

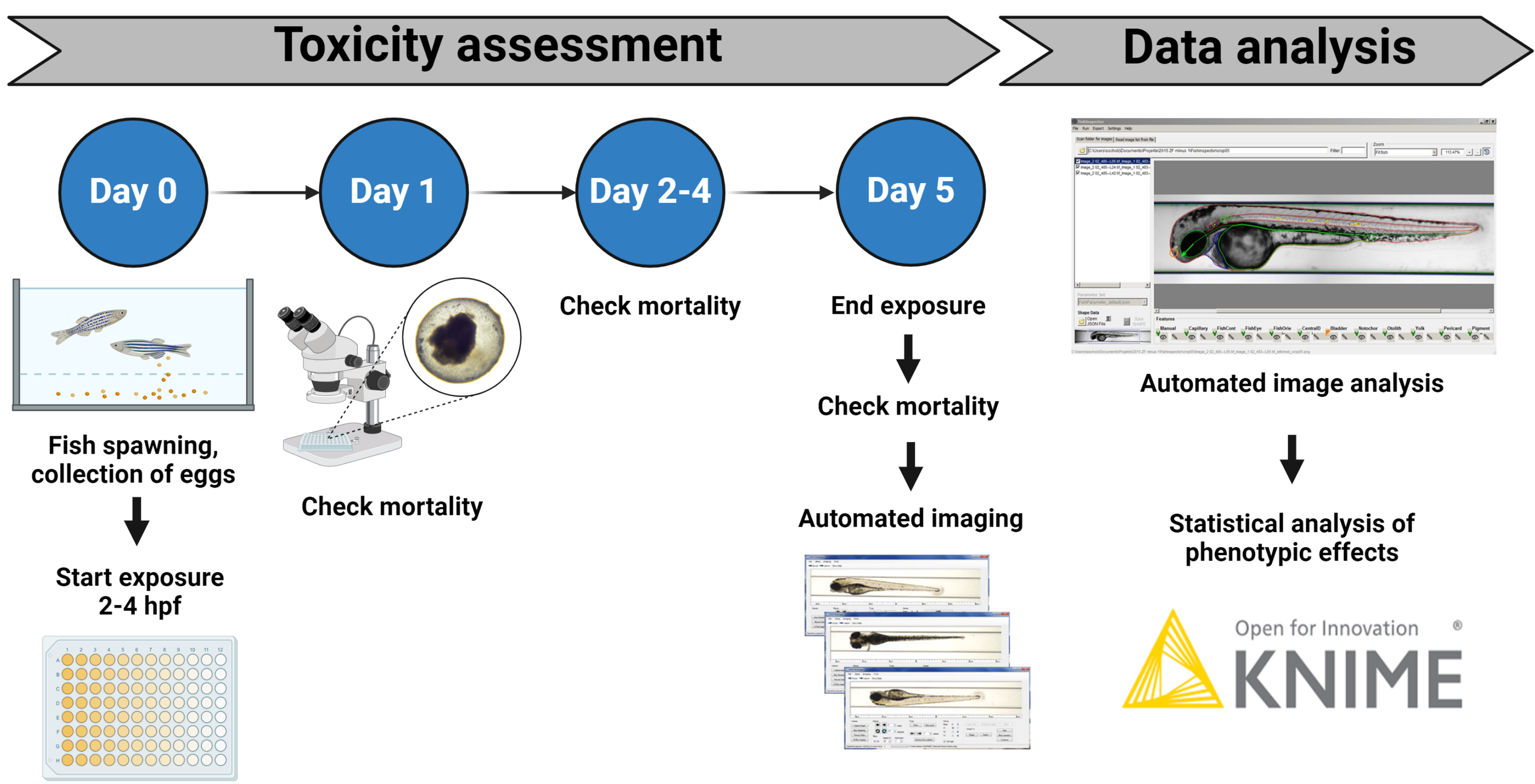
INTRODUCTION

OBJECTIVES

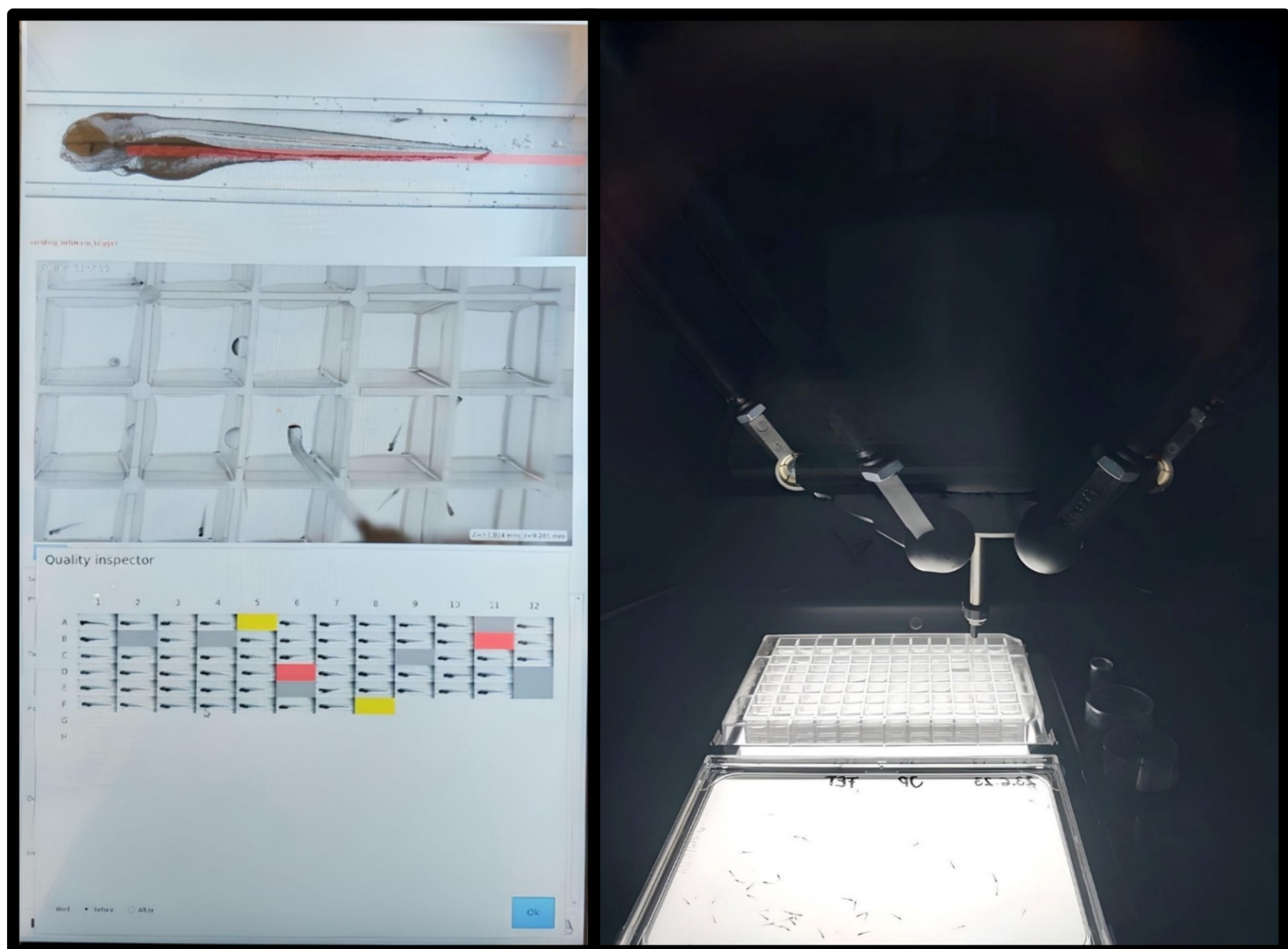
Extracts of six *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera et al. 2018 strains were evaluated for their potential lethal and sublethal effects on zebrafish embryos. The strains included two toxic (CS-505 and CS-506) and four nontoxic (UAM/DH-BiRr, UAM/DH-PnRr, UAM/DH-KmRr and KLL07). Zebrafish embryos were exposed from 2 hours post-fertilization (hpf) to 120 hpf at concentrations of 200, 100, 50, 25, and 12.5 mg/L diluted in E3 medium. Mortality rates were recorded at the end of the exposure period, and surviving embryos were phenotyped using a novel automated imaging-based method for quantitative assessment of morphological changes. The phenotyping method employed images analyzed via FishInspector (Hemholtz Environmental Research Center, Leipzig, Germany), a software leveraging deep-learning models to detect and annotate morphological features.

