

Life strategies of boreal aquatic plants
***Potamogeton alpinus* Balb. and *Ranunculus reptans* L.**
near the southern boundary of their geographical range
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In 1979, Grime hypothesised that plant life strategies depend on three main selection pressures, *i.e.* competition, stress and disturbances. According to this hypothesis, **disturbances** are factors reducing a specimen's mass because of partial or total destruction of its environment. As sessile organisms, plants cannot escape if environmental conditions have deteriorated and thus, they experience biotic and abiotic constraints. For most terrestrial plants, major environmental constraints are (i) limited availability of soil resources, light and space, or (ii) herbivores. A major factor shaping aquatic ecosystems is water motion, thus in addition to the previous constraints, aquatic plants are under the pressure of water flow or wave action, which may cause mechanical damage, and – indirectly – water turbidity, erosion or substrate deposition.

The resistance of plants to environmental disturbances depends on their **phenotypic plasticity**, *i.e.* the ability of a given genotype to morphologically and/or physiologically adapt to its environmental conditions (Schlichting 1986). In a dynamic environment, this might prevent the damage or destruction of shoots, leaves and reproductive organs. Plant response to disturbances can be interpreted in the context of trade-off life strategies, *i.e.* a compromise between avoidance and tolerance, as described by Puijalon et al. (2011).

Tolerance strategy enables the plant to cope with the impact of adverse conditions, whereas avoidance helps to prevent it. As far as hydrodynamic disturbances are concerned, tolerance strategy allows plants to survive mechanical constraints mainly by increasing tissue resistance and the cross-sectional area of their stem. This requires allocation of more biomass to radial growth and a greater content of resistant structural compounds like cellulose and lignin.

Avoidance strategy, on the other hand, is associated with features that allow plants to mitigate the mechanical constraints they encounter, *e.g.* by reconfiguring shoot architecture and/or reducing the area exposed to water flow, as well as the ability to form dense, protective patches and/or to change leaf structure and shape.

The present study deals with life strategies of two **boreal species of clonal aquatic plants**, *Potamogeton alpinus* Balb. and *Ranunculus reptans* L., near the southern edge of their geographical range in Europe. Both are scarce in most European countries. In north-western

and central Europe, they occur locally and, in some countries, they are vulnerable or endangered elements of the flora. The available literature disagrees as to the causes of their scarcity and extinction. It is suggested that the persistence of many of the populations is threatened by climate changes and human pressure on water bodies; other interpretations point to limited gene flow between local populations, which reduces genetic diversity, induces inbreeding and limits adaptability. Indeed, hydroengineering works reducing or eliminating water level fluctuations, coupled with eutrophication of freshwaters, drastically modify aquatic ecosystems (Fischer et al. 2000; van Kleunen et al. 2000b; Riis and Sand-Jensen 2001). A better understanding of plant behaviour in the naturally disturbed environment, in which they spontaneously occur, is a crucial step towards their more effective protection.

It should be noted that plastic reactions of boreal aquatic plants in the southern boundary of their geographical range (*i.e.*, the region I studied), have not yet been identified. Consequently, in European countries close to the southern coast of the Baltic, there is an urgent need to update the scientific basis of the legal and environmental protection of boreal aquatic plants and their habitats. My studies have been performed in north-western Poland, up to 120 km south of the Baltic Sea. This area is close to the south-eastern boundary of the geographical range of the two species and is separated by the Baltic Sea from their distribution centres in the Scandinavian Peninsula (Meusel et al. 1965; Hultén and Fries 1986).

