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Acetylcholinesterase activity in muscle tissue of Norway lobster *Nephrops norvegicus*: Importance of body size, season, sex and naturally occurring metals

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ABSTRACT

The aim of the present study was to determine the levels of acetylcholinesterase (AChE) activity in the tail muscle tissue of wild populations of *Nephrops norvegicus* from the Northern Adriatic, and correlate it to body size, seasons, sex and the content of mercury, arsenic, cadmium, lead and copper. The animals of both sexes were collected in spring and autumn from two relatively distant fishing grounds. A marked variability of muscle AChE activity was found (0.49 to 11.22 nmol/min/mg prot.), displaying the opposite seasonal trend between two sampling sites. Small, but significant negative correlation has been found between AChE activity and carapace length (r_s = - 0.35, $p < 0.05$). Data reported here provide an essential baseline for future studies of neurotoxicity in crustaceans. The study highlights the necessity for continuous monitoring of potentially toxic metals in edible marine species to avoid possible repercussions of seafood consumption on human health.

1. Introduction

The constantly increasing levels of pollutants introduced from various land-based sources represent serious threats for marine coastal ecosystems. Further intensification of anthropogenic activities and environmental degradation is foreseen in the near future due to human population increase and climate change related to global warming (Cramer et al., 2018). While the adverse effects of chemical pollutants in various marine organisms inhabiting particularly sensitive coastal and estuarine regions have been extensively studied in recent decades (Maulvault et al., 2019; Macleod et al., 2016), less is known on the susceptibility to these compounds of economically and ecologically important organisms in their offshore fishing grounds (Tursi et al., 2015).

The Norway lobster *Nephrops norvegicus* is among the most harvested species and used as a food source throughout the Mediterranean Sea and NE Atlantic. This decapod crustacean is both a predator and a scavenger that predominantly feeds on various small benthic invertebrates and fish (Zacchetti et al., 2022) and could be found even at depths of nearly 800 m (Johnson et al., 2013). Owing to the burrowing lifestyle, *N. norvegicus* may become susceptible to potentially harmful contaminants in the sediment but little is known on how the exposure to these chemicals affect the overall health of this organism (Cenov et al., 2018; Joyce et al., 2023; Stenton et al., 2022). Metals of natural and anthropogenic origin are of particular concern given their ubiquitous distribution, prolonged persistence in the seabed sediments and accumulation in benthic organisms (Komar et al., 2018; Qian et al., 2015).

The stress triggered by exposure to pollutants is quickly manifested

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in changes of molecular, biochemical, cellular and physiological functions coupled to maintenance of organisms' homeostasis. These signals serve as biomarkers for early detection of alterations that may eventually lead to irreversible damage at individual, population, and community level (Lagadic, 2002). Well-established standardised biomarkers have been routinely employed in environmental studies as an early diagnostic tool for the health of marine organisms residing in sensitive coastal and estuarine areas (Askem et al., 2018; Frías-Espericueta et al., 2022; Uluturhan et al., 2019) and have also been applied for commercially important species such as *N. norvegicus* (Antó et al., 2009; Carreras-Colom et al., 2022; Stenton et al., 2022). The enzyme acetylcholinesterase (AChE) is a biomarker which takes part in cholinergic transmission by hydrolysing the neurotransmitter acetylcholine into choline and acetic acid. Inhibition of AChE has predominantly been associated with the toxic effect of organophosphorus and carbamate pesticides in a wide range of marine invertebrates (Perić and Burić, 2019; Silva et al., 2019) including crustaceans (Butcherine et al., 2022; Dellali et al., 2021; Oliveira et al., 2013; Taylor et al., 2019). However, AChE in aquatic organisms may as well be affected by metals as shown in several experimental trials with mercury (Hg) in crayfish (Gunderson et al., 2018) and prawns (Harayashiki et al., 2016), cadmium (Cd) in oyster (Moncaleano-Niño et al., 2018) and amphipods (Dellali et al., 2021), lead (Pb) in clam (Bejaoui et al., 2020) and frog (Yologlu and Ozmen, 2015), copper (Cu) in fish (Pereira et al., 2019), mussel (Perić and Burić, 2019) and crab (Oliva et al., 2019) and metalloid arsenic (As) in mussel (Santos et al., 2022) and clam (Freitas et al., 2018). The neurotoxic effect of different metals was also reported for field exposed marine crabs (Capparelli et al., 2019; Rodrigues et al., 2014; van Oosterom et al., 2010) and bivalves (Moleiro et al., 2022; Vázquez-Boucard et al., 2014). Currently, the exact mechanism by which these compounds may interfere with AChE activity is not fully understood or is non-specific, as in case of mercury (Frasco et al., 2007).

