Characterization of molting disruptors using Adverse Outcome Pathway (AOP)- informed screening tests in crustaceans

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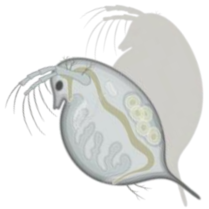
Importance of Molting



Molting is the shedding of the old exoskeleton (cuticle) of the previous life stage during growth. It provides several important functions for arthropods, including growth, repair, protection and reproduction

Growth & Survival

Molting is critical for arthropod growth, allowing them to shed exoskeletons and develop. Disruptions in molting can lead to developmental issues and increased mortality.

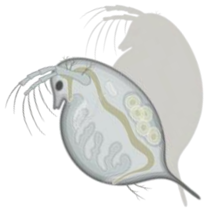
Sensitive

Many chemicals can interfere with molting hormones and exoskeleton formation. Pesticides and industrial chemicals pose significant risks to arthropods.

Regulatory Gaps

Current regulations often lack specific testing strategies for molting disruptors. This gap may leave many potentially harmful chemicals unregulated.

Complexity of Molting Disruptors Analysis



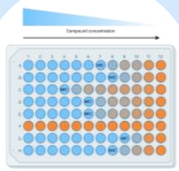
Diverse Mechanisms

Molting disruptors can act through various pathways, including hormone disruption and enzyme inhibition.

Different arthropods species may respond differently to the same disruptor.

Limited Detection Tools

Current tools for detecting molting disruptors are insufficient, often relying on broad toxicity endpoints (e.g. molting frequency). There is a need for more specific and sensitive methods.



Multi-tier Approaches for Identifying Molting Disruptors

Molting disruptor screening test

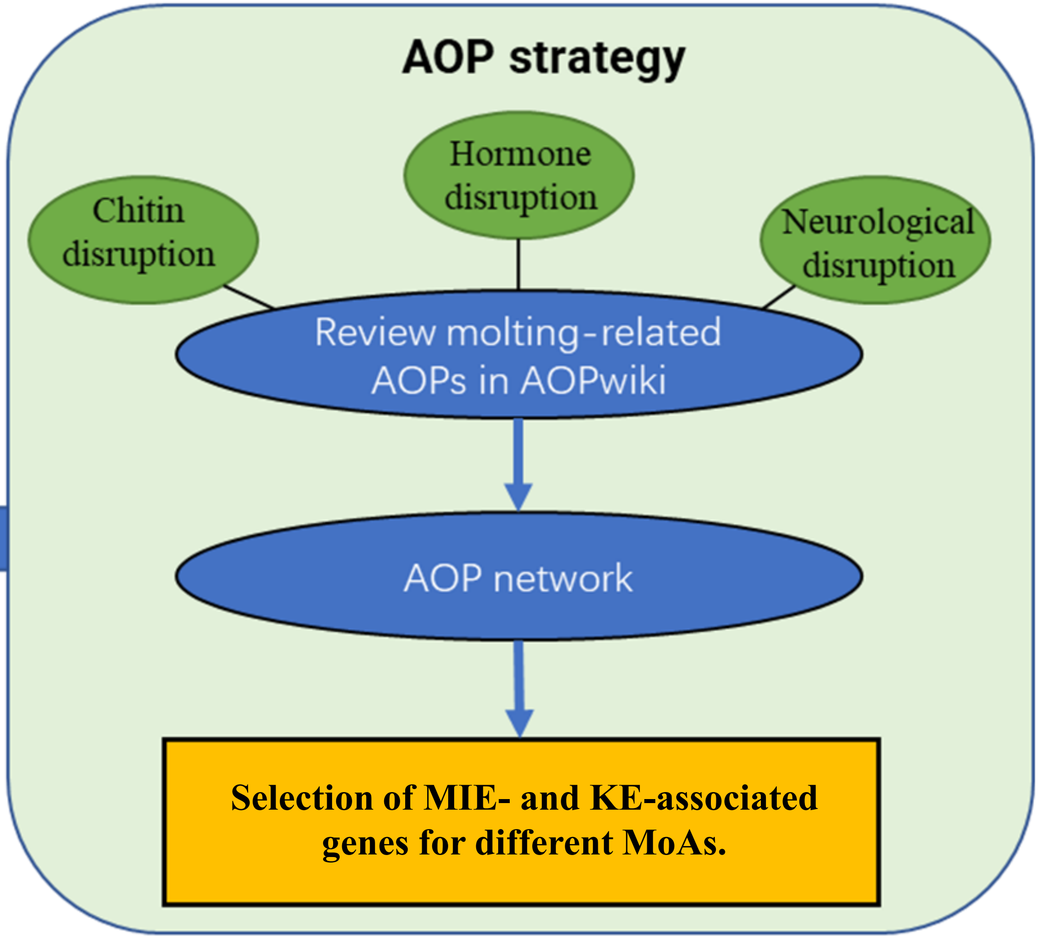
Develop and standardized methods for quantifying molting failure types and mortality in crustaceans

