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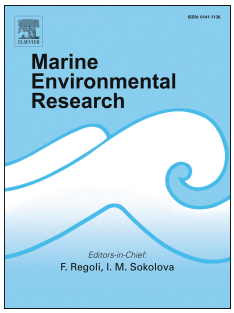

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PII: S0141-1136(18)30271-X

DOI: 10.1016/j.marenvres.2018.06.003

Reference: MERE 4539

To appear in: *Marine Environmental Research*

Received Date: 2 April 2018

Revised Date: 29 May 2018

Accepted Date: 4 June 2018

Please cite this article as: Moreira, A., Figueira, E., Libralato, G., Soares, A.M.V.M., Guida, M., Freitas, R., Comparative sensitivity of *Crassostrea angulata* and *Crassostrea gigas* embryo-larval development to As under varying salinity and temperature, *Marine Environmental Research* (2018), doi: 10.1016/j.marenvres.2018.06.003.

Comparative sensitivity of *Crassostrea angulata* and *Crassostrea gigas* embryo-larval development to As under varying salinity and temperature

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Abstract

Oysters are a diverse group of marine bivalves that inhabit coastal systems of the world's oceans, providing a variety of ecosystem services and represent a major socioeconomic resource. However, oyster reefs have become inevitably impacted from habitat destruction, overfishing, pollution and disease outbreaks that have pushed these structures to the break of extinction. In addition, the increased frequency of climate change related events promise to further challenge oyster species survival worldwide.

Oysters' early embryonic development is likely the most vulnerable stage to climate change related stressors (e.g. salinity and temperature shifts) as well as to pollutants (e.g. arsenic), and therefore can represent the most important bottleneck that define populations' survival in a changing environment. In light of this, the present study aimed to assess two important oyster species, *Crassostrea angulata* and *Crassostrea gigas* embryo-larval development, under combinations of salinity (20, 26 and 33), temperature (20, 24 and 28 ºC) and arsenic (As) exposure (0, 30, 60, 120, 240, 480, 960 and 1920 µg. As L^{-1}), to infer on different oyster species capacity to cope with these environmental stressors under the eminent threat of climate change and increase of pollution worldwide.

Results showed differences in each species range of salinity and temperature for successful embryonic development. For *C angulata*, embryo-larval development was successful at a narrower range of both salinity and temperature, compared to *C. gigas.*

Overall, As induced higher toxicity to *C. angulata* embryos, with calculated EC50 values at least an order of magnitude lower than those calculated for *C. gigas*.

The toxicity of As (measured as median effective concentration, EC50) showed to be influenced by both salinity and temperature in both species. Nonetheless, salinity

had a greater influence on embryos' sensitivity to As. This pattern was mostly noticed for *C. gigas*, with lower salinity inducing higher sensitivity to As. Results were discussed considering the existing literature and suggest that *C. angulata* populations are likely to become more vulnerable under near future predictions for temperature rise, salinity shifts and pollution.

Keywords: Arsenic, climate change, development, embryotoxicity, oyster, thermohaline.

1. INTRODUCTION

For centuries, oyster reefs formed the dominant structural habitat in temperate estuarine systems of the world. Such biogenic features are formed by one or few oyster species that provide the physical structure for entire ecosystems (Beck et al., 2009). Oyster reefs render a variety of ecosystem services, including the formation of habitat structure for commercially important fish species, water quality improvement, shoreline defence against coastal erosion and carbon dioxide storage (Coen et al., 2007; Ridge et al., 2017; Fodrie et al., 2017; Grabowski et al., 2012). Currently, these ecosystems are globally threatened, and the majority are classified as functionally extinct (Beck et al., 2011).

The main factors affecting the loss of important oyster reefs around the globe include overexploitation and habitat degradation (Cranfield et al., 1999; Leniham and Peterson, 1998), pollution (Ruano, 1997), exotic species introduction (Miossec et al., 2009; Ruesink et al., 2005), and disease outbreaks (Virvilis and Angelidis, 2006; Comps et al., 1988). In addition, climate change related impacts (i.e. temperature rise, seawater acidification and salinity regime alterations) will further challenge oyster species survival worldwide (Brander, 2007; Dekshenieks et al., 2000; Hoehg-Guldberg and Bruno, 2010; Knight, 2017; Levinton et al., 2011). The comprehension of the factors influencing oysters' environmental sensitivity can increase the background knowledge and the possibility to better protect and manage this important biological resource.

According to FAO (2015), oysters form an important global aquaculture shellfish resource. Particularly, *Crassostrea gigas* (Thunberg, 1793) a native species to Japan, is nowadays virtually present in coastal systems of the entire

93 planet (Miossec et al., 2009). In Europe, *C. gigas* is cultured in several
94 countries, with France leading *C. gigas* production and consumption statistics
95 (Heral, 1989; Buestel et al., 2009). However, wild populations have become
96 naturalized and established in several European countries, and reports on *C.*
97 *gigas* natural occurrences stretch from Norway to southern Portugal (Miossec et
98 al., 2009 and references therein).

99 Another important species contributing for global oyster fishery
100 landings, *Crassostrea angulata* (Lamarck, 1819), is the main species cultured in
101 southern China (Hsiao et al., 2016; Qin et al., 2012), also occurring in southern
102 Europe (Batista et al., 2015) and North Africa (Fabioux et al., 2002). At present,
103 the geographical distribution of *C. angulata* in Europe is limited to the Iberian
104 Peninsula (Buestel et al., 2009; Batista et al. 2015), where the most pristine
105 population naturally occurs in the Sado estuary (Portugal), making it an
106 important biological resource to be protected.

107 Under the increasing pressure of climate change related phenomena to
108 estuarine systems worldwide, the combined effects of stressors such as
109 temperature rise, salinity alterations and pollution (Harley et al., 2006; Lejeusne
110 et al., 2010; Robins et al., 2016), are likely to increase the selective pressure on
111 oyster species in the environment. Although adult *C. angulata* and *C. gigas* can
112 be fairly resilient to fluctuations of abiotic factors and to pollutants (Cross et al.,
113 2014; Moreira et al., 2016a,b; Zanette et al., 2011), early developmental stages
114 are more susceptible to physico-chemical environmental changes compared to
115 juveniles and adults (Beiras and His, 1994; His et al., 1999; MacInnes and
116 Calabrese, 1978; Ringwood, 1990). Thus, they represent the bottleneck that
117 define oyster species resilience to environmental change (Byrne et al., 2012),

and therefore are important to be studied to infer on different species sensitivities in a changing environment.

The aim of the present study was to compare the sensitivities of *C. angulata* and *C. gigas* embryo-larval development, considering various exposure scenarios including changes in arsenic (As) concentration, salinity and temperature. For this, *C. angulata* from the Sado estuary and *C. gigas* from Guernsey Island embryo-larval development were studied under different combinations of salinity and temperature to assess possible impacts of climate driven alterations, and the combined effect of these conditions with As. Arsenic was chosen as reference stressor due to: a) its toxicity to oyster embryos is strongly influenced by salinity and temperature variations (Moreira et al., 2018); b) its ubiquity in estuarine ecosystems (Neff, 2002) and tendency to remobilize from sediment (De Gieter et al., 2005; Ereira et al., 2015; Masson et al., 2007); and c) its propensity to increase in concentration from climatic events (Henke, 2009; Galloway et al., 2017). Arsenic concentrations in estuarine systems are therefore highly dynamic (Henke, 2009). For instance Ereira et al (2015) reported As concentrations of 5.2 μg L^{-1} in seawater, 20 mg kg^{-1} in sediment and 260 μg kg^{-1} in suspended particulate matter, evidencing the exposure of relatively high As concentrations to resident biota.

