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Effects of trace elements contaminations on the larval development of *Paracentrotus lividus* using an innovative experimental approach

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ABSTRACT

Several experiments were performed using larvae of *Paracentrotus lividus* (Lamarck, 1816) in order to determine the consequences of different chronic contamination with mixtures of (i) fifteen trace elements from concentrations measured in the world ocean seawater, and (ii) seven trace elements from contamination resulting from mining. To predict the impact of increased marine pollution, higher concentrations were also used. These bioassays were conducted using spawners collected from Calvi (reference site, Corsica), and Albo (mining area, Corsica). The effects of trace elements have been studied on the entire larval development. The results show wider arms and delayed development as the number and concentration of trace elements increases. Therefore, the synergy between the different trace elements is of paramount importance with regard to the impact on organisms. Probably due to a hormesis phenomenon, larvae contaminated with seven trace elements at average concentrations developed more quickly. This work also highlighted the importance of the origin of spawners in ecotoxicological studies. To our knowledge, this is the first study to investigate the effects of such a broad combination of trace elements for chronic contamination on the entire larval stage of *Paracentrotus lividus*.

1. Introduction

Since the beginning of the Industrial Revolution, anthropic pressures have increased due to strong population and economic growth. These pressures have led to the release of toxic substances into coastal and marine ecosystems (Bonanno and Di Martino, 2017). Trace elements are among the most common contaminants in the marine ecosystem leading to considerable long term ecological damage to aquatic organisms and the alteration of the dynamics of a system (Sandilyan and Kathiresan, 2014). The dispersion of these contaminants in the marine environment results mainly from anthropic sources but is also due to various geological phenomena (Baize, 2009). Trace elements can be classified as (i) "essential", allowing the growth and normal development of an organism within a well-defined interval, and (ii) "non-essential", having, in the present state of knowledge, no beneficial role in biological functions (Amiard, 2011). Essentiality is a characteristic which changes according to knowledge and whose sensitivity differs according to the authors. Beyond a critical threshold, any trace element, whether essential or not, causes disturbances at the molecular and cellular levels, thus impacting individual organisms, entire populations, and ultimately potentially ecosystems. (Amiard, 2011). Although marine organisms may have capacities of resistance and resilience to such abiotic stresses (Loizeau and Tusseau-Vuillemin, 2014), chronic contamination of the marine environment, particularly with trace elements, is a major issue for wildlife conservation (Amri et al., 2017).

The study of these contaminants is particularly important in the Mediterranean Sea because of its geomorphological and hydrodynamic characteristics (Bethoux et al., 1999). Within the Western Mediterranean basin, Corsica Island is often considered as a reference region on account of its high water quality and the low anthropic pressures (Richir et al., 2013). In spite of this, high levels of trace elements were observed in the gonads of sea urchins near an old asbestos mine at Canari (Corsica, France; El Idrissi et al., 2020; Ternengo et al., 2018). The high levels found in this area originate from the untreated excavated material discharged into the sea, representing about 11 million tons of sediment (Kantin and Pergent-Martini, 2007). The continuous leaching of mining

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residues, still present on the sides of the mine, contributes to the dispersion of nickel, chromium and cobalt along the coastline (Kantin and Pergent-Martini, 2007).

By its ecological features, high tolerance of contaminants and ability to bioaccumulate trace elements, *Paracentrotus lividus* is recognized as a bioindicator (El Idrissi *et al.*, 2020; Warnau et al., 1996). The embryo-larval and adult life stages of the sea urchin are the most studied and used in testing (Bielmyer et al., 2005). Adult sea urchins are extensively studied to assess the levels of trace elements contamination in coastal ecosystems (*e.g.,* El Idrissi *et al*., 2020; Ternengo et al., 2018). Although this allows determination of the degree and nature of pollution, no evidence regarding the biological consequences can be provided (Chapman et al., 1987). Bioassays enable the detection of these effects by measuring the biological responses of marine organisms (Fernandez and Beiras, 2001). Embryo-larval development of several species of sea urchins has been widely studied since the 1950s in order to monitor contaminants in the marine environment (*e.g.,* Okubo and Okubo, 1962; Tabata, 1956). Currently, bioassays using sea urchin larvae are among the most standardized in ecotoxicology (Pétinay et al., 2009).

