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Acute–Chronic Response Continuum Indicates Physiological Trajectories in *Daphnia* Under Salinity Stress

Yadi Oktariansyah(*), Arum Setiawan, Sri Maulina, Ferta Liza Putri, M. Wahyu Sobirin

Department of Biology Faculty of Mathematics and Natural Sciences Sriwijaya University, Palembang–Prabumulih Highway km 32, Indralaya, South Sumatra Indonesia

***Corresponding author:** yadioktariansyah@mipa.unsri.ac.id

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Abstract

Background: *Freshwater salinization is an emerging global stressor that disrupts osmotic regulation and energy balance in aquatic invertebrates. Although acute and chronic toxicity are traditionally evaluated separately, their biological relationship remains insufficiently integrated. This study aimed to determine whether salinity exposure in a laboratory-maintained population of Daphnia sp. is expressed as an acute–chronic response continuum, with physiological trajectories emerging across survival and reproductive endpoints.* **Methodology:** *Acute toxicity was assessed through a 24-hour sodium chloride exposure (0.00–3.80 g/L) to determine the LC50, followed by a 10-day chronic experiment using fractions of the LC50 (0–20%). Survival probability and reproductive onset were analyzed using Kaplan–Meier estimation and Cox proportional hazards models, while offspring production was evaluated using non-parametric tests.* **Findings:** *The 24-hour LC50 was estimated at 1.87 g/L. Chronic exposure significantly altered survival dynamics ($\chi^2 = 11.7$, $p = 0.02$) and reproductive onset ($\chi^2 = 10.3$, $p = 0.04$). Reproductive success declined from 100% in controls to 20–40% in exposed groups, while total offspring production differed significantly among treatments according to the Kruskal–Wallis test ($\chi^2 = 9.74$, $p = 0.045$) and the Jonckheere–Terpstra test indicated a significant decreasing trend across the salinity gradient ($JT = 79.5$, $p = 0.0148$). Sublethal reproductive impairment occurred at concentrations far below the acute lethal threshold.* **Contributions:** *Salinity stress in Daphnia sp. operates along a continuous and time-dependent physiological trajectory, where reproductive disruption precedes mortality. Integrating acute lethality with chronic survival and reproductive responses provides a more ecologically relevant framework for freshwater salinity risk assessment.*

Keywords: *Acute–Chronic Continuum*; *Daphnia*; *Kaplan–Meier Analysis*; *Physiological Trajectory*; *Salinity Stress*

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INTRODUCTION

Freshwater salinization has emerged as a growing environmental concern driven by agricultural runoff, aquaculture discharge, industrial effluents, and other anthropogenic activities. Increasing ionic concentrations in freshwater systems alter osmotic gradients and challenge organisms that are physiologically adapted to low salinity environments (Cunillera-Montcusí et al., 2022; Kaushal et al., 2021). Freshwater invertebrates, in particular, must actively regulate internal ion balance against external conditions (Cochran et al., 2023). Even moderate increases in salinity can therefore impose energetic costs, disrupt ion transport processes, and reduce overall biological performance (Popp et al., 2024).

Cladocerans of the genus *Daphnia* play a central ecological role in freshwater food webs and are widely used as model organisms in ecotoxicology. Their rapid life cycle and high reproductive capacity make them especially sensitive indicators of environmental stress (Reilly et al., 2023). In *Daphnia*, salinity stress primarily affects osmoregulatory function, increasing metabolic demand and potentially reallocating energy away from growth and reproduction (Sun et al., 2024). Such physiological disturbances may first manifest as delayed reproduction or reduced offspring output before progressing to elevated mortality risk (Mikulski & Mazurczak, 2023).

Conventional toxicity testing distinguishes sharply between acute and chronic responses. Acute assays quantify short-term lethality, commonly expressed as LC50 values, while chronic studies focus on longer-term endpoints such as survival and fecundity (Xu et al., 2020). Although this separation is methodologically convenient, it may not reflect biological reality. In natural systems, stressors act continuously, and sublethal impairments often precede lethal outcomes (Lee et al., 2023). However, it remains unclear how chronic physiological impairment develops relative to an experimentally defined acute lethal threshold, particularly whether survival and reproductive responses emerge at sublethal fractions of the acute LC50 and how these responses change over time. This study therefore integrates the acute LC50 with chronic assessments of survival, reproductive onset, and offspring production to characterize the temporal progression of physiological responses under salinity stress.

A trajectory-based perspective provides a useful conceptual framework for understanding this progression. Rather than treating mortality and reproduction as separate endpoints, survival probability and reproductive timing can be interpreted as dynamic indicators of physiological condition through time (Cribiu et al., 2020). Changes in age at first reproduction or cumulative offspring production may signal early physiological destabilization, while increasing mortality represents a later stage of functional collapse (Hansul et al., 2024). Integrating these endpoints allows examination of an acute–chronic response continuum that links lethal thresholds to sublethal life-history disruption.

This study investigates whether salinity exposure in *Daphnia* follows such a continuum. We hypothesize that salinity stress induces a progressive physiological trajectory in which sublethal effects on reproductive timing and offspring production occur at concentrations below the acute lethal threshold, and that increasing salinity fractions lead to a predictable escalation from reproductive impairment to elevated mortality risk. Under this framework, acute LC50 values do not represent isolated

toxicity metrics but instead mark the upper boundary of a continuous stress-response gradient shaping organism performance over time.