AChE features in different tissues of *N. norvegicus* were previously determined and its activity in tail muscle suggested as potentially useful biomarker of neurotoxicity in this crustacean species (Antó et al., 2009; Solé et al., 2006) and applied for health assessment of this benthic species (Carreras-Colom et al., 2022). To correctly evaluate the effect of anthropogenic stressors, a well-detailed knowledge is required on how intrinsic factors modulate the enzymatic activity across seasons and in individuals of different body size and sex.

The present investigation is a part of research looking into natural variations of biological indices in different tissues of wild *N. norvegicus* populations. The aim of this study was to determine the level of AChE activity in *N. norvegicus*, in relation to body size, seasons and sex. Alterations of AChE activity were also linked to body burden of potentially toxic metals (Hg, Cd, Pb, and Cu) and As in the muscle tissue. The results of this study will contribute to a better understanding of factors that may interfere with the evaluation of *N. norvegicus* health status by analyses of AChE activity during ecotoxicological risk assessment activities. This data will also upgrade currently available information on the safety of end consumers associated to exposure of *N. norvegicus* to metals in their habitats within Northern Adriatic offshore areas.

2. Materials and methods

2.1. Description of sampling locations

Two sampling sites within fishing grounds of the semi-enclosed Kvarner Bay (Northern Adriatic, Croatia) have been selected for this investigation (Fig. 1). The first sampling site S1 was positioned in the innermost part of the bay, at the distance of approximately 8–11 km from the city of Rijeka. The location of the second sampling site S2 was approximately 70 km south of Rijeka. Level of pollutants at the two offshore sampling sites is currently unknown. In general, concentration of various chemical pollutants may vary considerably along the coast of the Kvarner Bay, with the highest values recorded in the near-shore

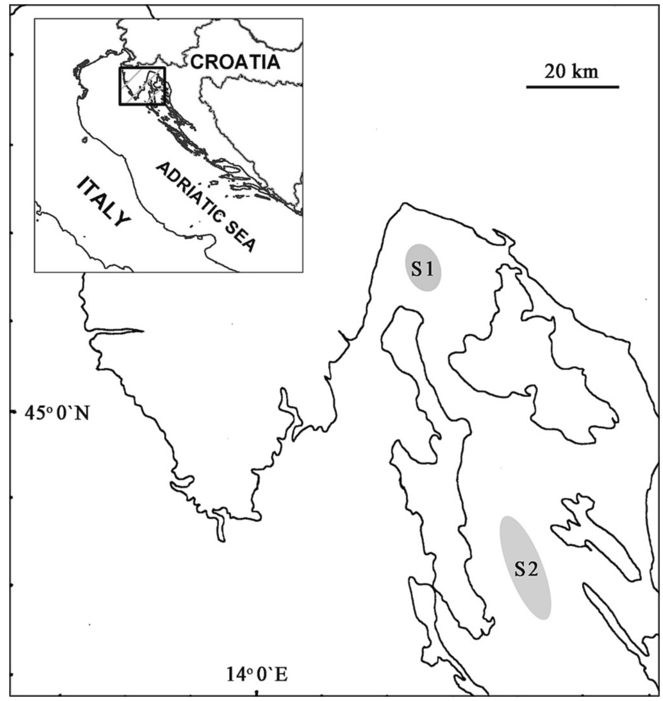

Fig. 1. Map of sampling sites in the Northern Adriatic.

sediments and biota next to industrial sites that are substantially reduced with an increasing distance from pollution sources (Bihari et al., 2007; Cukrov et al., 2011, 2014; Fafanđel et al., 2015; Perić et al., 2012). However, enrichment of sediments with mercury discharged from industrial sites and inland deposits was recorded on several occasions over wider areas of the Northern Adriatic (Covelli et al., 2011; Fitzgerald et al., 2007; Kotnik et al., 2015). Commercial fishing grounds that encompass both sampling sites are close to traditional routes for commercial vessels including oil tankers and numerous touristic boats.