Many studies have been carried out to test the influence of trace elements, in bioassays using sea urchin larvae. However, most studies tested one or cocktails of only few contaminants, most of time at high concentrations relative to the environment (*e.g.,* Bielmyer et al., 2005; Chiarelli et al., 2014). The single metal study allows to establish a metal-related effect but does not make it possible to understand the effects due to the mixtures of several trace elements. Since marine environments normally contain more than one trace element (Fernandez and Beiras, 2001), it's essential to carry out work on the effects of interactions of trace elements. In addition, studies have focused on the endotrophic stages (embryonic and first larval stage) and the effects of trace elements on the entire larval development has never been explored. The acquisition of knowledge through studies on *Paracentrotus lividus* is crucial in the context of degradation of the quality of coastal waters owing to its scientific, ecological and economic interest (Carballeira et al., 2012).

The aim of this study is to estimate the effects of chronic exposure to a mixture of trace elements on the development of *Paracentrotus lividus* – from the fertilized egg to the end of larval development – for (i) the world ocean seawater average concentration (mixture of fifteen trace elements), and (ii) contamination observed near the old asbestos mine of Canari (Corsica, France), resulting from mining activity (mixture of seven trace elements). This study will also enable us to understand the effects of an increase in marine pollution on the larvae of *Paracentrotus lividus* and to determine whether contamination of spawners has an influence on larval development in contaminated areas by using spawners collected from Calvi (reference site, Corsica), and Albo (mining area, Corsica). This will allow an assessment of whether there is some adaptation of the spawners that could be transmitted to the larval population and how this might have important implications in ecotoxicological studies.

2. Material and methods

2.1. Biological collection

Adult sea urchins were collected in a spawning period in Corsica (Mediterranean Sea) at two distinctive sites: (i) Albo (42°46.313 N, 9°20.150 E), contaminated due to its proximity to the old asbestos mine of Canari, and (ii) Calvi (42°34.916 N, 8°43.589 E), a reference site, with very low levels of trace elements contamination (El Idrissi *et al.*, 2020; Gobert and Richir, 2019; Ternengo et al., 2018). Individuals sampled were immediately transported in an insulated box to the laboratory and gametes were obtained by dissecting mature organisms. In order to optimize the genetic mixing, thirty males and thirty females for each site were used to consider the maximum of expected variability, that could be genetically driven, in a natural larval population. The spawning was carried out in reference seawater (control) in order to achieve *in vitro* fertilization (Buttino et al., 2016). One hour after fertilization, the embryos were observed to verify that the fertility rate is above 90% (Buttino et al., 2016; Pétinay et al., 2009).

2.2. Larval development bioassays

Three experiments were carried out using larvae produced by sea urchins from Albo or Calvi. A mixture of trace elements was produced in deionized water using analytical grade solutions (1 g L^{-1}; Certipur®, Merck, Germany; Table S.2) and was diluted in 10 mL of seawater. This mixture was poured into the rearing tank containing reference sea water. The concentration and the number of trace elements varied according to the experiment and treatment (Tables 1 and 2). Three replicates were performed per treatment and a control was carried out for each experiment. Temperature was adjusted at the beginning of the experiments (20 °C) and was continuously monitored using HOBO TidbiT® v2 loggers (accuracy: ± 0.21 °C). The sea water was renewed daily and food, composed of a mixture of phytoplankton, was provided *ad libitum*. This work was carried out in accordance with French regulations for animal experiments.

The first experiment was realized to assess the impact of the contamination measured in the world ocean seawater. The fertilized eggs from Calvi were placed in fifteen rearing tanks (120 000 eggs per tanks): three control and twelve contaminated. Four treatments at different concentrations were tested: world ocean seawater average concentration (treatment 1) and the same concentration multiplied by 5 (treatment 2), 10 (treatment 3) and 50 (treatment 4; Table 1). The average concentrations of world ocean seawater were calculated by compiling data from the literature (Richir et al., 2013; Table S.1). In total, fifteen trace elements were studied: silver (Ag), arsenic (As), aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), vanadium (V) and zinc (Zn).

The second and third experiments were carried out to assess the impact of the contamination measured in the seawater column in front of the old asbestos mine (Canari, Corsica, France). Seven trace elements were studied for three distinct purposes: (i) Co, Cr and Ni, measured in high concentrations; (ii) Fe, Cu and Zn, essential trace elements usually found in high levels in the gonads of sea urchins, and (iii) Hg, which has been the subject of frequent discussions for several years within the scientific community in view of its potential impact (Buttino et al., 2016; El Idrissi et al., 2020; Ternengo et al., 2018). Three treatments at different concentrations were tested: the average of concentrations measured in the seawater column in front of the old asbestos mine (treatment 1), and this same concentration multiplied by 5 (treatment 2) and 10 (treatment 3; Table 2). These treatments were applied to larvae produced by sea urchins from Calvi (experiment 2) and Albo (experiment 3) to verify whether the contamination status of spawners has an influence on larval development. The fertilized eggs from each site were placed in twelve distinct rearing tanks: three control and nine contaminated.

