

Time-dependent modulation of post-hatch cortisol and antioxidant responses in *Melanotaenia boesemani* embryos exposed to potassium derived from banana peel ash extract

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Abstract

Embryonic exposure to ionic disturbance can alter post hatch stress regulation in fish. This study examined whether immersion duration in potassium-rich banana peel ash extract affected endocrine and antioxidant responses in *Melanotaenia boesemani*. Embryos were immersed in a fixed potassium concentration of BPA extract (13.5 mg L⁻¹) for 15.5, 24, and 32.5 h, alongside untreated and KCl reference groups (three biological replicates per treatment). Cortisol, superoxide dismutase (SOD), and catalase (CAT) were quantified at 7 days post hatching, and survival was recorded at 60 days post-hatching. Data were analyzed using one-way ANOVA with Tukey HSD, Pearson correlation, and principal component analysis (PCA). All four endpoints (cortisol, SOD, CAT, and survival rate) differed significantly among treatments ($p < 0.05$). Cortisol was significantly higher in all BPA-exposed groups than in both controls, reaching its highest numerical value at 24 h, although the three BPA-duration groups did not differ significantly from one another. SOD and CAT activities increased with BPA exposure, reaching their highest values at 24 h. CAT remained significantly elevated relative to the controls and the 15.5 h group at both 24 and 32.5 h, whereas SOD was significantly elevated relative to the controls and the 15.5 h group only at 24 h. SR declined progressively with increasing immersion duration and was lowest at 32.5 h. Cortisol correlated positively with SOD ($r = 0.86$) and CAT ($r = 0.81$), and SOD correlated strongly with CAT ($r = 0.87$). Survival rate was negatively correlated with cortisol ($r = -0.59$) and CAT ($r = -0.75$). PCA reduced the four variables into two components explaining 94.3% of total variance and separated BPA exposed groups from controls along a biomarker dominated axis. These results indicate a coordinated endocrine and antioxidant response following embryonic BPA exposure, but the magnitude of the biomarkers was not directly related to survival outcome. The highest biomarker responses at 24 h coincided with intermediate survival, while the lowest survival at 32.5 h occurred alongside declining biomarker levels. This dissociation indicates that immersion duration itself, independent of potassium concentration, is an important variable to consider when evaluating biomass-derived potassium sources for use during early developmental stages.

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1. Introduction

The embryonic period is a particularly sensitive to many environmental stressors. During this period, teleosts establish their key regulatory mechanisms and physiological systems. Stressors during early development can influence embryogenesis and produce physiological consequences that persist beyond hatching. Exposure to stress in early life has been related to lowered survival, developmental abnormalities, and long-term changes in the control mechanisms of the stress response in teleost (Valcarce et al. 2024). *Melanotaenia boesemani*, Papua's native ornamental rainbowfish, is of considerable economic significance in the freshwater ornamental fish trade. The culture of this species in aquaculture systems is crucial for the effective development of the embryos and the survival of the larvae throughout the early life stages (Akhsan et al. 2020; Gupta and Roy 2024). Therefore, it is important to know the effects of the exposure conditions in the embryonic phase on the physiological state after hatching, to elucidate the mechanisms of developmental responses to stressors, and to support the optimization of the reproductive and production management of *M. boesemani* under controlled conditions.

Ion regulation is one physiological system that is especially important to this susceptibility as potassium is a major regulator of cellular homeostasis in early development. Potassium is the major intracellular ion and is involved in establishing the membrane potential, ion gradients across cells, neuromuscular activity, and Na⁺/K⁺ATPase-dependent transport. This balance in fish is maintained via coordinated interaction between ion transport mechanisms, energy metabolism, and endocrine signaling, and any change in the ionic conditions of the environment increases the physiological cost of maintaining homeostasis. This adjustment is mediated by the hypothalamic-pituitary-interrenal (HPI) axis, and cortisol mediates metabolic and ionoregulatory responses to environmental change (Culbert et al. 2022; Shaughnessy et al. 2023). Such changes can also induce a disturbance of the redox equilibrium at the cellular level and induce antioxidant defences, in particular superoxide dismutase (SOD) and catalase (CAT). These enzymes function sequentially to neutralize reactive oxygen species produced during physiological stress (Wang et al. 2022; Xu et al. 2022). The correlation between antioxidant enzymes and cortisol gives a more comprehensive picture of the response of growing fish to ionic challenges than either measure alone, since

ionoregulatory and oxidative stress pathway can be co-activated in environmental perturbation. The source of potassium itself however is not necessarily biologically neutral and may impact the response of these regulatory and antioxidant systems. Synthetic KCl is a more homogeneous source of potassium than potassium obtained from biomass. Banana peel is a potassium rich agricultural leftover. Thermal processing and aqueous extraction can give a fraction rich in minerals for resource recovery (Bai et al. 2025; Putra et al. 2022). However, the biological reaction to a biomass-extract cannot be presumed to be the same as that of synthetic potassium. Ash-derived material may contain other inorganic compounds, the identity and quantity of which is dependent on the raw material and processing conditions utilized. Therefore, biological assessment is important and should not be considered an afterthought before potassium rich banana peel ash extract may be evaluated for use in aquaculture application.