N. norvegicus samples were collected in autumn 2014 and spring 2015 by trawl fishing gear from the bottom at depths of 55–62 m (S1) and 70–78 m (S2). Following collection, the organisms were immediately inspected to select the healthy individuals without any signs of external damage. Males were discerned from females by increased thickness and higher rigidity of the first pair of abdominal swimmerets. The length between eye socket and carapace mid hind edge (carapace length, CL) was recorded for each individual. From each site, 72 individuals characterised by balanced sex ratio and heterogeneous length (Table 1) were dissected to obtain the tail muscle tissue for further analyses. Tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. Acetylcholinesterase activity

For acetylcholinesterase activity (AChE) analyses, a portion of tail muscle tissue was homogenized in 1:3 *w/V* of ice-cold 50 mM phosphate

Table 1
Carapace length values (mm) for *N. norvegicus* from sampling sites S1 and S2.

Site	Season	Sex	Avg	Min–Max	Median
S1	Aut	F (n = 18)	42.8 ± 9.0	30.8–57.2	43.5
		M (n = 18)	45.3 ± 10.1	31.2–61.1	46.1
	Spr	F (n = 18)	36.8 ± 7.3	26.2–47.2	38.0
		M (n = 18)	42.2 ± 11.3	26.8–58.6	44.8
S2	Aut	F (n = 18)	35.8 ± 6.1	27.9–49.1	36.4
		M (n = 18)	40.4 ± 5.8	32.6–53.4	40.6
	Spr	F (n = 18)	39.2 ± 8.0	26.8–52.8	39.5
		M (n = 18)	42.7 ± 8.4	30.5–57.6	41.9

buffer (pH 7.4) using a Teflon homogenizer. Homogenates were centrifuged for 30 min at 4 °C and 10,000 x *g*, after which the pellets were discarded, and the supernatants kept for further analyses. AChE activity was measured by the method of Bocquené and Galgani (1998). Diluted samples (50 μl) and 200 μl of 5,5-dithio-*bis*-2-nitrobenzoate solution (DTNB; 300 μM final) were added into micro plate wells. The enzymatic reactions in the wells were started by addition of substrate acetylcholine (ATC, 1 mM final). Measurements of enzymatic activity were carried out by recording the absorbance change at 405 nm occurring upon formation of coloured thionitrobenzoate anion, using a microplate reader (Lab-systems, Multiscan Ascent® and Ascent Software TM, 2.4. version). All samples were analysed in triplicates. Reaction of thiols with DTNB and spontaneous hydrolysis of substrate were determined in the absence of substrate and sample, respectively, and used for correction of absorbance change. The enzymatic activity in the sample was expressed as nmol of hydrolysed substrate per minute and mg of proteins. Concentrations of proteins in the samples were determined by the method of Bradford (1976) with bovine serum albumin as the standard.

2.3. Concentration of metals and arsenic in the muscle tissues

Muscle tissue samples were processed for metal analyses as described in Cenov et al. (2018). Briefly, subsamples of freeze-dried muscle (0.5 g dry weight) were microwave digested (Anton Paar Multivawe 3000, Perkin Elmer, USA) in 65 % nitric acid (HNO_3, Suprapur, Merck, Germany). Digested samples were diluted with ultrapure water (Siemens) and analysed for mercury (Hg), arsenic (As), cadmium (Cd), lead (Pb) and copper (Cu). With exception of Hg, all the analyses were carried out with inductively coupled plasma mass spectrometer (ICP MS NexION 300×) equipped with S10 autosampler (Perkin Elmer, USA). Analyses of Hg were performed with atomic absorption spectrometer (AMA 254, Advanced Mercury Analyser, Leco, USA). For analytical quality assurance, the appropriate blanks and certified reference materials were used, whereas the analyses were performed in triplicates. Method for measurement of As, Cd, Pb and Cu was validated with IAEA-407 (fish tissue; International Atomic energy Agency, Austria) and the mean recovery was between 89 % and 110 %. Metals were analysed according to the modified European standard methods EN 14084:2003 and EN 13804:2013. Furthermore, Pb and Cd were included into the accreditation scope of Croatian accreditation agency (Accreditation certificate 1127). NIST 2976 (mussel tissue, National Institute of Standards and Technology, USA) was used for validation of Hg measurements (mean recovery 104 %).

