Impairment of sensory organ development in petroleum-exposed zebrafish embryos – response of the lateral line system

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Background & Motivation

- **Increasing demand for crude oil** → huge and small diffuse **oil spills** pose a severe risk to the aquatic environment [1]
- **Dispersants:** oil spill response measure → formation of oil droplets → increased bioavailability
- Crude oil exposure → strong effects on the cardiovascular [2] and visual system [3] → **other sensory organs may be affected**
- **Lateral line system (LLS):** emerging endpoint in ecotoxicology & involvement in a multitude of behavioral traits [4]

- **Aim:** investigation of oil-induced impairments on the LLS as a sensitive endpoint in neuro-toxicological assessment

Materials

Water-accomodated fractions (WAF):

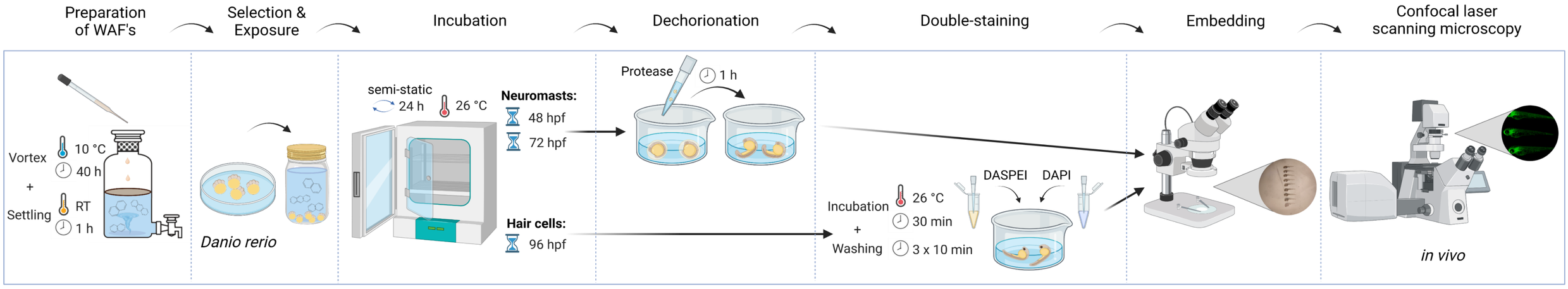
LEWAF
(low-energy WAF): Naphthenic North Sea (NNS) crude oil Stock 1:50 (w/v)

CEWAF
(chemically-enhanced WAF): Finasol® OSR 51/ NNS crude oil Stock 1:200 (w/v)

HEWAF
(high-energy WAF): Finasol® OSR 51 (dispersant) Stock 1:2000 (w/v)

(*Danio rerio* exposure concentrations ≤ EC_5)

