Uttar Pradesh Journal of Zoology

Volume 45, Issue 5, Page 161-174, 2024; Article no.UPJOZ.3305
ISSN: 0256-971X (P)

A Review and Meta-Analysis of the Studies Conducted on the Impact of Micropollutants on the Aggressive Behaviors of Some Crustaceans

Rosemary Patrick [a*] and Maureen Egbuatu [b]

[a] *School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW, Australia.*
[b] *Global Centre for Environmental Remediation, The University of Newcastle, Callaghan, NSW, Australia.*

Authors' contributions

This work was carried out in collaboration between both authors. Author RP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ME managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2024/v45i53942

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3305

Systematic Review Article

Received: 01/01/2024
Accepted: 05/03/2024
Published: 09/03/2024

ABSTRACT

The aquatic environment is constantly polluted by point and non-point sources of contaminants, endangering animals. For example, micropollutants and heavy metals have been demonstrated to influence the physiology and behavior of crustaceans. Some research has reported that the aggressive behavior of crustaceans tends to change as an early warning sign of environmental contamination. A meta-analysis of the effects of micropollutants and heavy metals on crustaceans' behavior. The results show that, for all investigated pollutant effects on fight duration across 18 datasets, the SMD range was -1.26 to 0.64 with a heterogeneity (Q) of 267.11 ($p < 0.01$), indicating high variability among studies. The publication bias p-value was 0.27, suggesting no significant publication bias for this endpoint. Specifically, for micropollutants affecting fight duration in 11

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*Corresponding author: Email: rosemary.patrick@fulokoja.edu.ng;

datasets, the SMD ranged from -1.41 to 1.02, with a heterogeneity (Q) of 182.82 ($p < 0.01$), again showing high variability. The publication bias p-value was 0.76, indicating no significant publication bias. Regarding the effects of all investigated pollutants on the number of fights in 10 datasets, the SMD ranged from -3.32 to -1.01, showing a statistically significant negative impact on aggressive interactions. The heterogeneity (Q) was 83.37 ($p < 0.01$), and the publication bias p-value was <0.01, indicating a significant publication bias for this endpoint. For micropollutants specifically affecting the number of fights in 7 datasets, the SMD ranged from -3.75 to -0.41, with a heterogeneity (Q) of 77.32 ($p < 0.01$) and a publication bias p-value of <0.01, again indicating a significant publication bias. When the size effect values and standard mean differences in confidence intervals of the different investigations were considered, the review revealed a high heterogeneity of published results and a tendency to find publication bias. The analysis concluded that aggressive behavior as an early warning sign for environmental contamination should be performed with caution because the levels required to trigger a response in some species are frequently insignificant regarding environmental relevance.

Keywords: Crustaceans; aggression; agonistic behavior; micropollutants, meta-analysis.

1. INTRODUCTION

Life depends on water, which covers a large portion of the Earth’s surface and is home to various plants, animals, and microorganisms. Unfortunately, because dangerous compounds emitted by anthropogenic activities sink to the bottom of the aquatic environment, rivers, reservoirs, lakes, and oceans are plagued by chemicals and other pollutants. Environmental pollution caused by human activity can harm biodiversity [1]. Since anthropogenic chemicals are pervasive in the ecosystem, they are likely to reduce aquatic diversity and cause public health problems. Recently, micropollutants, which consist of natural and anthropogenic substances, including pharmaceuticals, antibiotics, personal care products, pesticides, and industrial chemicals detected in the µg to below ng/l range, have raised global concern for their potentially damaging environmental impacts [2]. Micropollutants are ubiquitous and are generally meant to improve human life, but they are poorly removed during the wastewater treatment process, which means that they commonly end up in natural waters. Despite their presence in the environment at sub-microgram levels, they have been recognized to possess high biological activities, which have been investigated using multiple ecotoxicological endpoints [3].

Given the current levels of industrialization and growth in the pharmaceutical, agricultural, and medical industries, coupled with the increasing human population, there are various routes by which heavy metals and micropollutants enter aquatic environments, for instance, via municipal sewer systems or with runoff from fields. General resistance to degradation makes wastewater one of the primary sources of micropollutants and heavy metals. Most current wastewater treatment plants cannot effectively remove them [4,3]. Therefore, a vital part of the mitigation process is modern treatment technologies that utilize both abiotic and biotic methods. Additionally, it is necessary to compile information on the risk analysis of microcontaminants in the environment, particularly in aquatic environments used for multiple purposes.