Embryotoxicity tests on *C. angulata* and *C. gigas*, were carried out considering a range of As concentrations (30, 60, 120, 240, 480, 960 and 1920 μg L^{-1}), different salinity (20, 26 and 33) and temperature (20, 24 and 28 ºC) levels, as well as different time of exposure (24 and 48 h) to investigate: i) the effect of varying salinity and temperature on both species embryo-larval

development; ii) As embryotoxicity to both species; and iii) the effects of varying salinity and temperature on embryos sensitivity to As.

2. METHODS

2.1 Experimental setup

Experiments were conducted separately for each species, using previously cleaned and sterilised glassware for the entire experimental setup. Analytical grade artificial seawater (Tropic Marine Sea Salt) from the same batch was used for exposure media preparation and spawning, prepared according to the manufacturer's instructions using reverse osmosis (RO) water, 3 days before the experiments took place too achieve a salinity of 33 (i.e reference salinity) (Leverett and Thain, 2013). After complete salt dissolution and equilibration (24 h) seawater was filtered (0.2 µm) through cellulose acetate filters (Millipore) using a vacuum filtration unit. Seawater salinity was adjusted to obtain 3 separate batches at three different salinity levels (20, 26 and 33 ± 1) for exposure media preparation using RO water as dilution media (measured pH for all batches of seawater used ranged from 8.16 – 8.29 (Hanna-Instruments)). Salinity levels were in accordance to Moreira et al. (2018), that showed As toxicity to *C. gigas* embryos could restrict embryo-larval development within 20-33 of salinity.

A stock solution of sodium arsenate (Na_3AsO_4) (CAS no. 10048-95-0, Sigma-Aldrich, Missouri, USA) was prepared in ultra-pure water and spiked in separate volumetric vessels to achieve nominal As concentrations of 30, 60, 120, 240, 480, 960 and 1920 µg L^{-1}, for each target salinity. Exposure concentrations were chosen based on previous studies on *C. gigas* embryonic

development and As exposure (Martin et al., 1981; Mamindy-Pajany et al., 2016). Exposure solutions were distributed in 24-well sterile capped polystyrene microplates (VWR), giving one microplate per salinity level, 3 wells (3 ml each) per exposure condition (As concentration) including negative controls. Each microplate corresponding to salinity and As conditions, were 3 fold replicated for incubations at different temperatures (20, 24 and 28 ºC) to test two levels of 4 ºC increase of temperature within projected global surface temperature rise by the end of the 21st century relative to years (1986-2005) (RCP8.5) (IPCC, 2014), while testing a temperature range that would allow to infer on embryo development under As exposure based on previous data (Moreira et al., 2018). All the above stated microplates were further replicated twice, to test all conditions after two different timeframes of embryonic development, to allow for the assessment of As induced delayed development to oyster embryos (Moreira et al., 2018), while testing valid exposure time criteria of 24 h (His et al., 1999) and 48 h (Knezovich et al., 1981) for *C. gigas*. Each microplate was incubated at the desired temperature overnight in separate climatic chambers (±1 ºC) before spawning induction took place to stabilize testing media at the target temperature. Copper ($Cu(NO_3)_2$) was used as reference toxicant (positive control) (Libralato et at., 2009) at 3, 6, 12, 18 and, 30 µg L^{-1} of Cu, for which incubations took place at standard salinity 33 and temperature 24 ºC.

Each stock of seawater used for the embryotoxicity assays (at every combination of salinity and As concentration) were analysed by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) to determine effective As concentrations for each condition. Multi-Element Standard IV - 71A (Inorganic Ventures) was used as standard for As quantification, and calibration curve

192 verified with standard reference material (NIST SRM 1643f) (Supplementary
193 table S6).

194 2.2 Spawning and fertilization

195 *C. gigas* progenitors were obtained from Guernsey Sea Farms (UK), and
196 *C. angulata* progenitors were provided by an aquaculture facility operating in the
197 Sado estuary (Exporsado). *C. angulata* were collected during spawning season,
198 from intertidal growing tables, and stimulated to spawn one day after collection.
199 Spawning was induced by thermal stimulation, by consecutively changing
200 oysters from seawater baths set at 18 and 28 ºC in 30 min consecutive intervals
201 (Libralato et al., 2007). Gamete emission and quality (oocyte shape and sperm
202 motility) were visually inspected under a microscope. The number of male and
203 female oysters selected for fertilization were 4 and 3 (*C. gigas*) and 6 and 5 (*C.*
204 *angulata*), respectively.

205 Selected females were left to spawn in separate beakers, oocyte
206 suspensions were filtered through a 100 μm nylon mesh and mixed in a final
207 500 ml suspension (salinity 33, 24 ºC). Male gametes were collected in
208 separate, filtered through a 45 μm nylon mesh into a mixed suspension, and left
209 to activate for 20 min. Oocyte suspensions were fertilized by adding
210 approximately 1 to 10^6 oocyte-to-sperm ratios, and fertilization success verified
211 by microscopy.

212 Zygotes were immediately transferred to microplates containing the
213 exposure media to reach approximately 200 embryos per well. Microplates with
214 the exposure media (0, 30, 60, 120, 240, 480, 960 and 1920 μg L^{-1} of As) at
215 different salinities (20, 26, 33) and fertilized oocytes were left to incubate in the
216 dark, at different temperatures (20, 24 and 28 ±1 ºC) for 24 and 48 h post

fertilization. Embryo-larval development was stopped by adding buffered formalin (4%). Analysis followed visual inspection of embryo-larval development under an inverted microscope and camera (Leica: DMIL-1; MC170 HD) by counting 100 embryos per well, and characterizing the relative frequencies of different types of development (D-shape, pre-D, protruded mantle, kidney shape, indented shell and dead larvae) according to His et al. (1997) (Fig. 1).

The zygote suspensions from each species that remained unused from embryotoxicity assays were analysed for trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Sn and Zn), following an adaptation of Weng and Wang (2017) protocol as follows. Zygote suspensions of each species were centrifuged at 3000 *g* for 20 min (4 ºC) followed by pellet collection. The pellets consisting of each species zygotes were freeze dried, and posteriorly digested overnight with analytical grade nitric acid and hydrogen peroxide (30% w/w) (Chem-Lab NV), alongside with TORT-3 (Lobster Hepatopancreas Reference Material for Trace Metals, NRC Canada) for analytical control by elements recovery percentages calculation. The digested samples were analysed for As, Cd, Cr, Cu, Hg, Ni, Sn and Zn by ICP-MS, with IV- 71A (Inorganic Ventures) as standard, and calibration curves verified with standard reference material (NIST SRM 1643f), calculated measure of trueness over 90%. Recovery percentages based on TORT-3 reference values varied between 93.7 and 111.6%.

2.3 Data analysis

Results obtained were analysed as percentages of abnormal larvae (including pre-D and other malformations) according to His et al. (1999), and the validity of the experiments verified by results for negative and positive controls,

242 considering the acceptability ranges proposed by ASTM (2004) (>70% D-shape
243 larvae) for negative controls, and Libralato et al. (2009) (9.47 µg. L^{-1} ≤ EC50 ≤
244 21.72 µg. L^{-1}) for positive controls.

245 To represent embryo-larval development under different salinity and
246 temperature levels, frequencies of malformed larvae observed at different
247 salinities and temperature (negative controls), after 24 and 48 h development
248 were represented in Contour plots using SigmaPlot version 11.0.

