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Original article

Metal accumulation capacity of raphidascaridid nematode, *Hysterothylacium reliquens*, infecting the king soldier bream (*Argyrops spinifer*)

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ABSTRACT

There are several types of biological markers in the environment. The potential parasites' ability to serve as bio-indicators of pollution in marine ecosystems was the focus of the current study. Forty samples of the king soldier bream *Argyrops spinifer*, were collected from the Red Sea (Jeddah, Saudi Arabia) for this study, and they were examined for parasite infections. Only 25 fish samples included one naturally occurring nematode parasite, *Hysterothylacium reliquens*, which had three of its life-cycle stages (adults, L3, and L4 larvae). The levels of some metals (Fe, Cu, Cd, and Pb) were detected in the parasite stages and the tissues of the fish host tissues (liver, muscle, and intestine). These metals accumulated higher in non-infected fish tissues than in infected ones. The significant parasite capability for metal accumulation rather than fish tissues was validated by bioconcentration factors. Depending on the stage of a parasite's life, different metals were used. Larval stages are more prone to gathering essential elements (Fe and Cu). Although adults have a greater tolerance to toxic metals (Cd and Pb). The various metal absorption mechanisms were referred to as the differences between parasite stages. This proved that fish nematodes might serve as helpful bioindicators for assessing the extent of heavy metal contamination of aquatic ecosystems.

1. Introduction

It is possible to study the environmental changes in water quality directly using regular monitoring criteria or indirectly using bioindicators such as fish parasites (Holt and Miller, 2010; Hassan et al., 2016; Zaghloul et al., 2020). Such organisms react or adapt to a variety of applications, including pollution (Abdel-Gaber et al., 2017), environmental stress (Landsberg et al., 1998), and bioindicators of water quality (Framobi et al., 2007). The link between pollution and parasitism in aquatic species and parasites as indicators of water quality has drawn more attention during the last twenty years (Radwan et al., 2022). Pollutants harm fish by impairing their immune system, which exposes them to secondary parasitic infections (Khan and Thulin, 1991; Sitjà-Bobadilla et al., 2016).

Different parasite species can infect different types of marine fish. Nematodes are the most significant of the many parasites that infect fish (AlGabbani et al., 2021). One of the most common parasitic nematodes in marine fish, the genus *Hysterothylacium* Ward and Magath, 1917 (family Raphidascarididae) has more than 59 species (Abdel-Ghaffar et al., 2015). Compared to other parasite species, parasitic nematodes appear to have a more varied pattern

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of metal accumulation (Barus et al., 2007). All anisakid species' life cycles within *Hysterothylacium* probably have a lot of similarities. The fish's third larval stages (L3) are in the mesenteries, whilst sexually mature adults and the fourth larval stages (L4) are mostly found in the lumen of the fish's stomach and intestine. Because of the variations in metabolic processes, each developmental stage probably has a different pattern of metal accumulation (Sures and Siddall, 1999). Numerous research studies have been conducted on how the adult *Hysterothylacium* contributes to the accumulation of heavy metals in the environment (Dural et al., 2010; Abdel-Ghaffar et al., 2015; Alkan et al., 2015; Eissa et al., 2016; Leite et al., 2021). Little information is available about metal accumulation in larval fish nematodes (Khaleghzadeh-Ahangar et al., 2011; Sedaghat et al., 2022).

For a huge sector of the population, most sea breams, especially *Argyrops spinifer*, are regarded as great food fish. Few research studies have been done on its parasitic infections (Kritsky et al., 2000; AlGabbani et al., 2021; Mizher and Ali, 2021), nonetheless, little is known about the use of parasites in this host fish. To assess the impacts of bioindicators as a reflection of pollution, the current study set out to quantify the capacity differences of the parasite life-cycle stages that infected *Argyrops spinifer*.

2. Materials and methods

2.1. Fish samples and parasite collection

This study should be viewed as another analysis of the king soldier bream, *Argyrops spinifer*, and its nematode parasites by AlGabbani et al. (2021; Journal of King Saud University 33 (7): 101590). Fish specimens (n = 40) were randomly gathered from the Red Sea coast in Jeddah province off Saudi Arabia, between January to April 2021. Fish were dissected and examined for the presence of parasitic infections. The three life-cycle stages of the recovered nematode, *Hysterothalcium reliquensi,* were identified at both morphological and molecular levels.