Although widespread catastrophic pollutant events are uncommon, sublethal and chronic effects of exposure are frequent and can lead to decreased survival and reproductive success, both of which are crucial for the survival of aquatic animals [5]. Among aquatic animals, micro- and macro-crustaceans are very sensitive to micropollutants and heavy metals and have been repeatedly captured in several studies and reviews [6], [7], [8]. Crustaceans comprise a broad and varied group of arthropods, including amphipods, barnacles, branchiopods, copepods, decapods, fish lice, isopods, krill, mantis shrimp, remipedes, and seed shrimps [9]. Even in areas that appear to be free of pollution, pollutants have been detected in the tissues of several species or groups of crustaceans. They are known to bioaccumulate within tissues and can be passed down through generations, in addition to being transported and biomagnified across trophic levels [8]. To the best of our knowledge, pollutants impair the ecosystem health and overall well-being of crustaceans, even if pollution-induced extinction rates are unknown.

Additionally, there is evidence that certain pollutants, particularly those that disrupt hormones, contribute to crustacean loss.

Evidence suggests that pollutants are lowering many crustacean populations and modifying their evolutionary processes, in addition to placing some species on the verge of extinction [10]. The question then becomes: are there other early toxicological endpoints, such as behavioral alterations, that may significantly influence the survival and diversity of crustaceans in aquatic environments when exposure scenarios are chronic at low doses or acute at high concentrations? The objective of this study was to perform a systematic review and meta-analysis of aggressive behavior among crustacean populations during exposure to pollutants, such as micropollutants and heavy metals.

1.1 Aggressive Behavior of Crustaceans

Behavior was described by Scott and Sloman [11] as both an adaptive and occasionally maladaptive reaction to environmental cues and a fitness test. Therefore, it is essential for the existence and survival of the population and community of an organism. Additionally, from an ecological perspective, it is intuitively clear how crucial it is for population maintenance. Numerous investigations have shown that contaminants severely affect various behaviors in crustaceans [12-14]. Despite individual studies demonstrating that pollutants harm crustacean behavior, no analysis has attempted to explain the broader implications of these modifications on the health and well-being of aquatic ecosystems. Despite the recent growth in the subject, behavioral ecotoxicology has not been extensively studied [15,16].

The ability to predict various levels of biological outcomes is one of the advantages of including behavior in ecotoxicological investigations [11], [17]. Additionally, behavior is one of the most sensitive indicators of exposure impact, because there are observable changes in behavior at sublethal concentrations [18,19]. Given these advantages, the current review concentrates on aggression, a critical behavioral feature, to provide an early indicator of toxins in the aquatic environment. Toxicologists may observe reactions in aggressive behavior before they do so in other phenotypes or the genome since this behavior is constantly changing and adapting. This method is noninvasive, affordable, and does not always require a significant quantity of specialized equipment [20].

We acknowledge that there are some restrictions on employing this behavior in ecotoxicology. For instance, aggressive behavior can be flexible, making its analysis more time-consuming, less reproducible, and potentially more variable than physiological examinations. However, it is important to remember that physiological measurements frequently have similar limitations. Despite these drawbacks, aggressive behavior can be seen as a flexible reaction to an environmental stressor that helps crustaceans survive in changing and polluted ecosystems. In addition to other behavioral changes observed in crustaceans, aggressive behavior can be used to study how anthropogenic chemicals affect the populations and communities of crustaceans and how these changes may have a domino effect on the overall health of aquatic ecosystems. By including several levels of biological structures, this strategy guarantees the dissemination of knowledge about conservation and sustainability.

Resource holding potential, resource value, and aggressiveness are three attributes hypothesized to be correlated in game theory models of aggressive conflict behavior [21]. When the two species share a place and scarce resources, conflicts arise over which animals receive priority in terms of control and access. Most of the time, these disputes are settled through battles, fights, threats, and other violent actions. Individuals frequently engage in aggressive behavior in social connections, known as social dominance. Individuals engage in a well-researched sequential sequence of exchanges with the ability of either party to end the interaction or contest at any point [22]. Conspecifics frequently engage in antagonistic interactions that culminate in dominance, with one winning by forcing the other away and the other losing by retreating. The winner and loser effects can occur during agonistic interactions between animals. If an animal “wins” an agonistic conflict, they become dominant, and if they “lose,” they become subordinate. These exchanges affect future encounters, increasing the possibility of winning and losing [23, 24]. Altered aggressive behavior can have repercussions not only for individuals but also for the viability of populations and the survival of species. Changes in competitive ability can affect population dynamics by influencing key demographic parameters, resulting in a population decline. Such changes can affect species interactions and affect the structure and function of the ecological communities they inhabit.

2. METHODOLOGY

How reliable is aggressive or agonistic behavior an important early behavioral endpoint for ecotoxicological investigations?