Experimental Set-Up



Neuromasts

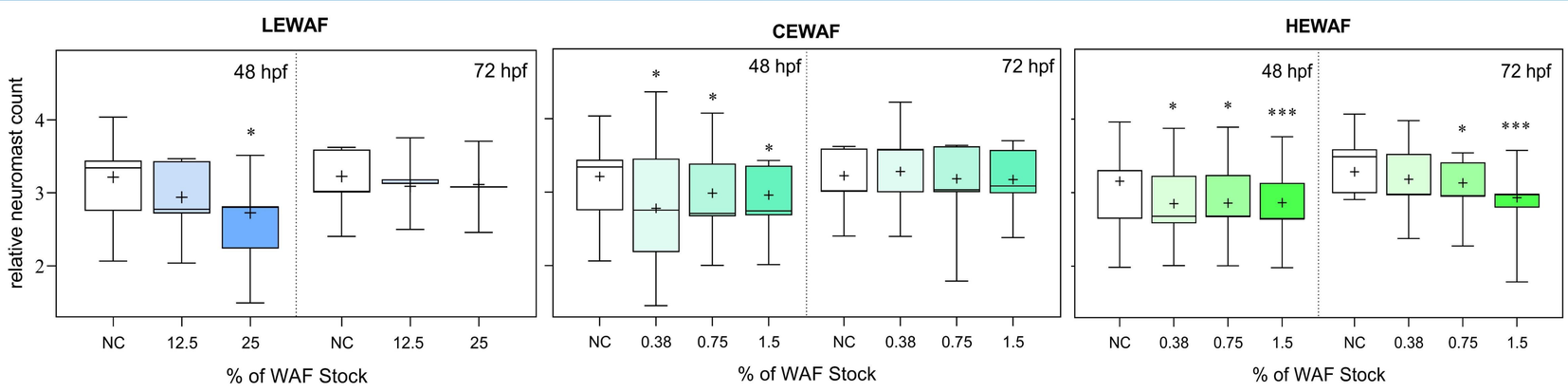


Fig. 1 Relative neuromast count of 48 and 72 hpf old *D. rerio (cldnb:lyn-eGFP)* embryos exposed to LEWAF, CEWAF or HEWAF. Neuromast count normalized against body size of corresponding treatments. Individual values (n = 24 - 49), mean (+), median (line) and SD (error bars). hpf = hours post fertilization.

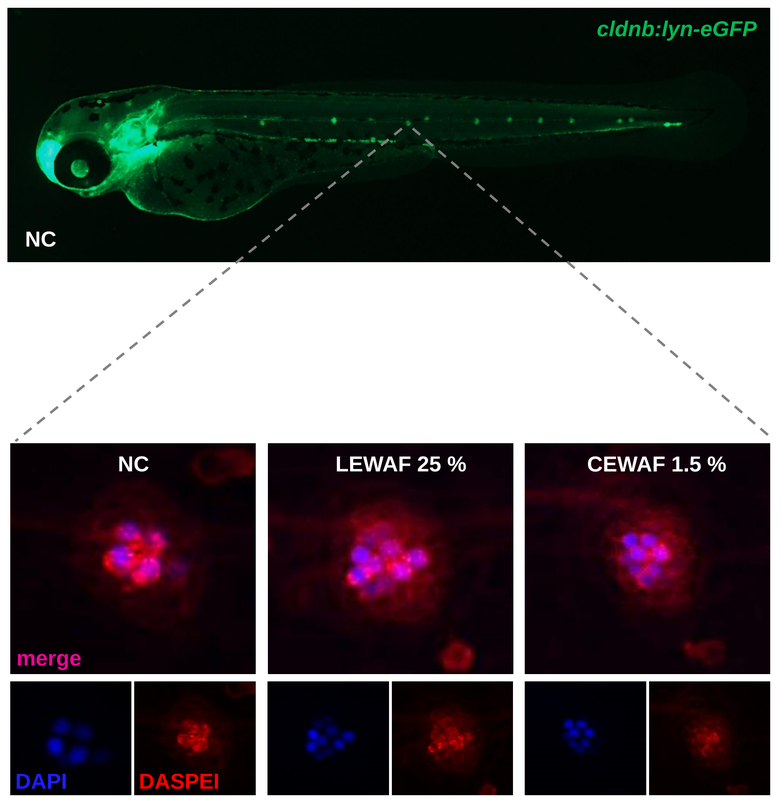


Fig. 3 Lateral line primordium and neuromast cells in *cldnb:lyn-eGFP* transgenic zebrafish (72 hpf; top) and DASPEI (mitochondria) and DAPI (nuclei) stained hair cells (96 hpf; bottom).

