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Climate-Related Changes in Salinity and Temperature Modulate Cadmium Bioaccumulation and Toxicity in the Haptophyte *Diacronema lutheri*

Esther U. Kadiene[1*], Baghdad Ouddane[2], Jiang-Shiou Hwang[3,4], Sami Souissi[4,5]

[1]Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria
[2]Laboratoire de Spectroscopie pour les Interactions, la Réactivité et l'Environnement, University of Lille, UMR CNRS 8516 LASIRE, Lille, France
[3]Institute of Marine Biology, NTOU, Keelung, Taiwan Region
[4]Center of Excellence for the Oceans, NTOU, Keelung, Taiwan Region
[5]Faculty of Sciences and Technologies, Univ. Lille, Lille, France
Email: *ekadiene@delsu.edu.ng, *estherkadiene@gmail.com

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Abstract

Coastal freshening and warming may modify the bioavailability and biological effects of persistent contaminants. This study examined how salinity and temperature affected cadmium (Cd) accumulation and Cd-related growth impairment in the haptophyte *Diacronema lutheri*. In a 21-day experiment, cultures at 15 or 35 PSU were exposed to 0 or 60 μg·L^{-1} Cd, and cell-density trajectories were analysed using mixed-design repeated-measures analysis of variance. Cadmium accumulation was also followed for seven days in Cd-exposed cultures at both salinities. Separate 72-h experiments quantified Cd accumulation across salinities of 15, 25 and 35 PSU or temperatures of 18˚C, 25˚C and 35˚C at nominal Cd concentrations of 36, 120, 600 and 1200 μg·L^{-1}. In the growth experiment, salinity and Cd significantly affected cell density (both $P < 0.001$), although the salinity × Cd × day interaction was not significant. Within Cd-exposed cultures, cell density was significantly lower at 15 PSU than at 35 PSU, and growth trajectories differed between salinities (salinity × day, $P = 0.007$). Seven-day Cd accumulation was greater at 15 PSU ($P < 0.001$), with a significant salinity × day interaction ($P = 0.003$). In the 72-h experiments, Cd accumulation increased with exposure concentration and was modified by salinity (salinity × Cd concentration, $P < 0.001$) and temperature (temperature × Cd concentration, $P = 0.010$). Reduced salinity strongly enhanced Cd accumulation, while elevated temperature modified short-term Cd accumulation in a

concentration-dependent manner. Reduced salinity and Cd exposure independently affected algal growth, but a salinity-dependent increase in Cd-specific growth toxicity was not statistically demonstrated. Climate-related coastal freshening and warming may therefore modify Cd accumulation and exposure risk in marine microalgae.

Keywords

Cadmium, Coastal Freshening, Warming, Bioaccumulation, *Diacronema lutheri*

1. Introduction

Coastal and estuarine ecosystems support fisheries, aquaculture, nutrient cycling, shoreline protection and carbon storage [1] [2], yet they are increasingly exposed to concurrent climate and contaminant pressures [3]. Cadmium (Cd) is a persistent, non-essential trace metal released to aquatic environments from mining, industrial activities, combustion, fertilizers and runoff. It can disturb essential-metal homeostasis, photosynthesis, enzyme function and cellular redox balance, thereby reducing microalgal growth and altering cellular metabolism [4]-[6]. In marine phytoplankton, Cd uptake and biological effects depend on concentration, nutrient status and chemical speciation [7]. At trace concentrations and under zinc limitation, Cd can partly substitute for zinc in some phytoplankton enzymes, whereas higher exposures become toxic [8] [9].

Climate-related changes in coastal waters can alter these controlling processes. Global salinity observations indicate an intensifying hydrological cycle, while marine heatwaves have become more frequent and prolonged [3] [10] [11]. In estuaries and nearshore waters, changing precipitation and river discharge can produce episodic or sustained freshening, and warming can increase the frequency of acute thermal stress [3] [12]. Lower salinity changes ionic strength and chloride availability, potentially altering Cd complexation, free-ion activity and competition with major ions at biological uptake sites [13]-[16]. Temperature can modify membrane properties, transport kinetics, metabolism and detoxification capacity, thereby changing both metal accumulation and organismal sensitivity [12] [17]. The direction and magnitude of these responses are species- and exposure-dependent, so contaminant effects measured at one fixed salinity and temperature may not represent variable coastal environments.