A systematic search and meta-analysis were conducted following the PRISMA criteria to assess whether pollutants significantly impact the aggressive behavior of crustaceans [25]. A systematic literature search was conducted in consecutive stages using keywords such as "micropollutants," "pharmaceuticals," "pesticides," "personal care products," "metals," "nutrients," "aggression," "agonistic behavior," and "crustaceans" to find studies that investigated the influence of pollutants on aggressive behavior. PUBMED, Web of Science Core, and Google Scholar were used for this search. The following conditions were applied to the search results: they had to be published theses or dissertations, full texts from books, or conference abstracts, and they had to be in English. The search was conducted between May 2022, and September 2022.

3. RESULTS

The first 70 studies discovered in this review were relevant. A second iteration was performed to search for more relevant research in the cited articles and the reference lists of the included papers. Following retrieval, only 18 studies were judged appropriate for inclusion based on the authors' criteria for effect sizes for the means and standard deviations for the number of assaults and the time in seconds of fights (Table 1).

Based on pertinent studies, sample sizes, and effect sizes were estimated. Some publications provided simple access to individual value tables, whereas others clearly stated the effect magnitude. Other research used graphs, such as plots, to represent individual values; in these instances, a web plot digitizer [26] was used to translate the graphs to numerical values. In a few studies, the effect sizes and individual statistics in tables or charts were irrelevant. How the results were presented also lacked consistency; we mainly used two options: the number of fights and the length of the contests.

The observed variation between studies may be due to both within-study variation and heterogeneity (i.e., differences in actual effect sizes between studies). To investigate the variation and heterogeneity among the effect sizes of the included studies, Q, significance, and I2 were computed. I2 levels of 25%, 50%, and 75% were considered poor, moderate, and high quality, respectively [27].

Meta-regression models were developed to account for the heterogeneity of the continuous moderator factors. Egger's regression test and visual inspection of a funnel plot were used to identify slight study bias, which included publication and research quality biases [28], [29]. A substantial test revealed bias in the small study ($p < 0.05$).

Eighteen studies with effect sizes were included in the meta-analysis (Table 1; Fig. 1) [30-46], [19]. The total number of candidate crustaceans or subjects across the studies was 1101. Among them, 20 were *Barytelphusa guerini*, 246 *Barytelphusa guerini*, 596 *Hemigrapsus oregonensis*, 96 *Macrobrachium carcinus*, 55 *Orconectes virilis*, 48 Uca pugilator, and 40 *Macrobrachium dayanum*.

Because a close examination of the forest plot revealed that crustaceans' responses to various pollutants varied, the standardized mean difference with a 95 percent confidence interval of -1.26 to 0.64 in the meta-analysis of all studies combined for fight duration was not significant (Fig. 1, Table 2). A heterogeneity test revealed that the dataset was heterogeneous. There was no publication bias, even though the stimulation and inhibition of aggressive behavior depended on the type of pollutant, exposure concentration, and crustacean species. The effects of micropollutants are highly variable; for instance, Tierney et al. [43] reported that *Orconectes rustic*us displayed less aggressive behavior, whereas Hossain et al. [32] discovered *that Fazonius viridis* displays more aggressive behavior after exposure to fluoxetine. Six of the 11 studies included in the micropollutant forest plot showed longer-lasting aggressive behaviors in various crustaceans, as indicated by positive SMDs and their corresponding CIs (Fig. 4).

The plot indicated that several studies had negative SMDs with large standard errors, whereas many others had negative SMDs with smaller standard errors. Egger's p-value = 0.244 and coeff = −0.30, p = 0.27, and coeff = -1.11 for micropollutants and all the pollutants combined, respectively, for fight duration as an aggression endpoint, support the finding that, despite the heterogeneity of the research, there was no significant publication bias. However, the same could not be said when the number of fights was used as an endpoint as it gave Egger's p-value < 0.0001 and coeff = −4.30 and p-value < 0.0001 and coeff = -5.29 for micropollutants and all the pollutants combined, respectively. These findings demonstrate a significant publishing bias when the total number of fights was used. Additionally, the Influence test revealed that Woodman et al. [46] study on the effect of sertraline significantly altered the results of micropollutants. This influence relied on the endpoints for aggressive conduct (Figs. 2 and 4) and was only significant when the endpoint for fighting was chosen.

4. DISCUSSION

The findings of this meta-analysis showed that water pollutants can increase or decrease individual aggression, even temporarily flipping the dominant status rank. The relationship between behavior and ecological processes makes behavioral indicators of toxicity suitable for determining how aquatic contaminants affect the behavior and population dynamics of crustaceans. This review addresses a critical question regarding whether exposure to low or high concentrations of micropollutants, nutrients, effluents, and heavy metals affects behavior and neural function differently. This is because, in the natural environment, these animals can be exposed to sudden changes in their ecosystems, frequent exposure to low and high concentrations of contaminants, and physical changes in water that could be detrimental. However, the lack of standardization in the techniques used to identify aggressive behavior in crustaceans makes it challenging to interpret the data.

