microsporophylls are produced preferentially in spring/early summer when the days are longest; they amount to more than 50% of the rosette leaves during this period, but only to some 10% in the remaining seasons. The spores mature and are released between late summer and early winter. It is concluded that not all spores mature in the year of their birth, and those that do not mature are released in the early summer of the following year, as well as the old empty leaves become detached.

Keywords: *Isoetes lacustris*; Rosette development; Climate; Seasons; Reproductive phenology

Introduction

Isoetes species are small-growing, evergreen water plants with thick, stiff leaves that form a basal rosette and have a proportionally large below ground biomass (Hutchinson, 1975). This growth form is typical for species in unproductive oligotrophic environments (Vitousek, 1982). *Isoetes lacustris* has developed particular adaptations to enable successful growth in carbon-limited lakes (Petersen & Sand-Jensen, 1995; Roelofs, Schuurkes, & Smits, 1984; Wium-Andersen & Andersen, 1972). Photosynthetically produced oxygen is released into the sediment through the roots, in this way stimulating mineralization and nitrification rates (Risgaard-Petersen & Jensen, 1997). As a result, the ecosystem can remain stable and oligotrophic for many centuries, *Isoetes* species performing a clear key-importance.

According to Karfalt and Eggert (1977) a qillwort plant produces the same number of leaves each year, after the first few years. Consistently, the annual leaf turnover of *I. lacustris* was established at about 1.0 leaf/leaf and year in Finnish sites (Kansanen & Niemi, 1974)

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and 0.85 or 1.0 leaves/leaf and year in the Wisconsin lakes (Boston & Adams, 1987). Hence, the leaf number does not explain the plant age. Bell and Hemsley (2000) stress that the plant stock, having reached its mature diameter, remains more or less the same size, as does the leaf number per year (Gacia & Ballesteros, 1994). In fact, the corm diameter of mature plants proved to be significantly correlated with the number of leaves (Vöge, 1997).

The leaf rosette of the quillwort consists of megasporophylls, microsporophylls, leaves in which the spores have been released, and leaves with undeveloped sporangia. The plants release many leaves and spores in autumn; however, they keep further leaves attached to the corm, during winter. Hence, the number of the different leaf types in a rosette appears to be a feature of a plant in a particular site in a particular season.

Though the quillwort has been studied from various aspects the phenology of the rosette structure is unknown. Because the typical reproductive phenology is a basic condition that enables the continued growth of the species, such studies were planned to be performed in a multitude of Scandinavian lakes in different climate regions, between late and early winter, with the following objectives:

(1) How does the leaf rosette develop: when do mega- and microsporophylls emerge during a year?
(2) Is a common pattern of the reproductive phenology recognized, accounting for different climate regions?

Materials and methods

The study subjects, area, and study time

The study subjects of the studies were the leaf rosettes of sporogenous *I. lacustris*.

The lakes which were visited in 2002/2003 are listed in Table 1, together with their coordinates, determined using GPS. They are situated between 59° and 70 ° n.l.; most lakes (17) are in Norway, with the rest in Sweden (7), and Finland (2). The altitude of the study lakes did not exceed 100 m, with the exception of one lake in Sweden near Kiruna (450 m). The electrolytic richness was measured (WTW) and the data are given in Table 1. The lakes possessed a Secchi depth of at least 5 m.

Two excursions were conducted: one each from late winter to early summer in 2002, and from late summer to early winter in 2003. Following Gacia and Ballesteros (1994) there is no noticeable leaf production between early and late winter; hence, the studies were interrupted during this period.

