

The Hidden Threat of Combined Stressors: The Influence of Temperature and pH on Antibiotic Toxicity in *Danio rerio*

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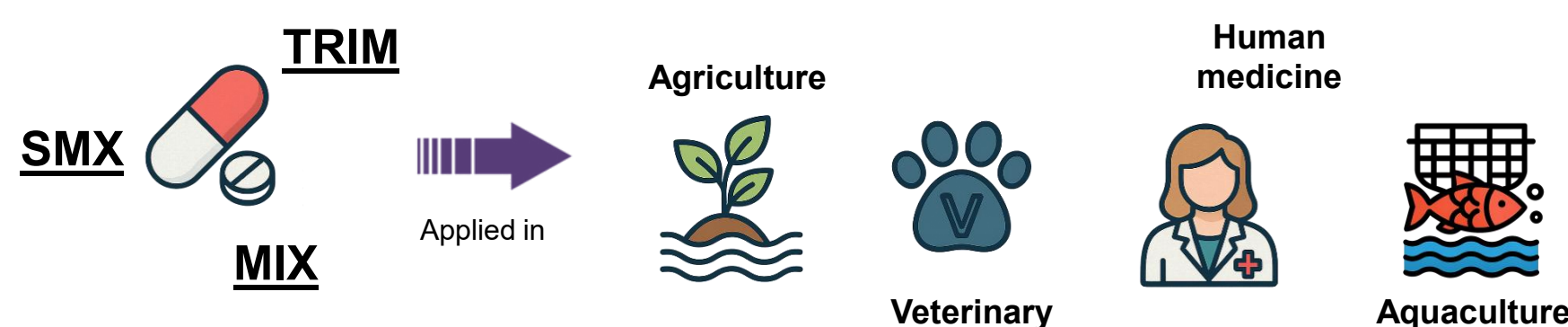
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Introduction



- **Aquatic ecosystems** are increasingly **subjected to multiple stressors**;
- **Climate change** drives variations in key abiotic factors, like **temperature and pH**;
- **These changes** can **alter the toxicity and absorption of antibiotics** in **aquatic organisms**;

- Sulfamethoxazole (**SMX**), Trimethoprim (**TRIM**) are two of the most used antibiotics in the last 50 years;



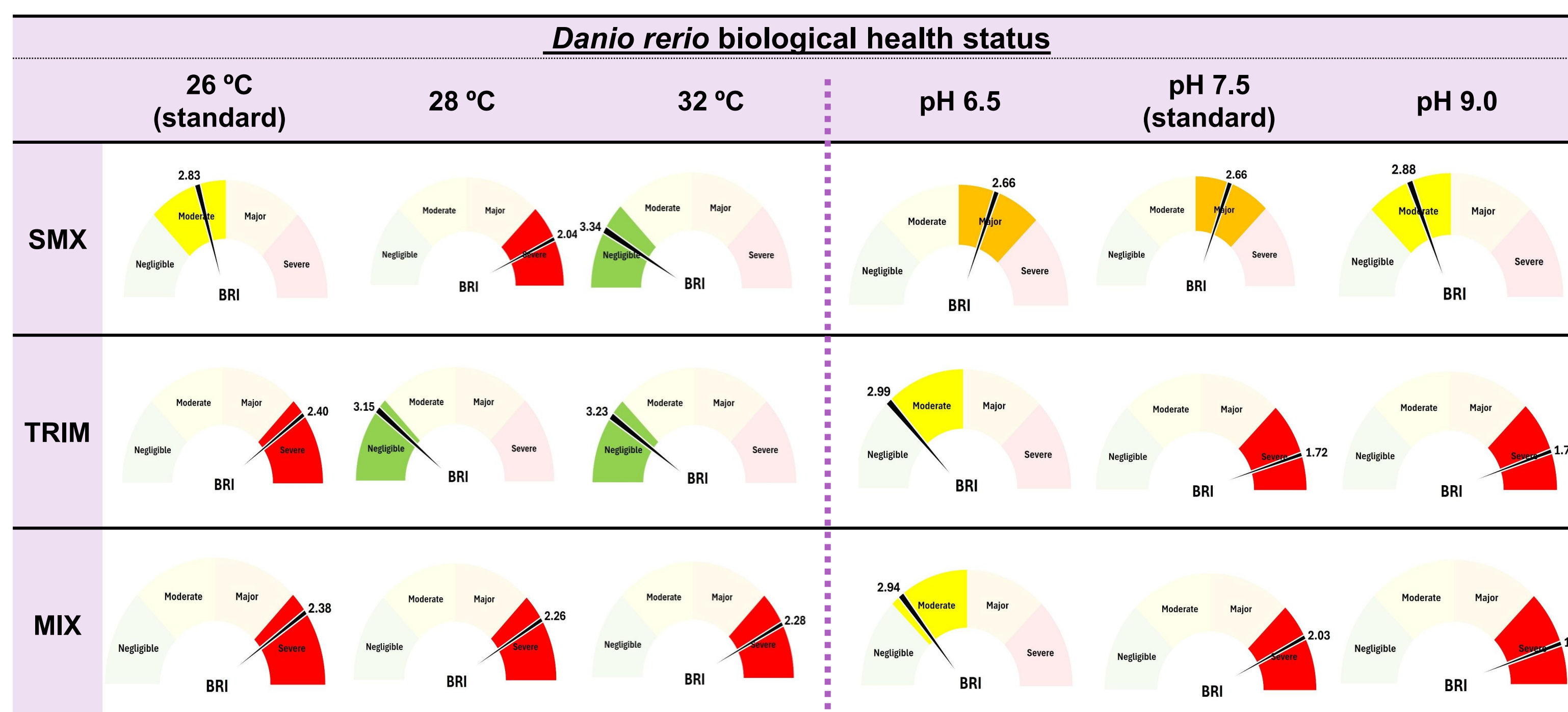
- These antibiotics and their mixture (**MIX**) are widely used to treat and prevent several bacterial infections¹;

