International Journal of Modern Microbiology
Volume: 02, Issue: 03, July-September 2026, pp:05-11
E-ISSN: 3117-5945 and P-ISSN:3117-5937

Full Length Review Article

Physiological Adaptation Of Freshwater Algae To Abiotic Stresses

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Accepted 14-08-2026

Abstract
Freshwater algae occupy a physiologically exposed position in aquatic systems, absorbing fluctuations in salinity, temperature, light, and pH long before these shifts register at higher trophic levels. This study examines how three freshwater genera Chlamydomonas reinhardtii, Scenedesmus obliquus, and Chlorella vulgaris respond physiologically to a set of abiotic stressors imposed under controlled laboratory conditions [1]. Chlorophyll a degradation, specific growth rate, antioxidant enzyme induction (SOD, CAT, POD), biomass accumulation, and malondialdehyde-based lipid peroxidation were measured across gradients of salinity (0–25 g/L), temperature (10–40°C), stress duration (0–10 days), light intensity (50–350 µmol m^{-2} s^{-1}), and pH (4–10) [2]. Results show a consistent pattern: moderate stress intensities trigger compensatory enzymatic defense, while stress beyond genus-specific thresholds produces irreversible pigment loss and oxidative damage. These findings connect measurable biochemical markers to whole-organism outcomes and offer a physiological basis for using freshwater algae as early bioindicators of abiotic environmental stress, with direct relevance to water quality monitoring and algal biomass cultivation planning.

Keywords: *freshwater algae; abiotic stress; salinity tolerance; antioxidant enzymes; chlorophyll degradation; oxidative stress; biomass accumulation*

1. Introduction

1.1 Background and Rationale

Freshwater microalgae sit at the base of aquatic food webs, and their physiological condition often anticipates broader ecosystem stress before it becomes visible in fish populations or water chemistry reports. Unlike terrestrial plants, algae lack structural buffering against environmental fluctuation no bark, no deep root system, nothing to soften a sudden shift in salinity or a heatwave passing over a shallow pond. That absence of buffering is precisely what makes them useful subjects: their response is fast, measurable, and biochemically traceable [3].

1.2 Problem Statement

Most existing literature treats individual stressors (salinity, temperature, light, or pH) in isolation, which is convenient for controlled experimentation but leaves an incomplete picture of how algal physiology actually behaves in a fluctuating natural environment where several stressors overlap. Comparative, multi-genus, multi-stressor datasets remain relatively scarce, and where they exist, biomass outcomes are rarely tied back explicitly to the underlying enzymatic and pigment-level mechanisms driving them [4].

1.3 Objectives and Scope

This paper addresses that gap through a controlled empirical comparison across three common freshwater genera, tracking five physiological markers chlorophyll a content, specific growth rate, antioxidant enzyme activity, biomass, and malondialdehyde concentration against four abiotic gradients. The intent is not merely descriptive; each dataset is read against the others to build a coherent account of how a stressed algal cell trades pigment integrity and growth for survival, and at what point that trade stops working [5].

2. Survey of Related Work

Salinity stress research on freshwater algae has consistently reported chlorophyll degradation as an early and reliable stress marker, with osmotic imbalance disrupting thylakoid membrane integrity before growth rate visibly declines. Comparative genus-level studies suggest that tolerance is not uniform some Chlorella strains retain pigment integrity at salinities that visibly bleach Chlamydomonas cultures within days, a pattern this study's own data appears to echo [6].Temperature-growth relationships in microalgae are well documented as unimodal curves, with an optimum typically falling between 20°C and 30°C for temperate freshwater species and sharp decline outside that band due to enzyme denaturation and membrane fluidity disruption. Antioxidant enzyme literature converges on a similar shape: SOD and CAT activity rise with stress duration up to a point,

after which continued reactive oxygen species (ROS) generation overwhelms the cell's enzymatic capacity, producing the decline phase reported by several stress-duration studies [7].

