Marine Environmental Research 155 (2020) 104878

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: http://www.elsevier.com/locate/marenvrev

Assessment of the relative sensitivity of the copepods *Acartia tonsa* and *Acartia clausi* exposed to sediment-derived elutriates from the Bagnoli-Coroglio industrial area

Ylenia Carotenuto [a,*], Valentina Vitiello [b], Alessandra Gallo [a], Giovanni Libralato [c], Marco Trifuoggi [d], Maria Toscanesi [d], Giusy Lofrano [e], Francesco Esposito [a], Isabella Buttino [a,b,**]

[a] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121, Napoli, Italy
[b] Istituto Superiore per la Protezione e Ricerca Ambientale, via del cedro 38, 57122, Livorno, Italy
[c] Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126, Naples, Italy
[d] Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126, Naples, Italy
[e] Centro Servizi Metrologici e Tecnologici Avanzati (CeSMA), Complesso Universitario di Monte Sant'Angelo, Via Cinthia 21, 80126, Naples, Italy

ARTICLE INFO

Keywords:
Zooplankton
Polycyclic aromatic hydrocarbons
Heavy metals
Ecotoxicology
Acute test
Chronic test
Mediterranean sea

ABSTRACT

The sensitivity of the copepods *Acartia tonsa*, commonly used in standardized tests for environmental risk assessment and *A. clausi,* the dominant autochthonous congener species in the Mediterranean Sea, was assessed using sediment-derived elutriates from the industrial area of Bagnoli-Coroglio and nickel chloride as referent toxicant. Acute *A. clausi* naupliar immobilization test showed EC_{50} for elutriates E25, E56 and E84 of 23.3%, 80.5% and >100%, respectively, compared to 59.5%, 66.6% and >100% in *A. tonsa*. In the 7 day sublethal test, a reduction in *A. clausi* egg production rates was observed in all elutriates, but only in E56 for *A. tonsa.* Elutriate 56, which contained the highest amount of polycyclic aromatic hydrocarbons, also induced 70% mortality in *A. clausi* females. Although *A. clausi* was more sensitive than *A. tonsa*, the two species had convergent responses to the three elutriates, thus opening the venue for a potential use of *A. clausi* in standardized ecotoxicity tests.

1. Introduction

The euryhaline calanoid copepod *Acartia tonsa* is a widespread species inhabiting coastal and estuarine waters of temperate and subtropical regions. Since 1970s, increased research efforts have been devoted to investigate the physiology, reproduction and feasibility to multi-generation rearing of this species (Jones et al., 2002; Støttrup et al., 1986; Zhang et al., 2013). Moreover, *A. tonsa* has been largely used as a model organism in ecotoxicology studies to evaluate the quality of marine matrices and to assess the presence of hazardous compounds in marine ecosystems (Andersen et al., 2001; Buttino et al., 2011; Kusk and Wollenberger, 2007). Presently, *A. tonsa* is included as a model organism in international standardized protocols for acute, semi-chronic (Gorbi et al., 2012; ISO, 1999) and chronic (Buttino et al., 2018; ISO, 2015) bioassays for environmental risk assessment in marine coastal area. Very recently, *A. tonsa* has also been included by the Italian Ministry of the Environment (DM, 173/2016) and by the Government of Canada (Environmental and Climate Change, 2019), in the battery of species to be used for the evaluation of sediment toxicity. Bioassays include either direct exposure of eggs to sediment or incubation into sediment-derived aqueous solutions (e.g. elutriate or pore water), and are based on the evaluation of specific end-points, such as egg hatching success and larval viability. However, a further chronic end-point, such as the reduction of *A. tonsa* fecundity, has been lately proposed in marine environmental risk assessment associated to the exposure of adults to emerging contaminants (i.e. nanomaterials) (Zhou et al., 2016a, 2018). Even if *A. tonsa* is a widespread species, in the Mediterranean Sea it is considered a non-indigenous species, as it was introduced in 1980' by ballast water. Nowadays, *A. tonsa* is confined to Northern Adriatic lagoons (Comaschi et al., 2000), Southern Italy (Belmonte and Potenza, 2001) and the Berre Lagoon in the Northern Mediterranean Sea (Gaudy et al., 2000). In these areas, it became the dominant species in the copepod assemblage, displacing or confining to more restricted areas other congeneric autochthonous species such as *A. margalefi* and

* Corresponding author. Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121, Napoli, Italy.
** Corresponding author. Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121, Napoli, Italy.
E-mail addresses: ylenia.carotenuto@szn.it (Y. Carotenuto), isabella.buttino@isprambiente.it (I. Buttino).

https://doi.org/10.1016/j.marenvres.2020.104878
Received 23 October 2019; Received in revised form 16 January 2020; Accepted 16 January 2020
Available online 21 January 2020

A. clausi (Acri et al., 2004). In the Northern Adriatic Sea, *A. tonsa* and *A. clausi* show a clear spatial separation, with *A. clausi* inhabiting areas with a higher seawater salinity than *A. tonsa* (Belmonte and Potenza, 2001; Bianchi et al., 2003). Both species are considered euryhaline as they are able to adapt to salinity ranges from 5‰ to 35‰ for *A. tonsa* (Miller and Marcus, 1994) and from 0‰ to 36‰ for *A. clausi* (Gaudy et al., 1989). They are also eurythermal and able to survive in waters ranging from −1 to 30 °C (Pagano et al., 2003). In the Mediterranean Sea, *A. clausi* is the most widespread Acartidae species and one of the dominant species in coastal waters. In particular, in the Gulf of Naples *A. clausi* is the second most important species of the copepod assemblage, in terms of annual averages, and the dominant one from spring to autumn (Mazzocchi et al., 2012); whereas in the Northern Adriatic Sea, population peaks occur during winter (Comaschi et al., 2000). Feeding ecology and reproductive biology of *A. clausi* are well known (Calliari and Tiselius, 2005; Guisande et al., 2002; Ianora et al., 2015; Sei et al., 2006), hence, considering its role as a link between phytoplankton and secondary consumers in the Mediterranean food webs (Calbet et al., 2002), it is important to gain more information about the sensitivity of *A. clausi* to environmental pollutants. This is especially relevant considering that the development of new bioassay protocols applied to ecologically relevant species is one of the key issues in ecotoxicology (Raisuddin et al., 2007). The use of non-indigenous species in areas where diffusion of *A. tonsa* poses a threat for the native copepod population is not advisable and local species should be proposed as alternative test species in ecotoxicity bioassays. For these reasons, *A. clausi* is indicated together with *A. tonsa* in the Italian guideline for marine sediment quality evaluation (ICRAM-APAT, 2007).

The occasion to use both co-generic species *A. tonsa* and *A. clausi* in ecotoxicology tests and to compare their sensitivity to characterize sediment quality was offered by the ABBaCo project (Morroni et al., 2020). The aim of the project was to propose new approaches/actions for environmental remediation and restoration of the marine area of Bagnoli-Coroglio, located in the Gulf of Pozzuoli (Naples, Italy). In this area, *A. clausi* is one of the most abundant copepod species, constituting more than 25% of the total copepod assemblage in spring (Margiotta et al., 2020). The industrial area of Bagnoli is classified as a Site of National Interest (SIN) due to its high contamination caused by a large steel plant operating for nearly one century (DM, August 31, 2001; Morroni et al., 2020). Therefore, the aim of the present study is to perform acute and chronic tests with *A. tonsa* and *A. clausi* exposed to elutriates derived from sediments collected in the area of Bagnoli-Coroglio, in order to evaluate the toxicity of these marine matrices and to compare the sensitivity of the two copepod species. The main goal of the study was to propose *A. clausi* as an alternative copepod species in bioassays.

2. Materials and methods

2.1. Sediment collection and elutriates preparation

Sediments were sampled with a vibrocorer during November–December 2017, at three sites within Bagnoli Bay located in the eastern area of the Gulf of Pozzuoli (Tyrrhenian Sea, Italy) during the ABBaCo project (Fig. 1). In particular, sites 25 (4517970N and 429171E), 56 (4517495N and 429047E) and 84 (4517070N and 429171E), were chosen among the stations sampled in the project, following a previous environmental characterization of the Bagnoli Bay (Ausili et al., 2012; Morroni et al., 2020). According to these studies, these sites belong to marine sediment areas whose toxicant concentration was higher (25),

Fig. 1. Map of the Bagnoli-Coroglio area with location of sediment collection sites, 25, 56 and 84.

extremely higher (56) and moderately higher (84), than established action values set for management of dredged marine sediments (Ausili et al., 2012; BoI-Pr-CA-BA-relazione-02.04, 2005; Morroni et al., 2020). For each core, the top 50 cm was used for elutriate preparation.

Elutriates were prepared according to standard procedures (ICRAM-APAT, 2007; USEPA, 1991). After the determination of the dry weight of sediment samples, elutriates were prepared by combining sediment and natural seawater with a salinity of 37‰, collected from uncontaminated areas in the Gulf of Naples, in a 1:4 solid-to-liquid volumetric ratio and stirring at 300 rpm for 1 h with an orbital shaker. After 1 h settling, the aqueous fraction was siphoned off without disturbing the settled material and centrifuged at 5100 *g* for 20 min at 4 °C. The supernatant was collected as elutriate and stored at −20 °C until bioassays and chemical analyses were performed (E25, E56 and E84).

2.2. Chemical analysis

2.2.1. Metals and metalloids

Elutriates, prepared as described above, were filtered using 0.45 μm regenerated cellulose membrane filters and acidified with 3% v/v HNO_3. Samples were analyzed with inductively coupled plasma with mass spectrometry (ICP-MS, Aurora M90, Bruker, USA) quantifying Al, Sb, As, Ba, Be, B, Cd, Co, Cr, Fe, Mn, Hg, Mo, Ni, Pb, Cu, Se, V and Zn. The quantitative analysis was performed using an external calibration curve built with five concentrations for each of the analyzed elements using multi-element standard solutions for ICP TraceCERT® in 5% nitric acid (Sigma-Aldrich, Milan, Italy) and ultrapure deionized water with conductivity < 0.06 μS/cm. The Limit Of Detection (LOD) and Limit Of Quantification (LOQ) for each metal are reported in Table S1.