Mode of action verification

Verify the MoA by identifying differentially expressed genes associated with MIEs and KEs

Identification of Potential Molting Disruptors

Applying the AOP-informed testing tools to identify molting disruptors among PARC-relevant chemicals



Current Work Tasks

AOP network development

Reviewed molting-relevant AOPs from AOPwiki and constructed an AOP network and characterize the most common modes of action (MoAs) associated with molting disruptors.

Daphnia-based Assays

Novel Daphnia-based assays has been developed to quantify molting disruption. Measured changes in molting frequency and different types of molting failure exposed to test chemicals.

Reference Chemicals

A set of reference chemicals with known MoAs has been tested.

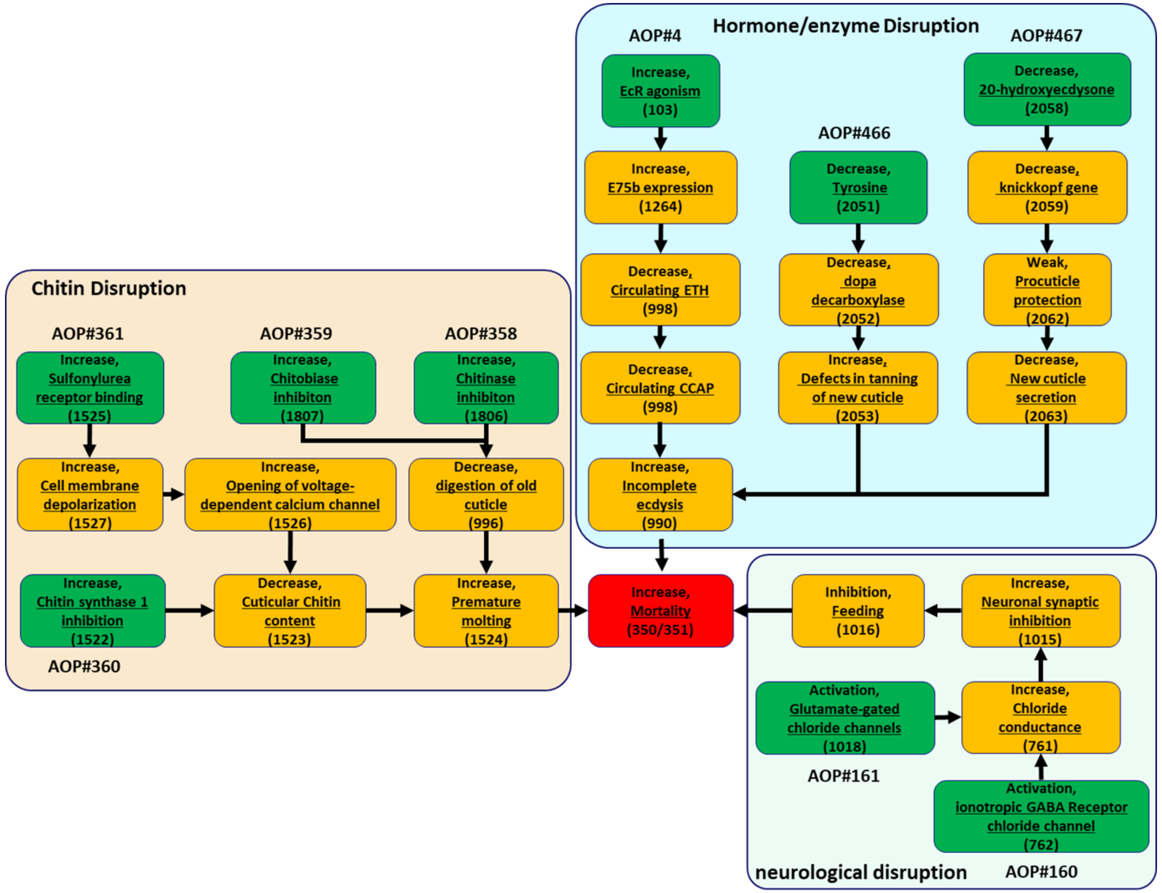
Chemical	Category	MoA	Relevant AOP
Spinosad	Molting disruptor	Chitobiase inhibitor	359
20-hydroxyecdysone	Hormone disruptor	Ecdysone Agonist	4, 467
Nikkomycin Z	Molting disruptor	Chitin synthesis inhibitor	360
Emamectin	Neurological disruptors	GluCls disruptor	161
Fipronil	Neurological disruption	Blocks GABAA-gated chloride channels	160