Hair Cells

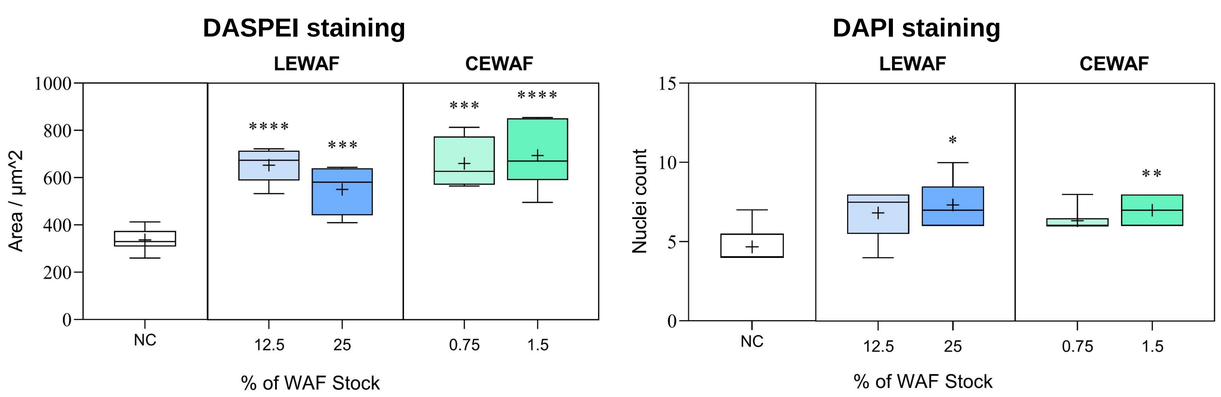


Fig. 2 DASPEI (mitochondria) and DAPI (nuclei) staining of 96 hpf old *D. rerio (cldnb:lyn-eGFP)* embryos exposed to LEWAF or CEWAF. Individual values (n = 6), mean (+), median (line) and SD (error bars).

Conclusion & Next Steps

Effects on the lateral line system of zebrafish embryos

- Reduced number of neuromasts after LEWAF/CEWAF (48 hpf) and HEWAF (48 & 72 hpf) exposure
- Increase in hair cell mitochondria area and hair cell nuclei
- Next step: completing hair cell examinations

→ **Contribution to a better understanding of specific sensory system impairments**

Future work: understanding crude oil impact on embryonic motor behavior

- Background: effects on spontanous tail coiling after LEWAF/HEWAF exposure (Fig. 4) → indicator for developmental neurotoxicity
- Next step: assessment of primary (Fig. 5) and secondary motorneuron development
- **Aim: deeper insight into xenobiotic-induced effects on neurodevelopment**

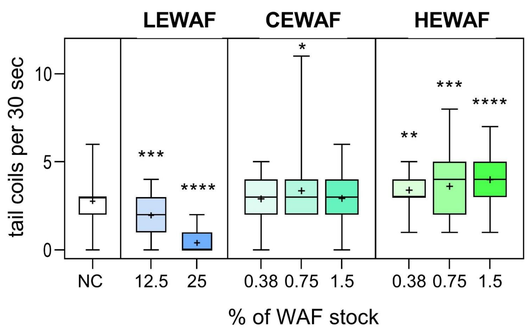


Fig. 4 Spontaneous tail coiling in 24 hpf old zebrafish embryos exposed to LEWAF, CEWAF or HEWAF. Individual values (n = 53 - 113), mean (+), median (line) and SD (error bars).

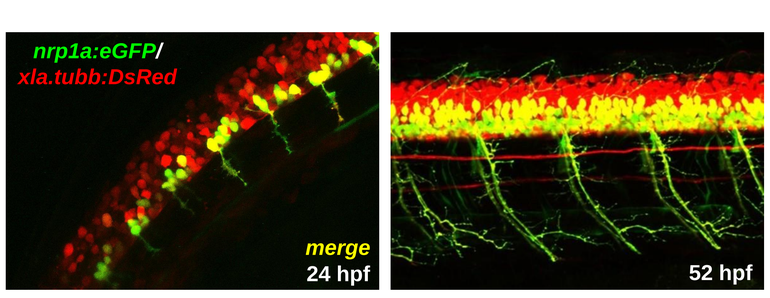


Fig. 5 Neuropilin 1a (*nrp1a*) expression highlights caudal primary motorneurons (green) in a pan-neuronal reporter (*xla.tubb*) background (red) in 24 and 52 hpf old *nrp1a:eGFP/xla.tubb:DsRed* double transgenic zebrafish embryos. Images of untreated *D. rerio* embryos.

[1] Fritt-Rasmussen, Janne, et al. "Heavy Fuel Oil (HFO): A review of fate and behaviour of HFO spills in cold seawater, including biodegradation, environmental effects and oil spill response." (2018). | [2] Pasparakis, Christina, et al. "Physiological impacts of Deepwater Horizon oil on fish." *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 224 (2019): 108558.| [3] Magnuson, Jason T., et al. "Effects of Deepwater Horizon crude oil on ocular development in two estuarine fish species, red drum (Sciaenops ocellatus) and sheepshead minnow (Cyprinodon variegatus)." *Ecotoxicology and environmental safety* 166 (2018): 186-191.| [4] Stengel, Daniel, Florian Zindler, and Thomas Braunbeck. "An optimized method to assess ototoxic effects in the lateral line of zebrafish (Danio rerio) embryos." *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 193 (2017): 18-29.

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Graphics made with BioRender.