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## **Disruption of oogenesis and molting by methoprene and glyphosate in *Gammarus fossarum*: involvement of retinoic acid?**

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### **Abstract**

In the last decade, the freshwater amphipod *Gammarus fossarum* proved to be a promising sentinel species in active biomonitoring programs to assess the effects of environmental contamination on non-target organisms. Given that the highly conserved retinoid (RETs) metabolism supports many biological functions and is perturbed by xenobiotics and used as biomarker for vertebrates, we explored the RETs functions in the crustacean model *Gammarus fossarum*. More specifically, we studied the implication of all-*trans* retinoic acid (atRA) in the reproduction (embryo, oocyte, and juvenile production) and development (success and delay of molting) by exposing *G. fossarum* females to atRA and citral (CIT), a known inhibitor of RA synthesis. In parallel, we exposed gammarids to methoprene (MET) and glyphosate (GLY), two pesticides suspected to interfere with atRA metabolism and signaling and frequently found in water systems. After 14 days of exposure, atRA, CIT, and MET reduced the number of oocytes, whereas only MET caused a reduced number of embryos. After 44 days, MET and GLY showed a tendency to decrease juvenile production. The duration of the molting cycle increased following the exposures to atRA and MET, while the treatment with CIT caused a typical endocrine disruptive inverted U-shaped curve. The exposure to GLY led to increased duration of the molting cycle at the lowest concentrations and lowered molting success at the highest concentration tested. This study highlights for the first time the implication of RA in the oogenesis and molting of *G. fossarum* and suggests that it may be a potential mediator of MET-induced effects on these processes. This study adds to the comprehension of the reproductive and developmental control in *G. fossarum* and opens new research avenues to study the effects of xenobiotics on the RET system in this sentinel species. Ultimately, our study will drive the development of RET-based biomarkers for non-target aquatic invertebrates exposed to xenobiotics.

### **Keywords**

Reprotoxicity ; Endocrine disruption ; Amphipod ; Vitamin A ; Juvenile hormone agonists ;  
Insecticide

### **Introduction**

Retinoids (RETs) are small molecules structurally similar to retinol (ROL, vitamin A) and are obtained from diet-derived carotenoids (CARs) or RET esters. Retinol is metabolized to retinoic acid (RA), the biologically active form of RETs. Retinoic acid plays crucial roles in cell growth, differentiation, and apoptosis, and is therefore implicated in embryonic development, growth, reproduction, and immunity through the activation of the Retinoic Acid Receptor (RAR) and the Retinoid X Receptor (RXR, Kam et al. 2012). For example, RA determines the onset of meiosis during gonadal development in mammals, birds, fish, and amphibians (Smith et al.

2008; Wallacides et al. 2009; Clagett-Dame and Knutson 2011; Peng et al. 2020). Because CARs are essential precursors of RETs, and knowing that imbalances of RA tissular concentrations have crucial implications for vertebrates, and their metabolism. CARs are involved in (1) protection against UV radiation and oxidative damages, (2) pigmentation, and (3) supply for bioconversion to RETs. In crustaceans, CARs are also well known for their essential role in reproduction (Wade et al. 2015). During early ovarian maturation, free or esterified forms of CARs are accumulated in the hepatopancreas, transported to the ovaries, and then incorporated into the developing oocytes in association with the vitellogenin (Wilder et al. 2010). However, to this date, it is still unknown if these functions are assigned to CARs or if they are carried out by RETs. This exemplifies the remarkable lack of understanding relative to the RET functions in crustaceans.

More recently, several endogenous RET isomers were studied in crustaceans tissues. More precisely, 13-*cis*/all-*trans* retinaldehyde (RALD) and 13-*cis* ROL were measured in the developing ovaries and all-*trans* ROL was measured in the eyestalks of *Litopenaeus vanamei* (Linan-Cabello and Paniagua-Michel 2004). The 9-*cis* and all-*trans* isomers of RA and the isomers 13-*cis* and all-*trans* of RALD were measured in the regenerating blastemas of *Uca pugilator* (Hopkins et al. 2008) and the isomers 9-*cis* and all-*trans* of RA were measured in the mature ovaries of *Scylla serrata* (Venkaiah et al. 2019). Isomers of RA (13-*cis* and all-*trans*), RA metabolites (13-*cis* 4-oxo and all-*trans* 4-oxo RA) and isomers of RALD (11-*cis*, 13-*cis* and all-*trans* RALD) were also found in whole-tissue homogenates of *G. fossarum* (Gauthier et al. 2023). In this study, it was also showed that RA metabolites and the sum of two RALD isomers (11- *cis* and all-*trans*) were modulated throughout the reproductive cycle (Gauthier et al. 2023). RXR is expressed in the eyestalks of *Callinectes sapidus*, *Oziotelphusa senex senex*, and *Macrobrachium nipponense* (Techa and Chung 2013; Li et al. 2014; Girish et al. 2015), in the ovaries of *U. pugilator*, *Gecarcinus lateralis*, *Marsupenaeus japonicus*, *Fenneropenaeus chinensis*, *Carcinus maenas*, *S. serrata*, and *Daphnia magna* (Durica et al. 2002; Kim et al. 2005; Asazuma et al. 2007; Wang et al. 2007; Priya et al. 2009; Nagaraju et al. 2011; Girish et al. 2015) and in homogenates of whole *Gammarus fossarum* (Gouveia et al. 2018). To ensure adequate RA tissular concentrations, the RET metabolism is regulated by a cascade of oxidase/reductase and isomerase as well as many intra- and extracellular binding proteins. Interestingly, citral (CIT), a compound known to inhibit the endogenous RA synthesis, induces limb deformities in developing chick embryos (Tanaka et al. 1996) and has similar effects in the regenerating limb bud of the crustacean *U. pugilator* (Hopkins 2001). However, studies reporting exposure of crustaceans to CIT are limited, and to our knowledge, none evaluated the effects of CIT on crustacean reproduction. On the other hand, exogenous exposures to RA in crustaceans were shown to influence circulating levels of glucose and hormones as well as reproduction and regeneration (Hopkins 2001; Reddy and Sainath 2008; Sreenivasula and Srilatha 2015; Girish et al. 2018; Venkaiah et al. 2019). Thus, the detection and variations of several endogenous isomers of RETs, the expression of RXR, and the responsiveness of crucial physiological processes to RA in crustaceans point toward important functions of RA in their physiology and deserve further research.

The massive usage of numerous pesticides for public health purposes (e.g., control of the mosquito populations) or in agriculture raises concern for aquatic non-target organisms (Lawler 2017; Zaller and Bruhl 2019). Among these, methoprene (MET) more specifically its

enantiomer S-MET, a juvenile hormone analog (JHA), is used to prevent the metamorphosis of mosquitoes in many countries. For vertebrates, both MET and its metabolite methoprenic acid are strong ligands for RXR (Harmon et al. 1995). Because xenobiotics can interfere with the RET system, RETs have served as the basis for developing biomarkers of environmental contamination in several species of mammals, birds, fish, and amphibians (Boily et al. 2004). A recent study observed perturbed RET concentrations in *G. fossarum* following exposure to the insecticide MET (Gauthier et al. 2023). Other studies included the insect *Apis mellifera* in this list. Honey bees exposed to thiamethoxam, glyphosate (GLY), or atrazine had altered levels of some CARs and RETs (Hedrei Helmer et al. 2014; Jumarie et al. 2017; Gauthier et al. 2018). Such investigations on the perturbation of the RET metabolism by pesticides were never undertaken in crustaceans and would provide avenues to suggest new RET-based biomarkers in crustaceans.