2.3. Monitoring of larval development

Larval development was monitored daily from the 4th day after fertilization. Each individual was photographed under a stereomicroscope (40-fold magnification) and measured using ImageJ software to determine total length, body width and length and arm length (Fig. 1). In total, more than 10 000 larvae were measured in this study in order to have a robust dataset. 100-fold magnification allowed determination of larval malformation according to the criteria of Carbaillera et al. (2012). To assess developmental delay, the larval stage was determined. The larval stages considered are the following: the 4-arm stage, the 6-arm stage and the 8-arm stage (Fig. 2); the shift to the next stage was determined from the appearance of the two additional arms.

Table 1
Trace elements concentrations (μg L^{-1}; world ocean seawater average concentration; *more details in Table S.1*), tested from larvae produced by sea urchins sampled at Calvi (experiment 1).

	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	V	Zn
Treatment 1: Average concentration	0.1	0.7	1.6	0.1	0.1	0.2	0.3	1.1	0.4	0.3	11.1	0.4	0.2	1.8	1.3
Treatment 2: 5– fold concentration	0.5	3.5	8	0.5	0.5	1	1.5	5.5	2	3	55.5	2	1	9	6.5
Treatment 3: 10– fold concentration	1	7	16	1	1	2	3	11	4	6	111	4	2	18	13
Treatment 4: 50– fold concentration	5	35	80	5	5	10	15	55	20	30	555	20	10	90	65

Table 2
Trace elements concentrations (μg L^{-1}; concentration measured near an old asbestos mine at Canari, Corsica, France; *more details in Table S.1*) tested from larvae produced by sea urchins sampled at Calvi (experiment 2) and Albo (experiment 3).

	Co	Cr	Cu	Fe	Hg	Ni	Zn
Treatment 1: Average concentration	0.02	0.15	0.30	1.10	0.40	1.38	1.30
Treatment 2: 5-fold concentration	0.1	0.75	1.5	5.5	2	6.9	6.5
Treatment 3: 10– fold concentration	2	1.5	3	11	4	13.8	13

2.4. Data presentation and statistical analyses

Larval growth was illustrated using a logarithmic trendline; this is the curved line with the best coefficient of determination (close to 1) and the most suitable for this dataset. Data were transformed when necessary, to satisfy the conditions of application of the parametric tests (data normality and homogeneity of variances). Growth data (*i.e.,* total length, body length, width and arm length) were analyzed using several repeated measures of analysis of variance (ANOVA) to evaluate differences between treatments and experiments. The significance level adopted was 95% ($\alpha < 0.05$). Analyses and graphical representations were performed using XLSTAT® software.

