ISSN:
3091-1796
Vol. 4 No. 10 PP. 1-16

DOI:
https://doi.org/10.56519/res44b81
Agosto 2026

RESPUESTA CONDUCTUAL DE POSTLARVAS DE CAMARÓN BLANCO *LITOPENAEUS VANNAMEI* FRENTE A LA PRESENCIA DE FILTROS SOLARES EN EL AGUA

BEHAVIORAL RESPONSE OF WHITE SHRIMP POSTLARVAE *LITOPENAEUS VANNAMEI* TO THE PRESENCE OF SUNSCREENS IN THE WATER

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Fecha de recepción: 19/06/2026 / Fecha de aceptación: 15/07/2026 / Fecha de publicación: 25/08/2026

RESUMEN: Los filtros solares son contaminantes emergentes que pueden llegar a los cuerpos de agua por el uso recreativo y por descargas domésticas. El comportamiento locomotor de las postlarvas de *Litopenaeus vannamei* expuestas a 25 y 300 mg/L de protector solar fue el objeto de estudio de esta investigación. Para ello se aplicó un bioensayo estático que contenía un control y dos tipos de tratamientos (25 y 300 mg/L) evaluados tras 24 h y 48 h de exposición. La actividad fue estimada por medio de la distancia recorrida y la estadística fue una complementación de la media de las distancias de desplazamiento, del coeficiente de variación (CV) y del análisis de densidad de Kernel. En cuanto a las diferencias, las pruebas de comparación para la distancia media de desplazamiento no mostraron diferencias estadísticamente significativas ($p > 0{,}05$). No obstante, a las 24 h el tratamiento de 25 mg/L mostró la mayor variabilidad (CV = 171,3%) y la mayor dispersión de los datos con valores extremos, mientras que a las 48 h los grupos de los tratamientos mostraron una mayor proporción de registros en distancias bajas, lo que anida con una reducción general de la actividad locomotora. En resumen, los resultados reflejan cambios en la variabilidad y en la distribución de la respuesta que no se perciben al comparar solamente las medias. Estos hallazgos aportan evidencia inicial sobre posibles efectos subletales de los filtros solares en postlarvas de *L. vannamei* y respaldan el uso de indicadores conductuales en estudios ecotoxicológicos.

Palabras clave: Litopenaeus vannamei, filtros solares, comportamiento locomotor, efectos subletales, ecotoxicología, variabilidad conductual

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ABSTRACT: Sunscreens are emerging pollutants that can enter water bodies through recreational use and domestic discharges. This study investigated the locomotor behavior of Litopenaeus vannamei postlarvae exposed to 25 and 300 mg/L of sunscreen. A static bioassay was conducted, consisting of a control and two treatment groups (25 and 300 mg/L), which were evaluated after 24 and 48 hours of exposure. Activity was estimated based on the distance traveled, and the statistical analysis included the mean displacement distances, the coefficient of variation (CV), and Kernel density analysis. As for the differences, the comparison tests for the mean displacement distance showed no statistically significant differences ($p > 0.05$). However, at 24 h, the 25 mg/L treatment showed the greatest variability (CV = 171.3%) and the widest dispersion of data with extreme values, whereas at 48 h, the treatment groups showed a higher proportion of records at short distances, which is consistent with an overall reduction in locomotor activity. In summary, the results reflect changes in the variability and distribution of the response that are not apparent when comparing only the means. These findings provide initial evidence of possible sublethal effects of sunscreens on L. vannamei postlarvae and support the use of behavioral indicators in ecotoxicological studies.

Keywords: Litopenaeus vannamei, sunscreens, locomotor behaviour, sublethal effects, ecotoxicology, behavioural variability

INTRODUCTION

The presence of emerging compounds in aquatic ecosystems is related to the daily use of chemical substances and the increase in human activities. Within this group are sunscreens, present in personal care products that can reach coastal and marine areas through recreational activities, domestic discharges and effluents from treatment plants (1, 2, 3, 4, 5). Some of these compounds persist in the environment and have been detected in water, sediments and biological tissues, which favors their interaction with aquatic organisms (1, 2, 6, 7). Sunscreens contain organic and inorganic ultraviolet filters designed to absorb or reflect UV radiation. However, several studies have reported adverse effects of certain compounds, including oxybenzone and octinoxate, on marine organisms (8, 9, 10, 11).

