Effects of Ammonia and Methanol spills on zooplankton in East Baltic and Bothnian Bay

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Introduction

The shipping industry is adapting to carbon-neutral technologies, including alternative fuels, like ammonia (**NH_3**) and methanol (**MeOH**). However, in case of spills, both ammonia and methanol can have **adverse effects** on **marine food webs** and particularly on **zooplankton**. Ammonia disrupts the osmoregulation of cells and promotes the synthesis of Reactive Oxygen Species (ROS), resulting in **mortality** and reduction of physiological rates, including **growth** and **feeding** (Han et al. 2022; Zhang et al. 2023). Methanol is considered less toxic, but still has diverse effects, such as on mortality (Kaviraj et al. 2004). Freshwater species might have a higher tolerance than marine species (Di Marzio et al. 2009), and even intra-species variation in ammonia tolerance has been demonstrated (Buttino 1994; Jespen 2015). However, the potential impacts of ammonia and methanol on aquatic food webs are poorly understood and quantified, especially under changing environmental conditions.

In this research we aimed at quantifying the responses of plankton organisms to ammonia and methanol pollution in the Gulf of Finland and Gulf of Bothnia, where the environment shows great variation in its hydrography and productivity.

Materials and methods

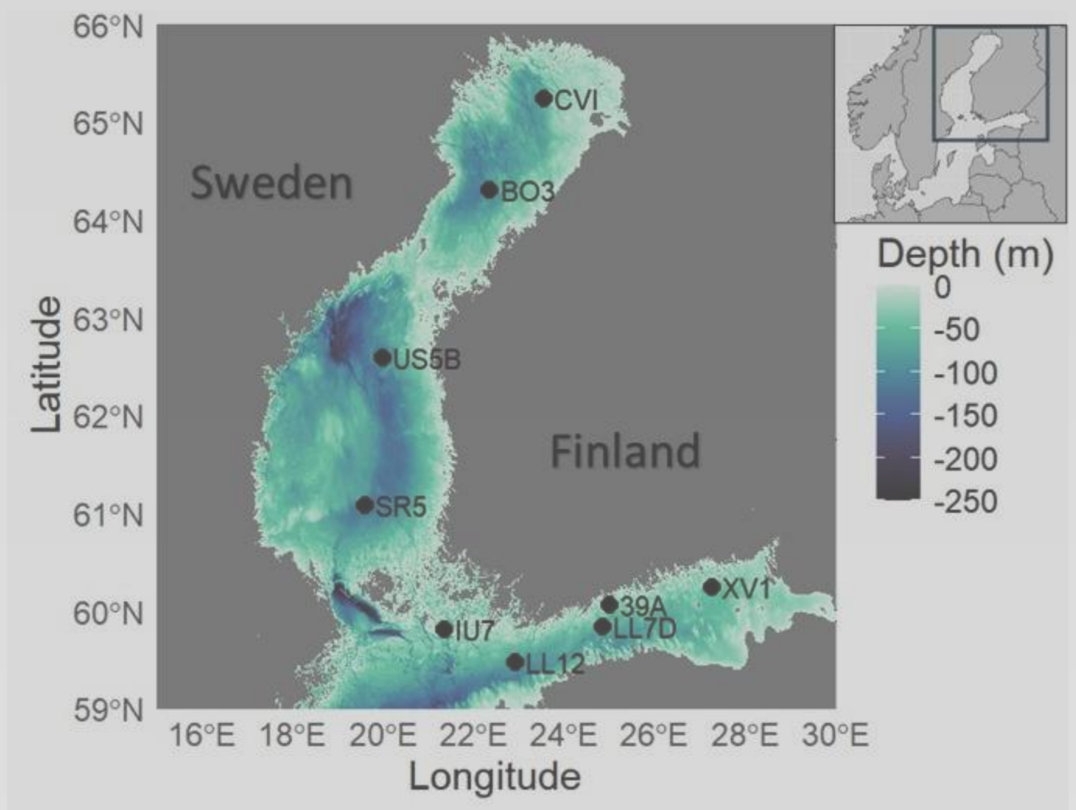
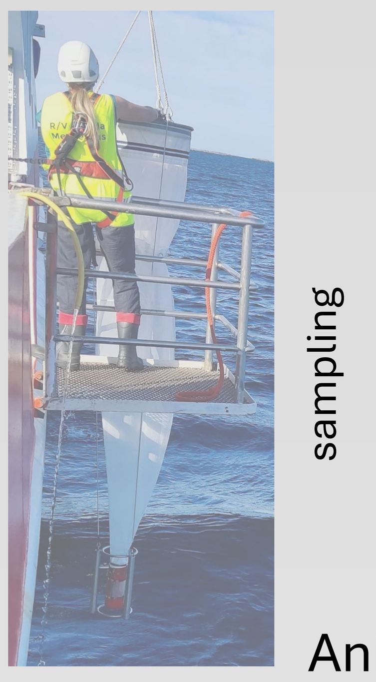
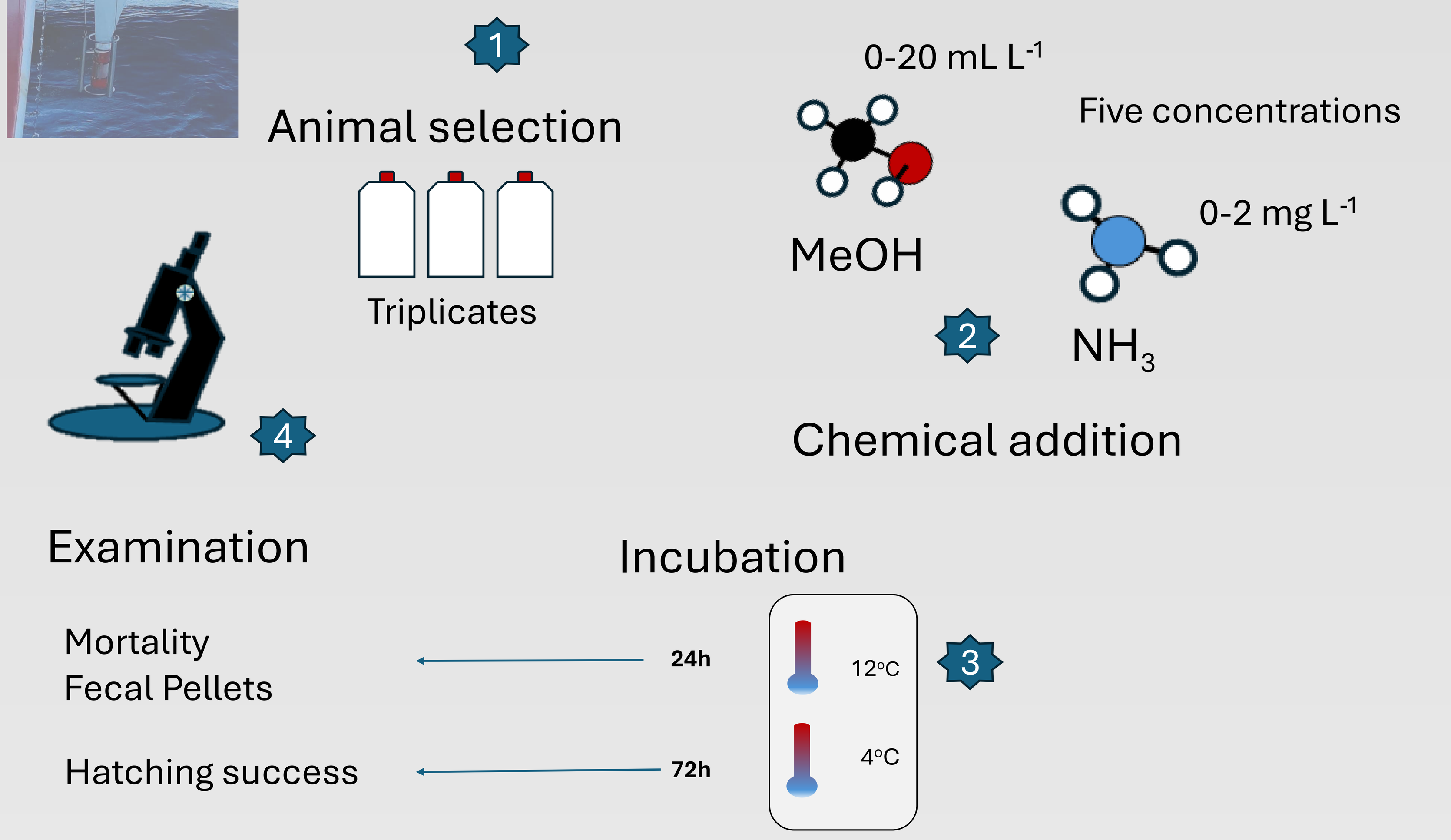


Figure 1. Sampling locations during the expedition. *Eurytemora affinis* 39A & SR5, *Thermocyclops sp.* XV1, *Temora longicornis* LL7D & IU7, *Acartia bifilosa* LL12 *Bosmina longispina* IU7 *Limnocalanus macrurus* US5B & CVI, *Daphnia cucullata* CVI