To establish dominant positions inside their territory, animals must act aggressively. According to DeVries et al. [47], many crustacean species exhibit pronounced territorial behavior, and the degree of dominance is observed in the phenotypic and physiological characteristics of each sex. However, exposure to contaminants by these species may cause considerable changes in aggression, which may significantly affect their dominance indices. Ortiz Lugo and Sosa Lloréns [38] noted that some modifications included not all chemicals interacting concurrently. Several pollutants may affect social behavior, depending on how rapidly they interpret social cues and whether they reach their target locations within the nervous system. Although the effects manifest at different times, as shown by variations in the duration of aggressive behavior, this may imply that the mechanisms of action of contaminants affecting the general level of activity and modulating the aggressiveness of these crustaceans are similar or closely related. Owing to the significant heterogeneity found in this meta-analysis, care should be taken when interpreting these results.

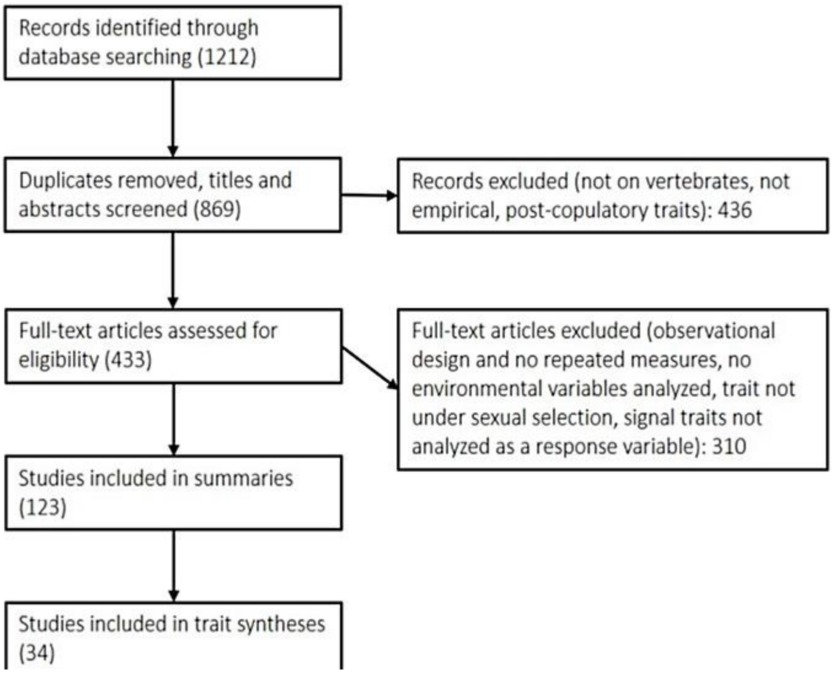

Chart 1. Study protocol

Table 1. An overview of studies included in the meta-analysis

Study	Pollutant	Concentration	Crustacean	Habitat	Treatment Aggression Response
Shirley 2021	17α-Ethinyl Estradiol	0.50 mg/l	*Uca pugilator*	Marine	Decreased
Peters et al. 2017	Fluoxetine	3.00 & 30 ng/l	*Hemigrapsus oregonensis*	Marine	Decreased
Dube 2019	Lead (Lead nitrate)	1.40 mg/l	*Barytelphusa guerini*	Freshwater	Increased
Dissanayake et al. 2009	Pyrene	200 µg/l	*Carcinus maenus*	Marine	Increased
Tripathi and Pandey 2014	Cadmium	37.50 µg/l	*Macrobrachium dayanum*	Freshwater	No significant change
Mishra et al. 2018	Copper, Potassium	0.37 mg/l, 0.016 mg/l	*Simocephalus vetulus*	Freshwater	Increased
Krang and Ekerholm 2006	Copper	100.00 & 500.00 µg/l	*Carcinus maenus*	Marine	Increased
Mamdouh et al. 2022	Zinc	46.03 & 92.06 mg/l	*Procambarus clarkii*		Increased
White et al. 2013	Copper	0.90 mg/l	*Pagurus bernhardus*		Decreased
Woodman et al. 2016	Sertraline	424.00 ng/l	*Orconectes virilis*		Increased
Reichmuth et al. 2011	Polluted sites	NA	*Callinectes sapidus*	Marine	Increased
Tierney et al. 2016	Fluoxetine	2.00, 200.00, & 500.00 µg/l	*Orconectes rusticus*		Decreased
Lugo and Llorens 2015	Dibuthyl phthalate	0.006 mg/l	*Macrobrachium carcinus*	Freshwater	Increased
	Chromium	0.10 mg/l	*Macrobrachium carcinus*	Freshwater	Increased
	Cadmium	0.005 mg/l	*Macrobrachium carcinus*	Freshwater	Decreased
	Manganese	0.005 mg/l	*Macrobrachium carcinus*	Freshwater	Decreased
	DEB	0.006 mg/l	*Macrobrachium carcinus*	Freshwater	Decreased
	BzBP	0.006 mg/l	*Macrobrachium carcinus*	Freshwater	Increased
Verma 2011	Nickel	65.77 mg/l	*Macrobrachium lamarrei*		Increased
			Macrobrachium dayanum		Decreased
Neal and Moore 2017	Naproxen	0.027-14.00 µg/l	*Orconectes virilis*	Freshwater	Increased
Hossain et al. 2020	Fluoxetine	0.05-100.00 µg/l	*Faxonius viridis*		Increased under dynamic conditions but decreased under static conditions
Mishra et al. 2020	Effluent	NA	*Simocephalus vetulus*	Freshwater	Increased
Stara et al. 2019	Calypso 480 SC (CAL)	1.00-100 µg/l	*Cherax destructor*		Increased
Hamilton et al. 2016	Fluoxetine	5 & 25 mg/l	*Pachygrapsus crassipes*		No significant change`