Behavioral ecotoxicology makes it possible to observe early signs of stress before lethal effects appear. Variables such as locomotor activity, swimming patterns, responsiveness, and erratic movements are useful for exploring sublethal effects of chemical contaminants (12, 13, 14, 15, 16). These changes can influence processes relevant to survival, such as foraging, predator evasion, and reproduction (17, 18). The Pacific white shrimp, Litopenaeus vannamei, is of great importance for Ecuadorian aquaculture. Their larval and postlarval stages are sensitive to variations in water quality, so they can be used as a model in toxicological and ecotoxicological tests (19, 20, 21).

Although interest in sunscreens in aquatic environments has increased, information on their behavioral effects on crustaceans of aquacultural importance remains limited (8, 21). The

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3091-1796
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available studies show that the response can change depending on the concentration, exposure time and sensitivity of the individuals, so the analysis of averages does not always allow us to describe all the variability of behavior (15, 21, 22, 23). Therefore, this work aimed to evaluate the effect of 25 and 300 mg/L of sunscreen on the locomotor behavior of L. vannamei postlarvae, by analyzing the distance traveled, behavioral variability and the distribution of the locomotor response. The study seeks to provide information on possible sublethal effects of sunscreens on aquatic organisms.

The effects observed in aquatic organisms are not the same for all sunscreens or for all species. They depend on the composition of the product, the concentration, the contact time and the water conditions (6, 7, 8, 11). Thus, laboratory tests should be considered as a controlled approach, not as an exact reproduction of what has happened in an ecosystem. Despite the limitation it entails, they do allow the detection of signals that would justify subsequent studies with environmentally representative concentrations and also greater physicochemical control.

Studying the movement of postlarvae is relevant because movement is part of basic activities such as exploration, feeding and response to environmental stimuli. The present investigation focused on describing whether the concentrations evaluated were associated with changes in distance traveled and behavioral variability. The aim was not to identify the ingredient responsible for the effect, but rather to provide a first description of the response of L. vannamei to the experimental exposure.

The analysis of this type of response is especially useful when organisms do not show immediate mortality, but do show changes that may anticipate an alteration in their performance. Distance traveled does not completely summarize behavior; That is why variation measures and distribution graphs were incorporated. This combination allows us to describe in greater detail how individuals behave within each treatment and prevents a single measurement or a single average from determining the interpretation of the study (12, 14, 15, 16).

MATERIALS AND METHODS

Organisms and experimental conditions

Postlarvae of Litopenaeus vannamei at stage PL13, obtained from a commercial larval culture laboratory, were used. They were transported to the experimental site avoiding sudden changes in temperature and salinity. In the laboratory they were kept in 2 L containers with filtered seawater, constant aeration and a cycle of 12 h of light and 12 h of darkness. Salinity was maintained at 33 PSU and temperature at 25 °C, suitable conditions for the cultivation of the species (19). During maintenance, siphoning was carried out every two days to remove organic waste.

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Before starting the test, the postlarvae remained in acclimatization for 48 h. This period sought to reduce the effect of the manipulation before the experimental exposure.

Preparation of solutions and concentrations

The solutions were prepared from a commercial sunscreen and seawater as a dilution medium. A static exposure design was used with three treatments: control (0 mg/L), low concentration (25 mg/L) and high concentration (300 mg/L) (24). For 300 mg/L, 1.8 g of product was dissolved in 6 L of seawater; for 25 mg/L, 0.3 g was dissolved in 12 L. The solutions were stirred for 10 min with a magnetic stirrer before use.

Table 1. Experimental treatments and calculation of sunscreen concentrations.