Target gene analysis

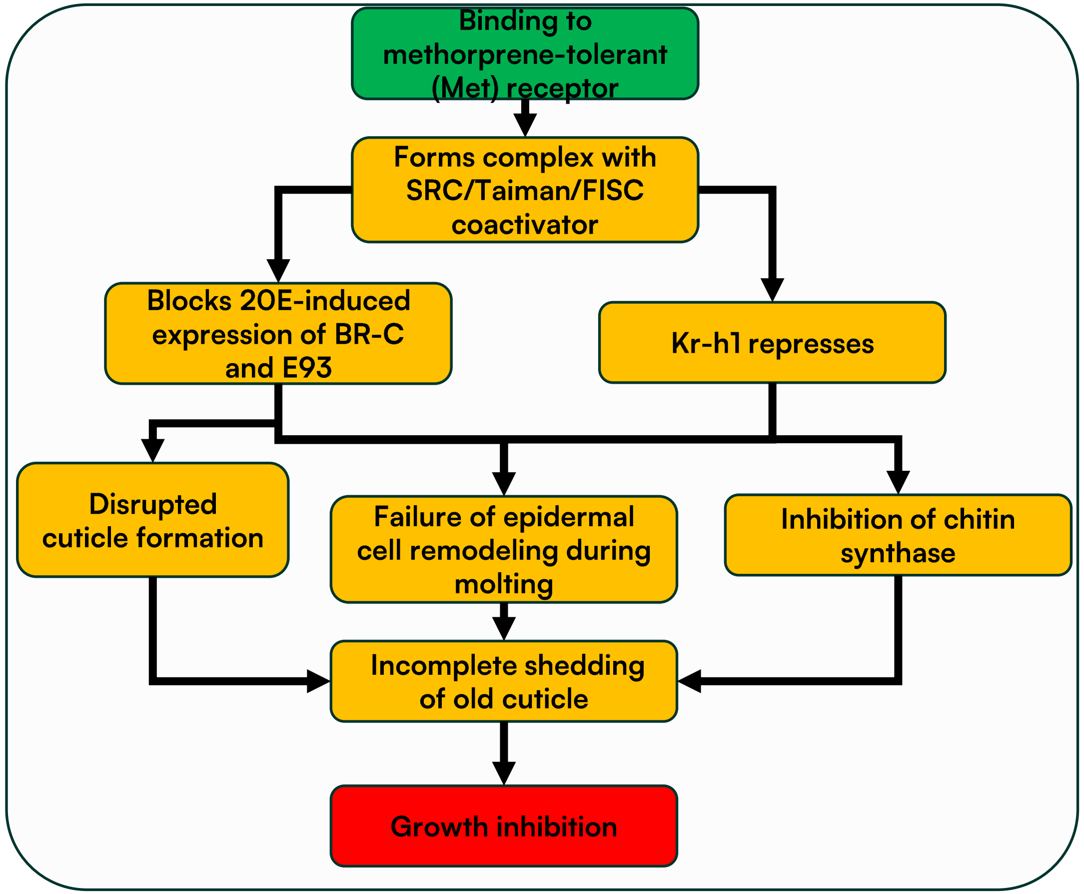
Early qPCR studies link phenotypic changes to specific gene expression patterns.

