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RESEARCH ARTICLE

Silver nanoparticles induce histopathological alterations in juvenile *Penaeus vannamei*

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Abstract

The objective of this study was to evaluate the histopathological alterations in juvenile *Penaeus vannamei* caused by silver nanoparticles (AgNPs) for two types of experiments: at sublethal concentrations of 3.6 to 7.1 μg/μL of metallic silver (Ag) for a short period up to 72 h and for 2.6 to 7.9 μg of Ag/μL for the long period up to 264 h. The severity degree of the changes was evaluated and the histopathological index (Hi) was determined in both experiments using the necrosis (cellular dead) as an indicator. The pathological changes in the striated muscle, gills, antennal gland, circulatory system, heart, lymphoid organ, and connective tissue are described. The histopathological effects were similar for the two experiments without a direct relationship with the concentrations. In the short-term experiment, the values of Hi were higher (2.34 ± 0.41 at 48 hpi and 1.91 ± 0.39 at 72 hpi) compared with the long-term experiment (values between 0.57 ± 0.36 to 1.74 ± 0.57 at 264 hpi). The observed pathologies are similar to those caused by other metals, with the exception of the agglomerations of black particles in the gills, lymphoid organ, and muscle, which has not been previously reported. This work shows that silver nanoparticles cause damage to shrimp in sublethal concentrations.

Keywords Silver nanoparticles · Pathological alterations · Sublethal concentrations · *Penaeus vannamei*

Introduction

The increase in aquaculture activity worldwide and globalization have augmented the possibility of the easy spread of various pathogens. Therefore, the industry faces diseases that limit its capacity for efficient production, which has caused detrimental effects on the industry worldwide (Banerjee et al. 2014). Nanotechnology shows important potential for

Highlights
- Sublethal concentrations of silver nanoparticles caused 23% mortality to shrimp
- Silver nanoparticles at sublethal levels cause pathological damage to shrimp
- Silver nanoparticles produce necrosis to different tissues and organs
- The treated shrimp could mitigate through their immune system the effects of AgNps

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revolutionizing agriculture, livestock, aquaculture, and fisheries (Rather et al. 2011). Nanotechnology is an emerging branch of science and engineering and has a high potential for application in aquaculture as a new tool for rapid disease detection and clinical examinations to increase the ability of organisms to absorb hormones, vaccines, and nutrients (Selvaraj et al. 2014; Huang et al. 2015) and possibly as a nanotechnology strategy for the control of disease (Sibaja-Luis et al. 2019). Silver nanoparticles (AgNPs) are the most commonly used in medicine due to their antimicrobial properties. In shrimp, various AgNPs have been used against bacteria of the genus *Vibrio* with encouraging results (Vaseeharan et al. 2010, 2012; Kandasamy et al. 2013; Ramamoorthy et al. 2013; Sivaramasamy et al. 2016; Bahabadi et al. 2017) or against the bacteria that cause necrotizing hepatopancreatitis (NHP-B) (Acedo-Valdez et al. 2017). Juarez-Moreno et al. (2017) used AgNPs of the Argovit™ brand at very low concentrations and showed that they did not cause stress or changes in blood parameters; moreover, 80% of shrimp infected with the virus and treated with the AgNPs survived, indicating their potential for their use as of AgNPs as an antiviral strategy. Ochoa-Meza et al. (2019) assessed the capacity of non-toxic PVP (Polyvinylpyrrolidone)-coated Argovit™ AgNPs to stimulate the response of the immune system of WSSV-infected shrimps with or without an excess of iron ions. They showed that the AgNp concentrations used did not reduce the viral infection of WSSV. Romo-Quiñonez et al. (2020) evaluated a new silver nanoparticle formulation named Argovit-4 (Argovit®) in four experiments to determine the protective effect against WSSV. Their results show that the new Argovit-4 formulation has potential as an antiviral additive in feed against WSSV.

Gambardella et al. (2015) and Canesi and Corsi (2016) studied the effect of AgNPs on marine plants and animals belonging to different trophic levels; their results show that nanoparticles accumulation mainly occurs in the digestive tract and gills, so AgNP exposure can influence different trophic levels within the marine ecosystem. They concluded that more information on eco/biological interactions of nanoparticles (NPs) in the marine environment is needed. In a systematic review on silver nanoparticle-induced cytotoxicity, Akter et al. (2018) concluded that the studies are not enough to obtain a concrete idea on the cytotoxicity of AgNPs, and the mechanism behind the toxicity can be considered dependent on different kinds of properties such as nanoparticle size, shape, dose, agglomeration or aggregation, and also, the organism variation, which plays a vital role. This work aims to determine the pathological changes that occur in the shrimp *Penaeus vannamei* with the application of sublethal concentrations of Argovit™ AgNPs in 72 and 264 h period and to evaluate whether the shrimp are able to recover during a longer exposure period.

Materials and methods

Silver nanoparticles

Argovit™ preparation of highly dispersed AgNPs with a concentration of 200 mg/mL (20%) of Ag covered with Polyvinylpyrrolidone (PVP) in water. PVP and all components to prepare phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 pH 7.4) were purchased from Sigma-Aldrich (St. Louis MO, USA).

Argovit™ AgNPs are spheroids with a diameter of 35 ± 15 nm. The Ag content in the Argovit preparation is 12 mg/mL (equivalent to 0.11 mol of Ag/L) and is stabilized with 188 mg/mL of PVP (Juarez-Moreno et al. 2017). These particles have been shown to act against bacteria, fungi (Vazquez-Muñoz et al., 2014) and viruses (Borrego et al. 2016; Juarez-Moreno et al. 2017). The AgNPs of the Argovit™ brand is non-toxic, and with their design, they have met international and national sanitary filters due to their multiple applications and a wide spectrum of extraordinary properties. Argovit has international certificates for use in veterinary and human surgery (Bogdanchikova et al. 2016; Borrego et al. 2016).

Nanoparticle suspension

The concentrations were selected according to the results of 6 preliminary experiments and calculated on a logarithmic scale, in which we seek to identify concentrations that did not kill the shrimp but for which pathological alterations could be identified. The concentrations were calculated according to the metallic silver content in the Argovit particles (12 mg/mL). The solutions were prepared in a 25 μL volume of PBS and kept at 4 °C in darkness.

Experimental design

Two experiments were designed to evaluate the effects of nanoparticles in shrimp during a short period of 72-h post-injection (hpi) and long-term exposure of 264 hpi. The first short-term experiment consisted of evaluation of the following seven concentrations (3.6, 4.1, 4.5, 5.1, 5.7, 6.4, and 7.1 μg/μL) of metallic silver and control with 116.6 μg/μL of polyvinylpyrrolidone (PVP), which was applied for stabilization of AgNPs in Argovit product. We obtained 80 healthy juvenile *Penaeus vannamei* shrimp with an average weight of 5.3 ± 0.00 g from a farm. The organisms were distributed in batches of ten organisms in eight 15-L aquariums with a continuous flow of seawater (35 practical salinity units (PSU)) and were maintained at a temperature of 28 °C. In the second experiment (long-term), 140 healthy juvenile shrimp from a shrimp larvae producer laboratory with an average weight of 5.15 ± 0.88 g were used. Twenty-eight shrimp were placed in each

one of 5 aquariums. The treatment consisted of the application of three AgNP metallic silver concentrations (2.6, 5.3 and 7.9 μg of Ag/μL) and control with 125.5 μg/μL of PVP. The organisms in both experiments were injected with an AgNP volume of 25 μL in the ventral sinus at the height of the fifth pair of pereiopods with unique treatment only at the beginning of the experiment. After the injections, the shrimp were returned to their aquariums.