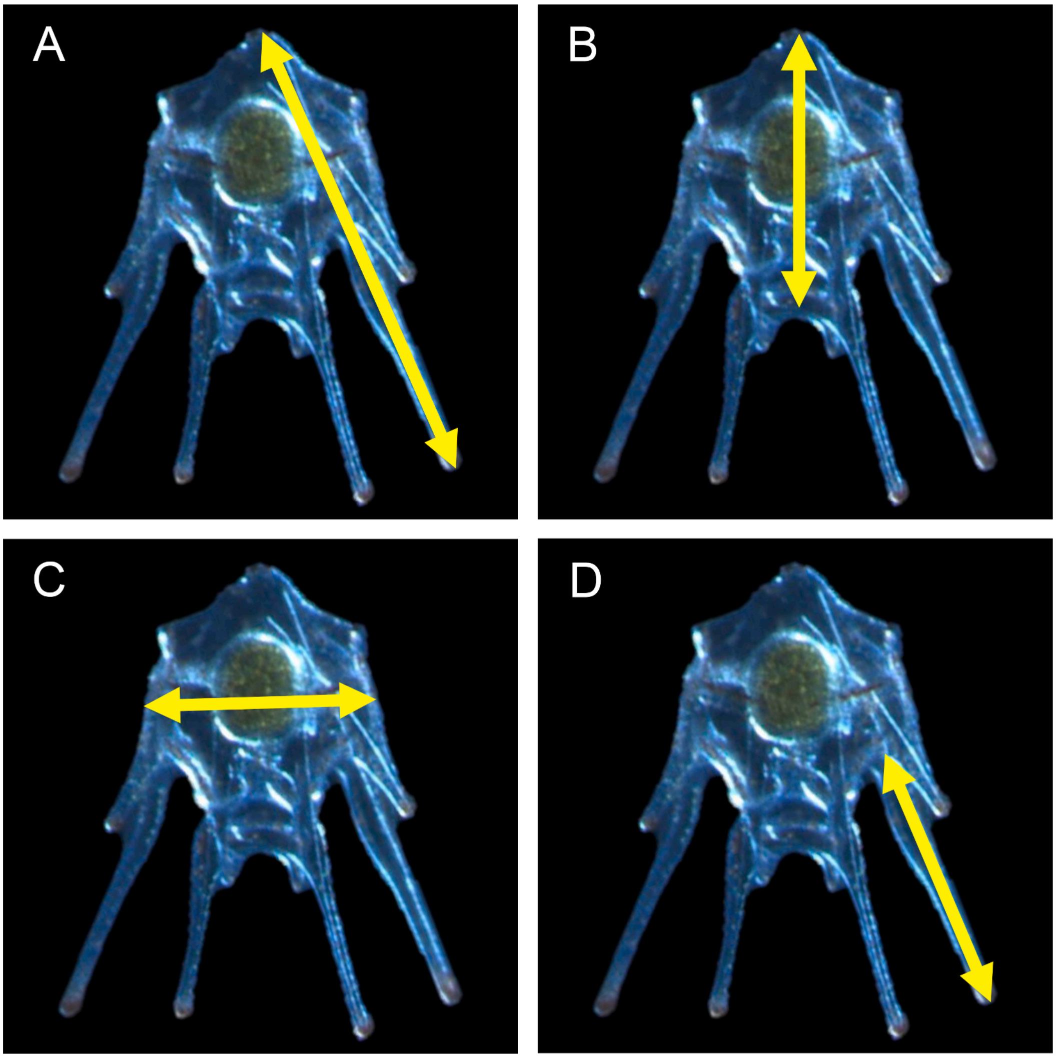

Fig. 1. Measurements of total length (A). body length (B). body width (C) and arm length (D) performed on *Paracentrotus lividus* larvae photographed under a stereomicroscope (40-fold magnification).

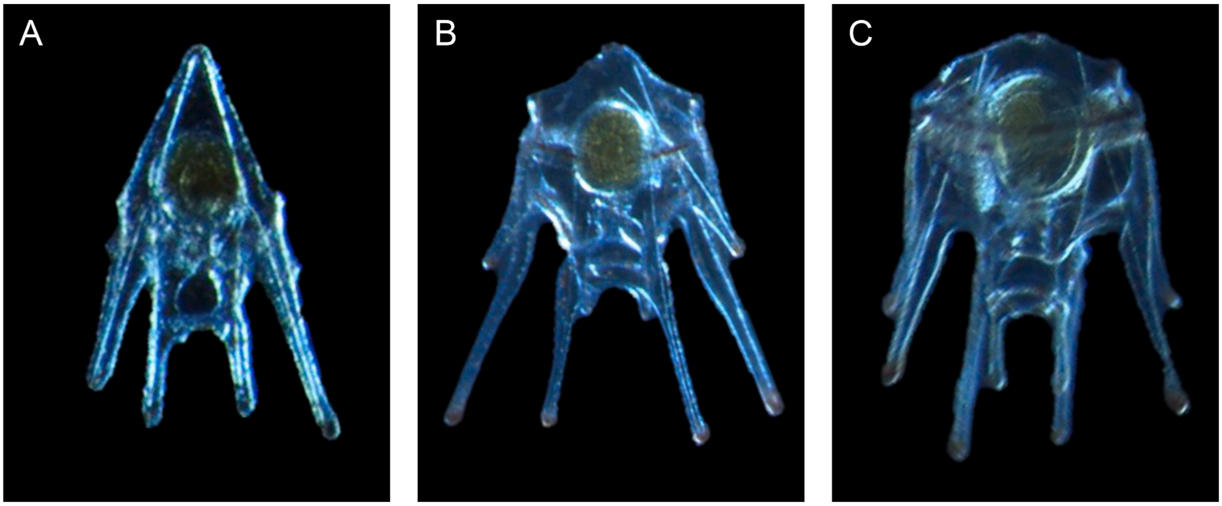

Fig. 2. Larval stages studied in this study with 4-arm (A). 6-arm (B) and 8-arm (C) performed on *Paracentrotus lividus* larvae photographed under a stereomicroscope (40-fold magnification).

