Marine Pollution Bulletin 227 (2026) 119486

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

miRNome profiling in *Tritia mutabilis* embryos exposed to tributyltin: insights into developmental toxicity

Paolo Cocci, Gilberto Mosconi, Francesco Alessandro Palermo *

School of Biosciences and Veterinary Medicine, University of Camerino, Italy

ARTICLE INFO

Keywords:
Tributyltin
Endocrine disruptors
Tritia mutabilis
miRNA
developmental toxicity

ABSTRACT

Tributyltin (TBT) is a widespread marine contaminant that affects the development and reproduction of marine invertebrates; however, its molecular impacts on early embryonic development remain poorly understood. In this study, we investigated the effects of environmental concentrations of TBT on the miRNA expression profiles of the marine gastropod *Tritia mutabilis* during intracapsular embryogenesis. Embryos were exposed for 10 days to low (10^{-12} M) and high (10^{-10} M) concentrations of TBT, and the differential expression of miRNAs was assessed by high-throughput sequencing. Obtained results revealed a significant modulation of several miRNAs across treatments, with a set of 11 miRNAs responding to both low and high TBT concentrations, while miR-486-5p and miR-183-5p were specifically modulated under the low TBT concentration, and miR-263b, miR-184, and miR-100-5p were exclusive to the highest concentration. Pathway analyses identified a range of biological processes affected, including nervous system development, cellular functions such as proliferation and cell growth, signal transduction, cellular component assembly and cell-cell interactions. Notably, several pathways were highly enriched (i.e., ≥100 regulated target genes) under both conditions, including focal adhesion, Ras signaling, regulation of actin cytoskeleton, Rap1 signaling, cAMP signaling, calcium signaling, and cGMP-PKG signaling pathways, highlighting the vulnerability of developmental and cellular communication networks, and further supported by the expression analysis of the corresponding miRNA-regulated target genes. These findings demonstrate that TBT contributes to the developmental abnormalities of marine gastropod embryos by modulating miRNA-mediated control of gene transcription. Our results contribute to advancing the understanding of miRNAs' potential utility as biomarkers for environmental monitoring.

1. Introduction

Tributyltin (TBT) is a highly toxic chemical compound that has been widely used in antifouling paints on ships to prevent the growth of marine organisms such as barnacles, algae, and molluscs (Kroon et al., 2020; Cocci et al., 2021a). However, despite its effectiveness, TBT has raised significant environmental concerns due to its persistence, bioaccumulation, and detrimental effects also on non-target marine organisms, particularly gastropods. The impact of TBT on marine gastropods, a class of invertebrates that includes various species of slugs and sea snails, has been a subject of extensive scientific investigation. Marine snails play crucial roles in ecosystems as both prey and predators, and any disturbances in their populations may affect ecological communities (Hu et al., 2022). TBT exposure in marine gastropods has been associated with a range of adverse effects across different developmental or adult stages. Even at low concentrations, TBT can disrupt normal physiological functions such as growth and immune system, cause imposex and lead to reproductive failures, ultimately affecting population dynamics (Cocci et al., 2021a; Beyer et al., 2022; Hu et al., 2022; Øystein Hjermann et al., 2022; Chi-Ho Ip et al., 2024). It can also induce genetic and epigenetic alterations, affecting gene expression patterns and disrupting developmental pathways critical for the proper growth and survival of these organisms (Cocci et al., 2021a; Srut et al., 2023). During critical developmental stages, such as exposure of encapsulated embryos within protective egg masses, exposure to TBT has been shown to disrupt normal embryonic development, leading to various adverse effects (Cocci et al., 2021a). Toxic effects of TBT can impair organogenesis, hinder proper growth and differentiation, and induce abnormalities in developing embryos (Choi et al., 2023; Svigruha et al., 2024). In this regard, it has been recently demonstrated that exposure to environmental relevant concentrations of TBT induce time-dependent changes in RXR gene transcription and is associated with

* Corresponding author at: School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III Da Varano, I-62032, Camerino, MC, Italy.
E-mail address: francesco.palermo@unicam.it (F.A. Palermo).

https://doi.org/10.1016/j.marpolbul.2026.119486
Received 1 July 2025; Received in revised form 15 November 2025; Accepted 24 February 2026
Available online 4 March 2026

global DNA demethylation, reduced DNA-methyltransferase I (DNMT1) expression and activity levels (Cocci et al., 2021a). In molluscs, miRNAs have emerged as essential regulators of numerous biological processes, including development, immune response, reproduction, and stress adaptation (Abo-Al-Ela and Faggio, 2021; Huang et al., 2021). Small RNA-Seq is considered a powerful tool for unravelling transcriptome complexity and identifying genes and regulatory non-coding RNAs. In this contest, it has been demonstrated that the dysregulation of specific miRNAs involved in the regeneration of the central nervous system, such as miR-124, could potentially disrupt neural development processes in *Lymnaea stagnalis*. This mechanism leads to impaired neurological function or altered behavior in later life stages (Walker et al., 2018; Piazza et al., 2024). Furthermore, it has been reported that specific miRNAs act as biomarkers for biotic and abiotic stress. These latter burdens include changes in water physical/chemical parameters and exposure to chemical pollutants. In the marine gastropod *Littorina littorea*, exposure to temperature stress results in differential expression of miRNAs involved in stress responsive signaling pathways (Biggar et al., 2012; Zhang et al., 2020). MiR-1, which target genes were associated with shell biomineralization (Zhu et al., 2020), show differential expression patterns in response to changes in seawater pH in *Strongylocentrotus intermedius* (Yin et al., 2025). Studies on the *Tegillarca granosa* have shown that exposure to heavy metals, such as cadmium and copper, alters the expression profiles of specific miRNAs involved in detoxification pathways (miR−21 and let-7) (Abo-Al-Ela and Faggio, 2021). Based on our best knowledge, there are no studies on miRNA expression profiles and their target genes in marine gastropods exposed to organotin compounds, especially during embryonic development. To gain an understanding of the impact of TBT on the marine snail *Tritia mutabilis*, we conducted high-throughput small RNA sequencing and profiling of miRNAs during intracapsular development. Moreover, we carried out functional analyses aimed at identifying the main signaling pathways influenced by differentially expressed miRNAs.