249 To assess As embryotoxicity, data from As exposures were corrected for
250 the effects observed in the respective negative control (salinity and
251 temperature) using the Abbott's formula (ASTM, 2004). Data were submitted to
252 non-linear regression analysis using GraphPad Prism version 6.01. Effective As
253 concentrations retrieved from ICP-MS for each condition were used for toxicity
254 calculations, after lognormal transformation. The best fit dose-response curve
255 values allowed to estimate the median effect concentrations (EC50s) and
256 respective 95% confidence intervals for conditions where larvae development
257 was successful. To test the effects of salinity and temperature on embryos
258 sensitivities to As, the following null hypotheses were tested: i) H_0': no
259 differences existed among EC50s at different salinity levels considering fixed
260 temperature; ii) H_0'': no differences existed among EC50s at different
261 temperature levels considering fixed salinity; iii) H_0''': no differences existed
262 between EC50s at 24 and 48 h for each combination of salinity and
263 temperature. To test for differences among EC50 values between conditions,
264 analysis of variance was performed using one-way ANOVA, followed by
265 Tukey's multiple comparisons tests, based on values of Log(EC50) and the
266 associated standard error returned by the software for each estimated curve

(GraphPad Prism version 6.01). EC50 values are provided for each species in separate graphs showing the relative 95% confidence intervals, and significant differences (*p*-values ≤ 0.05) were represented with different letters: lower-case letters for comparisons of EC50s at different temperature and different salinities after 24 h exposure; upper-case letters for comparisons of EC50s at different temperature levels and different salinities, after 48 h exposure; asterisks (*) when statistical differences between 24 h and 48 h exposures at each fixed combination of salinity and temperature were observed.

3. RESULTS and DISCUSSION

3.1 Embryonic development, salinity and temperature

Crassostrea angulata

C. angulata embryo-larval development under different combinations of salinity and temperature are depicted in Fig. 2A and B (24 and 48 h post fertilization respectively). Contour plots show that *C. angulata* presented low frequencies of malformed larvae (<20%) at intermediate and high salinity levels (26 and 32) and temperatures of 24 and 28 ºC, after 24 h development (Fig. 2B). On the other hand, *C. angulata* presented high frequencies of malformed larvae at every salinity level combined to low temperature (20 ºC) (100% identified as Pre-D) after 24 h development (Fig. 2A). At higher temperatures and low salinity (20), results showed higher percentages of malformed larvae of 90% (28 ºC) and 75% (24 ºC).

Studies concerning this species teratogenesis date back to the 19th century, when the first trials on artificial fecundation of *C. angulata* were described for full salinity and 22 ºC (Bouchon-Brandely, 1882), however scarce

292 information is available on the effects of varying salinity and temperature on this
293 species embryonic development in laboratory conditions. Nonetheless, early
294 studies by Amemiya (1926) found that the lowest limit of salinity for *C. angulata*
295 embryonic development was 21, and optimum salinity to be between 28 and 35
296 (studies performed at 20-23,5 ºC, 24 h). Similarly, our data showed that low
297 salinity (20) induced poor embryonic development (75-100% malformations) at
298 all tested temperatures, and malformation frequency decreased with the
299 increase of salinity.

300 After 48 h of embryo-larval development, *C. angulata* showed lower
301 frequencies of malformed larvae at the intermediate salinity (26) at all
302 temperature levels (Fig. 2B), while relatively higher malformation percentages
303 were observed at the highest salinity (33), at 26 and 28 ºC at 48 h, compared to
304 results obtained at 24 h (Fig. 2A).

305 The relative increase of the range of temperature and salinity at which
306 embryos successfully developed into D-shape larvae observed between 24 and
307 48 h, reflected differences in developing rates that depend on the surrounding
308 media characteristics, namely temperature and salinity. Because increasing the
309 time of exposure allowed for embryo development completion, at suboptimal
310 temperature and salinity conditions. Both these environmental factors affect
311 oyster embryo development rate. For instance, studies on *Pinctata imbricata*
312 pearl oysters from the tropical Atlantic (Urban, 2000) showed that suboptimal
313 temperature and salinity delayed embryonic development (O'Connor and
314 Lawler, 2004). This delayed effect is also observed in later stages, namely D-
315 shape larvae of *C. angulata* (Thiyagarajan and Ko, 2012), *C. gigas* (His et al.,
316 1989) and *P. imbricata* (Dove and O'Connor et al. 2007). Moreover, data

obtained in the present study show that intermediate salinity (26) is likely closest to the optimum salinity for this species under laboratory conditions, the salinity at which we observed the widest thermal tolerance range (Le Dantec, 1868) (most noticed after 48 h development). This is in accordance with studies by Thiyagarajan and Ko, (2012) that described optimum salinity for *C. angulata* D-shape larvae development between 24 and 27. Also in line with environmental studies showing higher survival rates of *C. angulata* larvae at temperatures over 22 ºC and salinities between 28 and 32 (Le Dantec, 1868).

Crassostrea gigas

C. gigas embryo-larval development under different salinity and temperature combinations is depicted in Figure 2C and D (24 and 48 h respectively). Contour plots show that *C. gigas* presented low frequencies of malformed larvae (<30%) at all combinations of salinity and temperature after 24 h development, except for embryos exposed to low salinity (20) and low temperature (20 ºC), where high percentages of malformed larvae were observed (100% identified as pre-D, supplementary table S2) (Fig. 2C).

Previous studies showed low percentages of D-shape *C. gigas* at 20 ºC and full salinity (Gamain et al., 2017) (40% D-shape larvae after 24 h post fertilization), or even complete arrest of development at salinities ranging from 15-32 at 20 ºC (100% trochophore stage at 24 h) (Moreira et al., 2018).

C. gigas embryos exposed to the same conditions for 48 h, showed high frequencies of developing D-shape larvae at all combinations of salinity and temperature (<35% malformed larvae), including low salinity (20) and low temperature (20 ºC) (Fig. 2D).

These results indicate some degree of variability in the developing rate of *C. gigas* at suboptimal temperature (20 ºC) and are likely related to differences in development rates at suboptimal temperature. This is corroborated by results obtained at 48 h post-fertilization, for which high frequencies of D-shape larvae were observed in the present study, and in accordance with results from Parker et al. (2010) that also observed high rates of embryonic development at 48 h and low temperature (20 ºC). Differences in methodology during the embryo-larval assay, namely the origin of seawater source (His et al., 1997; Beiras and His, 2004), as well as phenotypic plasticity and genetic variability (Pace et al., 2006; Taris et al., 2006 and references therein), could explain the differences observed between our findings and some of the results reported in literature.

Comparison between species

Differences between species successful embryonic development under different combinations of salinity and temperature were evident, with *C. angulata* embryos presenting a narrower range of salinity and temperature for complete development than *C. gigas*. Implications of such findings under the eminence of climate change could mean differentiated resilience capacities between these species to cope with near future shifts in salinity and temperature regimes, and possibly constrain *C. angulata* populations' geographical distribution comparing to that of *C. gigas*.

3.2 Arsenic embryotoxicity

Results obtained from the embryotoxicity assay for each species showed that negative controls for both species were within the accepted values

described for *Crassostrea gigas* embryotoxicity standard protocols (ASTM, 2004; His et al., 1999), with frequencies of normal developed larvae (D-shape) after 24 h development exceeding 70%, at standard salinity (33) and temperature (24 ºC) for both species (*C. gigas* 75% and *C. angulata* 90%). Moreover, results obtained regarding positive controls (Cu) were within previously reported values (9.47 µg. L^{-1} ≤ EC50 ≤ 21.72 µg. L^{-1}) (Libralato et al., 2009), and were similar for both species (10.07 (8.98-1.30) µg L^{-1} of Cu for *C. angulata* and 11.40 (9.59-13.54) µg L^{-1} of Cu for *C. gigas*. These results confirm the validity of the experiment and show that the sensitivity of both species to Cu were identical.