2.2. Heavy metals analysis

Along with the parasites, different fish tissues (liver, muscle, and intestine) from infected and uninfected specimens were gathered and examined to assess the concentrations of the chosen heavy metals. In brief, all samples were dried in a thermal oven (Memmert, GmbH, Germany) for 1 hr at 105 °C, ignited in a furnace for 6 hr at 550 °C to remove all organic compounds present, and then weighed with a microbalance. By adding 0.5 g of the dry material to 5 ml of concentrated HCl and continuing stirring to dissolve the ash, the samples were digested. Following thorough digestion, samples were diluted to a predetermined volume (25 ml) using double-distilled H_2O before being filtered in clean bottles to remove any impurities. The inductively coupled plasma-optical emission spectroscopy (ICP-OES Varian 720/730-ES Series Spectrometers) was used to measure the metal content. Determination of heavy metals was done as (Reading of ICP-OES $\times$ dilution factor) / Dry weight of sample. Values of all monitored heavy metals were given as mean ± standard deviation (SD). Based on Sures et al. (1999), the ratios $C_{[parasite]}/C_{[host\ tissue]}$ (bioconcentration factors - BCF) were calculated to represent the parasites' ability for accumulation. The ratio of adult to larval concentration was also determined.

2.3. Statistical analysis

A one-way analysis of variance (ANOVA) was carried out to assess the significant difference between the tissues and the parasites. To investigate potential correlations/interactions between the elemental concentrations of various organs in infected fish and parasites, spearman rank correlation (r) was performed. All statistical methods were performed using SigmaPlot® version 11.0 (Systat Software, Inc., Chicago, IL, USA). A value was considered statistically significant if the p-value ≤ 0.05.

3. Results

A total of 25 (83.33%) fish specimens were discovered to have three stages (adult, L4, and L3) of the nematode parasite *Hysterothylacium reliquens* naturally present in their bodies. The intestine of the infected fish had both adult and L4 stages, while, the pyloric ceca contained the L3 stage. The current study concentrated on four metals that were found in fish tissues (liver, intestine, and muscles) as well as parasites when they were tested using standard solutions of metals. These metals were classified as essential metals (Fe and Cu) and non-essential metals (Cd and Pb).

Tables 1–3 demonstrate the distribution of metals in the host-parasite relationship. The US Environmental Protective Agency's allowable limits for the metals were found to be exceeded at risky levels. In the tissues of the infected fish compared to those of the non-infected ones, the rate of deposition of the metals found was noticeably lower. Additionally, all metals were found in the parasites' tissues in significantly higher concentrations than in the tissues of their infected fish host.

The element concentrations between parasite stages showed distinct variations (Table 4). With BCF up to 1.680 and 1.785, respectively, larval nematode parasites were shown to have significantly higher concentrations of the essential elements of Fe and Cu. While, toxic elements Pb and Cd with BCF up to 1.802 and 2.250, respectively, were significantly detected in the adult stage. As a result, the ratios ($C_{[larval\ stage]}/C_{[adult\ stage]}$) for essential elements were higher than 1. While, the ratios ($C_{[larval\ stage]}/C_{[adult\ stage]}$) for non-essential elements were lower than 1.

Highly significant relationships were found when element concentrations between host and parasite stages were analyzed (Table 5). For the parasitic nematode stages' Fe and Cu, some highly significant positive correlations were found. Pb and Cd concentrations in the fish intestine and larval nematode stages (r = -0.97 and r = -0.94; respectively) were shown to be strongly negatively correlated. For Fe and Cu. There was a substantial positive correlation between host muscle and larval nematode stages (r = 0.91 and r = 0.90, respectively). Additionally, a highly significant correlation (r = 0.97 and r = 0.96; respectively) between Cd and Pb was found between the adult nematode stage and host intestine.

4. Discussion

One of the biggest issues facing the globe today is environmental pollution. To identify pollutants in specific contaminants, biological indicators including plants, animals, and microorganisms are exploited (Parmar et al., 2016; Motlagh and Yang, 2019; Zaghloul et al., 2020). The use of fish parasites as a method for assessing the degree of pollution in the marine environment of the studied area is the main topic of this study which is different and questionable from other ecosystems. Our findings supported previous research in that the bioaccumulation of several metals in various fish organs was higher in uninfected fish than in infected fish (Dural et al., 2011; Eissa et al., 2012). The highest metal concentrations were found in fish intestines, supporting the hypothesis of Azmat et al. (2008) that some heavy metals are primarily accessible to intestinal parasites. The lowest detected metal levels were detected in muscles. This data is consistent with suggesting

Table 1
Concentrations of heavy metals in the intestine of the *Argyrops spinifer* and its nematode parasites.