Table 2. Meta-analysis of aggressive or agonistic behavior of crustaceans exposed to common pollutants

Meta-analysis performed	**No. of data sets**	**Effect size (95% CI)**	**SE of summary Effect Size**	**Effect size *p***	**Heterogeneity**		**Publication Bias *p***
					Q	*p*	
All investigated pollutant effects fight duration	18	-1.26 – 0.64	0.48	0.52	267.11	<0.01	0.27
Micropollutants (fight duration)	11	-1.41 – 1.02	0.62	0.75	182.82	<0.01	0.76
All investigated pollutant effects on number of fight	10	-3.32 – -1.01	0.59	0.0002	83.37	<0.01	<0.01
Micropollutants (number of fights)	7	-3.75 – -0.41	0.85	0.0144	77.32	<0.01	<0.01

CI: Confidence Interval

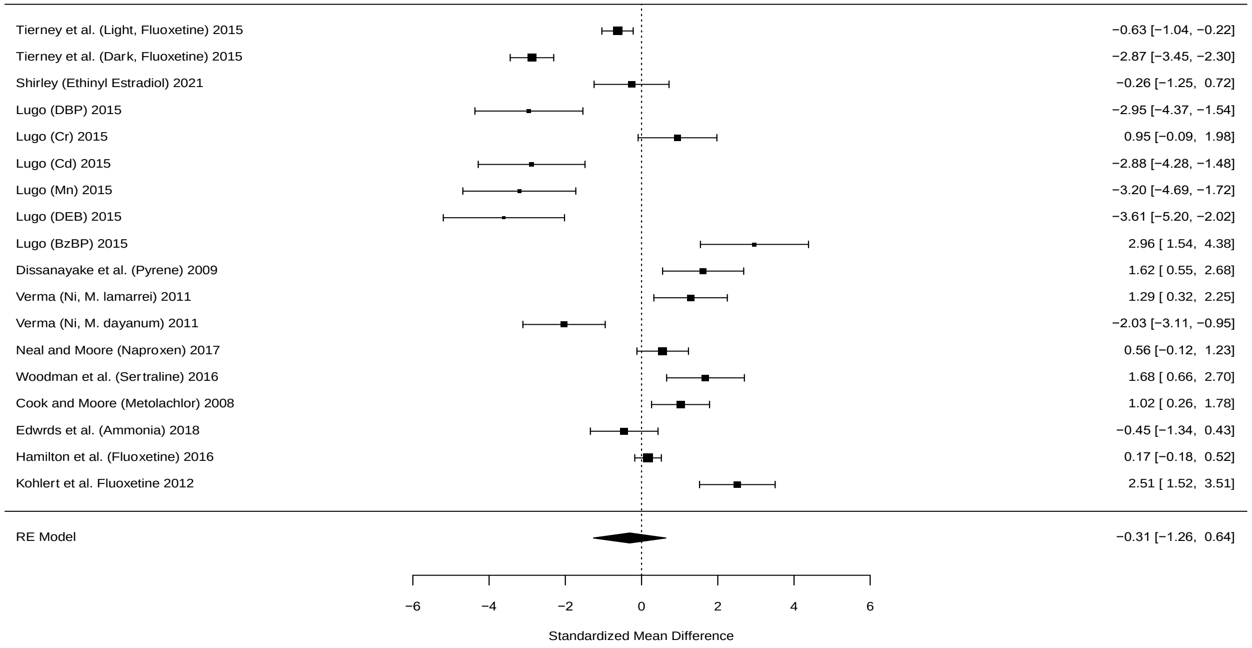

Fig. 1. The differences in the aggressive behavior of crustaceans following exposure to different pollutants. For total fight duration data, Standard Mean Difference (SMD) estimations are shown as filled squares, with square sizes denoting the relative importance of each study's effect size estimate in the analysis. The total summary effect size is shown by the filled diamond [SMD = 0.31, 95% CI (-1.26 to 0.64), p > 0.05]. Error bars and diamond width indicate 95% CIs. RE = Random Effects Model