Reproduction is a meaningful and critical endpoint from an ecological perspective as it allows the maintenance of a population. For this reason and because it was shown to be useful to study the effects of environmental contaminants, *in vitro* tests assessing the reproduction of non-target aquatic species gained much scientific interest. The reproductive toxicity bioassay developed by Geffard et al. (2010) for the non-target crustacean amphipod *G. fossarum* is particularly interesting for several reasons. *G. fossarum* plays a significant role in wild ecosystems: it is a scavenger, thus participates in the degradation of organic matter, and is a feeding source for many organisms. It is widely distributed in Europe (and, to some extent, in America), and easy to collect and maintain under laboratory conditions. Furthermore, gammarids are non-target organisms and are sensitive to chemicals and xenobiotics, such as pesticides (Bundschuh et al. 2013). The bioassay allows the identification of reproduction and molting defects in *G. fossarum*. In healthy amphipods, ovarian maturation is synchronized with the molting cycle and happens concurrently. In this context, the synchronization of ovarian maturation and molting offers a biological framework and is exploited to allow not only the observation the identification of endocrine disruptive compounds (EDC). Besides, the assay offers an interesting experimental context to acquire new knowledge on the molecular mediators of these hormone-regulated processes. For vertebrates, the RET signaling pathway is currently being examined and discussed as a potential candidate to evaluate EDC (Organisation for Economic Cooperation and Development 2021). Considering the evolutionary conservation of RET signaling throughout many invertebrate phyla, the study of RET functions in crustaceans may prove helpful in identifying new mechanisms of EDC, which pose a contemporary challenge for non-target aquatic crustaceans.

The objective of this study was to assess the potential use of RETs as biomarkers in the sentinel freshwater amphipod model *G. fossarum*. Thus, for the first time, the implication of RA in the reproduction and development of this model was assessed using the *in vitro* bioassay developed by Geffard et al. (2010). In the first experiment, we investigated its physiological involvement by monitoring parameters of reproduction (embryo, oocyte, and juvenile production) and development (success and delay of molting) following the exposure to atRA and citral (CIT), a known RA synthesis inhibitor. In the second experiment, to probe the RET metabolism for biomarker development, we studied the toxicological effects of the two following model pesticides: methoprene (MET), a typical EDC in crustaceans, and glyphosate (GLY). Both are known or suspected to intervene with the RA metabolism or signaling pathway (Harmon et al.

1995; Paganelli et al. 2010; Hedrei Helmer et al. 2014; Gauthier et al. 2018) and are frequently found in water systems (Lawler 2017; Matozzo et al. 2020).

## Materials and methods

### Chemicals and exposure solutions

Acetone, analytical grade all-*trans* RA, CIT, GLY (n-(phosphomethyl)glycine), and MET (PESTANAL®) were purchased from Sigma Aldrich (St. Quentin Fallavier, France). All-*trans* RA, CIT, and MET were dissolved in acetone, and GLY was dissolved in groundwater. We used acetone which is the solvent routinely used in our lab for similar experiences because it was previously demonstrated as having no effects on reproductive parameters in *G. fossarum* (Gouveia et al. 2018). Glyphosate readily dissolved in groundwater and, therefore, no vehicle was needed. For both experiments, atRA, CIT, and MET solutions were further diluted with acetone to constitute the working solutions (WS) while GLY solution was diluted serially with groundwater to obtain the WS. WSs for atRA, CIT, MET, and GLY were kept in a – 80 °C freezer and used daily to expose gammarids. Nominal concentrations were used in the text; however, concentrations of the first dilution for each compound were confirmed by chemical analysis and were all within 10% of the theoretical values for both experiments (Table S1). Analyses for atRA, CIT, and MET were conducted by the TRACES research team of the Institut des Sciences Analytiques de Lyon UMR5280 (Rhone, France) whereas GLY analyses were performed by La Drome Laboratoire (Drome, France).

### Gammarid collection and acclimatization

Gammarids used in experiments 1 and 2 were collected in February and in May 2020, respectively, in La Cressonniere du Bugey, a watercress bed (Ain, France) known for its very low anthropogenic pressure and used as a control site. Gammarids were selected by sieving (2–2.5 mm) were brought back to the laboratory for a 10-day acclimation period (aerated 30 L aquariums, 16 °C ± 0.5 °C, 16/8 h light/dark cycles), fed ad libitum with *Alnus* spp. leaves and provided with continuous fresh groundwater. Groundwater was obtained from a forage well connected to the water table in Villeurbanne (Rhone, France), filtered through sand and a 10 µm filter with activated charcoal. In amphipods, the reproductive cycle of the female is synchronized with molting. After molting, eggs are laid in the marsupium and fertilized by a male later during the reproductive amplexus. The embryos will continue to develop, and the hatched eggs will be released from the marsupium before the upcoming molt (Geffard et al. 2010). For both experiments, to ensure that females used were all in the reproductive stage A and within 24 h of each other, females were selected as follows(see experiment 2 for stage description). A large number of couples (± 1200 for experiment 1, and ± 1600 for experiment 2) were isolated. All females that have laid their eggs were discarded after 48 h. The following day, the same process was carried out. This time, freshly molted females in the reproductive cycle A were pooled and distributed randomly in each exposure condition (see the “[Experiments](#)” section).

## Experiments

We conducted two experiments based on the bioassay developed by Geffard et al. (2010). For both experiments, we monitored survival daily (dead individuals were discarded) to ensure the observation of sublethal effects and confirmed that no significant differences were noted between CTRL and SOLV groups (Figs. S1 and S2). The first experiment lasted 14 days and was realized to observe the effects of atRA, CIT, and MET on the reproduction parameters of female *G. fossarum* (production of embryos and oocytes). These two parameters were observed for each females. For this reason, we tested a range of concentrations of atRA and For both pesticides, we also wanted to encompass concentrations that are found in the environment (up to 45 µg/L for MET and up to 105 mg/L for GLY, Brovini et al. 2021; Hershey et al. 1995) and selected only the active ingredients alone given the annual shifts in the commercial formulations employed. In our design, MET was also selected as a reference chemical to illustrate the effects of a typical EDC. In the second experiment, we wished to evaluate the effects of atRA, CIT, MET, and GLY on juvenile production and molting. Therefore, gammarids were exposed for 44 days, which allowed the observation of a whole reproduction cycle. That allowed us to monitor the juvenile production, success of molting, and duration of the molting cycle.

