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Mechanism of Duckweed Morphological Plasticity under Gradient Phosphate Concentrations

Zifei Li

Collège du Léman, Geneva, Switzerland
uliana.perrotti@cdl.ch

Abstract. Phosphorus is a key limiting nutrient triggering aquatic eutrophication, and duckweed with simple structure and sensitive phenotypic changes is an ideal model to explore plant phosphorus adaptive responses. This paper focuses on the morphological plasticity of duckweed exposed to low, moderate and high phosphate gradients and constructs a multi-index evaluation system for its growth status. Low phosphate restrains duckweed cell division and chlorophyll synthesis, leading to shrunken pale fronds and elongated roots for nutrient capture. Moderate phosphate supports balanced frond proliferation, stable root development and continuous biomass accumulation to form optimal growth state. High phosphate brings short-term rapid propagation but induces intraspecific competition and long-term aquatic ecological risks. Single frond counting generates biased analysis, while joint assessment of frond area, leaf color, root length and dry weight can accurately judge phosphorus stress. This study provides theoretical support for applying duckweed as bio-indicator and ecological remediation material in phosphorus-polluted water bodies.

Keywords: Duckweed, phosphate gradient, morphological plasticity, multi-index evaluation

1. Introduction

Phosphorus is an essential macronutrient for aquatic primary producers, mainly absorbed as inorganic phosphate to synthesize ATP, nucleic acids and functional proteins, which directly regulates plant metabolic activity, cell proliferation and tissue development [1]. As a floating aquatic macrophyte with simple structure and rapid asexual reproduction, duckweed is highly sensitive to fluctuations of water phosphate levels. Variations in phosphate concentration not only alter duckweed growth rate and biomass accumulation, but also reshape aquatic microbial communities and break the balance of lake or pond eutrophication [2]. Existing aquatic ecological trials have verified that phosphate, together with nitrogen, constitutes the core limiting nutrient for duckweed proliferation; moderate phosphate supply can significantly boost biomass yield, while extreme low or high phosphate concentrations impose obvious growth restrictions on duckweed populations. Owing to its strong nutrient absorption capacity, duckweed has been widely adopted in the treatment of phosphate-polluted wastewater, and it also serves as an ideal model organism for aquatic plant nutrition research.

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Overseas research mainly focuses on quantitative modeling of duckweed phosphate uptake efficiency and single growth indicators such as frond number. Most foreign controlled trials only count frond quantity to judge growth performance, ignoring morphological traits including root length and leaf pigment content, and lack systematic multi-indicator correlation analysis under continuous phosphate gradients [3]. Western teams have established mathematical models of duckweed phosphorus removal but rarely discuss morphological adaptive plasticity induced by phosphorus stress.

Domestic studies pay more attention to the practical application of duckweed in rural sewage remediation, and preliminarily record leaf yellowing, root elongation and biomass changes under phosphorus deficiency [4]. However, most domestic experiments separate single morphological indexes without integrating frond area, pigment, root trait and fresh weight for comprehensive evaluation. Globally, few literatures systematically summarize the complete morphological response chain of duckweed from low to high phosphate environment, and there is no unified multi-index evaluation system for phosphorus stress response, which restricts its application in water eutrophication monitoring.

Current research generally relies on frond number alone to evaluate duckweed growth status, which cannot fully reflect the comprehensive adaptive changes of individuals under different phosphate gradients. Single indicator evaluation easily generates biased experimental conclusions, while the coordinated variation rules of frond morphology, root traits and biomass remain unclear. To fill this research gap, this paper divides phosphate environments into low, moderate and high concentration groups, and systematically compares duckweed's frond quantity, leaf area, chlorophyll content, root length and fresh weight under three phosphorus levels. This work further elaborates the internal regulatory logic of phosphate on duckweed physiological morphology, and clarifies the application potential of multi-morphological indicators in water phosphorus monitoring and eutrophication control.

2. Basic biological characteristics of duckweed

Duckweed distributes widely in static water and slow flowing water areas such as lakes and artificial ponds across temperate and subtropical zones. The growth cycle of duckweed is far shorter than other higher aquatic plants, and the whole strain can complete population renewal within several weeks under suitable temperature and illumination conditions. Different from large submerged and emergent aquatic plants with complex rhizome systems, duckweed has simplified tissue composition, which only consists of flat fronds and a single slender adventitious root without differentiated stem tissues. Fronds bear two core physiological functions including light energy capture for photosynthesis and generation of new propagules through asexual reproduction. Thin root structures stretch vertically into the water column to absorb dissolved inorganic phosphorus and nitrogen, and provide mild anchoring force to fix floating duckweed individuals on water surface without rooting into sediment layers. The special living mode of duckweed determines its close connection with water body nutrient conditions. All mineral nutrients required for physiological activities are obtained from aqueous solution instead of bottom mud, so subtle fluctuation of phosphate content will trigger continuous adjustment of duckweed tissue structure [3].