2. Materials and methods

2.1. Egg capsules and TBT treatments

Egg capsules of the marine snail (*Tritia mutabilis*) were collected from artificial substrates placed along the western coasts of the Central Adriatic Sea, as previously described (Cocci et al., 2021a, 2021b). Only capsules containing embryos of a single developmental stage were selected for the experiment (i.e., egg capsules with early developing embryos, 3 days post-deposition; <300 μm; (Borysko and Ross, 2014). Embryos were individually placed in 24-well culture plates containing filtered seawater and exposed to two environmentally relevant concentrations of TBT: 10^{-12} M (TBT1) and 10^{-10} M (TBT2) for 10 days according to Cocci et al. (2021a). Embryonic mortality and morphological changes were monitored daily with a stereomicroscope (Carl Zeiss Stemi™) and images were examined by a USB Camera (Optika B Series) using the Optika ProView software.

2.2. Total RNA isolation

Total RNA was isolated from *T. mutabilis* samples, consisting of a pool of 12 eggs for each treatment (negative control, TBT1, TBT2), adding 1 ml of Trizol reagent (Invitrogen, Carlsbad, CA, USA). The pooling of samples is a method already utilized in miRNA studies on gastropods to enhance sequencing capacity and efficiency, as the isolated material alone is often insufficient for comprehensive analysis (Walker et al., 2018; Yang et al., 2023; Dametto et al., 2025). The quantitation of RNA was achieved with the Qubit RNA HS Assay Kit, and the RNA integrity and quality were assessed with the Qubit RNA IQ Assay Kit using a Qubit™ 4 Fluorometer (Thermo Fisher Scientific).

2.3. Libraries construction

For sequencing, 1 μg of total RNA was used to generate the miRNA sequencing library. Extracted RNAs were pooled together in each group to produce homogenous sample material (Assefa et al., 2020) and to provide sufficient amounts of RNA for both sequencing and qPCR analysis. In this study, nine cDNA libraries (three groups each including three replicates) were prepared. Briefly, a pair of adaptors was ligated sequentially to the 3′ and 5′ ends of miRNA (RA3: 5′ TGGAATTCTCGGGTGCCAAGG; RA5: 5′ GUUCAGAGUUCUACAGUCCGACGAUC; Illumina), and following ligation with adaptors, a library was prepared by reverse transcription and PCR pre-amplification using TruSeq® Small RNA Library Preparation Kits (Illumina). The quality of the library was measured by the Qubit™ RNA Assay High Sensitivity Kits using Qubit 4.0 Fluorometer (ThermoFisher).

2.4. Illumina sequencing for miRNOME analysis

The normalized library (concentration of 2 nM) was loaded into the Illumina iSeq100 system (Illumina), using a cartridge based on iSeq 100 i1 Reagent v2 chemistry (single read: 80 cycles; read length 1 × 75 bp). Raw sequence data (.bcl files) generated from Illumina iSeq100 system were converted into fastq files and demultiplexed by Local Run Manager (Illumina). Quality control check was verified (Table 1) using FastQC (Illumina). Sequences were analyzed using a bioinformatics approach to miRNA-sequencing analysis described by Potla et al. (2021) and Song et al. (2017). Sequences were trimmed to remove adapters using CLC Genomics Workbench 24.0.1 (trim settings: automatic removal of read-through adapter sequences; removal of low-quality sequences. Reads with poly-N were also filtered to obtain clean reads. Other detected RNAs (e.g., repeat sequences, rRNA, tRNA, snRNA, and IsomiR) were deleted after blasting against the GenBank database, according to Song et al. (2017). Reads shorter than 18 bp and longer than 30 bp were discarded. Cleaned miRNA sequences were aligned using BaseSpace Small RNA v1.0.0 (Illumina) and CLC Genomics Workbench 24.0.1 (setting parameters: maximum mismatches 2, additional/missing bases 2). Due to the absence of an annotated genome for *Tritia* species, conserved miRNA identification was performed using BLAST analyses against the human genome (reference Illumina library) and against molluscan known miRNAs available in miRbase (i.e, *Lottia gigantea* and *Haliotis rufescens*). Differential expression for small RNA-Seq and heat map was performed using CLC Genomics Workbench 24.0.1 [normalization method: TMM; (Robinson and Oshlack, 2010)]. miRNA was considered differentially expressed with a fold change (FC) of ≥1.5 (or ≤−1.5), with an FDR-adjusted *p*-value ≤0.05.

2.5. Quantitative real-time PCR (qRT-PCR) validation

To confirm the accuracy of miRNA-seq results the polyadenylation of RNA samples has been carried out using the Poly(A) tailing of RNA kit by Applied Biological Materials (ABM). Briefly, RNA (1 μg), ATP (10 mM), Poly (A) polymerase (1 U/μl), 10× Poly (A) polymerase reaction buffer and RNAse-free ddH_2O have been added to a sterile tube sitting on ice, centrifugated and incubated at 37 °C for 30 min. cDNA synthesis was performed using the OneScript® Hot Reverse Transcriptase (ABM). Briefly, the reaction was developed in a final volume of 20 μl containing universal poly-T-adapter primer (2 μM; 5′-GCGAGCACAGAATTAATACGACTCACTATAGGT$_{12}$VN–3′; Eurofins Genomics), dNTPs, 5× RT Buffer, OneScript® Hot RTase, nuclease-free H2O, and polyadenylated RNA. The tubes were incubated at 60 °C for 40 min and 85 °C for 3 min. In order to measure the expression of miRNAs, a qRT-PCR has been carried out on the 7300 RealTime PCR system by Applied Biosystem using the yourSIAL® Green Mix 2× reagent (Sial Group) in a final volume of 20 μl containing 2 μl cDNA together with the 0.8 μl forward (i.e., the mature miRNA sequence) and 0.8 μl reverse primer (i.e., the universal adapter sequence, 5′-

Table 1
Quality control checks on the total raw sequence data using FastQC (Illumina).