Helps establish Adverse Outcome Pathways (AOPs) for molting disruption.

AOPs associated to molting disruption

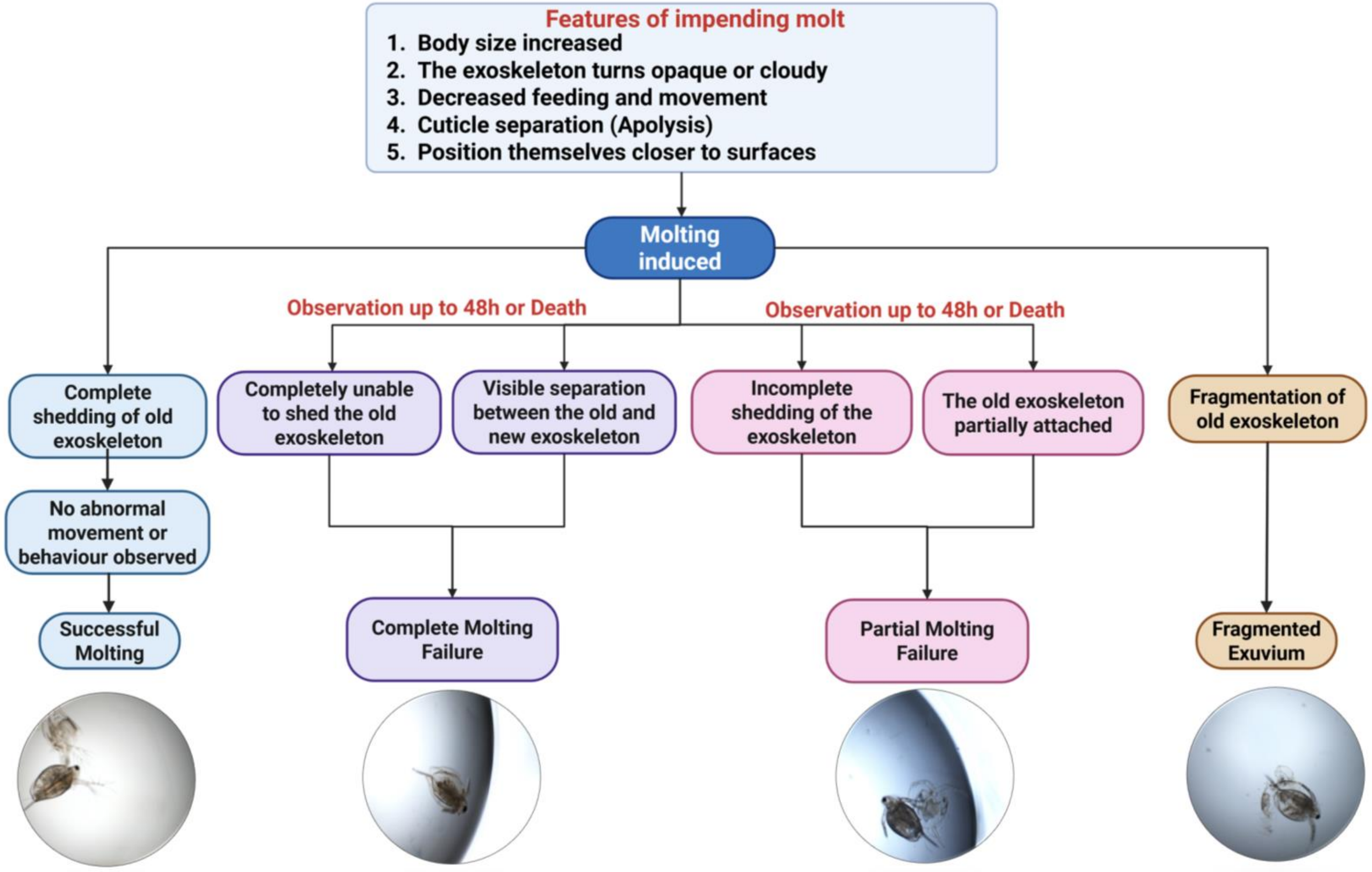


AOP of Juvenile Hormone (JH) on Molting disruption (under development)