Samples for histology

Ten organisms were collected at random from each lot to evaluate the initial health condition by histology. During the two experiments, five randomly selected organisms were collected during each sampling period (at 48 and 72 hpi for the short-term experiment and at 48, 120, 192, and 264 hpi for the long-term experiment). The collected organisms were fixed with Davidson's solution and processed histologically following the methodology of Bell and Lighter (1988). The stained sections were analysed with a compound microscope (Karl Zeiss Axioscop), and images were taken with an Olympus digital Qimagine camera.

Histopathological index

In each experiment, the severity of the histological damage (Sd) in the tissues was determined. Values were assigned from 0 to 4, with 0 indicating the absence of tissue damage and 4 indicating a maximum damage effect according to a classification method modified from Lightner (1996). For the tissues included in the histological index calculations, values (V) according to their function (Sonnenholzner et al. 2002) were assigned, being "3" for antennal gland and lymphoid organ, since they are excretion organs and are related to the immune system; a value of "2" was given to the heart, gills, and circulatory tissue, because these organs are important for the transport of haemocytes and foreign particles; and finally, a value of "1" was given to the muscle and connective tissue. Initially, the Sd value was divided by 4 normalizing each pathological measurement for identification as a clinical sign of AgNP exposure. Each pathological measurement was catalogued semi-quantitatively. In this study to determine the histological index (Hi) per organism, the necrosis as the main pathological lesion in cells was used. The Hi was calculated with the equation: $Hi = \Sigma\ (Sd \times V/4)$, with global histological index range of 0 to 6.17.

Data from both experiments were compared individually by two-way ANOVA and multiple comparisons with Holm Sidak tests. In the case of the long-term experiment, the distribution of data was not normal (Kolmogorov-Smirnov and Bartlett's tests); therefore, the R1 rank transformation (Conover and Iman 1981; Conover 2012) was used. The significance level was $P < 0.05$ in all cases. The graphic was constructed with photoshop.

Confirmation of silver in the histological sections

To identify (presence/absence) whether the particles observed in the shrimp tissues were silver particles, a modification of the normal staining technique used by Bell and Lighter (1988) was applied after omitting the haematoxylin application step (eosin-phloxine (E-F) staining method), which allowed the silver to be differentiated by its black colour in contrast to the brown-coloured melanin.

Results

Mortality

During the short-term experiment (concentrations of 3.6, 4.1, 4.5, 5.1, 5.7, 6.4, and 7.1 μg/μL), a maximum mortality of 20% was observed in the treatments with 3.6, 5.7, and 7.1 μg of Ag/μL, and 10% mortality was observed in the other treatments except for 5.1 μg of Ag/μL, for which no mortality was observed (similar to PVP).

In the long-term experiment, the mortality was 23% and 20% for the 5.3 and 7.9 μg of Ag/μL treatments, respectively. No mortality was observed for the 2.6 μg of Ag/μL treatment or in the PVP organisms.

Clinical observations of shrimp injected with AgNPs into the ventral sinus

During the injection process for each animal, a brown liquid flowing into the ventral sinus towards the gills and the heart was observed. The shrimp were returned to the corresponding aquariums after the injection, where they lay on one side without moving for a few minutes due to the stress of handling and the injection itself before they began to swim normally. Those who did not die immediately exhibited normal swimming and feeding behaviour throughout the experiment.

Pathological alterations

In both experiments, the organisms injected with the AgNPs always showed necrosis in all tissues evaluated with similar histopathological damage in all concentrations, such as haemocyte infiltration, the formation of haemocytic nodules, accompanied by melanisation, and the presence of dark deposits in response to the presence of AgNPs.

Because histopathological damage in both experiments was similar, the results are presented together; however, there is no intention to compare the severity and the Hi between experiments because the origin of the shrimp was different.

The muscle of shrimp from the control groups (PVP) did not show dark deposits in either experiment (Fig. 1a); however, necrosis and haemocytic infiltration occurred only in small foci in the short-term experiment PVP with a Hi range between 0.03 and 0.13 at 48 and 72 hpi (respectively) (Fig. 2a) and between 0.00 and 0.13, in the long-term experiment PVP (Fig. 2b).

The organisms injected with the AgNPs in the short- and long-term experiments always showed focal necrosis of muscle bundles. Most of these effects were located near the injection site, although sometimes they were found in other areas of the shrimp. Infiltration of haemocytes was evident with dark deposits delimited by haemocytic nodules and melanisation, pyknosis, and fibroblast infiltration (Fig. 1b, c). The size and extension of the nodules and the melanisation did not have a direct relationship with the concentration. The severity in muscle necrosis varied in the short-term experiment in all AgNP-treated organisms with Hi values between 0.19 and 0.33 (Fig. 2a). In AgNP treatments of the long-term experiment, the severity values ranged between 0.03 and 0.38 Hi, being the highest severity at 192 hpi (0.22 and 0.38 Hi, respectively) in the 5.3 and 7.9 μg of Ag/μL (Fig. 2b).

The gills of all treated organisms in the two experiments presented necrosis in pillar cells with haemocyte infiltration and formation of haemocytic nodules accompanied by melanisation and dark deposits (Fig. 1d). Nephrocyte pyknosis was also observed (Fig. 1e) in the short-term experiment and varied from focal to extensive; with Hi of 0.27 and 0.56, and no direct relationship was found with the nanoparticle concentrations (Fig. 2a). In the long-term experiment, necrotic pillar cells were observed at all-time points with the maximum value of Hi = 0.29 at 192 hpi, and only pyknotic nephrocytes were present at the highest concentrations (7.9 μg of Ag/μL). The gills of the shrimp from the PVP groups of the two experiments showed no dark deposits (Fig. 1f) and show the lowest necrosis index (Fig. 2a, b).