A previous study on *M. boesemani* embryos has shown that the biological effects of biobased and synthetic potassium are very sensitive to the circumstances of exposure. The length of immersion plays an important role in toxicity effects (Hismayasari et al. 2026). However, mortality and developmental endpoints limited information about physiological changes post exposure. It is not known whether variations in the duration of embryonic exposure result in permanent alterations in endocrine stress control and antioxidant defence after hatching, a gap that is important since biochemical and survival responses may not correlate. For this purpose, an integrated evaluation is required which combines endocrine, antioxidant and organism level endpoints to allow physiological activation to be separated from actual adverse effects. Beyond its physiological relevance, understanding the post hatch consequences of embryonic potassium exposure has practical implications for aquaculture. Banana peel, an abundant agricultural byproduct, represents a potentially low cost, renewable source of potassium that could reduce reliance on synthetic reagents as KCl in hatchery management. However, this benefit can only be realized if the biological safety of biomass-derived potassium is established across the exposure conditions likely to occur in practice, including variation in immersion duration. The present work therefore addresses both a mechanistic question of how immersion duration during embryonic development shaped post hatch physiological state and a practical one, namely whether BPA derived potassium can be used safely as a component of *M. boesemani* hatchery protocols. The effects of different embryonic immersion durations in potassium-rich banana peel ash extract on the post-hatch physiological responses of *M. boesemani* were evaluated in present study. It was hypothesized that immersion duration would not necessarily correspond with survival outcome. This design offers a framework for evaluating the time dependent carry over effect of biomass-derived potassium exposure across multiple levels of biological organization.

2. Materials and Methods

2.1 Experimental fish and embryo preparation

Embryos used in this study were obtained from broodstock of *Melanotaenia boesemani* sourced from Center for Independent Marine and Fisheries Training, Mina Mulya. Broodstock were mass-spawned at a male-to-female ratio of 1:1. Black raffia strands fitted with weights were used as spawning substrates to facilitate egg attachment. Embryos were collected and maintained until they reached the eye stage at approximately 48 hours post-fertilization (hpf), after which point they

were subjected to the immersion treatments (Akhsan et al. 2020; Gupta and Roy 2024).

2.2 Preparation of banana peel ash-derived potassium

Kepok banana (*Musa paradisiaca*) peels were chopped, oven dried, and subsequently incinerated in a furnace at 600 °C for 2 – 4 h. The resulting white ash was extracted using a hotplate stirrer and filtered through Whatman No.1 filter paper. The resulting filtrate was hereafter referred to as banana peel ash extract (BPA). Potassium concentration in BPA filtrate was quantified using a Shimadzu inductively coupled plasma – atomic emission spectrometer (ICP-AES) at the Integrated Research Laboratory, Brawijaya University.

2.3 Experimental design

The experiment was conducted using a completely randomized design comprising five treatment groups: an untreated control (K0), a synthetic potassium control using KCl (K1), and three BPA-derived potassium treatments differing in embryo immersion duration, namely A (15.5 h), B (24 h), and C (32.5 h). A total of 3,750 embryos were used in this study, distributed equally across the five treatment groups (750 embryos per treatment). Each treatment consisted of three replicate tanks, with 250 embryos stocked per replicate tank. The potassium concentration was maintained constant at 13.5 mg L⁻¹ across the BPA treatment groups. Physiological responses were evaluated by measuring stress related biomarkers including cortisol, SOD, and CAT to assess endocrine stress responses and antioxidant defense status following potassium exposure. Developmental performance was additionally assessed through survival rate (SR) as an indicator of early developmental success.

2.4 Cortisol and antioxidant enzyme measurement

Cortisol was extracted from *M. boesemani* tissue at 7 days post hatching (dph) following the protocol described by Aldurrah et al. (2023). Briefly, samples were subjected to cryogenic freezing, homogenization, liquid-liquid extraction with diethyl ether, centrifugation, evaporation under a stream of nitrogen, and subsequent reconstitution in phosphate buffered saline (PBS). Cortisol concentration in the extracts was quantified using a commercial enzyme-linked immunosorbent assay kit (EIAgen™Cortisol Test, Biochem ImmunoSystem) according to the manufacturer's instructions. Measurements were performed in triplicate. Final cortisol concentrations were corrected using factor of 1.0604 (Mezzomo et al. 2019).

For SOD and CAT analysis, larvae from each independent experimental replicate were pooled separately. Twenty larvae from each replicate were used to obtain sufficient biological material for enzyme analysis, resulting in three independent pooled biological samples per treatment (n = 3). Each pooled sample was processed and analyzed independently. Technical measurements, when performed in replicate, were averaged to obtain a single value for each biological replicate. Catalase (CAT) and superoxide dismutase (SOD) activities were determined using commercial assay kits (MedikBio) according to the manufacturer's instructions, with slight modification based on Alasmari et al. (2020). For CAT analysis, samples were homogenized in ice-cold PBS and centrifuged at 14,000 rpm for 10 min at 4 °C. For SOD analysis, samples were homogenized in lysis buffer containing 50 mM potassium phosphate, 0.1 mM EDTA, and 0.5% Triton X-100, and centrifuged at 12,000 x g for 5 min at 4 °C. The supernatant was taken for the respective enzyme assay. CAT activity was determined based on

the enzymatic decomposition of hydrogen peroxide (H₂O₂), followed by the addition of the detection reagent. Absorbance was measured at 570 nm and quantified using an H₂O₂ standard curve. SOD activity was determined using the xanthine oxidase-WST-1 assay, where SOD in the sample competes with WST-1 for reduction. Absorbance was measured at 440 nm before and after 60 min of incubation, and SOD activity was calculated from the corresponding SOD standard curve.