METHOD

Test organism and culture conditions

This study was conducted in the Physiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indonesia. The test organisms were freshwater cladocerans identified as *Daphnia* sp., which were obtained commercially from a local ornamental-aquatic animal supplier and subsequently maintained and propagated under laboratory conditions. Because species-level taxonomic or molecular identification was not performed, the experimental animals are referred to as *Daphnia* sp. throughout this manuscript. Neonates (< 24 h old) produced from parthenogenetic females were used in all experiments to ensure uniform physiological condition. Organisms were cultured in aerated freshwater medium under controlled conditions (25 ± 1°C; 16 h light : 8 h dark photoperiod) and fed daily with a suspension of green microalgae, *Chlorella* sp., at non-limiting rations. Water-quality parameters, including pH, dissolved oxygen (DO), and electrical conductivity, were not quantitatively measured during the exposure period and therefore were not included as covariates in the statistical analyses. The culture medium was renewed every two days to maintain stable environmental conditions, following standardized cladoceran culture procedures commonly applied in laboratory toxicity testing. The same green microalgal food source used for routine culture maintenance was also provided during the chronic exposure experiment, with feeding conditions kept consistent among treatments (OECD, 2004; Toma et al., 2021).

Research Procedures

Acute toxicity test

Salinity treatments were prepared using analytical grade sodium chloride (NaCl) dissolved in freshwater medium. Six concentrations were tested: 0, 15, 40, 65, 90, 125, 140, 165 and 190 mg/50 mL (equivalent to 0.00–3.80 g/L). Each concentration consisted of three independent replicates, with five neonates per replicate maintained in 50 mL solution under static conditions without feeding. Acute toxicity was assessed using a 24-h static exposure to sodium chloride (NaCl). The 24-h exposure period was selected to obtain an early acute lethality endpoint that could subsequently be used as the reference point for establishing the chronic exposure gradient. Thus, the acute assay was designed specifically to estimate a 24-h LC50 rather than to reproduce the 48-h exposure duration specified for the OECD TG 202 *Daphnia* immobilisation test (OECD, 2004). These data were used to estimate the median lethal concentration (LC50), which served as the basis for defining sublethal chronic treatments linking acute and chronic responses (Toma et al., 2021).

Chronic exposure experiment

Chronic exposure lasted 10 days using fractions of the LC50 (0%, 5%, 10%, 15%, and 20%). The chronic assay was conducted using five independent experimental units for each treatment, with one *Daphnia* individual maintained individually in each unit (n = 5 per treatment). Individual housing was used to enable independent monitoring

of survival, reproductive onset, and offspring production throughout the 10-day exposure period. During the 10-day chronic exposure, all individuals were provided with the same green microalgal food source under consistent feeding conditions across treatments. Feeding was maintained throughout the exposure period to avoid differences in nutritional availability among treatments. Organisms were maintained in 50 mL medium under semi-static conditions and transferred daily into freshly prepared solution while being fed once daily. Observations were conducted every 24 h at approximately the same time to maintain temporal consistency in biological measurements (Suarez et al., 2025; Rašković et al., 2023).

Biological endpoints

Three endpoints were recorded: survival, age at first reproduction (appearance of eggs or neonates), and total offspring production. All offspring were counted and removed after observation. Individuals not reproducing during the experiment were treated as censored observations. These endpoints represent sensitive indicators of chronic physiological disturbance in *Daphnia* and are widely used to detect sublethal ecological effects preceding mortality (Landes et al., 2020; Salvatore et al., 2024).

Statistical analysis

All analyses were conducted using Rstudio 4.4.3 (R Foundation for Statistical Computing). Acute mortality differences were analysed using one-way analysis of variance followed by Tukey post-hoc comparisons, and LC50 values were estimated using probit regression implemented in the *MASS* package (Venables & Ripley, 2002). For chronic responses, time-to-death and time-to-first reproduction were analysed using Kaplan–Meier estimation to describe temporal biological response trajectories (Landes et al., 2020; Schober & Vetter, 2018). Differences among treatments were evaluated using the log-rank test and Cox proportional hazards regression using the *survival* (Therneau, 2021) and *survminer* package (Kassambara et al., 2016). Offspring production was analysed using the Kruskal–Wallis test and ordered responses along the salinity gradient were tested using the Jonckheere–Terpstra trend test implemented in the *clinfun* package (Seshan & Whiting, 2023) with supporting comparisons performed using the *FSA* package (Ogle et al., 2026). *Kaplan–Meier* curves were interpreted as physiological response trajectories describing time-dependent organism performance (Asharam et al., 2024) under salinity stress. Statistical significance was accepted at $p < 0.05$.

RESULT AND DISCUSSION

Acute Salinity Toxicity

Acute exposure to increasing salinity concentrations significantly reduced *Daphnia* sp. survival after 24 h (Table 1). Survival remained relatively high (77.8 – 88.9 %) at lower concentrations (0.30 – 1.80 g/L), suggesting that individuals were able to maintain osmotic homeostasis within this range. However, a marked decline in survival was observed at concentrations ≥ 2.50 g/L, where survival dropped to 33.3% and further decreased to 11.1 % at the highest treatment (3.80 g/L). One-way ANOVA confirmed significant differences among treatments ($F(8,18)= 8.95$, $p < 0.001$), and Tukey HSD comparisons separated the concentrations

into two statistically distinct groups: lower concentrations (0.30 – 1.80 g/L) and higher concentrations (≥ 2.50 g/L).

Table 1. Acute toxicity of salinity to *Daphnia*

Concentration (g/L)	Survival Rate (%)	Group
0.00	100.0	a
0.30	88.9	a
0.80	77.8	a
1.30	88.9	a
1.80	77.8	a
2.50	33.3	b
2.80	22.2	b
3.30	22.2	b
3.80	11.1	b

Note: ANOVA: F(8,18)= 8.95, p= 0.0000646. Tukey HSD: different letters indicate significant differences ($p < 0.05$)

This threshold-like response suggests that acute mortality increases sharply once compensatory osmoregulatory mechanisms are overwhelmed. Freshwater cladocerans actively regulate hemolymph ion concentrations through epithelial ion transport systems, primarily involving Na^+/K^+ exchange (Morris & O'Donnell, 2021). At moderate salinity levels, these mechanisms can sustain internal balance; however, beyond a critical concentration, ion exchange capacity becomes insufficient, leading to osmotic stress, metabolic disruption, and eventual mortality (Mantovani & McNamara, 2021). Comparable patterns have been reported in *Daphnia* exposed to chloride salts, where acute lethality was associated with failure of ion regulation and increased energetic demand (Morris et al., 2020).