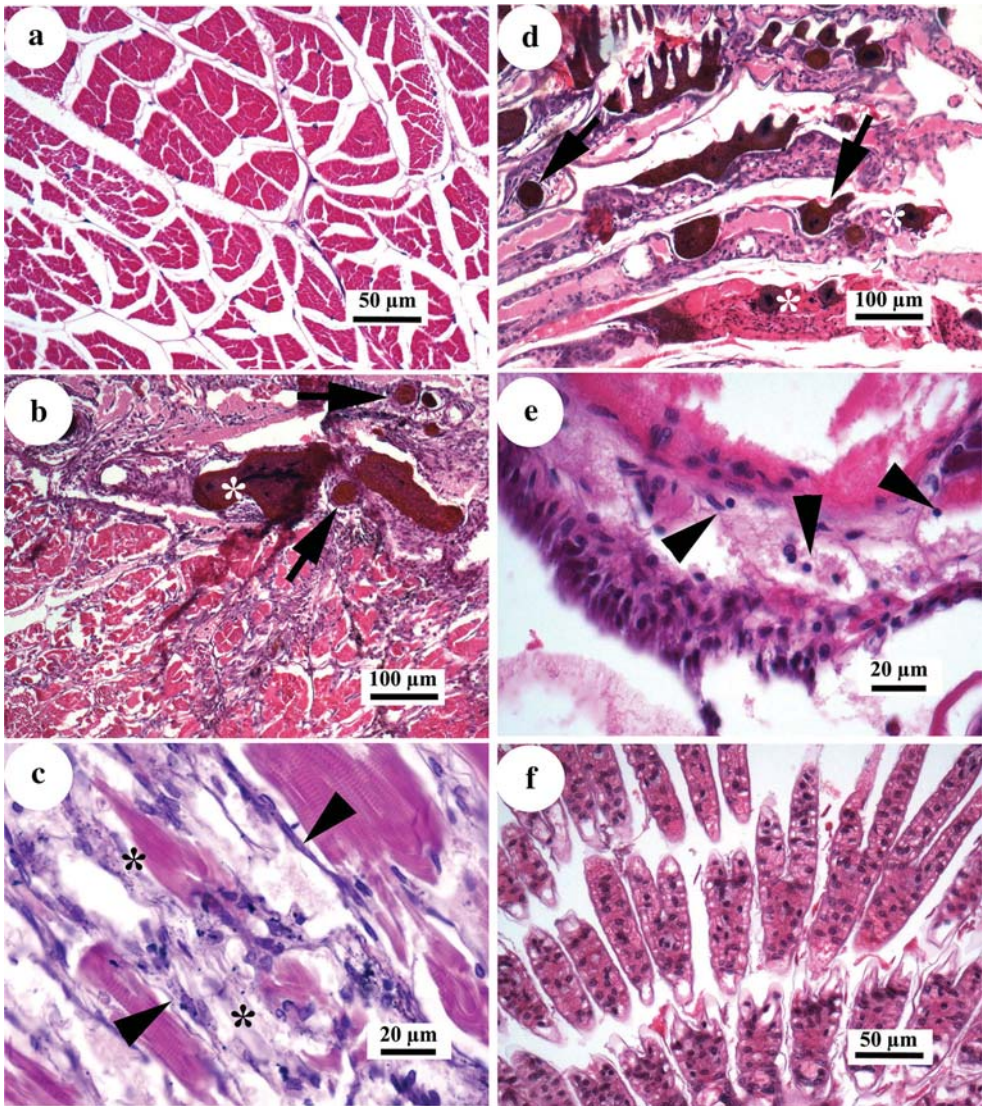

Fig. 1 Longitudinal cut of striated muscle (**a**–**c**) and gills (**d**–**f**) of *Penaeus vannamei*. Muscle of PVP shrimp (**a**) and muscle of shrimp exposed to metallic silver (**b**, **c**). Gills of treated shrimp exposed to AgNPs (**d**, **e**) and gills from PVP shrimp (**f**). Arrows indicate melanised haemocytic nodules; *Dark Ag deposits; arrowheads point to granulation fibrous tissue (**c**) and nephrocyte pyknosis (**e**). H&E stain

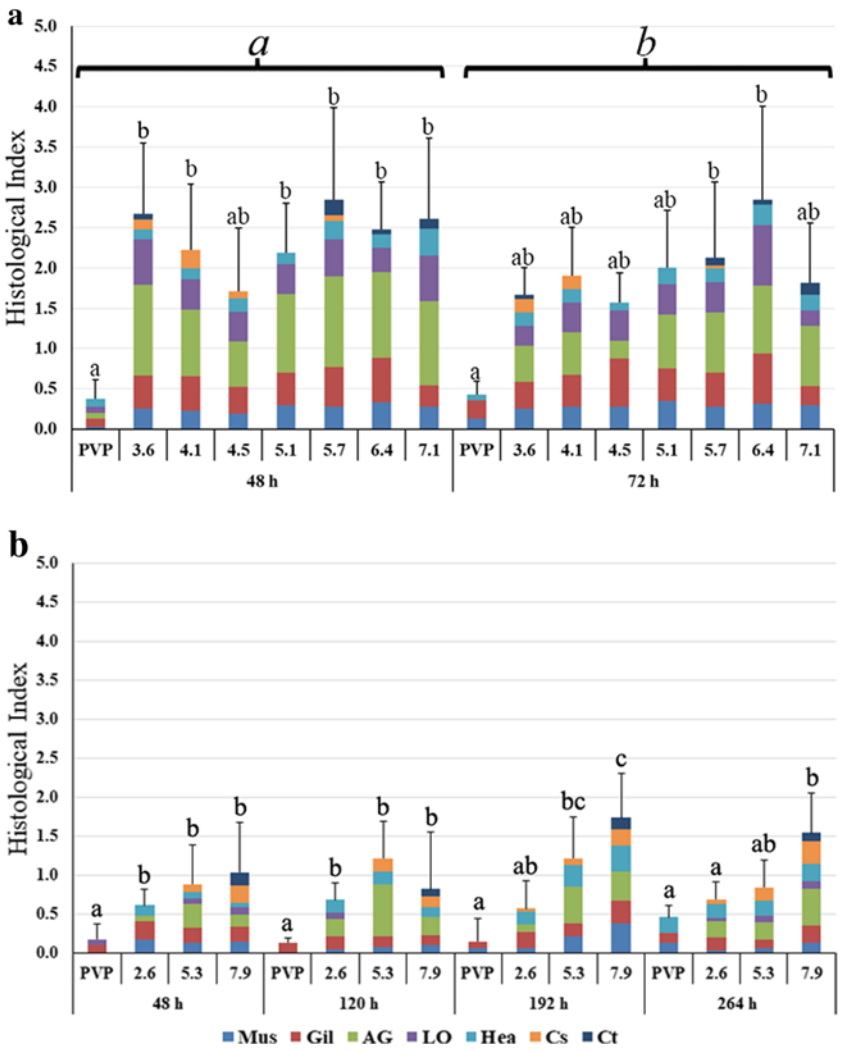

Fig. 2 Mean values (±SD) of the histological index of necrosis in *Penaeus vannamei* in the short-term experiment (**a**) at 48 and 72 h and long-term experiment (**b**) at 48–264 h after injection with metallic silver concentrations (from 3.6 to 7.1 in A and from 2.6 to 7.9 in B μg of Ag/μL) and PVP. Mus, muscle; Gil, gills; AG, antennal gland; LO, lymphoid organ; Hea, heart; Cs, circulatory system; Ct, connective tissue. Different letters indicate significant differences (two-ways ANOVA, $P < 0.05$)

No dark deposits were identified in the antennal gland of the PVP organisms in both experiments (Fig. 3a); however, only in the short-term experiment, shrimps from the PVP also had necrosis and atrophy in the antennal gland; however, the severity was low (HI = 0.08) and occurred in small foci and only in some organisms (Fig. 2a). The opposite was observed in the epithelial cells of the antennal gland of all treated organisms with AgNPs in both experiments, since this was the most affected organ compared to the other organs and tissues evaluated. This tissue exhibited different degrees of necrosis (Fig. 3b), haemocytic infiltration, atrophy, and fibroblast infiltration (fibrosis) (Fig. 3c). The Hi values of necrosis were between 0.56 and 1.13 (48 hpi) and between 0.23 and 0.84 (72 hpi) in the short-term experiment (Fig. 2a), and Hi values between 0.09 and 0.47 (192 hpi) and between 0.20 and 0.47 (246 hpi) in the long-term experiment (Fig. 2B).

The stromal tissue of the lymphoid organ of both experiments lost its normal structure with the cell necrosis (Fig. 3d), haemocytic infiltration, development of nodules that at times were melanised (Fig. 3e), and with focal dark deposits (Fig. 3f). The lymphoid organs from the PVP organisms did not present dark deposits. The Hi value in shrimp treated with AgNPs ranged in between 0.38 and 0.56 at 48 hpi, and 0.19 and 0.75 at 72 hpi in the short-term (Fig. 2a), and in the long-term experiment, the Hi was lower (≤ 0.09) at all times (Fig. 2b).