Crassostrea angulata

As toxicity measured in terms of EC50 considering standard toxicity assay conditions with oyster embryos (24 ºC, 33 salinity, 24 h) for *C. angulata* was 39.2 µg L^{-1} As (18.7 µg L^{-1} As at 48 h) (Table I). To our knowledge, this is the first study on the effects of contaminants to *C. angulata* embryonic development. The toxicity of As (EC50) measured in the present study for *C. angulata* was in the lower range of previously reported toxicity threshold values for related oyster species such as *Crassostrea virginica* (with LC50 of 7.2 mg L^{-1}) (Calabrese et al., 1973) and *C. gigas* (EC50 ranging from ca. 1 to 920 µg L^{-1} of As) (Moreira et al., 2018 and Mamindy-Pajany et al., 2013).

Crassostrea gigas

EC50 considering standard conditions for *C. gigas* (24 ºC, 33 salinity, 24 h) of 452 µg L^{-1} As (663.5 µg L^{-1} As at 48 h) (Table I), was within previously

392 reported EC50 values (920 µg L^{-1} As from Mamindy-Pajany et al. (2013) and
393 326 µg L^{-1} As from Martin et al. (1981)). Moreover, in a previous study (Moreira
394 et al., 2018) As toxicity determined at standard salinity and temperature assay
395 conditions revealed an EC50 lower than 1 µg L^{-1} As.

396 Among the few available data on As toxicity to bivalve embryos, a great
397 variability is observed, in contrast to other types of pollutants (His et al., 1999).
398 These discrepancies could be related to different reference seawater
399 characteristics (Beiras and Albentosa, 2004), as observed in a previous study
400 (Moreira et al., 2018), or different methodological approaches (Martin et al.
401 (1981), that used different incubation temperature (20 ºC), different time (48 h)
402 and As form AsO_5, no reference to real concentrations (Mamindy-Pajany et al.,
403 2013).

404

405 ## Comparisons between species

406 Our results suggest that *C. angulata* is likely more sensitive to As than
407 *C. gigas*, with at least an order of magnitude lower EC50 values (Table I),
408 despite that positive control for Cu toxicity showed similar results for both
409 species, indicating sensitivity to As should be different between species, and
410 was not related to batch's overall sensitivity (confirmed by results obtained for
411 Cu). Several factors could be hypothesised to have influenced these results, but
412 the most important one could be a differentiated species-specific sensitivity to
413 As.

414 Indeed, species specific tolerance to As seemed to be the main reason
415 explaining the differentiated toxicity observed. Moreover, these results are
416 supported by other studies, namely those that showed taxon related differences

417 between *C. angulata* (Spanish origin) and *C. gigas* (French origin), regarding
418 each progenies' maturation traits as well as mortality (Soletchnik et al., 2002).
419 Also, Huvet et al., (2002) suggested that *C. gigas* could present overall better
420 gamete quality than *C. angulata*, while studying hybrid crosses between both
421 species. To our knowledge this is the first study on *C. angulata* embryotoxicity,
422 and our data add to the body of evidence that show differences in
423 ecophysiological traits between these closely related species (Soletchnik et al.,
424 2002; His et al., 1972; Goulletquer et al., 1999; Heral, 1996; Moreira et al.,
425 2016).

426 Other factors such as broodstock origin or parental exposure history
427 could also have influenced each species sensitivity to As but were unlikely
428 given our results and the existing literature as follows.

429 Gamain and co-workers (2017) studied *C. gigas* from different origins
430 (hatchery, cultivated and wild) and found no major differences in embryotoxicity
431 of metolachlor to embryos of different parental origins. However, and in contrast
432 with our study, progeny from hatchery oysters were more sensitive to Cu than
433 wild and cultivated ones. Considering this, it would be unlikely that oysters
434 (hatchery *C. gigas*, and cultivated *C. angulata*) from our study, would have
435 presented the observed differentiated response to As, solely based on
436 broodstock origin (hatchery and cultivated).

437 A recent study by Weng and Wang (2017) demonstrated maternal
438 transfer of trace metals in *Crassostrea hongkongensis* adults to their progeny
439 (oocytes and larvae) reflecting parental exposure from contaminated sites, with
440 negative impacts on the most contaminated embryos' development. Therefore,
441 parental transfer of trace elements to zygotes in *C. angulata* could have

influenced final As toxicity, knowing that the Sado estuary is anthropogenically polluted (Costa et al., 2009). However, the results obtained in the present study on trace element content in fertilized oocytes showed only marginal differences in trace element concentrations between species, except for Cr and Ni (higher in *C. angulata*) and Zn (higher in *C. gigas*) (Table II). Trace element content from both *C. angulata* and *C. gigas* were in the lower range of those described by Weng and Wang (2017) for *C. hongkongensis* embryos, considering the lowest reported values (lowest contaminated sites) for As, Cd, Cr, Cu, Ni and Zn (4.26 ± 0.83; 0.21 ± 0.11; 0.71 ± 0.39; 45.3 ± 21.0; 1,42 ± 1.03; 300 ± 87.8 μg g^{-1} dry weight, respectively). Hence, in the present study, parental element transfer to embryos was not likely to have influenced final As toxicity in either species, given that we observed overall low contamination, and few differences in element concentrations in embryos between species.

3.3 Effects of salinity and temperature on species sensitivity to As

Crassostrea angulata

EC50 values determined for *C. angulata* at all tested combinations of salinity and temperature levels, at both 24 and 48 h of exposure are depicted in Fig. 3. For *C. angulata*, EC50 was not possible to calculate at the lowest salinity (20), regardless of temperature and duration of exposure. Moreover, at higher salinities and 20 ºC, EC50 was only possible to determine for the intermediate salinity (26) and only at 48 h development (Fig. 3). These findings reflected the observed thermohaline tolerance of this species, with overall lower toxicity at optimum salinity (26).

To assess the effects of salinity on embryo sensitivity to As, pairwise comparisons among EC50 values obtained at each salinity were performed considering fixed temperature. Pairwise comparisons showed that at 24 h, EC50 values between salinities were significantly different only at 24 ºC, for which EC50 was higher at salinity 33 than at salinity 26. On the other hand, results for *C. angulata* embryos exposed for 48 h, showed significantly higher EC50 values at salinity 26 at both compared temperature levels (24 and 28 ºC) (Fig. 3). These findings illustrate that different salinities as well as time of exposure altered the sensitivity of embryos to As.

Salinity can influence oyster embryo development rate, by increasing the rate with the increase of salinity (O'Connor and Lawler, 2004), and therefore the retardation effect of As previously described (Moreira et al., 2018) at intermediate salinity (26) could be more noticeable than at higher salinity (33), as observed after 24 h development. However, these differences were counteracted after 48 h exposure, for which significantly higher EC50 values were observed at salinity 26 comparing to salinity 33, for both comparable temperature levels (24 and 28 ºC). These results, showed that extending the exposure period from 24 to 48 h had a beneficial effect at salinity 26 (significant increase of EC50 at 24 ºC), corroborating the above stated hypothesis that developing rates influenced final As toxicity. On the other hand, sensitivity to As (lower EC50) increased significantly at higher salinity (33) comparing 24 and 48 h exposures, likely resulting from poor physiological status at higher salinities (Thiyagarajan and Ko, 2012), also supported by results obtained in negative controls at the same conditions for which we observed relatively high frequencies of dead larvae (data not shown).