Results

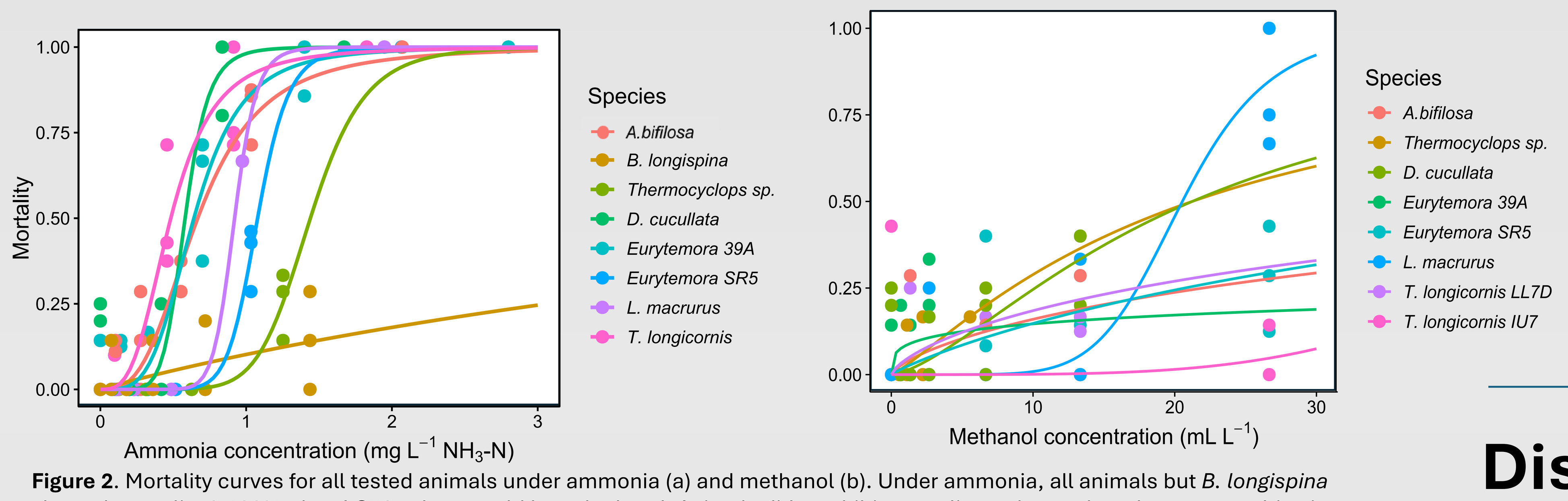


Figure 2. Mortality curves for all tested animals under ammonia (a) and methanol (b). Under ammonia, all animals but *B. longispina* showed mortality 0-100%, thus LC50 values could be calculated. Animals didnt exhibit mortality under methanol stress, resulting in the calculation of LC50 only for *L. macrurus*

Table 1. Average length (prosome for copepods) in um, number of tested animals (N), carbon content (ug ind-1), and LC50 values at 24h (mg L-1 and mL L-1 for ammonia (red) and methanol (blue), respectively)

Species	Mean Size (um)	N	Carbon Content (ug)	LC50 24h	LC50-SD
E. affinis 39A	621	147	1.70	0.64	4.3e-02
E. affinis SR5	669	226	2.18	1.08	6.2e-02
T. longicornis	635	207	2.89	0.48	3.3e-02
A. bifilosa	702	216	1. 50	0.67	4.0e-02
Thermocyclops sp.	431	125	1.13	1.44	3.7e-01
L. macrurus	1886	108	9.05	0.92	7.4e-04
D. cucullata	787	158	1.92	0.58	5.7e-02
L. Macrurus	1769	111	8.24	20.54	1.04