Asexual reproduction serves as the dominant population expansion mode of duckweed under warm temperature and balanced nutrition supply. New small daughter fronds germinate continuously from the marginal meristem zone of mature parent fronds, and separate from maternal tissues after reaching a certain volume to form independent individuals. This convenient propagation characteristic makes frond number become the most intuitive and easily counted observation index

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in indoor laboratory culture and field sampling work. Frond quantity alone cannot represent the complete real growth condition of single duckweed individuals under varied phosphorus gradients. Frond expansion area, internal chlorophyll storage level and root stretching degree are equally important auxiliary evaluation standards to reflect physiological health state. For example, long term phosphorus deficiency will slow down the synthesis rate of chlorophyll precursor substances inside frond cells and bring obvious frond chlorosis phenomenon. Insufficient phosphorus supply also stimulates continuous root stretching of duckweed to expand the contact range between root surface and nutrient containing water [5, 6]. Water phosphate concentration can simultaneously adjust frond development state and root morphological characteristics of duckweed, and form a set of coordinated integrated morphological adaptation responses to adapt to different nutritional environments. The simple anatomical structure and sensitive phenotypic change feature make duckweed an ideal experimental material to reveal the interaction rule between aquatic plants and phosphorus nutrition.

3. Morphological responses of duckweed under three phosphate gradient groups

3.1. Growth and morphological changes under low phosphate environment

Water bodies marked by low phosphate content lack enough inorganic phosphorus raw materials to maintain normal biochemical reaction inside duckweed cells. Phosphorus acts as core component of energy carrier ATP and genetic substances DNA and RNA, and phosphorus shortage will seriously restrain synthesis progress of these key compounds, further block cell division and intracellular material active transportation process [7]. Under stable low phosphorus culture conditions, the horizontal expansion speed of frond cells drops obviously, and the overall single frond area presents continuous shrinking trend. The generation quantity of new daughter fronds also decreases month by month with the extension of culture cycle, and the whole population expansion rate slows down significantly at the same time. Insufficient phosphorus supply restricts the complete synthetic pathway of chlorophyll a and chlorophyll b inside frond mesophyll cells. The total pigment content reduces steadily, and frond color turns from uniform deep green to pale yellow with scattered light color patches showing uneven pigment distribution state. High definition image recording technology can capture this visible stress feature for long term comparative analysis of phosphorus stress degree among experimental groups.

Root morphological adjustment acts as the most core adaptive strategy of duckweed coping with long term phosphorus deficiency. To expand the contact area between root epidermis and nutrient containing water, duckweed will extend the total length of adventitious roots remarkably within one to two weeks after being transferred into low phosphorus culture solution. Root stretching trait belongs to a set of conserved phenotypic response shared by most aquatic and terrestrial plants under phosphorus stress, which aims to increase the probability of capturing limited dissolved phosphate molecules in surrounding water. The specific morphological performance of duckweed under low phosphorus environment will not remain fixed. Multiple coexisting environmental factors can interfere with the adjustment amplitude of frond and root tissues, including water nitrogen phosphorus ratio, daily illumination duration and average culture temperature. Under identical low phosphorus concentration standard, different genetic strains of duckweed show obvious individual differences in frond shrinkage range and root elongation multiple. Researchers cannot judge the real phosphorus stress degree only through simple frond counting statistics data. Root length measurement, frond area scanning and fresh weight weighing data need to be incorporated into

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integrated multi dimensional data analysis work to avoid one-sided experimental conclusion deviation [8].

Apart from visible frond and root phenotype, low phosphate environment also brings hidden influence on organic matter accumulation of duckweed populations. The overall fresh weight growth rate of experimental groups under phosphorus limitation is far lower than that of medium phosphorus groups. Even if the frond number of low phosphorus duckweed still maintains slow growth speed, the average dry weight of single individual will keep decreasing because of insufficient material synthesis supply. This hidden biomass change cannot be found only by observing surface frond quantity, which further proves the necessity of combining multiple morphological indicators for comprehensive evaluation.