Parameter	**Treatment 1**	**Treatment 2**	**Treatment 3**
Treatment type	Control	Low	High
SPF	100	100	100
Concentration (mg/L)	0	25	300
Relative concentration (%)	0	8,3	100
Sunscreen quantity (g)	0	0,3	1,8
Volume (L)	6	12	6
Ratio (g/L)	0	0,025	0,3

Note. Concentrations were calculated from the mass/volume ratio and expressed in mg/L. The relative concentration took the treatment of 300 mg/L (100%) as a reference.

Experimental design

The test was carried out in 200 mL transparent containers, with 90 mL of the corresponding solution in each unit. Postlarvae of similar size were selected to reduce biological variation. The system was direct exposure and did not include spatial gradients; Therefore, it allowed us to evaluate changes in locomotor activity, but not avoidance responses (12, 13, 14, 15, 16).

One postlarva was placed per experimental unit and 12 replicates per treatment were used. The assignment was random. Before recording, each of the organisms was conditioned for 2 min; Next, a 3-min continuous recording was performed. Observations were made after 24 and 48 hours of exposure.

Study variables

The main variable was the total distance traveled by each of the postlarvae, used as an indicator of locomotor activity. Qualitative observations, such as erratic movements, immobility, decreased activity, and possible escape responses, were also recorded (3, 12).

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Data analysis

The distance traveled was obtained from video recordings processed with ImageJ and Tracker. Statistical analysis was performed in R (v. 4.5.3). Mean, median, standard deviation and coefficient of variation (CV%) were calculated to describe the dispersion of the data.

Normality and homogeneity of variances were evaluated with the Shapiro-Wilk and Levene tests. Due to the dispersion and the presence of outliers, the non-parametric Kruskal-Wallis test was used. As a descriptive complement, histograms and Kernel density estimates were prepared with ggplot2 (25, 26).

RESULTS

3.1 Locomotor activity and travel distance

The effect of two concentrations of sunscreen (25 mg/L and 300 mg/L) on the locomotor activity of Litopenaeus vannamei postlarvae was evaluated. Analysis of the behavioral response using the non-parametric Kruskal-Wallis test did not reveal statistically significant variations in the net movement distance at any of the evaluation times. After 24 hours of exposure, the organisms showed movement patterns comparable to the control group ($p = 0.28$). The average values recorded for the low (55.6 ± 94.7 mm) and high concentration (46.3 ± 62.8 mm) treatments were higher than the control (18.0 ± 22.4 mm). However, the high dispersion of the data prevented establishing a statistical difference (Figure 1). At 48 hours, no significant differences were detected ($p = 0.58$). However, the exposed groups presented lower values than the control. The postlarvae under the low concentration treatment traveled an average of 14.6 mm and those under the high concentration 18.2 mm, while the control group reached 32.3 mm. Boxplots show median, interquartile range, and individual variability ($n = 12$ per treatment). The Kruskal-Wallis test did not show significant differences between groups ($p > 0.05$).

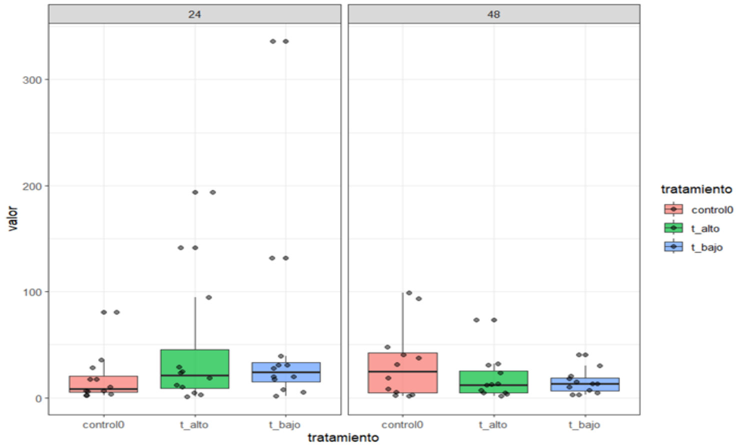

Figure 1. Distance traveled (mm) by L. vannamei postlarvae exposed to different concentrations of sunscreens for 24 and 48 h.