The concentrations in muscle tissue were expressed as μg/g dry weight (d.w.). A dry-to-weight conversion factor of five was used to express the wet weight (w.w.), based on the weight difference between wet and freeze-dried samples, indicating that the average proportion of moisture content in the tissue was equal to 80 % (Cresson et al., 2014; Mille et al., 2018).

2.4. Data processing

Shapiro-Wilk and Levene's test were used to verify the normality and homogeneity of data, respectively. For testing of difference between the responses of two groups by Student's *t*-test, data for AChE activity corresponding to each tested group were log transformed to achieve normality. Since the normality assumption across variables could not be met by transformation operation, correlations were determined using the non – parametric Spearman's rank correlation analysis. The significance level was set to $p < 0.05$. All statistical analyses were carried out using RStudio software, version 1.0.153 (RStudio Team, 2017).

3. Results and discussion

The activity of acetylcholinesterase (AChE) in the muscle of *Nephrops norvegicus* for two sites, seasons and sex, is shown in Fig. 2. The values for AChE activity at sites S1 and S2 ranged between 0.49 and 11.22 and 0.64 and 9.71 nmol/min/mg of proteins, respectively, and were generally in line to those previously reported for muscle tissue of *N. norvegicus* from NW Mediterranean (Antó et al., 2009; Carreras-Colom et al., 2022; Solé et al., 2006).

Natural variability of AChE activity in marine invertebrates has been associated to seasonal fluctuations of environmental and biotic factors, including the reproductive cycle (Capparelli et al., 2019; Perić and Petrović, 2011; Ramos et al., 2016). Considering that the reproductive activity of *N. norvegicus* in the Mediterranean intensifies during spring to summer period and becomes suppressed in autumn (Aguzzi and Sardà, 2008; Orsi Relini et al., 1998) a strong seasonality of AChE activity was expected. Results of previous study on AChE activity changes in the muscle tissue of *N. norvegicus* over different seasons were inconclusive due to a low number and unbalanced sex ratio of individuals (Antó et al., 2009). In the present work, a larger sample size containing equal number of males and females allowed detection of the opposite, site-dependent seasonal patterns. At site S1, the enzymatic activity was significantly higher in autumn in female lobsters only ($t(34) = 3.34$, $p < 0.01$), while at S2 significantly higher AChE activity was recorded in spring in both males ($t(34) = -4.29$, $p < 0.001$) and females ($t(34) = -0.32$, $p < 0.01$) (Fig. 2). This contrasting seasonal trend for two sampling fishing grounds indicates the influence of local, site-related factors that could have modulated the enzyme activity in spatially distant populations. Previous studies reported that the enrichment with metals in the investigated region was in line with other off-shore regions of the Adriatic Sea (Cukrov et al., 2011, 2014).

The differences in AChE activity between males and females were significant only in autumn at site S2 ($t(34) = 2.72$, $p < 0.05$) (Fig. 2). In the study of Solé et al. (2006), a reduced AChE activity in *N. norvegicus* females was recorded in spring and was explained by sex-dependent specificities of hormonal status during the period of intensive reproductive activity. Contrastingly, in the present work, males displayed reduced AChE activity with respect to females, and this effect was seen only in autumn, following the peak of reproductive season in the Adriatic (Orsi Relini et al., 1998; Marković et al., 2016). The inconsistency between the two studies and the inability to discern a clear sex-related AChE activity pattern of sampled *N. norvegicus* could have resulted