Phytoplankton are particularly relevant to climate-contaminant interactions because they respond rapidly to environmental change and form the base of aquatic food webs. Their small size and large surface-area-to-volume ratio facilitate exchange with the surrounding medium, while accumulated metals may impair primary productivity and become available to consumers [7] [18]-[20]. Importantly, total dissolved metal concentration is not always equivalent to the fraction available for biological uptake. High-frequency observations in estuarine

coastal waters have shown that dynamic, potentially bioavailable trace-metal fractions can vary with physicochemical conditions and phytoplankton activity [21]. Laboratory studies that separate the effects of salinity, temperature and exposure concentration are therefore needed to identify conditions that enhance Cd accumulation and biological effects. Previous studies from our group have shown that Cd can impair copepod development and reproduction, induce sex-specific molecular responses, and accumulate through dissolved, dietary and multigenerational exposure pathways [22]-[25].

Diacronema lutheri (formerly *Pavlova lutheri*) is a marine haptophyte of ecological and commercial importance. Taxonomic reassessment placed the species within the genus *Diacronema* [26]. It has been used in trace-metal ecotoxicology [27] and is widely cultured as live feed in aquaculture because of its favourable biochemical composition, including nutritionally important long-chain polyunsaturated fatty acids [28]-[31]. These attributes make it a useful model for examining whether climate-related environmental variability changes metal exposure in a microalga relevant to both coastal food webs and hatchery production.

The present study investigated the effects of salinity and temperature on Cd accumulation and growth in *D. lutheri*. The objectives were to: 1) determine how reduced salinity and Cd exposure affected 21-day growth trajectories; 2) characterize the seven-day pattern of Cd accumulation at low and marine salinity; and 3) quantify, in separate 72-h experiments, how salinity or temperature modified Cd accumulation across a concentration gradient. We hypothesized that reduced salinity would enhance Cd accumulation, that reduced salinity and Cd would each affect algal growth, and that elevated temperature would modify short-term Cd accumulation.

2. Materials and Methods

2.1. Culture Maintenance and Experimental Conditions

The marine haptophyte *Diacronema lutheri* (formerly *Pavlova lutheri*) was obtained from the Roscoff Culture Collection, France. Stock cultures were maintained in sterile Conway medium supplemented with f/2 nutrients [32] [33]. Cultures were grown in 1000-mL borosilicate Erlenmeyer flasks containing 800 mL of autoclaved natural seawater at 33 - 35 PSU and 18˚C under a 12:12 h light:dark photoperiod provided by cool-white fluorescent lamps. Cultures were gently aerated with filtered ambient air and subcultured weekly to maintain active growth. Stock-culture pH ranged from 7.8 to 8.1.

2.2. Experiment 1: Effects of Salinity and Cd on Growth and Daily Cd Accumulation

Exponential-phase cells were inoculated at an initial density of 3.17×10^6 cells·mL^{-1} into 2-L borosilicate flasks containing 1.5 L of medium adjusted to 15 or 35 PSU. Salinity was adjusted before nutrient enrichment. The 15-PSU medium was prepared by diluting autoclaved natural seawater with Milli-Q water, whereas full-

strength seawater was used for the 35-PSU treatment. Following salinity adjustment, identical nutrient concentrations were added to all treatment media. Per litre of salinity-adjusted autoclaved seawater, the medium contained 100 mg $NaNO_3$, 20 mg NaH_2PO_4, 45 mg Na_2EDTA, 33.6 mg H_3BO_3, 0.36 mg $MnCl_2$, 1.3 mg $FeCl_3$, 0.021 mg $ZnCl_2$, 0.02 mg $CoCl_2{\cdot}6H_2O$, 0.02 mg $CuSO_4{\cdot}5H_2O$, 0.09 mg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.2 mg thiamine hydrochloride and 0.01 mg cyanocobalamin. Consequently, the nominal concentrations of added nutrients, trace elements and vitamins were equivalent across salinity treatments. Cultures were exposed to 0 $\mu g{\cdot}L^{-1}$ Cd (control) or 60 $\mu g{\cdot}L^{-1}$ Cd supplied as $CdCl_2$. Each salinity × Cd combination comprised three replicates. The experiment was conducted at 18˚C under the same photoperiod used for culture maintenance.