Total cluster	%PF	% ≥ Q30	Sample	Illumina Index	Input reads	Reads with adapters	Read passed (>18 and <30 bp)
3,891,718	58.5	85.5	Control	RPI04	1,186,740	999,265	754,546
			TBT1	RPI08	1,148,111	987,021	712,650
			TBT2	RPI10	1,081,061	924,784	685,489

Q30: Quality score 99.9%.
%PF: Cluster Passing Filter.

GCGAGCACAGAATTAATACGAC-3′). Expression levels of miRNA in samples were normalized with respect to the housekeeping miR-16-5p. The amplification reaction has been reached with the following cycle: 95 °C for 2 min, followed by 5 s at 95 °C and 30 s at 60 °C (repeated 40 times). At the end of the process, a melting curve was performed for each reaction to discriminate among non-specific amplicons. To confirm qPCR products a gel electrophoresis method has been used. The relative mRNA expression levels were analyzed with the method of $2^{-\Delta\Delta Ct}$.

2.6. miRNA target sequence computational analysis

To investigate the pathways that could be regulated by the miRNAs whose expression changed in our samples, we used DIANA-mirPath v3 (https://dianalab.e-ce.uth.gr/html/mirpathv3/index.php? r = mirpath). The parameters that have been set were micro-T CDS as database (micro-T threshold: 0.5), the *p*-value threshold to 0.05 with the FDR correction and Kyoto Encyclopaedia of Genes and Genomes (KEGG) database show pathways in which at least eight miRNAs regulated target genes. This tool allowed us to determine the functional implications of miRNA modulation under TBT exposure.

3. Results and discussion

3.1. miRNome analysis of T. mutabilis embryos

The miRNA-sequencing dataset is presented in the Supplementary data. The results and the quality of sequencing are shown in Table 1.

An average of 1.1 million raw reads was obtained from this analysis. After filtering, around 80% of the raw reads passed the quality control inspection, and the reads in the 18–30 nucleotide range represented 72% to 76% of the total. The analysis of length distribution shows that the majority of miRNAs are 20–25 nt in size, revealing that the mappable reads are aligned with the characteristic length of mature miRNAs. After alignment with the known 2127 miRNAs, a total of 922 known miRNAs were detected in the control group, and 678 and 608 for TBT1 and TBT2, respectively, at counts ≥1 (Supplementary data). Approximately 90% of the miRNA reads were concentrated on 27 most abundant mature miRNAs. Among them, the most represented miRNAs were miR-3672

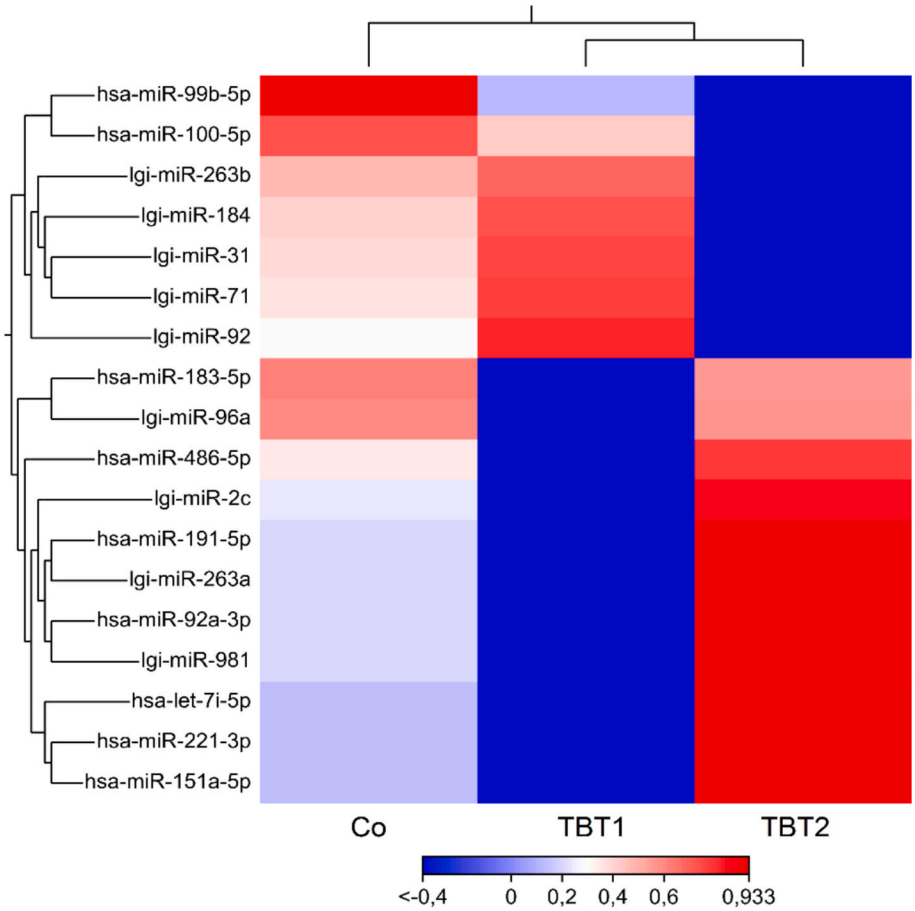

Fig. 1. Two-dimensional heat map of miRNA expression identified in marine snail *T. mutabilis* embryos exposed for 10 days to TBT (10^{-10} M and 10^{-12} M). The heatmap was obtained using CLC Genomics Workbench 24.0.1 (Scale bar: z-score normalization).