The heart tissue of the treated organisms in both experiments had dark deposits in small foci in most treatments (except for the lowest concentrations 2.6 and 3.6 μg of Ag/μL groups in both experiments). In addition, some organisms show focal necrosis, haemocytic infiltration, and less frequently small nodule formation and melanisation. Necrosis and haemocytic infiltration identified in the PVP organisms of the short-term experiment had Hi values of 0.10 at 48 hpi and 0.07 at 72 hpi; however, the severity in necrosis was higher in the organisms treated with the Ag (Hi between 0.13 and 0.33 at 48 hpi and 0.17 and 0.25 at 72 hpi) (Fig. 2a). In the long-term experiment, the Hi values oscillated between 0.07 and 0.33 in all treatment with Ag, being the severity higher at 192 hpi. The PVP organisms did not show damage in all times, except at 264 hpi (Fig. 2b).

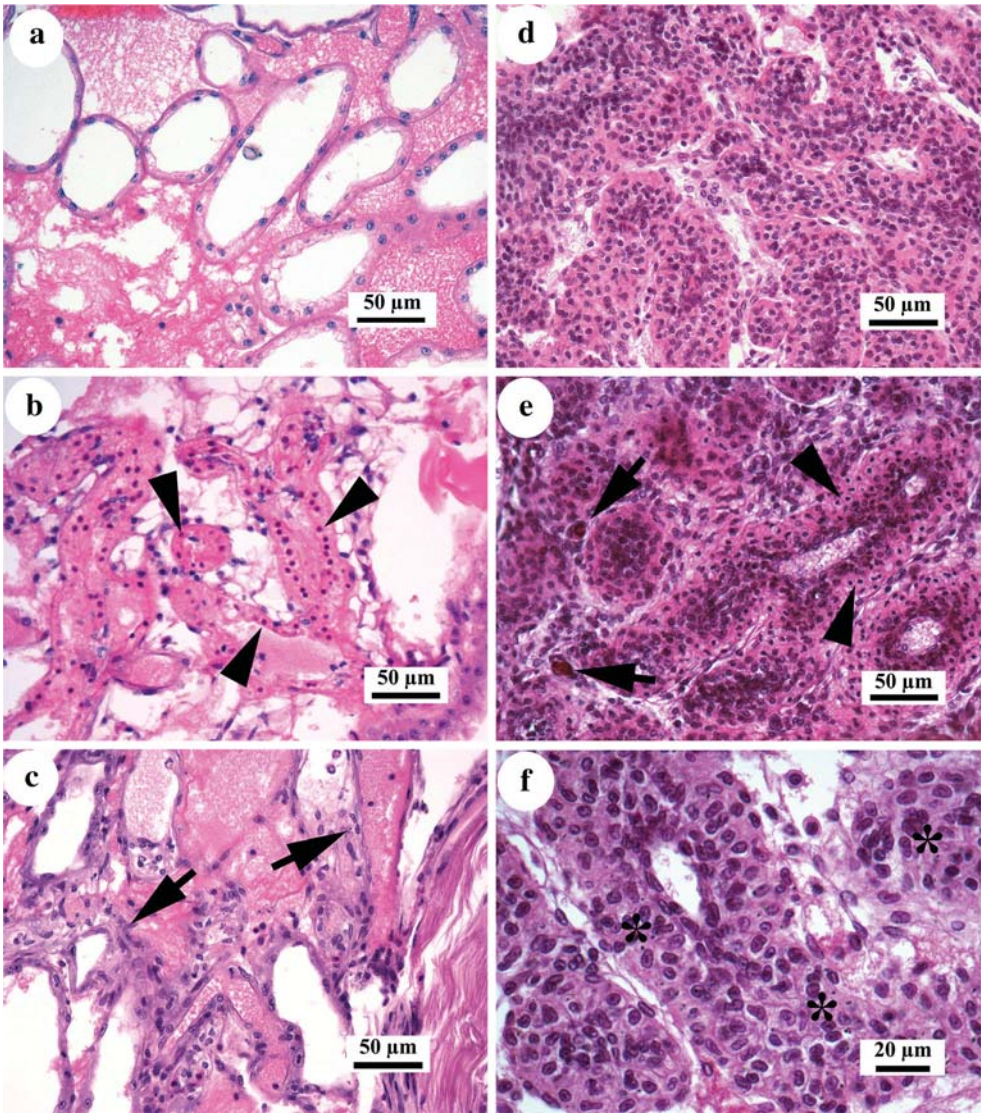

Fig. 3 Longitudinal cut of the antennal gland and lymphoid organ of *Penaeus vannamei*. The antennal gland of shrimp from the control group (**a**) and antennal gland exposed to the metallic silver (**b**, **c**). Arrowheads indicate necrosis of the antennal gland epithelia and the arrow shows granular tissue infiltration. Lymphoid organ from the PVP group (**d**) and cut of the lymphoid organ exposed to metallic silver (**e**, **f**). The arrowheads show necrosis of the stromal matrix; the arrows indicate haemocytic melanised nodules with black deposits (*). H&E staining

Agglutination and necrosis of haemocytes were present in the circulatory system of the treated organisms, however, while necrosis was not present for the concentrations 5.1, 6.4, and 7.1 μg of Ag/μL of the first experiment (Fig. 2a) and the same was observed in the long-term experiment for 2.6 μg of Ag/μL at 48 and 120 hpi (Fig. 2b). These haemocytes appeared in areas where the necrosis was observed in the gills, heart, muscle, connective tissue, and antennal gland. The PVP organisms did not show damage in the haemocytes at all times.

The connective tissue of some treated shrimps in the short-term experiment had necrosis, haemocytic infiltration, haemocytic nodules (some of which were melanised), and dark deposits on one side of the ventral nerve cord, whereas in shrimps from the PVP presented only mild haemocytic infiltration. Shrimp from the long-term experiment injected with different concentrations showed similar damage only for the 7.9 μg of Ag/μL, whereas the shrimp treated with 2.6 and 5.3 μg of Ag/μL and the PVP organisms did not show these pathologies (Fig. 2a, b).

Statistical results

In the short-term experiment (Fig. 2a), the variation in the severity of the necrosis of organs and tissues is evident. In this experiment, the AgNP-exposed shrimp showed a Hi (2.34 ± 0.41 at 48 hpi and 1.91 ± 0.39 at 72 hpi) than in the PVP (0.38 ± 0.24 and 0.43 ± 0.17 at 48 and 72 hpi, respectively) with significant difference between PVP and all treatments ($P < 0.001$), except of 4.5 μg of Ag/μL at 48 hpi, being the 3.6, 5.7, 6.4, and 7.1 μg of Ag/μL which reflected the highest values; meanwhile, at 72 hpi, only 5.7 and 6.4 μg of Ag/μL were significantly different from PVP. The Hi values recorded at 48 hpi were significantly higher ($P < 0.05$) than those registered at 72 hpi for all treatments. It is also clear that the most

affected tissue was the antennal gland followed by the gills and muscle.

In the long-term experiment (Fig. 2b), the necrotic effect in the organs and tissues was minor (0.57 ± 0.36 to 1.74 ± 0.57) compared with that in the short-term experiment. The Hi values tended to be higher with respect to the increment in AgNPs concentrations independently of time elapsed, showing a significant effect between PVP and treatments.

The statistical analysis shows significant differences between PVP and all treatments with Ag in 48 and 120 hpi, but in the last two samplings, there are significant differences only between PVP and 5.3 and 7.9 μg of Ag/μL at 192 hpi and between PVP and 7.9 μg of Ag/μL at 264 hpi.

Confirmation of the presence of AgNPs in tissues

The E-F staining method showed dark deposits in the tissues and haemocytic nodules corresponding to silver nanoparticle deposits. This finding differs from melanisation by the brown colouration of melanin, with various tones representing melanin accumulation. Figure 4 a illustrates this phenomenon; the figures show the muscle and lymphoid organ with different amounts of accumulated silver.