To evaluate the effect of temperature on embryos sensitivity to As, pairwise comparisons between EC50 values obtained at different temperatures, within each salinity level were performed. Results showed significant increases of EC50s with the increase of temperature (24 and 28 ºC) at salinity 26 for both 24 and 48 h exposures. At salinity 33 no significant differences were observed between temperature levels, at either 24 and 48 h exposures (Fig 3).

Studies on the effects of temperature on the toxicity of pollutants to bivalve embryos, have reported contrasting results. For instance, increased toxicity with the increase of temperature has been shown for Cu and Ag (Boukadida et al. (2016), and for Pb (Hrs-Brecko, 1977) in *Mytillus galloprovincialis*. However, studies on *C. virginica* showed that Cu was more toxic at lower temperatures, presumably because closer to suboptimal temperature conditions would induce higher toxicity (MacInnes and Calabrese, 1979). Assuming that higher temperature increases the rate of chemical reactions in the media, the uptake of contaminants through biological membranes can be promoted at higher temperatures (Hazel, 1997), hence toxicity could be expected to increase at higher temperatures.

In the present study results obtained for *C. angulata* at optimum salinity (26) and both exposure periods (24 and 48 h) showed the opposite trend, for which the effect of increasing temperature resulted in lower As toxicity. These results were likely related to a counteractive effect of increased rates of embryo development at higher temperatures, with the retarding effect of As (Moreira et al., 2018), thus resulting in higher EC50s at higher temperatures.

517 *Crassostrea gigas*

518 Median effect concentrations (EC50's) of As under different salinity and
519 temperature conditions after 24 and 48 h incubation for *C. gigas* are presented
520 in Fig. 4. Results obtained show a clear increase of EC50's with the increase of
521 salinity, considering both 24 and 48 h larval development, considering fixed
522 temperature levels (Fig. 4).

523 These results are in line with previous studies using *C. gigas* exposed
524 to As (Moreira et al., 2018), Ag and Cu (Coglianese, 1982), Cu and metalochlor
525 (Gamain et al., 2016) at different salinities. The increased sensitivity to As
526 (lower EC50s) with the decrease of salinity is likely related to different
527 osmoregulation status of oyster embryos in response to lower salinities. Under
528 hypoosmotic conditions, the ion flux between the embryos and surrounding
529 media may increase, inducing higher uptake of soluble contaminant through
530 active transport processes (Connel, 1989; Grosell et al., 2007). Another factor
531 contributing to higher sensitivity to As at lower salinities, could be related to
532 differences in As speciation with salinity variation, however we discarded such
533 possibility, because under a similar range of salinity (and temperature)
534 conditions, As speciation analysis showed no important differences in prevailing
535 As species and bioavailability with varying salinity and temperature (Moreira et
536 al., 2018).

537 The effect of varying temperature on *C. gigas* embryos' sensitivity to As,
538 inferred by pairwise comparisons of EC50 values among temperature levels,
539 were variable within each salinity level and incubation period (Fig. 4). At the
540 lowest salinity (20), no significant differences were observed among EC50's
541 obtained for all temperature levels (at both 24 and 48 h). At salinity 26, EC50

542 was significantly lower at 20 ºC, comparing to the remaining temperature levels,
543 after 24 h incubation. At salinity 33 after 24 h embryo development, the highest
544 EC50 value was observed at 24 ºC, with significant differences towards that
545 obtained at 20 ºC. (Fig. 4).

546 These findings suggest that higher sensitivity (lower EC50s) observed at
547 lower temperatures (20 ºC, 24 hours) for all tested salinity levels likely resulted
548 from an additive effect of delayed development induced by both As and low
549 temperature, considering that the developing rate of oyster larvae decrease at
550 lower temperatures (His et al., 1989; Dove and O´Conner, 2007), and that As
551 induces a retarding effect on embryo development (Moreira et al., 2018)

552 Results further revealed that after 48 h no significant differences were
553 observed among temperature levels at salinity 26 and 33 (Fig 4). These findings
554 demonstrate that the arresting effect of As on *C. gigas* larvae development
555 previously described is not permanent, because extending the exposure period
556 to 48 h revealed higher frequencies of embryo-larval development completion
557 (lower frequencies of Pre-D larvae) (Supplementary table S2), which in turn
558 resulted in similar toxicity effects to larvae exposed at different temperature
559 levels at 48 h, unlike results obtained at 24 h for which temperature showed a
560 higher effect.

561 Comparisons between EC50 values obtained for each combination of
562 salinity and temperature at 24 h incubation and the corresponding conditions
563 after 48 h exposure (Fig. 4), revealed no differences in EC50 values at any
564 combination of low (20) and intermediate salinities (26) at every temperature
565 level between 24 and 48 h exposures (Fig. 4 and Table S3). However,
566 significant differences were observed at all temperature levels tested at salinity

33, where significantly higher EC50 values were observed after 48 h comparing to values obtained after 24 h. It is important to note that at salinity 20 at 20 ºC, EC50 was only possible to calculate after 48 h exposure, since after 24 h embryo development was not completed in these conditions (Fig. 2B and D).

Comparisons between results obtained for *C. gigas* at 24 and 48 h further revealed a significant increase of EC50s at the highest salinity (33), and all combinations of temperature, with the increase of exposure time. These results illustrated that toxicity threshold determination can depend on time of exposure for toxicants that affect embryonic development by retarding development such as As.

4. Concluding remarks

The present study allowed to assess the embryotoxicity of As under different thermohaline conditions to *C. angulata* and to compare results with those from an important worldwide distributed species *C. gigas*. Results obtained showed marked differences on each species embryo-development capacity, namely concerning the tolerance range to varying salinity and temperature, which further reflected in the pattern of As toxicity observed. *C. angulata* presented a narrower range of salinity and temperature than *C. gigas* for which embryo-development successfully occurred, considering 24 and 48 h post fertilization. *C. angulata* presented better embryonic development at intermediate salinity (26) and at temperatures above 20 ºC, while *C. gigas* presented high frequencies of developing embryos at all combinations of salinity and temperatures tested. Overall, these results suggest that early ontogeny of *C. angulata* may be limited to a narrower range of abiotic factors (salinity and

592 temperature) compared to *C. gigas*, with possible implications at the population
593 level. Considering that early life stages generally constitute the most susceptible
594 stage of oysters' life cycle, the thermohaline differences observed for embryo
595 development may dictate species competitive advantages towards one another
596 under the projected scenarios of climate driven alterations of temperature and
597 salinity regimes in estuarine systems worldwide. Hence, *C. angulata* may be
598 more susceptible to environmental change than *C. gigas*.

599 Concerning As toxicity, EC50 values for *C. angulata* showed that the
600 effect of As was overall lower at the intermediate salinity 26, however
601 temperature also showed to influence As toxicity in an antagonistic manner,
602 with higher temperatures inducing lower toxicity effects. For *C. gigas* the effect
603 of As was highly dependent on salinity, with sensitivity to As showing to
604 decrease with the increase of salinity. The effect of temperature was only
605 noticeable after 24 h exposure, when low temperatures induced higher
606 sensitivity, while after 48 h development the effect of temperature was
607 mitigated.

608 Comparisons on As toxicity measured as EC50 values between
609 species, showed that *C. angulata* was at least 10 times more sensitive to As
610 than *C. gigas* and these differences were likely species related. Our results
611 suggest that the survival of *C. angulata* strongly depends on a narrower range
612 of abiotic factors compared to its closely related congener, and therefore the
613 future of this population may be endangered considering the future projections
614 on climate change and pollution worldwide.