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3.2 Behavioral stability and coefficient of variation (CV%)

Because the magnitude of the displacement did not show significant differences, the coefficient of variation (CV%) was used as a descriptive support to observe the stability of the response. At 24 h, the low concentration group reached the highest value (CV = 171.3%), above the control and the 300 mg/L treatment. At 48 h, CV values decreased, especially by 25 mg/L (77.6%), which coincides with a more concentrated response at low distances.

Table 2. Descriptive parameters of the locomotor displacement of L. vannamei.

Time (h)	Treatment	Average (mm)	Std. Standard (±)	Variability (CV%)
24	Control	18.0	22.4	125.7%
24	Low (25 mg/L)	55.6	94.7	171.3%
24	High (300 mg/L)	46.3	62.8	136.6%
48	Control	32.3	33.9	105.7%
48	Low (25 mg/L)	14.6	11.4	77.6%
48	High (300 mg/L)	18.2	20.3	111.6%

Note. Table 2 shows that, at 24 h, the sunscreen treatments presented average values higher than the control, while at 48 h a decrease in the exposed organisms was recorded. High coefficients of variation show a dispersed response, so the data should be interpreted in conjunction with distribution plots and not just averages.

3.3 Qualitative observations and movement patterns

Complementing the quantitative analysis of locomotor activity, qualitative observations allowed us to identify relevant behavioral alterations that were not completely evident through distance traveled metrics. Throughout the experimental days, progressive alterations in the locomotor behavior of the postlarvae subjected to sunscreens were recorded, particularly experienced in the 25 and 300 mg/L treatments, in response to mechanisms linked to chemical stress and/or perception of the pollutant.

In the first measurement carried out at 24 h, the postlarvae that received the 25 mg/L treatment presented episodes of increased activity, developing rapid and sudden movements within the experimental container; In several postlarvae, escape movements, sudden accelerations and erratic trajectories alternating with short episodes of rest were recorded. This pattern can be characterized as behavior as an initial response to the stimulation of the exposure.

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In the context of 300 mg/L, the behavioral responses were more heterogeneous: although there were cases of individuals who presented in their movements (initial activation), the majority showed less coordinated shoulders and a behavior relatively associated with remaining in certain places in the container. Circular trajectories, spiral movements and erratic movements, without a specific direction, were recorded, which are considered a sign of the neurobehavioral alteration of aquatic organisms exposed to toxic conditions; In addition, partial loss of stability in movement could be observed, which was visible through sudden changes in body orientation, and difficulties in maintaining continuous swimming patterns.

After 48 hours, behavioral failures became evident in both treatments. After these 48 hours, the exposed postlarvae showed a general decrease in locomotor activity, accompanied by long periods of immobility and a reduction in the frequency of movements; This behavior was more evident in the 300 mg/L treatment, since many individuals remained in a state of immobility for long periods, which was interrupted only by short and infrequent movements. This state suggests a state of motor depression or drowsiness associated with the time of exposure to the pollutant.

Likewise, within some exposed organisms we have seen symptoms of disorientation, evidenced by successive collisions against the walls of the container and sudden changes in the direction of movement. These behaviors reflect, in principle, that the space perception and motor skills systems may be altered. On the contrary, the control group had a clearly and very consolidated pattern over the course of the experiment, characterized not only by more regular displacement trajectories, but also by a lower frequency of the presence of periods of immobility and a more homogeneous displacement between individuals.

Qualitative observations also indicate a notable intraspecific variability in the response to the pollutant: thus, while some organisms respond with very intense activity, others immediately respond by inhibiting movement, which reveals individual differences in physiological sensitivity and tolerance to chemical stress. This heterogeneity is consistent with the high values observed for the coefficient of variation at 24 h, which corroborates the existence of behavioral fragmentation among the exposed populations.

The observed patterns describe behavioral changes in postlarvae exposed to sunscreens. The response was not uniform between individuals and changed over time, going from more dispersed records at 24 h to a greater concentration of low distances at 48 h. Therefore, rather than talking about a definitive effect, the data allow us to recognize behavioral signals that must be confirmed in subsequent studies.