### Experiment 1

Previously selected and synchronized females were randomly allocated to beakers (500 mL) containing groundwater in triplicates to three concentrations of atRA (7, 70, and 210 µg/L), CIT (11, 110, and 330 µg/L), and MET (6, 60, and 600 µg/L). Controls (CTRL) and control for acetone (SOLV) were also made in triplicates. In this experiment, we used 7 synchronized females per beaker. To avoid atRA photodegradation, exposures to this compound were made in beakers wrapped in aluminum foil. To ensure constant exposure throughout both experiments, we renewed the media daily using groundwater (aerated for 48 h) and 25 µL of freshly thawed WS for each compound. According to Gouveia et al. (2018), the acetone concentration we used (0.005% v/v) has no effects on gammarid reproduction. A square of Nitex nylon mesh fabric (5 . 10 cm) was placed in each beaker for enrichment and to allow movement and dispersion of gammarids. After 14 days, females typically in the C2-D1 stage readily allow the observation of the production of embryos (number of embryos in the marsupium) and oocytes (number of oocytes located dorsally). Hence, using a binocular, embryos were emptied from the marsupium and they were counted for each female. Oocytes were counted with a microscope. Given the direct influence of female size on the production of embryos and oocytes, both parameters were normalized by their size (mm) which were measured on photographs (. 10) by image analysis using ImageJ (v1.53a). Because only part of the primary oocytes (stage B and C1) goes through the secondary vitellogenesis (stage C2) as shown by Geffard et al. (2010), the count of oocytes in the present experiment was performed on females that showed no delay in their development (C2 and D1). We assessed molting stages of females (A, B, C1, C2, D1, and D2) based on the observation of the integument changes on the dactylopodite and protopodite of individuals as described by Geffard et al. (2010). The postmolt stage A is characterized by a fine and soft cuticle that hardens during stage B, easily observable on the dactylopodite setae. During the intermolt stage (C), there is progressive epidermal retraction. In stage C1, the setae retract along the protopodite, while in stage C2, the new dactylopodite is completely retracted. Premolt stage (D) is characterized by the synthesis of a

new cuticle visible on the new dactylopodite (D1) and the protopodite setae (D2). Given the short duration of stages A and B, they were merged as stage AB.

## Experiment 2

To further investigate the sub-lethal effects of atRA, CIT, and MET on ecdysis and reproduction over a long period, gammarids were exposed for 44 days to the same compounds following similar exposure conditions. In addition to survival, temperature, conductivity, and dissolved oxygen concentration (mg/L) were monitored daily (Table S2). According to the results from the first experiment and to optimize survival for a longer exposure period, concentrations of the compounds for the second experiment were adjusted as follows: atRA (0.11, 1.1, and 11  $\mu\text{g/L}$ ), CIT (6, 60, and 600  $\mu\text{g/L}$ ), and MET (0.25, 2.5, and 25  $\mu\text{g/L}$ ). We also investigated the effects of GLY at the following concentrations: 0.45, 4.5, and 45 mg/L. In this experiment, we used 10 synchronized females per beaker (triplicates). When females reached the C2 stage (after 17 days), one ready-to-mate male (in copulating amplexus) per female was added to each beaker and left for copulation. They were removed when no more dyads were found which corresponds, in our study, to 32 days. The reproductive stage of every female was registered daily to estimate the molting cycle duration. We measured the duration (days) of the female reproductive cycle from the beginning of the exposure (day 1) until the female was freed from copulating amplexus and had fertilized eggs in their marsupium (AB stage). During this period, the biological endpoints observed were (1) the number of produced juveniles per female, (2) the number of females succeeding to complete molting and egg-laying, and (3) the duration of the molting cycle. For the juvenile production, the newly hatched juveniles were counted every day and standardized by the number of females in that beaker. Up to 10 juveniles per beaker were photographed daily using a binocular. Juveniles were also measured by image analysis using ImageJ (v1.53a). On day 32, females who had not started mating or molting were registered and removed from experimental units.

## Statistical analyses

Survival was tested with Log-rank (Mantel-Cox) tests comparing curves from each condition to their control group, respectively. Results for gammarids exposed to atRA, CIT, and MET were compared with the SOLV group (to assess the effect of solvent), whereas the ones exposed to GLY were compared with the CTRL group. Embryo and oocyte count and duration of the reproductive cycle were tested using the GLM (General Linear Model) one-way analyses of variance. We verified the normality of data distribution and the homogeneity of variance with Shapiro-Wilk and Levene tests respectively. Outliers were identified and discarded following a Grubb's test (< 2% of data for all parametric tests). Significant models ( $p < 0.05$ ) were followed by a Dunnett "t" test against the respective control group. For mean juvenile production (data per beaker) and when normality and homogeneity of variance were not met, the non-parametric Kruskal-Wallis (KW) test was used, followed by Dunn's test (pairwise comparison with the respective control group). The non-parametric Jonckheere-Terpstra (JT) test, based on the medians ordered in a particular direction, was performed to detect a possible trend related to a concentration–response relationship when neither parametric nor KW detected significant effects. When applicable, unilateral statistical tests were employed. Moulting success was analyzed with Fisher's exact test by comparing predicted and observed events of molting. The

*p*-values were considered significant when they were lower than 0.5 and marginally significant when between 0.5 and 1. All statistical analyses were performed with RStudio (version 1.4.1717) except for survival analyses that were performed with GraphPad Prism (version 9.3.1).

## Results

### Experiment 1

In the first experiment, we did not find significant differences in survival among groups (Fig. S1). Embryo count of atRAexposed females showed a significant dose-dependent diminution. The mean diminution of embryo count was equivalent to – 35% and – 25% at the two highest concentrations of atRA, respectively (70 µg/L and 210 µg/L, Fig. 1). Embryo count following CIT exposure did not show any significant differences. On contrary, MET caused a clear diminution of embryo count for all concentrations tested (6 µg/L, 60 µg/L, and 600 µg/L) and represented – 53%, – 50%, and 64.5% of the embryo count in unexposed females. AtRA, CIT, and MET all caused clear oocyte count diminutions at every concentration tested.

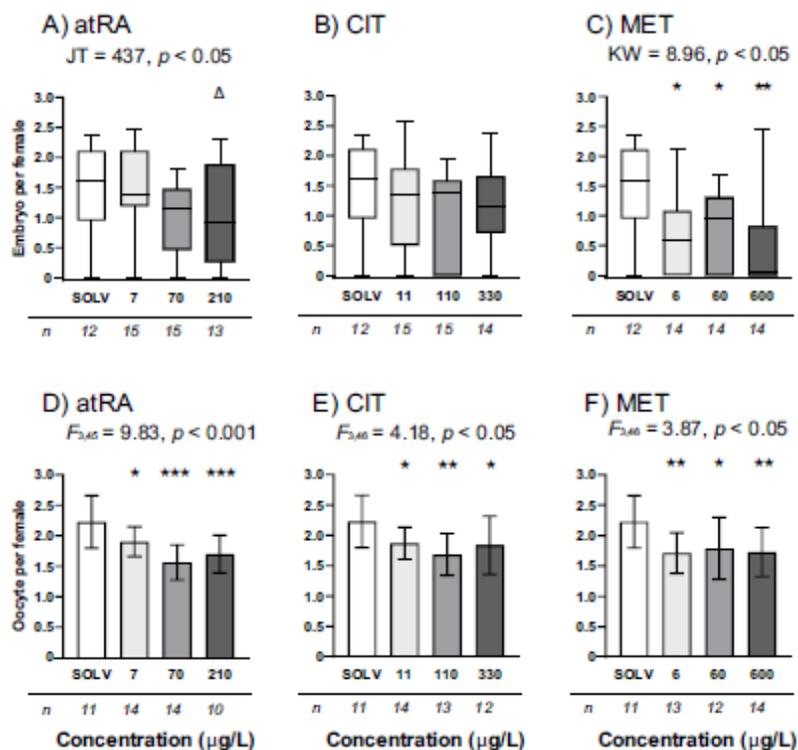


Fig. 1 Production of embryos (A–C) and oocytes (D–F) for females *G. fossarum* exposed for 14 days to increasing concentrations of atRA, CIT, and MET. Production of embryos and oocytes was calculated by normalizing the number of embryos or oocytes per female by the dorsal length of each female. For oocyte count, only females that were non-delayed in their development were selected (C2 and D1). Δ Significant JT trend test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