In view of the above, **the aim of this doctoral dissertation is to identify the life strategies of two boreal aquatic plants.** The specific goals of the dissertation include:

1. the description of **environmental conditions** (physico-chemical features of the water and the substrate, photosynthetically active radiation, water flow, etc.) as well as the nature and intensity of environmental disturbances in the populations;
2. the examination of **specimen architecture** depending on environmental conditions as well as the nature and intensity of hydrodynamic constraints (the flow in the watercourses and wave motion in the littoral of lakes);
3. the description of **phenotypic reactions to hydrodynamic constraints** based on *in situ* research of permanent plots in water bodies and on *ex situ* laboratory experiments;
4. the identification of the species' **life strategy** in response to hydrodynamic disturbances.

Potamogeton alpinus (alpine or red pondweed; Potamogetonaceae), is a submerged aquatic plant with a boreal-mountain type distribution in the Northern Hemisphere (Meusel et al. 1965). Its presence in wetland deposits was found in fossil materials from the beginning or the end of interglacial periods, mainly from cooler climate stages (Velichkevich and Zastawniak 2006). Today, the species occurs mostly in the Scandinavian peninsula and much less frequently in central and north-west Europe. It is most common in small, well-lit, not very fertile and shallow watercourses, slightly acidic or alkaline, and in oligotrophic flow-through lakes (Wiegleb and Todeskino 1983; Zalewska-Gałosz 2008).

I conducted my studies of *P. alpinus* in 15 populations in rivers and flow-through lakes in Pomorskie Lake District (**Robionek et al. 2015**). I determined environmental conditions in the watercourses on the basis of 18 physico-chemical features of water and sediment. Environmental conditions in slow flowing (*i.e.* water flow < 0.2 m/s) and fast flowing watercourses (*i.e.* 0.2 – 0.7 m/s) differed mainly in terms of water, mineral and organic matter content in the sediments, but each of the features is directly dependent on water flow itself. In the slow streams, there are two types of sites significantly differing in terms of light intensity: well illuminated (PAR 82.7 ± 13.5 %) or shaded (PAR 41.2 ± 5.0 %).

I described the architecture of *P. alpinus* on the basis of 20 traits in a total of 411 plant samples, *i.e.* modules of this clonal species. Modules are repeating structural units of growth, consisting of above-ground stem (with leaves, shoot and inflorescence) and a fragment of the rhizome connecting the stem with the next module (or with more flanking modules). I observed two types of flowering modules: one with floating and submerged leaves, the other with submerged leaves only. I assessed the influence of 18 environmental factors on 17 plant traits. Furthermore, I analysed the variability of five architectural features of modules. For most of the traits compared, the modules differed significantly in the two types of watercourses; moreover, I demonstrated a greater variability of module features among fast than among slow watercourses.

I also checked if, in the absence of hydrodynamic constraints, other environmental factors affect the architecture of *P. alpinus*. I found that plants with floating leaves occurred not only in fast watercourses, but also in slowly flowing and shaded waters. Plants with no floating leaves, on the other hand, occur in slow and illuminated waters. Light intensity may be an environmental factor determining the presence or absence of floating leaves where water flow is slow.

I interpreted the data in terms of **the life strategies facing hydrodynamic disturbances**. *Potamogeton alpinus* displays a number of phenotypic responses to fast-water flow that exemplify the strategy of avoidance rather than of tolerance. Firstly, plants produce floating leaves which absorb CO₂ and O₂ from the air, help to keep the stem as well as the inflorescence in an upright position preventing flower immersion, thus enabling pollination. Another instance of avoidance strategy is shoot elongation. It allows flowering plants in fast flowing water, where they often tilt, to stay above water surface. It also places photosynthetically active leaves closer to the water surface, preventing light deficiency in turbulent flow. In fast flowing waters, submerged leaves are more elongated and, as a result, less exposed to destruction, because of lower hydraulic resistance. Smaller shoot diameter also mitigates hydrodynamic forces. Another expression of avoidance strategy is the strengthening of the shoot, *i.e.* a higher stem-specific density and a greater participation of the stele in the cross section of the stem, resulting in higher resistance of the stem in fast water flow. When hydrodynamic constraints are absent, *P. alpinus* morphology mainly responds to insufficient light intensity. Avoidance strategy is then reflected by the presence of floating leaves in slow and shaded streams, where the floating leaves mitigate the effects of insufficient light intensity. Stems without floating leaves are only observed in well-lit slow streams, where floating leaves are not needed and where their absence can be interpreted as an avoidance of unnecessary expenditure of biomass and energy.