2.2.2. Determination of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)

PAHs and PCBs were determined in elutriates according to USEPA methods 3535A (USEPA, 2007) and 8270D (USEPA, 2014). Sixteen PAHs were investigated: acenaphthene (ACE), acenaphthylene (ACY), anthracene (ANT), benzo(a)anthracene (BaA), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(ghi)perylene (BgP), benzo(a)pyrene (BaP), chrysene (CHR), dibenz(ah)anthracene (DhA), fluoranthene (FLT), fluorene (FLR), indeno[1,2,3-cd]pyrene (IND), naphthalene (NAP), phenanthrene (PHE), pyrene (PYR). Moreover, nineteen PCBs congeners were analyzed: PCB1, PCB5, PCB18, PCB31, PCB44, PCB52, PCB66, PCB87, PCB101, PCB110, PCB138, PCB141, PCB151, PCB153, PCB170, PCB180, PCB183, PCB187, and PCB206. For both PAHs and PCBs determination, samples were pre-treated as follows: Solid-phase extraction (SPE) was carried out on 0.5 L of elutriates, filtered and pre-concentrated on C18-disk (ENVITM, −18 DSK SPE Disk, diam. 47 mm). Before starting the extraction process, samples were spiked with 25 μl of decachlorobiphenyl (100 μg/L standard solution) or with an internal standard constituted by a mixture of deuterated PAHs for PCBs and for PAHs determination, respectively. Disks were pre-conditioned with methanol and, subsequently, with distilled water. Analytes were eluted with a solution of 1:1 dichloromethane and n-hexane. The extract was then concentrated to 1 mL in multivap under nitrogen flow (Multivap8, LabTech, Italy). The extract was injected into a gas chromatography-mass spectrometer (GC-MS, MS-TQ8030-Shimadzu, Japan). The quantitative analysis was carried out in Selected Ion Monitoring (SIM) mode. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the range method of prediction to 95% of linear regressions for each investigated PAHs. The calculated average values of LOD and LOQ were 0.02 and 0.05 μg L^{-1}, respectively for PCBs, and 0.004 and 0.01 μg L^{-1} for PAHs. The data quality was ensured by certified reference materials (ERM-CA100 (European Commission) for PAHs and QC1033 (Supelco) for PCBs). The recovery percentage was 70–110% for PAHs and 65–120% for PCBs.

2.3. Copepod collection and culture maintenance

Zooplankton samples were collected during June–July 2018 from the Gulf of Naples using a 200 μm Nansen net and transferred to the laboratory within 1 h. In the laboratory, wild *A. clausi* females and males (N = 100 couples) were sorted under a Leica stereomicroscope and incubated in two 1 L glass beakers containing 0.22-μm filtered seawater (FSW) at 37‰ salinity and enriched with unialgal culture of *Rhinomonas reticulata* (CCAP995/2, SZN_FE208) and *Isochrysis galbana* (CCMP1323, SZN_FE207) in the exponential growth phase, at a concentration of 1.8 × 10^4 cells mL^{-1} and 5.0 × 10^4 cells mL^{-1}, respectively (total 2 mg C L^{-1}). *Acartia clausi* culture was maintained for up to two weeks in a climate-controlled room at 20 °C and with a 12:12 h light:dark photoperiod; copepods were fed three times a week and the water was partially renewed every week.

Acartia tonsa culture was reared at the ISPRA laboratory in Livorno (Italy). Copepods were maintained in two 1 L glass beakers containing FSW at 30‰ salinity and under the same food, temperature and light conditions as *A. clausi* (Zhang et al., 2013).

The algal strains *R. reticulata* and *I. galbana* were cultured as batch cultures in the exponential growth phase using F/2 medium without silicate, as previously reported (Zhang et al., 2013).

2.4. Acute test

Fifty couples of *A. clausi* or *A. tonsa* were sorted from the main culture the day before the acute test and incubated in 1 L beaker containing FSW and *R. reticulata* at a final concentration of 3 × 10^4 cells mL^{-1} (2 mg C L^{-1}). After 20 h, eggs at the bottom of the beakers were siphoned, filtered through a 50 μm mesh net and incubated for the 48h acute test. This bioassay was performed following previous methods (UNICHIM, 2012; Vitiello et al., 2016). Briefly, groups of <24 h old eggs were transferred into 10 mL crystallizing dishes containing FSW at 37‰ or 30‰ salinity, for *A. clausi* or *A. tonsa*, respectively (negative control), nickel chloride ($NiCl_2$) at different concentrations (positive control) or elutriates E25, E56 and E84. For both copepod species, two eggs were transferred into each of 18-well plates containing 2.5 mL of different treatments. Three replicates of five wells for each treatment were carried out, for a total of 30 eggs. Plates were incubated in a temperature controlled room at 20 °C and 12:12 light:dark photoperiod.

Acute tests with *A. clausi* incubated in elutriates E25, E56 and E84 were carried out using undiluted elutriate (100%) and three dilutions of each elutriate, obtained adding FSW at 37‰ salinity (v:v) and corresponding to final 25%, 50% and 75% elutriate dilutions. Acute tests with *A. tonsa* were performed with 20%, 40%, 60% and 80% (v:v) dilutions. Undiluted elutriates could not be tested since they were originally prepared using FSW at 37‰ salinity collected in the Gulf of Naples, which required adjustment to 30‰ salinity.

For positive control, nickel chloride hexahydrate stock solution of 1000 mg L^{-1} (Sigma-Aldrich Milan, Italy) was diluted in bi-distilled water (BDW) to obtain a nominal concentration of 10 mg L^{-1}, corresponding to a measured value of 10 ± 0.09 mg Ni L^{-1} analyzed with ICP OES 720 (Agilent Technologies, Santa Clara, CA, USA). Dilutions in FSW were prepared to obtain final concentrations of 0.0125, 0.025, 0.05, 0.10, 0.20 and 0.40 mg Ni L^{-1} for *A. tonsa* (Buttino et al., 2018). For *A. clausi* the test was initially performed using similar $NiCl_2$ concentrations as for *A. tonsa* (0.05, 0.10, 0.20 and 0.40 mg Ni L^{-1}). However, due to a high immobilization of the first naupliar stage at 0.05 mg Ni L^{-1}, a second set of concentrations was tested (0.01, 0.02, 0.04, 0.08 and 0.16 mg Ni L^{-1}).

Egg hatching success and naupliar immobilization or mortality were evaluated after 48h of incubation under an inverted microscope (Zeiss, Milan, Italy) at 50x magnification. The percentage of egg hatching success (HS) was calculated by counting the number of empty egg membranes with respect to the number of incubated eggs, whereas the percentage of naupliar immobilization was assessed by counting the

number of immobile/dead nauplii on the bottom of the well with respect to the number of hatched eggs.

2.5. Chronic test

Acartia tonsa adults were collected from the same multi-generation culture used for the acute test, whereas *A. clausi* adults were sorted from wild zooplankton sample collected two days before the experiment in the Gulf of Naples (see above).

Ten male and female couples of both *A. tonsa* and *A. clausi* were incubated into 60 mL crystallizing dishes containing 50 mL of 80% (v:v) elutriate concentrations of E25, E56 and E84 at 30‰ or 37‰ salinity, respectively. Each couple was fed with *R. reticulata* at 3×10^4 cells mL^{-1} final concentration. A group of 10 couples for each species was incubated in FSW without the elutriate, as controls, at the same salinity and algal concentration indicated above. Every day and up to 7 days, elutriates solutions and copepods were transferred into new crystallizing dishes and fed with *R. reticulata* at 3×10^4 cells mL^{-1}, whereas spawned eggs were counted and incubated in FSW at 30‰ or 37‰ salinity, depending on the copepod species, and left to hatch at 20 °C and 12:12 light:dark photoperiod. The percentage of egg hatching success was assessed after 48h counting the number of empty egg membranes under an inverted microscope (Zeiss, Milan, Italy) at 25x magnification with respect to the total number of eggs.

2.6. Effect concentration statistical analysis

Effect concentrations (EC_{50}) of reference toxicant $NiCl_2$ and elutriates inducing 50% naupliar immobilization/mortality in both *A. tonsa* and *A. clausi* acute tests were calculated using Probit Analysis version 1.5. Significant differences between treatments for each copepod species, and between copepod species exposed to the same treatment, were determined using One-way Analysis of Variance (ANOVA) and multiple *t*-test analysis, respectively. Female survival curves in chronic tests were compared using the Log-rank Mantel-Cox test. In addition, two-way ANOVA followed by Fisher's LSD post-hoc test was also used to test the effects of both treatment (elutriate) and copepod species (*A. clausi* and *A. tonsa*) on egg production and hatching success in chronic tests. All statistical analyses were performed using GraphPad Prism version 6. The relationships between variables and the variation present in the dataset matrix were determined by biplotting both the ordination component scores and the variable loading coefficients through Principal Component Analysis (PCA) based on the Pearson's correlation matrix (XLSTAT, v. 2019.3.1) (Addinsoft, 2019).

3. Results

3.1. Chemical analysis

Elutriates E25, E56 and E84 prepared from sediments collected in the Bagnoli-Coroglio area were analyzed for their content of heavy metals (HMs), Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs). Elutriate E84 contained the highest concentration of HMs (12.8 mg L^{-1}), whereas lower and similar amount were detected in E25 and E56 (about 5 mg L^{-1}). Relative concentration of each trace metals detected in the three elutriates is indicated in Table 1. Of these, boron (B) (4–5 mg L^{-1}), manganese (Mn) (1–7 mg L^{-1}) and vanadium (V) (0.2 mg L^{-1}), were the most abundant elements, followed by barium (Ba), molybdenum (Mo), selenium (Se), arsenic (As), zinc (Zn) and chromium (Cr). Nickel (Ni) was always < 10 μg L^{-1}. The total concentration of PAHs was higher in E56 (8.62 μg L^{-1}), compared to E25 (1.84 μg L^{-1}) and E84 (0.13 μg L^{-1}), with naphthalene, acenaphthylene and acenaphthene being the dominant hydrocarbons in E56 (Table 1). Polychlorinated biphenyls were always below the limit of detection (<0.05 μg L^{-1}).

Table 1
Chemical analysis of elutriates prepared from sediments of the Bagnoli-Coroglio area and Environmental Quality Standards (EQS) set by the European Commission (annual average, μg L^{-1}) (EC, 2008). Concentration of Heavy metals (HMs) and Polycyclic Aromatic Hydrocarbons (PAHs) expressed as μg L^{-1}. For HMs values are given as mean ± standard deviation (SD). No replicate measures for PAHs. In bold, values exceeding EQS values.

Chemical	E25	E56	E84	EQS
Heavy metal (μg L^{-1})				
Al	<10	<10	<10	
Sb	<2	4.9 ± 1.0	3.14 ± 0.7	
As	14.2 ± 3.0	19.1 ± 4.0	29.4 ± 6.2	
Ba	25.6 ± 5.1	123 ± 24.7	48.8 ± 9.8	
Be	<5	<5	<5	
B	4219 ± 928	3492 ± 768	5203 ± 1145	
Cd[a]	<5	<5	<5	0.2
Co	<2	<2	<2	
Cr	17.9 ± 4.1	10.4 ± 2.4	24.2 ± 5.6	
Fe	<50	94.1 ± 19.8	<50	
Mn	5.2 ± 1.0	1073 ± 2.15	7078 ± 1416	
Hg[a]	**0.74 ± 0.19**	**0.63 ± 0.2**	**0.84 ± 0.2**	0.05
Mo	24.0 ± 5.5	37.5 ± 8.6	35.0 ± 8.1	
Ni	<10	<10	<10	
Pb	<1	1.53 ± 0.4	<1	
Cu	14.0 ± 0.0	<10	<10	
Se	38.7 ± 8.5	31.3 ± 6.9	13.4 ± 2.9	
V	218 ± 46	172 ± 36	298 ± 63	
Zn	18.7 ± 3.7	<10	31.4 ± 6.3	
Total	*4596.56*	*5059.44*	*12765.20*	
PAH (μg L^{-1})				
Naphthalene	0.07	**4.00**	<0.01	1.2
Acenaphthylene	0.02	2.06	<0.01	
Acenaphthene	<0.01	1.08	<0.01	
Fluorene	0.02	0.02	<0.01	
Anthracene[a]	0.09	**0.20**	0.01	0.1
Phenanthrene	<0.01	0.29	<0.01	
Fluoranthene	<0.01	0.05	0.05	
Pyrene	0.70	0.16	0.03	
Benz[a]anthracene	0.08	0.12	<0.01	
Dibenz(a,h)anthracene	<0.01	<0.01	<0.01	
Chrysene	0.24	0.13	0.02	
Benzo(b)fluorantene[a]	**0.21**	**0.13**	0.02	0.03
Benzo(k)fluoranthene[a]	**0.15**	**0.10**	<0.01	
Benzo(a)pyrene[a]	<0.01	**0.10**	<0.01	0.05
Indeno(1,2,3-cd)pyrene[a]	**0.14**	**0.09**	<0.01	0.002
Benzo(g,h,i)perylene[a]	**0.13**	**0.07**	<0.01	
Total	*1.84*	*8.62*	*0.13*	

[a] denotes priority hazardous substance defined by the EC.