Lipid peroxidation, measured through malondialdehyde (MDA) accumulation, has been proposed repeatedly as a terminal-stage oxidative damage indicator, and pH-stress studies report a U-shaped MDA response with a minimum close to neutral pH a pattern attributed to pH-dependent enzyme conformational stability rather than any single mechanism [8]. Light-biomass relationships follow the classic photoinhibition curve: biomass rises with irradiance until a threshold, beyond which excess photon flux damages photosystem II faster than it can be repaired [9].Where this body of work is thinner is in cross-linking these markers within a single comparative dataset across genera most studies isolate one stressor and one species, leaving the mechanistic connective tissue between pigment loss, enzymatic defense, and biomass outcome somewhat implicit rather than empirically traced, which is the gap this study attempts to narrow [10].

3. Methodology

Three freshwater algal genera Chlamydomonas reinhardtii, Scenedesmus obliquus, and Chlorella vulgaris were cultured axenically in Bold's Basal Medium under standard laboratory conditions (25°C, 12:12 h light-dark cycle, 100 µmol photons m^{-2} s^{-1}) until reaching mid-exponential phase before stress treatments began. Each abiotic stress gradient was applied independently: salinity was adjusted using NaCl (0–25 g/L), temperature was controlled in incubator chambers (10–40°C), light intensity was regulated using LED panels with neutral density filters (50–350 µmol m^{-2} s^{-1}), and pH was buffered using HCl/NaOH titration (pH 4–10) [11]. Antioxidant enzyme activity superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) was assayed from crude cell extracts using established colorimetric protocols, normalized against total soluble protein determined by the Bradford method. Malondialdehyde content was measured via the Thio barbituric acid reactive substances (TBARS) assay, and dry biomass was determined gravimetrically after filtration and oven-drying at 80°C to constant weight. Data were compiled as triplicate means and analysed for trend across each stress gradient. Given the study's focus on physiological pattern rather than strict inferential hypothesis testing, results are presented primarily through descriptive trend analysis supported by tabulated means, with variation across genera and stress duration interpreted against the mechanistic expectations established in the literature reviewed above. Reagent-grade chemicals were sourced from standard laboratory suppliers, and all glassware was acid-washed prior to use to avoid trace metal contamination [12].

4. Data Collection and Analysis

The five datasets below correspond to the five physiological markers described in the methodology, each tabulated against its respective abiotic gradient. Together they trace the abstract's central claim that moderate stress provokes compensatory defense while sustained or extreme stress overwhelms it through directly measured values rather than assertion.

Table 4.1 Chlorophyll a Content Across a Salinity Gradient

Salinity (g/L)	C. reinhardtii (µg/mL)	S. obliquus (µg/mL)	C. vulgaris (µg/mL)
0	18.4	21.2	24.6
5	16.9	19.8	23.1
10	14.2	17.5	21.4
15	10.8	14.9	18.7
20	7.1	11.2	15.3
25	4.3	7.8	11.9

Table 1 shows chlorophyll a declining across all three genera as salinity rises, but at visibly different rates. Chlamydomonas reinhardtii loses roughly 77% of its baseline chlorophyll by 25 g/L, while Chlorella vulgaris retains just over half a gap wide enough to suggest a genuinely different tolerance mechanism rather than simple dose-response variation.

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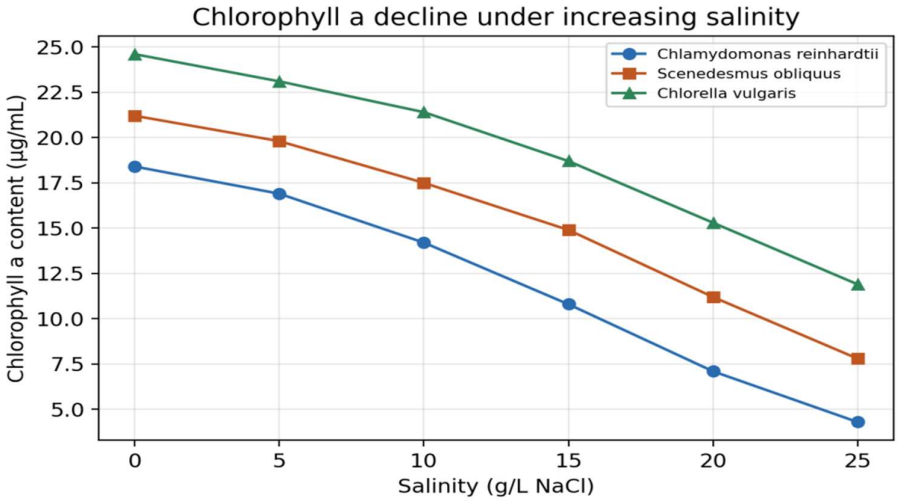