Table 2. Probit model of LC50 estimation

Parameter	Estimate ± SE	Statistic	p value
Intercept	−4.848 ± 1.271	z = −3.813	<0.001***
log10(Dose [mg/50 mL])	2.460 ± 0.633	z = 3.887	<0.001***
LC50, log10[mg/50 mL]	1.970 ± 0.067	—	—
LC50 [mg/50 mL]	93.40	—	—
LC50 [g/L]	1.87	—	—
95% CI [g/L]	1.60–2.18	—	—

Note: The LC50 was estimated using a binomial probit regression with log10-transformed dose expressed in mg/50 mL. The estimated log10 LC50 was back-transformed to obtain the LC50 on the original concentration scale. *** $p < 0.001$.

Probit regression analysis confirmed a significant dose-dependent relationship between salinity and mortality (Table 2). The log10-transformed concentration coefficient was positive and highly significant (2.460 ± 0.633, $p < 0.001$), indicating increasing mortality probability with increasing salinity concentration. The estimated 24-h LC50 was 93.4 mg/50 mL, equivalent to 1.87 g/L. On the log-transformed

dose scale used in the probit model, the estimated LC50 was 1.970 ± 0.067 log10[mg/50 mL]. This value falls between the statistically distinct survival groups, reinforcing its biological relevance as a transition point between physiological compensation and lethal failure.

Importantly, survival remained high at concentrations below the LC50, suggesting that acute mortality alone may underestimate early physiological disturbance. Sublethal salinity exposure has been shown to alter energy allocation and reproductive performance even in the absence of immediate lethality (Huang et al., 2022; Yuslan et al., 2021). Therefore, the LC50 should be interpreted not as an isolated threshold but as the upper boundary of tolerance within a broader acute–chronic response continuum.

Chronic Survival: Time-Dependent Mortality Patterns

Chronic exposure to increasing fractions of the acute LC50 significantly altered survival dynamics over the 10-day period (Table 3). Control individuals (0% LC50) maintained complete survival (100%), indicating stable physiological performance under non-stress conditions. In contrast, exposure to salinity fractions resulted in progressive mortality, with complete mortality observed at 5% LC50 by Day 10. Intermediate responses were recorded at 10% LC50 (60% survival), while higher fractions (15% and 20%) reduced survival to 20%.

Table 3. Chronic survival of *Daphnia* under fractions of LC50 salinity

LC50 Fraction (%)	Mortality (n)	Survival Rate (%)
0	0	100
5	5	0
10	2	60
15	4	20
20	4	20

Note: Log-rank: $\chi^2 = 11.7$, df = 4, p = 0.02

Log-rank analysis confirmed significant differences among survival curves ($\chi^2 = 11.7$, df = 4, p = 0.02), demonstrating that salinity fractions significantly affected time-dependent mortality patterns. This temporal structure is critical, as it reflects progressive physiological destabilization rather than immediate toxic collapse.

Chronic exposure to sublethal fractions of the acute LC50 demonstrates that mortality under salinity stress is fundamentally time-dependent rather than concentration-dependent alone. Although LC50 values are derived from short-term assays, the present results show that even 5–20% of that concentration can substantially reduce survival over a 10-day period. This pattern supports the concept of cumulative physiological stress, where sustained osmotic imbalance progressively increases energetic demands for ion regulation, evidenced by elevated metabolic rate and Na^+/K^+-ATPase activity under prolonged salinity exposure in *Daphnia* (Huang et al., 2022). In freshwater organisms such as *Daphnia*, active transport mechanisms must continuously compensate for altered ionic gradients, diverting energy from maintenance and repair processes (Lee et al., 2023; Sun et al., 2024).

Over time, this energetic reallocation likely narrows the margin of homeostatic stability, ultimately increasing mortality risk even in the absence of acute osmotic shock (Venâncio et al., 2023).

Interestingly, the 5% LC50 treatment exhibited greater mortality than the 10% fraction, suggesting a non-linear or stochastic component in chronic tolerance. Such variability may reflect individual-level differences in osmoregulatory efficiency, energy reserves, or early-life condition. Chronic stress responses often involve complex energetic trade-offs, where subtle differences in physiological capacity can generate heterogeneous survival outcomes under low-dose exposure (Barrett et al., 2024). This suggests that chronic salinity does not trigger immediate toxic collapse but instead initiates a gradual destabilization process (Barrett et al., 2024; Xiao et al., 2025). The non-linear response observed between 5% and 10% LC50 may reflect biological heterogeneity among individuals, including variation in osmoregulatory efficiency, metabolic reserves, or early-life condition. Such variability is common under low-dose chronic stress, where small physiological differences can lead to divergent survival outcomes (Lambret et al., 2021). Collectively, these findings reinforce the acute–chronic continuum concept, highlighting that lethal thresholds derived from short-term tests may underestimate ecological risk under prolonged exposure scenarios.

Table 4. Cox model for chronic survival

Variable	Hazard Ratio	95% Confidence Interval	p value
LC50 fraction (%)	1.059	0.987 – 1.136	0.108

Cox proportional hazards regression indicated a positive association between increasing LC50 fraction and mortality risk (Table 4). Although the linear trend was not statistically significant ($p = 0.108$), the direction of the effect suggests that each incremental increase in LC50 fraction slightly elevated mortality hazard. The lack of statistical significance likely reflects limited sample size rather than absence of biological effect, as supported by the significant log-rank test.