Discussion

As reported by Kachenton et al. (2018), the examination of Ag toxicity and its tissue pathology is uncommon, and thus, few studies have reported lesions induced by Ag use and its risks. Acedo-Valdez et al. (2017) administrated AgNPs to *P. vannamei* juveniles by forced feeding to determine the effectiveness of controlling the bacteria that caused necrotising hepatopancreatitis (NHP-B) and reported pathologies which were caused by bacteria and not by the AgNPs. Ochoa-Meza et al. (2019) reported that after the exposition to 120 and 12 ng/μL AgNps and iron ions, respectively, it did not present significant damage after 96 hpi in shrimp tissues. The brown colouration (observed in the ventral sinus) flowing towards the cephalothorax until reaching the gills observed at the time of injection in this work was also reported for injection with various metals, including cadmium in *Penaeus vulgaris* (Nimmo et al. 1977) and *Penaeus semisulcatus* (Sabu et al. 2017); copper in *Metapenaeus japonicus* (Bambang et al. 1995), *Penaeus japonicus* (Soegianto et al. 1999), and *Macrobrachium rosenbergii* (Li et al. 2007); and Cd and Zn in *Litopenaeus vannamei* (Wu et al. 2009). The shrimp injected in the present study with PBS (data not presented) and PVP did not show either melanisation or this dark colouration at the time of the injection.

Mortalities caused by the use of silver in crustacean has been described by Ishwarya et al. (2016) when determining the toxic effects of AgNO3, *Cissus quadrangularis* (Cq)-synthesized AgNPs, and Cq extract in the micro-crustacean *Ceriodaphnia cornuta*. AgNO3 and Cq-AgNPs showed high mortality rates even if tested at very low doses. Banumathi et al. (2017) in a study with silver nanoparticles coated by *Camellia sinensis* (Cs) and AgN03 to determine its toxicity observed 100% mortality of *C. cornuta* post-exposure to AgNO3 (40 μg/mL) if compared to the Cs leaf extract and Cs-AgNPs, showing 30 and 56% mortality at the same concentration, respectively. The metallic silver at low concentrations does not cause high mortality in shrimps; this was demonstrated by Juarez-Moreno et al. (2017) when they reached a survival of 98.7% at 96 hpi in injected shrimps with 100 μL of lower concentrations of 0.5, 5, and 20 ng/μL AgNPs. Acedo-Valdez et al. (2017) did not observe mortality when shrimps were treated with 35 μg of AgNPs. Ochoa-Meza et al. (2019) administered 12 and 120 ng/μL of metallic silver by injection together with an inoculum of WSSV, but they did not have a negative control containing only silver nanoparticles; thus, their mortality results are only due to the effect of WSSV and no mortality due to silver nanoparticles alone was reported. In this study, our results showed cumulative mortality at 72 and 264 h (20 to 23% respectively) and pathological changes in the tissues for all sublethal concentrations applied. We had no mortality at the concentration of 5.1 μg of Ag/μL. However, at low and higher concentration of AgNPs than 5.1 μg of Ag/μL expressed mortality. We did not expect

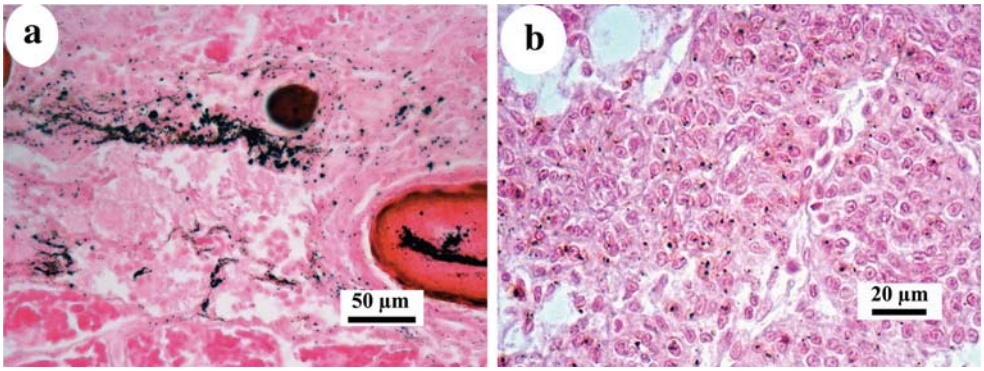

Fig. 4 Longitudinal cuts of the tissues striated muscle (**a**) and lymphoid organ (**b**) of *Penaeus vannamei* exposed to metallic silver. Dark colourations indicate the presence of silver. E-F staining method

mortality at these concentrations, so we can only assume some aspect of individual immune susceptibility derived from an anaphylactic shock derived from the introduction of nanoparticles to the body's circulatory system. Another possible explanation is the ability of nanoparticles to form aggregates. Lekamge et al. (2018) mention in their study that AgNPs have high capacity to form aggregates in the aquatic environment and suggest that the deposition of particles makes them less bioavailable. In this sense, it is possible that when inoculating the nanoparticles in the shrimp, they also form aggregates when interacting with the haemolymph and shrimp tissues. Therefore, in the shrimp inoculated with the lower concentrations (3.6 at 4.5 μg of Ag/μL), the formation of aggregates in the shrimp could be less, which caused a greater release of Ag^+ in the shrimp circulatory system, causing increased toxicity and consequently shrimp death. Unlike the higher concentrations (5.1 at 7.1 μg of Ag/μL) where possibly the release of Ag^+ in the circulatory system was less derived from the formation of aggregates in the tissues, limiting its toxicity. This hypothesis is supported by histological observations where shrimp exposed to concentrations of 5.1 to 7.1 μg of Ag/μL showed greater accumulation of nanoparticles in the tissues (e.g. Figs. 1b, d and 4a, b), which was accompanied with the formation of haemocytic nodules. Future work will be directed to evaluate the effect of the formation of AgNP aggregates in shrimp tissues and the concentration of Ag^+ in shrimp haemolymph.

The pathological manifestations observed in the various affected tissues or organs in the present study were not pathognomonic except for the agglomerations of black particles observed in the gills, lymphoid organ, and muscle, which were not reported in other works with different pollutants. The accumulation of Cs-AgNPs was also observed in the intestinal tract of *C. cornuta* as dark spots indicating the nanomaterial accumulation and organ damages at the concentration of 40 μg/mL under light and confocal laser microscopic analysis (Banumathi et al. (2017).

It is known that AgNPs, due to their size, are able to penetrate the biological membranes of eukaryote cells (Brooking et al. 2001) and affect the physiology of the exposed cells as mitochondrial function and cell viability (Braydich-Stolle et al. 2005). In the present study, the histological alterations observed in shrimp tissues exposed to all concentrations indicate necrosis in tissues as principal damage by Ag (Hi 7 times higher in the short-term experiment and 8 times higher in the long-term experiment than the control). The values obtained are variable in both experiments but with a tendency to increase with the AgNP concentration in the long-term experiment.

We did not find reports assessing the histopathological effects caused by Ag in shrimp. In this study, the Hi values indicate an important effect in organisms treated with Ag that compromises the normal organ function, mainly in the antennal gland. In addition, tissue damage was accompanied by activation of defence system to counter to AgNPs and maintain organism haemostasis. The recognition of foreign agents stimulates the cellular response through haemocyte activation; this phenomenon was observed from the zero hour (i.e. immediately after the injection). In the present study, it was unclear, whether the hyalinocytes phagocytosed the silver nanoparticles or were simply participating in some way in recognition of the AgNPs as a foreign agent; however, from a histological perspective, no accumulation of material was observed in the haemocytes per se. An electron microscopy study is necessary to evaluate the effect of silver nanoparticles on the hyaline haemocytes of shrimp. After possible phagocytosis by the hyalinocytes, the blockage of foreign particles was complemented by the formation of haemocytic nodules and encapsulation of lumps of silver nanoparticles, as also mentioned by Battistella et al. (1996). The encapsulation of silver particles into melanised haemocytic nodules began to appear after 48 h in the muscle, gills, and heart of the shrimp in both experiments.