3.2. Acute test

Acute test with *Acartia tonsa* eggs exposed for 48h to increasing concentrations of the reference toxicant from 0.0125 to 0.40 mg Ni L^{-1}, showed a dose-dependent effect with increasing percentage of naupliar immobilization, ranging from 6.67% to 100% (ANOVA $F_{6,14} = 200.1$; $p < 0.0001$) (Fig. 2a). EC_{50} calculated for *A. tonsa* was 0.131 mg Ni L^{-1} (95% Confidence Interval = 0.108–0.155 mg Ni L^{-1}), well within the expected values. Egg hatching success (86.7%) and naupliar immobilization (0%) of the control group were in the range of expected values (Gorbi et al., 2012; ISO, 1999).

Acute tests with *Acartia clausi* showed that $NiCl_2$ at concentrations ranging from 0.05 to 0.40 mg Ni L^{-1} were extremely toxic for this species, inducing 82.6% naupliar immobilization already at 0.05 mg Ni L^{-1} (Fig. 2b); whereas when incubated in 0.01–0.160 mg Ni L^{-1}, it ranged from 73.3 to 93.3% (Fig. 2c). In control groups, egg hatching success was 90.3–90.8% and naupliar immobilization rates ranged from 11.7% to 12.5%. In both tests, percentage of naupliar immobilization was statistically different between treatments (ANOVA $F_{4,10} = 52.93$; $p < 0.0001$; ANOVA $F_{5,12} = 26.23$; $p < 0.0001$), although in the second experiment all Ni concentrations elicited similar responses and the significance of the difference was only ascribable to the control. EC_{50} for *A. clausi* exposed to $NiCl_2$ was extrapolated to 0.005 mg Ni L^{-1} (95% Confidence

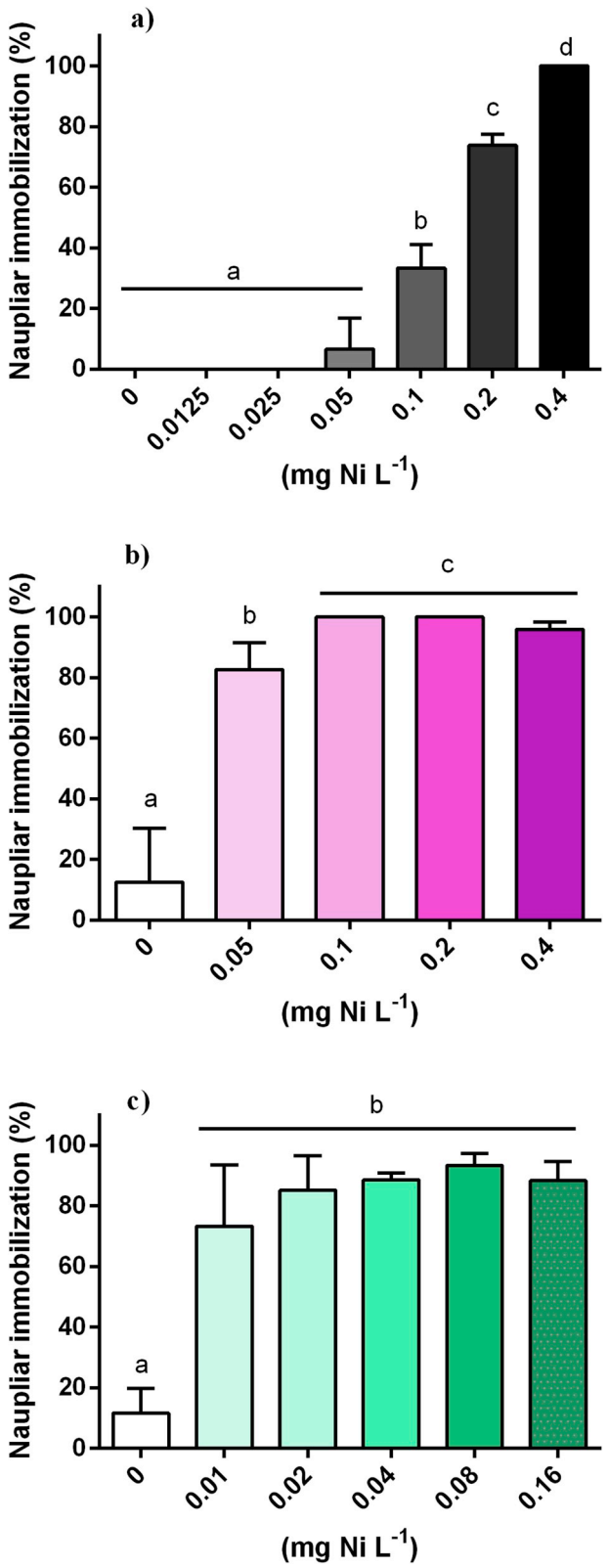

Fig. 2. Acute toxicity test (48h) with $NiCl_2$ (mg Ni L^{-1}). Naupliar immobilization (%) of *Acartia tonsa* (a) and *Acartia clausi* (b, c). Values are mean ± standard deviation (SD). Treatments with different letters are significantly different (ANOVA $p < 0.05$).

Interval = 0.004–0.006 mg Ni L^{-1}).

Multiple *t*-test analysis performed between the two copepod species exposed to the same concentrations of Ni, showed that naupliar immobilization was always significantly higher in *A. clausi* as compared to *A. tonsa* (for 0.2 mg Ni L^{-1}, $t_4 = 12.46$, $p < 0.001$; for 0.1 mg Ni L^{-1}, $t_4 = 14.99$, $p < 0.001$; for 0.05 mg Ni L^{-1}, $t_4 = 9.75$, $p < 0.001$).

Nauplii born from *A. tonsa* eggs incubated for 48h in elutriates E25 and E56 at the highest concentration (80% elutriate) underwent increased percentage of naupliar immobilization with respect to the lowest concentrations; in particular, 69.0% and 59.4% naupliar immobilization were recorded, respectively (E25: ANOVA $F_{4,10} = 8.14$; $p < 0.01$; E56: ANOVA $F_{4,10} = 14.01$; $p < 0.001$) (Fig. 3a and b). Elutriate E84 did not affect naupliar mobility/viability even at the highest concentration, and this value was not statistically different from controls (8.3% vs 3.7%, respectively) (ANOVA $F_{4,10} = 1.29$; $p > 0.05$) (Fig. 3c) (Table 2). EC_{50} in *A. tonsa* exposed to elutriates were 59.5%, 66.6% and >100% for E25, E56 and E84, respectively (Table 2). According to the toxicity ranking indicated by the guidelines for the management of dredged marine sediments (ICRAM-APAT, 2007), E25 and E56 were considered highly toxic, whereas E84 was not toxic.

Acartia clausi nauplii were strongly impaired by undiluted elutriates E25 and E56, with 100% and 94.4% immobilization, respectively (E25: ANOVA $F_{4,10} = 103.0$; $p < 0.0001$; E56: ANOVA $F_{4,10} = 18.92$; $p < 0.001$) (Fig. 4a and b). Naupliar mobility/viability was not affected by Elutriate 84, which induced 29.5% immobilization at the highest concentration, but was not statistically different from the control (3.7%) (ANOVA $F_{4,10} = 1.70$; $p > 0.05$) (Fig. 4c) (Table 2). EC_{50} of naupliar immobilization for *A. clausi* exposed to elutriates were 23.3%, 80.5% and >100% for E25, E56 and E84, respectively (Table 2). In the latter case, we also calculated the EC_{20}, which was <90%. According to the mentioned toxicity ranking, E25 was considered extremely toxic, E56 highly toxic, whereas E84 was moderately toxic.

3.3. Chronic test

Acartia tonsa females incubated in elutriates E25, E56 and E84 produced on average 15.7, 10.3 and 14.3 eggs $female^{-1}$, respectively, during the first day of incubation, similar to the control (ANOVA $F_{3,32} = 1.791$; $p > 0.05$). Egg production remained stable with time in control and elutriates E84 and E25, whereas it decreased slightly in E56 during day 2 and 3 (Fig. 5a). Average egg production over the 7 day period was significantly lower in E56 (8.3 eggs $female^{-1}$), with respect to control (16.8 eggs $female^{-1}$), E25 (22.6 eggs $female^{-1}$), and E84 (19.4 eggs $female^{-1}$) treatments (ANOVA $F_{3,24} = 15.95$; $p < 0.0001$, Fisher's LSD post-hoc test $p < 0.01$) (Table 3). Egg hatching success of *A. tonsa* after one day of incubation ranged from 62.3% in E56, 64.9% in E84 and 68.1% in E25 and was not statistically different from control (77.1%) and between treatments (ANOVA $F_{3,32} = 0.498$; $p > 0.05$). Although an occasionally low egg hatching success was observed on day 3 for E56 and E84 treatments, these values remained stable over the whole incubation period (Fig. 5c), with an average hatching success of 65.6%, 45.5% and 55.9% in E25, E56 and E84, and 70.0% in control (ANOVA $F_{3,24} = 1.094$; $p > 0.05$) (Table 3). Survival of *A. tonsa* females during the 7 d chronic test decreased slightly with respect to the control, reaching a minimum of 62.5% in E84, compared to 88.9% in control FSW (Fig. 5e) (Table 3). However, female survival curves over the whole test were not statistically different between treatments (Log-rank Mantel-Cox test, Chi square = 1.73, df = 3, $p > 0.05$).