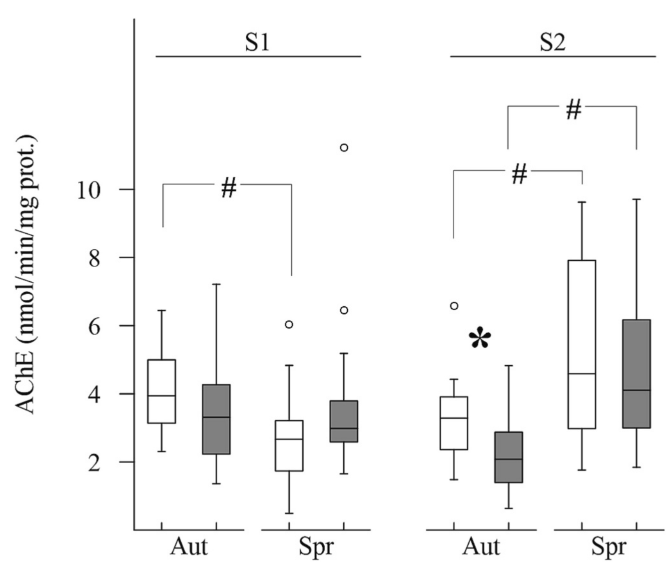

Fig. 2. Acetylcholinesterase activity (AChE) in the tail muscle of female (□) and male (■) Norway lobsters *Nephrops norvegicus* from sites S1 and S2 in the Northern Adriatic in Autumn (Aut) and Spring (Spr). Significant differences ($p < 0.05$) between sex and seasons are indicated by asterisks and hashes, respectively.

from a well-known reproductive cycle asynchrony of this species (Marković et al., 2016; Orsi Relini et al., 1998; Sardà, 1995). The maturation stage of gonads was not investigated in this study, except of visual inspection of sampled organisms that revealed 22 and 30 % of females bearing a mature ovary on their abdomen in spring and autumn, respectively. In addition, considering the role of AChE in cholinergic neurotransmission, differences in the activity of AChE observed between females and males, might also reflect a sex-related specificities of behaviour, such as locomotion, feeding or burrowing activity. For instance, *N. norvegicus* females displayed a reduced emergence period in comparison to males, for better predator protection and variable dynamics of food intake over reproductive cycle (Aguzzi et al., 2007). This issue requires further investigation.

Mild but significant negative correlation of AChE with carapace length ($r_s = -0.35$, $p < 0.05$) (Table 2) is in concordance with previous reports for *N. norvegicus* from NW Mediterranean coast (Antó et al., 2009; Solé et al., 2006). These results might suggest alterations and remodelling of neuromuscular system or physiological requirements over different life stages as previously described for crustaceans (Atwood, 1992). The moulting process, when old exoskeleton is discarded and new one of increased size is formed, determines the development, growth and concomitant innervation of lobster muscle tissue (Govind, 1992). Thus, more intensive moulting frequency typically seen in young *N. norvegicus*, could explain somewhat higher AChE activity in individuals of lower body size. In addition, changes of cell surface to volume ratio naturally occurring during growth that affect cells metabolism, fitness and functionality (Bodenstein et al., 2023; Hermaniuk et al., 2021; Miettinen et al., 2017) could be hypothesised as another factor involved in AChE activity modulation across various life stages.

Alternatively, lower AChE activity measured in larger *N. norvegicus* samples could also be an indication of longer exposure to contaminants in their habitat. Of all potentially toxic metals analysed here, only mercury (Hg) was significantly, albeit mildly, correlated to AChE activity ($r_s = -0.32$, $p < 0.05$) (Table 2). The sensitivity and susceptibility of AChE to Hg is species-specific (Frasco et al., 2007) and pieces of evidence for Hg inhibitory effect in crustacean species are still relatively rare. However, an inhibition of AChE activity after Hg exposure was previously reported for muscle tissue of tiger prawn *Penaeus monodon* (Harayashiki et al., 2018) and signal crayfish *Pacifastacus leniusculus* (Gunderson et al., 2018). Neurotoxic AChE inhibitory effect of Hg was also observed in fish (Araújo et al., 2018; Barboza et al., 2018).

As shown in Table 2, Hg displayed a strong and significant positive correlation to body size ($r_s = 0.80$, $p < 0.05$), with the best fit for the relationship obtained using the power fit equation ln (carapace length) = 0.3025612208 * ln (Hg concentration) + 3.265349541, $R^2 = 0.62$ (Fig. 3), indicating that its accumulation in *N. norvegicus* increases with age. This is consistent with previous reports for *N. norvegicus* (Barghigiani et al., 2000; Canli and Furness, 1993; Capelli et al., 2004; Di Lena et al., 2018). The average content of 4.63 ± 2.75 μg Hg/g d.w. found in the muscle tissue (Table 3) was comparable to the levels previously established for Central Adriatic (Di Lena et al., 2018; Perugini et al., 2009), and higher than in samples from Tyrrhenian sea (Di Lena et al., 2018), NW Mediterranean area (Cresson et al., 2014) and in particular from Atlantic coasts (Canli and Furness, 1993; Lourenço et al., 2009) and North Sea (Wiech et al., 2021).