In the present work, we observed important pathological alterations in the muscle of the shrimp in areas close to the injection site; nevertheless, similar pathologies were observed in other muscular sections of the shrimp. Bianchini et al. (2007) observed that after applying 1 to 10 μg of Ag/L to *Penaeus duorarum* for 48 h, the muscle tissue accumulated less silver; however, they did not mention whether pathological alterations occurred.

The gills play an important role in toxicology and the immune system by removing bacteria and smaller materials during moulting (Henry et al. 2012). The same authors reported that the biological interactions of metals in the gills were based on their wide range of redox potentials and complex formations. They indicate that several metals can potentially substitute for an essential metal of lower affinity and distort the geometry of the natural molecules, resulting in numerous morphological and physiological dysfunctions; however, to date, a role has not been understood for Ag^+. The pathological alterations observed in the gills in the present work were responses for the dispersion of silver particles in the circulatory system, since in several cases, we observed dark colouration of the haemolymph addressing the gill through the exoskeleton at the time of injection, which in some cases, together with the stress of the injection, caused the almost immediate death of some organisms. Martin et al. (2000) suggested that the gill epithelium "encloses like a sandwich" the foreign material between the exoskeleton and the epithelium and this is lost during the next moult. In the present work, we did not extend the experiment to 1 month, and thus, no moults occurred in either of the two experiments, although a similar response was expected.

Juveniles of *Penaeus vannamei* subjected to silver toxicity in the present work presented necrosis in the gill filaments

with development of melanised haemocytic nodules and nephrocyte necrosis. Bianchini et al. (2007) indicated that when the shrimp *Penaeus duorarum* was exposed to sublethal concentrations of silver (1 to 10 μg/L) in seawater, the gills showed high silver levels, which were associated with high silver levels in the haemolymph. These authors suggest that this result indicates that the gills are an important route for the uptake of silver in marine invertebrates. In this sense, Martin et al. (1993) and Battistella et al. (1996) mentioned that the gills were the main site for cleaning foreign particles and that the ever-present nephrocytes were capable of sequestering larger molecules and particles. In the present work, the gills and in particular the nephrocytes possibly acted similarly as silver catchers.

In most decapods, the antennal gland plays an important role analogous to the vertebrate kidney (Tsai and Lin 2014). In this work, this organ was the most strongly affected, since focal or extensive pyknotic haemocytes, atrophy, and necrosis were observed; in the latter case, the organ could barely carry out its functions. Shrimp with focal pathological alterations could survive without presenting major problems; in both experiments, shrimp presented normal swimming and feeding behaviour and did not show clinical signs of presenting any problems. The explanation might be consistent with the reports of Gerhardt et al. (2002) and Untersteiner et al. (2005), in which they indicated that crustaceans had a plasticity strategy and were able to change to another stress response behaviour to avoid the effects of toxins.

The lymphoid organ is another excretory organ that functions as a haemolymph filter (Bell and Lighter 1988; Kondo et al. 1994), since it is the largest phagocytic organ in shrimp (Rusaini and Owens 2010) and functions mainly in clearing viral and bacterial infections and removal of foreign particles from the haemolymph (Martin et al. 1996; Van de Braak et al. 2002). The organisms injected with the AgNPs showed black inclusions inside the stromal tissue of the tubules of the lymphoid organ, which proved that this organ plays an important role in cleaning and possibly eliminating the nanoparticles. The pathological alterations observed in the organs in shrimps of this study affected by silver were similar to those previously reported to be caused by other metals, except for the accumulation of black silver particles. The shrimp treated could mitigate through their immune system the effects; however, we did not extend the experiment to 1 month, so we were unable to evaluate whether the shrimp is capable to recover during a chronic exposure period.

Silver is thinking in to use it in aquatic ecosystem as control of pathogens and parasites such as other metals as Cu. In this sense, Gopi et al. (2019) in a study to evaluate the toxic effect of Cu accumulation of *Oreochromis niloticus* as well as the effect of chronic Cu exposure on the immune parameters, biochemical responses and oxidative stress in this fish concluded that discharging trace metal elements into aquatic bodies must be controlled, and remedial techniques should be applied to remove metal pollution from freshwater ecosystems. They also concluded that fish under metal stress can be used as biomarker of metal pollution. With further studies, shrimp can also be used as biomarker of silver pollution. We recommend more studies to establish higher evidence on the effects the AgNPs in shrimp such as the non-immune and biochemical response parameters and the pathogen susceptibility. We further recommend carrying out the use of silver particles without buffer and an electron microscopy study to determine the effects of nanoparticles on shrimp haemocytes. However, histological assessments are recommended when considering silver nanoparticles as a preventive or therapeutic potential in shrimp culture, especially to evaluate their viability and to ensure that shrimp are able to eliminate all silver nanoparticles after the moulting period. In this regard, an oral route of administration is suggested whilst long-term monitoring may prove the absence of tissue damage and bioaccumulation for food safety purposes.

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Authors' contributions MCCHS: leadership responsible for the research activity planning and execution, including mentorship external to the core team. Management and coordination responsibility for research activity planning. Planning with the group the experimental design and methodology. Responsible together with the histology group for the histopathology results. Preparation and translation of the work to be published together with the histopathology group

SAR: from the histopathology group. Responsible for the histology processing as well as for the analysis and description of the histopathology observed in the treated shrimps. Responsible for the analysis of the data and application of statistics. Preparation of the work to be published in conjunction with the leader and RLO

RLO: histopathology group. Responsible for the histology processing as well as for the analysis and description of the histopathology observed in the treated shrimps, together SAR and MCCHS. Analysis of the data and application of statistics. Preparation of the work to be published together with the leader and SAR

LMR: participate actively in the design of the experiments and in the execution of the tests. Contributes in obtaining the best shrimp juveniles and its better management during the experiments, provision of study materials, reagents, materials, and instrumentation

MAFN: participate actively in the design of the experiments and in the execution of the tests. Verification, as a part of the activity of the overall replication/reproducibility of results/experiments. Analysis of the data and application of statistics. Specially, critical review, commentary, and revision

CHRM: participate actively in the design of the experiments and in the execution of the tests. Critical review, commentary, or revision of the paper

AP: donation of the silver nanoparticles

NB: critical review, commentary, and revision of the paper

Cristina Chávez led the project, got the financial, formal analysis, investigation, data curation, writing original draft, and visualization, and changes was realized by all the authors.

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures were performed according to Mexican guidelines and policies stated in the NOM-062-ZOO-1999 (these guidelines apply mostly to mammalian species but we applied the same principles regarding animal welfare and care) and British guidelines for fish welfare reported by Ashley (2007).