As shown above, *P. alpinus* is characterised by high phenotypic plasticity, which together with its phenological reactions enables it to adapt to environmental changes. **Phenology** is the study of periodic events in biological life cycles and of the determinism of seasonal climate variations and habitat factors. The timing of periodic events in the case of water plants mainly depends on temperature; its rise may cause shifts in phenology. Therefore, phenological data are useful for the estimation of biological effects of both present and future climate changes. Recording the timing of periodic events in plant populations, especially at the periphery of their geographical range where climate may be less suitable, becomes vitally important in view of the increasing human pressure and, most of all, climate warming. Phenological analysis of aquatic populations has already showed that the timing of life history events responds to changes in global environmental conditions (Gałka and Szmeja 2013). In the future, phenological data on *P. alpinus* may be crucial for assessing the impact of global warming on the phenology of alpine pondweed. They may also be used for the protection of boreal aquatic plant species in this part of Europe, for analytical comparison with Scandinavian

population, and even for data interpretation in the framework of palaeoecological reconstructions.

The first step in the phenological studies I have conducted (**Robionek et al. 2018**) was to identify in detail the age stages (morphological development stages) of *P. alpinus*, on the basis of seven traits of its architecture and biomass allocation to particular parts of the plant. On the basis of 728 modules, I described five age stages: juvenile, mature, generative, senile and winter bud (which plays the functional role of overwintering turion). Thereafter, I collected phenological data from permanent plots. Every two weeks, I measured the environmental conditions, the number of modules and their age stages, without plant removal. On the basis of the dominance of the age stages, I identified five phenological phases: growth, maturation, reproduction, senescence and winter dormancy (= resting stage). I described the time of their emergence, the duration as well as mean water temperature and shoot density associated with each stage. I found water temperature to be the environmental trait discriminating the phenological phases.

For many species, clonal reproduction, as well as shifts in the duration of age stages, offers a safe escape route under suboptimal environmental conditions. Climate warming might disturb the development timing of *P. alpinus*, decreasing the probability of its sexual reproduction and the phenological distance between populations from Central European Plain and from Scandinavia.

The second species that I have studied is *Ranunculus reptans* (creeping spearwort; Ranunculaceae), which has a circumpolar distribution in temperate to boreal-sub-arctic zones of Europe, Asia and North America (Hultén and Fries 1986). This amphibious plant usually lives in shallow and periodically flooded shores of oligotrophic lakes (van Kleunen et al. 2000a), and in temporarily flooded habitats that are essentially free of reed, woody species, or graminoids. It has a perennial clonal structure, and reproduces mainly in a vegetative way by the growth of offspring modules (*i.e.* as above, a repeated structural growth unit, consisting of above-ground stem and a fragment of stolon linking to the flanking module/modules). *Ranunculus reptans* produces seeds only on the emerged shores (Prati and Peintinger 2000).

I studied *R. reptans* (**Robionek et al. 2020**) in 18 lakes, with one population each. I described the environmental conditions in the lakes on the basis of eleven major physico-chemical features of water and sediment (but *a posteriori*, the lakes did not differ in this respect). I described plant architecture on the basis of 13 traits documented in 1455 modules,

either aquatic (permanently submerged; 57% of plants) or terrestrial (out of water throughout the year; 43%). I documented radical differences in *R. reptans* architecture and reproduction. Reduction of plant size, elongation of leaves and stolons, as well as vegetative reproduction only are characteristic of immersed modules. Terrestrial modules are larger, with wider lanceolate leaves and shorter stolons; they reproduce both vegetatively and generatively.