2.5 Survival rate assessment

Survival rate (SR) was assessed at 60 days post-hatching (60 dph) by recording the number of surviving larvae in each experimental replicate. Larvae were considered alive when spontaneous movement or a response to gentle mechanical stimulation was observed. Survival rate (SR) was calculated as follows:

$$SR (\%) = (\text{Number of surviving larvae} / \text{Total Larvae}) \times 100$$

2.6 Statistical analysis

One-way ANOVA was used to evaluate the effects of immersion duration on the measured physiological responses using IBM SPSS Statistics version 27. When significant differences were detected ($p < 0.05$), Tukey's HSD post hoc test was applied for multiple comparisons. Relationships among the measured responses were assessed using Pearson's correlation analysis. Principal component analysis (PCA) was performed to evaluate the contribution of individual response variables to the overall multivariate pattern. PCA results were visualized using score plots and loading biplots to illustrate sample distribution and the contribution of each variable. Pearson correlation and PCA analysis were conducted in RStudio version 2026.07.1 using stats, FactoMineR, factoextra, and corrplot packages. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Effects of exposure duration on measured parameters

The early post-hatch physiological stress responses of *M. boesemani* larvae following BPA exposure were evaluated by measuring cortisol, superoxide dismutase (SOD), catalase (CAT), and survival rate (SR) at three immersion durations (15.5, 24, and 32.5 hours) at a fixed BPA concentration of 13.5 mg L⁻¹ with an unexposed control (K0) and a synthetic control (K1) in a fully randomized design with three independent tank replicates per treatment. Table 1 presents group averages and one-way ANOVA results for all four parameters. Corresponding multiple comparisons are indicated in Fig. 1A-D.

3.1.1 Cortisol

Cortisol concentration differed significantly among treatments. The untreated control K0 (1.80 ± 0.12 ng mL⁻¹) and KCl control K1 (1.49 ±

0.06 ng mL⁻¹) were statistically comparable. In contrast, cortisol was significantly higher in all BPA-exposed groups than in both controls, reaching 2.82 ± 0.23 ng mL⁻¹ in treatment A, a maximum of 3.17 ± 0.25 ng mL⁻¹ in treatment B, and 2.69 ± 0.19 ng mL⁻¹ in treatment C. Although the response increased from 15.5 to 24 h and then declined at 32.5 h, the three BPA-duration groups did not differ significantly from one another according to Tukey's HSD test ($p < 0.05$)

3.1.2 Antioxidant enzyme activity (SOD and CAT)

There was significant variation in antioxidant enzyme activities across the treatments. The SOD activity was 0.88 ± 0.21 U mL⁻¹ in K0, 2.10 ± 0.22 U mL⁻¹ in K1, and 2.35 ± 0.14 U mL⁻¹ in treatment A. The maximum SOD activity was observed in treatment B (2.95 ± 0.22 U mL⁻¹), followed by treatment C (2.62 ± 0.15 U mL⁻¹). SOD activity was significantly higher in treatment B than K0, K1, and treatment A. Treatment C was significantly higher than K0 but not different from K1, A, and B. CAT activity showed a similar trend, increasing from 0.12 ± 0.03 U mL⁻¹ in K0 and 0.15 ± 0.02 U mL⁻¹ in K1 to 0.21 ± 0.03 U mL⁻¹ in A, peaking at 0.31 ± 0.02 U mL⁻¹ in B, and declining slightly to 0.27 ± 0.02 U mL⁻¹ in C. CAT activity in treatment A was higher than that in K0, while B and C were significantly higher than K0, K1, and A and did not differ from each other.

3.1.3 Survival Rate (SR)

Survival rate differed significantly among treatments ($F_{4, 10} = 9.66$; $P = 0.002$), with the lowest SR observed in treatment C (75.2 ± 1.51%). However, pairwise comparisons indicated that treatment C did not differ significantly from treatment A (79.2 ± 1.51%) and untreated control (K0 = 80 ± 1.61 %), whereas it only differed significantly from the KCl control (K1 = 82 ± 1.41%), indicating that the synthetic control did not impair larval survival under the tested conditions. The SR of treatment A (15.5 h BPA exposure) was 79.2 ± 1.51 % and was similarly not significantly different from either control groups. Treatment A showed a survival rate comparable to both controls under the tested conditions. Compared to K1 control, survival was significantly lower at 24 h (treatment B = 76.8 ± 1.41%) and 32.5 h (treatment C = 75.2 ± 1.51%). The SR of treatment C was significantly lower than that of treatment A ($p < 0.05$), indicating that survival declined with increasing immersion duration within the range tested.