From a mechanistic perspective, chronic mortality under sublethal fractions of the LC50 indicates that prolonged osmotic stress progressively compromises physiological homeostasis. Even when concentrations remain well below the acute lethal threshold ($LC50 \approx 1.87$ g/L), sustained exposure may increase metabolic demand for ion regulation, reduce energy available for maintenance, and gradually elevate mortality risk. This pattern aligns with previous findings showing that chronic salinity exposure can induce delayed mortality through cumulative energetic stress rather than immediate osmotic shock (Evans & Kültz, 2020; Yao et al., 2025).

Importantly, these results support the concept of an acute–chronic response continuum. The LC50 defines the concentration at which 50% mortality occurs within 24 h, yet chronic exposure to fractions as low as 5–20% of that value produced substantial mortality over time. Thus, lethal thresholds derived from acute tests represent only the upper boundary of tolerance.

Kaplan–Meier survival curves clearly illustrate the temporal structure of mortality under chronic salinity exposure (Figure 1). In the control group, the survival

curve remained horizontal at probability = 1.0 throughout the experiment, reflecting absence of mortality. In exposed groups, survival probability declined stepwise over time, with downward shifts corresponding to mortality events. The earlier and steeper the decline in the curve, the greater the mortality risk and the faster the physiological destabilization. Notably, curves corresponding to higher LC50 fractions showed earlier divergence from the control, indicating that mortality risk accumulated progressively under increasing salinity stress.

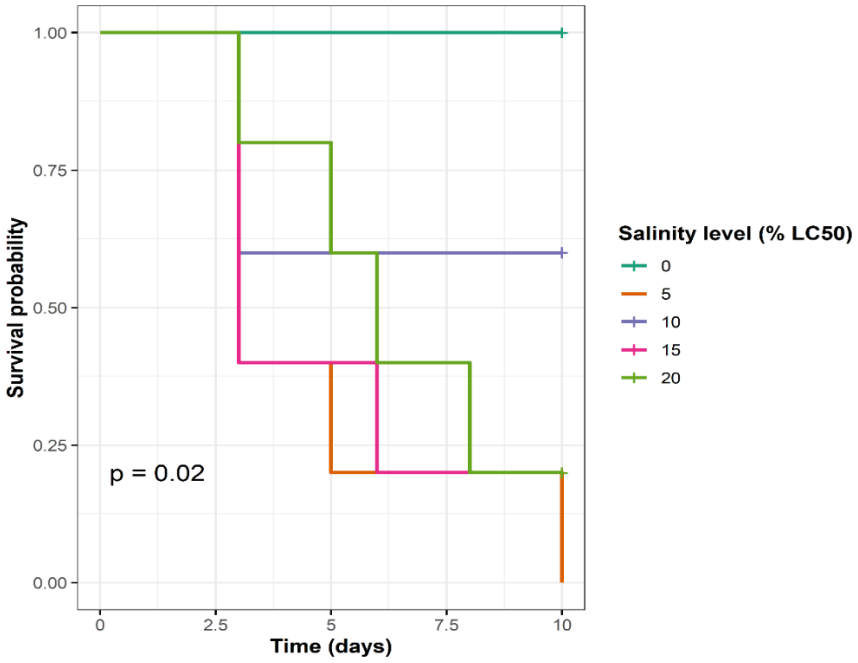

Figure 1. *Kaplan–Meier* survival trajectories of *Daphnia* sp. under chronic salinity exposure

The *Kaplan–Meier* approach is particularly informative because it does not simply quantify final mortality percentages; rather, it describes how survival probability changes through time. In this study, mortality did not occur simultaneously across treatments but emerged at different time points depending on exposure level. This time-dependent separation of curves demonstrates that chronic salinity exposure induces gradual physiological decline rather than abrupt toxic collapse. Thus, the survival curves represent dynamic physiological trajectories, mapping the progression from compensated homeostasis to functional failure.

In this framework, Kaplan–Meier survival curves can be interpreted as physiological trajectories, illustrating how organism performance deteriorates through time under increasing salinity stress. Mortality does not appear as a binary outcome but as the final stage of a time-dependent destabilization process linking sublethal stress to lethal consequences.

Reproductive Onset and Offspring Production: Progressive Reduction in Population Output

Chronic salinity exposure significantly altered both reproductive timing and overall population output (Tables 5 and 6). All control individuals reproduced during the 10-day period (100% success), with dominant age at first reproduction (AFR) occurring between Days 5–7, reflecting synchronized maturation under optimal conditions. In contrast, exposure to LC50 fractions markedly reduced reproductive success. Only 20% of individuals reproduced at 5%, 15%, and 20% LC50, while 40% reproduced at 10% LC50. Reproductive timing also shifted under stress, with delayed onset observed at higher fractions (e.g., ~Day 10 at 15% LC50 and ≥ Day 7 at 20% LC50).

Table 5. Reproductive onset under LC50 fractions

LC50 Fraction(%)	Reproducing Success Rate (%)	AFR Range (Day)
0	100	5 – 7
5	20	~5
10	40	3 – 7
15	20	~10
20	20	≥ 7

Note: Log-rank: $\chi^2 = 10.3$, $p = 0.04$

Log-rank analysis confirmed significant differences among treatments ($\chi^2 = 10.3$, $p = 0.04$), demonstrating that salinity fractions altered the temporal distribution of reproductive events (Table 5). Although the *Cox* model showed only a marginal linear trend (HR= 0.915, p= 0.066), the hazard ratio below 1 indicates that increasing salinity fraction reduced the instantaneous probability of reproductive onset. Biologically, this suggests a progressive decline in the likelihood of early maturation as osmotic stress intensifies.