References

Acedo-Valdez MR, Grijalva-Chon JM, Larios-Rodríguez E, Maldonado-Arce AD, Mendoza-Cano F, Sánchez-Paz JA, Castro-Longoria R (2017) Antibacterial effect of biosynthesized silver nanoparticles in Pacific white shrimp *Litopenaeus vannamei* (Boone) infected with necrotizing hepatopancreatitis bacterium (NHP-B) Lat. Am J Aquat Res 45(2):421–430. https://doi.org/10.3856/vol45-issue2-fulltext-17

Akter M, Sikder MDT, Rahman MDM, Ullah AKMA, Hossain KFB, Banik S, Hosokawa T, Saito T, Kurasaki M (2018) Review. A systematic review of silver nanoparticles-induced cytotoxicity: physicochemical properties and perspectives. J Advan Res 9:1–16. https://doi.org/10.1016/j.jare.2017.10.008

Ashley P (2007) Fish welfare: current issues in aquaculture. Appl. Animal Behave Sci 104:199.235. https://doi.org/10.1016/J.applanim.206.09.001

Bahabadi MN, Delavar FH, Mirbakhsh M, Niknam K, Johari SA (2017) Assessment of antibacterial activity of two different sizes of colloidal silver nanoparticle (AgNPs) against *Vibrio harveyi* isolated from shrimp *Litopenaeus vannamei*. Aquac Int 25:463–472. https://doi.org/10.1007/s10499-016-0043-8

Bambang Y, Thuet P, Charmantier-Daures M, Trilles JP, Charmantier G (1995) Effect of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus*. Aquat Toxicol 33:125–139. https://doi.org/10.1016/0166-445X(95)00011

Banerjee S, Kumar V, Pathak AN (2014) Drug effects on aquaculture and it's remediation. JDDT 4(1):117–122. https://doi.org/10.22270/jddt.v4i1.750

Banumathi B, Vaseeharan B, Suganya P, Citarasu T, Govindarajan M, Alharbi NS, Kadaikunnan S, Khaled JM, Benelli G (2017) Toxicity of Camellia sinensis-fabricated silver nanoparticles on invertebrate and vertebrate organisms: morphological abnormalities and DNA damages. J Clust Sci 28:2027–2040. https://doi.org/10.1007/s10876-017-1201-5

Battistella S, Bonivento P, Amirante GA (1996) Hemocytes and immunological reactions in crustaceans. Ital J Zool 63(4):337–343. https://doi.org/10.1080/11250009609356156

Bell TA, Lighter DV (1988) A handbook of normal penaeid shrimp histology. Allen Press, Lawrence, p 114

Bianchini A, Playle EC, Wood CM, Walsh OJ (2007) Short-term silver accumulation in tissues of three marine invertebrates: shrimp *Penaeus duorarum*, sea hare *Aplysia californica*, and sea urchin *Diadema antillarum*. Aquat Toxicol 84:182–189. https://doi.org/10.1016/j.aquatox.2007.02.021

Bogdanchikova N, Vázquez-Muñoz R, Huerta-Saquero A, Pena-Jasso A, Aguilar-Uzcanga G, Picos-Díaz PL, Pestryakov A, Burmistrov V, Martynyuk O, Luna-Vázquez-Gómez R, Almanza H (2016) Silver nanoparticles composition for treatment of distemper in dogs. Int J Nanotechnol 13(1–3):227–237. https://doi.org/10.1504/IJNT.2016.074536

Borrego B, Lorenzo G, Mota-Morales J, Almanza-Reyes H, Mateos F, López-Gil E, de la Losa N, Burmistrov VA, Pestryakov AN, Brun A, Bogdanchikova N (2016) Potential application of silver nanoparticles to control the infectivity of Rift Valley fever virus *in vitro* and *in vivo*. Nanomedicine. 12(5):1185–1192. https://doi.org/10.1016/j.nano.2016.01.021

Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC (2005) *In vitro* cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicol Sci 88(2):412–419. https://doi.org/10.1093/toxsci/kfi256

Brooking J, Davis SS, Illum L (2001) Transport of nanoparticles across the rat nasal mucosa. J Drug Target 9:267–279. https://doi.org/10.3109/10611860108997935

Canesi L, Corsi I (2016) Effects of nanomaterials on marine invertebrates. Sci Total Environ 565:933–940. https://doi.org/10.1016/j.scitotenv.2016.01.085

Conover WJ (2012) The rank transformation-an easy and intuitive way to connect many nonparametric methods to their parametric counterparts for seamless teaching introductory statistics courses. Wires Comput Stat 4:432–438. https://doi.org/10.1002/wics.1216

Conover WJ, Iman RL (1981) Rank transformations as a bridge between parametric and nonparametric statistics. Am Stat 35:124–129. https://doi.org/10.2307/2683975

Gambardella C, Costa E, Piazza V, Fabbrocini A, Magi E, Faimali M, Garaventa F (2015) Effect of silver nanoparticles on marine organisms belonging to different trophic levels. Mar Environ Res 111:41–49. https://doi.org/10.1016/j.marenvres.2015.06.001

Gerhardt A, de Bisthoven LJ, Mo Z, Wang C, Yang M, Wang Z (2002) Short-term responses of *Oryzias latipes* (Pisces: Adrianichthyidae) and *Macrobrachium nipponense* (Crustacea: Palaemonidae) to municipal and pharmaceutical waste water in Beijing, China: survival, behaviour, biochemical biomarker. Chemosphere 47:35–47. https://doi.org/10.1016/s0045-6535(01)00223-5

Gopi N, Vijayakumara S, Thayab R, Govindarajan M, Alharbie NS, Kadaikunnane S, Khalede MJ, Al-Anbre MN, Vaseeharana B (2019) Chronic exposure of *Oreochromis niloticus* to sub-lethal copper concentrations: effects on growth, antioxidant, non-enzymatic antioxidant, oxidative stress and non-specific immune responses. J Trace Elements Med Biol 55:170–179. https://doi.org/10.1016/j.jtemb.2019.06.011

Henry RP, Lucu CE, Onken H, Weihrauch D (2012) Multiple functions of the crustacean gill: osmotic/ionic regulation, acid-base balance, ammonia excretion, and bioaccumulation of toxic metals. Front Physiol 3(431):1–33. https://doi.org/10.3389/fphys.2012.00431

Huang S, Wang L, Liu L, Hou Y, Li L (2015) Nanotechnology in agriculture, livestock, and aquaculture in China. A review. Agron Sustain Dev 35:369–400. https://doi.org/10.1007/s13593-014-0274-x

Ishwarya R, Vaseeharan B, Shanthi S, Ramesh S, Manogari P, Dhanalakshmi K, Vijayakumar S, Benelli G (2016) Green synthesized silver nanoparticles: toxicity against Poecilia reticulata fishes

and Ceriodaphnia cornuta crustaceans. J Clust Sci. https://doi.org/10.1007/s10876-016-1126-4

Juarez-Moreno K, Mejía-Ruiz CH, Díaz F, Reyna-Verdugo H, Red AD, Vazquez-Felix EF, Sánchez-Castrejón E, Mota-Morales JD, Pestryakovf A, Bogdanchikova N (2017) Effect of silver nanoparticles on metabolic rate, hematological response and survival of juvenile white shrimp *Litopenaeus vannamei* (Boone). Chemosphere 169:716–724. https://doi.org/10.1016/j.chemosphere.2016.11.054

Kachenton S, Whangpurikul V, Kangwanrangsan N, Tansatit T, Jiraungkoorskul W (2018) Silver nanoparticles toxicity in brine shrimp and its histopathological analysis. Int J Nanosci 1-5: 1850007. https://doi.org/10.1142/S0219581X18500072

Kandasamy K, Alikunhi NM, Manickaswami G, Nabikhan A, Ayyavu G (2013) Synthesis of silver nanoparticles by coastal plant *Prosopis chilensis* (L.) and their efficacy in controlling vibriosis in shrimp *Penaeus monodon*. Appl Nanosci 3:65–73. https://doi.org/10.1007/s13204-012-0064-1

Kondo M, Itami T, Takahashi Y, Fujii R, Tomonaga S (1994) Structure and function of the lymphoid organ in the kuruma prawn. Dev Comp Immunol 18(Suppl l):S109