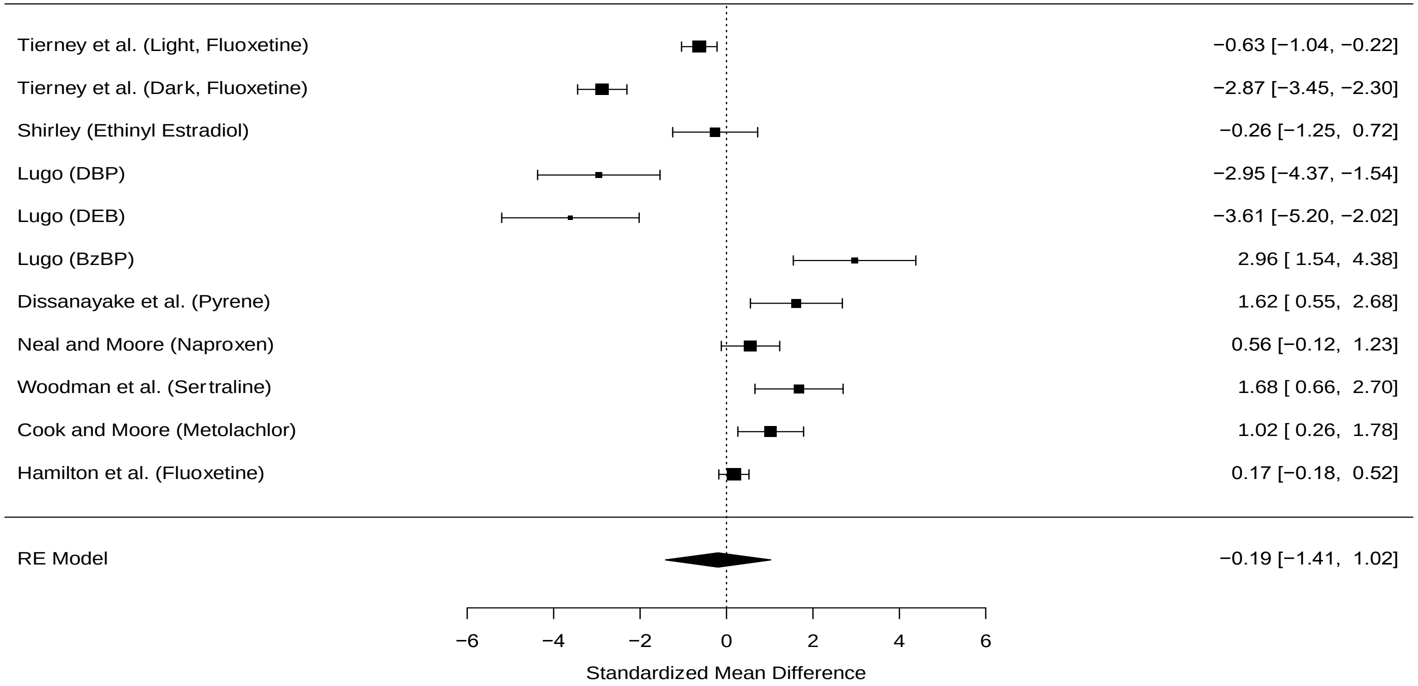

Fig. 2. The differences in the aggressive behavior of crustaceans following exposure to micropollutants. Standard Mean Difference (SMD) estimates for total fight duration data are depicted by filled squares, with square sizes indicating the relative weight of each study's effect size estimate in the analysis. The filled diamond reflects the overall summary effect size [SMD = -0.19, 95% CI (-1.41 to 1.02), p = 0.75]. Error bars and diamond width indicate 95% CIs. RE = Random effects model