Cell density was determined daily for 21 days. Lugol-fixed subsamples were counted in a Malassez haemocytometer (0.1-mm depth) using an inverted microscope (Olympus IX71, Tokyo, Japan). Four technical counts were made from each flask and averaged to obtain one cell-density value for each biological replicate on each sampling day.

During days 1 - 7, a 20 - mL subsample was collected daily for Cd analysis. Removed volume was not replaced, producing a cumulative reduction of 140 mL, equivalent to 9.3% of the initial 1.5-L culture volume. Each subsample was filtered through a pre-weighed 0.45-µm polycarbonate membrane filter (Whatman Nuclepore, 47-mm diameter), rinsed with 10 mL of metal-free isotonic solution, dried at 60˚C for 24 h and weighed to determine dry biomass. Filters were digested in concentrated HNO_3:HCl (3:1, v/v; ultrapure grade) at 120˚C for 3 h in PTFE vessels. Digests were cooled, diluted to 10 mL with Milli-Q water and analysed for Cd by inductively coupled plasma optical emission spectrometry (ICP-OES; Agilent 5100 SVDV). Analytical accuracy was checked using the certified reference materials PACS-3 and HISS-1. Cadmium accumulation was expressed as $\mu g\ Cd{\cdot}g^{-1}$ dry weight (DW).

2.3. Experiment 2: Effects of Salinity or Temperature on 72-Hour Cd Accumulation

Two separate short-term uptake experiments were conducted. In the salinity experiment, cultures were maintained at 15, 25 or 35 PSU at a fixed temperature of 18˚C. In the temperature experiment, cultures were maintained at 18˚C, 25˚C or 35˚C at a fixed salinity of 35 PSU. Each environmental treatment was exposed to nominal Cd concentrations of 36, 120, 600 or 1200 $\mu g{\cdot}L^{-1}$. Salinity was adjusted as described for Experiment 1, and temperature treatments were maintained in a temperature-controlled cabinet.

Exponential-phase cells were inoculated at approximately 3.17×10^6 $cells{\cdot}mL^{-1}$ into sterile 100-mL borosilicate beakers containing 80 mL of the corresponding medium. Each treatment comprised three independent biological replicates. After 72 h, entire cultures were filtered, rinsed, dried, weighed, digested and analysed for Cd using the procedure described in Section 2.2.

The pH, salinity and temperature of each treatment medium were measured at the beginning of the experiments after preparation of the exposure media. Repeated measurements were not conducted during exposure in order to minimize culture disturbance, sample loss and the risk of contamination. Cadmium exposure concentrations are reported as nominal concentrations added to the culture medium. Dissolved Cd concentrations were not computed. The experiments were designed as preliminary uptake-screening studies to determine relative Cd accumulation by *D. lutheri* under different salinity, temperature and nominal exposure-concentration conditions and to provide information for the design of subsequent experiments.

2.4. Statistical Analysis

Data are presented as mean ± standard deviation (SD), and statistical significance was accepted at $P < 0.05$. Analyses were conducted in R [34]. The complete 21-day cell-density dataset was analysed using a mixed-design repeated-measures analysis of variance (ANOVA). Salinity (15 and 35 PSU) and Cd exposure (0 and 60 $\mu g \cdot L^{-1}$) were between-subject factors, and sampling day (days 1 - 21) was the within-subject factor. Individual flasks were specified as subjects nested within the salinity × Cd treatment combination. The model tested the main effects of salinity, Cd and day and all corresponding two- and three-way interactions. A planned mixed-design repeated-measures comparison was also conducted using only Cd-exposed cultures to test the primary question of whether growth during Cd exposure differed between salinities.