Egg production of *A. clausi* on day 1 was significantly lower in the three elutriate treatments (3.2, 2.1 and 1.4 eggs $female^{-1}$ for E25, E56 and E84, respectively), compared to the control (7.8 eggs $female^{-1}$) (ANOVA $F_{3,33} = 4.686$; $p < 0.01$) (Fig. 5b). This trend remained stable over the whole experiment, with an average egg production of 1.7, 1.6 and 2.0 eggs $female^{-1}$, for E25, E56 and E84, respectively, and 7.2 eggs $female^{-1}$ in the control (ANOVA $F_{3,24} = 43.17$; $p < 0.0001$) (Table 3). Chronic exposure of *A. clausi* females for up to 7 days to different

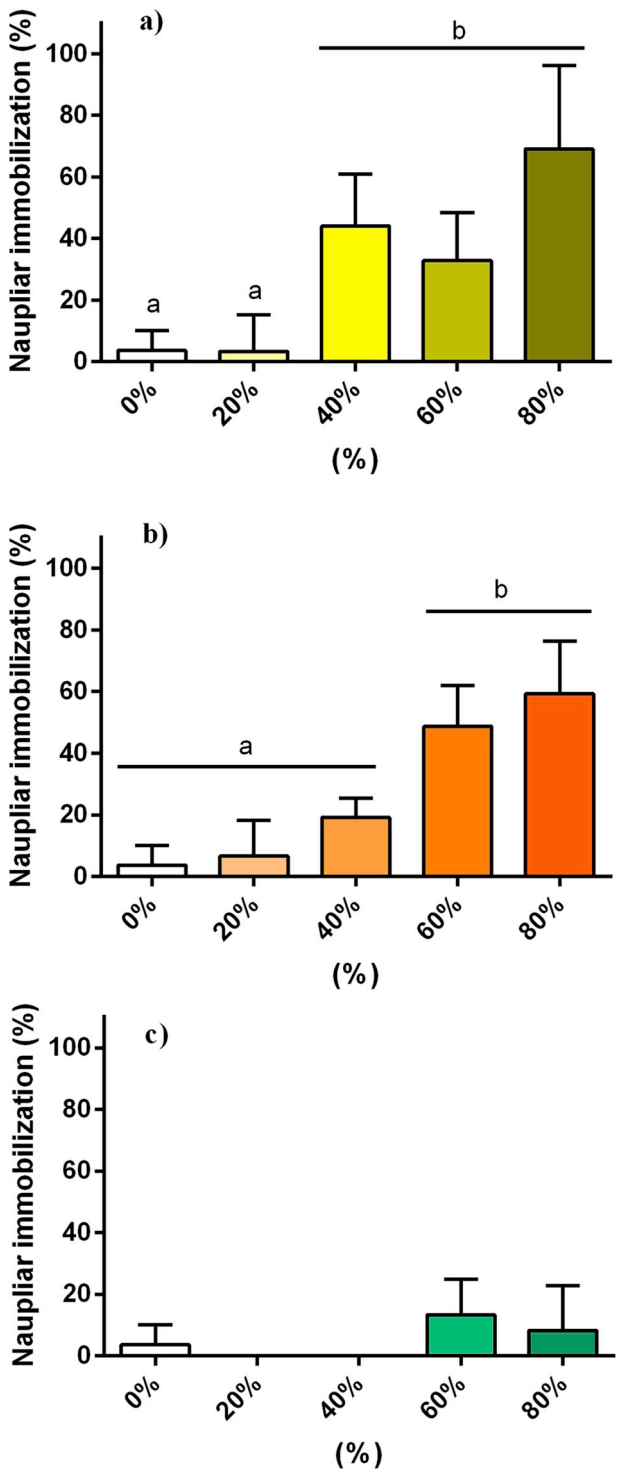

Fig. 3. Acute toxicity test (48h) of *Acartia tonsa* in elutriates. Naupliar immobilization (%) in serial dilution of a) E25, b) E56 and c) E84. Control treatment (0%) is FSW at 30‰. Values are mean ± standard deviation (SD). Treatments with different letters are significantly different (ANOVA $p < 0.05$).

elutriates did not reduce egg hatching success, which was on average 79.3%, 72.1% and 77.4% in E25, E56 and E84, respectively, and 82.1% in the control (Fig. 5d) (ANOVA $F_{3,24} = 0.282$; $p > 0.05$) (Table 3). Interestingly, elutriates influenced female survival with time starting from the second day of incubation. In particular, after 4 days of incubation *A. clausi* survival was 77.8% in E84, 66.7% in E25 and 30% in E56, compared to 88.9% in control, and remained stable until day 7

Table 2
Acartia tonsa and *Acartia clausi* acute tests. Naupliar immobilization (%) observed after egg incubation in elutriates E25, E56 and E84. Control group corresponded to filtered seawater. Values are mean ± standard deviation (SD). EC_{50} for naupliar immobilization was calculated using Probit/Logit analysis (95% Confidence Interval, CI).

Copepod	Elutriate dilution (%)	Naupliar immobilization (%)			
		Control	**E25**	**E56**	**E84**
Acartia tonsa	80		69.0 ± 27.0	59.4 ± 16.9	8.3 ± 14.4
	60		32.9 ± 15.4	48.7 ± 13.3	13.3 ± 11.5
	40		44.0 ± 16.9	19.2 ± 6.3	0 ± 0
	20		3.4 ± 12.0	6.7 ± 11.5	0 ± 0
	0	3.7 ± 6.4	–	–	–
			EC_{50}		
			59.5 (39.5–79.5)	66.6 (51.4–81.8)	>100
Acartia clausi	100		100 ± 0	94.4 ± 9.6	29.5 ± 28.9
	75		100 ± 0	31.7 ± 16.1	35.7 ± 30.3
	50		100 ± 0	22.4 ± 20.4	6.25 ± 8.4
	25		49.5 ± 15.2	13.7 ± 14.3	7.1 ± 10.1
	0	3.7 ± 6.4	–	–	–
			EC_{50}		
			23.3 (10.5–36.1)	80.5 (52–108.7)	>100 (132–250)

(Fig. 5f) (Table 3). Female survival curves over the whole test were statistically different between treatments, with E56 significantly different from control and E84 (Log-rank Mantel-Cox test, Chi square = 8.79, df = 2, $p < 0.05$), but similar to E25 (Log-rank Mantel-Cox test, Chi square = 3.24, df = 1, $p > 0.05$).

A two-way ANOVA was applied to compare egg production, hatching success and female survival between *A. tonsa* and *A. clausi* and among elutriates. The analysis showed that the copepod species accounted for 65% of the total variation in egg production ($F_{1,48} = 296.8$, $p < 0.0001$); whereas the treatment explained only 12.2% of the total variation ($F_{3,48} = 18.38$, $p < 0.0001$), as was the interaction between species and treatment (11.6% of the total variation) ($F_{3,48} = 17.44$, $p < 0.0001$). As for egg hatching success, the copepod species had a significant effect on the results, contributing to 30.1% of the total variation ($F_{1,48} = 23.79$, $p < 0.0001$), which was due to differences between *A. tonsa* and *A. clausi* exposed to E25 and E84 (Sidak's multiple comparisons test, $p < 0.05$). On the contrary, no treatment effect (9.0% of the total variation, $F_{3,48} = 2.39$, $p > 0.05$) and no interaction between species and treatment (0.2% of variation, $F_{3,48} = 0.05$, $p > 0.05$) was observed on hatching success. Finally, neither species (4.4% of variation, $F_{1,48} = 3.43$, $p > 0.05$), or interaction (8.3% of variation, $F_{3,48} = 2.134$, $p > 0.05$) had any significant effect on female survival, whereas the treatment significantly accounted for 25.1% of the variation ($F_{3,48} = 6.46$, $p < 0.001$). Overall, egg production during the chronic test was both copepod- and treatment-specific, hatching success was species-specific for elutriate E25 and E84 and female survival was only treatment-specific.

3.4. Multiple parameters statistical analysis

A biplot summarizing PCA results including chemical and ecotoxicological data is shown in Fig. 6. The first two principal components accounted for 55.13% and 37.61% of the variation, respectively. Therefore 92.73% of the variation can be depicted by a two-axis ordination diagram. The biplot regarding components loadings suggested that the F1 scores are influenced by values of B, Cr, Fe, Mn, Hg, Mo, Pb, Cu, Se, V, Zn, Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Anthracene, Phenanthrene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo(b)Fluorantene, Benzo[k]fluoranthene, Benzo[a]

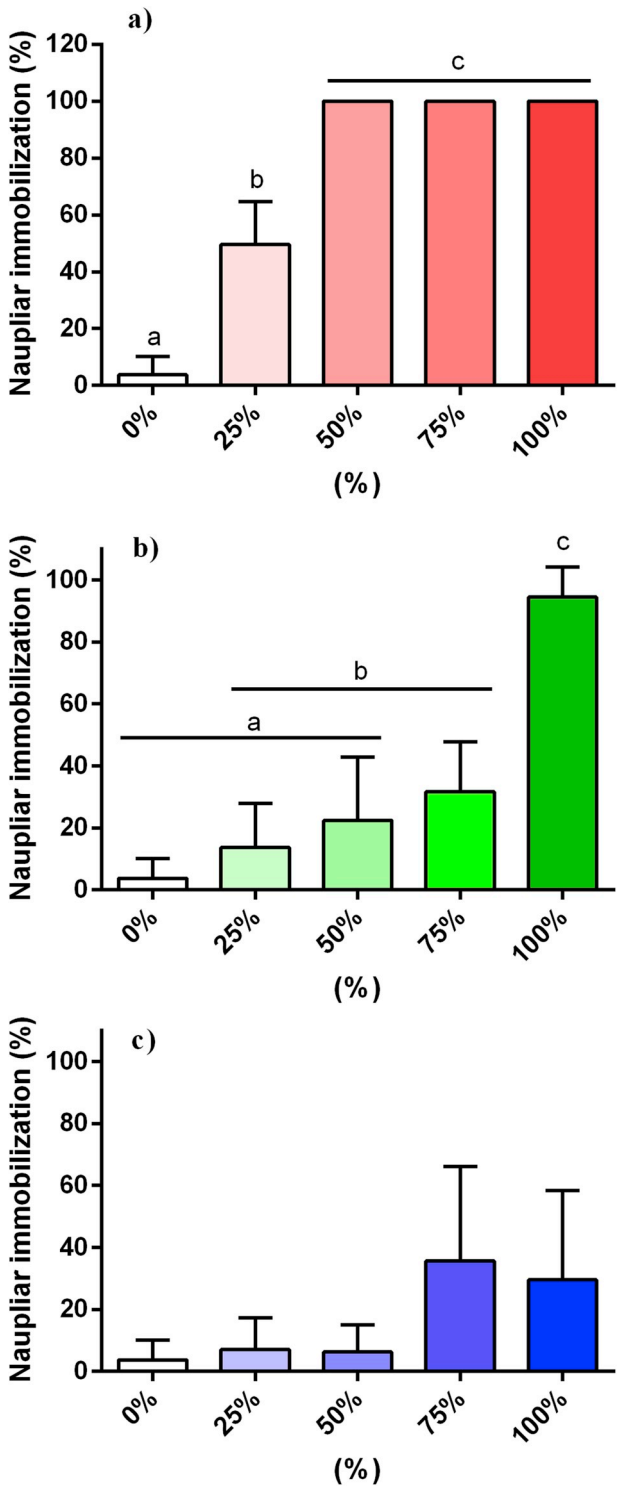

Fig. 4. Acute toxicity test (48h) of *Acartia clausi* in elutriates. Naupliar immobilization (%) in serial dilution of a) E25, b) E56 and c) E84. Control treatment (0%) is FSW at 37‰. Values are mean ± standard deviation (SD). Treatments with different letters are significantly different (ANOVA $p < 0.05$).

pyrene, which are clustered together and have positive loadings on the first axis. In addition, the loading of Sb, As, Ba, B, Cr, Fe, Mn, Hg, Mo, Pb, Cu, Se, V, Zn, Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Anthracene, Phenanthrene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo(b)Fluorantene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene, Benzo[ghi]perylene on the F2 suggested that the second component scores could reflect the concentrations of these compounds in the samples. Egg production, egg hatching success (F3), female survival (F4), and EC_{50} (F5) accounted for a minor part of the variability (7%). Looking at the ordination plot of component scores in the F1–F2 biplot, elutriate samples were scattered in three quadrants, suggesting that each sample presents specific characteristics due to the combination of the considered variables. Apparently, *A. tonsa* and *A. clausi* seemed to present a convergent sensitivity considering the targeted sample.