3.2 Correlation among stress and antioxidant biomarkers

Pearson correlation analysis showed that endocrine stress responses were correlated with antioxidant responses in *M. boesemani* larvae (Fig. 2). A strong positive correlation was found between the cortisol concentration and SOD activity ($r = 0.86$, $p < 0.001$) and CAT ($r = 0.81$, $p < 0.001$). The strongest positive association was between SOD and CAT ($r = 0.87$, $p < 0.001$). Conversely, SR was negatively associated with

Table 1. Cortisol concentration, SOD and CAT activities, and survival rate of <i>M. boesemani</i> larvae immersed in BPA for different immersion durations							
Treatment	K0	K1	A (15.5 h)	B (24 h)	C (32.5 h)	F-value	p-value
Cortisol (ng mL ⁻¹)	1.80±0.12 ^a	1.49±0.06 ^a	2.82±0.23 ^b	3.17±0.25 ^b	2.69±0.19 ^b	44.79	< 0.001
SOD (U mL ⁻¹)	0.88±0.21 ^a	2.10±0.22 ^{ab}	2.35±0.14 ^{ab}	2.95±0.22 ^c	2.62±0.15 ^{bc}	14.35	< 0.001
CAT (U mL ⁻¹)	0.12±0.03 ^a	0.15±0.02 ^{ab}	0.21±0.03 ^b	0.31±0.02 ^c	0.27±0.02 ^c	37.50	< 0.001
SR (%)	80±1.61 ^{bc}	82±1.41 ^c	79.2±1.51 ^{abc}	76.8±1.41 ^{ab}	75.2±1.51 ^a	9.66	0.002
Values are presented as mean ± SD (n = 3); Different superscripts indicate significant differences according to Tukey's HSD test (p < 0.05); K0: untreated control; K1: KCl control; A: BPA immersion for 15.5 h; B: BPA immersion for 24 h; C: BPA immersion for 32.5 h at BPA concentration of 13.5 mg L ⁻¹							

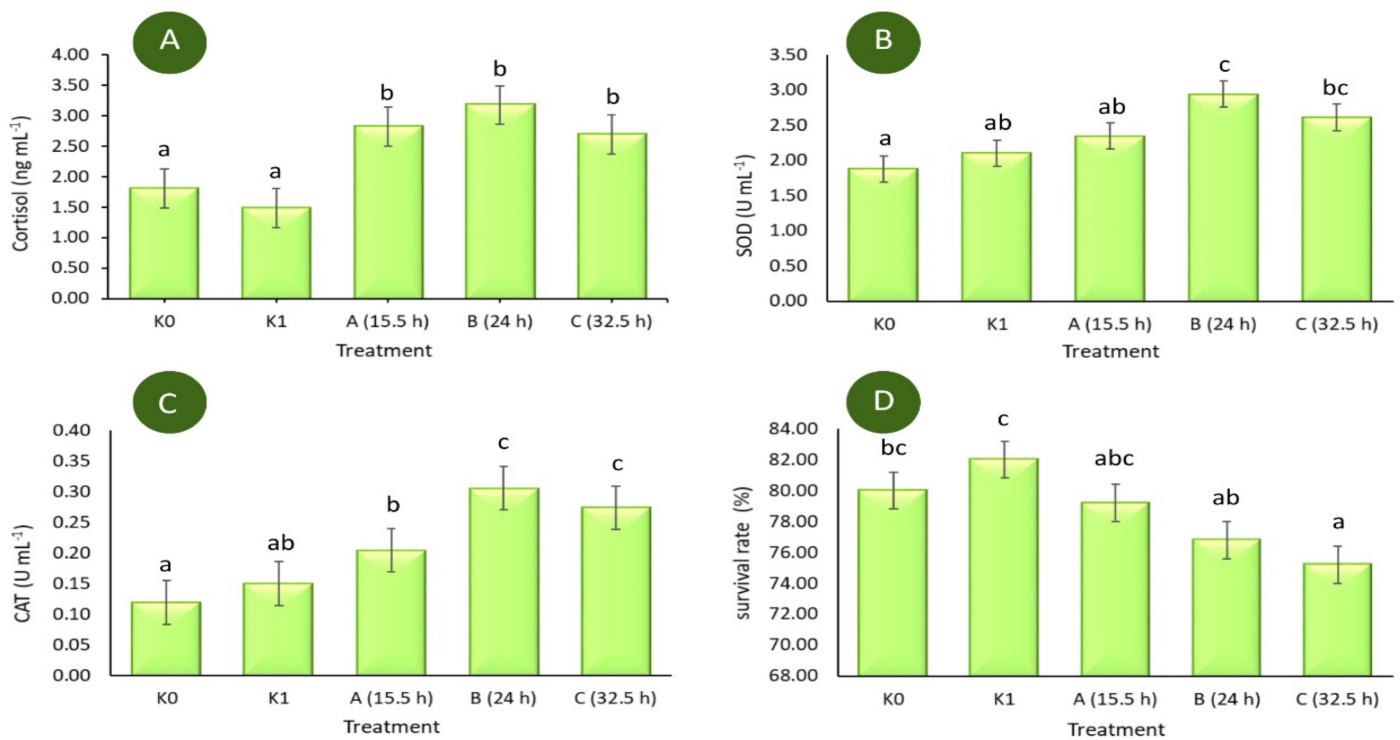


Fig. 1. Effect of BPA immersion durations on physiological stress biomarkers and survival rate of *M. boesemani* (A) cortisol concentration, (B) superoxide dismutase (SOD) activity, (C) catalase (CAT) activity, and (D) survival rate (SR) across treatment groups: K0 (unexposed control), K1 (chemical control), and BPA immersion. Data are presented as mean $\pm$ SD and bars with different superscript letters indicate statistically significant differences among treatments according to Tukey's HSD test ($p < 0.05$)

cortisol ($r = -0.59$, $p < 0.05$) and CAT activity ($r = -0.75$, $p < 0.001$). A negative relationship between SOD activity and SR was also observed