Seven-day Cd accumulation was analysed using mixed-design repeated-measures ANOVA, with salinity as the between-subject factor, day as the within-subject factor and flask as the subject. Following a significant salinity × day interaction, salinities were compared within each day using Welch's independent-samples tests with Holm adjustment. Greenhouse-Geisser corrections were applied to within-subject effects when sphericity was violated, and corrected degrees of freedom and P-values are reported.

The two 72-h experiments were analysed separately by two-way ANOVA. The salinity experiment included salinity (15, 25 and 35 PSU) and nominal Cd concentration (36, 120, 600 and 1200 $\mu g \cdot L^{-1}$) as fixed factors. The temperature experiment included temperature (18˚C, 25˚C and 35˚C) and nominal Cd concentration as fixed factors.

3. Results

3.1. Salinity- and Cd-Dependent Growth Trajectories

Cell density increased during the first half of the 21-day experiment, reached maximum values between approximately days 11 and 15, and subsequently declined in all treatments (**Figure 1**). Maximum cell density was $28.47 \pm 0.17 \times 10^6$ cells$\cdot mL^{-1}$ in the 15-PSU control, $24.25 \pm 0.56 \times 10^6$ cells$\cdot mL^{-1}$ at 15 PSU + Cd, $33.26 \pm 0.34 \times 10^6$ cells$\cdot mL^{-1}$ in the 35-PSU control and $30.16 \pm 0.87 \times 10^6$

cells·mL^{-1} at 35 PSU + Cd. Thus, the highest maximum density occurred in the 35-PSU control and the lowest in the 15 PSU + Cd treatment.

The complete repeated-measures model showed significant effects of salinity ($F(1, 8) = 135.85$, $P < 0.001$) and Cd exposure ($F(1, 8) = 58.70$, $P < 0.001$), whereas the salinity × Cd interaction was not significant ($F(1, 8) = 0.45$, $P = 0.522$). Cell density changed significantly with day (Greenhouse-Geisser-corrected $F(3.68, 29.47) = 422.23$, $P < 0.001$). Salinity × day ($F(3.68, 29.47) = 18.14$, $P < 0.001$) and Cd × day ($F(3.68, 29.47) = 13.14$, $P < 0.001$) interactions were significant, but the salinity × Cd × day interaction was not ($F(3.68, 29.47) = 1.67$, $P = 0.187$).

In the analysis of Cd-exposed cultures, cell density differed significantly between salinities ($F(1, 4) = 170.99$, $P < 0.001$), changed with day (Greenhouse-Geisser-corrected $F(2.13, 8.53) = 140.31$, $P < 0.001$), and showed a significant salinity × day interaction ($F(2.13, 8.53) = 9.12$, $P = 0.007$). Maximum cell density at 15 PSU + Cd was 19.6% lower than at 35 PSU + Cd, demonstrating poorer overall culture performance at reduced salinity within the Cd-exposed treatments. However, because low salinity also affected growth in the absence of Cd and the salinity × Cd and salinity × Cd × day interactions were not significant, this comparison does not demonstrate that the Cd-specific growth effect was significantly greater at 15 PSU.

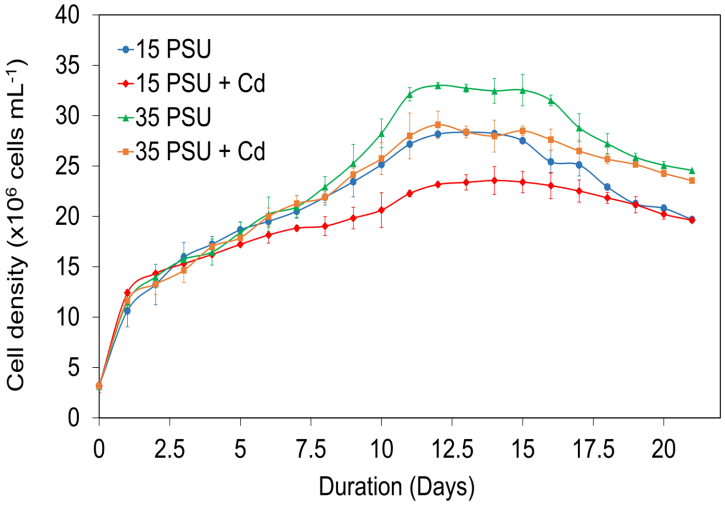

Figure 1. Cell-density trajectories of *Diacronema lutheri* cultured for 21 days at 15 or 35 PSU in the absence or presence of 60 µg·L^{-1} Cd at 18˚C. Values are mean ± SD (n = 3). All treatments were inoculated at a common day-0 density of 3.17 × 10^6 cells·mL^{-1}.