(70,500 counts), miR-512-5p (25,709 counts) and miR-499a-5p (25,234 counts).

3.2. miRNome regulation after exposing egg capsules to TBT

Differential expression analysis of abundant miRNA (with counts ≥1000; (Presslauer et al., 2017; Feng et al., 2020) performed using computational tools and statistics by CLC Genomics Workbench 24.0.1 generated the heatmap plot describing the significant ($P < 0.05$) upregulation and downregulation of miRNAs during treatments (Fig. 1). Eighteen differentially expressed miRNAs were considered for further analysis based on fold change values. These miRNAs detected by the iSeq100 system were validated by qPCR analysis and the results indicated consistent expression level (Supplementary data Figs. 2, 3).

Among them, 13 miRNAs in the TBT1 group and 14 miRNAs in the TBT2 group met the threshold criteria of FC ≥ 1.5. Interestingly, a total of 11 miRNAs were commonly regulated in embryos during the treatment with both TBT1 and TBT2 while miR-486-5p and miR-183-5p were specifically modulated under TBT1 treatment, and miR-263b, miR-184, and miR-100-5p were exclusive to TBT2 (Fig. 2). This differential pattern suggests that although a core set of miRNAs is involved in a general response to TBT exposure, specific miRNAs may be selectively activated depending on the chemical concentration of TBT. This miRNA specificity might reflect distinct response mechanisms, metabolic adjustments, or toxicity pathways generated by each dose.

Exposure of marine gastropod egg capsules to TBT (10^{-12} and 10^{-10} M) resulted in significant, dose-dependent alterations in miRNA expression. In eggs exposed to a lower concentration of TBT (10^{-12} M), three miRNAs were upregulated - miR-31, miR-71, and miR-92 - with low fold-change increases of 1.52, 1.51, and 1.56, respectively ($p < 0.01$). Conversely, the majority of miRNAs showed significant downregulation under TBT1 conditions. Notably, miR-221-3p, miR-151a-5p, and let-7i-5p showed approximately 8.0-fold decreases, while miR-191-5p, miR-263a, miR-92a-3p, and miR-981 demonstrated fold-change decreases more than 14-fold. Among these, miR-2c was the most down-regulated miRNA, with a fold-change decrease of approximately 21.6 ($p = 0.003$). Regarding miRNA selectively regulated by only the low dose (TBT1), miR-183-5p and miR-486-5p displayed a significant downregulation of approximately 1.6-fold. These regulatory patterns suggest that environmental concentrations of TBT may interfere with the development of embryos by regulating miRNAs essential for normal embryogenesis. Similarly, our recent data indicated that exposure to low doses of TBT (10^{-12} M) alters important functions during the early stages of *T. mutabilis* embryo development and induce potential epigenetic alterations (Cocci et al., 2021a). In this work, we demonstrated that environmentally relevant concentrations of TBT negatively affected embryo growth, developmental timing, and induced molecular alterations such as time-dependent changes in RXR transcription, global DNA hypomethylation, and reduced DNMT1 expression and activity (Cocci et al., 2021a). In embryos exposed to the highest TBT concentration (10^{-10} M), an opposite trend was observed for specific miRNAs (Fig. 2). Several miRNAs that were downregulated at the lowest dose of TBT, such as miR-221-3p, miR-151a-5p, and let-7i-5p, showed significant upregulation, with fold-change increases of approximately 1.90. This trend extends to include miR-191-5p, miR-263a, miR-92a-3p, miR-981, and miR-2c, which exhibited fold-change increases between 1.93 and 1.99. On the other hand, miR-31, miR-71, and miR-92, which were upregulated at TBT1, displayed significant downregulation at TBT2. Specifically, miR-31 decreased by 29.49-fold, while miR-71 and miR-100-5p were downregulated by 22.44-fold and 15.39-fold, respectively. Currently, there is a lack of studies specifically investigating the effects of TBT on miRNA expression in marine invertebrates. Our study is the first that highlights the intricate relationship between specific miRNA and molecular regulatory mechanisms under TBT exposure