Quantification of molting types in *Daphnia magna*

Molting type and assessment Criteria



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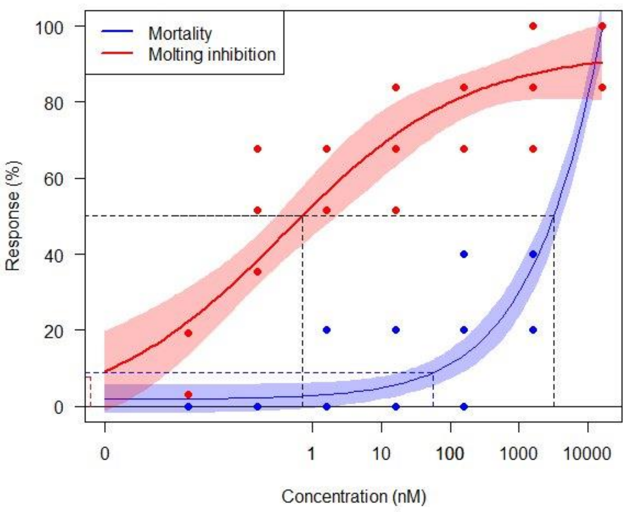
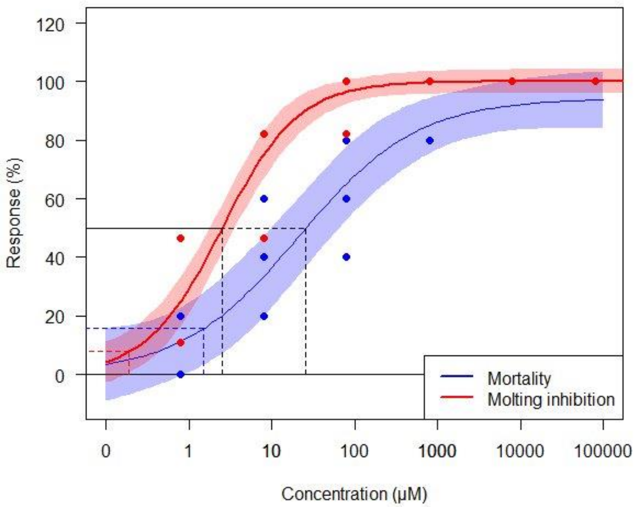
Results (screening test)