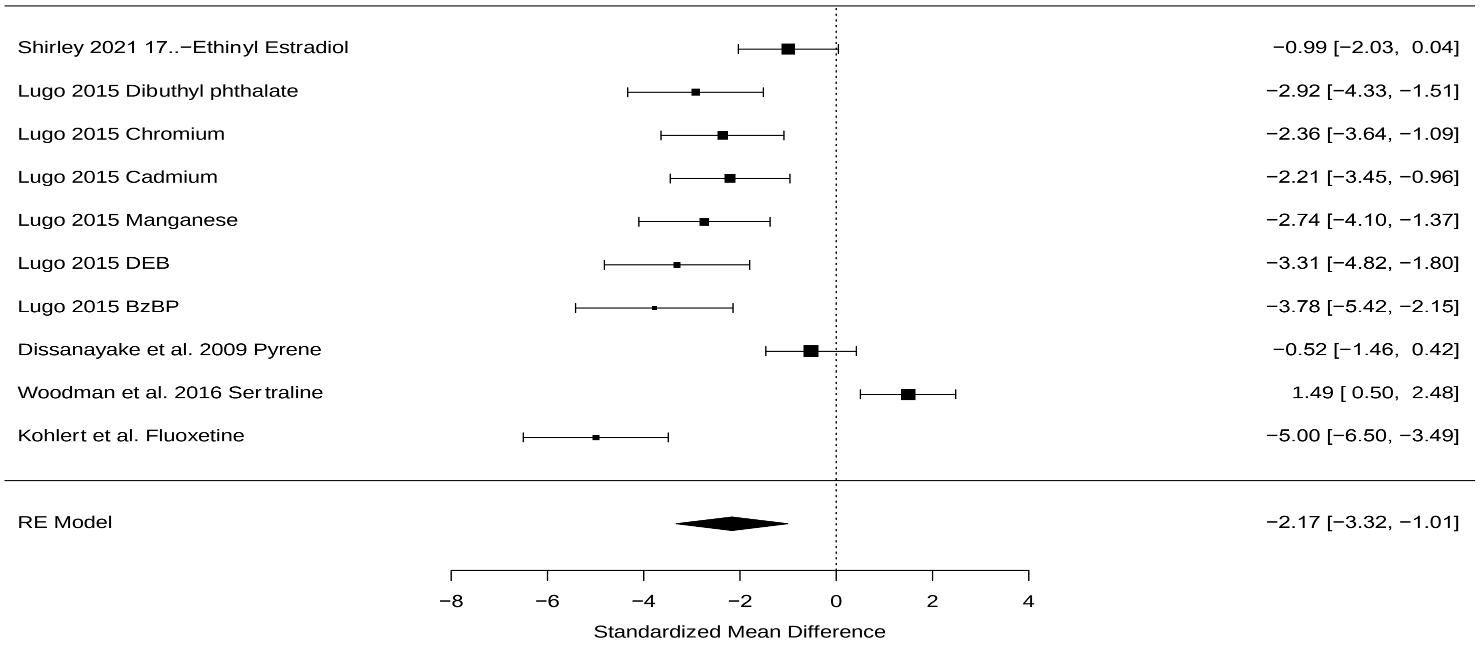

Fig. 3. The differences in the aggressive behavior of crustaceans following exposure to different pollutants. Standard Mean Difference (SMD) estimates for the total number of fight data are depicted by filled squares, with square sizes indicating the relative weight of each study's effect size estimate in the analysis. The filled diamond reflects the overall summary effect size [SMD = -2.17, 95% CI (-3.22 to -1.01), $p < 0.01$]. Error bars and diamond width indicate 95% CIs. RE = Random effects model