Considering the biplot analyzing only ecotoxicological data (Fig. 7), the main discriminating endpoint between treatments for *A. tonsa* and *A. clausi* are egg production and egg hatching success, in that order, rather than EC_{50} or female survival. It is worth noting that the endpoint naupliar immobilization, which is considered in standardized protocols to asses marine matrices quality, is not a discriminating variable between *A. tonsa* and *A. clausi*.

4. Discussion

Acartia tonsa is one of the species included in the Italian legislation to detect the potential toxicity of marine dredged sediments (DM, 173/2016). Despite *Acartia clausi* is a dominant species in most temperate coastal areas, and is indicated in the ICRAM-APAT guidelines (ICRAM-APAT, 2007) as an alternative model species to *A. tonsa,* it has rarely been employed in ecotoxicology bioassays. Toxic effects induced by ammonia and phenol on *A. clausi*, for example, were firstly investigated by Buttino (1994), who estimated daily fecundity, egg hatching success and fecal pellet production after 10-day exposure. More recently, acute and sublethal exposure to pesticides (Willis and Ling, 2003, 2004), phenolic compounds and chemical additives present in plastics (Beiras et al., 2019; Tato et al., 2018), have been investigated in *A. clausi*, showing that these compounds induced high naupliar and copepodid immobilization, as well as low egg production in the adult stage.

To the best of our knowledge, no studies have been conducted on sediment elutriate samples using A. *clausi*, until now. Therefore, to first assess the relative sensitivity of *A. clausi* with respect to *A. tonsa* we performed acute tests using the reference toxicant $NiCl_2$. Although the EC_{50} for *A. clausi* was an extrapolated value, being lower than the lowest tested concentration in this species (0.01 mg Ni L^{-1}), the responses of the two species to the same concentrations of Ni (0.2–0.1-0.05 mg Ni L^{-1}) were significantly different, thus demonstrating that nauplius I larval stage of *A. clausi* was comparatively more sensitive to this toxicant than *A. tonsa*. Nickel is reported to be toxic for several calanoid and harpacticoid copepod species (Buttino et al., 2011; Jiang et al., 2013; Wang and Wang, 2010; Zhou et al., 2016b), either in dissolved form as $NiCl_2$ or as nanoparticle. In particular in *A. tonsa*, Zhou et al. (2016b) reported that $NiCl_2$ induced 30% naupliar immobilization at 0.1 mg Ni L^{-1} (EC_{50} 0.164 mg Ni L^{-1}), whereas nickel nanoparticles (NiNPs) administered at 50 mg L^{-1} induced 80% naupliar immobilization (EC_{50} = 22.14 mgL^{-1} NiNPs).

The higher relative sensitivity of early naupliar stages of *A. clausi* with respect to *A. tonsa* is further confirmed by the acute toxicity test performed with the elutriates obtained from sediments collected in the Bagnoli-Coroglio area. Our results, in fact, indicated that although the two copepod species showed convergent sensitivity toward the elutriates, EC_{50} inducing naupliar immobilization in *A. clausi* were comparatively lower than those recorded in *A. tonsa* (except for E56). According to the ranking defined by ICRAM-APAT (ICRAM-APAT, 2007) an EC_{50} <40% indicates 'very high' toxicity, 40% ≤ EC_{50}<100% 'high' toxicity, EC_{50} >100% and EC_{20} <90%, 'moderate' toxicity and, finally, $EC_{50} \geq$ 100% and $EC_{20} \geq$ 90%, no toxicity. Hence, E25 is considered highly toxic for *A. tonsa* and very highly toxic for *A. clausi*, E56 is highly toxic for both species, and E84 not toxic for *A. tonsa* but moderately toxic for *A. clausi*. Differences in copepod sensitivity to the three elutriates were also confirmed in the 7-day chronic tests. After long-term exposure, all three elutriates significantly reduced egg production in *A. clausi*, whereas *A. tonsa* fecundity was affected only by E56. This elutriate also

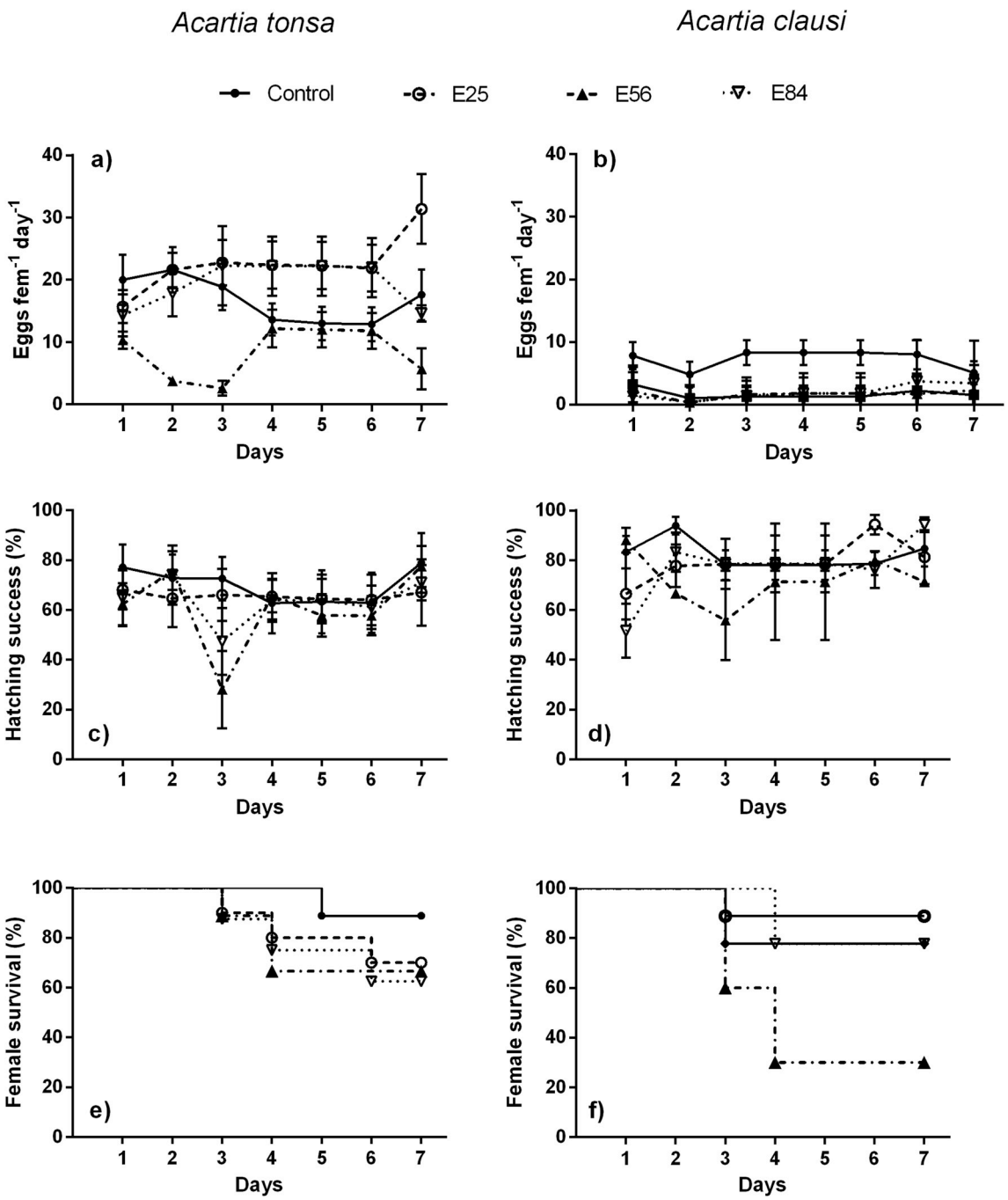

Fig. 5. Chronic toxicity test (7 days) of *Acartia tonsa* and *Acartia clausi* in elutriates. Egg production (eggs fem^{-1} day^{-1}) (a, b), hatching success (%) (c, d), and female survival curves (%) (e, f). For a-d values are mean ± standard error (SE).

reduced the survival of *A. clausi* females but had no effect on the same endpoint in *A. tonsa*. Species-specific or population-specific response to natural or anthropogenic toxicants are frequently reported in copepods (Hansen et al., 2011; Lauritano et al., 2012), and they might be related to the detoxification machinery of each species to counteract the harmful effect of the contaminant. Recently, Zhou et al. (2016a) analyzed gene expression levels of the heat shock protein 70 (HSP 70) in *A. tonsa* adults exposed to the emerging contaminant Quantum Dots and recorded an up-regulation after 3 days of exposure, when neither egg production nor egg hatching success were affected. The authors suggested that HSP 70 protein could play a role in the detoxification ability of *A. tonsa*. It is interesting to note that in the present study *A. tonsa* exposed to E56 recovered its productivity, in terms of egg production and egg hatching success, after 3 days exposure (Fig. 5a, c), and a similar recovery occurred for egg hatching success in *A. clausi* exposed to the same elutriate (Fig. 5d). It is likely that a similar defensive mechanism against contaminants could also have occurred in both species.

Toxicity of chemicals may also depend on physic-chemical characteristics of the experimental conditions, such as temperature (Kusk et al., 2011), salinity (Kusk et al., 2011; Seo et al., 2006) or UV light regimes (Bellas and Thor, 2007), possibly related to changes in metabolic activity, delayed development rate, higher energy allocation to re-establish animal homeostasis under additional stressful conditions, or photo-enhanced toxicity of the toxicants. In the present study, we used a salinity of 37‰ for *A. clausi*, which is near the salinity range experienced by this species in the Gulf of Naples during its maximum abundance in

Table 3
Acartia tonsa and *Acartia clausi* adult chronic tests. Mean egg production (eggs female^{-1}) and egg hatching success (%) over the 7 days exposure and female survival (%) on day 7, measured after incubation in elutriates E25, E56 and E84. Control group corresponded to copepods incubated in FSW and *Rhinomonas baltica*. Values are mean ± standard deviation (SD), except for female survival, where one cumulative value was available at the end of the experiment. For ANOVA, different letters indicate significantly different treatments.