Table 6. The *Cox* model for Reproductive onset

Variable	Hazard Ratio	95% Confidence Interval	p value
LC50 fraction (%)	0.915	0.832–1.1006	0.066

Kaplan–Meier curves for reproductive onset clearly illustrate these temporal differences. In the control group, the cumulative probability of reproduction increased steeply between Days 5–7. Under salinity exposure, curves rose more slowly or plateaued at lower probabilities, indicating both delayed onset and reduced likelihood of reproduction. Unlike mortality curves, where survival probability declines, the reproductive *Kaplan–Meier* curve represents the cumulative probability of initiating reproduction. A slower or flatter curve therefore reflects impaired physiological readiness for maturation.

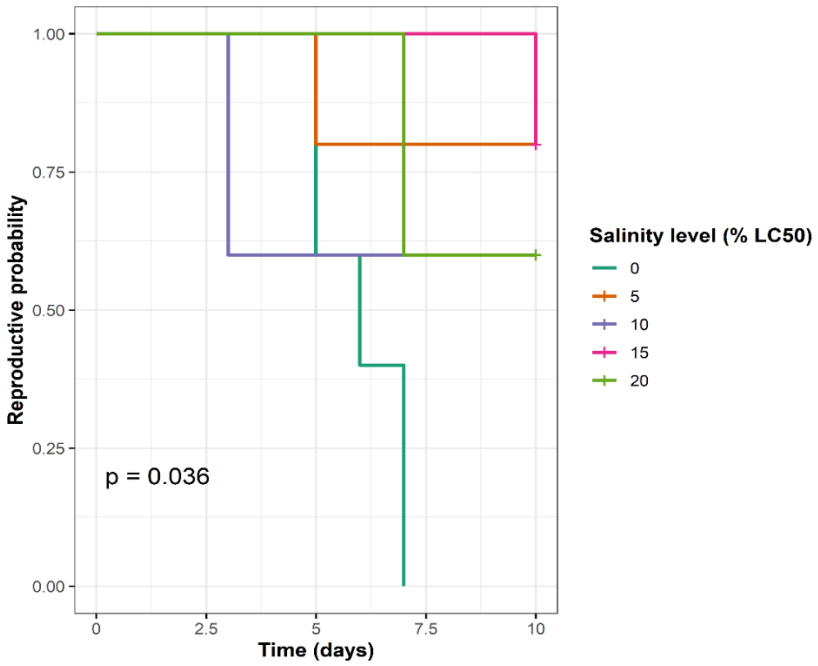

Figure 2. *Kaplan–Meier* reproduction trajectories of *Daphnia* sp. under chronic salinity exposure

These shifts in reproductive timing were accompanied by substantial reductions in cumulative offspring production (Table 7). Control individuals produced a cumulative total of 20 offspring, with a mean of 4.00 ± 2.55 and a median of 5 (IQR: 4–5). In contrast, offspring production was strongly suppressed in all salinity-exposed groups. At 5% LC50, total production decreased to 2 offspring (0.40 ± 0.89; median 0 [IQR: 0–0]), while the 10% LC50 treatment produced 7 offspring in total (1.40 ± 2.61; median 0 [IQR: 0–1]). Production was further reduced at 15% LC50 to one offspring (0.20 ± 0.45; median 0 [IQR: 0–0]) and was completely absent at 20% LC50. The predominance of zero values among exposed individuals indicates that the reduction in reproductive output resulted primarily from failure to reproduce rather than a uniform reduction in brood size among reproducing individuals.

Table 7. Offspring production under LC50 fractions

LC50 Fraction (%)	*n*	Total offspring	Mean ± SD	Median (IQR)
0	5	20	4.00 ± 2.55	5 (4–5)
5	5	2	0.40 ± 0.89	0 (0–0)
10	5	7	1.40 ± 2.61	0 (0–1)
15	5	1	0.20 ± 0.45	0 (0–0)
20	5	0	0.00 ± 0.00	0 (0–0)

Note: *Kruskal–Wallis*: $\chi^2 = 9.74$, $p = 0.045$ *. *Jonckheere–Terpstra*: JT = 79.5, $p = 0.0148$ (*significance at $\alpha = 0.05$). Values are based on cumulative offspring production per individual during the 10-day chronic exposure. Median (IQR) represents the median and first–third quartile range.

The *Kruskal–Wallis* test confirmed significant differences in offspring production among treatments ($\chi^2 = 9.74$, $p = 0.045$). Furthermore, the *Jonckheere–Terpstra* test detected a significant decreasing trend along the ordered salinity gradient (JT= 79.5, p= 0.0148), demonstrating a directional and progressive decline in reproductive output with increasing LC50 fraction.

From a physiological perspective, these patterns suggest energy reallocation under chronic osmotic stress. Increased metabolic demand for ion regulation likely reduces energy available for gametogenesis and embryonic development. Delayed AFR combined with reduced brood production indicates disruption of multiple life-history components simultaneously. Importantly, reproductive suppression occurred at fractions far below the acute lethal threshold (LC50 ≈ 1.87 g/L), highlighting that population-level consequences emerge prior to substantial mortality.

Within the acute–chronic response continuum framework, reproductive impairment represents an earlier stage of physiological destabilization, whereas mortality reflects its terminal outcome. The reproductive *Kaplan–Meier* trajectories and the monotonic decline in offspring production collectively describe a graded physiological deterioration rather than an abrupt toxic event. Thus, chronic salinity exposure compromises population sustainability at concentrations substantially lower than those causing acute lethality, reinforcing the interpretation of a continuous and time-dependent physiological trajectory in *Daphnia* sp.