## Experiment 2

In this experiment, no statistical differences were found between the CTRL and the SOLV group for survival, respectively (Fig. S2). Similarly, for gammarids exposed to atRA, CIT, and GLY, no statistically significant differences were found between the respective control and the compounds. However, it was observed a marginally significant model for gammarids exposed to MET ( $p = 0.053$ ). This result was associated with significantly higher survival of gammarids exposed to the highest concentration of MET (25  $\mu\text{g/L}$ ). The juvenile hatching kinetic showed very similar rates among all conditions (Fig. 3A–D). It was also reported that newly hatched juveniles were significantly smaller following exposure to atRA, CIT, MET, and GLY (Fig. 3E–H). Although statistically significant in some cases, the effects of atRA, CIT, MET, and GLY on juvenile size were minute ( $< 3\%$ ) and will not be further discussed. In the same experiment, the majority of females molted successfully (Table S3), except for females exposed to GLY at the highest concentration (45  $\text{mg/L}$ ). At this concentration, a significant reduction in molting success was observed where 58.1% of females molted successfully compared to 91.7% in the CTRL group (odds ratio = 7.85,  $n = 45$ ,  $p < 0.05$ ).

Mean juvenile production from females exposed to atRA and CIT was not influenced by the exposure, but it showed a tendency to be decreased dose-dependently following exposure to MET and GLY (Fig. 2A–D). These diminutions for MET ( $-24.8\%$ , 0.25  $\mu\text{g/L}$ ;  $-13.8\%$ , 2.5  $\mu\text{g/L}$ ; and  $-29.7\%$ , 25  $\mu\text{g/L}$ ) and GLY ( $-18.6\%$ , 0.45  $\text{mg/L}$ ;  $-21.6\%$ , 4.5  $\text{mg/L}$ ; and  $-17.4\%$ , 45  $\text{mg/L}$ ) were substantial compared to their respective control. For gammarids exposed to CTRL and SOLV, the mean duration of their reproductive cycles was 26.3 days  $\pm$  1.8 and 25.1 days  $\pm$  1.4, respectively, and did not display significant differences when compared to one another (Fig. 2E–H). Among the different tested compounds, different patterns of effects were observed. AtRA increased the duration of the molting cycle specifically at 0.11  $\mu\text{g/L}$  and 11  $\mu\text{g/L}$ , but not at the intermediate concentration (1.1  $\mu\text{g/L}$ ). Similarly, females exposed to MET displayed longer cycles at the lowest (0.25  $\mu\text{g/L}$ ) and the highest concentration (25  $\mu\text{g/L}$ ), whereas they were only marginally significant at the intermediate concentration (2.5  $\mu\text{g/L}$ ,  $p = 0.076$ ). CIT and GLY had significant effects at the lowest concentrations whereas CIT showed a U-shape response. CIT increased very clearly the duration of cycles for females exposed to the two lowest concentrations (6  $\mu\text{g/L}$  and 600  $\mu\text{g/L}$ ) but had no effect at the highest concentration (600  $\mu\text{g/L}$ ). Similarly, GLY showed a significant increase in the duration of the cycle, but only at the lowest concentration (0.45  $\text{mg/L}$ ).

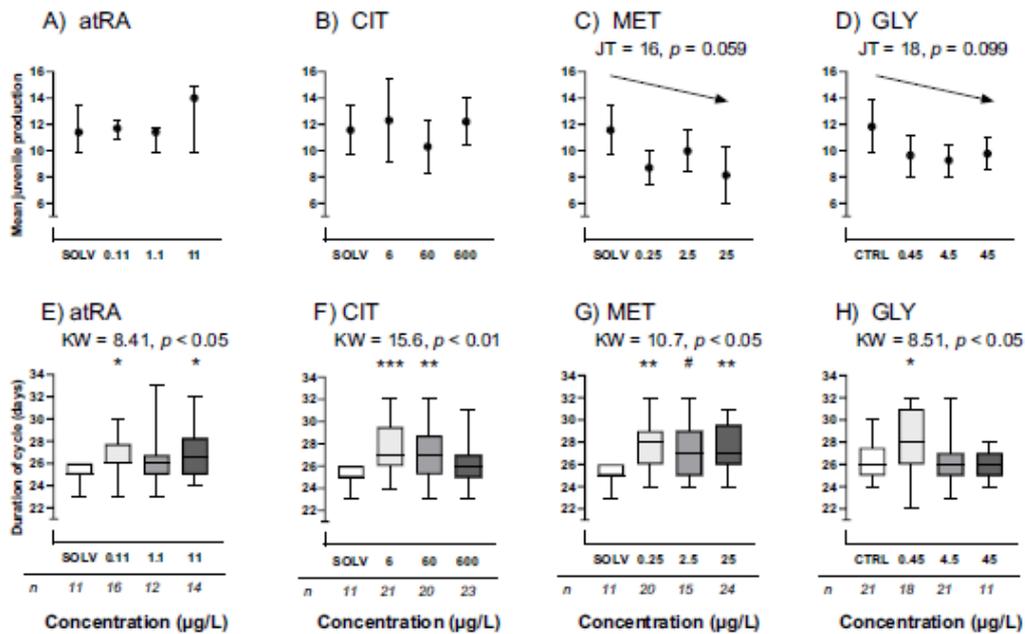


Fig. 2 Production of juvenile per female (A–D) and duration of molting cycle (E–H) for gammarids exposed for 44 days to increasing concentrations of atRA, CIT, MET, and GLY. Concentrations are displayed in  $\mu\text{g/L}$ , except for GLY (mg/L). See the “Materials and methods” section for estimations of juvenile production and reproductive cycles. Arrows represent dose-dependent relationship, #  $0.05 > p < 0.1$ , \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

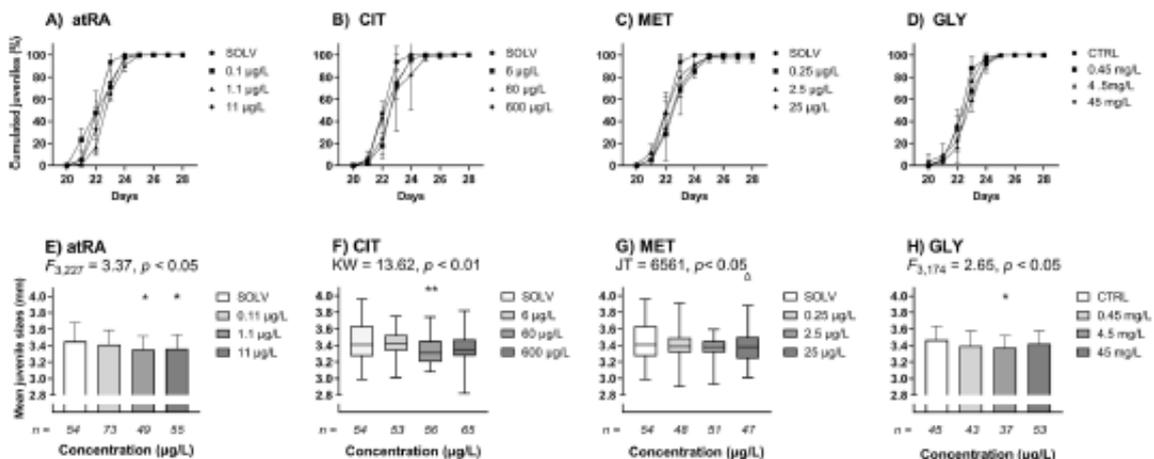


Fig. 3 Cumulative proportion of juvenile hatching (A–D) and sizes of newly hatched juveniles (E–H) following exposures to increasing concentrations of atRA, CIT, MET, and GLY. The hatched embryos that had been released for the marsupial pouch of females were counted daily and reported in daily percentages of the total cumulated juvenile production.  $\Delta$  Significant JT trend test, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

## Discussion

In this study, we investigated the implication of RA on the reproductive and developmental physiology of *G. fossarum*. We exposed gravid females to atRA and CIT to simulate an excess or a deficiency of RA. We also monitored the toxicological effects of two pesticides commonly

used and frequently found in surface water, MET and GLY that were either known or suspected to affect the RA metabolism and signaling.