3.2. Seven-Day Pattern of Cd Accumulation

Cadmium accumulation was consistently greater at 15 PSU than at 35 PSU throughout the seven-day exposure (**Figure 2**). At 15 PSU, mean accumulation increased from 9.08 ± 0.70 µg·g^{-1} DW on day 1 to 11.82 ± 0.42 µg·g^{-1} DW on day 3, before declining to 8.22 ± 0.32 µg·g^{-1} DW on day 7. At 35 PSU, accumulation increased more gradually from 4.03 ± 0.41 µg·g^{-1} DW on day 1 to 5.68 ± 0.96 µg·g^{-1} DW on day 5 and was 5.02 ± 0.60 µg·g^{-1} DW on day 7.

Repeated-measures ANOVA showed significant effects of salinity ($F(1, 4) = 502.98$, $P < 0.001$) and sampling day (Greenhouse-Geisser-corrected $F(2.77, 11.08) = 10.63$, $P = 0.002$), together with a significant salinity × day interaction ($F(2.77, 11.08) = 9.27$, $P = 0.003$). Holm-adjusted day-specific comparisons confirmed greater Cd accumulation at 15 PSU on all seven sampling days (adjusted $P = 0.0006$ - 0.0109).

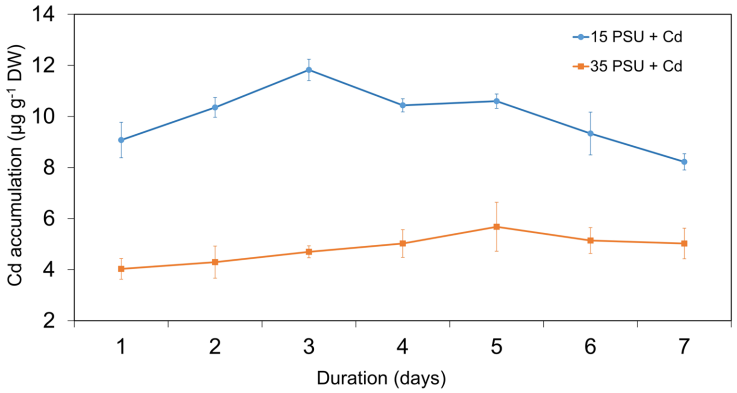

Figure 2. Cadmium accumulation by *Diacronema lutheri* during seven days of exposure to 60 µg·L^{-1} Cd at 15 or 35 PSU and 18˚C. Values are mean ± SD of three biological replicates. Cadmium accumulation was significantly higher at 15 PSU on every sampling day after Holm adjustment ($P < 0.05$).

3.3. Effects of Salinity and Cd Concentration on 72-Hour Accumulation

Cadmium accumulation increased with nominal exposure concentration and was consistently greater at reduced salinity (**Figure 3**). Two-way ANOVA showed significant effects of salinity ($F(2, 24) = 41.87$, $P < 0.001$) and Cd concentration ($F(3, 24) = 84.50$, $P < 0.001$), together with a significant salinity × Cd-concentration interaction ($F(6, 24) = 7.28$, $P < 0.001$).