Collectively, the integration of acute lethality, chronic survival dynamics, reproductive onset, and offspring production provides strong evidence for an acute–chronic response continuum under salinity stress. The 24-h LC50 defines the upper boundary of short-term physiological tolerance; however, chronic exposure to fractions as low as 5 – 20% of this threshold produced measurable disruption in survival trajectories and reproductive performance. Notably, reproductive impairment occurred at concentrations well below those causing acute mortality, indicating that lethal endpoints underestimate early biological disturbance. Similar patterns have been reported in freshwater cladocerans, where sublethal salinity exposure altered energy allocation, reduced reproductive output, and impaired life-history traits before significant mortality occurred (Salvatore et al., 2024).These findings support the view that acute and chronic effects are mechanistically connected through progressive physiological destabilization rather than representing discrete toxic categories.

From a mechanistic perspective, increasing salinity imposes osmotic stress that elevates energetic demand for ion regulation and homeostasis. As metabolic resources are diverted toward maintenance, less energy remains available for growth and reproduction, leading to delayed age at first reproduction and reduced brood production. Over time, sustained energetic imbalance may culminate in increased mortality risk. Such sequential progression from sublethal impairment to lethal outcome aligns with theoretical models of stress physiology in aquatic invertebrates, where cumulative energetic trade-offs drive declining performance across multiple life-history endpoints (Toma et al., 2021). The Kaplan–Meier survival and reproductive curves in this study visually represent these time-dependent physiological trajectories, illustrating how organism performance deteriorates progressively under increasing salinity fractions. Thus, the observed patterns reinforce the interpretation that salinity

stress in *Daphnia* sp. operates along a graded and continuous response gradient, linking acute toxicity to chronic population-level consequences.

Study Limitations and Future Prospects

Despite providing evidence for an acute–chronic response continuum under salinity stress, several limitations should be acknowledged. First, the experimental duration for chronic exposure was limited to 10 days, which captures early life-history responses but does not encompass full reproductive lifespan or multigenerational effects. In *Daphnia sp.*, cumulative reproductive output and population growth rate may continue to diverge over longer exposure periods. Second, the relatively small sample size per treatment may have reduced statistical power, particularly in Cox proportional hazards analyses where trends were evident but not always statistically significant. Increasing replication could improve detection of linear hazard gradients and reduce variability observed in low-dose treatments. Third, this study focused on phenotypic life-history endpoints without direct measurement of physiological or biochemical markers. While survival and reproduction reflect integrative organismal performance, underlying mechanisms such as ion transporter activity, metabolic rate, oxidative stress, or gene expression were not quantified. Incorporating physiological biomarkers would strengthen causal interpretation of the proposed energetic trade-off framework. Additionally, salinity was represented solely by NaCl, whereas natural salinization often involves complex ionic mixtures that may produce additive or synergistic effects.

Future research should extend this trajectory-based framework in several directions. Long-term and multigenerational experiments would clarify whether sublethal reproductive suppression translates into reduced intrinsic population growth rates and altered adaptive capacity. Integrating metabolic, transcriptomic, or ion-regulatory measurements could directly link energetic allocation to observed life-history shifts. Comparative studies across *Daphnia sp.* species or genotypes would also help determine whether the acute–chronic continuum reflects conserved physiological constraints or population-specific tolerance strategies.

Finally, applying time-to-event modelling in combination with population projection models may provide stronger ecological relevance by translating physiological trajectories into predicted population dynamics under freshwater salinization scenarios. By addressing these limitations, future studies can refine the conceptual model of physiological trajectories and improve ecological risk assessment for freshwater systems experiencing increasing salinity pressure. A further limitation is that water-quality parameters such as pH, dissolved oxygen, and electrical conductivity were not quantitatively monitored during the exposure period. Future experiments should incorporate routine measurements of these parameters to distinguish the direct effects of NaCl-derived salinity from potential variation in the physicochemical conditions of the exposure medium. Furthermore, The relatively small number of independent individuals per treatment (n = 5) represents an important limitation of the chronic experiment and may have reduced statistical power, particularly for hazard-based analyses. Future studies should increase the number of independent replicates to improve statistical power and provide more robust estimates of population-level responses

CONCLUSION

This study demonstrates that salinity stress in a laboratory-maintained population of *Daphnia sp.* sp. is expressed as an acute–chronic response continuum rather than as an isolated lethal response. The 24-h LC50 was estimated at 1.87 g/L, providing an experimentally derived reference point for the chronic exposure gradient. The principal contribution of this study is the integration of acute lethality with time-dependent chronic survival, reproductive onset, and offspring production, revealing that substantial reproductive impairment emerged at only 5–20% of the acute LC50, well below the concentration associated with acute lethality. Kaplan–Meier analyses further demonstrated distinct temporal trajectories of survival and reproductive onset, while offspring production showed a significant decreasing trend along the ordered salinity gradient. These findings indicate that reproductive dysfunction can emerge before pronounced mortality and therefore provides an earlier indication of physiological disturbance than mortality alone. The study nevertheless has several limitations, including the use of *Daphnia sp.* sp. without species-level confirmation, the relatively small number of independent individuals in the chronic assay (n = 5 per treatment), the 10-day exposure period, and the absence of quantitative monitoring of pH, dissolved oxygen, and conductivity. In addition, the use of NaCl as a model salt limits direct extrapolation to salinization involving more complex ionic mixtures. Future studies incorporating species-confirmed populations, larger replication, longer exposure periods, and comprehensive water-quality monitoring are therefore needed to validate and extend the proposed acute–chronic physiological trajectory framework.