Table 1. The lakes studied, geographical position, and water conductivity

No.	Lake name	Country	Latitude	Longitude	$\mu S\,cm^{-1}$
1	Stordalsvatnet	S	N 59.6904°	E 006.0189°	25
2	Jölstravatnet	N	N 61.5109°	E 006.1569°	16
3	Honnan	S	N 62.0197°	E 015.9022°	32
4	Kilspollen	N	N 62.0256°	E 006.0398°	23
5	Stugusjön	N	N 62.3965°	E 010.4272°	42
6	Revsundsjön	S	N 62.7201°	E 015.4408°	107
7	Näkten	S	N 62.9612°	E 014.5548°	100
8	Krinsvatn	N	N 63.8024°	E 010.2299°	31
9	Non-available	N	N 63.8793°	E 010.2165°	40
10	Non-available	N	N 64.2312°	E 010.8297°	35
11	Grongstadvatnet	N	N 64.5760°	E 012.2445°	32
12	Store Svenningsvatn	N	N 65.3257°	E 013.3782°	25
13	Norr Svergeträsket	S	N 65.5410°	E 017.6449°	26
14	Storavan	S	N 65.6138°	E 018.3613°	18
15	Ömmervatn	N	N 65.9875°	E 013.4413°	35
16	Kvitblikvatnet	N	N 67.3306°	E 015.4863°	122
17	Non-available	S	N 67.8135°	E 020.5848°	30
18	Sandnesvatnet	N	N 67.8506°	E 016.0163°	30
19	Skillvatnet	N	N 68.0697°	E 015.9264°	35
20	Non-available	N	N 69.2617°	E 016.0788°	71
21	Inarijärvi	FIN	N 69.2793°	E 028.0134°	22
22	Boogvannet	N	N 69.5725°	E 030.0396°	40
23	Ukonselka	FIN	N 69.5811°	E 028.7032°	25
24	Rundvann	N	N 69.6790°	E 030.0854°	64
25	Abborvann	N	N 69.6822°	E 030.0374°	36
26	Jansvannet	N	N 70.6498°	E 023.6680°	117

With the aid of SCUBA, in 26 lakes without perceptible impact each 20 fertile plants were selected at random from *Isoetes* populations characterized by similar density at a depth of about 2 m. In order to protect the population, neighbouring plants were spared. The study lakes were situated in temperate, boreal or subarctic regions. The short seasonal growth in cold regions results in a small number of leaves, compared with temperate climate conditions (Vöge, 1997, 2003). Hence, when describing the particular leaf rosette, percentage terms of the different leaf types had to be calculated.

The leaf types

Six leaf types were distinguished when describing the seasonal rosette structure. Sporophylls are called immature, as long as their spores are immature. The following six leaf types were distinguished: mature and immature megasporophylls, mature and immature microsporophylls, leaves with an empty sporangium, and leaves with an undeveloped sporangium. Spores are described as immature, when their colour is typically light. It is stressed, that spores mature gradually, thus, separating mature and immature sporophylls cannot be considered error free. Leaves with a sporangium containing residual mega- or microspores were assigned to the group of mega- or microsporophylls. Leaves with an empty sporangium had released all their spores. Vegetative leaves plus the innermost, youngest leaves, which may be recognized as mega- or microsporophylls later on, are described now as undeveloped sporangia. The sporangia proved to develop early and may be recognized as mega- or microsporangia also on rather young leaves.

The study seasons

Six study seasons were distinguished in this study: late winter, spring, early summer, late summer, autumn, and early winter. They are described by common events that are observed everywhere in the study region independent of climate, such as:

(1) *late winter*: the ice cover of lakes disappears,
(2) *spring*: flowering of *Tussilago farfara*,
(3) *early summer*: rising water temperature,
(4) *late summer*: decreasing water temperature,
(5) *autumn*: yellowing of leaves,
(6) *early winter*: ice cover develops.

The development of quillwort plants depends on altitude, northern latitude and, possibly, on microclimate conditions of the lake inhabited. The climate conditions in boreal and subarctic regions, compared with a temperate region, result in a long winter, a short summer and a relatively small number of leaves per rosette. It is assumed that the water temperature in a particular season reflects the shares of the different leaf types in the rosettes. Table 2 shows how the leaf data, which describe the rosette structure of a certain plant, are assigned to one of the six study seasons. The actual study area and month plus the concerning guide water temperature explain the study season. The guide water temperatures are means from earlier studies, which were performed in the regarding regions and months (unpubl. data). So, the data established in a subarctic region in May at less than 10 °C water temperature are assigned to "late winter", the plant data gained in a temperate region in May at more than 10 °C belongs to "spring".