Copepod	Life-history parameter	Control	E25	E56	E84	ANOVA
Acartia tonsa	Egg production	16.8^{a} ± 3.6	22.6^{a} ± 4.6	8.3^{b} ± 4.2	19.4^{a} ± 3.7	$p < 0.0001$
	Egg hatching success	70.0 ± 7.0	65.7 ± 1.5	60.7 ± 16.4	63.7 ± 8.5	$p > 0.05$
	Female survival	88.9	70.0	66.7	62.5	–
Acartia clausi	Egg production	7.2^{a} ± 1.6	1.7^{b} ± 0.7	1.6^{b} ± 0.6	2.0^{b} ± 1.2	$p < 0.0001$
	Egg hatching success	82.1 ± 5.8	79.3 ± 8.2	72.1 ± 10.1	77.4 ± 12.8	$p > 0.05$
	Female survival	88.9	66.7	30.0	77.8	–

spring-summer (37.7–37.9‰) (Margiotta et al., 2020; Mazzocchi et al., 2012). Therefore, it cannot be considered a 'stressful' condition for this species to induce a higher relative sensitivity of *A. clausi* with respect to *A. tonsa*.

Adult survival is a common reference endpoint in most ecotoxicology bioassays (ISO, 1999). However, our results indicated that evaluation of this endpoint alone might underestimate the toxic impact of sediment-derived aqueous matrices on copepods. Survival of *A. clausi* females, for example, was reduced to less than 40% after 4 days of incubation in E56, whereas all nauplius I larval stages incubated in the same elutriate died after only 2 days. Age-specific sensitivity to toxicants has also been reported in copepods (Kadiene et al., 2017). Adult stages are usually orders of magnitude less sensitive than larval stages to toxicants (Medina et al., 2002), and lethal effects on adults usually occur at higher concentrations than those observed on naupliar stages (Barata et al., 2002; Willis and Ling, 2004). This could be due to the higher surface contact area in relation to volume (Rand, 1995) or to different detoxification abilities of nauplii and adults (Green et al., 1996).

Our study also showed that egg production and egg hatching success in both copepod species were affected by the tested elutriates. Similar sublethal tests based on such chronic endpoints have been applied to assess the toxicity of nickel in *A. tonsa* (Zhou et al., 2016b) and of the aquaculture pesticide emamectin benzoate in *A. clausi* (Willis and Ling, 2003). Our findings, thus, confirmed that a 7-day chronic test is a valid instrument to evaluate the sublethal impact of contaminated matrices on population recruitment of herbivorous zooplankton.

Although it is difficult to ascribe the observed toxic effect on *A. clausi* and *A. tonsa* to a specific compound or class of contaminants, given the high chemical heterogeneity of the elutriates, the recurrently toxic effect of E25 and E56 on nauplii of both species, and of E56 on *A. clausi* female survival and *A. tonsa* egg production, could be related to the relatively higher PAH content of these samples. Toxic effects of PAHs on *A. tonsa* naupliar stages and adults have been previously reported (Bellas and Thor, 2007; Krause et al., 2017; Medina et al., 2002), whereas no information is currently available for *A. clausi*. Similarly to PAHs, heavy metals have also been reported to induce high naupliar or adult immobilization in copepods (Kadiene et al., 2017; Seo et al., 2006). In our experiments, E84 had the highest concentration of total and relative HMs with respect to other elutriates, yet it did not induce any toxic effect

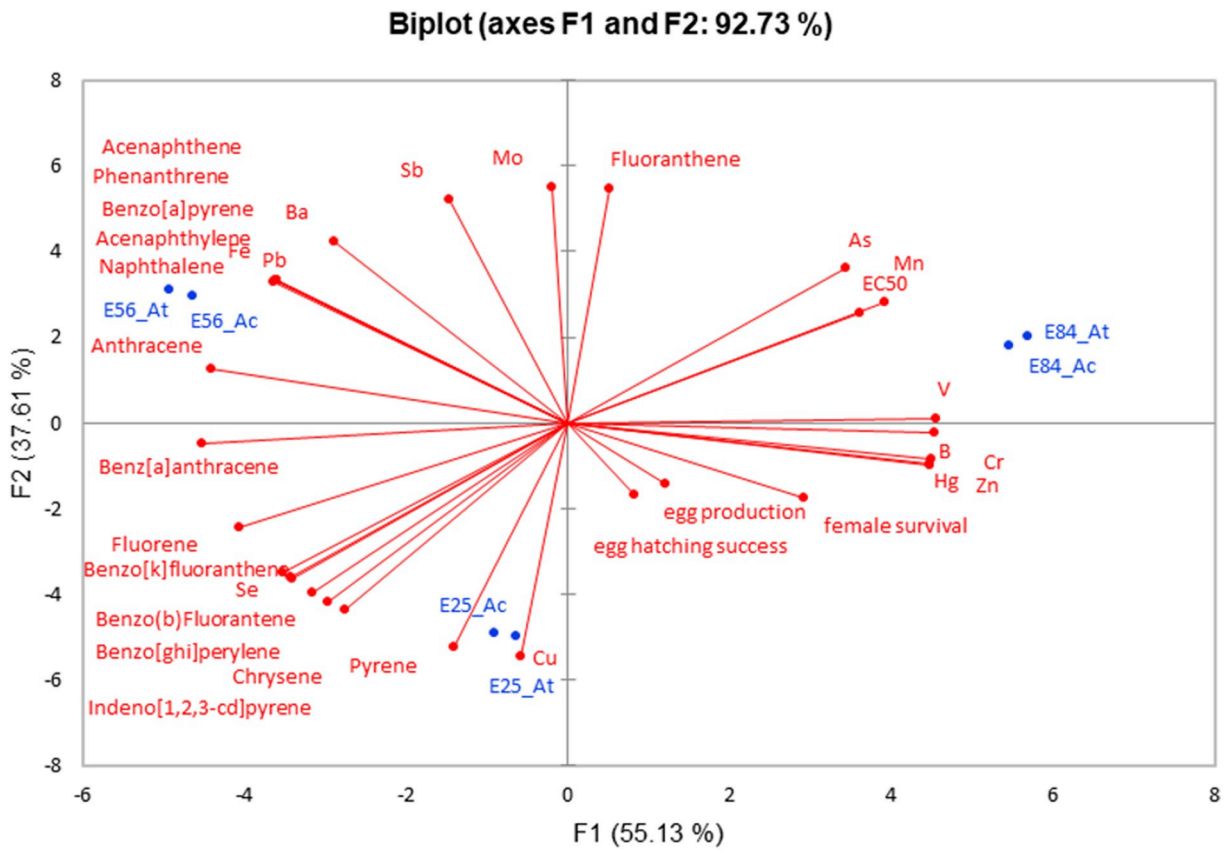

Fig. 6. Principal Component Analysis (PCA) plot considering all chemical and ecotoxicological parameters. The first two axes are shown and the percentage of variance explained is indicated. At = *Acartia tonsa*; Ac = *Acartia clausi*.

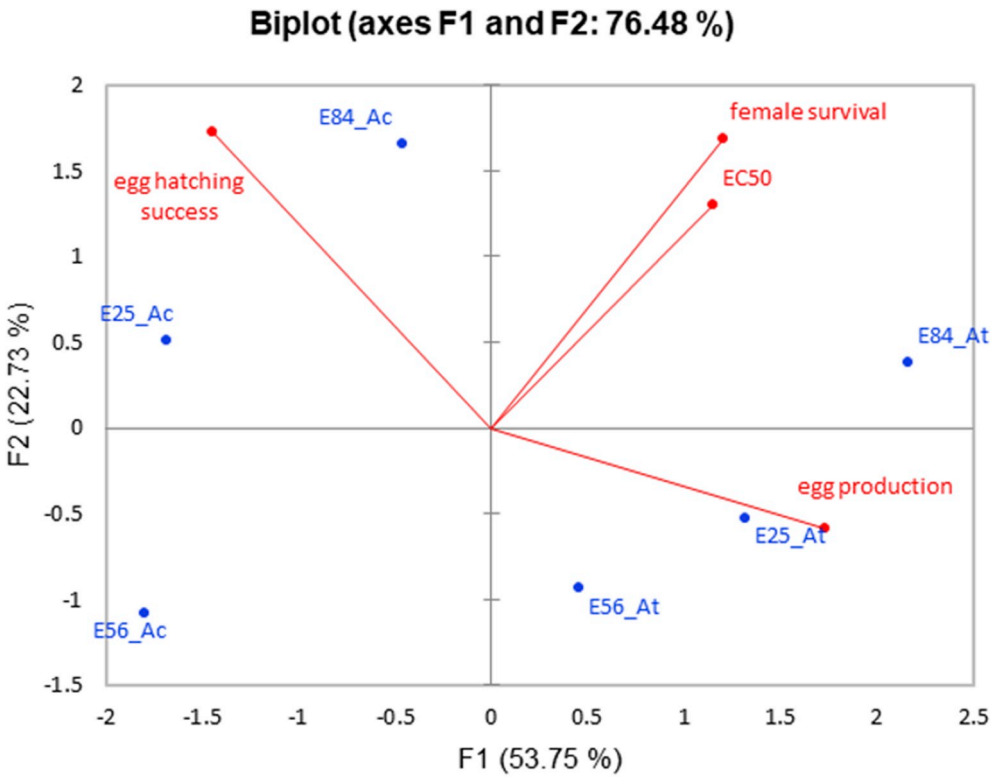

Fig. 7. Principal Component Analysis (PCA) plot considering only ecotoxicological parameters. The first two axes are shown and the percentage of variance explained is indicated. At = *Acartia tonsa*; Ac = *Acartia clausi*.

on both *Acartia* species when all endpoints were considered. However, we cannot exclude longer term effects on population recruitment or potential synergistic effects related to the high number of chemical components in the mixture. The PCA considering all chemical and ecotoxicological data, in fact, showed that the three elutriates were distributed in three different quadrants, suggesting that each sample presented a specific combination of PAHs and HMs. The stronger toxic effect of E56, hence, might be related to additive effects between PAHs and HMs.

The high level of chemicals measured in the three elutriates is in agreement with the industrial nature of the Bagnoli-Coroglio area, and well reflected the previously reported chemical composition of sediments (Ausili et al., 2012; BoI-Pr-CA-BA-relazione-02.04, 2005; Morroni et al., 2020). In particular, PAHs and metals such as Cd, Cu, Hg, Pb and Zn, have an anthropogenic origin related to the steel plant previously active in the area. However, high content of specific elements, such as As, for example, are possibly due to underwater hydrothermal activities in the seabed (Romano et al., 2018). Overall, individual values of HMs measured in the three elutriates, except mercury (Hg), were below the EQS concentrations indicated by the European Commission for surface waters (EC, 2008). In contrast, concentrations of several PAHs were well above the EQS values. In particular, naphthalene, benzo(b)- and benzo (k)fluorantene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene and benzo(g,h, i)perylene, were between 4–100x above the reference concentrations in E56 and E25 (see Table 1). These values were measured in sediment-derived elutriates and not directly in surface waters above the sediments. However, we cannot exclude that higher concentrations of PAHs and HMs would be naturally released from sediments into the water column over longer time periods, thus representing a potential threat for co-occurring herbivorous zooplankton species and other consumers in that area (Chiarore et al., 2020; Gambi et al., 2020; Hay Mele et al., 2020; Liberti et al., 2020; Ruocco et al., 2020; Santella et al., 2020; Tangherlini et al., 2020). Chronic exposure to naphthalene at concentrations between 10 and 50 μg L^{-1} and up to 2 mg L^{-1}, for example, resulted in a significant reduction in life span, egg production, numbers of nauplii produced and mean brood size of the calanoid copepods *Eurytemora affinis* (Ott et al., 1978) and *Paracartia grani* (Calbet et al., 2007). Similarly, it has been suggested that exposure to pyrene and fluoranthene at around 3 μg L^{-1} may pose a threat to *A. tonsa* populations living in industrialized estuaries (Bellas and Thor, 2007).