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REFERENCES

Asharam, K., Mitku, A. A. A., Ramsay, L., Jeena, P. M., & Naidoo, R. N. (2024). Environmental exposures associated with early childhood recurrent wheezing in the mother and child in the environment birth cohort: a time-to-event study. *Thorax*, *79*(10), 953–960. https://doi.org/10.1136/thorax-2023-221150

Barrett, N. J., Harper, E. M., Last, K. S., Reinardy, H. C., & Peck, L. S. (2024). Behavioural and physiological impacts of low salinity on the sea urchin *Echinus esculentus*. *Journal of Experimental Biology*, *227*(2), jeb246707. https://doi.org/10.1242/jeb.246707

Cochran, J. K., Banks, C., & Buchwalter, D. B. (2023). Respirometry reveals major lineage-based differences in the energetics of osmoregulation in aquatic invertebrates. *Journal of Experimental Biology*, *226*(20), jeb246376. https://doi.org/10.1242/jeb.246376

Cribiu, P., Devaux, A., Garnero, L., Abbaci, K., Bastide, T., Delorme, N., Quéau, H., Degli Esposti, D., Ravanat, J.-L., Geffard, O., Bony, S., & Chaumot, A. (2020). A "Population Dynamics" Perspective on the Delayed Life-History Effects of Environmental Contaminations: An Illustration with a Preliminary Study of Cadmium Transgenerational Effects over Three Generations in the Crustacean Gammarus. *International Journal of Molecular Sciences*, *21*(13), 4704. https://doi.org/10.3390/ijms21134704

Cunillera-Montcusí, D., Beklioğlu, M., Cañedo-Argüelles, M., Jeppesen, E., Ptacnik, R., Amorim, C. A., Arnott, S. E., Berger, S. A., Brucet, S., Dugan, H. A., Gerhard, M., Horváth, Z., Langenheder, S., Nejstgaard, J. C., Reinikainen, M., Striebel, M., Urrutia-Cordero, P., Vad, C. F., Zadereev, E., & Matias, M. (2022). Freshwater salinisation: a research agenda for a saltier world. *Trends in Ecology & Evolution*, *37*(5), 440–453. https://doi.org/10.1016/j.tree.2021.12.005

Evans, T. G., & Kültz, D. (2020). The cellular stress response in fish exposed to salinity fluctuations. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *333*(6), 421–435. https://doi.org/10.1002/jez.2350

Hansul, S., Fettweis, A., Smolders, E., & Schamphelaere, K. De. (2024). Extrapolating Metal (Cu, Ni, Zn) Toxicity from Individuals to Populations Across *Daphnia* Species Using Mechanistic Models: The Roles of Uncertainty Propagation and Combined Physiological Modes of Action. *Environmental Toxicology and Chemistry*, *43*(2), 338–358. https://doi.org/10.1002/etc.5782

Huang, J., Li, Y., Sun, Y., Zhang, L., Lyu, K., & Yang, Z. (2022). Size-specific sensitivity of cladocerans to freshwater salinization: Evidences from the changes in life history and population dynamics. *Environmental Pollution*, *296*, 118770. https://doi.org/10.1016/j.envpol.2021.118770

Kassambara, A., Kosinski, M., & Biecek, P. (2016). survminer: Drawing Survival Curves using "ggplot2." In *CRAN: Contributed Packages*. https://doi.org/10.32614/CRAN.package.survminer

Kaushal, S. S., Likens, G. E., Pace, M. L., Reimer, J. E., Maas, C. M., Galella, J. G., Utz, R. M., Duan, S., Kryger, J. R., Yaculak, A. M., Boger, W. L., Bailey, N. W., Haq, S., Wood, K. L., Wessel, B. M., Park, C. E., Collison, D. C., Aisin, B. Y. 'aaqob I., Gedeon, T. M., … Woglo, S. A. (2021). Freshwater salinization syndrome: from emerging global problem to managing risks. *Biogeochemistry*, *154*(2), 255–292. https://doi.org/10.1007/s10533-021-00784-w

Lambret, P., Janssens, L., & Stoks, R. (2021). The impact of salinity on a saline water insect: Contrasting survival and energy budget. *Journal of Insect Physiology*, *131*, 104224. https://doi.org/10.1016/j.jinsphys.2021.104224

Landes, J., Engelhardt, S. C., & Pelletier, F. (2020). An introduction to event history analyses for ecologists. *Ecosphere*, *11*(10), e03238. https://doi.org/10.1002/ecs2.3238

Lee, S., Haque, Md. N., Lee, D.-H., & Rhee, J.-S. (2023). Comparison of the effects of sublethal concentrations of biofoulants, copper pyrithione and zinc pyrithione on a marine mysid - A multigenerational study. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *271*, 109694. https://doi.org/10.1016/j.cbpc.2023.109694

Mantovani, M., & McNamara, J. C. (2021). Contrasting strategies of osmotic and ionic regulation in freshwater crabs and shrimps: gene expression of gill ion transporters. *Journal of Experimental Biology*, 224(3), jeb233890. https://doi.org/10.1242/jeb.233890

Mikulski, A., & Mazurczak, D. (2023). Maternal effect in salinity tolerance of Daphnia–One species, various patterns? *PLOS ONE*, *18*(4), e0283546. https://doi.org/10.1371/journal.pone.0283546

Morris, C., & O'Donnell, M. J. (2021). Vacuolar H+-ATPase and Na+/K+-ATPase energize Na+ uptake mechanisms in the nuchal organ of the hyperregulating freshwater crustacean *Daphnia magna*. *Journal of Experimental Biology*, *224*(12), jeb242205. https://doi.org/10.1242/jeb.242205

Morris, C., Sakarya, M., Koh, O., & O'Donnell, M. (2020). Alterations in Hemolymph Ion Concentrations and pH in Adult *Daphnia magna* in Response to Elevations in Major Ion Concentrations in Freshwater. *Environmental Toxicology and Chemistry*, *40*(2), 366–379. https://doi.org/10.1002/etc.4919

OECD. (2004). *Test No. 202: Daphnia sp. Acute Immobilisation Test*. OECD Publishing. https://doi.org/10.1787/9789264069947-en. Retrieved March 8Th, 2026.