I also determined the population density and distribution of the four developmental stages in fourteen lakes at *R. reptans* flowering time, by comparing measurements from immersed (390 samples) *versus* emerged (120 samples) populations. Aquatic *R. reptans* populations are on average less dense than the terrestrial ones. As for developmental stages, I observed lower density of generative modules and higher density of rooted juvenile and mature modules under water than in terrestrial conditions.

In order to better understand the terrestrial *versus* aquatic adaptations, my work also included an experimental approach. I determined the *ex situ* persistence of seven architectural features of *R. reptans* in a laboratory culture in which 480 modules were grown for nine weeks in four different, controlled conditions. During the experiment, changes in most of the studied traits were consistent with the *in situ* tendencies. In terrestrial conditions, plants tend to be bigger, their leaves wider and lanceolate leaves more numerous; growth of new modules is faster in terrestrial conditions and leads to higher population density on land. However, some experimental results were less consistent with *in situ* observations, suggesting the contribution of other factors, such as different light availability, the presence of epiphyton, and the divergent extent of hydrodynamic disturbances.

I have interpreted all the data collected as the result of the life strategy in the face of submersion and hydrodynamic disturbance. The changes of *R. reptans* traits under water have been consistent with two main characteristics of avoidance, *i.e.* smaller size and longer, acicular and thinner leaves than on land. All these features minimise (i) the frontal area, (ii) the risk of uprooting, (iii) hydraulic resistance, and (iv) mechanical damage. Another manifestation of avoidance in *R. reptans* is its high capacity for architectural reconfiguration when environmental conditions change. This involves modifications of module architecture and even organ reshaping, as clearly demonstrated by the transplantation experiments. Moreover, the formation of short but complex individuals with multiple axes rooted in many points of the substrate decreases the risk of uprooting and is also connected with a strategy of tolerance. I therefore do not exclude a certain combination of tolerance and avoidance strategies in *R. reptans*.

In conclusion, **both studied species have showed many features indicative of avoidance strategy**, but only a few features of tolerance strategy. *Potamogeton alpinus* and *R. reptans* differ significantly in many respects, e.g. their evolutionary history, a preference for running vs. standing waters, architecture type (caulescent with nonlinear leaves vs. a rosette with linear leaves). Despite these marked differences, I have observed in both species similar trait changes in reaction to hydrodynamic constraints, which are consistent with avoidance strategy. Thus, the presented series of publications contributes to a better understanding of life strategies of aquatic plants and broadens our knowledge concerning the ecology of boreal aquatic plants in the south-east of their geographical range.

The research conducted is an analysis of life strategies of both plant species, showing tendencies which are partly supported by the literature quoted or completely new. The results presented here may soon be applied in the protection of boreal plant species and their habitats in Central Europe. They are also a preliminary research for a wider comparative analysis of life strategies of sensitive boreal plants in view of the imminent climate changes. An assessment of the full diversification of life strategies of *P. alpinus* and *R. reptans* requires comparative studies in the centres of their geographical range, particularly in the Scandinavian Peninsula and in mountainous sites, mainly in the Alps.

CONCLUSIONS

The following features displayed by *Potamogeton alpinus* and *Ranunculus reptans* have pointed to their life strategy of avoidance:

1. high capacity for architectural reconfiguration in a dynamic environment;
2. frontal area minimisation by plant size and stem diameter reduction;
3. increased shoot elasticity thanks to its elongation, greater density and stele proportion;
4. reduced hydraulic resistance, drag coefficient and mechanical damage to leaves thanks to thinner, elongated submerged leaves;
5. keeping flowers above water surface by developing floating leaves in watercourses, which stabilises the stem and raises the inflorescence, or by flowering only on land, out of water;
6. in the absence of hydrodynamic disturbance, *P. alpinus* has also revealed a strategy of avoiding low light intensity by producing floating leaves in shaded streams.

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