In a previous study by Geffard et al. (2010), the oocyte and embryo count per female for unexposed gammarids ranged from  $12.4 \pm 2.8$  to  $21.5 \pm 3.4$  and from  $10.9 \pm 1.5$  to  $12.9 \pm 2.0$ , respectively, throughout the reproductive cycle. In our first experiment, the reported mean oocyte counts per female for CTRL and SOLV were  $14.4 \pm 2.5$  and  $21.8 \pm 4.5$ , respectively. When oocyte count was standardized by the size of females, our values also resembled the ones obtained by Cribeu et al. (2020) for unexposed gammarids. For embryo count per female, we reported values of  $7.6 \pm 9.2$  and  $14.0 \pm 7.9$  for CTRL and SOLV, respectively, which roughly compares to reference values from Geffard et al. (2010). In the first experiment, the CTRL group had a high proportion of females who were unexplainably not bearing embryos and caused very high standard deviations. For this reason, we excluded the CTRL group for the first experiment of our study. Results for gammarids exposed to atRA, CIT, and MET were then compared to SOLV, a condition in which > 80% of females were bearing embryos and showed similar values to what was obtained by Cribeu et al. (2020) once corrected by female sizes. In the second experiment, females in the CTRL and SOLV group generated  $11.8 \pm 2.0$  and  $11.6 \pm 1.8$  juveniles, respectively. According to the observations of Geffard et al. (2010), these numbers are in the same range as the embryo count of healthy females in C2 and D1 in the first experiment (results not shown). In the CTRL and SOLV groups, the newly hatched juveniles had mean sizes of  $3.47 \text{ mm} \pm 0.17$  and  $3.45 \text{ mm} \pm 0.23$ , respectively, and hatched in a very synchronized manner for all conditions. At the end of the second experiment, we estimated the duration of the female molting cycles for the CTRL and SOLV groups to be around  $26.3 \text{ days} \pm 1.8$  and  $25.1 \text{ days} \pm 0.9$ , respectively, which were expected according to the predicted development time modeled by Chaumot et al. (2020) for *G. fossarum* at 16 °C.

### **Implication of RA in reproduction and development**

According to our results, atRA reduced oocyte production in gravid *G. fossarum* females and increased the duration of the molting cycle. As stated before, in a wide variety of vertebrates, the onset of the gonadic germ cells meiosis is dictated by RA levels through the activation of RXR, which could also be the case in crustaceans. Impaired differentiation of blastemal cells following exposure to emulsions of atRA was reported in the crustacean *U. pugilator* in conjunction with an increased UpRXR expression (Hopkins and Durica 1995; Chung et al. 1998). As RXR is also measured in whole-tissue homogenates of *G. fossarum* throughout the reproductive cycle (Gouveia et al. 2018), it is plausible that exposure to atRA perturbed the oogonial differentiation as a result of disrupted RA tissular concentrations (not observed in our study). Recent studies demonstrated the modulatory action of 13-*cis* and 9-*cis* RA on the production and release of hormones in secretory organs together with enhanced ovarian maturation in the crustacean *O. senex senex* and *S. serrata* (Girish et al. 2018; Venkaiah et al. 2019). These studies highlighted the endocrine control of some RA isomers on ovarian cell processes notably vitellogenesis (Girish et al. 2018; Venkaiah et al. 2019). However, atRA did not induce ovarian maturation in *L. vannamei* (Linan-Cabello and Paniagua- Michel 2004). Having evidence of the complex crosstalk regulation network implicating RAs and RXRs and the differential affinities of -*cis* and -*trans* isomers of RA to RXR for vertebrates, the nature of

the RA isomer could influence the outcome in crustaceans and should thus be considered carefully. The apparent crucial importance of the molecular configuration of RA draws attention to the several isomerization processes that occur in crustacean secretory and target tissues, such as in the ovaries where RA isomerization from *-trans* to *-cis* forms was suggested to occur (Linan-Cabello and Paniagua-Michel 2004). To gain mechanistic insights into this complex biological network, effective extraction procedures coupled with discriminant analytical methods for the separation and quantification of a wide variety of RA isomers in crustacean matrices will be necessary. We could, in a future study, analyze the tissue contents of RETs, in particular, the isomers of RA in gammarids.

In our study, CIT reduced the oocyte production of gravid *G. fossarum* females. CIT also delayed molting, however in a very distinct manner compared to atRA. Citral displayed a typical U-shaped non-monotonic dose-response curve (NMDRCs) which are typical for EDC in many animal models (see review by Vandenberg et al. 2012). To this date, there is limited knowledge on the mechanism of action and the various effects of CIT in the animal kingdom. Yet, its RA synthesis-inhibiting properties have been confirmed in many vertebrates and linked to perturbed oogonial meiosis or limb bud cell differentiation in the fish *Oryzias latipes* and the crustacean *U. pugilator*, respectively (Hopkins 2001; Adolphi et al. 2016). In *G. fossarum*, we previously reported that CIT reduces the tissular RA concentrations and successfully perturb the ratios between RALD and RA (Gauthier et al. 2023). If CIT inhibits the RA biosynthesis in the target and the secretory organs of *G. fossarum*, deficiency in RA could result in RA signaling disorders, which, in turn, could have detrimental effects on oogenesis either directly or via the endocrine system.

In 2007, Abramson et al. reported that molting of the insect *Rhodnius prolixus* was delayed following exposure to CIT vapors. Interestingly, NMDRCs are more likely to occur with chemicals that act through more than one mechanism of action, which, by exerting an effect on RA tissular levels, may be the case for CIT. Retinoic acid is already known to induce certain effects when deficient or in excess such as teratogenicity for vertebrates. In our study, it is important to acknowledge that neither CIT nor atRA reduced molting success. Nevertheless, atRA delayed molting without displaying a clear dose-response relation. Taken together, the similar effects of atRA and CIT on oocyte production support the hypothesis that RA plays a non-negligible role in crustacean oogenesis, potentially through an effect on the oogonial differentiation. It also reinforces the hypothesis that RA has a certain regulatory role in the crustacean molting process. As the exposure to RA synthesis inhibitor CIT elicited a more pronounced effect than the exposure to atRA, we hypothesized that gammarids managed RA excess better than a RA deficiency.

