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SHORT RESEARCH AND DISCUSSION ARTICLE

# Can inorganic elements affect herpesvirus infections in European eels?

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**Abstract**
In combination, pollution and pathogens represent a serious threat to the health of European eels that has been increasingly recognized. Thus, the impact of contaminants, cadmium, lead, mercury, and selenium, on anguillid herpesvirus 1 infection in wild European eels has been evaluated. Despite the small sample size, results indicate that selenium and mercury concentrations may compromise the European eel immune system as herpesvirus infection was more prevalent in specimens with higher Hg and Se hepatic concentrations.

**Keywords** European eels · Herpesvirus · Selenium · Mercury · Contaminants

## Introduction

Pollution, even at low concentrations, can have drastic effects on the physiology, immunology and ecology of European eels (*Anguilla anguilla*) populations, being this species listed as "Critically Endangered" in the Red List of the International Union for Conservation of Nature (Jacoby et al. 2014). At the same time eels, like all animals, are subjected to a wide range of infectious diseases that can have significant effects on host ecology and physiology and are therefore a source of natural stress to populations. One of these infections is caused by anguillid herpesvirus 1 (AngHV-1) which produces a hemorrhagic disease with increased mortality in wild and farmed eels (Haenen et al. 2009) and plays a contributory role in the decline of the wild European eel stocks (Haenen et al., 2012). In combination, these two kinds of stressors, pollutants and pathogens, potentially may be synergistically detrimental to affected populations. The interactions between infectious diseases and pollutants in eels have therefore become an increasing area of concern. In 2007, the Joint EIFAC/ICES Working Group on Eels initiated the set up of a European Eel Quality Database to collect recent data on contaminants and diseases over the eel habitats (Belpaire et al. 2011). So far, some studies have been focused on the joint effects of pollutants and parasites on eel health (Quadroni et al. 2012; Sures 2006; Sures et al. 2006; Sures and Knopf 2004). On the contrary, to our knowledge, no studies regarding viral and pollutants combined effects in European eels have been published. Thus, we aimed to investigate the impact of several inorganic elements, well known as environmental stressors (Geeraerts and Belpaire 2010), on wild European eel AngHV-1 infections.

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## Material and methods

### Sampling

A total of 75 European eels (mean weight = 343.780 ± 22.498 g, mean lenght = 591.05 ± 11.24 mm) from Mar Menor lagoon, South-eastern Iberian Peninsula (37° 38′ N, 0° 42′ W), were sampled between October 2015 and February 2016. After being anesthetized, and then killed with a lethal dose

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of tricaine methane sulfonate (*MS222*) at 100 mg/l, eels were measured to the nearest millimeter and weighed. In order to determine individual silvering stage, a combination of two quantitative criterion (ocular index "OI" (Pankurst 1982) and pectoral fin length (Durif et al. 2005)) as well as two qualitative criteria (state of differentiation of the lateral line and colour contrast (Acou et al. 2005)) was used. Eels were then dissected and portions (0.2–0.5 g) from muscle, from behind the anal cavity and without skin, and liver were obtained and stored at -20 °C until processed for inorganic element analyses.

Body condition was calculated as scaled mass index (SMI) according to Peig and Green (2009).

## Virological study

Pools of spleen, liver, kidney, and gills (approximately 50 mg of each organ) from each eel were obtained and stored at − 80 °C until processed. Tissue pools were sent to the National Reference Laboratory for Fish, Crustacean and Shellfish Diseases (The Netherlands) where the presence of AngHV-1 was determined by real time-PCR. Samples were analyzed in pools of maximum 10 eels. If any pool was positive then, the specimens comprising the pool were individually tested.