Table 2
Spearman's correlation coefficients (r_s) between AChE, metals and carapace length (CL) in *N. norvegicus* from the Northern Adriatic. Bold values denote statistical significance at the $p < 0.05$ level.

	Hg	As	Cd	Pb	Cu	CL
AChE	**−0.32**	−0.16	−0.10	−0.36	−0.24	**−0.35**
Hg		**0.33**	**−0.17**	0.05	0.14	**0.80**
As	0.32		−0.10	**−0.36**	**−0.24**	**0.34**
Cd	−0.18	−0.10		**0.42**	0.11	−0.11
Pb	0.05	−0.36	0.42		**0.36**	0.04
Cu	0.14	−0.24	0.11	0.35		0.09

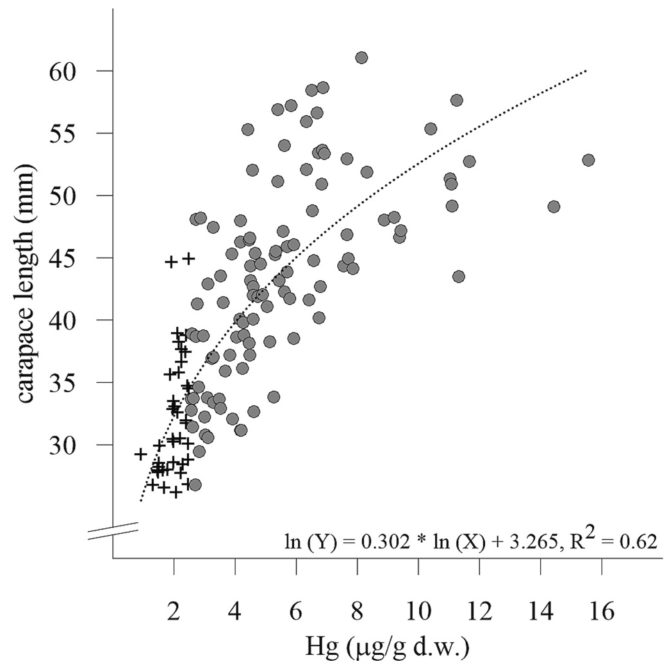

Fig. 3. Relation between carapace length (mm) and mercury concentration (μg/g d.w.) in the tail muscle of Norway lobster *Nephrops norvegicus* ($N = 144$). Dashed line denotes the power – fit. Symbols indicate samples with Hg levels of ≥0.5 μg/g w.w. (●) and < 0.5 μg/g w.w. (+) when a dry-to-wet tissue conversion factor of 5 is used (Cresson et al., 2014; Mille et al., 2018).

Bioaccumulation of Hg over longer period is typically associated with demersal and predatory pelagic fish and marine mammals (Delgado-Suarez et al., 2023; Erasmus et al., 2022). However, relatively high Hg concentrations with respect to other marine areas that were found in muscle tissue indicate that *N. norvegicus* in the Northern Adriatic Sea could be exposed to an elevated background level of Hg in sediment surface related to long-term riverine input and dispersal from Hg mining (Covelli et al., 2011; Fitzgerald et al., 2007; Kotnik et al., 2015).

The concentrations of Hg in the muscle of *N. norvegicus* were in agreement with those previously reported for digestive gland tissue (Cenov et al., 2018). Moreover, a positive correlation of Hg with metallothioneins, the key proteins that participate in metal detoxification and storage in digestive gland, was determined as well (Cenov et al., 2018). These findings, together with the current results, might suggest the occurrence of physiological stress in *N. norvegicus* caused by Hg exposure in their immediate environment. The ecological consequences at higher level of biological organisation could be also anticipated, based on correlations between AChE inhibition and behavioural impairments previously observed in decapod crustaceans after exposure to Hg (Harayashiki et al., 2016) or organic chemicals (Mesquita et al., 2011).