615 Other issues rise from the present study, namely the observations that
616 the delay effects of As on *Crassostrea* embryo development is not permanent

(noted by differences between 24 and 48 h exposures) and add to recent findings that showed that sea urchin embryos can recover from toxicity induced by different trace metals (Morroni et al., 2018). These results are also important for future studies considering differentiated effects under varying physico-chemical parameters that may consider the 48-h criterion. For future studies relying on bivalves collected in the environment we suggest trace elements analysis to be performed on oocyte suspensions. All together these issues should be considered for future ecotoxicological studies and water quality framework directives relying on embryotoxicity assays.

Acknowledgements

This study was supported by the Portuguese Science Foundation (FCT) through CESAM (UID/AMB/50017), by FCT/MEC through national funds, and the co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020. Anthony Moreira benefited from PhD grant SFRH/BD/93107/2013. Rosa Freitas benefited from a research position funded by the Integrated Programme of SR&TD "Smart Valorization of Endogenous Marine Biological Resources Under a Changing Climate" (reference Centro-01-0145-FEDER-000018), co-funded by Centro 2020 program, Portugal 2020, European Union, through the European Regional Development Fund. The authors would like to thank Mr. Pedro Ferreira from Exporsado for providing *C. angulata* progenitors.

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Figure 1 – Photographic record of embryo-larvae types observed in *Crassostrea angulata* after embryotoxicity exposures: a) D-shape; b) pre-D; c) protruded mantle; d) kidney shape; e) indented shell; and f) dead larvae after His et al. (1997).

Figure 2 – Contour plots representing the observed percentage of malformations at different combinations of salinity and temperature, for *C. angulata* after 24 h (A) and 48 h (B) development; and *C. gigas* after 24 h (C) and 48 h (D) development. Each colour represents a different category of percent frequency malformation.

Figure 3 – Median effect concentrations (EC50s) of As and relative 95% confidence intervals for *C. angulata* at different salinity and temperature conditions, after 24 and 48 h exposure. Different letters represent statistical differences between tested groups ($p \leq 0.05$): lower-case letters for comparisons between combinations of salinity and temperature conditions after 24 h exposure; upper-case letters for comparisons between combinations of salinity and temperature conditions after 48 h exposure; asterisks (*) for statistical differences between 24 and 48-h exposures at each combination of salinity and temperature.

Figure 4 – Median effect concentrations (EC50s) of As and relative 95% confidence intervals for *C. gigas* at different salinity and temperature conditions, after 24 and 48 h exposure. Different letters represent statistical differences between tested groups ($p \leq 0.05$): lower-case letters for comparisons between combinations of salinity and temperature conditions after 24 h exposure; upper-case letters for comparisons between combinations of salinity and temperature conditions after 48 h exposure; asterisks (*) for statistical differences between 24 and 48-h exposures at each combination of salinity and temperature.

Table I – Summary table of median effect concentrations (EC50s) of As and relative 95% confidence intervals for *C. gigas* and *C. angulata* at different combinations of salinity and temperature, after 24 and 48 h exposures.

T (ºC)	*Salinity*	***C. angulata***		***C. gigas***	
		24 h	48 h	24 h	48 h
20	20	n.c	n.c	n.c	**101.1** (96.9-105.5)
	26	n.c	**31.0** (n.d)	**119.0** (114.6-123.5)	**161.9** (147.2-178.0)
	33	n.c	n.c	**285.5** (267.3-305.0)	**530.0** (465.2-603.8)
24	20	n.c	n.c	**101.9** (101.7-102.0)	**80.71** (80.70-80.73)
	26	**29.8** (29.8-29.8)	**41.8** (38.7-45.0)	**179.7** (178.6-180.7)	**214.3** (206.1-222.9)
	33	**39.2** (38.8-39.6)	**18.7** (17.1-20.5)	**451.5** (312.6-652.2)	**663.5** (516.5-852.3)
28	20	n.c	n.c	**103.4** (90.64-117.9)	**103.3** (103.0-103.5)
	26	**39.6** (39.5-39.7)	**50.5** (44.0-58.1)	**190.1** (180.9-199.8)	**198.9** (185.5-213.3)
	33	**36.2** (28.5-46.1)	**15.9** (14.6-17.3)	**303.7** (229.6-401.5)	**493.7** (429.9-567.0)

n.c - EC50 value not calculated due to low or zero percentage of developed larvae (D-shape)

n.d – 95% confidence interval not determined due to few points for curve fit parameter estimation

Table II – Trace element concentration (µg.g^{-1} dry weight) determined for each *C. angulata* and *C. gigas* fertilized oocytes suspensions used for embryotoxicity experiments (mean ± RSD).

	C. angulata	*C. gigas*
As	3.43±0.031	3.29±0.035
Cd	0.11±0.0046	0.10±0.0054
Cr	3,69±0.049	0,36±0.0047
Cu	7.75±0.078	7.06±0.078
Hg	0,04±0.0051	0,03±0.0031
Ni	1,91±0.026	0,76±0.012
Sn	0,46±0.0085	0,40±0.0051
Zn	56,69±0.58	78,94±0.86

Supplementary table S1 – Mean percentage of *C. angulata* embryos presenting delayed development (Pre-D) at different combinations of salinity, temperature and As concentrations, after 24 and 48-hour exposures. Conditions for which 100 % embryos were classified as Pre-D's are highlighted in dark grey.

			µg L^{-1} of As							
	Salinity	**Temperature (ºC)**	**0**	**30**	**60**	**120**	**240**	**480**	**960**	**1920**
24 h	**20**	**20**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		24	75,3	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		28	75,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
	26	**20**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		24	4,0	24,0	95,7	100,0	100,0	100,0	100,0	100,0
		28	8,0	19,0	93,0	99,3	100,0	100,0	100,0	100,0
	33	**20**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		24	4,3	5,0	97,7	98,0	99,3	100,0	100,0	100,0
		28	5,0	5,3	62,0	88,7	99,3	100,0	100,0	100,0
48 h	**Salinity**	**Temperature (ºC)**								
	20	**20**	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		26	35,5	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		28	62,5	100,0	100,0	100,0	100,0	100,0	100,0	100,0
	26	**20**	10,3	48,3	100,0	100,0	100,0	100,0	100,0	100,0
		24	0,3	3,0	93,3	100,0	100,0	100,0	100,0	100,0
		28	4,7	6,3	87,0	15,0	100,0	100,0	100,0	100,0
	33	**20**	97,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		24	1,3	1,5	43,0	90,3	99,0	100,0	100,0	100,0
		28	39,0	76,7	63,0	33,7	1,0	0,3	100,0	100,0

Supplementary table S2 – Mean percentage of *C. gigas* embryos presenting delayed development (Pre-D) at different combinations of salinity, temperature and As concentrations, after 24 and 48-h exposures. Conditions for which 100 % embryos were classified as Pre-D's are highlighted in dark grey.