Overall, the convergent sensitivity of the two species to the elutriates, as also supported by the PCA analysis, confirms the robustness of the acute test based on naupliar immobilization as endpoint, in evaluating the impact of sediment-derived aqueous matrices on copepods. Additional endpoints, such as egg production and adult survival, can also add information on the long term impact of contaminants on zooplankton demography. On the other hand, to improve the ecological relevance of the test results, our findings also suggest that *A. clausi* can be used as autochthonous species more representative of the zooplankton community (Margiotta et al., 2020).

In order to perform such tests year-round, and overcome limitations related to seasonal occurrence of the local species, it would be useful to set up multi-generation cultures under laboratory-controlled conditions. Considering previous results on larval development of *A. clausi* in the presence of different algal diets (Frangóupolous et al., 2000; Sei et al., 2006), and the similarity of this species with *A. tonsa* in terms of feeding preferences, tolerance to temperature and salinity ranges, it is likely that *A. clausi* might also be amenable to long-term cultivation under controlled experimental conditions.

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.

CRediT authorship contribution statement

Ylenia Carotenuto: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Data curation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Valentina Vitiello:** Formal analysis, Resources, Writing - original draft, Writing - review & editing. **Alessandra Gallo:** Resources, Visualization, Writing - original draft, Writing - review & editing. **Giovanni Libralato:** Investigation, Formal analysis, Validation, Resources, Writing - original draft, Writing - review & editing, Visualization. **Marco Trifuoggi:** Resources, Validation, Writing - review & editing. **Maria Toscanesi:** Formal analysis, Validation, Writing - review & editing. **Giusy Lofrano:** Resources, Validation, Writing - review & editing. **Francesco Esposito:** Resources, Writing - review & editing. **Isabella Buttino:** Conceptualization, Methodology, Formal analysis, Validation, Resources, Writing - original draft, Writing - review & editing, Visualization.

Acknowledgements

This study was supported by the project ABBaCo funded by the Italian Ministry for Education, University and Research (grant number C62F16000170001).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.104878.

References

Acri, F., Aubry, F.B., Berton, A., Bianchi, F., Boldrin, A., Camatti, E., Comaschi, A., Rabitti, S., Socal, G., 2004. Plankton communities and nutrients in the Venice Lagoon: comparison between current and old data. J. Mar. Syst. 51, 321–329.

Addinsoft, 2019. XLSTAT Statistical and Data Analysis Solution. Boston, USA. https://www.xlstat.com.

Andersen, H.R., Wollenberger, L., Halling-Sorensen, B., Kusk, K.O., 2001. Development of copepod nauplii to copepodites - a parameter for chronic toxicity including endocrine disruption. Environ. Toxicol. Chem. 20, 2821–2829.

Ausili, A., Romano, E., Mumelter, A., Tornato, A., 2012. Stato dell'arte sulle bonifiche delle aree marine e di transizione interne ai SIN, SiCon 2012. Workshop su Siti Contaminati. Esperienze megli interventi di risanamento. Taormina (Italy).

Barata, C., Medina, M., Telfer, T., Baird, D.J., 2002. Determining demographic effects of cypermethrin in the marine copepod Acartia tonsa: stage-specific short tests versus life-table tests. Arch. Environ. Contam. Toxicol. 43, 373–378.

Beiras, R., Tato, T., Lopez-Ibanez, S., 2019. A 2-Tier standard method to test the toxicity of microplastics in marine water using Paracentrotus lividus and Acartia clausi larvae. Environ. Toxicol. Chem. 38, 630–637.

Bellas, J., Thor, P., 2007. Effects of selected PAHs on reproduction and survival of the calanoid copepod Acartia tonsa. Ecotoxicology 16, 465–474.

Belmonte, G., Potenza, D., 2001. Biogeography of the family Acartiidae (Calanoida) in the Ponto-Mediterranean Province. Hydrobiologia 453–454, 171–176.

Bianchi, F., Acri, F., Aubry, F.B., Berton, A., Boldrin, A., Camatti, E., Cassin, D., Comaschi, A., 2003. Can plankton communities be considered as bio-indicators of water quality in the Lagoon of Venice? Mar. Pollut. Bull. 46, 964–971.

BoI-Pr-CA-BA-relazione-02.04, 2005. Caratterizzazione ambientale dei fondali e degli arenili inclusi nella perimetrazione del sito di bonifica di Napoli Bagnoli-Coroglio. Relazione tecnica conclusiva. ICRAM-SZN. Ottobre 2005.

Buttino, I., 1994. The effect of low concentrations of phenol and ammonia on egg production rates, fecal pellet production and egg viability of the calanoid copepod Acartia clausi. Mar. Biol. 119, 629–634.

Buttino, I., Pellegrini, D., Romano, G., Hwang, J.-S., Liu, T.-M., Sartori, D., Sun, C.-K., Macchia, S., Ianora, A., 2011. Study of apoptosis induction using fluorescent and higher harmonic generation microscopy techniques in Acartia tonsa nauplii exposed to chronic concentrations of nickel. Chem. Ecol. 27, 97–104.

Buttino, I., Vitiello, V., Macchia, S., Scuderi, A., Pellegrini, D., 2018. Larval development ratio test with the calanoid copepod Acartia tonsa as a new bioassay to assess marine sediment quality. Ecotoxicol. Environ. Saf. 149, 1–9.

Calbet, A., Saiz, E., Alcaraz, M., 2002. Copepod egg production in the NW Mediterranean: effects of winter environmental conditions. Mar. Ecol. Prog. Ser. 237, 173–184.

Calbet, A., Saiz, E., Barata, C., 2007. Lethal and sublethal effects of naphthalene and 1,2-dimethylnaphthalene on the marine copepod Paracartia grani. Mar. Biol. 151, 195–204.

Calliari, D., Tiselius, P., 2005. Feeding and reproduction in a small calanoid copepod: Acartia clausi can compensate quality with quantity. Mar. Ecol. Prog. Ser. 27, 557–568.

Chiarore, A., Musco, L., Bertocci, I., Gallo, A., Cannavacciuolo, A., mutalipassi, M., Caramiello, D., Giomi, F., Fusi, M., Danovaro, R., Munari, M., 2020. Sea urchin chronicles. The effect of oxygen super-saturation and marine polluted sediments from Bagnoli-Coroglio Bay on different life stages of the sea urchin *Paracentrotus lividus*. Mar. Environ. Res. (this issue).

Comaschi, A., Acri, F., Bianchi, F., Bressan, M., Camatti, E., 2000. Temporal changes of species belonging to Acartia genus (Copepoda: Calanoida) in the Northern basin of the Venice lagoon. Boll. Mus. Civ. Stor. Nat. Venezia 50, 189–193.

DM, 31st August 2001. Ministerial Decree, Gazzetta Ufficiale Della Repubblica Italiana, Serie Generale - N, p. 250.

DM, 173/2016. Ministerial Decree, Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Supplemento ordinario alla Gazzetta Ufficiale, n. 208 6 settembre 2016. Serie generale. Regolamento recante modalità e criteri tecnici per l'autorizzazione all'immersione in mare dei materiali di escavo di fondali marini, p. 77.

EC, 2008. Directive 2008/105/EC of the European Parliament and of the Council, 16 December 2008, on Environmental Quality Standards in the Field of Water Policy.

Environmental, Climate Change, 2019. Biological Test Method: Reference Method for Determining Acute Lethality Using Acartia tonsa, p. 48. Reference Method STB 1/RM/60 June 2019.

Frangóupolous, M., Guisande, C., Maneiro, I., Riveiro, I., Franco, J., 2000. Short-term and long-term effects of the toxic dinoflagellate Alexandrium minutum on the copepod Acartia clausi. Mar. Ecol.: Prog. Ser. 203, 161–169.

Gambi, C., Dell'Anno, A., Corinaldesi, C., Lo martire, M., Musco, L., Da Ros, Z., Armiento, G., Danovaro, R., 2020. Impact of historical contamination on meiofaunal assemblages: the case study of the Bagnoli-Coroglio Bay (Southern Tyrrhenian Sea). Mar. Environ. Res. (this issue).

Gaudy, R., Cervetto, G., Pagano, M., 2000. Comparison of the metabolism of Acartia clausi and A. tonsa: influence of temperature and salinity. J. Exp. Mar. Biol. Ecol. 247, 51–65.

Gaudy, R., Moraitou Apostolopoulou, M., Pagano, M., Saint Jean, L., 1989. Salinity as a decisive factor in the length of cephalothorax of Acartia clausi from three different areas (Greece and Ivory Coast). Rapp. Comm. Int. Mer. Mediterr. 31, 233.

Gorbi, G., Invidia, M., Savorelli, F., Faraponova, O., Giacco, E., Cigar, M., Buttino, I., Leoni, T., Prato, E., Lacchetti, I., Sei, S., 2012. Standardized methods for acute and semichronic toxicity tests with the copepod Acartia tonsa. Environ. Toxicol. Chem. 31, 2023–2028.

Green, A.S., Chandler, T.G., Piegorsch, W.W., 1996. Life-stage-specific toxicity of sediment-associated chlorpyrifos to a marine, infaunal copepod. Environ. Toxicol. Chem. 15, 1182–1188.

Guisande, C., Frangopulos, M., Carotenuto, Y., Maneiro, I., Riveiro, I., Vergara, A.R., 2002. Fate of paralytic shellfish poisoning toxins ingested by the copepod Acartia clausi. Mar. Ecol.: Prog. Ser. 240, 105–115.

Hansen, B.H., Altin, D., Rørvik, S.F., Øverjordet, I.B., Olsen, A.J., Nordtug, T., 2011. Comparative study on acute effects of water accommodated fractions of an artificially weathered crude oil on Calanus finmarchicus and Calanus glacialis (Crustacea: Copepoda). Sci. Total Environ. 409, 704–709.

Hay Mele, B., Russo, L., Crocetta, F., Gambi, C., Dell'Anno, A., Danovaro, R., Guglielmo, R., Musco, L., Patti, F.P., Riginella, E., Tangherlini, M., Ribera d'Alcala', M., D'Alelio, D., 2020. Ecological assessment of anthropogenic impact in marine ecosystems: the case of Bagnoli Bay. Mar. Environ. Res. (this issue).