In this study, arsenic (As) was the most abundant element, displaying concentrations in the range between 49.40 and 280.13 μg/g d.w. (Table 3). These values were slightly higher than those reported previously for tail muscle of *N. norvegicus* from the same fishing area in the Northern Adriatic (Klarić et al., 2004; Sekulić et al., 1993), Central Adriatic (Visciano et al., 2013) and Southern Adriatic (Storelli and Marcotrigiano, 2001). Inactivation of AChE by arsenic (As) was previously demonstrated in laboratory experiments with bivalve mussels (Chakraborty et al., 2013; Freitas et al., 2018). It was also reported that decreased AChE activity found in mud crab *Scylla serrata* from a riverine estuary could be associated to high total As burden (van Oosterom et al., 2010). Nevertheless, while As concentrations in the muscle of *N. norvegicus* were also positively correlated to body length ($r_s = 0.34$, p

Table 3
Concentrations of total mercury (tHg), arsenic (As), cadmium (Cd), lead (Pb) and copper (Cu) (mean ± s.d., μg/g d.w.) in the white tail muscle of *Nephrops norvegicus* from different locations in the Mediterranean Sea and Atlantic Ocean. Range of concentrations is indicated in parenthesis (where available). Asterisks indicate values originally reported on a wet weight basis and recalculated using dry-to-wet conversion factor five (Cresson et al., 2014; Mille et al., 2018); Hashes indicate maximum values.

Location	tHg	As	Cd	Pb	Cu	Reference
Mediterranean Sea						
	4.63 ± 2.75	112.31 ± 42.29	0.08 ± 0.07	0.29 ± 0.33	21.37 ± 5.93	present work
	(0.92–15.57)	(49.40–280.13)	(<0.001–0.41)	(0.04–2.74)	(5.09–51.52)	
		(26.85–106.95*)				Sekulić et al., 1993
Northern Adriatic		(85.55 ± 22.40*)				Klarić et al., 2004
	5.985 ± 2.30*					Di Lena et al., 2018
	(2.735–11.705*)					
	4.85 ± 4.35*					Perugini et al., 2009
	(1.45–16.35*)					
			0.05 ± 0.05*	0.05 ± 0.05*		Perugini et al., 2014
Central Adriatic		80.45 ± 3.05*				Visciano et al., 2013
		121.9*	<0.05*	0.185*	22.15*	Iamiceli et al., 2015
		43.48 ± 14.21				Storelli and Marcotrigiano, 2001
Southern Adriatic		(35.63–69.15)				
		270*	<0.05*	0.16*	20.5*	Iamiceli et al., 2015
	1.345 ± 0.60*					Di Lena et al., 2018
Thyrenian Sea	(0.69–3.05*)					
			0.336 ± 0.266	0.138 ± 0.136	61.9 ± 25.1	Mille et al., 2018
			(0.030–0.630)	(0.04–0.350)	(30.2–93.1)	
	1.80 ± 0.97					Cresson et al., 2014
Gulph of Lyons	4.14#					
	2.55*		0.150*		27.35*	Capelli et al., 2004
Ligurian Sea	0.6–8.5*		0.015–0.755*		10.75–83.10*	
Atlantic Ocean						
			0.10 ± 0.05	0.124 ± 0.038	12.6 ± 4.7	Mille et al., 2018
Bay of Biscay			(0.040–0.160)	(0.100–0.190)	(8.2–18.4)	
West Scotland	0.60 ± 0.30		1.74 ± 0.71	1.70 ± 2.07	26.0 ± 9.0	Canli and Furness, 1993
	2.0 ± 0.8*		0.50 ± 0.2*	0.25 ± 0.05*	53.0 ± 11.0*	Lourenço et al., 2009
SW Portugal	(1.05–3.65*)		(0.15–0.75*)	(0.2–0.3*)	(41.0 ± 77.5*)	
	0.5 ± 0.25*					Wiech et al., 2021
West Norway	(0.13–1.45*)					

< 0.05), no correlation with AChE activity was detected (Table 2) possibly because the percentage of inorganic As, which represents the more toxic form of As (Ventura-Lima et al., 2011), was low with respect to the total As. The exact ratio of As forms in the muscle tissue was not evaluated in this study, however it was previously shown that inorganic As in the *N. norvegicus* tail muscle from Southern Adriatic Sea accounts for <10 % of the total As (Storelli and Marcotrigiano, 2001). It could be also speculated that *N. norvegicus* AChE may not contain structural features required for As binding and subsequent inactivation (Ventura-Lima et al., 2011).