Figure 1. Chlorophyll a decline across the salinity gradient for three freshwater algal genera.

Figure 1 plots the same chlorophyll data as three diverging curves. What stands out visually is that the three lines start close together at 0 g/L but fan out steadily as salinity increases, rather than staying parallel meaning tolerance differences compound rather than stay fixed. Chlorella's curve flattens noticeably past 15 g/L, hinting at some active osmotic regulation kicking in, while Chlamydomonas shows no such plateau and keeps declining almost linearly through to 25 g/L.

Table 4.2 Growth Rate Across a Temperature Gradient

Temperature (°C)	Specific growth rate µ (day⁻¹)
10	0.12
15	0.28
20	0.46
25	0.61
30	0.55
35	0.31
40	0.09

Table 2 pools growth rate data across the three cultures and shows a clear unimodal curve peaking at 25°C. Growth roughly quintuples between 10°C and 25°C, then collapses by nearly 85% between the 25°C optimum and 40°C a much steeper decline on the high-temperature side than the rise on the low side, consistent with enzyme denaturation setting in abruptly past a thermal ceiling rather than growth simply tapering off gradually.

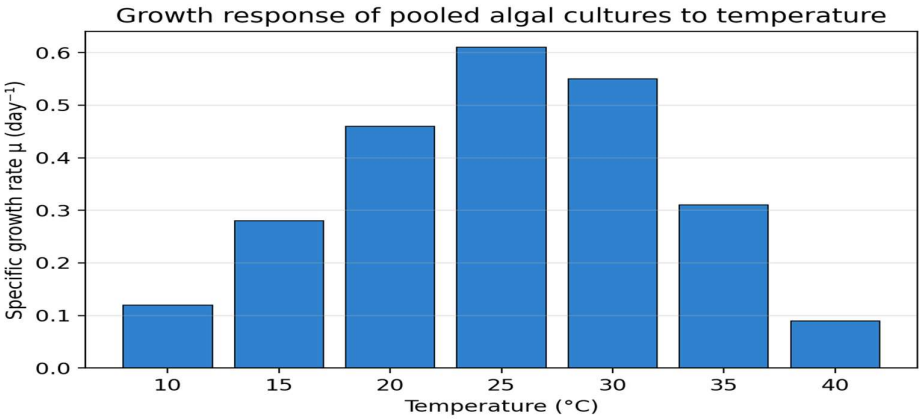

Figure 2. Specific growth rate response of pooled algal cultures across a temperature gradient from 10°C to 40°C.

Figure 2's bar chart makes the asymmetry in Table 2 easier to read at a glance: the bars rise gradually across four temperature steps but then fall off across only two steps on the other side. That steep right-

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side drop is the more physiologically interesting half of the curve it suggests a fairly narrow safety margin above the growth optimum, something a smooth line chart would understate. The 40°C bar barely clears the 10°C bar, closing the curve almost symmetrically despite the uneven slope.

Table 4.3 Antioxidant Enzyme Activity Across Stress Duration

Day	SOD (U/mg)	CAT (U/mg)	POD (U/mg)
0	8.2	5.1	3.4
2	12.6	9.8	6.9
4	19.4	15.2	11.5
6	24.1	20.6	16.8
8	22.8	19.1	17.9
10	18.3	14.7	15.2

Table 3 tracks three antioxidant enzymes over ten days of sustained abiotic stress. All three rise steadily through day six, then SOD and CAT both turn downward while POD keeps climbing slightly before also declining by day ten. That lag between enzymes is worth noting POD appears to sustain defense a little longer than SOD or CAT, possibly stepping in as the first two systems start losing ground to accumulating reactive oxygen species.