Evaluation of the plant samples

For each 20 plants sampled in the 26 study lakes, the mean numbers of each leaf type were calculated and their shares were given in percentage terms. They were assigned to the corresponding study season. Excel was used to calculate the standard deviation. Thus, from the phenology of the six leaf types, the reproductive phenology of the quillwort plant may be concluded.

Table 2. Assigning of rosette data to the study season

Boreal/subarctic study area			Season		Temperate study area	
Guide temperature (°C)	Month				Month	Guide Temperature (°C)
8.0	September/October	→	Early winter	←	November	7.6
4.0	October–March	→	Winter	←	December–February	4.5
6.8	April/May	→	Late winter	←	March	6.1
12.5	May/June	→	Spring	←	April/May	12.0
18.0	June/July	→	Early summer	←	June/July	17.0
18.7	July/August	→	Late summer	←	August/September	18.0
13.0	August/September	→	Autumn	←	October	11.9

Results and discussion

Growth conditions in the study lakes

Rørslett and Brettum (1989) have established the water conductivity for 387 Scandinavian lakes supporting quillwort. The mean conductivity was 46 $\mu S\,cm^{-1}$, the minimum value 3 $\mu S\,cm^{-1}$ and the maximum value was 265 $\mu S\,cm^{-1}$. The corresponding data, gained in this study, is listed in Table 1: mean conductivity is 45 $\mu S\,cm^{-1}$, minimum value is 16 $\mu S\,cm^{-1}$ and the maximum value is 122 $\mu S\,cm^{-1}$; the span of the data, describing the electrolyte content, is rather small and similar to the data given above (Rørslett & Brettum, 1989). Together with the overall sufficient water transparency all study lakes offer good growth conditions for the quillwort.

The rosette structure within a year

The rosette structure, that is the share of each of the six leaf types during the six seasons, is depicted in Fig. 1a–d. The division into immature and mature spores is a source of error, because the spores mature gradually, explaining the standard deviation, which however, appears reasonable (Table 3).

Excepting winter, the share of mature megasporophylls amounts to some 10% and that of immature megasporophylls make up some 20% between spring and autumn. In early winter, both mature and immature megasporophylls constitute about 10%, it is the same for immature megasporophylls in late winter, but the share of mature megasporophylls is lowest at that time: about 4%. It has to be emphasized, that in particular the immature megasporophylls make up a considerable part in the leaf rosette throughout the year (Fig. 1a). The megasporophyll development is interrupted during winter, due to low water temperature. The main development of microsporophylls, however, is a rather short event between spring and early summer (Fig. 1b). Leaves with empty sporangia are at their minimum in early summer (Fig. 1c). Hence, in early summer the leaves, originating from last year, are detached when