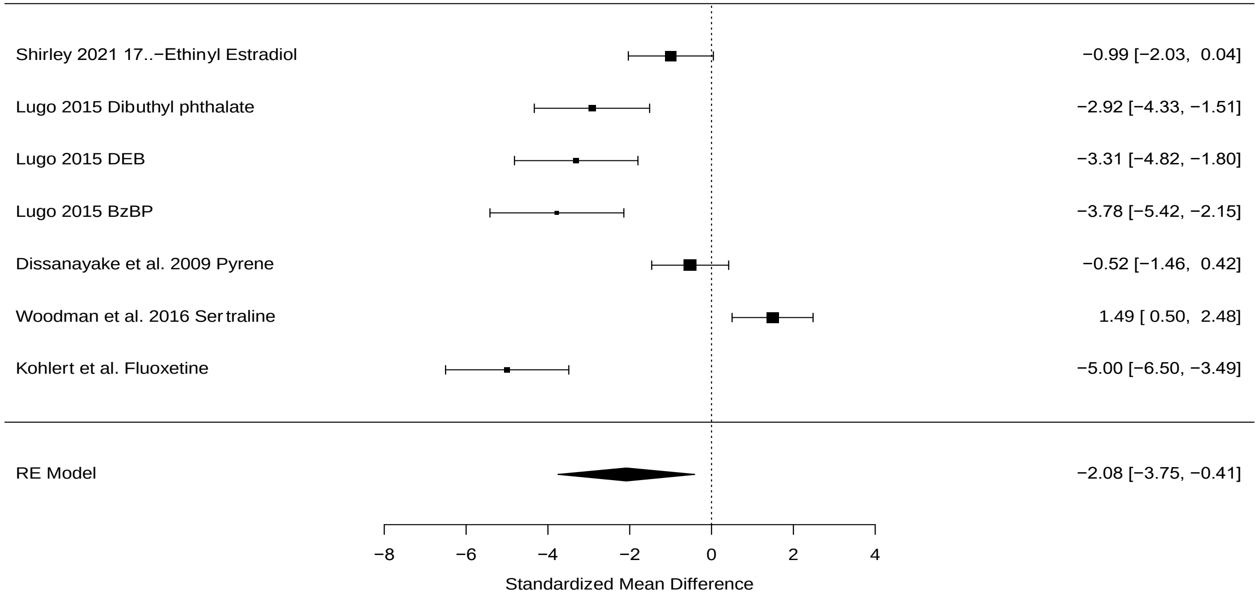

Fig. 4. The differences in the aggressive behavior of crustaceans following exposure to different micropollutants. Standard Mean Difference (SMD) estimates for the total number of fight data are depicted by filled squares, with square sizes indicating the relative weight of each study's effect size estimate in the analysis. The filled diamond reflects the overall summary effect size [SMD = -2.08, 95% CI (-3.75 to -0.41), p < 0.01]. Error bars and diamond width indicate 95% CIs. RE = Random effects mode

Interestingly, the meta-analysis performed in this review supports the idea that exposure to contaminants in aquatic habitats may affect agonistic dynamics and hierarchy in crustaceans. This is particularly true for crustaceans exposed to various heavy metals, which display lower levels of aggression than non-exposed animals [38,44]. A population-level impact is likely to occur if exposure to heavy metals results in various distinct qualities such as a decreased degree of aggression, and population dynamics may shift as a result. Conspecific competing strategies that have changed because of contamination and social behaviors that have changed may have an enormous impact on the behavioral ecology of the species because they disturb individual genetic responses and the neurological system.

Fluoxetine concentrations in freshwater habitats have been observed to range from 0.012 to 1.4 µg/L [48]. Because significant increases in aggressive behavior occurred at exposure doses reaching the µg/l range, the substance is classified as an endocrine disruptive agent if detected in sufficient amounts in the environment [33]. Fluoxetine alters intracellular signaling pathways, memory, cognitive function, activity, and development at environmentally relevant concentrations. These changes can collectively influence the behavior of aquatic animals [49]. By increasing foraging and locomotor activity in the presence of predators, particularly during the day when these crabs are typically hidden, exposure of shore crabs *Hemigrapsus oregonensis* to fluoxetine at environmental concentrations of 0.03 µg/L influenced both diurnal and nocturnal prey risk behaviors, according to Peters et al. [38]. The current study showed that variations in the concentrations and species of exposed crustaceans greatly influence changes in aggressive behavior, and the crustacean response cannot be generalized. Therefore, such inferences should be made with caution. The unexpected finding of Hamilton et al. [50] discovered that the aggressive behavior of exposed crustacean populations was significantly unaffected by doses up to the mg/L level. It is possible that fluoxetine might increase crab activity levels and cause them to act aggressively by modifying serotonin levels. However, this activity varies across studies [51].

The observed high heterogeneity was most likely due to differences in exposure lengths, as some trials were acute, whereas others were chronic, which could have different behavioral repercussions. Other potential causes for the disparities between studies include neuronal tolerance to chronic fluoxetine and other pollutants, mechanisms of action of pollutants, species-specific changes in behavior and reaction to contaminants, and test settings. In a circular arena, for example, a crab cannot engage in ‘cornering’ behavior, which has previously been associated with dominant-subordinate classes in crayfish, with dominants cornering substantially more [52]. Thus, if investigators employ arenas with corners, crabs cannot move across the arena, resulting in biased and incorrect results.

5. CONCLUSIONS AND RECOMMENDATIONS

As this crucial component is currently unknown, it is necessary to assess the impact of pollutant mixes on the aggressive behavior of crustaceans. Aggressive behavior as an early warning sign for environmental contamination should be performed with caution because the amounts needed to elicit a response in some species are often not environmentally relevant. Despite these flaws, pollutants have a significant but variable impact on aggressive crustacean behavior, impacting their chances of survival in contaminated aquatic habitats. The goal is to connect environmental neuroscience with documented changes in aggression within the context of dynamic behavioral mechanisms. This research helps us to understand the mechanisms involved in mediating and modifying aggressive behaviors linked to hyperactivity, anger, and violence. However, aggressive behavioral monitoring procedures must be standardized for this to happen.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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