- Evaluate the lethal and sublethal effects of methanolic extracts from six *Raphidiopsis raciborskii* strains on zebrafish embryos.
- Determine mortality rates (LC₅₀) and sensitivity ratios for phenotypic endpoints.
- Identify key developmental abnormalities induced by cyanobacterial metabolites.

METHODS



Automated Imaging Robot
by Life Science Methods

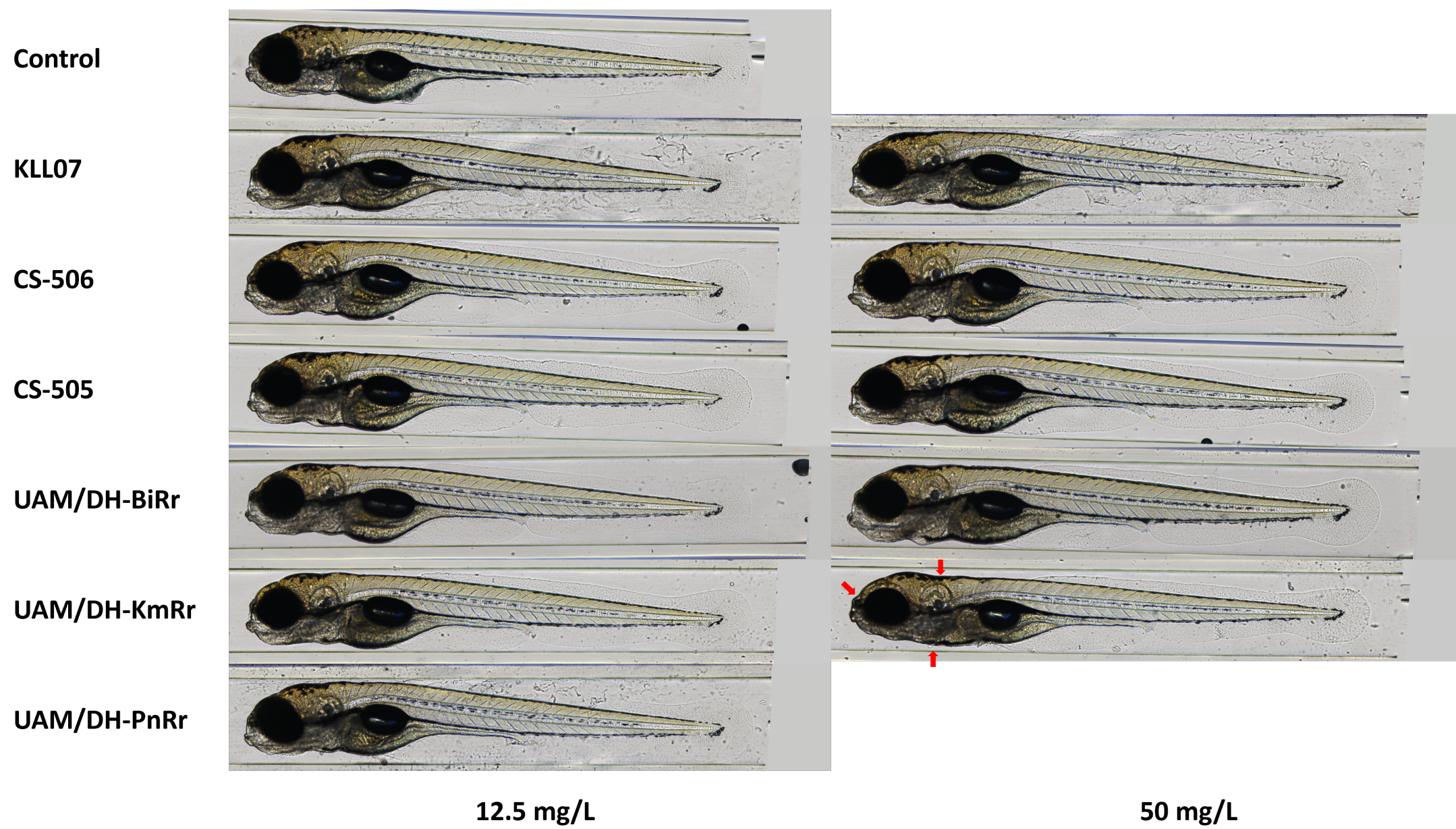
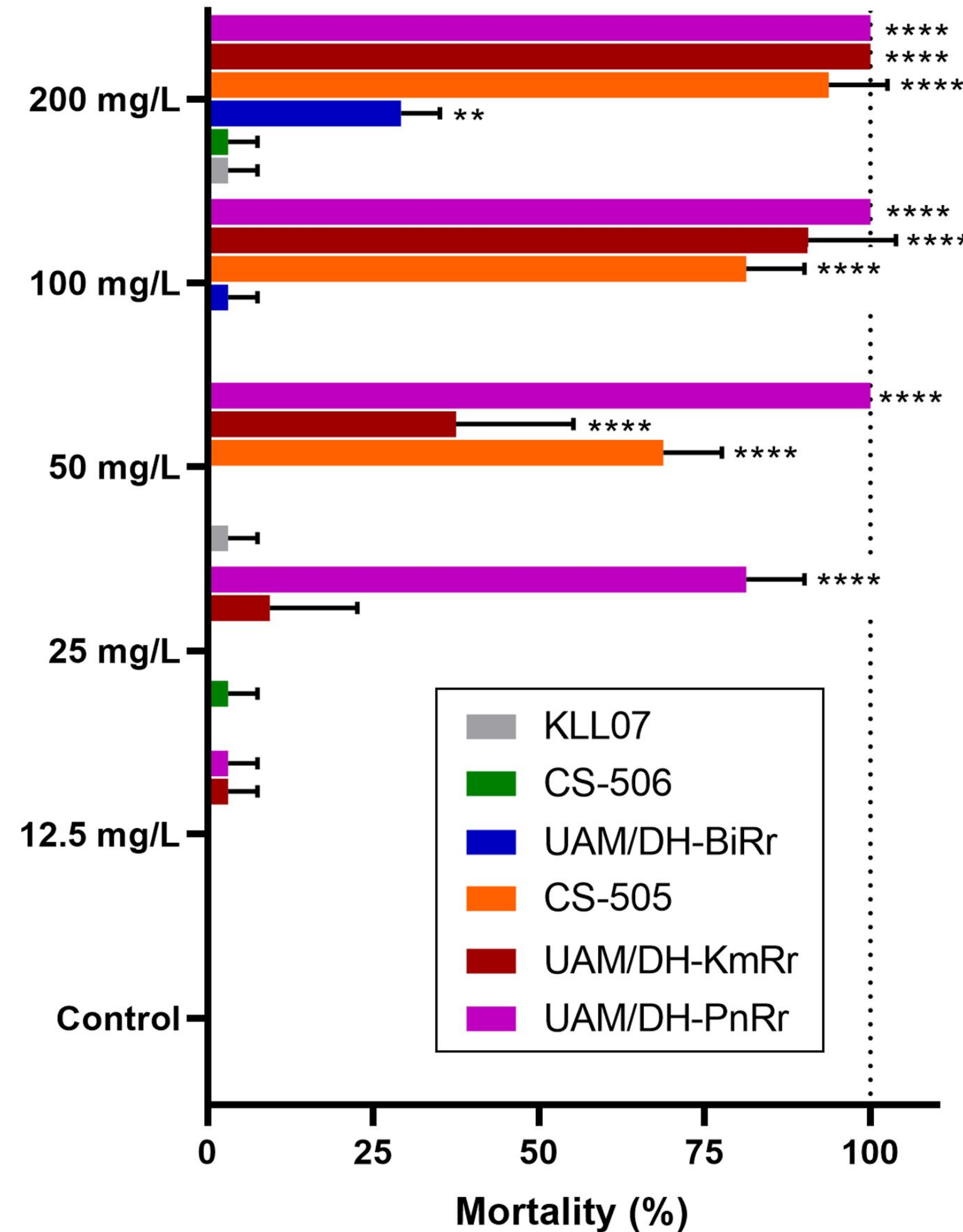