3.4 Nonlinear dose-response relationship

The descriptive data did not show a clear linear dose-response relationship (Figure 2). The 25 mg/L treatment at 24 h presented a higher average distance traveled than the 300 mg/L treatment, suggesting a greater initial activity for the low concentration. This trend was

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reversed after 48 h, since the organisms exposed to this concentrate moved less, especially in the treatment with a higher concentration. This result indicates that exposure time has had an effect on the observed response.

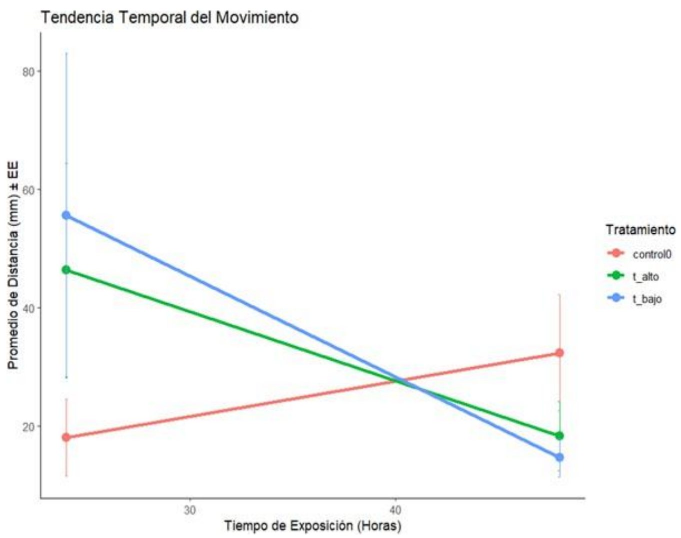

Figure 2. Interaction graph showing changes in locomotor activity between treatments and exposure times.

3.5 Density analysis and frequency distribution

The analysis of frequency histograms allowed us to evaluate the structure of the locomotor response at the population level, as seen in Figure 3. After 24 h of exposure, the control group exhibited values concentrated in low displacements, which demonstrates a relatively homogeneous behavior. On the contrary, the tested treatments exhibited wider and more dispersed distributions, in addition to the presence of extreme data, in the case of the 25 mg/L treatment, the presence of individuals with large displacements was identified, thus causing the distribution to show a "long tail" in it.

This type of behavior is a non-homogeneous response, where organisms with high and low locomotor activity coexist, which gives rise to a fragmentation of the population response to the pollutant. At 48 h, the histograms show a general shift of the distributions towards low ranges of movement (0-50 mm), reflecting that there is a greater proportion of organisms with reduced activity. This type of pattern suggests a move towards a state of motor inhibition or depression.

The control group has a broader representation of the distributions, which highlights greater stability of locomotor activity. The graphic representation allows us to detect variations in the pattern and dispersion of the responses in the distributions that are not detected with the averages, emphasizing a temporal dynamic in the behavioral response and taking into consideration the importance of evaluating the shape of the population structure to detect sublethal effects.

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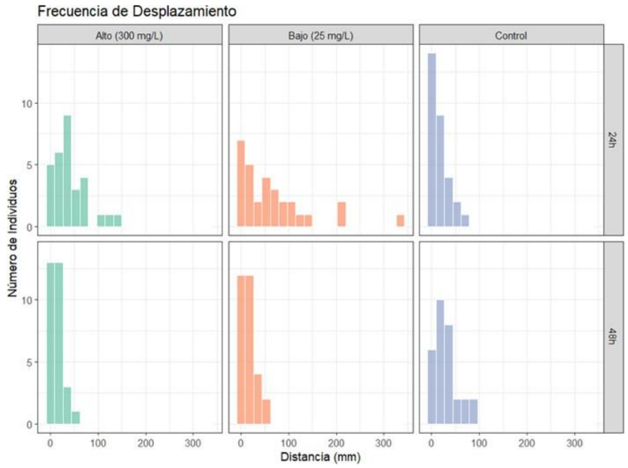

Figure 3. Histograms of displacement frequency (mm) in L. vannamei postlarvae exposed to different concentrations of sunscreen.