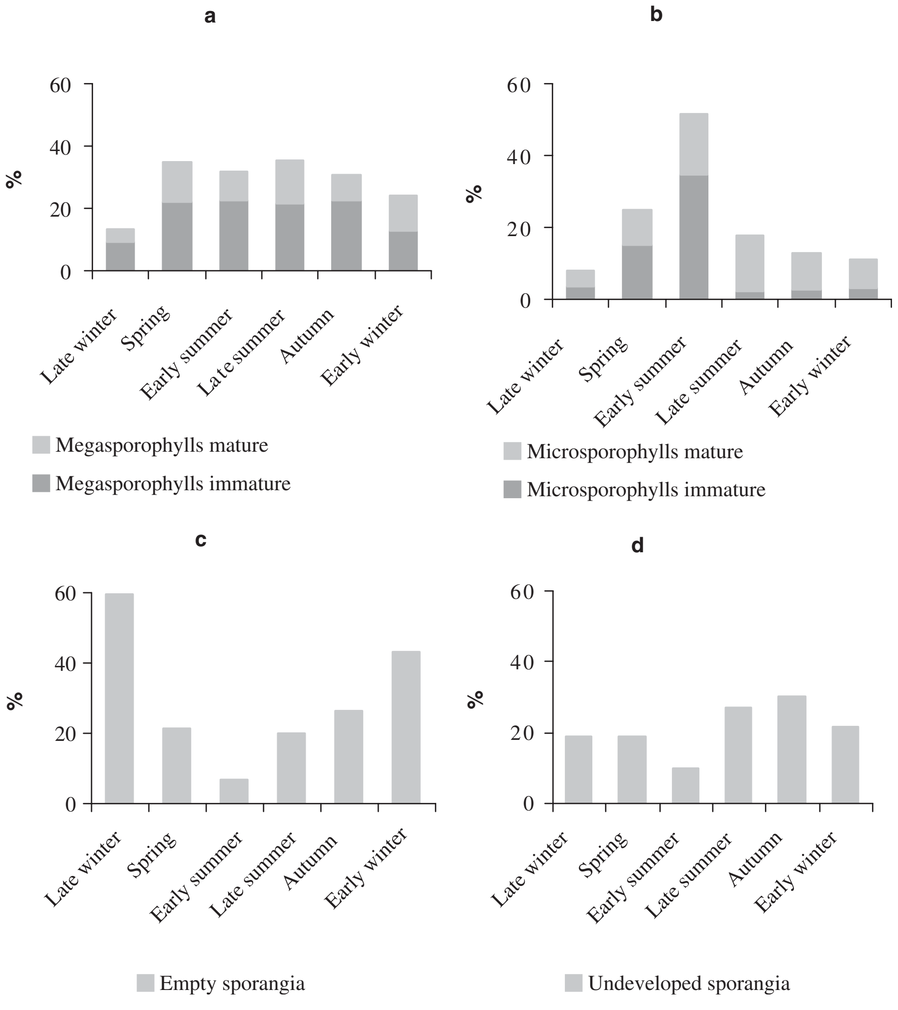

Fig. 1. (a–d) Mean percents of leaves per rosette during a year, (a) mature and immature megasporophylls; (b) mature and immature microsporophylls; (c) leaves with empty sporangia; and (d) leaves with undeveloped sporangia.

Table 3. Rosette structure during the seasons studied

% Leaves per rosette	Late winter	Spring	Early summer	Late summer	Autumn	Early winter
Megasporophylls, mature	4.3 ± 1.7	12.8 ± 1.9	9.5 ± 2.6	14.3 ± 5.2	8.2 ± 3.8	11.0 ± 0.6
Megasporophylls, immature	9.1 ± 8.8	22.0 ± 6.2	22.2 ± 4.6	21.3 ± 5.6	22.5 ± 4.6	12.9 ± 2.1
Microsporophylls, mature	4.4 ± 3.4	9.5 ± 5.0	17.1 ± 2.8	15.4 ± 4.5	10.1 ± 9.5	7.7 ± 1.3
Microsporophylls, immature	3.5 ± 2.9	15.3 ± 4.5	34.6 ± 6.7	2.2 ± 1.5	2.6 ± 2.6	3.3 ± 2.6
Empty sporangia	59.7 ± 12.5	21.4 ± 10.6	6.9 ± 5.1	19.8 ± 2.4	26.5 ± 6.8	43.3 ± 14.0
Undeveloped sporangia	19.1 ± 2.4	19.0 ± 6.5	9.7 ± 9.1	27.1 ± 5.7	30.1 ± 12.1	21.7 ± 5.4