3.2. Growth and morphological changes under moderate phosphate environment

Moderate phosphate supply level can fully meet the daily material consumption of duckweed cell division and continuous tissue construction. Sufficient dissolved phosphorus in water supports stable synthesis of intracellular ATP and nucleotide substances, and maintains high efficiency of asexual reproduction of frond propagules [9]. Under medium phosphorus gradient culture conditions, duckweed populations grow in balanced and stable states with uniform dark green frond tissues without scattered yellow spots. The total population fresh weight accumulates steadily with the extension of culture cycle, and the dry weight growth curve presents smooth upward trend without obvious stagnation period. Dry weight data can reflect the real organic accumulation capacity of duckweed populations more accurately compared with fresh weight, because fresh samples carry variable free water attached on frond surface, and the weight measurement result will be interfered by water absorption degree during sampling operation. Multiple parallel control culture experiments under different temperature gradients confirm that within the effective optimal phosphate range, the rising concentration of water phosphorus presents positive correlation with the total biomass of duckweed populations. Medium phosphorus environment can form a balanced trade off state between population reproduction speed and single individual development quality.

Sufficient dissolved phosphorus existing in water will remove the driving force that pushes duckweed to stretch root tissues for nutrient capture. The average root length of duckweed individuals maintains stable medium level without excessive stretching growth under moderate phosphate culture conditions. Duckweed will distribute most absorbed energy substances to frond cell proliferation and organic matter accumulation instead of consuming extra nutrition on root tissue development and elongation. Frond expansion space can be fully released under this nutritional state, and single frond can obtain larger photosynthetic area to capture more light energy for carbon fixation reaction. More light energy captured by fronds will be converted into carbohydrate compounds and stored inside plant tissues, laying material foundation for continuous population proliferation. It is necessary to clarify that the so-called moderate phosphate threshold does not represent a fixed unified concentration numerical standard applicable to all duckweed varieties. Different duckweed strains have different phosphorus demand characteristics, and culture temperature and daily light intensity will also change the optimal phosphorus supply range of duckweed populations. Researchers cannot copy a single fixed phosphorus concentration value to all experimental culture systems without strain and environmental adjustment.

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3.3. Growth and morphological changes under high phosphate environment

Excessively high phosphate concentration in water can provide abundant nutrition raw materials in a short term, which accelerates the cell division frequency and new frond formation speed of duckweed populations. Within the first two to four weeks after being transferred into high phosphorus culture solution, the frond number and water surface coverage rate of duckweed rise rapidly, and the overall fresh weight of experimental groups presents sharp growth trend in this stage [6]. If only observing the short term proliferation performance of single duckweed individual, high phosphorus water condition shows obvious growth promoting effect compared with low phosphorus environment. However, the long term ecological risk hidden behind rapid duckweed propagation cannot be ignored from the perspective of complete aquatic ecosystem circulation. Massive duckweed populations formed under high phosphorus load will cover the whole water surface layer densely, block the penetration path of sunlight into the lower water layer, and seriously restrain the photosynthesis efficiency of submerged aquatic plants growing at the bottom of water. A large amount of dead duckweed residues will be produced with the extension of culture time, and these plant tissues will be decomposed by aquatic heterotrophic microorganisms. The whole decomposition process will consume a great deal of dissolved oxygen dissolved in water body, and compress the living space of fish and other aquatic organisms that rely on sufficient oxygen supply [10].

High phosphate culture environment will also bring obvious adjustment to individual morphological characteristics of duckweed instead of simply making all individuals bigger and healthier. Continuous mass propagation of duckweed under high phosphorus load will trigger fierce intraspecific competition among individuals for limited light resources and floating space. Although the total frond quantity of the whole group keeps increasing, the average expansion area of single frond will shrink gradually, and the average root length of each duckweed individual also shows obvious shortening trend compared with moderate phosphorus experimental groups. When analyzing the growth state of duckweed under high phosphorus gradient, researchers need to take both the short term nutrition promotion effect and long term intraspecific resource competition pressure into comprehensive consideration instead of only relying on frond counting data to draw one sided judgment.

Duckweed cultured under high phosphorus environment has strong biological enrichment capacity for phosphate ions dissolved in water. Regular artificial harvesting of mature duckweed populations can take the absorbed phosphorus elements out of water together with plant tissues, and effectively reduce the overall nutrient load of eutrophic water bodies [11]. If the dense floating duckweed populations are not salvaged and cleaned up in time after mass propagation, the residual plant residues will sink to the bottom and decay slowly, and the phosphorus elements stored inside tissues will be released back into water again, which triggers secondary nutrient pollution of the whole water environment. The dual property of duckweed under high phosphate condition needs special attention in practical water restoration work. Scientific artificial management and periodic harvesting can give full play to its phosphorus removal function, while uncontrolled long term propagation will become typical early warning signal of water eutrophication deterioration.