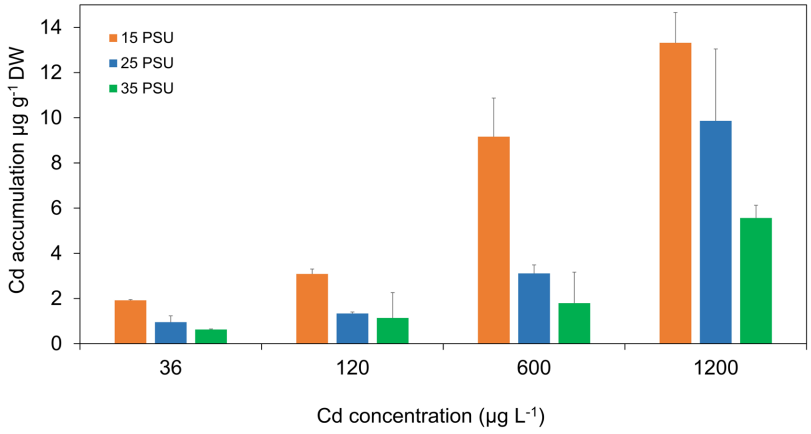

Figure 3. Cadmium accumulation by *Diacronema lutheri* after 72 h of exposure to 36, 120, 600 or 1200 µg·L^{-1} Cd at 15, 25 or 35 PSU and 18˚C. Values are mean ± SD (n = 3).

The largest relative contrast between 15 and 35 PSU occurred at 600 μg·L^{-1} Cd, where accumulation was 9.16 ± 1.71 and 1.79 ± 1.37 μg·g^{-1} DW, respectively, corresponding to a 5.11-fold difference. The highest biomass Cd concentration in the salinity experiment was 13.32 ± 1.34 μg·g^{-1} DW at 15 PSU and 1200 μg·L^{-1} Cd, compared with 5.56 ± 0.56 μg·g^{-1} DW at 35 PSU at the same exposure concentration.

3.4. Effects of Temperature and Cd Concentration on 72-Hour Accumulation

Cadmium accumulation generally increased with temperature and nominal exposure concentration (Figure 4). Two-way ANOVA showed significant effects of temperature ($F(2, 24) = 31.47$, $P < 0.001$) and Cd concentration ($F(3, 24) = 71.68$, $P < 0.001$). The temperature × Cd-concentration interaction was significant ($F(6, 24) = 3.66$, $P = 0.010$), indicating that the temperature effect differed across the exposure gradient.

At 600 μg·L^{-1} Cd, accumulation increased from 1.79 ± 1.37 μg·g^{-1} DW at 18˚C to 7.71 ± 0.48 μg·g^{-1} DW at 35˚C, representing a 4.30-fold difference. The highest biomass Cd concentration in the temperature experiment was 11.53 ± 2.17 μg·g^{-1} DW at 35˚C and 1200 μg·L^{-1} Cd.

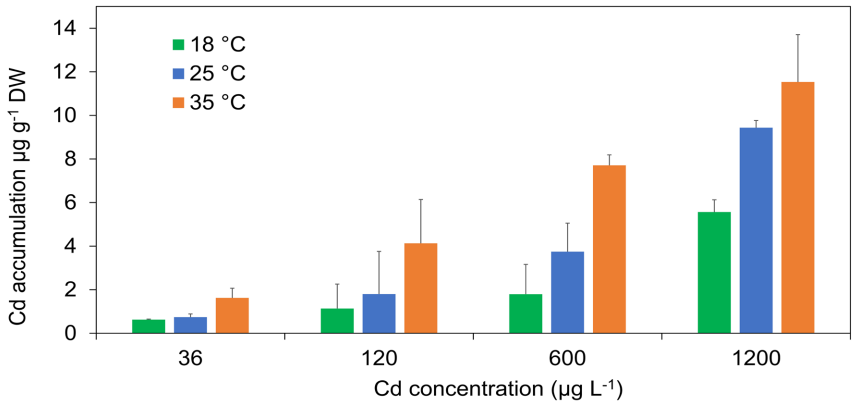

Figure 4. Cadmium accumulation by *Diacronema lutheri* after 72 h of exposure to 36, 120, 600 or 1200 μg·L^{-1} Cd at 18˚C, 25˚C or 35˚C and 35 PSU. Values are mean ± SD of three biological replicates.

4. Discussion

4.1. Growth Responses under Reduced Salinity and Cd Exposure

The 21-day experiment showed that reduced salinity and Cd exposure each altered the growth of *D. lutheri*. Their significant interactions with day indicate that the magnitude of each effect changed as cultures passed through active growth, maximum density and decline. The 15 PSU + Cd treatment had the lowest maximum density, whereas the 35-PSU control had the highest, demonstrating that the combined low-salinity and Cd condition was least favourable for culture performance.