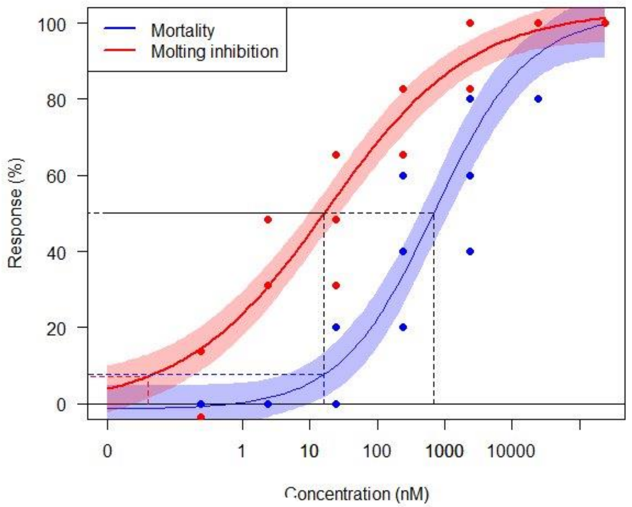
Spinosad



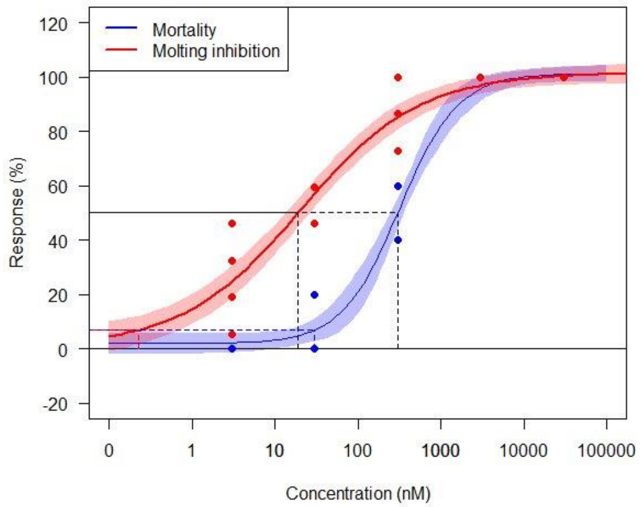
20-hydroxyecdysone



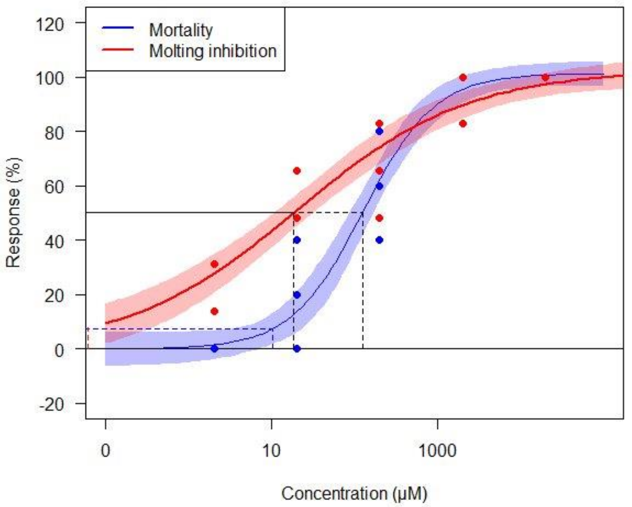
Nikkomycin_Z



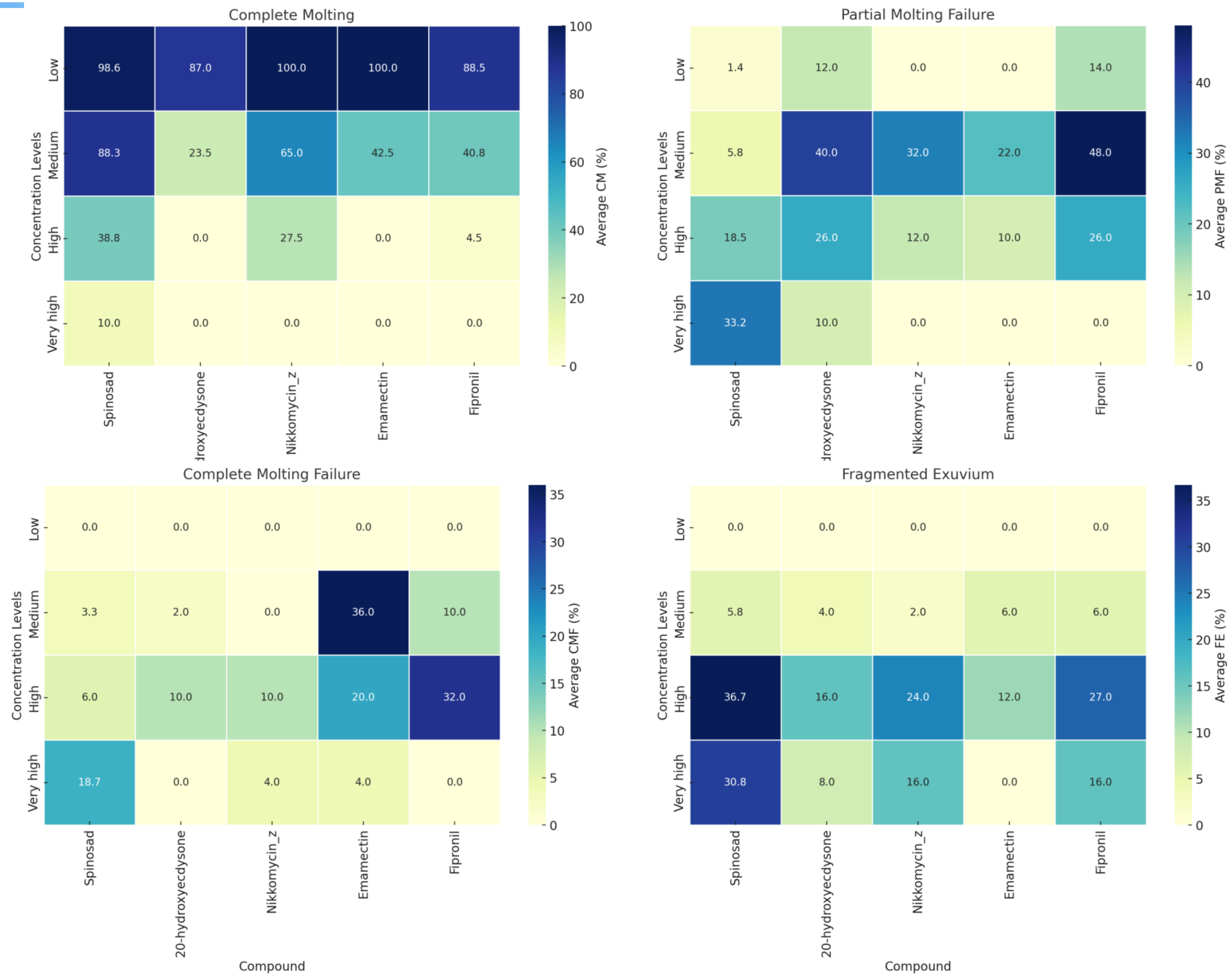
Emmaectin



Fipronil



Results (Molting types quantification after exposure)



Vision for the New Project (MOLTDISRUPT) In PARC

Expanded the work !!!!

Additional organisms

Expand screening to other relevant arthropod models, such as copepods, amphipods, and insects. Provides a broader understanding of molting disruption across different species.

01

Toolbox development

Develop a toolbox links phenotypic responses and gene expression data with AOP/qAOP frameworks for mechanistic interpretation, incorporating New Approach Methods (NAMs) and advanced omics technologies.

02

Screen Chemicals

Use the toolbox to screen chemicals of emerging concern. Prioritize chemicals based on potential risks to arthropod populations.

03

Regulatory translation

Develop quantitative AOP (qAOP) to model the relationship between chemical exposure and adverse outcomes. Translate qAOPs into risk assessment to support decision-making.

04

Data management: Ensure data is reported in a standardized format and shared following FAIR principles (using FAIRification hub "qData"). Facilitates collaboration and knowledge exchange among researchers and regulators