4. Comprehensive multi index evaluation system for duckweed growth state

Judging the real physiological growth state of duckweed only through simple frond number statistics has prominent limitation in experimental analysis and field monitoring work. Frond counting index can only reflect the population reproduction speed within a fixed period, and cannot cover the

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development volume of single individual, chlorophyll health status and total biomass storage capacity of duckweed populations. Under identical phosphate gradient culture conditions, two parallel experimental groups may reach the same frond counting quantity but hold completely different average frond area and dry weight data. Simple single index analysis mode will produce random deviation and one sided error for subsequent experimental conclusion deduction. Combining multiple mutually complementary morphological indicators to carry out joint evaluation can effectively avoid the above analysis defects and improve the objectivity of research results.

Frond area measurement serves as the most important supplementary evaluation standard to make up the deficiency of frond counting statistics. Multiple groups of parallel comparative culture experiments under different phosphorus gradients show that adopting frond quantity or frond area alone to calculate the relative growth rate of duckweed will generate two sets of distinct analysis results. Frond area data can reflect the photosynthetic potential of single duckweed individual, and make up the information blank brought by only counting frond quantity without measuring individual size. Frond area scanning can be completed through image software after fixed photographing, and the measured numerical value can be used for quantitative comparison among different phosphate treatment groups.

Frond color state can directly reflect the chlorophyll synthesis level and overall health degree of duckweed photosynthetic system. Healthy duckweed individuals growing under moderate phosphate conditions maintain uniform deep green frond tissues with stable pigment synthesis efficiency. Duckweed cultured under long term low phosphate environment will present light yellow frond phenotype because of blocked chlorophyll production path. Image comparison can record the color difference of fronds under different phosphorus stress for long term tracking observation.

Root length measurement data reflects the active adaptive adjustment ability of duckweed responding to varied water phosphate concentration levels. Under phosphorus shortage state, duckweed will stretch root tissues to expand nutrient absorption range. When dissolved phosphorus in water reaches moderate sufficient level, duckweed will reduce energy input on root stretching development and transfer more substances to frond proliferation and organic accumulation. Root morphological data provides unique auxiliary judgment basis for distinguishing different phosphorus stress intensities.

Fresh weight and dry weight two sets of biomass indicators can quantify the total organic matter accumulation level of duckweed populations under each phosphate gradient. When measuring fresh weight, researchers need to use filter paper to fully absorb residual free water attached on frond surface before weighing to reduce measurement error. When testing dry weight, collected duckweed samples need to be placed in constant temperature drying oven until the weight remains unchanged for two consecutive measurements. Joint application of fresh weight and dry weight data can eliminate the interference of surface free water and accurately reflect the real material accumulation difference of duckweed under low, moderate and high phosphorus culture environments.

5. Conclusion

This paper investigates how different phosphate concentration affects the growth status and morphology of duckweed, with low, moderate, and high concentration of phosphate environment. Under the low phosphate concentration environment, the growth rate of duckweed is limited by the insufficient volume of phosphorus. Therefore, the ATP, DNA, and protein synthesis may get reduced, eliminating cell division, formation of the new leaves, accumulation of the biomass. In the morphological field, low phosphorus condition can lead to fewer number of leaves, lighter colour of leaves. Also, the duckweed may increase the length of root, as the duckweeds has to increase the

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absorption of nutrients in surrounding environment. Under moderate-phosphate condition, duckweed usually present the most balanced growth response, with increased number of leaves, greener leaves, faster rate of photosynthesis, and large volume of biomass. Additionally, in high-phosphate condition, the duckweeds may perform a a very rapid growth in a short period. But this does not show a healthier morphology, as the high density of duckweeds may cause excessive converage of leaves, leading to the competition of the natural resources, such as sunlight, dissolved oxygen, and growing sapce. In this case, the excessive growth of duckweed result in a eutrophication of the surrounding environment, menacing the normal growth of the other aquatic plants. The significance of the research dependents not merely on number and wideness of leaves, but also lies in multiple indicators including fresh, dry mass and other factors. Moreover, by harvesting duckweed regularly, duckweed can be used as a tool for ecological restoration, serving as an indicator of response to the phosphate gradient.

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