### **MET- and GLY-induced reproductive and developmental toxicity**

Because MET is known to alter embryonic development and metamorphosis of insects, we expected a strong effect on the functions under the endocrine control, specifically the reproduction and development of *G. fossarum*. Unsurprisingly, MET reduced the production of embryos, oocytes, and juveniles and increased the duration of the molting cycle. Embryotoxicity was observed by Kim and Hwang (2002) following 3-week exposure to approximately 15.5 µg/L of MET. In parallel, repeated injections of sublethal MET concentrations (15 µg/L, one-tenth of the 48 h-LD50) in the freshwater crab *Travancoriana*

*schirnerae* also induced lowered diameters of the oogonia and the oocytes in all phases of the vitellogenesis and important histopathological anomalies in the ovaries (Padmanabhan et al. 2017). In another study, juveniles of *Neocaridina davidi* molted 21.1% and 25.3% less frequently following 56-day exposure to 100 and 200 µg/L of MET, respectively, resulting in smaller individuals compared to the controls (Hu et al. 2019). To this date, the precise molecular targets explaining the MET-induced physiological perturbations in crustaceans are in isomers of RA in favor of a more transcriptionally active isomer all-*trans* retinoic acid (Gauthier et al. 2023). Also, the fact that RA, MET, and its metabolite methoprenic acid are potent ligands for RXR in vertebrates (Harmon et al. 1995) raises interrogation relative to the interaction of MET and RXR in crustaceans. This interaction was suggested as an explanation for developmental abnormalities observed in the regenerating limb of *U. pugilator* (Stueckle et al. 2008). Given our results, although RA, CIT, and MET all showed clear effects on the oocyte production and, to some extent on molting, they had different effects on embryo production. Our results support a potential implication of RA in the MET-induced effect on oogenesis and molting. However, it is not the case for embryotoxicity. Although both MET and RA may share RXR as a ligand in some secretory or target tissues, it is not known whether the interaction of MET and RXR influences the affinity of RXR for RA isomers, or even for the ecdysone receptor (EcR), and if such mechanism could lead to RA or ecdysteroid signaling disorders in crustaceans. As free and conjugated ecdysteroids are known to orchestrate vitellogenesis and ultimately carry the oocyte throughout various developmental checkpoints, disruption of the hormonal system could lead to the oogenesis and molting defects observed in our study.

In our study, we showed that GLY affects molting in female *G. fossarum*. Whereas the lowest concentration caused significant molting delays, the highest concentrations completely inhibited the molting process, and consequently, the molting success. GLY also caused a trend of decreased juvenile production by females. Similar effects of GLY and GLY-based herbicides (GBH) on reproduction and development have also been shown in other crustaceans (Mensah et al. 2012; Cuhra et al. 2013). Disruption of gene expression implicated in the endocrine system in the crustacean *Macrobrachium potiuana* has been observed and may explain some effects on crustacean reproduction and development (de Melo et al. 2019). However, the toxicodynamics of GLY in crustaceans is not well understood. As exposures to atRA and CIT were unsuccessful to reduce molting success, reduced molting success at high concentrations of GLY is not likely to be mediated by RA. However, the distinct effect of GLY on molting recorded at the lowest concentration could be the result of perturbed RA tissular levels. Glyphosate has already been associated to perturbation of RET concentrations in invertebrates. In the honeybee *Apis mellifera*, a 10-day exposure to GLY caused a diminution of a RA precursor, all-*trans* ROL (Hedrei Helmer et al. 2014). At this point, our results are not refuting a possible mediation of RA in the GLY-induced molting delays observed at the lowest concentration tested. However, additional research will be needed to further study the effects of GLY on tissular RET levels in crustaceans.

Following a 3-month trial study where MET was applied for mosquito control in lakes following the recommended application rates, MET was detected in 50% of water surface samples with a mean concentration of 26 µg/L and ranging at up to 45 µg/L (Hershey et al. 1995). Because we observed reproductive and developmental defects in *G. fossarum* females following exposure to low concentrations of MET (respectively 0,6 µg/L and 0.25 µg/L), MET

may pose risks to non-target invertebrates. GLY is also frequently found in surface waters draining farming areas. For example, in Canada and Brazil, GLY was found at concentrations ranging up to 140 µg/L and 2.02 mg/L, respectively (Freire et al. 2012); the latter being close to the concentrations investigated in our study and susceptible to induce molting delays. If the extent of the molting delays (from 30 to 55 h) and the reduced oocyte count (from 14.6 to 64.5% of reduction) were to be observed in situ, a lowered reproductive success in the following cycles would be expected and could severely hinder the population dynamics. The results obtained in our study relative to the environmental risks posed by pesticides are conservative as pesticides are rarely used alone, and most of the time associated with surfactants that enhance their toxicity in live organisms. It is important to highlight that the observed molting delays following exposure to all compounds occurred in conditions that did not influence the juvenile hatching kinetics (Fig. 3) which attest to the synchronization of reproductive cycles in exposed females. This also further confirms the effects occurring specifically on reproduction or molting.

## Conclusion

In this study, we investigated the influence of four compounds of physiological and toxicological interest (atRA, CIT, MET, and GLY) on reproductive and developmental markers in gravid *G. fossarum* females. First, our results showed the implication of RA on two endocrine-controlled processes, notably oogenesis and molting, with CIT eliciting a typical EDC NMDRC on molting. Secondly, we confirmed that MET, known for its ED properties in crustaceans, has important negative effects on the production of oocytes, embryos, juveniles, and molting in *G. fossarum*. In parallel, we showed that GLY delays or completely stops molting at low and high concentrations, respectively. That led us to hypothesize that RA could be a mediator of MET-induced effects on oogenesis and molting whereas it is unlikely to be implicated in the effects on embryos. In addition, we also hypothesize that RA may be implicated in the GLY-induced inhibition found at high concentration. RETs are currently investigated as potential EDC targets for vertebrates. This raises interrogation relatively to the modulation of RETs in crustacean's neuroendocrine system and may be an avenue to decipher this complex regulatory network. Also, these results suggest that the RET metabolism leading to the formation of RA is an interesting candidate for biomarker development in *G. fossarum* and merits further research. A better understanding of the implication of RETs in the reproductive and developmental physiology of *G. fossarum* paves the way to the finding of new biomarkers in this model, which ultimately will profit biomonitoring programs.

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Author contribution M.G. codesigned the experiments, carried out the laboratory work and statistical analyses, interpreted the results, built the corresponding tables and figures, and wrote

the manuscript. J.D. participated to the laboratory work and preliminary statistical analyses. E.V. supervised the chemical analyses and approved the final version of the manuscript. O.G. codesigned the experiments supervised the project and revised the manuscript. M.B., C.J., and J.G. revised and approved the final version of the manuscript.

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**Data availability** Data will be made available upon reasonable request.