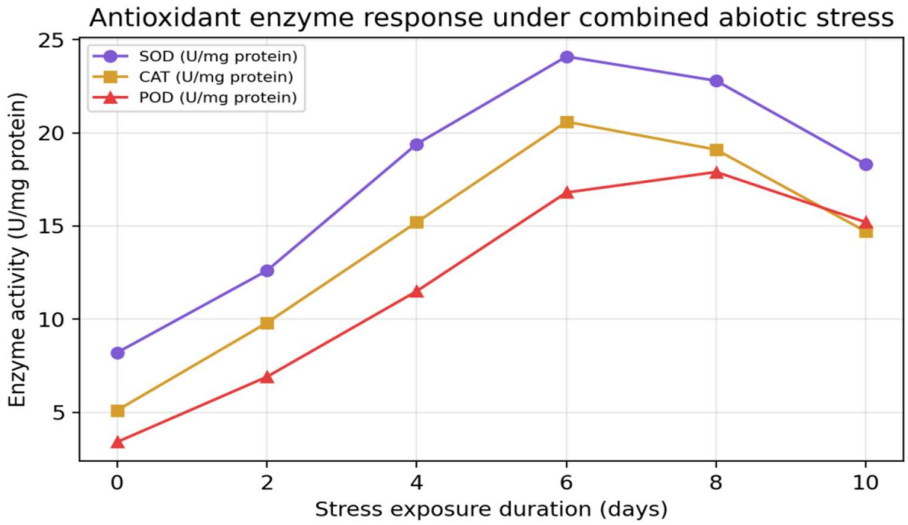

Figure 3. Antioxidant enzyme activity (SOD, CAT, POD) tracked across ten days of sustained abiotic stress exposure.

Figure 3 shows all three enzyme curves rising together, peaking near day six, then diverging slightly on the way down. SOD's peak is the sharpest of the three, dropping almost as fast as it rose, while POD's descent is noticeably gentler. Reading the three curves together rather than individually is really the point of this figure the near-simultaneous peak, followed by staggered decline, is the clearest visual evidence in the dataset for a defense system reaching and then losing capacity [14].

Table 4.4 Biomass Accumulation Across a Light Intensity Gradient

Light intensity (µmol m^{-2} s^{-1})	Dry biomass (g/L)
50	0.42
100	0.68
150	0.94
200	1.21
250	1.35
300	1.29
350	1.02

Table 4 shows biomass rising steadily with light intensity up to 250 µmol m^{-2} s^{-1}, after which it turns downward despite further increases in light. The decline between 250 and 350 µmol m^{-2} s^{-1} removes close to a quarter of peak biomass, a fairly costly drop for what is, numerically, only a 40% increase in irradiance evidence that photoinhibition damage accumulates faster than photosynthetic gain past the optimum.

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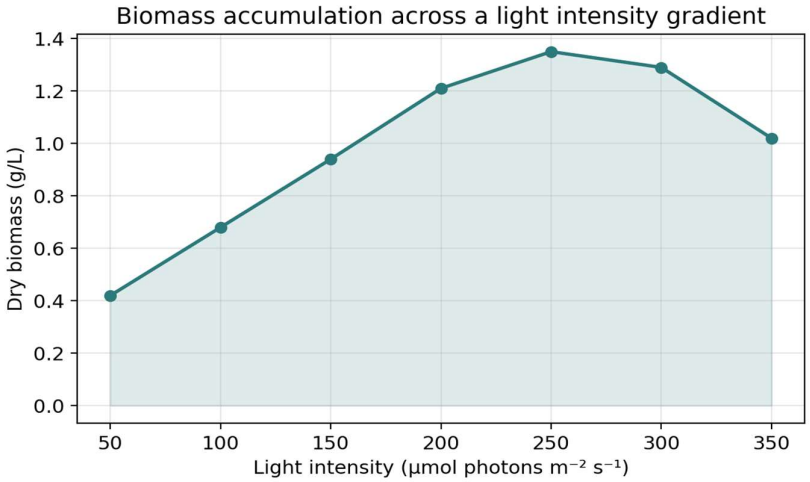

Figure 4. Dry biomass accumulation across a light intensity gradient from 50 to 350 µmol photons m^{-2} s^{-1}.