RESULTS

CONCLUSION

From the mortality rates of zebrafish embryos, 120 h LC₅₀ values were calculated for the three strains (UAM/DH-PnRr, UAM/DH-KmRr, and CS-505), that led to high mortalities, which were determined to be 20.3 mg/L, 42.2 mg/L, and 63.2 mg/L, respectively. The strain UAM/DH-BiRr caused significant mortality only at the highest test concentration. The KLL07 and CS-506 strains caused no notable mortality on any of the tested concentrations in the applied zebrafish embryo model.

Mortalities of zebrafish embryos after 120 h exposure :



The CS-505, CS-506, KLL07, and UAM/DH-BiRr strains caused no quantifiable phenotypic effects on concentrations where embryos survived. In the case of the UAM/DH-PnRr strain, embryos survived only at 12.5 mg/L, with no calculable phenotypic effects. The UAM/DH-KmRr strain caused significant sublethal effects at 50 mg/L concentration. EC₅₀ values were calculated, as well as sensitivity ratios for each affected endpoints:
SR = EC₅₀[endpoint] / LC₅₀[experimental]

Effected organ	Phenotypic endpoint	EC50 (mg/L)	SR
Yolk sac	Yolk size reduction	88,1	2,09
Notochord	Notochord curvature	103,3	2,45
Embryonic head	Mouth increased angle to eye posterior otolith	65,0	1,54
	Eye decreased distance to mandibular arch	74,5	1,77

The most sensitive phenotypic endpoints affected the embryonic head, while the less sensitive endpoints affected the yolk sac and the notochord. The head phenotypes being more sensitive means the toxin likely targets early neural crest or cranial developmental processes, while effects on yolk and notochord require higher concentrations, suggesting those are secondary or systemic effects at elevated exposures.

The *Raphidiopsis raciborskii* strains UAM/DH-PnRr, UAM/DH-KmRr, and CS-505 proved to be slightly toxic in the acute zebrafish embryo toxicity assay, while the UAM/DH-BiRr, KLL07, and CS-506 strains were practically nontoxic in this model system. Craniofacial malformations are highly sensitive developmental endpoints, implying the chemical is interfering with early neural crest cell migration or differentiation, since craniofacial structures (jaws, eyes, otic vesicles) are largely derived from neural crest cells. This points toward disruption of signaling pathways crucial for craniofacial morphogenesis, such as Wnt signaling, Hedgehog signaling (especially Sonic hedgehog, Shh), FGF (Fibroblast growth factor) signaling, or BMP (Bone morphogenetic protein) signaling. Yolk size reduction indicates altered nutrient absorption/metabolism or yolk utilization, possibly affecting energy balance or lipid metabolism. Notochord curvature suggests disruption of axial development, which could also involve the Shh signalling pathway. Overall, certain strains exhibited slight developmental toxicity in zebrafish embryos, likely through disruption of neural crest cell migration and key morphogenetic signaling pathways involved in craniofacial, metabolic, and axial development.

ACKNOWLEDGEMENTS

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CYAN META