Ogle, D. H., Doll, J. C., Wheeler, A. P., & Dinno, A. (2026). FSA: Simple Fisheries Stock Assessment Methods. In *CRAN: Contributed Packages*. https://doi.org/10.32614/CRAN.package.FSA. Retrieved March 12, 2026.

Popp, T. E., Hermet, S., Fredette-Roman, J., McKeel, E., Zozaya, W., Baumlin, C., Charmantier, G., Lee, C. E., & Lorin-Nebel, C. (2024). Evolution of ion transporter Na+/K+-ATPase expression in the osmoregulatory maxillary glands of an invasive copepod. *IScience*, *27*(7), 110278. https://doi.org/10.1016/j.isci.2024.110278

Rašković, B., Poleksić, V., Vuković, G., Špirović Trifunović, B., Božić, G., Ćupić Miladinović, D., Marković, Z., & Brkić, D. (2023). Acute and Subchronic Exposure of the Common Carp (*Cyprinus carpio*) to Herbicide S-Metolachlor. *Water*, *15*(23), 4182. https://doi.org/10.3390/w15234182

Reilly, K., Ellis, L.-J. A., Davoudi, H. H., Supian, S., Maia, M. T., Silva, G. H., Guo, Z., Martinez, D. S. T., & Lynch, I. (2023). *Daphnia* as a model organism to probe biological responses to nanomaterials—from individual to population effects via adverse outcome pathways. *Frontiers in Toxicology*, *5,* 1178482. https://doi.org/10.3389/ftox.2023.1178482

Salvatore, M. M., Pappalardo, C., Suarez, E. G. P., Salvatore, F., Andolfi, A., Gesuele, R., Galdiero, E., Libralato, G., Guida, M., & Siciliano, A. (2024). Ecotoxicological and metabolomic investigation of chronic exposure of *Daphnia*

magna (Straus, 1820) to yttrium environmental concentrations. *Aquatic Toxicology*, *276*, 107117. https://doi.org/10.1016/j.aquatox.2024.107117

Schober, P., & Vetter, T. R. (2018). Survival Analysis and Interpretation of Time-to-Event Data: The Tortoise and the Hare. *Anesthesia & Analgesia*, *127*(3), 792–798. https://doi.org/10.1213/ANE.0000000000003653

Seshan, V. E., & Whiting, K. (2023). Clinfun: Clinical Trial Design and Data Analysis Functions. In *CRAN: Contributed Packages*. https://doi.org/10.32614/CRAN.package.clinfun. Retrieved March 14, 2026.

Suarez, E. G. P., Revel, M., Libralato, G., Guida, M., & Heise, S. (2025). Sublethal and lethal toxicity assessment of lanthanum and gadolinium to *Daphnia magna* in a 7-day test method. *Environmental Science and Pollution Research*, *32*(5), 2467–2478. https://doi.org/10.1007/s11356-024-35854-7

Sun, X., Arnott, S. E., & Little, A. G. (2024). Impacts of sequential salinity and heat stress are recovery time-specific in freshwater crustacean, *Daphnia pulicaria*. *Ecotoxicology and Environmental Safety*, *269*, 115899. https://doi.org/10.1016/j.ecoenv.2023.115899

Therneau, T. M. (2021). Survival: Survival Analysis. In *CRAN: Contributed Packages*. https://doi.org/10.32614/CRAN.package.survival. Retrieved March 14, 2026.

Toma, C., Cappelli, C. I., Manganaro, A., Lombardo, A., Arning, J., & Benfenati, E. (2021). New Models to Predict the Acute and Chronic Toxicities of Representative Species of the Main Trophic Levels of Aquatic Environments. *Molecules*, *26*(22), 6983. https://doi.org/10.3390/molecules26226983

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th edition). Springer. 495 page. https://doi.org/10.1007/978-0-387-21706-2

Venâncio, C., Wijewardene, L., Ribeiro, R., & Lopes, I. (2023). Combined effects of two abiotic stressors (salinity and temperature) on a laboratory-simulated population of *Daphnia longispina*. *Hydrobiologia*, *850*(14), 3197–3208. https://doi.org/10.1007/s10750-023-05249-9

Xiao, H., Jin, X., Wang, Z., Ye, Q., Li, W., Han, L., & Ding, J. (2025). Effects of Chronic Low-Salinity Stress on Growth, Survival, Antioxidant Capacity, and Gene Expression in *Mizuhopecten yessoensis*. *Biology*, *14*(7), 759. https://doi.org/10.3390/biology14070759

Xu, E. G., Cheong, R. S., Liu, L., Hernandez, L. M., Azimzada, A., Bayen, S., & Tufenkji, N. (2020). Primary and Secondary Plastic Particles Exhibit Limited Acute Toxicity but Chronic Effects on *Daphnia magna*. *Environmental Science & Technology*, *54*(11), 6859–6868. https://doi.org/10.1021/acs.est.0c00245

Yao, T., Masanja, F., Lu, J., Fu, S., Luo, W., Shija, V. M., Ye, L., & Zhao, L. (2025). Lasting impacts of rapid salinity change on physiological energetics of estuarine oysters (*Crassostrea hongkongensis*). *Marine Environmental Research*, *207*, 107076. https://doi.org/10.1016/j.marenvres.2025.107076

Yuslan, A., Najuwa, S., Hagiwara, A., Ghaffar, M. A., Suhaimi, H., & Rasdi, N. W. (2021). Production Performance of *Moina macrocopa* (Straus 1820) (Crustacea, Cladocera) Cultured in Different Salinities: The Effect on Growth, Survival, Reproduction, and Fatty Acid Composition of the Neonates. *Diversity*, *13*(3), 105. https://doi.org/10.3390/d13030105

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