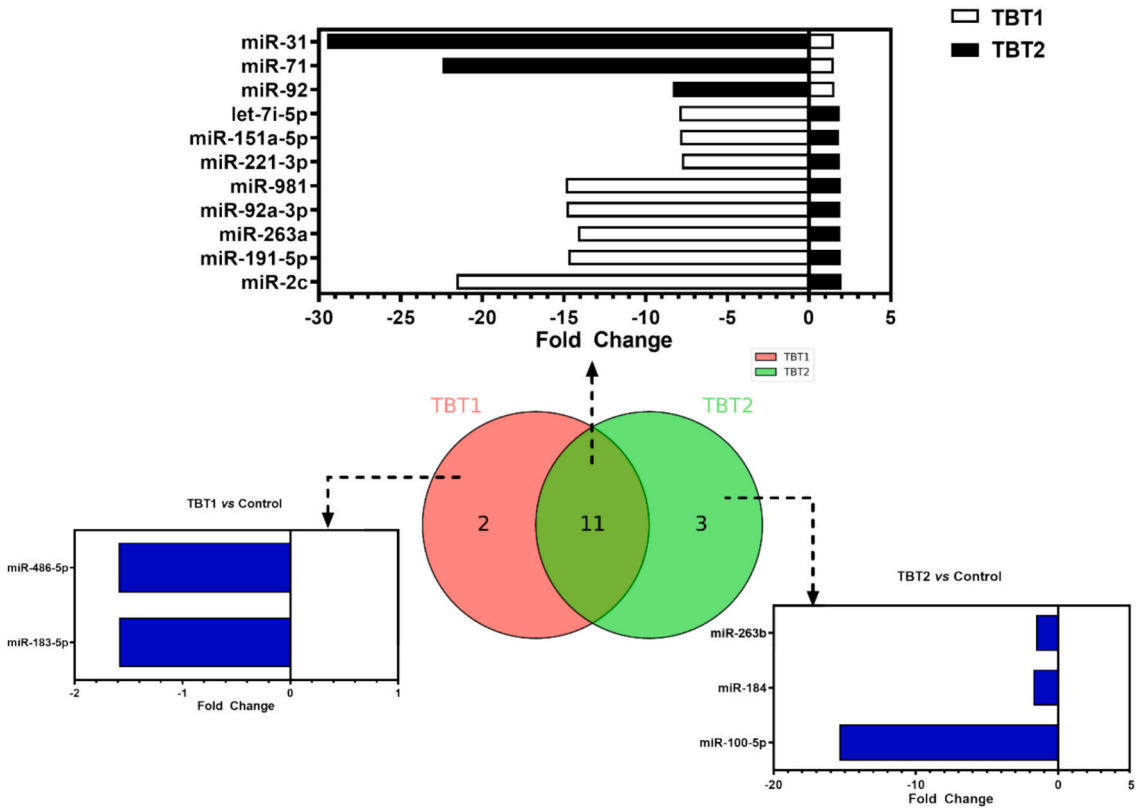

Fig. 2. Venn diagrams of differentially expressed miRNAs among the embryos of *T. mutabilis* exposed to environmental concentrations of TBT. Black and white bars represent the fold-change values expressed in linear scale, with negative values indicating down-regulation, respectively for shared miRNAs. Blu bar represents the fold-change values for the exclusive miRNAs for each TBT dose.

during embryogenesis. For instance, it has been demonstrated that miR-2 is an invertebrate-specific family of miRNAs involved in neural development and maintenance among invertebrates (Marco et al., 2012), and its dysregulation could lead to issues in the development of the nervous system. The let-7 family of miRNAs has been implicated in the regulation of digestion-related genes, suggesting its role in digestive system remodeling during veined rapa whelk (*Rapana venosa*) metamorphosis (Song et al., 2017). Several evolutionarily conserved miRNAs with a small number of isoforms, such as miR-184, miR-100, miR-92 are important immune regulators in molecular responses serving as biomarkers for various biotic and abiotic stressors in molluscs (Huang et al., 2021). These findings support the hypothesis that organotin compounds may pose a significant threat to the early developmental stages of *Tritia mutabilis* embryos.

3.3. Pathways regulated by differentially expressed miRNAs

The analysis of the KEGG pathway demonstrated significant modulation of key miRNAs associated with important cellular processes. However, studies on the miRNA profiles of non-target species questioned difficulties in pathway analysis due to the absence of species-specific pathway databases. To address these limitations, it has been validated the use of cross-species comparisons, utilizing pathway information from model organisms such as humans or mice to infer potential functions of miRNAs in non-model species and to limit the inaccurate or non-representative pathway associations. The target prediction analysis identified 2508 and 2243 target genes following exposure to TBT1 and TBT2, respectively. A core set of miRNAs, including miR-92a-3p, let-7i-5p, miR-221-3p, miR-31-5p, miR-151a-5p, and miR-191-5p, was commonly affected under both TBT1 and TBT2 treatments, suggesting their broad involvement in multiple signaling