Ianora, A., Bastianini, M., Carotenuto, Y., Casotti, R., Roncalli, V., Miralto, A., Romano, G., Gerecht, A., Fontana, A., Turner, J.T., 2015. Non-volatile oxylipins can render some diatom blooms more toxic for copepod reproduction. Harmful Algae 44, 1–7.

ICRAM-APAT, 2007. Manuale per la movimentazione di sedimenti marini. Manual for the handling of marine sediments, p. 77. http://www.apat.gov.it/site/_files/manualeSedimentiMarini.pdf.

ISO, 1999. International Organization for Standardization. Water Quality—Determination of Acute Lethal Toxicity to Marine Copepods (Copepoda, Crustacea). Draft International Standard ISO/DIS 14669. ISO, Geneva.

ISO, 2015. International Organization for Standardization. Water Quality-Calanoid Copepod Development Test with Acartia Tonsa. ISO/FDIS 16778. Calanoid Copepod Early-Life Stage Test with Acartia Tonsa. ISO, Geneva.

Jiang, J.-L., Wang, G.-Z., Mao, M.-G., Wang, K.-J., Li, S.-J., Zeng, C.-S., 2013. Differential gene expression profile of the calanoid copepod, *Pseudodiaptomus annandalei*, in response to nickel exposure. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 157, 203–211.

Jones, R.H., Flynn, K.J., Anderson, T.R., 2002. Effect of food quality on carbon and nitrogen growth efficiency in the copepod Acartia tonsa. Mar. Ecol. Prog. Ser. 235, 147–156.

Kadiene, E.U., Bialais, C., Ouddane, B., Hwang, J.-S., Souissi, S., 2017. Differences in lethal response between male and female calanoid copepods and life cycle traits to cadmium toxicity. Ecotoxicology 26, 1227–1239.

Krause, K.E., Dinh, K.V., Nielsen, T.G., 2017. Increased tolerance to oil exposure by the cosmopolitan marine copepod Acartia tonsa. Sci. Total Environ. 607–608, 87–94.

Kusk, K., Wollenberger, L., 2007. Towards an internationally harmonized test method for reproductive and developmental effects of endocrine disrupters in marine copepods. Ecotoxicology 16, 183–195.

Kusk, K.O., Avdolli, M., Wollenberger, L., 2011. Effect of 2,4-dihydroxybenzophenone (BP1) on early life-stage development of the marine copepod Acartia tonsa at different temperatures and salinities. Environ. Toxicol. Chem. 30, 959–966.

Lauritano, C., Carotenuto, Y., Miralto, A., Procaccini, G., Ianora, A., 2012. Copepod population-specific response to a toxic diatom diet. PloS One, e47262.

Liberti, A., Bertocci, I., Pollet, A., Musco, L., Locascio, A., Ristoratore, F., Spagnuolo, A., Sordino, P., 2020. An indoor mesocosm study of the combined effect of industrial

pollution and turbulent events on the gut environment in a marine invertebrate. Mar. Environ. Res. (this issue).
Margiotta, F., Balestra, C., Buondonno, A., Casotti, R., D'Ambra, I., Di Capua, I., Gallia, R., Mazzocchi, M.G., Merquiol, L., Pepi, M., Percopo, I., Saggiomo, M., Sarno, D., Zingone, A., 2020. Do plankton reflect the environmental quality status? The case of a post-industrial Mediterranean bay. Mar. Environ. Res. (this issue).
Mazzocchi, M.G., Dubroca, L., García-Comas, C., Capua, I.D., Ribera d'Alcalà, M., 2012. Stability and resilience in coastal copepod assemblages: the case of the Mediterranean long-term ecological research at Station MC (LTER-MC). Prog. Oceanogr. 97–100, 135–151.
Medina, M., Barata, C., Telfer, T., Baird, D.J., 2002. Age- and sex-related variation in sensitivity to the pyrethroid cypermethrin in the marine copepod Acartia tonsa Dana. Arch. Environ. Contam. Toxicol. 42, 17–22.
Miller, D.D., Marcus, N.H., 1994. The effects of salinity and temperature on the density and sinking velocity of eggs of the calanoid copepod Acartia tonsa Dana. J. Exp. Mar. Biol. Ecol. 179, 235–252.
Morroni, L., d'Errico, G., Sacchi, M., Molisso, F., Armiento, G., Chiavarini, S., Rimauro, J., Guida, M., Siciliano, A., Ceparano, M.T., Aliberti, F., Gallo, A., Libralato, G., Patti, F.P., Gorbi, S., Fattorini, D., Nardi, A., Di Carlo, M., Mezzelani, M., Benedetti, M., Pellegrini, D., Musco, L., Danovaro, R., Dell'Anno, A., Regoli, F., 2020. Integrated characterization and risk management of marine sediments: the case study of the industrialized Bagnoli area (Naples, Italy). Mar. Environ. Res. (this issue).
Ott, F.S., Harris, R.P., O'Hara, S.C.M., 1978. Acute and sublethal toxicity of naphthalene and three methylated derivatives to the estuarine copepod, Eurytemora affinis. Mar. Environ. Res. 1, 49–58.
Pagano, M., Kouassi, E., Saint-Jean, L., Arfi, R., Bouvy, M., 2003. Feeding of Acartia clausi and Pseudodiaptomus hessei (Copepoda: Calanoida) on natural particles in a tropical lagoon (Ebrié, Côte d'Ivoire). Estuar. Coast Shelf Sci. 56.
Raisuddin, S., Kwok, K.W.H., Leung, K.M.Y., Schlenk, D., Lee, J.-S., 2007. The copepod Tigriopus: a promising marine model organism for ecotoxicology and environmental genomics. Aquat. Toxicol. 83, 161–173.
Rand, G.M., 1995. Fundamentals of Aquatic Toxicology : Effects, Environmental Fate, and Risk Assessment. Taylor & Francis, Washington, D.C.
Romano, E., Bergamin, L., Celia Magno, M., Pierfranceschi, G., Ausili, A., 2018. Temporal changes of metal and trace element contamination in marine sediments due to a steel plant: the case study of Bagnoli (Naples, Italy). Appl. Geochem. 88, 85–94.
Ruocco, N., Bertocci, I., Munari, M., Musco, L., Caramiello, D., Danovaro, R., Zupo, V., Costantini, M., 2020. Morphological and molecular responses of the sea urchin *Paracentrotus lividus* to highly contaminated marine sediments: the case study of Bagnoli-Coroglio brownfield (Mediterranean Sea). Mar. Environ. Res. (in press), this issue: 104865.
Santella, L., Limatola, N., Bertocci, I., Chun, J., Musco, L., Munari, M., Caramiello, D., Danovaro, R., 2020. Oxygen supersaturation mitigates the impact of the regime of contaminated sediment reworking on sea urchin fertilization process. Mar. Environ. Res. (this issue).
Sei, S., Invidia, M., Gorbi, G., 2006. Near anoxia and sulfide as possible factors influencing the spatial distribution of Acartia tonsa and Acartia clausi: comparative evaluation of egg tolerance. J. Exp. Mar. Biol. Ecol. 337, 121–130.
Seo, J.S., Lee, K.-W., Rhee, J.-S., Hwang, D.-S., Lee, Y.-M., Park, H.G., Ahn, I.-Y., Lee, J.-S., 2006. Environmental stressors (salinity, heavy metals, H2O2) modulate expression of glutathione reductase (GR) gene from the intertidal copepod Tigriopus japonicus. Aquat. Toxicol. 80, 281–289.
Støttrup, J.G., Richardson, K., Kirkegaard, E., Pihl, N.J., 1986. The cultivation of Acartia tonsa Dana for use as a live food source for marine fish larvae. Aquaculture 52, 87–96.
Tangherlini, M., Corinaldesi, C., Rastelli, E., Musco, L., Armiento, G., Danovaro, R., Dell'Anno, A., 2020. Chemical contamination can promote turnover diversity of benthic prokaryotic assemblages: the case study of the Bagnoli-Coroglio bay (Southern Tyrrhenian Sea). Mar. Environ. Res. (this issue).
Tato, T., Salgueiro-Gonzalez, N., Leon, V.M., Gonzalez, S., Beiras, R., 2018. Ecotoxicological evaluation of the risk posed by bisphenol A, triclosan, and 4-nonylphenol in coastal waters using early life stages of marine organisms (Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus, and Acartia clausi). Environ. Pollut. 232, 173–182.
UNICHIM, 2012. Metodo n. 2365. Qualità dell'acqua. Determinazione dell'inibizione della mobilità di nauplii di Acartia tonsa Dana (Crustacea:Copepoda) 24h e 48h di esposizione. Asssociazione per l'Unificazione nel Settore dell'Industria Chimica-Ente Federato UNI.
USEPA, 1991. USEPA/COE (US Army Corps of Engineers), 1991. Evaluation of Dredged Material Proposed for Ocean Disposal Testing Manual. EPA-503-508-91/001. US EPA Office of Water (WH-556F).
USEPA, 2007. Method 3535A (SW-846): Solid-phase Extraction (SPE)," Revision 1. Washington, DC. (USA).
USEPA, 2014. Method 8270D (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). Revision 5.
Vitiello, V., Zhou, C., Scuderi, A., Pellegrini, D., Buttino, I., 2016. Cold storage of Acartia tonsa eggs: a practical use in ecotoxicological studies. Ecotoxicology 25, 1033–1039.
Wang, M., Wang, G., 2010. Oxidative damage effects in the copepod *Tigriopus japonicus* Mori experimentally exposed to nickel. Ecotoxicology 19, 273–284.
Willis, K.J., Ling, N., 2003. The toxicity of emamectin benzoate, an aquaculture pesticide, to planktonic marine copepods. Aquaculture 221, 289–297.
Willis, K.J., Ling, N., 2004. Toxicity of the aquaculture pesticide cypermethrin to planktonic marine copepods. Aquacult. Res. 35, 263–270.
Zhang, J., Wu, C., Pellegrini, D., Romano, G., Esposito, F., Ianora, A., Buttino, I., 2013. Effects of different monoalgal diets on egg production, hatching success and apoptosis induction in a Mediterranean population of the calanoid copepod Acartia tonsa (Dana). Aquaculture 400–401, 65–72.
Zhou, C., Carotenuto, Y., Vitiello, V., Wu, C., Zhang, J., Buttino, I., 2018. De novo transcriptome assembly and differential gene expression analysis of the calanoid copepod Acartia tonsa exposed to nickel nanoparticles. Chemosphere 209, 163–172.
Zhou, C., Vitiello, V., Casals, E., Puntes, V., Iamunno, F., Pellegrini, D., Changwen, W., Benvenuto, G., Buttino, I., 2016b. Toxicity of nickel in the marine calanoid copepod Acartia tonsa: nickel chloride versus nanoparticles. Aquat. Toxicol. 170, 1–12.
Zhou, C., Vitiello, V., Pellegrini, D., Wu, C., Morelli, E., Buttino, I., 2016a. Toxicological effects of CdSe/ZnS quantum dots on marine planktonic organisms. Ecotoxicol. Environ. Saf. 123, 26–31.