The planned comparison between Cd-exposed cultures showed that growth

during Cd exposure differed between salinities. Maximum density at 15 PSU + Cd was almost one-fifth lower than at 35 PSU + Cd, and the temporal trajectories of the two treatments differed significantly. However, this comparison evaluates overall culture performance under the two combined treatment conditions. It does not isolate the additional growth reduction attributable specifically to Cd. In the complete four-treatment model, neither the salinity × Cd interaction nor the salinity × Cd × day interaction was statistically significant. Furthermore, low salinity reduced growth in the absence of Cd. The poorer growth observed in the 15 PSU + Cd treatment therefore cannot be interpreted as statistical evidence that reduced salinity enhanced Cd-specific growth toxicity. Instead, the results demonstrate strong independent effects of salinity and Cd exposure, with their combined occurrence producing the lowest overall culture performance. Salinity stress can alter osmotic adjustment and growth in marine algae [35], while Cd can interfere with photosynthesis, essential-metal acquisition and cellular metabolism [5]-[7] [36]. Trace-metal exposure has also previously reduced abundance and altered physiological performance in *D. lutheri* [27]. Related studies from our group demonstrated that Cd exposure delayed development and reduced reproductive performance in *Pseudodiaptomus annandalei*, while molecular and multigenerational responses involved pathways associated with growth, metabolism and stress regulation [22] [24] [25]. These studies provide broader evidence of Cd toxicity but do not demonstrate the mechanism underlying the growth responses observed in the present experiment.

4.2. Salinity-Dependent Cd Accumulation

The accumulation experiments provided the strongest evidence that salinity modified Cd exposure in *D. lutheri*. Cd accumulation was greater at 15 PSU on every day of the seven-day experiment, and the significant salinity × day interaction showed that salinity affected both the amount accumulated and its temporal pattern. Cd accumulation was greater at 15 PSU on every sampling day, and the significant salinity × day interaction showed that salinity affected both the amount accumulated and its temporal pattern. At 15 PSU, biomass-associated Cd reached its highest value on day 3 and subsequently declined, whereas accumulation at 35 PSU remained lower and reached a later maximum. Salinity-dependent changes in Cd speciation and ion competition provide possible explanations reported in the wider literature. Increasing chloride concentration promotes the formation of Cd-chloride complexes, while changes in ionic strength and major-ion concentrations can alter metal activity and competition at cellular uptake sites [13]-[16]. Experimental work in marine invertebrates has similarly shown salinity-dependent Cd uptake [16] [37]. However, dissolved Cd concentrations, free Cd ions, chloride complexes and dissolved organic-metal complexes were not measured in the present study. Therefore, the observed salinity effect cannot be assigned to a specific speciation or uptake mechanism.

The cause of the decline in biomass-associated Cd after day 3 at 15 PSU cannot

be determined from the present measurements. Dissolved Cd concentrations, intracellular partitioning, efflux, metal-binding compounds and biochemical detoxification responses were not measured. The temporal pattern should therefore be interpreted as a phenomenological change in Cd associated with the washed algal biomass rather than as evidence of acclimation, intracellular sequestration or another specific physiological mechanism. The significant salinity × Cd-concentration interaction in the 72-h experiment demonstrates that the magnitude of the salinity effect varied with nominal Cd concentration and was particularly large at 600 $\mu g \cdot L^{-1}$. However, because the exposure medium and cellular partitioning were not analysed through time, the experiment demonstrates relative treatment differences rather than equilibrium partition coefficients or a particular uptake mechanism [18].