Lekamge S, Miranda AF, Abraham A, Li V, Shukla R, Bansal V, Nugegoda D (2018) The toxicity of silver nanoparticles (AgNPs) to three freshwater invertebrates with different life strategies; *Hydra vulgaris, daphnia carinata,* and *Paratya autraliensis*. Front Environ Sci 6:162. https://doi.org/10.3389/fenvs.2018.00152

Li N, Zhao Y, Yang J (2007) Impact of waterborne copper on the structure of gills and hepatopancreas and its impact on the content of metallothionein in juvenile giant freshwater prawn *Macrobrachium rosenbergii* (Crustacea: Decapoda). Arch Environ Contam Toxicol 52:73–79. https://doi.org/10.1007/s00244-005-0214-5

Lightner DV (1996) A handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. J. World Aquacult. Soc., Baton Rouge

Martin GG, Poole D, Poole C, Hose JE, Arias M, Reynolds L, McKrell N, Whang A (1993) Clearance of bacteria injected into the hemolymph of the penaeid shrimp, *Sicyonia ingentis*. J Invertebr Pathol 62(3): 308–315. https://doi.org/10.1006/jipa.1993.1118

Martin GG, Hose JE, Minka G, Rosenberg S (1996) Clearance of bacteria injected into the hemolymph of the ridgeback prawn, *Sicyonia ingentis* (Crustacea: Decapoda): role of hematopoietic tissue. J Morphol 227:227–233. https://doi.org/10.1002/(SICI)1097-4687(199602)227:2<227:AID-JMOR8>3.0.CO;2-5

Martin GG, Quintero M, Quigley M, Khosrovian H (2000) Elimination of sequestered material from the gills of decapod crustaceans. J Crustac Biol 20:209–217. https://doi.org/10.1163/20021975-99990032

Nimmo DWR, Lightner DV, Bahner LH (1977) Effects of cadmium on shrimps *Penaeus duodarum, Palaemonetes pugio* and *Palaemonetes vulgaris*. In: Vernberg FJ, Calabresse A, Thurberg FP, Vernberg WB (eds) Physiological responses of marine biota to pollutants. Academic Press, New York, pp 131–184

Ochoa-Meza AR, Álvarez-Sánchez AR, Romo-Quiñonez CR, Barraza A, Magallón-Barajas FJ, Chávez-Sánchez A, García-Ramos JC, Toledano-Magaña Y, Bogdanchikova N, Pestryakov A, Mejía-Ruiz CH (2019) Silver nanoparticles enhance the survival of white spot syndrome virus infected *Penaeus vannamei* shrimps by activation of its immunological system. Fish Shellfish Immonol 84:1083–1089. https://doi.org/10.1016/j.fsi.2018.10.007

Ramamoorthy S, Kannaiyan P, Moturi M, Devadas T, Muthuramalingam J, Natarajan L, Arunachalam N, Ponniah AG (2013) Antibacterial activity of zinc oxide nanoparticles against *Vibrio harveyi*. Indian J Fish:107–112

Rather MA, Sharma R, Aklakur M, Ahmad S, Kumar N, Khan M, Ramya VL (2011) Nanotechnology: a novel tool for aquaculture and fisheries development. A prospective mini-review. Fish Aquac J. (2011): FAJ-16

Romo-Quiñonez CR, Álvarez-Sánchez AR, Álvarez-Ruiz P, Chávez-Sánchez MC, Bogdanchikova N, Pestryakov A, Mejia-Ruiz CH (2020) Evaluation of a new Argovit as an antiviral agent included in feed to protect the shrimp *Litopenaeus vannamei* against White Spot Syndrome Virus infection. PeerJ 8:e8446. https://doi.org/10.7717/peerj.8446

Rusaini, Owens L (2010) Insight into the lymphoid organ of penaeid prawns: a review. Fish Shellfish Immunol 29:367–377. https://doi.org/10.1016/j.fsi.2010.05.011

Sabu AS, Sanil NK, Nammalwar P (2017) Effect of cadmium in the gills of green tiger prawn, *Penaeus semisulcatus*. Int J Fish Aquac 5(1): 467–478

Selvaraj B, Subramanian K, Gopal S, Renuga PS (2014) Nanotechnology as a novel tool for aquaculture industry: a review. World J Pharma Sci 2(9):1089–1096

Sibaja-Luis AI, Ramos-Campos EV, Luiz de Oliveira J, Fernandes-Fraceto L (2019) Trends in aquaculture sciences: from now to use of nanotechnology for disease control. Rev Aquac 11:119–132. https://doi.org/10.1111/r.aq.12229

Sivaramasamy E, Zhiwei W, Li F, Xiang J (2016) Enhancement of vibriosis resistance in *Litopenaeus vannamei* by supplementation of biomastered silver nanoparticles by *Bacillus subtilis*. J Nanomed Nanotechnol 7:1–10. https://doi.org/10.4172/2157-7439.1000352

Soegianto A, Charmantier-Daures M, Trilles J, Charmantier G (1999) Impact of copper on the structure of gills and epipodites of the shrimp *Penaeus japonicus* (Crustacea: Decapoda). J Crustac Biol 19:209–223. https://doi.org/10.2307/1549227

Sonnenholzner S, Rodríguez J, Pérez F, Betancourt I, Echeverría F, Calderón J (2002) Supervivencia y respuesta inmune de camarones juveniles *L. vannamei* desafiador por vía oral a WSSV a diferentes temperaturas. El Mundo Acuícola 8:50–55

Tsai JR, Lin HC (2014) Functional anatomy and ion regulatory mechanisms of the antennal gland in a semi-terrestrial crab, *Ocypode stimpsoni*. Biol Open 3:409–417. https://doi.org/10.1242/bio.20147336

Untersteiner H, Gretschel G, Puchner T, Napetschnig S, Kaiser H (2005) Monitoring behavioral responses to the heavy metal cadmium in the marine shrimp *Hippolyte inermis* leach (Crustacea: Decapoda) with video imaging. Zool Stud 44(1):71–80

Van de Braak CBT, Botterblom MHA, Taverne N, van Muiswinkel WB, Rombout JHWM, van der Knaap WPW (2002) The roles of the haemocytes and the lymphoid organ in the clearance of injected *Vibrio* bacteria in *Penaeus monodon* shrimp. Fish Shellfish Immunol 13:293–309. https://doi.org/10.1006/fsim.2002.0409

Vaseeharan B, Ramasamy P, Chen JC (2010) Antibacterial activity of silver nanoparticles (AgNPs) synthesized by tea leaf extracts against pathogenic *Vibrio harveyi* and its protective efficacy on juvenile *Feneropenaeus indicus*. Lett Appl Microbiol 50(4):352–356. https://doi.org/10.1111/j.1472-765X.2010.02799.x

Vaseeharan B, Sargunar GC, Lin CY, Chen CJ (2012) Green synthesis of silver nanoparticles through *Calotropis gigantea* leaf extracts and evaluation of antibacterial activity against *Vibrio alginolyticus*. Nanotech Dev 2(1):12–16. https://doi.org/10.4081/nd.2012.e3

Vazquez-Muñoz R, Avalos-Borja M, Castro-Longoria E (2014) Ultrastructural analysis of *Candida albicans* when exposed to silver nanoparticles. PLoS One 9:e108876. https://doi.org/10.1371/journal.pone.0108876

Wu JP, Chen HC, Huang DJ (2009) Histopathological alterations in gills of white shrimp, *Litopenaeus vannamei* (Boone) after acute exposure to cadmium and zinc. Bull Environ Contam Toxicol 82:90–95. https://doi.org/10.1007/s00128-008-9582-7