## Inorganic element analysis

Cadmium (Cd), lead (Pb), mercury (Hg), and selenium (Se) were determined in liver and muscle samples. To find out Pb, Cd, and Se contents, samples were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES, ICAP 6500 Duo, Thermo Scientific, with One Fast System) following descriptions from Cortés-Gómez et al. (2018). Briefly, samples were treated with trace mineral grade nitric acid (69% Suprapure, Merck) and 33% H2O2 (Suprapure, Merck), in special Teflon reaction tubes, heated for 20 min at 220 °C in a microwave digestion system (UltraClave-Microwave Milestone®), and finally diluted to 10 ml with double deionized water (MilliQ). The wavelengths were 220.353 nm (Pb), 214.438 nm (Cd), and 196.090–203.985 (Se), and the uncertainty percentages were 6.14 (Pb), 4.56 (Cd), and 6.43 (Se). The recovery percentages for reference materials were 96.44 (Pb), 95.32 (Cd), and 106.43 (Se). To determine total Hg content, samples were analyzed using an atomic absorption spectrometer AMA254 Advanced Mercury Analyzer (Leco), without sample pre-treatment or sample preconcentration. Inorganic element concentrations were expressed in micrograms per gram in wet weight ($\mu g\ g^{-1}$ ww). The detection limits (DL) were 0.001 $\mu g\ g^{-1}$ (Pb, Cd, and Se) and 0.003 $\mu g\ g^{-1}$ (Hg).

## Statistical procedures

The software R 3.4.4 (R Core Team 2018) was used to analyze the data. For each element, geometric mean, standard error (SE), minimum and maximum were obtained. Data below the DL were expressed as half of this (0.0005 for Pb, Cd, and Se, and 0.0015 for Hg) to perform the statistical analysis. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the data distribution according to the number of samples. Mann-Whitney *U* test was used as nonparametric statistical method. The significance level was set as 0.05.

# Results and discussion

According to the silvering stage evaluated parameters, all specimens were yellow eels. Results of the present study showed an AngHV-1 prevalence of 22.67 % (17/75). No clinical sign of viral infection was observed. AngHV-1 is frequently detected in wild European eel in Europe with no clinical signs, which means that some individuals reveal a natural resistance but can act as carrier hosts (Haenen et al. 2009). Although these eels appear healthy, stress (e.g., high water temperatures, low oxygen concentrations) might trigger a disease outbreak with high mortalities (Haenen et al. 2010). Statistically higher SMI and total length were observed in AngHV-1 positive eels compared to negative ones (374.39 ± 13.03 vs 241.45 ± 15.42, and 638.82 ± 15.51 vs 577.73 ± 13.44 mm respectively). These results are consistent with previous findings indicating that an increase in individual total length is accompanied by an increased risk AngHV-1 loads (Kullmann et al. 2017).

Table 1 shows detected concentrations of Pb, Cd, Hg, and Se in the evaluated eel tissues. The percentages of samples above the DL were 6.7, 38.7, 5.3 and 0 for Pb, Cd, Hg, and Se detection respectively in muscle, as well as 1.3, 16.0, 9.3, and 2.7 for Pb, Cd, Hg, and Se detection respectively in the liver. No significant differences were found between AngHv-1 infection and Cd or Pb concentrations in the evaluated eels. On the contrary, Hg concentrations in the muscle and liver as well as Se concentrations in the muscle were significantly higher in specimens positive to AngHV-1.

Regarding Hg, significantly higher concentrations of this toxic element were detected in the liver (0.013 vs 0.005 $\mu g\ g^{-1}$) and muscle (0.015 vs 0.009 $\mu g\ g^{-1}$) of virus-positive eels. Christensen et al. (1996) demonstrated that Hg exposure exacerbates infection with virulent strains of herpes simplex virus type 2 in mice (Christensen et al. 1996). According to these authors, Hg potentiates this herpes virus infection because of increased viral replication, inhibition of cytokine production, and decreased macrophage activity. The mechanism by which Hg exacerbates AngHV-1 infection in European eels remains unknown but inhibition of cytokine production

**Table 1** Concentrations of Pb, Cd, Hg and Se (μg g$^{-1}$, wet weight) in European eels from Mar Menor lagoon (Spain). Same lowercase letter indicates statistically significant differences between positive and negative specimens to AngHV-1 infection (Mann-Whitney *U* test)