3.6 Behavioral instability index (CV%)

The analysis of the coefficient of variation (CV%) made it possible to evaluate the behavioral variability between treatments and exposure times, as seen in Figure 4. At 24 hours, the 25 mg/L treatment presented the highest level of instability (CV = 171.3%), surpassing both the control and the 300 mg/L treatment. mg/L, indicating a high heterogeneity in the locomotor response of individuals exposed to sublethal concentrations. This trend suggests that organisms show different responses to first exposures, in response to the perception of the harmful substance.

After 48 hours, it can be seen that all treatments show a general decrease in the coefficient of variation (CV%), with the 25 mg/L treatment showing the most pronounced decrease (77.6%). The decrease in variability seems to be a trend towards a more homogeneous general behavior, associated with a decrease in locomotor activity. This pattern reveals the tendency from an initial state of excitement (characterized by high variability) to one of motor depression (where organisms show more similar and scarce movements).

The figure reveals the temporality of the behavioral response, where the high initial variability can be understood as one of the first evidences of stress, and the stabilization of variability as the component of the decrease in activity. In this context, the results support the use of CV% as a sensitive biomarker for the detection of sublethal effects in aquatic organisms.

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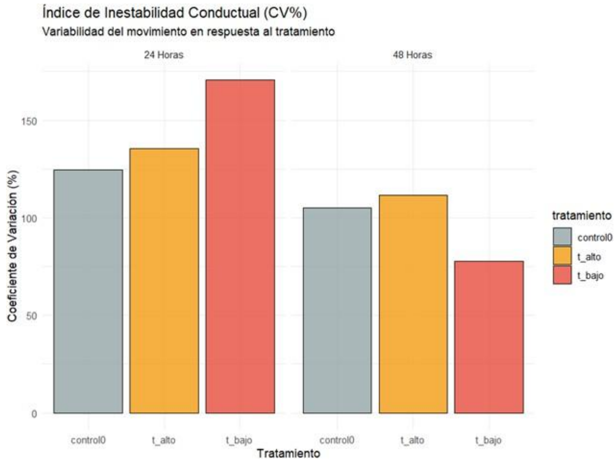

Figure 4. Behavioral instability index expressed as coefficient of variation (CV%) in L. vannamei postlarvae.

3.7 Detailed analysis of density and behavioral structure

3.7.1 Distribution and dispersion at 24 hours

After 24 h of exposure, the analysis of density and frequency distribution showed descriptive differences in the organization of the locomotor response between the groups (Figure 5). The control showed narrower distributions and distributed in smaller displacements, in contrast, the tested treatments showed broader curves.

Treatments exposed to sunscreens showed broader and more dispersed distributions, showing a not so homogeneous response. In the 25 mg/L treatment, the treatment showed the widest density curve and notable heterogeneity between individuals.

At the same time, treatment at 25 mg/L showed evidence of secondary peaks in the intermediate ranges of displacement (150-200 mm), but indication of a multimodal distribution in the population. In this case, this distribution reflects a fragmentation of the behavioral response into repeated subgroups, as well as the existence of different levels of sensitivity to the pollutant. The existence of these subpopulations is evidence of a certain loss of behavioral synchronization, as well as group homeostasis in the face of chemical stress.

On the other hand, the treatment at 300 mg/L yielded samples with a more compact distribution than that of the treatment at 25 mg/L, but always showing a broader distribution than that presented by the control group. In this case, locomotor activity was concentrated mainly in moderate displacement ranges, with less presence of extreme values, which would indicate that higher concentrations of the contaminant could induce an earlier inhibition of locomotor activity, limiting the presence of hyperactive responses present in the treatment at 25 mg/L.

The widening of the treatment density curves reflects the existence of greater intraspecific variability and a less predictable behavioral response to sunscreen exposure. This type of behavior would be consistent with the high values of the coefficient of variation at 24 hours,

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particularly in the treatment with the lowest concentration (CV = 171.3%), which again corroborates that the variability of movement behavior can be a sensitive biomarker of sublethal stress.