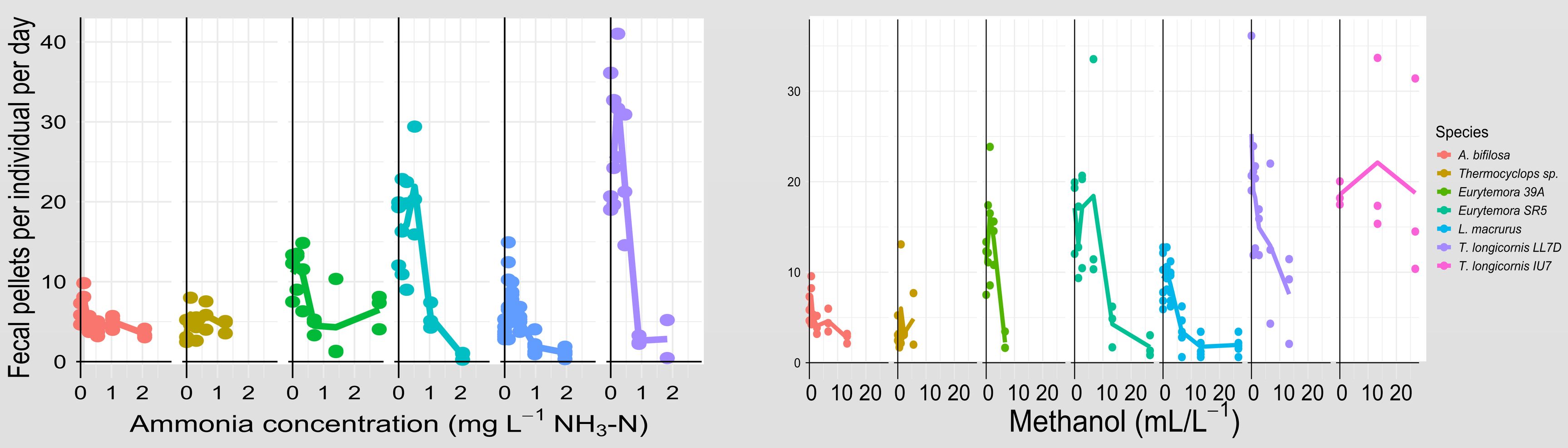


Figure 3. Fecal pellet production per individual per day (FP) under the tested concentrations. A. bifilosa and Thermocyclops sp. Showed less variation than the other species. Overall, small doses of the chemicals seem to positively affect FP, while FP is reduced in higher concentrations.

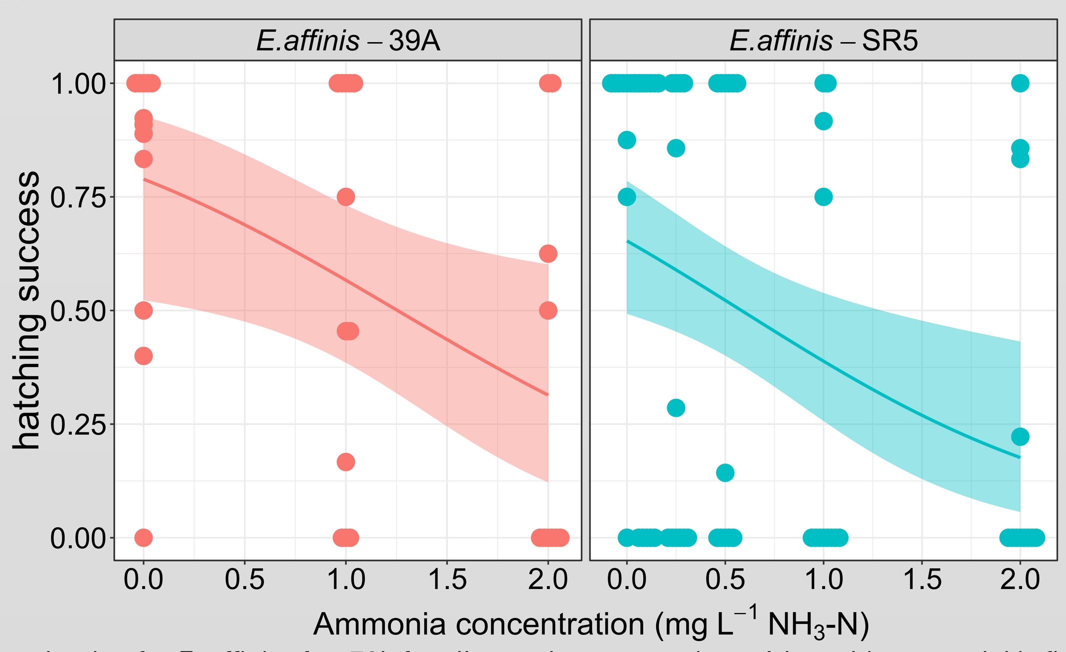


Figure 4. Fecal pellet production for *E. affinis* after 72h for all tested concentrations. A base binary model is fitted to data. 39A controls had higher success than the ones from SR5. Nevertheless, in both cases ammonia exposure negatively affected the hatching success

Discussion

Physiological Response → **Environment & Traits**
We observed both species and population-specific differences that could be related to both species' traits and the environment

Temperature, pH: increase the overall footprint of **NH_3** spills by driving a higher percentage of the un-ionised form being present in the water environment.

Population-specific differences (**mortality** and **hatching**). The LC50 values and the baseline hatching success of *E. affinis* indicate there are differences in toxicity tolerance between species and populations. These differences may be due to adaptations to higher background levels of ammonia.

Feeding mode (suspension vs. Ambush feeding) could have affected the response to NH_3 as we observed in the

Next steps: development of **Damage Models** with integration of known affecting **traits**.

Acknowledgements

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