4.3. Temperature-Dependent Cd Accumulation

Temperature modified short-term Cd accumulation, and the significant temperature × Cd-concentration interaction showed that the magnitude of the response depended on nominal exposure concentration. Similar temperature-dependent changes in Cd accumulation and sensitivity have been reported in marine microalgae [17]. However, membrane properties, transport activity, metabolic rate, oxidative stress, intracellular partitioning and biochemical detoxification were not measured in the present study. The mechanism responsible for the temperature-dependent accumulation pattern therefore cannot be determined. The 35˚C treatment represented an acute thermal-stress condition for *D. lutheri* rather than an optimal culture temperature. The observed increase in biomass-associated Cd at this temperature should therefore be described as a phenomenological association between exposure temperature and accumulation rather than as evidence of increased transport, diffusion or impaired cellular regulation. This study did not establish whether simultaneous warming and freshening would produce additive, synergistic or antagonistic effects; a fully crossed salinity × temperature × Cd design would be required in the future. Ocean warming and marine heatwaves are intensifying, while changes in the hydrological cycle can modify coastal salinity [3] [10]-[12]. These processes may alter contaminant uptake and organismal sensitivity even when nominal contaminant concentration is unchanged. The present findings therefore support incorporating realistic salinity and temperature variability into coastal metal-risk assessment.

Together, the experiments revealed related but statistically distinct responses. Reduced salinity significantly increased Cd accumulation and also independently reduced algal growth. The 15 PSU + Cd treatment consequently combined the lowest salinity condition with Cd exposure and produced the poorest overall growth. However, because neither the salinity × Cd nor the salinity × Cd × day interaction was significant, the growth results do not demonstrate that the additional effect of Cd was greater at 15 PSU. The accumulation measurements establish that biomass-associated Cd was greater at reduced salinity, but they do not

establish a direct causal relationship between accumulated Cd and growth impairment. Photosynthetic efficiency, pigments, oxidative-stress markers, mortality, intracellular metal localization and dissolved Cd concentrations were not measured. The findings should therefore be interpreted as separate evidence that salinity modified Cd accumulation and that salinity and Cd independently affected growth.

The findings are relevant to coastal ecology and aquaculture. *D. lutheri* is used as live feed and valued for long-chain polyunsaturated fatty acids [28]-[31]. Increased Cd accumulation could reduce algal productivity and increase exposure of organisms consuming the biomass. Metal transfer through aquatic food webs depends on cellular retention, assimilation efficiency, loss rates and consumer physiology [19] [20]. The results suggest that climate-related warming and freshening may modify the biological availability of existing Cd contamination at the base of coastal food webs. Our previous work with calanoid copepods further showed that metal uptake can differ markedly between dissolved and dietary exposure routes [23] and that continued Cd exposure can produce generational bioaccumulation and fitness effects [25].

The experiments were designed as preliminary assessments of relative Cd accumulation and algal growth under nominal exposure conditions. Dissolved Cd concentrations were not computed; therefore, Cd losses or changes arising from adsorption to experimental vessels, precipitation, complexation or cellular removal was not quantified. Initial pH, salinity and temperature were recorded, but repeated measurements were avoided to minimize culture disturbance and contamination. Future experiments should incorporate measured dissolved Cd concentrations, contamination-controlled physicochemical monitoring, cellular metal partitioning and physiological biomarkers.

5. Conclusion

Reduced salinity and Cd exposure independently affected the growth of *Diacronema lutheri*. Although the 15 PSU + Cd treatment produced the lowest growth, the salinity × Cd and salinity × Cd × day interactions were not significant. A salinity-dependent increase in Cd-specific growth toxicity was therefore not statistically demonstrated. In contrast, the accumulation experiments showed clear salinity dependence. Reduced salinity increased biomass-associated Cd, modified its seven-day temporal pattern and altered the concentration-accumulation relationship. Elevated temperature also modified short-term Cd accumulation in a concentration-dependent manner. Because the experiments used nominal Cd concentrations and did not include dissolved-metal, biochemical or physiological measurements, the mechanisms underlying these patterns could not be determined. The findings provide preliminary evidence that climate-related coastal freshening and warming may modify Cd accumulation in marine microalgae and support the need for subsequent mechanistic studies using measured dissolved exposure concentrations.

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Author Contributions

Esther U. Kadiene: Conceptualization, methodology, investigation, formal analysis and writing-original draft. Baghdad Ouddane: Resources, chemical analysis and validation. Jiang-Shiou Hwang: Supervision and funding acquisition. Sami Souissi: Conceptualization, supervision, project administration, funding acquisition and writing-review and editing. All authors read and approved the final manuscript.

Data Availability

The data from this study are present in this report; additional information needed will be made available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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