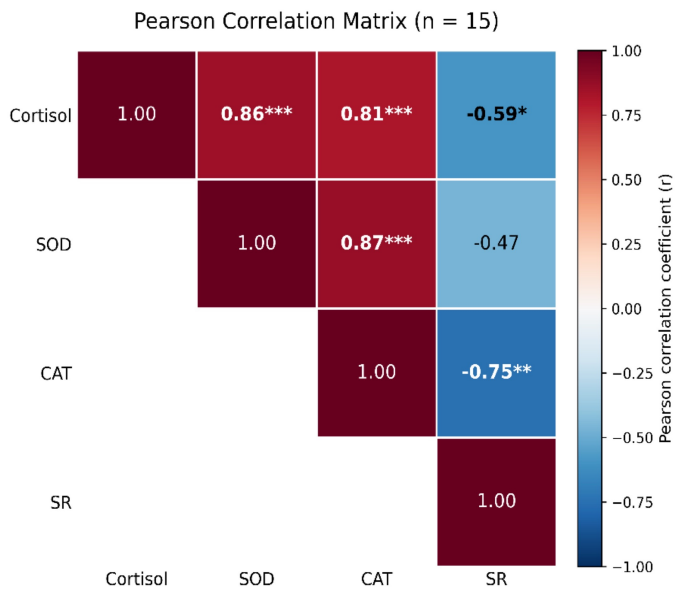


Fig. 2. Pearson correlation matrix among physiological stress biomarker and survival rate of *M. boesemani* larvae. Correlation coefficients (r) are shown for cortisol, superoxide dismutase (SOD), catalase (CAT), and survival rate (SR) based on 15 observations. Red and blue color indicate positive and negative correlations, respectively, with color intensity representing correlation strength. Statistical significance is denoted as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***)

($r = -0.47$) but was not significant ($p > 0.05$). Antioxidant enzyme activities were positively correlated with cortisol levels. Overall, physiological biomarker responses were negatively correlated with survival. The pattern of correlations indicated that higher cortisol was related to higher antioxidant enzyme activities, and higher physiological biomarker responses were related to lower survival. The relationships complement the treatment dependent responses observed for cortisol, SOD, CAT, and SR in the experimental groups.

3.3 Principal Component Analysis of physiological responses

Principal component analysis performed on individual replicates ($n = 15$) reduced the four physiological variables into two components explaining 94.3% of total variance ($PC1 = 79.8\%$, $PC2 = 14.5\%$) (Fig. 3). Loading vectors for cortisol, SOD, and CAT were oriented toward the positive PC1 axis, with SOD and cortisol displaying comparable positive loadings on PC2 while CAT loaded almost exclusively along PC1. SR showed an opposing orientation, loading toward the negative PC1 and positive PC2 quadrant, consistent with the inverse pooled correlation reported between SR and the stress biomarkers (Fig. 2). Along PC1, replicates from the untreated (K0) and synthetic control (K1) groups occupied the negative region and formed overlapping 95% confidence ellipses, indicating similar multivariate biomarker profiles between the two control conditions. Replicates from 15.5 h immersion group were positioned near the PC1 origin with the widest dispersion along PC2, while the 32.5 h and 24 h groups showed the most positive PC1 scores among all treatment groups. This ordering along PC1 was non-monotonic with respect to exposure duration, as the intermediate 24 h duration rather than the longest 32.5 h duration occupied the extreme end of the biomarker dominated axis.

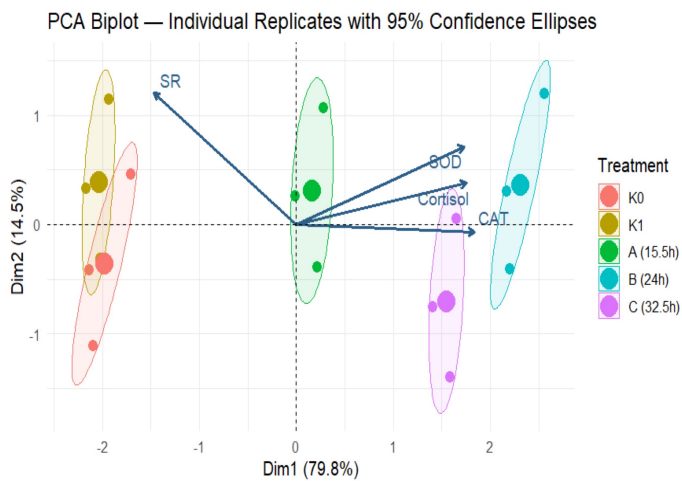


Fig. 3. Principal component analysis (PCA) biplot of individual replicate scores ($n = 15$) for four physiological stress-response variables (cortisol, SOD, CAT, and SR) alongside untreated (K0) and synthetic (K1) control groups. Analysis based on scaled variables; PC1 and PC2 explained 79.8% and 14.5% of variance (94.3% cumulative). Large points denote group centroids; ellipses show 95% confidence regions per group. Arrows indicate variable loading vectors