their spores have been released. During summer, the share of leaves with empty sporangia strongly increases again, when this year's sporophylls and spores mature and are detached. The percentages of leaves with undeveloped sporangia (this group of leaves proved to consist nearly exclusively of very young leaves) vary less distinctly in the course of a year (Fig. 1d), indicating together with the quick, continued increase of the empty sporangia, that leaves, megasporophylls preferably, are born rather continuously. They are most numerous in late summer and autumn. Not all spores mature in the year in which the sporophylls were born, explaining the remarkable share of such leaves during winter and spring. The development is finished until early summer, a turning point in quillwort life. The standard deviation (Table 3) is remarkably high for leaves with empty and undeveloped sporangia. Both kinds of leaves are in their minimum in early summer but increase clearly in late summer. This rapid change may explain the high deviation of the early summer data. It is remarkably high, too, for the microsporophylls in autumn. During the last years it was difficult to categorize the results to a certain season; possibly due to climate change – an "autumn" may appear more as a "late summer".

Which leaves persist during winter? Regarding the rosette structure in early winter, the plants spend winter with nearly half of the leaves possessing empty sporangia; their share has increased during winter (60%): hence, further mega- and microsporophylls have released their spores. The shares of leaves with immature and undeveloped sporangia have remained nearly the same during winter, as had been expected.

Sporophyte and gametophyte

The most important results that were established in this study are the following:

A common pattern of the reproductive phenology of *Isoetes* was recognized, independent of the different climate conditions of the sites studied. However, a small number of plants in each population follow their own way in the annual rosette development, which possibly may be advantageous regarding continued survival.

The life history of *I. lacustris* sporophytes appears to be an annually continued growth of megasporophylls, interrupted by a short period of microsporophyll development near the time of the longest days in the year. However, a small amount of microsprorphylls is produced throughout the year if the water is warm enough. The steady development of megasporophylls may be connected with the low number (nearly 100) of rather large spores contained in a megasporangium. The more sporadic appearance of microsporophylls may be related to the high number (nearly 100,000) of tiny spores in a microsporangium. In fact, the plant overwinters with a small reserve supply of each immature and mature micro- and megasporophylls. While ice developing might damage a corm without leaves, a more or less complete plant may follow its typical life cycle.

Following the course of life of the quillwort from a megaspore to a sporophyte, an embryo develops in a megaprothallium. The embryo does not possess chlorophyll, so it must live on megaprothallium substance. In case the megaspore is very small – if the spore producing plant lives under sustained environmental impacts – the prothallium is very small too and the embryo may suffer from food shortage. The sporophyte that unrolls later on may be limited too in its further development. Sporelings are often observed in contact with megaspores, possessing each up to four leaves and roots. On juvenile plants, which are often observed on the corm of fertile plants, up to nine leaves are developed on the young corm. In their second year, the leaf number per rosette is at least ten and the plant becomes fertile (Vöge, 2003; Szmeja, 1994). In the course of a year, new leaves displace the old ones together with roots and corm tissue, which are sloughed off (Kott & Britton, 1983). After an unknown number of the cycles, as characterized above, the plants attain the senile stage, when some few infertile sporangia only make part of the leaf rosette (Vöge, 2003). Plants in a senile stage are observed rarely in a population; it is assumed that quillwort plants may become rather old. Further to this, quillwort plants may disappear within 1 or 2 years, because vegetative parts decompose very rapidly

(Hanlon, 1982). According to Rørslett (1985) the dead plants decay so fast, that only an impression in the surficial sediment layer was left.

Conclusions

Following Rørslett and Brettum (1989) it is emphasized that quillworts are victims of their own, insufficiently flexible growth strategy under sustained environmental impacts. The lacking flexibility appears to be due to the positive feedback that was mentioned above. In eutrophicated lakes, the water transparency depends on the plankton density, which varies seasonally. Usually in early summer, a "plankton window" is recognized. The results of the recent studies suggest that the corresponding high light availability may favour the microsporophyll development. Frequently, during summer, the plankton density is high and the lack of light may affect the formation of megasporophylls and, possibly, the spore maturity. A particularly long, warm autumn, due to climate change, may encourage the leaf development, if the water is clear enough. Hence, the rosette structure may vary within certain limits.

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