| | | Whole population = 75 | | | | AngHV-1 negative (*n* = 58) | | | | AngHV-1 positive (*n* = 17) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± SE | Min | Max | Median | Mean ± SE | Min | Max | Median | Mean ± SE | Min | Max | Mean |
| Muscle | Pb | 0.099 ± 0.030 | nd | 1.434 | 0.175 | 0.106 ± 0.030 | nd | 1.129 | 0.208 | 0.078 ± 0.083 | nd | 1.434 | 0.086 |
| | Cd | 0.004 ± 0.001 | nd | 0.044 | 0.008 | 0.004 ± 0.001 | nd | 0.038 | 0.008 | 0.004 ± 0.003 | nd | 0.044 | 0.008 |
| | Hg | 0.010 ± 0.002 | nd | 0.177 | 0.001 | 0.009 ± 0.001[a] | nd | 0.040 | 0.01 | 0.015 ± 0.010[a] | 0.003 | 0.177 | 0.015 |
| | Se | 0,497 ± 0,019 | 0.200 | 1.220 | 0.049 | 0.509 ± 0.023 | 0.200 | 1.220 | 0.52 | 0.459 ± 0.030 | 0.229 | 0.758 | 0.468 |
| Liver | Pb | 1.788 ± 0.157 | nd | 7.976 | 2.151 | 1.843 ± 0.191 | nd | 7.976 | 2.199 | 1.613 ± 0.199 | 0.380 | 3.182 | 1.827 |
| | Cd | 0.034 ± 0.009 | nd | 0.458 | 0.072 | 0.036 ± 0.011 | nd | 0.458 | 0.071 | 0.028 ± 0.015 | nd | 0.188 | 0.984 |
| | Hg | 0.007 ± 0.002 | nd | 0.155 | 0.006 | 0.005 ± 0.001[b] | nd | 0.026 | 0.005 | 0.013 ± 0.009[b] | 0.005 | 0.155 | 0.013 |
| | Se | 6.098 ± 0.490 | nd | 18.168 | 7.266 | 5.286 ± 0.568[c] | nd | 18.168 | 6.835 | 9.926 ± 0.817[c] | 4.460 | 13.951 | 11.797 |

*SE* standard error, *AngHV-1* anguillid herpesvirus 1, *nd* nondetected; [a] $p = 0.035$ and $Z = -2.114$, [b] $p < 0.001$ and $Z = -3.551$, [c] $p = 0.016$ and $Z = -2.404$

(Morcillo et al. 2015) and decreased macrophage activity (Sarmento et al. 2004) due to in vitro Hg exposure have been also reported in some fish species.

Se is an essential micronutrient that, through its incorporation into selenoproteins, participates in several vital metabolic pathways, the antioxidant defense system and the functioning of the immune system. In fact, Se appears to be a key nutrient in counteracting the development of virulence and inhibiting the progression of some viral infections (Gómez et al. 2001). In this context, Se-enriched feed additives have become an attractive resource for formulation of functional feeds for animal farming, including aquaculture (Pacitti et al. 2016). According to this, higher Se bioaccumulation rates could have been expected in virus-negative eels. The opposite results were observed in the present study as significantly higher concentrations of Se in the liver of virus-positive eels (9.926 vs 5.286 μg g$^{-1}$) have been detected.

Several can be the explanations to these results. On one hand, although present in the liver, Se availability might have been impaired. Hg presents a high affinity for Se and, therefore, reduces its bioavailability (Sugiura et al. 1978; Spiller et al. 2017). Once Se atom is bound to a Hg atom, the Se atom becomes unavailable to participate in selenoprotein formation inhibiting their function, disrupting the intracellular redox environment (Spiller et al 2017) and impairing the beneficial effects on the immune system. Se might have also been sequestered by AngHV-1 present in liver, one of the main target tissues of AngHV-1. Incorporation of Se by viral selenoproteins has been previously reported (Taylor et al. 1994a, b). On the other hand, at elevated concentrations Se has the potential to adversely affect the immune system (Janz et al. 2010) being the optimal and toxic levels of Se very close (Borba et al. 2013). Finally, Se does not affect all viruses to the same extent. According to Chaturvedi et al. (2004), low Se rates mainly favors RNA virus replication. Regarding specifically herpes virus infections, these authors reported that heart damage caused by human herpes simplex virus, a DNA virus, is significantly higher in Se adequate than in Se-deficient mice.

These findings emphasize that Se and Hg bioaccumulation influence the AngHV-1 infection process. Hopefully, these results will encourage additional studies to elucidate further the role of toxic element bioaccumulation on European eel viral pathogenesis.

## Conclusion

Se and Hg concentrations in the Mar Menor lagoon seems to have compromised the European eel immune system as susceptibility to AngHV-1 virus was enhanced in specimens with higher bioaccumulation rates.

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