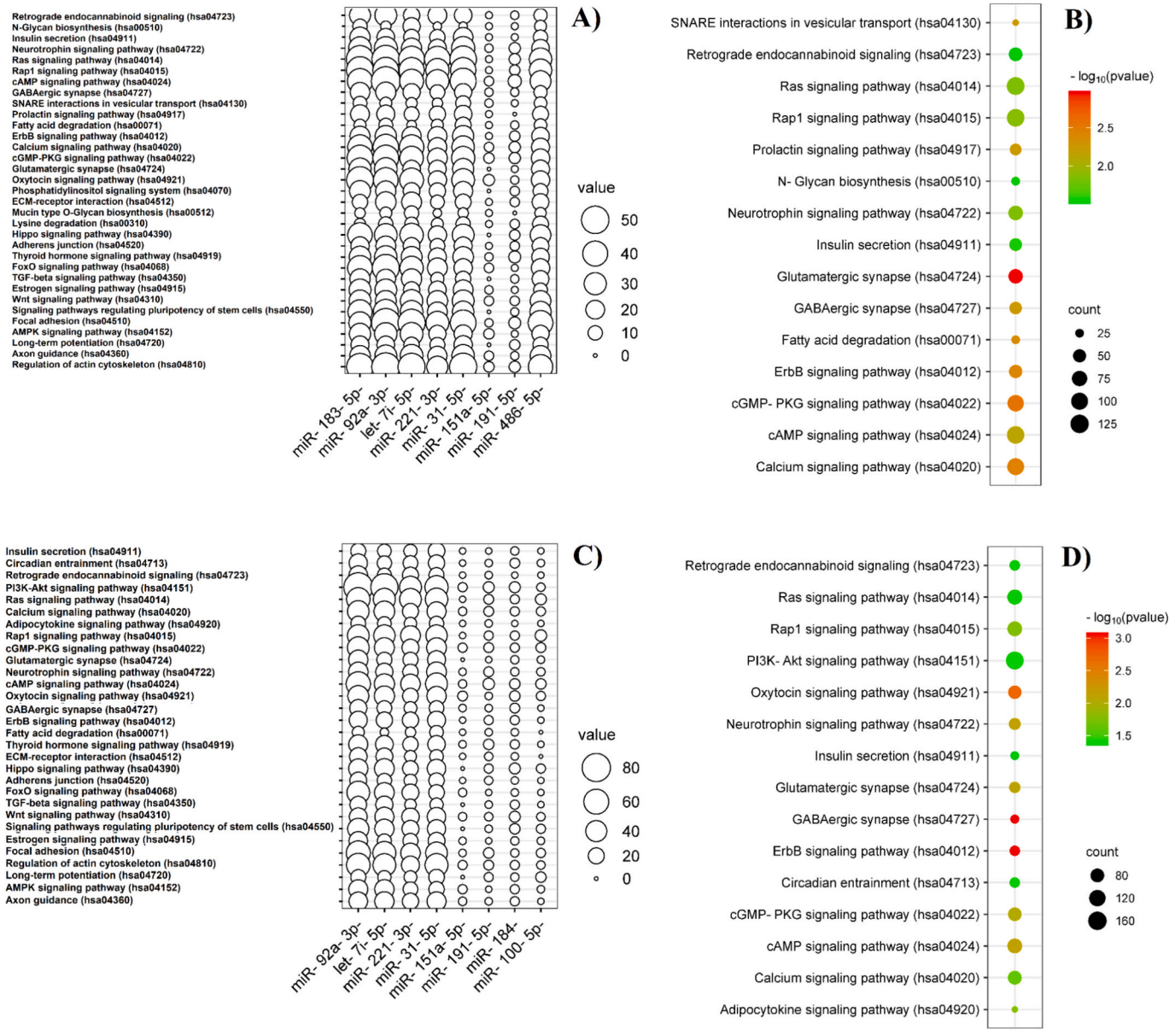

Fig. 3. The prediction of potential pathways regulated by the differentially expressed miRNAs in embryos of *T. mutabilis* exposed to A) TBT1 (10^{-12} M) and C) TBT2 (10^{-10} M). The size of the circles indicates the reliability of the prediction results. The names of the differentially expressed miRNAs are on the bottom, while the potentially regulated pathways are on the left (DIANA-mirPath v3 analysis). B-D) Simplified KEGG enrichment analysis based on the number of target genes associated with TBT exposure. Circle size represents the number of modulated target genes, and the color scale represents the *p*-value calculated by the DIANA-mirPath v3 server.

pathways (Fig. 3A, C). Among these, miR-92a-3p, let-7i-5p, miR-221-3p, and miR-31-5p exhibited regulation of more than 1000 target genes, whereas miR-151a-5p and miR-191-5p influenced a more limited set of genes (i.e., 112 and 197 counts respectively), indicating a sort of marginal impact for these last miRNAs on pathway regulation (Fig. 3A, C) (Supplementary data Table 1). For these four miRNAs, predicted target genes were identified through consensus analysis and subsequently mapped onto the three most significantly enriched pathways (i.e., Ras signaling, Regulation of actin cytoskeleton, and Focal adhesion). The analysis revealed high-confidence target matches exhibiting strong seed complementarity (6-mer, 7-mer, 8-mer, or 9-mer) and a microT-CDS score > 0.60, including RAC1, PIK3CA, β-ACT, IL6R, AKT3, PTEN, and TLR4, all of which also displayed significantly altered expression patterns (Supplementary data).

3.4. Dose-dependent molecular pathways underlying TBT effects

Furthermore, specific differences were observed between the two treatments. In the TBT1 group, miR-183-5 and miR-486-5p selectively modulated 905 and 581 target genes (Fig. 3A), whereas miR-184 and miR-100-5p exclusively regulated a limited number of target genes under TBT2 exposure. (i.e., 117 and 82, respectively) (Fig. 3C). The most highly represented miRNA in terms of regulated genes was miR-92a-3p, which showed the highest number of interactions across multiple pathways (i.e, 2006 counts), followed by let-7i-5p and miR-31-5p that exhibit more than 1500 gene regulations. The pathway analysis summarized in Fig. 3 revealed that the significantly modulated miRNAs were implicated in a variety of KEGG pathways, covering nervous system development (e.g., neurotrophin signaling, GABAergic synapse, glutamatergic synapse pathways), cellular functions such as proliferation and cell growth (e.g., PI3K-Akt, FoxO, Wnt, Hippo signaling pathways), signal transduction (cAMP, calcium, cGMP-PKG, AMPK signaling pathways), cellular component assembly (e.g., focal adhesion, regulation of actin cytoskeleton) and cell-cell interactions (e.g., ECM-receptor interaction, adherens junction). All these functions are essential for the appropriate development of the *T. mutabilis* embryos, ensuring growth and cellular differentiation. The disruption of these pathways following TBT exposure suggests potential adverse effects on embryogenesis, which could impair normal developmental processes and impact overall viability. For instance, the Wnt pathway is integral to cell fate determination and tissue development, and its dysregulation can result, for example, in congenital malformations (Goldbeter and Pourquié, 2008; Vincan, 2008). Thus, the modulation of the Wnt signaling pathway by miRNAs indicates potential impacts on embryonic patterning and organogenesis.