Understanding how these **stressors interact** is **essential for assessing the risks to biodiversity and ecosystem functioning**, reinforcing the importance of **integrative ecotoxicological approaches** in **environmental risk assessment**.

Aim

Evaluate the chronic toxicity of **environmentally relevant concentrations** of SMX, TRIM, and their mixture (**MIX**) under **different temperatures (26, 28, and 32 °C)** and **pH conditions (6.5, 7.5, and 9.0)** on *Danio rerio*, using a multi-biomarker approach to assess the organism's biological health status.

Main results

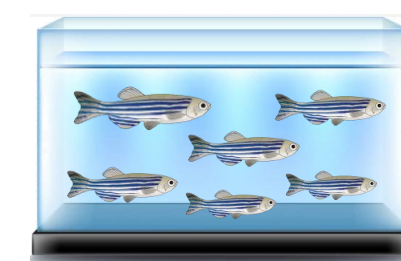


- At **28 °C**, **SMX** and **MIX** exhibited increased toxicity inducing **severe biological alterations** (e.g., neurotoxicity and DNA damage);
- **TRIM** decrease toxicity with increase in temperature, causing **negligible alterations** in *D. rerio* (e.g., antioxidant defense mechanisms alterations);
- Antibiotics **mixture was the most toxic** and overall, causing **severe alterations** in *D. rerio* (including pronounced genotoxic and histological damage) across all the temperature scenarios;

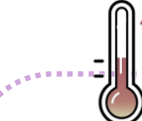
- **SMX** demonstrated **higher effects under pH 6.5**, causing **major alterations** in *D. rerio* (e.g., oxidative stress, lipid peroxidation, and DNA damage);
- The toxicity of **TRIM** and **MIX decreased at pH 6.5**, while **pH 9.0** remained similar to the standard condition, leading to impairment of antioxidant defenses as well as DNA and histological injuries (**severe alterations**);

Methodology

Chronic assays OECD 2000 Test N° 215²



Danio rerio juveniles



Temperature variations

IPCC acidification projections for aquatic ecosystems³

26 °C (Standard temperature)

28 °C (Moderately high temperature +2°C)

32 °C (High temperature +6 °C)

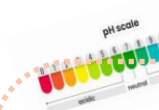


Antibiotics

Sulfamethoxazole
(150 µg SMX/L)

Trimethoprim
(30 µg TRIM/L)

Mixture
(150 µg SMX/L +
30 µg TRIM/L)



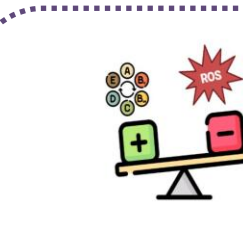
pH variations

pH 6.5 (IPCC acidification projections for marine ecosystems³)

pH 7.5 (Standard pH)

pH 9.0 (Alkaline freshwater projections⁴)

After 28 days of exposure



Antioxidant defense mechanisms
SOD, CAT, GRed, GPx, GSTs activities
GSH content



Lipid peroxidation
TBARS levels



Energetic metabolism alterations
Cellular energy allocation
LDH activity



Cholinergic neurotransmission
AChE activity



Genotoxicity
Gills comet assay



Histology
Liver pathological index

Danio rerio biological health status⁵

Biomarker Response Index (BRI)				
Alterations percentage	≥ 101	50-100	19-49	≤ 18
	Score 1	Score 2	Score 3	Score 4
BRI	1.00- 2.50	2.51-2.75	2.76-3.00	3.01-4.00
Biological health status	Severe alterations	Major alterations	Moderate alterations	Negligible alterations

Conclusions

- **Antibiotic mixture** was consistently the most toxic treatment;
- **Environmental changes** (temperature or pH) **amplified antibiotic toxicity** in *D. rerio*;
- **Combined environmental stressors** and antibiotics severely **affect *D. rerio* health**;
- These results highlight the **complex interactions between chemical contaminants and climate-driven abiotic stressors**, underlining the need for **integrated environmental assessments**.

References

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