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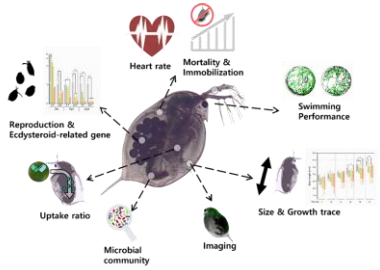
Collaboration

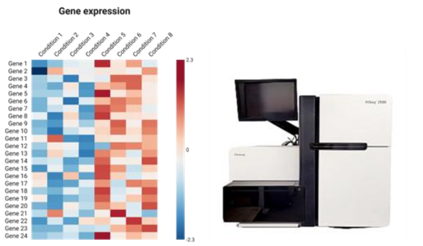


Partner with labs specializing in transcriptomics, omics and high content/High-throughput imaging.

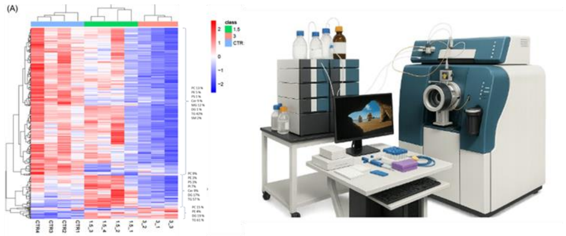
Use advanced technologies to identify molecular and morphological changes associated with molting disruption

Omics/Histological Capacity

Transcriptomics analysis



Lipidomics/metabolimics analysis



Work with modeling experts to develop qAOPs and validate their predictive power. Use qAOPs to support regulatory decision- making and risk management.

Modeling Expertise

Arthropod Models

Collaborate with insect and crustacean labs to expand the range of arthropod models.

Share expertise and resources to optimize assays for different species.

AOP Development and refinement

Collaborate with AOP development experts to integrate mechanistic data into AOP frameworks.

Regulatory Partners

Engage with regulatory partners to align project outcomes with regulatory needs.

Test qAOPs and other tools in regulatory use cases to ensure their practical application.

Partnership for the Assessment of Risks from Chemicals

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NCTP

EXPECT Project

PARC Project

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