4. Discussion

Embryos exposed to banana peel ash extract derived potassium (BPA) for different immersion durations showed distinct profiles that were still detectable at 7 days post hatching (dph). Cortisol was significantly higher in all BPA exposed groups than in both the untreated control and the KCl (K1) reference group. Within the BPA treatments, cortisol reached its highest numerical value after 24 h of embryonic exposure and was lower after 32.5 h, although the three BPA groups did not differ statistically from one another. These results should not be interpreted as a cortisol time course during immersion. Instead, elevated cortisol relative to both controls was detectable across all three BPA duration groups at 7 dph, with the highest numerical value at 24 h. However, because the BPA duration groups did not differ significantly from one another, these data do not establish that immersion duration determined a specific endocrine phenotype.

A plausible mechanism involves disturbance of ionic homeostasis during development. Potassium maintains membrane potential, cellular ion gradients and Na^+/K^+ -ATPase dependent transport, and altering environmental ionic composition raises the regulatory burden needed to keep these gradients stable. Cortisol is closely tied to ionoregulation through the hypothalamic-pituitary-interrenal (HPI) in teleost. Cortisol and HPI axis components change markedly during seawater acclimation in Atlantic Salmon (Culbert et al. 2022), Atlantic sturgeon and sterlet (Shaughnessy et al. 2023), supporting a general role for glucocorticoid signalling in adjusting to ionic challenge. The elevated cortisol seen at 7 dph in BPA – exposed *M. boesemani* fits this pattern of carry-over endocrine regulation following embryonic ionic disturbance, though HPI-axis activity during the immersion period itself was not measured here.

The lower cortisol value after the longest BPA exposure needs cautious interpretation. It does not show that cortisol rose through 24 h and then fell during exposure, since all measurements were taken at the same post-hatch age, and a lower value does not necessarily mean full

recovery. Cortisol is regulated through glucocorticoid receptor mediated negative feedback adjustment (Castillo-Ramírez et al. 2024). The lower cortisol in larvae from the 32.5 h treatment could therefore reflect altered feedback regulation, an acclimatory process, or simply a different post-exposure trajectory. Confirming HPI-axis exhaustion or impairment would require serial cortisol sampling alongside CRE, ACTH, steroidogenic enzyme, or glucocorticoid receptor data, none of which were collected in the present study. The co-occurrence of the lowest cortisol and the lowest survival in the 32.5 h treatment is a notable pattern, but this single co-occurrence does not by itself establish an inverse relationship between cortisol and lower survival. The pooled correlation across treatments ($r = -0.59$) indicates a moderate association at best, and correlation across grouped treatment means cannot distinguish a causal link from both variables independently responding to exposure duration. Cortisol magnitude alone is unlikely to adequately represent physiological impairment because it is a single time-point measurement of an organism level endpoint that is itself cumulative. Survival at 60 dph integrates all physiological costs accumulation from the embryonic period onward, whereas cortisol, SOD, and CAT were measured once at 7 dph. A biomarker profile that appears more subdued at 7 dph does not rule out physiological costs that manifest later, nor does an elevated biomarker profile necessarily predict a proportional cost to survival. Consistent with this recent work in zebrafish found that stress-related metabolic and biochemical changes can occur without a concomitant rise in cortisol, supporting the interpretation of endocrine, biochemical and organism level endpoints jointly rather than inferring impairment from any single measure (Vieira et al. 2025).

This antioxidant profile post hatch was broadly in agreement with the endocrine profile. In BPA treated larvae SOD and CAT activity increased. Both showed peak activity in the 24 h group and declined considerably by 32.5 h. Because SOD and CAT activities were assessed after 7 dph, they indicate antioxidant status after exposure and not during the embryonic immersion itself. SOD and CAT act as related components of cellular antioxidant defence: SOD transforms superoxide radicals to hydrogen peroxide, while CAT helps eliminate that peroxide. Ionic or osmotic disturbance is known to reshape antioxidant defences in fish and broadly, salinity and temperature manipulation in turbot significantly altered SOD, CAT, and GPx activities (Wang et al. 2022). The exposure of crucian carp to saline alkaline stress has been linked to shifts in SOD, CAT, and broader metabolic regulation (Han et al. 2025). Here the correlation pattern also supports coordinated regulation: cortisol was substantially and positively associated with both SOD and CAT, and the extent of correlation was greatest between SOD and CAT. That tight SOD-CAT relationship makes physiological sense, since the peroxide generated by SOD becomes the substrate for CAT and removes it downstream, which is consistent with reports that SOD and CAT activation tends to regulate ROS status under stress in fish (Dagoudo et al. 2023; de Fátima Pereira de Faria et al. 2025).