Heavy metals	Non-infected fish	Infected fish	Parasite (1) [Larval stage]	Parasite (2) [Adult stage]
Fe	3.705 ± 0.03	1.235 ± 0.03 [a]	1.544 ± 0.03 [ab]	1.301 ± 0.03 [ab]
Cu	2.215 ± 0.04	0.638 ± 0.001 [a]	0.923 ± 0.002 [ab]	0.799 ± 0.002 [ab]
Pb	0.220 ± 0.001	0.120 ± 0.001 [a]	0.149 ± 0.001 [ab]	0.155 ± 0.001 [ab]
Cd	0.009 ± 0.001	0.004 ± 0.001 [a]	0.005 ± 0.001 [ab]	0.009 ± 0.001 [ab]

Values are means ± SD. [a] significance at $p \leq 0.05$ against the control group, [b] significant at $p \leq 0.05$ against the infected group.

Table 2
Concentrations of heavy metals in the muscle of the *Argyrops spinifer* and its nematode parasites.

Heavy metals	Non-infected fish	Infected fish	Parasite (1) [Larval stage]	Parasite (2) [Adult stage]
Fe	1.632 ± 0.02	0.989 ± 0.002 [a]	1.544 ± 0.03 [ab]	1.301 ± 0.03 [ab]
Cu	0.937 ± 0.002	0.517 ± 0.001 [a]	0.923 ± 0.002 [ab]	0.799 ± 0.002 [ab]
Pb	0.200 ± 0.001	0.092 ± 0.001 [a]	0.149 ± 0.001 [ab]	0.155 ± 0.001 [ab]
Cd	0.012 ± 0.02	0.004 ± 0.002 [a]	0.005 ± 0.001 [ab]	0.009 ± 0.001 [ab]

Values are means ± SD. [a] significance at $p \leq 0.05$ against the control group, [b] significant at $p \leq 0.05$ against the infected group.

Table 3
Concentrations of heavy metals in the liver of the *Argyrops spinifer* and its nematode parasites.

Heavy metals	Non-infected fish	Infected fish	Parasite (1) [Larval stage]	Parasite (2) [Adult stage]
Fe	1.905 ± 0.03	0.919 ± 0.001 [a]	1.544 ± 0.03 [ab]	1.301 ± 0.03 [ab]
Cu	1.709 ± 0.01	0.534 ± 0.002 [a]	0.923 ± 0.002 [ab]	0.799 ± 0.002 [ab]
Pb	0.191 ± 0.001	0.086 ± 0.001 [a]	0.149 ± 0.001 [ab]	0.155 ± 0.001 [ab]
Cd	0.010 ± 0.001	0.004 ± 0.001 [a]	0.005 ± 0.001 [ab]	0.009 ± 0.001 [ab]

Values are means ± SD. [a] significance at $p \leq 0.05$ against the control group, [b] significant at $p \leq 0.05$ against the infected group.

Table 4
Bioconcentration factors $C_{[parasite]}/C_{[fish\ tissue]}$ for larval and adult stages of *H. reliquens* and ratios $C_{[Larval\ stage]}/C_{[Adult\ stage]}$.

Heavy metals	*Hysterothylacium reliquens* $C_{[Larval\ stage]}/C_{[organ]}$ ± SD			*Hysterothylacium reliquens* $C_{[Adult\ stage]}/C_{[organ]}$ ± SD			$C_{[Larval\ stage]}/C_{[Adult\ stage]}$
	Intestine	Muscles	Liver	Intestine	Muscles	Liver	
Fe	1.250	1.561	1.680	1.053	1.315	1.415	1.186
Cu	1.446	1.785	1.728	1.252	1.545	1.496	1.155
Pb	1.241	1.619	1.732	1.291	1.684	1.802	0.960
Cd	1.250	1.239	1.243	2.250	2.223	2.230	0.555

Table 5
Spearman rank correlation coefficients (r) for the significant relationships between metal concentrations in both parasitic stages and fish tissues.