The levels of cadmium, lead and copper in the muscle of *N. norvegicus* were generally in the same order of magnitude as those previously reported for other Mediterranean and Atlantic coastal regions (Table 3) and none of these metals displayed correlations with AChE activity (Table 2). This result could suggest that concentrations of these metals were too low to create an effect on AChE and that these compounds are consequently of little relevance for muscle AChE activity fluctuations in *N. norvegicus* from the Northern Adriatic.

Anthropogenic activity in coastal areas usually produces rather complex mixture of pollutants in marine ecosystems. Therefore, it is plausible that the ability to discern a clearer pattern of AChE activity was limited by the presence of other types of pollutants, not assessed in this study. Pesticides containing organophosphates and carbamates as active ingredient are commonly applied for pest control in agricultural fields, from where these compounds may be delivered to coastal zones by run-offs, riverine input and air deposition and then transferred from water column to sediments (Triassi et al., 2019).

Organophosphorous and carbamate pesticides reportedly inhibited AChE activity in various crustaceans (Bertrand et al., 2016; Oliveira et al., 2013; Taylor et al., 2019). Nevertheless, the landscapes adjacent to sampling sites have contained agricultural activity of low intensity and the levels of pesticides in environmental matrices sampled from Northern Adriatic offshore locations are yet to be confirmed. AChE activity modulation in crustaceans and other invertebrates was also linked to other classes of marine pollutants, such as polybrominated diphenyl ether (Chen et al., 2015), various antifoulants (Lee et al., 2017) and herbicides (Juhel et al., 2017).

High Hg content detected in *N. norvegicus* muscle tissue raises questions on the overall safety for its consumption. By using a dry-to-wet tissue conversion factor of 5, the mean total Hg concentration of 4.68 μg/g d.w. determined in this study is converted to the value of 0.93 μg/g w.w. that is almost two-fold above the maximum level of 0.5 μg/g w.w. in foodstuff as set by EU standards (EC, 2006). Moreover, the value has been exceeded in >75 % of individuals (Fig. 3).

One previous study indicated that frequent consumption of *N. norvegicus* captured at some presumed Hg hot spots in the Adriatic might not be safe since the EU threshold level of total Hg content was exceeded in virtually all samples of *N. norvegicus* muscle (Di Lena et al., 2018). Thus, it is essential to carry on with investigations in the Adriatic in order to gain more knowledge on the Hg content in *N. norvegicus* and increase public awareness of the risks associated to its consumption (Di Lena et al., 2018; Perugini et al., 2009).

4. Conclusions

In this study, the range of AChE activity was determined in the muscle of *Nephrops norvegicus* from two fishing grounds in the Northern Adriatic. In the present study differences in baseline AChE activity with respect to season and sex were determined. Most importantly, careful selection of individuals of homogeneous body size is needed to increase the power and reliability of AChE activity evaluation. The present data indicate that benthic organisms in the Northern Adriatic may be exposed to natural, but relatively high background levels of Hg raising concerns for possible adverse effects in wild caught *N. norvegicus*. However, controlled laboratory experiments using individuals of varying length and age are needed to verify the influence of Hg on AChE activity in *N. norvegicus* muscle. Full discrimination potential of AChE activity in the muscle of *N. norvegicus* should be evaluated by examination of organisms collected from regions with contrasting levels of metals. Finally, this study underlines the importance of continuous monitoring in order to evaluate Hg availability trends and the overall safety of seafood consumption.

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Ethical statement

This work did not require institutional ethical review or approval.

CRediT authorship contribution statement

Arijana Cenov: Writing – original draft, Methodology, Investigation. **Dijana Tomić Linšak:** Writing – review & editing, Supervision. **Lorena Perić:** Writing – original draft, Conceptualization. **Paula Žurga:** Validation, Methodology, Formal analysis. **Darija Vukić Lušić:** Visualization, Conceptualization. **Luka Traven:** Software, Data curation. **Željko Linšak:** Resources, Investigation. **Sandra Marinac Pupavac:** Validation, Methodology. **Bojan Hamer:** Resources, Conceptualization. **Jadranka Pelikan:** Methodology, Investigation. **Marin Glad:** Visualization, Supervision, Software, Investigation.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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