On the other hand, from an ecotoxicological perspective, the dispersion we observed may also indicate that the postlarvae must have modified their type of movement upon exposure. The existence of high-glass and low-glass individuals also indicates that the response is not uniform and that therefore it is convenient to have the total distribution of the data.

3.7.2 Density shift at 48 hours

At 48 h of exposure, the density analysis showed a visible modification in the structure of the locomotor response of Litopenaeus vannamei postlarvae exposed to sunscreens. Compared to 24 h, the treatment curves shifted toward lower displacement values.

The frequency histograms showed a predominant concentration of organisms in the range of 0 to 10 mm of displacement, particularly in the 300 mg/L treatment. The suggested behavior also indicates that there is a tendency towards states of very low movement, such as, for example, partial immobility or lethargy and the limited movement that characterizes them, short journeys and the existence of long periods of rest. The decrease in the amplitude of the distributions manifests the loss of hyperactivity observed in the first twenty-four hours and reveals the transition towards motor depression responses, consequences of the increase in exposure time.

In the 25 mg/L treatment, the measurements still determine some individuals with moderate movements, but the majority were characterized by very reduced levels of locomotor activity, with a more concentrated (less dispersed) distribution than the initial one, which suggests a progressive homogenization of behavior within the experimental group, where individual responses tend to converge on low levels of mobility, a process that would also be accompanied by the reduction in dispersion observed at 48 hours, which corresponds to the period of time in which a stabilization of the physiological response of individuals to temporary exposure to chemical stress is determined.

On the contrary, the control group offered a broader and more balanced distribution, with individuals distributed in different displacement ranges and without a predominant accumulation at values close to zero. This allows us to conclude that unexposed postlarvae maintained more active and more continuous locomotor patterns, as well as a relatively stable behavioral organization during the development of the trial.

The shift of the density curves towards low values can be interpreted as a decrease in the locomotor performance of the animals under test conditions, but this reading must be cautious, because the bioassay did not yield physiological biomarkers and an analysis of the measurement of the components present in the water was not carried out.

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Likewise, the change from broad and heterogeneous distributions to narrower and more concentrated curves shows a temporal variation in the response. In descriptive terms, first more dispersed recordings were observed and then greater grouping at low distances, which coincides with the general reduction in movement.

3.8 Relationship between density and variability (CV%)

The structure of the density curves is consistent with the high values of the coefficient of variation observed at 24 hours. The extensive distributions, together with the existence of extreme values, are indicative that individuals did not respond homogeneously, but rather of a high dispersion between individuals.

The curves suggest the probability of occurrence of individuals under different levels of activity, and broader information can be observed in the distributions at 24h of the treatments and a shift towards low values of movement at 48h.

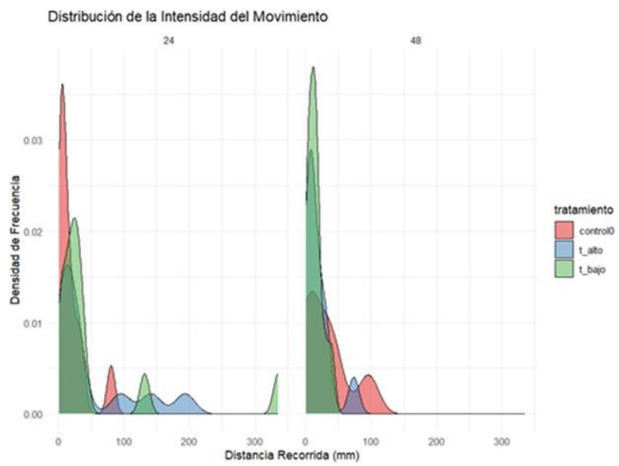

Figure 5. Density distribution of locomotor activity (mm) in L. vannamei postlarvae.