However, the positive correlations between cortisol, SOD, and CAT should not be interpreted as evidence that cortisol directly caused oxidative stress. These correlations were based on only 15 observations across several treatments and so treatment level differentiation is likely to contribute substantially to the observed pattern. A more accurate characterization is that the endocrine and antioxidant responses changed in concert as part of a single integrated phenotype, rather than

a simple causal link between cortisol and reactive oxygen species. The decrease in SOD and CAT at 32.5 h cannot be taken as a depletion of the antioxidant without further support. In the present study, ROS, hydrogen peroxide, lipid peroxidation, protein oxidation, GPx, and glutathione redox balance were not evaluated. This is of importance as a full evaluation of fish stress usually comprises SOD/CAT in addition to direct markers of oxidative damage. Simultaneously measured reactive oxygen species, antioxidant enzymes, lipid peroxidation, protein carbonylation, glutathione levels, and metabolic indicators in zebrafish larvae and concluded that oxidative damage cannot be determined by enzyme activity alone (Vieira et al. 2025). The SOD/CAT remained relatively constant throughout the 24 and 32.5 h treatments (CAT/SOD = 0.105 and 0.103, respectively) suggesting that there is no selective CAT impairment or peroxide accumulation from insufficient CAT activity. The most tenable interpretation is that BPA immersion duration altered the intensity of a synchronized post hatch antioxidant response rather than its internal equilibrium.

In this study, physiological biomarkers and survival followed divergent trajectories. Cortisol, SOD, and CAT all peaked at 24 h of BPA exposure and declined at 32.5 h, while survival declined progressively across the same duration and was lowest at 32.5 h treatment. The BPA groups did not differ significantly in survival, yet the coincidence of the lowest survival with a post peak drop in biomarker activity points to a broader pattern: the size of a biomarker response does not automatically track the eventual biological outcome. One plausible explanation for the cortisol increase at 24 h is activation of the hypothalamic-pituitary-interrenal (HPI) axis in response to physiological disturbance, which would be expected to mobilize energy to sustain homeostasis. However, HPI axis activity was not directly measured in this study, this remains an inferred rather than a demonstrated mechanism. It would not be expected to persist under continued stress if HPI axis activation did occur. In zebrafish larvae, cortisol rises quickly after a stressor and then falls as glucocorticoid receptor-mediated negative feedback dampens the HPI Axis (Castillo-Ramírez et al. 2024). A comparable disconnect between cortisol level and physiological state has been reported in Atlantic salmon under chronic stress where found lower cortisol in chronically stressed fish despite impaired growth and cardiac changes (Opinion et al. 2023). This offer one plausible explanation for the lower mean cortisol at 32.5 h in this study.

The SOD and CAT trajectories are compatible with a compensatory antioxidant response and not direct evidence of damage. SOD converts superoxide radicals to hydrogen peroxide which is further broken down by CAT, thereby limiting oxidative harm. Similar changes in SOD and CAT activity and increased ROS were observed in glyphosate exposed zebrafish larvae (Liu et al. 2022), and oxidative stress preconditioning in zebrafish larvae activates antioxidant and detoxification pathways like Keap1/Nrf2 and MAPK linked responses, which confer tolerance to later oxidative stress (Xu et al. 2022). The comparatively high SOD and CAT activity at 24 h in the present data are in agreement with this picture of active defence and not of harm already in progress. Less obvious is why they fall off after 32.5 h. Alternatively, the antioxidant response may have reached a peak during the intermediate duration and then declined with extended immersion. With continual stress ROS generation could at some point exceed or limit enzymatic defence capabilities such that oxidative damage accumulates although enzyme activity itself no longer

increases. The SOD and CAT data alone cannot validate this process, although it is physiologically plausible, given ROS levels, lipid peroxidation, glutathione status and direct oxidative damage were not examined in this study.

This trend also does not generalize across species or stressor. By contrast, in the present observation, cortisol, SOD, and CAT did not decrease following acute stress in hybrid yellow catfish but remained elevated (Dagoudo et al. 2023). This difference suggests that biomarker kinetics vary strongly with the type of stressor, the intensity and the duration of exposure, the developmental stage, the species, and the sampling time. Distinct developmental consequences and oxidative stress in arsenate exposed zebrafish embryo even at doses that did not substantially impair survival (Vieira et al. 2025). Together these studies suggest that biochemical biomarkers may precede, be independent of, or be opposite to survival outcomes.

Survival data of *M. boesemani* offer another depth to this hypothesis. Survival is reduced and developmental abnormalities is increased in zebrafish exposed to repeated stress during early development. Thus, stress accumulated at sensitive developmental stages may result in consequences that are not fully detected by a single biochemical marker (Valcarce et al. 2024). Since BPA concentration was held constant at 13.5 mg L⁻¹ while only duration varied, the lower survival at the longest exposure suggests immersion duration itself may contribute to the cumulative biological effect even without a change in nominal potassium concentration. This is consistent with a related toxicity assay in the same species, in which immersion duration rather than potassium concentration or source was identified as the dominant predictor of embryonic mortality risk (Hismayasari et al. 2026). However, because survival did not differ significantly across all BPA immersion durations in the present study, this remains duration-related trend rather than a statistically confirmed concentration-time relationship. Considered jointly, biochemical stress responses and survival outcomes appear to sit at different levels of biological organization. Strong biomarker activation at 24 h may mark an active compensatory phase, the weaker responses at 32.5 h, paired with the lowest survival, does not indicate that recovery has occurred.