A comparison of pathways affected by TBT1 and TBT2 showed both shared and distinct regulatory patterns (Fig. 3B, D). The consistency of these pathways across both treatments suggests a common response mechanism to TBT exposure. Several pathways were highly enriched (i.e., $\geq$100 regulated target genes) under both conditions, including focal adhesion, Ras signaling, regulation of actin cytoskeleton, Rap1 signaling, cAMP signaling, calcium signaling, cGMP-PKG signaling pathways. These highly regulated pathways play fundamental roles in the intracapsular development of this marine gastropod by regulating key cellular processes necessary for embryogenesis. In fact, Focal adhesion and regulation of the actin cytoskeleton are essential for cellular adhesion, migration, and morphogenesis, which are critical during early development when cells must establish structural integrity (Ciobanasu et al., 2012; Ramos et al., 2022). Ras and Rap1 signaling pathways mediate cell proliferation and differentiation, influencing developmental timing and patterning (Boettner and Van Aelst, 2009). cAMP, calcium, and cGMP-PKG signaling pathways are key regulators of intracellular communication and synaptic signaling, which may be particularly relevant for the formation of sensory systems in developing embryos (Gomez and Nasi, 2005). Despite the high number of shared pathways, notable differences emerged between the two TBT doses. The PI3K-Akt signaling pathway was significantly regulated under TBT2 but not TBT1, suggesting a dose-dependent modulation of this critical pathway involved in the signal transduction network. In general, the PI3K-Akt signaling pathway is crucial for cell survival, proliferation, and differentiation, ensuring proper tissue formation (Wei et al., 2020; Glaviano et al., 2023). Similarly, the adipocytokine signaling and circadian entrainment pathways were specifically enriched in TBT2, reinforcing its role in metabolic regulation at higher exposure levels. Conversely, pathways such as mucin-type O-glycan biosynthesis, lysine degradation, Phosphatidylinositol signaling system, Prolactin signaling, N-Glycan biosynthesis and SNARE interactions in vesicular transport pathways exhibited significant regulation already at the lowest concentration of TBT1.

4. Conclusion

In summary, our results indicate that exposure to TBT induces significant alterations in miRNA expression profiles, which in turn modulate multiple signaling pathways involved in cell proliferation, neurogenesis, and cellular differentiation. These molecular perturbations are likely to contribute to the developmental abnormalities observed in TBT-exposed gastropod embryos. Further studies are required to validate the regulatory interactions between differentially expressed miRNAs and their predicted gene targets, as well as to elucidate their functional roles in TBT-induced toxicity. Moreover, the potential of miRNAs as biomarkers for environmental monitoring should be explored, as it may offer effective tools for assessing ecosystem health in response to chemical pollutants.

CRediT authorship contribution statement

Paolo Cocci: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Gilberto Mosconi:** Writing – review & editing, Investigation, Formal analysis. **Francesco Alessandro Palermo:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2026.119486.

Data availability

Data will be made available on request.

References

Abo-Al-Ela, H.G., Faggio, C., 2021. MicroRNA-mediated stress response in bivalve species. Ecotoxicol. Environ. Saf. 208, 111442.
Assefa, A.T., Vandesompele, J., Thas, O., 2020. On the utility of RNA sample pooling to optimize cost and statistical power in RNA sequencing experiments (vol 21, 312, 2020). BMC Genomics 21.
Beyer, J., Song, Y., Tollefsen, K.E., Berge, J.A., Tveiten, L., Helland, A., Oxnevad, S., Schoyen, M., 2022. The ecotoxicology of marine tributyltin (TBT) hotspots: a review. Mar. Environ. Res. 179.
Biggar, K.K., Kornfeld, S.F., Maistrovski, Y., Storey, K.B., 2012. MicroRNA regulation in extreme environments: differential expression of microRNAs in the intertidal snail Littorina littorea during extended periods of freezing and anoxia. Genom. Proteom. Bioinforma. 10, 302–309.
Boettner, B., Van Aelst, L., 2009. Control of cell adhesion dynamics by Rap1 signaling. Curr. Opin. Cell Biol. 21, 684–693.