			µg L^{-1} of As							
	Salinity	**Temperature (ºC)**	**0**	**30**	**60**	**120**	**240**	**480**	**960**	**1920**
24 h	**20**	**20**	99,3	100,0	100,0	100,0	100,0	100,0	100,0	100,0
		24	20,7	6,7	88,0	100,0	100,0	100,0	100,0	100,0
		28	14,7	23,0	25,3	12,0	0,3	100,0	100,0	100,0
	26	**20**	6,3	1,7	2,3	28,0	89,7	99,7	100,0	100,0
		24	1,0	1,3	22,7	79,7	99,3	100,0	100,0	100,0
		28	5,0	3,7	4,3	18,7	79,0	99,3	100,0	100,0
	33	**20**	10,0	12,0	4,3	6,3	17,3	40,7	77,7	96,0
		24	5,3	1,7	1,3	2,3	4,3	20,0	84,7	83,0
		28	4,0	1,3	2,7	2,7	4,3	41,0	81,0	92,7
48 h	**Salinity**	**Temperature (ºC)**								
	20	**20**	4,3	4,0	14,3	60,0	100,0	100,0	100,0	100,0
		24	11,0	8,0	27,0	99,0	100,0	100,0	100,0	100,0
		28	1,3	3,0	11,3	73,0	100,0	100,0	100,0	100,0
	26	**20**	0,3	3,3	1,3	14,3	53,0	99,7	100,0	100,0
		24	1,5	2,7	0,7	7,7	67,0	96,3	100,0	100,0
		32	6,0	8,0	14,7	21,3	13,7	0,7	100,0	100,0
	33	**20**	7,3	0,3	1,3	2,3	0,3	42,7	86,7	96,0
		24	0,0	0,3	0,7	0,0	0,0	20,0	58,7	74,0
		28	25,3	21,0	30,0	29,7	27,7	35,3	23,3	5,3

Supplementary table S3 – Results on Tukey's multiple comparisons tests between conditions for 24-h exposures.

	C. gigas		*C. angulata*	
Tukey's multiple comparisons	**Adjusted *p*-value**	**Summary**	**Adjusted *p*-value**	**Summary**
20 ºC sal 26 vs. 20 ºC sal 33	< 0,0001	****	-	-
20 ºC sal 26 vs. 28 ºC sal 26	0,0004	***	-	-
20 ºC sal 33 vs. 28 ºC sal 33	> 0,9999	ns	-	-
24 ºC sal 33 vs. 24 ºC sal 26	< 0,0001	****	0,0259	*
24 ºC sal 33 vs. 24 ºC sal 20	< 0,0001	****	-	-
24 ºC sal 33 vs. 20 ºC sal 33	0,0023	**	-	-
24 ºC sal 33 vs. 28 ºC sal 33	0,0152	*	0,9695	ns
24 ºC sal 26 vs. 24 ºC sal 20	< 0,0001	****	-	-
24 ºC sal 26 vs. 20 ºC sal 26	0,0094	**	-	-
24 ºC sal 20 vs. 28 ºC sal 20	> 0,9999	ns	-	-
24 ºC sal 26 vs. 28 ºC sal 26	> 0,9999	ns	0,0201	*
24 ºC sal 20 vs. 28 ºC sal 20	> 0,9999	ns	-	-
28 ºC sal 20 vs. 28 ºC sal 26	< 0,0001	****	-	-
28 ºC sal 20 vs. 28 ºC sal 33	< 0,0001	****	-	-
28 ºC sal 26 vs. 28 ºC sal 33	0,0011	**	0,9524	ns

Supplementary table S4 – Results on Tukey's multiple comparisons tests between conditions for 48-hour exposures.

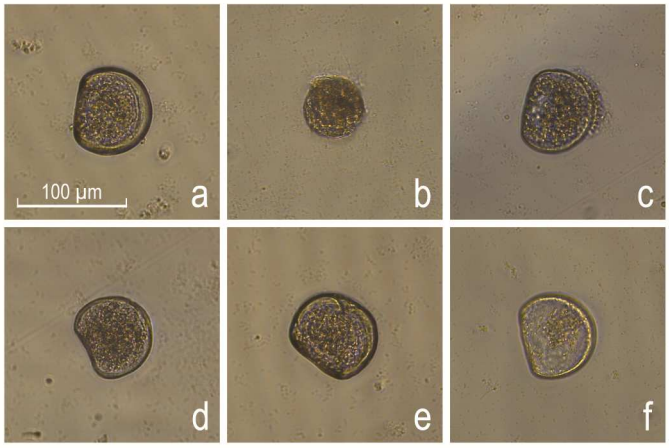
	C. gigas		*C. angulata*	
Tukey's multiple comparisons	**Adjusted *p*-value**	**Summary**	**Adjusted *p*-value**	**Summary**
20 ºC sal 20 vs. 20 ºC sal 26	0,0004	***	-	-
20 ºC sal 20 vs. 20 ºC sal 33	< 0,0001	****	-	-
20 ºC sal 20 vs. 24 ºC sal 26	0,9054	ns	-	-
20 ºC sal 20 vs. 24 ºC sal 20	> 0,9999	ns	-	-
20 ºC sal 20 vs. 28 ºC sal 20	< 0,0001	****	-	-
20 ºC sal 26 vs. 20 ºC sal 33	0,2288	ns	-	-
20 ºC sal 26 vs. 24 ºC sal 26	0,7887	ns	-	-
20 ºC sal 26 vs. 28 ºC sal 26	0,6726	ns	-	-
20 ºC sal 33 vs. 24 ºC sal 33	> 0,9999	ns	-	-
20 ºC sal 33 vs. 28 ºC sal 33	< 0,0001	****	-	-
24 ºC sal 20 vs. 24 ºC sal 26	< 0,0001	****	-	-
24 ºC sal 20 vs. 24 ºC sal 33	0,8974	ns	-	-
24 ºC sal 20 vs. 28 ºC sal 20	< 0,0001	****	-	-
24 ºC sal 26 vs. 24 ºC sal 33	> 0,9999	ns	< 0,0001	****
24 ºC sal 26 vs. 28 ºC sal 26	0,1538	ns	0,1246	ns
24 ºC sal 33 vs. 28 ºC sal 33	< 0,0001	****	0,2685	ns
28 ºC sal 20 vs. 28 ºC sal 26	< 0,0001	****	-	-
28 ºC sal 20 vs. 28 ºC sal 33	< 0,0001	****	< 0,0001	****
28 ºC sal 26 vs. 28 ºC sal 33	0,0004	***	-	