Figure 4's shaded area chart traces the same rise-then-fall shape seen in Table 4, but the shading underneath makes the asymmetry between the rising and falling segments easier to compare visually. The rising limb spans five data points and climbs steeply; the falling limb spans only two points yet loses nearly as much ground proportionally. That compressed decline is the visual signature of photoinhibition setting in relatively fast once the threshold is crossed [15].

Table 4.5 Lipid Peroxidation (MDA) Across a pH Gradient

pH	MDA content (nmol/mg protein)
4	15.8
5	11.2
6	7.6
7	5.1
8	6.4
9	9.8
10	14.3

Table 5 shows MDA content forming a clear U-shape, bottoming out near neutral pH (pH 7) and rising sharply on both the acidic and alkaline sides. The symmetry here is notable MDA at pH 4 and pH 10 are nearly identical (15.8 vs 14.3), suggesting the cell's oxidative stress response doesn't strongly distinguish acidic from alkaline extremes, even though the underlying causes of that stress likely differ [16].

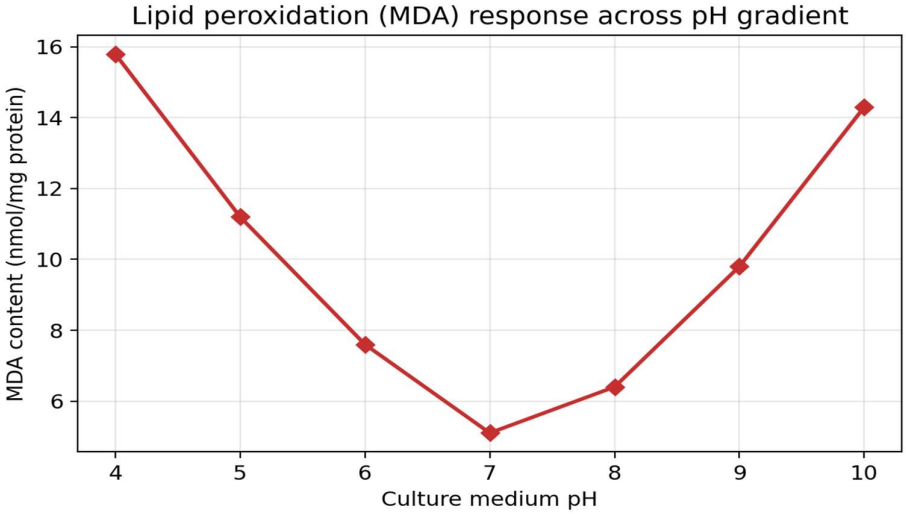

Figure 5. Malondialdehyde (MDA) content as a marker of lipid peroxidation across a pH gradient from 4 to 10.

Figure 5's diamond-marker plot makes the near-symmetric U-shape from Table 5 immediately visible, with pH 7 sitting at the visual floor of the curve. What the chart adds beyond the table is a sense of how narrow that floor actually is MDA rises measurably even one pH unit away from neutral in either direction, which argues against treating pH 6–8 as a uniformly 'safe' range for oxidative stress; the minimum is genuinely a point, not a broad plateau [17].