Borysko, L., Ross, P.M., 2014. Egg capsules and larval development of Nassarius burchardi (Philippi, 1849) and Nassarius jonasii (Dunker, 1846) and comparisons with other Nassariidae (Caenogastropoda). Molluscan Res. 34, 213–221.
Chi-Ho Ip, J., P, T.Y.L., K, K.Y.H., Qiu, J.W., K, M.Y.L., 2024. Transcriptomic analysis reveals the endocrine toxicity of tributyltin and triphenyltin on the whelk *Reishia clavigera* and mechanisms of imposex formation. Environ. Int. 190, 108867.
Choi, H.C., Lee, J.W., Hwang, U.K., Jeon, H.J., Oh, S.Y., Kim, C.W., Kang, H.S., 2023. Effects of tributyltin-contaminated aquatic environments and remediated water on early development of sea urchin (*Hemisentrotus pulcherrimus*). Animals (Basel) 13.
Ciobanasu, C., Faivre, B., Le Clainche, C., 2012. Actin dynamics associated with focal adhesions. Int. J. Cell Biol. 2012, 941292.
Cocci, P., Mosconi, G., Palermo, F.A., 2021a. Effects of tributyltin on retinoid X receptor gene expression and global DNA methylation during intracapsular development of the gastropod T.ia mutabilis (Linnaeus, 1758). Environ. Toxicol. Pharmacol. 88, 103753.
Cocci, P., Troli, E., Angeletti, M., Palermo, F.A., 2021b. Field monitoring of Tritia mutabilis (Linnaeus, 1758) egg capsule deposition and intracapsular embryonic patterns using artificial substrates and machine learning-based approaches. Front. Mar. Sci. 8.
Dametto, S., Gourbal, B., Chaparro, C., Pinaud, S., Duval, D., 2025. Unveiling the hemolymphatic miRNome composition of the schistosomiasis vector snail *Biomphalaria glabrata*. Curr. Res. Parasitol. 7.
Feng, D., Li, Q., Yu, H., Liu, S., Kong, L., Du, S., 2020. Integrated analysis of microRNA and mRNA expression profiles in Crassostrea gigas to reveal functional miRNA and miRNA-targets regulating shell pigmentation. Sci. Rep. 10, 20238.
Glaviano, A., Foo, A.S.C., Lam, H.Y., Yap, K.C.H., Jacot, W., Jones, R.H., Eng, H., Nair, M. G., Makvandi, P., Geoerger, B., Kulke, M.H., Baird, R.D., Prabhu, J.S., Carbone, D., Pecoraro, C., Teh, D.B.L., Sethi, G., Cavalieri, V., Lin, K.H., Javidi-Sharifi, N.R., Toska, E., Davids, M.S., Brown, J.R., Diana, P., Stebbing, J., Fruman, D.A., Kumar, A. P., 2023. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. Mol. Cancer 22.
Goldbeter, A., Pourquié, O., 2008. Modeling the segmentation clock as a network of coupled oscillations in the Notch, Wnt and FGF signaling pathways. J. Theor. Biol. 252, 574–585.
Gomez, M.D., Nasi, E., 2005. Calcium-independent, cGMP-mediated light adaptation in invertebrate ciliary photoreceptors. J. Neurosci. 25, 2042–2049.
Hu, N., Brönmark, C., Bourdeau, P.E., Hollander, J., 2022. Marine gastropods at higher trophic level show stronger tolerance to ocean acidification. Oikos 2022.
Huang, S., Yoshitake, K., Asaduzzaman, M., Kinoshita, S., Watabe, S., Asakawa, S., 2021. Discovery and functional understanding of MiRNAs in molluscs: a genome-wide profiling approach. RNA Biol. 18, 1702–1715.
Kroon, F.J., Berry, K.L.E., Brinkman, D.L., Kookana, R., Leusch, F.D.L., Melvin, S.D., Neale, P.A., Negri, A.P., Puotinen, M., Tsang, J.J., van de Merwe, J.P., Williams, M., 2020. Sources, presence and potential effects of contaminants of emerging concern in the marine environments of the Great Barrier Reef and Torres Strait, Australia. Sci. Total Environ. 719, 135140.
Marco, A., Hooks, K.B., Griffiths-Jones, S., 2012. Evolution and function of the extended miR-2 microRNA family. RNA Biol. 9, 242–248.
Øystein Hjermann, D., Galante-Oliveira, S., McHugh, B., Fryer, R., 2022. Status and trends in the levels of imposex in marine gastropods (TBT in shellfish). In: OSPAR, 2023: The 2023 Quality Status Report for the North-East Atlantic. OSPAR Commission, London.
Piazza, A., Carlone, R., Spencer, G.E., 2024. Non-canonical retinoid signaling in neural development, regeneration and synaptic function. Front. Mol. Neurosci. 17, 1371135.
Potla, P., Ali, S.A., Kapoor, M., 2021. A bioinformatics approach to microRNA-sequencing analysis. Osteoarthr. Cartil. Open 3, 100131.
Presslauer, C., Bizuayehu, T.T., Kopp, M., Fernandes, J.M.O., Babiak, I., 2017. Dynamics of miRNA transcriptome during gonadal development of zebrafish. Sci. Rep. 7.
Ramos, L., Yousaf, Y., Kelsell, D., Blaydon, D., 2022. Identification of a role for aquaporin 5 in regulation of the actin cytoskeleton and cell-cell adhesion in keratinocytes. Brit. J. Dermatol. 186, E221.
Robinson, M.D., Oshlack, A., 2010. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 11, R25.
Song, H., Qi, L., Zhang, T., Wang, H.Y., 2017. Understanding microRNA Regulation Involved in the Metamorphosis of the Veined Rapa Whelk (Rapana venosa). G3 (Bethesda) 7, 3999–4008.
Srut, M., Sabolic, I., Erdelez, A., Grbin, D., Turk, M.F., Bakaric, R., Peharda, M., Stambuk, A., 2023. Marine pollutant tributyltin affects DNA methylation and fitness of banded murex populations. Toxics 11.
Svigruha, R., Molnar, L., Elekes, K., Pirger, Z., Fodor, I., 2024. Effect of tributyltin exposure on the embryonic development and behavior of a molluscan model species, Lymnaea stagnalis. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 285, 109996.
Vincan, E., 2008. Wnt Signaling: Volume 2: Pathway Models. Methods in Molecular Biology, 469. Humana Press, New Jersey.
Walker, S.E., Spencer, G.E., Necakov, A., Carlone, R.L., 2018. Identification and characterization of microRNAs during retinoic acid-induced regeneration of a molluscan central nervous system. Int. J. Mol. Sci. 19.
Wei, J., Gou, Z., Wen, Y., Luo, Q., Huang, Z., 2020. Marine compounds targeting the PI3K/Akt signaling pathway in cancer therapy. Biomed. Pharmacother. 129, 110484.
Yang, M.J., Song, H., Shi, P., Liang, J., Hu, Z., Zhou, C., Hu, P.P., Yu, Z.L., Zhang, T., 2023. Integrated mRNA and miRNA transcriptomic analysis reveals the response of Rapana venosa to the metamorphic inducer (juvenile oysters). Comput. Struct. Biotechnol. J. 21, 702–715.
Yin, W.L., Mai, W.H., Cui, D.Y., Zhao, T.J., Song, J., Zhang, W.J., Chang, Y.Q., Zhan, Y.Y., 2025. Dynamic responses during early development of the sea urchin to CO2-driven ocean acidification: a microRNA-mRNA integrated analysis. Mar. Pollut. Bull. 212.
Zhang, J., Hadj-Moussa, H., Storey, K.B., 2020. Marine periwinkle stress-responsive microRNAs: a potential factor to reflect anoxia and freezing survival adaptations. Genomics 112, 4385–4398.
Zhu, X., Chen, Y., Zhang, Z., Zhao, S., Xie, L., Zhang, R., 2020. A species-specific miRNA participates in biomineralization by targeting CDS regions of Prisilkin-39 and ACCBP in Pinctada fucata. Sci. Rep. 10, 8971.