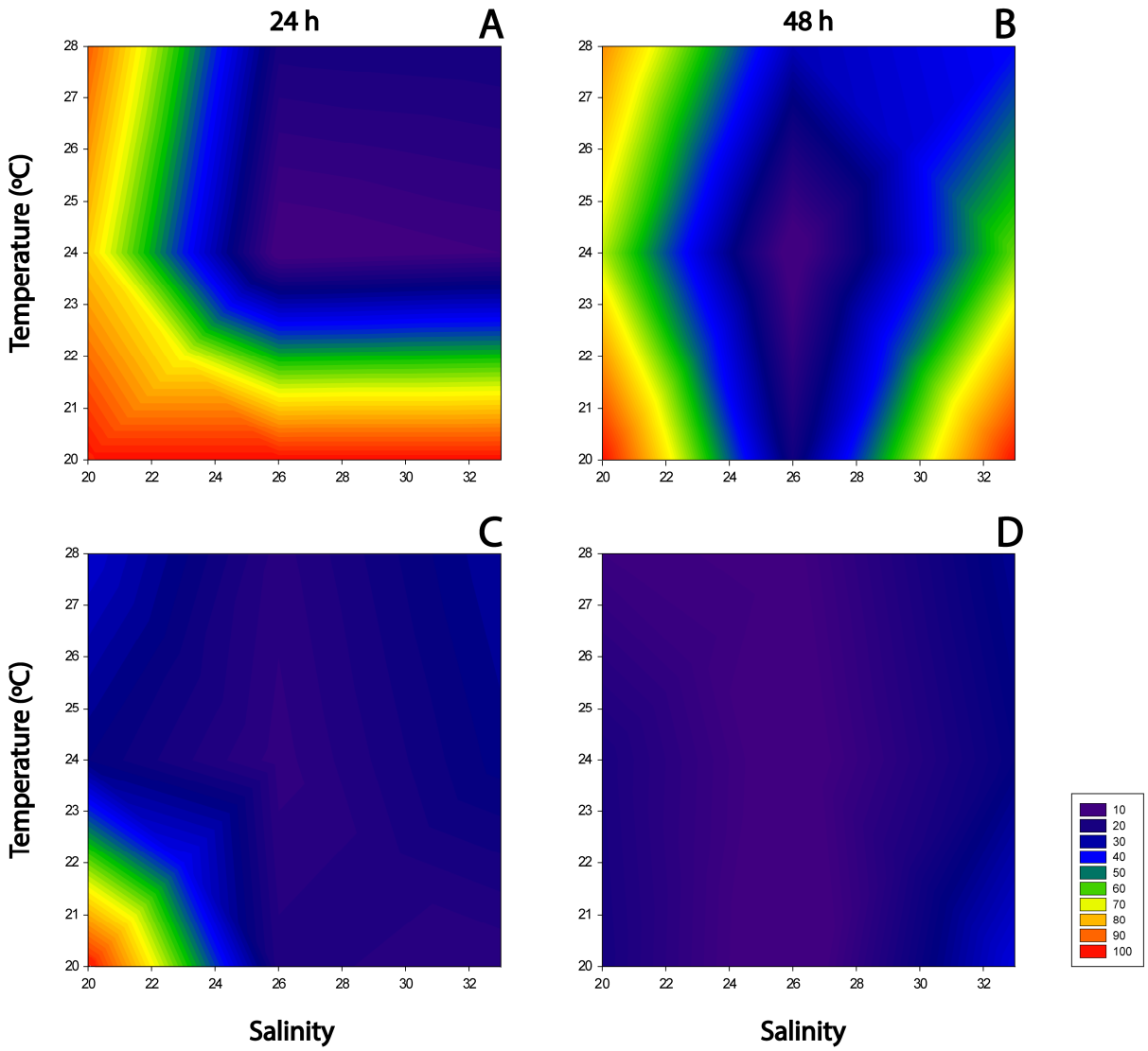
Supplementary table S5 – Results on Tukey's multiple comparisons tests for each condition between 24 and for 48-h exposures.

	C. gigas		*C. angulata*	
Tukey's multiple comparisons	**Adjusted *p*-value**	**Summary**	**Adjusted *p*-value**	**Summary**
20 ºC sal 26 24 hrs vs. 48 hrs	0,1395	ns	-	-
20 ºC sal 33 24 hrs vs. 48 hrs	< 0,0001	****	-	-
24 ºC sal 20 24 hrs vs. 48 hrs	0,9069	ns	-	-
24 ºC sal 26 24 hrs vs. 48 hrs	0,9426	ns	0,001	**
24 ºC sal 33 24 hrs vs. 48 hrs	0,0088	**	< 0,0001	****
28 ºC sal 20 24 hrs vs. 48 hrs	> 0,9999	ns	-	-
28 ºC sal 26 24 hrs vs. 48 hrs	> 0,9999	ns	0,0257	*
28 ºC sal 33 24 hrs vs. 48 hrs	0,0005	***	< 0,0001	****

Supplementary table S6 - Effective arsenic (As) concentrations (µg L^{-1}) prepared for each salinity level for each exposure concentration (mean ± RSD).

	Nominal As (µg L^{-1})	Salinity		
		20	26	33
C. angulata	30	36±0.3	28±0.3	20±0.2
	60	68±0.6	72±0.7	74±0.7
	120	136±1.2	158±1.5	149±1.4
	240	256±2.4	274±2.5	262±2.4
	480	530±4.9	522±4.8	525±4.8
	960	1050±9.7	1046±9.6	1037±9.6
	1920	2052±18.9	2097±19.3	2040±18.8
C. gigas	30	47±0.4	46±0.4	43±0.4
	60	79±0.7	81±0.7	86±0.8
	120	158±1.5	160±1.5	166±1.5
	240	256±2.4	258±2.4	254±2.3
	480	503±4.6	502±4.6	493±4.5
	960	1039±9.6	1008±9.3	1025±9.6
	1920	1932±17.8	1997±18.4	1966±18.1




24 h
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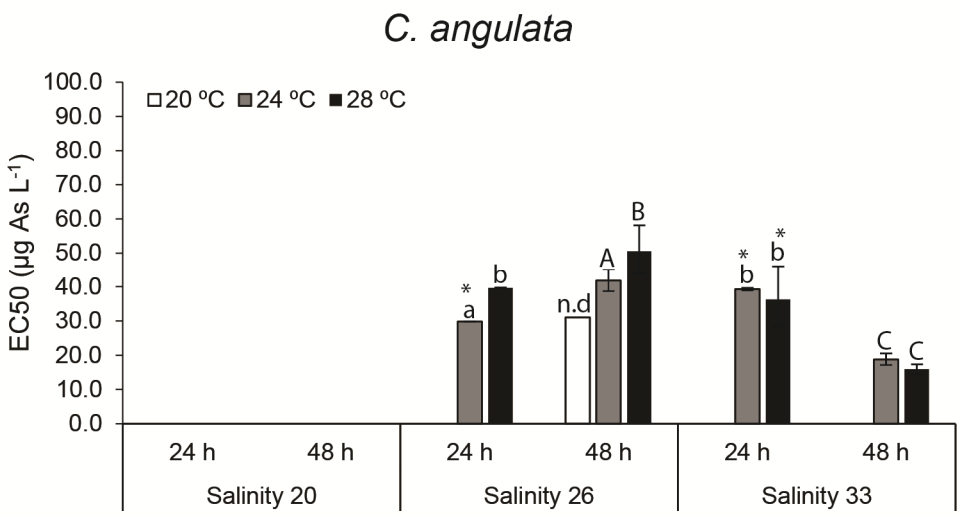

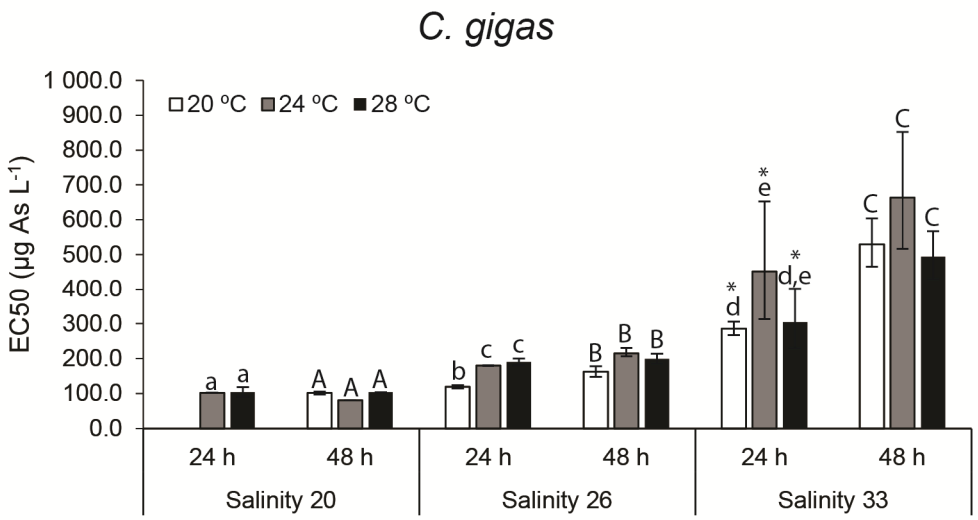

Highlights

- Thermohaline range for embryo-larval development was wider in *C. gigas*

- *C. angulata* embryo development was more sensitive to As

- As induced a delayed but not permanent effect on embryo development

- Salinity and temperature influenced both species sensitivity to As

- *C. angulata* may become most impacted by climate change and pollution than *C. gigas*