Heavy metal	Parasite stage *vs.* organ/parasite	R	*p*-value
Fe	Larval stage ↔ Fish liver	0.84	< 0.05
	Larval stage ↔ Fish muscle	0.91	< 0.05
Cu	Adult stage ↔ Fish muscle	0.93	< 0.05
	Adult stage ↔ Fish intestine	0.91	< 0.05
	Larval stage ↔ Adult stage	0.92	< 0.05
	Larval stage ↔ Fish muscle	0.90	< 0.05
Cd	Larval stage ↔ Fish intestine	– 0.94	< 0.05
	Adult stage ↔ Fish intestine	0.97	< 0.05
Pb	Larval stage ↔ Fish intestine	– 0.97	< 0.05
	Adult stage ↔ Fish intestine	0.96	< 0.05

that fish muscles have a low ability to accumulate heavily exposed heavy metals (Yilmaz, 2005; Bashir et al., 2012; Olgunoğlu et al., 2015).

Metal monitoring with parasitic nematodes has only been the subject of very few studies. Most of the nematode literature focused on the adult parasites' capacity for accumulation, while information on the larval stages' capacity for accumulation has received less attention (Nachev et al., 2013). Our study compares and displays the metal accumulation potential in various stages of fish nematodes (adult and larvae). Four metals (Fe, Cu, Cd, and Pb) were found in significantly higher concentrations in parasitic species (*H. reliquens* in both adult and larval stages) than in host tissues. According to Sedaghat et al. (2022), some parasites can collect heavy metals at higher concentrations than in the tissues and organs of their hosts. However, the range of elements varied among the parasite stages studied.

The adult nematode parasite primarily accumulated elements known for their toxicity to many organisms, including Cd and Pb. This is consistent with the statement of Barus et al. (2007), Abdel-Ghaffar et al. (2015), Leite et al. (2017), and Hassan et al. (2018) that metals bound in bile complexes excreted in the intestine are taken up by adult nematodes and thus are not available for reabsorption by the host intestine. Thus, toxic elements could be unavailable to host target tissues and simultaneously present in the parasites.

The larvae of nematodes mainly contain elements that are components of various enzymes and other macromolecules and are therefore considered essential elements (Fe and Cu) for a variety of species. Sures (2003) and Merian (2004) have demonstrated that metal absorption begins at an early stage of parasite development. Given that the cuticle of larval worms is less complex than that of adults, the higher content of essential elements may be related to the biology and morphology of nematodes (Nachev et al., 2013). Following the infection of the fish, the larva migrates through the intestinal wall into the body cavity and starts feeding on blood and tissue before encapsulating (Khaleghzadeh-Ahangar et al., 2011). Additionally, the correlation between Fe concentration in host tissues and nematode content supported the findings of Nachev et al. (2013) who discovered that Fe is a crucial part of the blood pigment hemoglobin and is almost certainly absorbed by host blood. As a cofactor of numerous enzymes, the element Cu is extremely abundant in organisms and may have comparable uptake sources. The larval stage contained relatively low levels of toxic elements, which could be explained by the different pathways used by nematode parasites at different stages of development or by competition between the larval and adult stages for nutrients and metals with host tissues, as suggested by Mazhar et al. (2014).

In agreement with Malek et al. (2007), it was suggested that the bioaccumulation of helminths might reflect the higher capacity of the host to excrete heavy metals and could be considered advantageous to the parasites, which could act as heavy metal sanitizers for the host. Bioconcentration factors determined in this study for Fe and Cu confirmed the high accumulation capacity of parasites for a given pollutant compared to fish tissues. Numerous internal and external variables, including physicochemical features and the physiological and behavioral characteristics of parasites and their hosts, might be blamed for the association between heavy metals (Ravera et al., 2007). Cu levels in the nematodes and the host muscle and gut correlated positively in the current study, demonstrating once more that the parasites may profit from the high levels in the host and not adversely alter the balance of microelements in their host. This runs counter to the findings of Nachev et al. (2013), who discovered that the synergistic impact of both metals on their absorption and bioconcentration by fish tissues and their parasites was suggested by the positive correlation between the metals. However, contrary to Szefer et al. (1998), Cd and Pb levels in the adult of *H. reliquens* were positively associated with those in the host gut, indicating that these metals were taken up from the tissues where the parasites were present. Additionally, Nachev et al. (2013) who discovered the antagonistic action of these metals on their bioconcentration in fish tissues supported the detrimental relevance for the larval stage.

5. Conclusion

Fish nematodes are useful bioindicators for determining the extent of heavy metal contamination of the aquatic studied environments. Furthermore, nematodes in their larval stage accumulate critical metals more readily than their adult counterparts can harmful ones.

Declaration of Competing Interest

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

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