DISCUSSION

The comparison of the mean distances did not show statistically significant differences between treatments. We should not be held responsible for the effect of the concentrations we have evaluated on the average displacement. The descriptive results suggest that the response was not uniform: at 24 h the 25 mg/L treatment produced the highest coefficient of variation and the distance was more distributed, and at 48 h the exposed groups concentrated more recordings at low distances but without revealing the same degree of variability as the control group. In behavioral ecotoxicology studies, these changes in variability can complement, but not replace, the statistical test on means (12, 14, 15, 16).

The extreme values and dispersion observed at 24 h indicate that some organisms moved more than others under the same exposure condition. This heterogeneity may be related

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to individual differences, manipulation or exposure to the product. For this reason, it is not possible to attribute variability alone to a specific mechanism. Even so, the use of histograms and Kernel density allowed us to describe an aspect of the data that the average does not clearly show (23, 25, 26).

The general reduction in movement at 48 h deserves cautious interpretation. It may be compatible with lower locomotor activity, but the study did not measure physiological biomarkers or the effective concentration of sunscreen components. It also does not allow the effect of UV filters to be separated from that of other ingredients in the formulation. Therefore, the results should be read as a behavioral response observed under the conditions of the bioassay and not as evidence of a specific neurological, endocrine or metabolic mechanism (8, 9, 10, 11).

The absence of a linear dose-response relationship in the descriptive data should also not be interpreted as evidence of a biphasic response. The greater dispersion at 25 mg/L may be due to a combination of individual variation, sample size, and product exposure. Therefore, the observed pattern must be confirmed with independent replicates and with analyzes designed to simultaneously evaluate concentration and exposure time (22, 23, 25).

The design also does not allow the environmental risk to be estimated by itself. The concentrations were nominal and the study was carried out under controlled conditions, while in natural environments dilution, currents, light, mixing with other contaminants and variations in temperature and salinity intervene. The differences do not invalidate the bioassay because they do not allow us to establish the conclusion that the product tried to cause permanent alterations, but they become a limit to the best conclusions and improvements for subsequent work.

The perception that there is no statistical difference does not indicate that there is no effect, but it does not allow us to conclude that the product has tried to cause permanent alterations. The result must be interpreted within the framework of limits of our design, which we contextualize given the high variability and the convenience of replicating the trial.

From an environmental perspective, locomotor behavior is relevant because it participates in foraging, predator evasion, and habitat use (12, 13, 18). The findings justify continuing with trials that incorporate physicochemical control of water, analytical verification of concentrations, larger sample size and exposures closer to environmental scenarios. It would also be useful to integrate physiological or biochemical variables to relate behavioral changes to other stress indicators (3, 7, 14).

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CONCLUSIONS

Under the conditions of this bioassay, concentrations of 25 and 300 mg/L of sunscreen do not cause significant differences in the average displacement of L. vannamei postlarvae at 24 h or 48 h.

The 25 mg/L treatment presented the greatest variability at 24 (CV = 171.3%), and the distribution analyzes showed a more dispersed response than that of the control group. At 48 h, the exposed groups had a greater concentration of recordings at low distances. These descriptive patterns suggest behavioral changes that require confirmation in new trials.

The mean distance, the coefficient of variation, histograms and Kernel density provided complementary information to describe the locomotor response. To establish the ecological importance of the observed patterns, studies with complete physicochemical control, analysis of the components present in the water and greater replication are needed.

For all that has been said, the results should be evaluated as exploratory evidence and not as proof of specific toxicity. The value of the study lies in pointing out that, even when the means do not differ significantly, the form of data distribution can offer useful clues to determine which questions need to be analyzed in a next stage.

ACKNOWLEDGEMENTS

We thank Jorge Cedeño for his collaboration in data collection; to the Virgen del Cisne laboratory for the provision of the postlarvae; and to the laboratory of the Universidad Laica Eloy Alfaro for providing the equipment and instruments used. The support of the Eloy Alfaro Lay University, the Milagro State University, the teachers and the tutors who accompanied the research process are also recognized.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest related to the publication of this article. Likewise, no external financing was received that could influence the results obtained.

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3091-1796
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