## References

- Abramson CI, Aldana E, Sulbaran E (2007) Exposure to citral, cinnamon and ruda disrupts the life cycle of a vector of Chagas disease. *Am J Environ Sci* 3(1):7–8. [https:// doi. org/ 10.3844/ ajessp.2007.7.8](https://doi.org/10.3844/ajessp.2007.7.8)
- Adolfi MC et al (2016) Retinoic acid and meiosis induction in adult versus embryonic gonads of medaka. *Sci Rep* 6(September):1–13. [https:// doi. org/ 10. 1038/ srep3 4281](https://doi.org/10.1038/srep34281). (Nature Publishing Group)
- Asazuma H et al (2007) Molecular cloning and expression analysis of ecdysone receptor and retinoid X receptor from the kuruma prawn, *Marsupenaeus japonicus*. *Comp Biochem Physiol - B Biochem Mol Biol* 148(2):139–150. [https:// doi. org/ 10. 1016/j. cbpb. 2007. 05. 002](https://doi.org/10.1016/j.cbpb.2007.05.002)
- Boily M, Marjolaine B, Spear PA (2004) Retinoïdes : biomarqueurs et base moléculaire d'effets de substances toxiques. *Ecotoxicologie Moléculaire : Principes fondamentaux et perspectives de développements*. Presses de l'Université du Québec, Sainte-Foy, pp 197–256
- Brovini EM et al (2021) Glyphosate concentrations in global freshwaters: are aquatic organisms at risk? *Environ Sci Pollut Res*. [https://doi. org/ 10. 1007/ s11356- 021- 14609-8](https://doi.org/10.1007/s11356-021-14609-8)
- Bundschuh M et al (2013) 'Effects of peak exposure scenarios on *Gammarus fossarum* using field relevant pesticide mixtures.' *Ecotoxicol Environ Saf* 95:137–143. [https:// doi. org/ 10. 1016/j. ecoenv.2013. 05. 025](https://doi.org/10.1016/j.ecoenv.2013.05.025). (Elsevier)
- Chaumot A et al (2020) 'In situ reproductive bioassay with caged *Gammarus fossarum* (Crustacea): part 1—gauging the confounding influence of temperature and water hardness.' *Environ Toxicol Chem* 39(3):667–677. [https:// doi. org/ 10. 1002/ etc. 4655](https://doi.org/10.1002/etc.4655). (John Wiley & Sons Ltd)
- Chung AC et al (1998) Cloning of crustacean ecdysteroid receptor and retinoid-X receptor gene homologs and elevation of retinoid-X receptor mRNA by retinoic acid. *Mol Cell Endocrinol* 139:209–227 Clagett-Dame M, Knutson D (2011) Vitamin A in reproduction and development. *Nutrients* 3(4):385–428. [https:// doi. org/ 10. 3390/ nu304 0385](https://doi.org/10.3390/nu3040385)

- Cribiu P, Devaux A, Garnero L (2020) A “ population dynamics ” perspective on the delayed life-history effects of environmental contaminations : an illustration with a preliminary study of cadmium transgenerational effects over three generations in the crustacean *Gammarus*. *Int J Mol Sci* 21(4704):1–17
- Cuhra M, Traavik T, Bohn T (2013) Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. *Ecotoxicology* 22(2):251–262. [https:// doi. org/10. 1007/ s10646- 012- 1021-1](https://doi.org/10.1007/s10646-012-1021-1)
- de Melo MS et al (2019) ‘Effects of low glyphosate-based herbicide concentrations on endocrine-related gene expression in the decapoda *Macrobrachium potiuna*.’ *Environ Sci Pollut Res* 26(21):21535–21545. [https:// doi. org/ 10. 1007/ s11356- 019- 05496-1](https://doi.org/10.1007/s11356-019-05496-1)
- Durica DS et al (2002) Characterization of crab EcR and RXR homologs and expression during limb regeneration and oocyte maturation. *Mol Cell Endocrinol* 189(1–2):59–76. [https:// doi. org/10. 1016/ S0303- 7207\(01\) 00740-7](https://doi.org/10.1016/S0303-7207(01)00740-7)
- Freire R, Schneider RM, de Freitas FH, Bonifacio CM, Tavares CRG (2012) Monitoring of toxic chemical in the basin of Maringa stream. *Acta Sci Technol* 34(3):295–302. [https:// doi. org/ 10. 4025/ actas citec hnol. v34i3. 10302](https://doi.org/10.4025/actas.citec.hnol.v34i3.10302)
- Gauthier M et al (2018) ‘Chronic exposure to imidacloprid or thiamethoxam neonicotinoid causes oxidative damages and alters carotenoid-retinoid levels in caged honey bees (*Apis mellifera*).’ *Sci Rep* 8(16274):1–11. [https:// doi. org/ 10. 1038/ s41598- 018- 34625-y](https://doi.org/10.1038/s41598-018-34625-y). (Springer, US)
- Gauthier M et al (2023) The retinoid metabolism of *Gammarus fossarum* is disrupted by exogenous all-trans retinoic acid, citral, and methoprene but not by the technical formulation of glyphosate. *Ecotoxicol Environ Saf* 252(February):0–9. [https:// doi. org/ 10.1016/j. ecoenv. 2023. 114602](https://doi.org/10.1016/j.ecoenv.2023.114602)
- Geffard O et al (2010) ‘Ovarian cycle and embryonic development in *Gammarus fossarum*: application for reproductive toxicity assessment.’ *Environ Toxicol Chem* 29(10):2249–2259. [https://doi. org/ 10. 1002/ etc. 268](https://doi.org/10.1002/etc.268). (John Wiley & Sons Ltd)
- Girish BP, Swetha CH, Reddy PS (2015) Expression of RXR, EcR, E75 and VtG mRNA levels in the hepatopancreas and ovary of the freshwater edible crab, *Oziotelphusa senex senex* (Fabricius, 1798) during different vitellogenic stages. *Science of Nature* 102(3–4):1–10. [https:// doi. org/ 10. 1007/ s00114- 015- 1272-9](https://doi.org/10.1007/s00114-015-1272-9)
- Girish BP et al (2018) ‘Evidence for retinoic acid involvement in the regulation of vitellogenesis in the fresh water edible crab, *Oziotelphusa senex senex*.’ *Comp Biochem Physiol Part A* 222(April):1–6. [https:// doi. org/ 10. 1016/j. cbpa. 2018. 04. 003](https://doi.org/10.1016/j.cbpa.2018.04.003). (Elsevier)
- Gouveia D et al (2018) Identification, expression, and endocrine-disruption of three ecdysone-responsive genes in the sentinel species *Gammarus fossarum*. *Sci Rep* 8(1):1–13. [https:// doi. org/ 10. 1038/ s41598- 018- 22235-7](https://doi.org/10.1038/s41598-018-22235-7)
- Harmon M, a, et al (1995) Activation of mammalian retinoid X receptors by the insect growth regulator methoprene. *Proc Natl Acad Sci USA* 92(13):6157–6160. [https:// doi. org/ 10. 1073/ pnas. 92. 13. 6157](https://doi.org/10.1073/pnas.92.13.6157)