The principal component analysis provides an integrative view of the four physiological endpoints that complements, but does not simply restate, the univariate and correlation analysis. The concentration of variance in PC1 indicates that cortisol, SOD, and CAT are not behaving as physiologically independent endpoints but as co-varying outputs of a shared upstream process, consistent with the established sequence in which glucocorticoid signalling upregulates antioxidant enzyme activity as a secondary response to oxidative load generated during the primary stress response (Yuan et al. 2025). This pattern is consistent with a mechanistic interpretation, whereby the increase in SOD and CAT in *M. boesemani* larvae following embryonic exposure reflects a downstream consequence of HPI axis activation rather than a distinct toxicological endpoint in its own right. Testing this interpretation directly would require concurrent measurement of HPI axis components alongside antioxidant enzyme activity, which was beyond the scope of the present study. The association of cortisol with SOD and CAT fits with a concerted endocrine and antioxidant response to BPA exposure: stimulation of the stress axis increases energy demand and can alter redox balance in cells while antioxidant enzymes counteract the consequent oxidative burden. This does not indicate higher harm with increased biomarkers in fish can respond in diverse

directions depending on stress severity and time, which enables the interpretation of these biomarkers in a collaborative manner rather than in isolation (Ullah et al. 2026). Multivariate structure also differentiates moderate and prolonged BPA exposure. The significant coordinated biomarker response at the intermediate duration is consistent with active physiological compensation. This shift at the longest duration is attributable to a change in that coordination rather than an increase in all biomarkers. A similar pattern was reported in *Oreochromis niloticus* exposed to a prolonged heatwave (Nathaniel et al. 2026), where cortisol and antioxidant enzymes changed in different directions as the exposure continued. This reinforces the notion that longer exposure alters the stress response, rather than simply enhancing it.

A likely mechanism is down regulation of cortisol responses after prolonged or repetitive stimulation. Glucocorticoid receptor mediated feedback reduces cortisol following stress activation and modulates sensitivity to subsequent stressors in zebrafish larvae (Castillo-Ramírez et al. 2024). Similarly, chronically stressed Atlantic salmon had lower cortisol despite impaired growth, showing that reduced cortisol does not necessarily indicate recovery (Opinion et al. 2023). The negative correlation between survival and the molecular indicators supports this perspective. Survival is a cumulative, organism level endpoint, while cortisol, SOD, and CAT capture the physiological state at a single development stage. Significant biochemical activation may therefore reflect an active attempt to maintain homeostasis without preventing downstream costs, and reduced activation after prolonged should not be read as evidence of recovery on its own (Raposo de Magalhães et al. 2022). These results point to a duration-dependent shift in physiological state rather than a linear intensification of stress with longer BPA exposure. Endocrine activation, antioxidant defence, and survival are related to one another, but they do not change in the same way or to the same degree, a pattern that emerges only when the multivariate analysis is considered alongside the univariate and correlation result.

Exposure duration emerged as a key determinant of the physiological response during the early life stage *M. boesemani*. At a fixed potassium concentration, longer BPA exposure shifted the balance between endocrine activation, antioxidant defence, and survival; indicating the biological effect depends not only on medium concentration but also on immersion duration, a period during which regulatory system in developing fish are especially vulnerable to disturbance (Valcarce et al. 2024). This duration effect also acts as a caveat to assuming that biochemical activity is either fundamentally protective or harmful. An increase of antioxidant activity can represent an active response to oxidative stress, but a decrease of this activity after extended exposure does not always mean recovery. A similar temporal dissociation was seen in marine medaka, with an initial SOD/CAT stimulation followed by decreased antioxidant capacity and negative developmental effects (Li et al. 2025), and in *Astronotus ocellatus*, where poor recovery between stress events affected cortisol and antioxidant responses and compromised physiological condition (Zare et al. 2023). The present results suggest that various physiological endpoints need to be assessed instead of a single marker in addition to survival. These results are also relevant to banana peel valorization as a potassium source: thermally processed banana peel biomass yields potassium-rich fractions such as K_2O and K_2CO_3 (Bai et al. 2025), making BPA chemically plausible as a recoverable mineral resource (Putra et al. 2022). However, the present data should not be read as evidence that BPA-derived potassium is ready for aquaculture use.

Unlike reagent grade KCl, ash-derived extracts are a compositionally variable matrix shaped by raw material, combustion temperature, extraction method, and post-processing variability that produced measurable physiological effects here even at a fixed potassium concentration.

5. Conclusions

Exposure to banana peel ash derived potassium produced a duration-dependent physiological response in the early life stage of *M. boesemani*. Cortisol, SOD, and CAT activity peaked at 24 h and declined at 32.5 h, whereas a numerical declining trend was observed in survival with increasing immersion duration, reaching its lowest value at the longest exposure. This divergence indicates that biomarker activation followed a non-monotonic trajectory, whereas survival was not governed by a simple linear concentration-duration relationship. Instead, the pattern suggests a change from active physiological compensation at intermediate exposure to altered biomarker reactivity at the longest immersion duration, despite continuous decline in survival. As the potassium content was fixed, the differences observed in responses may be attributed to the immersion durations. These findings support evaluating biomass-derived potassium sources using integrated physiological and organism-level endpoints, and indicate that safe use of BPA requires optimizing exposure duration together with concentration rather than assuming safety from potassium content alone.

Declarations

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