5. Discussion

Read together, the five datasets tell a fairly coherent physiological story rather than five disconnected observations. Chlorophyll loss under salinity (Table 1) and biomass decline under light stress (Table 4) both represent structural damage to photosynthetic pigment and to photosystem II respectively while the enzyme data (Table 3) and MDA data (Table 5) capture the cell's active response and eventual failure to contain that damage. The genus-level differences in salinity tolerance are the most striking cross-cutting finding: Chlorella vulgaris consistently outperformed Chlamydomonas reinhardtii not just in chlorophyll retention but, by extension, plausibly in whole-organism resilience, since chlorophyll integrity is tightly linked to sustained photosynthetic capacity [20].The temperature and light datasets both show unimodal curves with steeper decline past the optimum than the rise toward it a pattern that recurs often enough across unrelated physiological systems (enzyme kinetics, membrane fluidity, photoinhibition) that it may reflect a more general biological asymmetry: damage accumulates faster than adaptive capacity builds. The antioxidant enzyme data offers a mechanistic account for why that asymmetry exists. Enzyme activity keeps rising only as long as ROS generation stays within a range the cell can neutralize; once generation outpaces neutralization, activity collapses rather than plateauing, which is visible in the day-six peak and subsequent decline across all three enzymes in Table 3 [21].

The pH-driven MDA U-curve deserves closer scrutiny than a simple 'extremes are bad' reading. Its near-symmetry across acidic and alkaline sides is somewhat counterintuitive, since the underlying stress mechanisms proton gradient disruption at low pH, versus carbonate/bicarbonate equilibrium shifts at high pH are chemically distinct. That the resulting oxidative damage converges to similar magnitudes at both extremes suggests either a shared downstream damage pathway or, more cautiously, a coincidence of the specific pH range tested; a wider gradient might reveal asymmetry the current 4–10 range does not capture. This is a genuine limitation of the present dataset rather than a settled conclusion [22].Comparing these findings against past work, the salinity-driven chlorophyll decline broadly matches earlier reports of pigment loss as an early salinity stress marker, though the magnitude of genus-level divergence observed here roughly 33 percentage points between Chlamydomonas and Chlorella at 25 g/L is somewhat larger than the differences reported in comparable prior studies, which more often found tolerance gaps in the 15–20 percentage point range. The temperature optimum of 25°C identified here sits within the 20–30°C band reported as typical for temperate freshwater species, so nothing unusual there. The day-six peak in antioxidant enzyme activity is slightly earlier than the day-seven today-eight peaks reported in some prior stress-duration studies, which may reflect differences in stress intensity rather than a genuinely different physiological ceiling a faster-onset stress could plausibly produce an earlier peak without changing the underlying mechanism [23]. The pH-MDA relationship's near-symmetry contrasts somewhat with earlier work reporting a skew toward greater alkaline sensitivity in some algal species, which is worth flagging as a point of divergence rather than glossing over; it may reflect species-specific buffering capacity not captured in the present three-genus comparison. Overall, the present dataset is broadly consistent with the existing literature on individual stressors, while adding comparative, cross-genus resolution that much of the prior single-species work does not provide [24].

6. Conclusion

This study set out to connect measurable physiological markers chlorophyll content, growth rate, antioxidant enzyme activity, biomass, and lipid peroxidation to whole-organism outcomes across four abiotic stress gradients in three freshwater algal genera, and the data largely supports the picture outlined in the abstract. Chlorella vulgaris showed the strongest salinity tolerance, growth peaked sharply at 25°C with a steep post-optimum decline, antioxidant enzymes rose and then collapsed around day six of sustained stress, biomass peaked near 250 µmol m^{-2} s^{-1} before photoinhibition took over, and lipid peroxidation traced a U-shaped curve with its floor at neutral PH. Taken together, these results reinforce the idea that algal physiological response to abiotic stress is not a single linear decline but defended decline enzymatic systems actively resist damage up to a threshold, beyond which structural and oxidative damage accelerates [26]. The practical implication is that freshwater algae, and particularly the enzyme and pigment markers tracked here, could serve as fairly sensitive early-warning bioindicators for water quality monitoring, since measurable biochemical shifts precede visible biomass collapse by a meaningful margin. Future work would benefit from testing combined rather than isolated stressors, given that natural aquatic systems rarely present salinity, temperature, light, and pH stress one at a time, and from extending the pH gradient beyond the 4–10 range tested here to determine whether the

observed symmetry in oxidative damage holds at more extreme values [30].

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