- Hedrei Helmer S et al (2014) Effects of realistic doses of atrazine, metolachlor, and glyphosate on lipid peroxidation and diet-derived antioxidants in caged honey bees (*Apis mellifera*). *Environ Sci Pollut Res Int* 22:8010–8021. <https://doi.org/10.1007/s11356-014-2879-7>
- Hershey AE et al (1995) Effects of methoprene and Bti (*Bacillus thuringiensis* var. *israelensis*) on non-target insects. *Hydrobiologia* 308:219–227
- Hopkins PM (2001) Limb regeneration in the Fiddler Crab, *Uca pugilator*: hormonal and growth factor control. *Am Zool* 41(3):389–398. <https://doi.org/10.1093/icb/41.3.389>
- Hopkins PM, Durica DS (1995) Effects of all-trans retinoic acid on regenerating limbs of the fiddler crab, *Uca pugilator*. *J Exp Zool* 272(6):455–463
- Hopkins PM, Durica D, Washington T (2008) ‘RXR isoforms and endogenous retinoids in the fiddler crab, *Uca pugilator*.’ *Comp Biochem Physiol Part A: Mol Integr Physiol* 151(4):602–614. <https://doi.org/10.1016/J.CBPA.2008.07.021>. (Pergamon)
- Hu XL et al (2019) ‘Effects of two juvenile hormone analogue insecticides, fenoxycarb and methoprene, on *Neocaridina davidi*.’ *Environ Pollut* 253:89–99. <https://doi.org/10.1016/j.envpol.2019.06.120>. (Elsevier Ltd)
- Jumarie C, Aras P, Boily M (2017) ‘Mixtures of herbicides and metals affect the redox system of honey bees.’ *Chemosphere* 168:163–170. <https://doi.org/10.1016/j.chemosphere.2016.10.056>. (Elsevier Ltd)
- Kam RKT et al (2012) Retinoic acid synthesis and functions in early embryonic development. *Cell Biosci* 2(1):11. <https://doi.org/10.1186/2045-3701-2-11>. (Biomed Central Ltd)
- Kim HW, Sung GL, Mykles DL (2005) Ecdysteroid-responsive genes, RXR and E75, in the tropical land crab, *Gecarcinus lateralis*: differential tissue expression of multiple RXR isoforms generated at three alternative splicing sites in the hinge and ligand-binding domains. *Mol Cell Endocrinol* 242(1–2):80–95. <https://doi.org/10.1016/j.mce.2005.08.001>
- Kim PG, Hwang SH (2002) Endocrine disruptors in developing embryo on *Daphnia magna*. *Korean J Environ Health* 28(4):17–22
- Lawler SP (2017) Environmental safety review of methoprene and bacterially-derived pesticides commonly used for sustained mosquito control. *Ecotoxicol Environ Saf* 139(December 2016):335–343. <https://doi.org/10.1016/j.ecoenv.2016.12.038>. (Elsevier Inc)
- Li Z et al (2014) Identification of spliced mRNA isoforms of retinoid X receptor (RXR) in the Oriental freshwater prawn *Macrobrachium nipponense*. *Genet Mol Res* 13(2):3914–3926. <https://doi.org/10.4238/2014.May.23.2>
- Linan-Cabello MA, Paniagua-Michel J (2004) Induction factors derived from carotenoids and vitamin A during the ovarian maturation of *Litopenaeus vannamei*. *Aquacult Int* 12:583–592
- Matozzo V, Fabrello J, Marin MG (2020) ‘The effects of glyphosate and its commercial formulations to marine invertebrates: A review’. *J Mar Sci Eng* 8(6). <https://doi.org/10.3390/JMSE8060399>

Mensah PK, Muller WJ, Palmer CG (2012) 'Using growth measures in the freshwater shrimp *Caridina nilotica* as biomarkers of Roundup® pollution of South African freshwater systems.' *Phys Chem Earth* 50–52:262–268. <https://doi.org/10.1016/j.pce.2012.08.003>. (Elsevier Ltd)

Nagaraju GPC, Rajitha B, Borst DW (2011) Molecular cloning and sequence of retinoid X receptor in the green crab *Carcinus maenas*: a possible role in female reproduction. *J Endocrinol* 210(3):379–390. <https://doi.org/10.1530/JOE-11-0154>

OECD (Organisation for Economic Cooperation and Development) (2021) Detailed review paper on the retinoid system. Series on Testing and Assessment, No. 343

Padmanabhan LN, Raghavan SDA, Ayanath A (2017) Impact of methoprene on oogenesis of the freshwater crab *Travancoriana schirnerae* Bott, 1969 (Decapoda: Gecarcinucidae). *Braz J Biol Sci* 4(7):165–179. <https://doi.org/10.21472/bjbs.040717>

Paganelli A et al (2010) Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. *Chem Res Toxicol* 23(10):1586–1595. <https://doi.org/10.1021/tx1001749>

Peng C et al (2020) 'Retinoic acid and androgen influence germ cells development and meiotic initiation in juvenile orangespotted grouper, *Epinephelus coioides*.' *Gen Comp Endocrinol* 289(August 2019):113379. <https://doi.org/10.1016/j.ygcen.2019.113379>. (Elsevier)

Priya TAJ et al (2009) Molecular characterization and effect of RNA interference of retinoid X receptor (RXR) on E75 and chitinase gene expression in Chinese shrimp *Fenneropenaeus chinensis*. *Comp Biochem Physiol - B Biochem Mol Biol* 153(1):121–129. <https://doi.org/10.1016/j.cbpb.2009.02.009>. (Elsevier B.V)

Reddy PS, Sainath SB (2008) Effect of retinoic acid on hemolymph glucose regulation in the fresh water edible crab *Oziotelphusa senex senex*. *Gen Comp Endocrinol* 155(3):496–502. <https://doi.org/10.1016/j.ygcen.2007.09.013>

Smith CA et al (2008) Onset of meiosis in the chicken embryo; evidence of a role for retinoic acid. *BMC Dev Biol* 8:1–19. <https://doi.org/10.1186/1471-213X-8-85>

Sreenivasula RP, Srilatha M (2015) 13-Cis-retinoic acid-induced hyperglycemia in the fresh water edible crab, *Oziothelphusa senex senex* is mediated by triggering release of hyperglycemic hormone from eyestalks. *J Aquac* 6(8):6–10. <https://doi.org/10.4172/2155-9546.1000356>

Stueckle TA, Likens J, Foran CM (2008) Limb regeneration and molting processes under chronic methoprene exposure in the mud fiddler crab, *Uca pugnax*. *Comp Biochem Physiol, Part C: Toxicol Pharmacol* 147(3):366–377. <https://doi.org/10.1016/j.cbpc.2008.01.004>

Tanaka M, Tamura K, Ide H (1996) Citral, an inhibitor of retinoic acid synthesis, modifies chick limb development. *Dev Biol* 175(2):239–247. <https://doi.org/10.1006/dbio.1996.0111>

Techa S, Chung JS (2013) Ecdysone and retinoid-X receptors of the blue crab, *Callinectes sapidus*: cloning and their expression patterns in eyestalks and Y-organs during the molt cycle. *Gene* 527(1):139–153. <https://doi.org/10.1016/j.gene.2013.05.035>. (Elsevier B.V)

- Vandenberg LN et al (2012) Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev* 33(3):378–455. <https://doi.org/10.1210/er.2011-1050>
- Venkaiah K, Thathapudi D, Sainath SB (2019) ‘Detection and mode of action of retinoids on ovarian development in the mud crab *Scylla Serrata*.’ *Int J Aquat Biol* 7:245–253
- Wade NM, Gabaudan J, Glencross BD (2015) A review of carotenoid utilisation and function in crustacean aquaculture. *Rev Aquac* 9(2):141–156. <https://doi.org/10.1111/raq.12109>
- Wallacides A et al (2009) Evidence for a conserved role of retinoic acid in urodele amphibian meiosis onset. *Dev Dyn* 238(6):1389–1398. <https://doi.org/10.1002/dvdy.21948>
- Wang YH, Wang G, LeBlanc GA (2007) Cloning and characterization of the retinoid X receptor from a primitive crustacean *Daphnia*
- Wilder M, Okumura T, Tsutsui N (2010) Reproductive mechanisms in Crustacea focusing on selected prawn species: vitellogenin structure, processing and synthetic control. *Aqua BioSci Monogr* 3(3):73–110. <https://doi.org/10.5047/absm.2010.00303.0073>
- Zaller JG, Bruhl CA (2019) Editorial: non-target effects of pesticides on organisms inhabiting agroecosystems. *Front Environ Sci* 7(May):1–3. <https://doi.org/10.3389/fenvs.2019.00075>