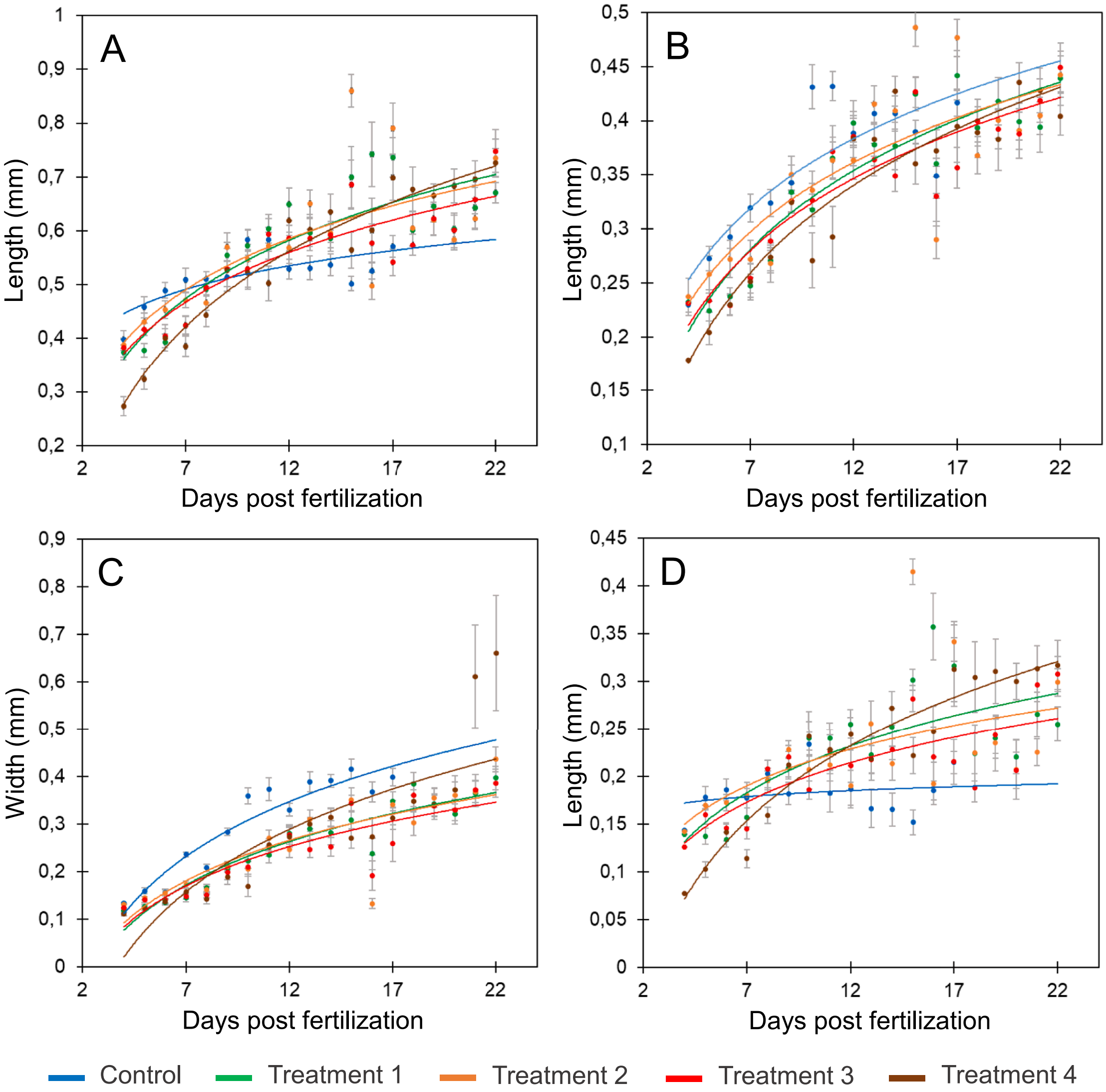

Fig. 3. Effects of chronic exposure to a mixture of trace elements on development of *Paracentrotus lividus* larvae produced by sea urchins from Calvi (A: total length; B: body length; C: body width and D: arm length) for world ocean seawater average concentration and 5- to 50-fold higher concentrations (experiment 1).

3. Results

3.1. *Effects of world ocean seawater average concentration*

The aim of the first experiment was to determine the effects of chronic exposure to a mixture of trace elements on the development of *Paracentrotus lividus* for concentrations measured in seas and oceans and with concentrations from 5- to 50-fold higher.

No malformation was observed under the stereomicroscope but larvae exhibited a significantly greater total length (from 11th day) in contaminated rearing tanks compared to the control (p-value < 0.001; Fig. 3A). A differential influence among metals concentration could be observed as the highest concentration generally resulted in lower total length at early development times, and to higher total length at the end of development. In contrast, the general trend of body length and width variation are similar to the control, although control larvae had a greater body length and width than contaminated larvae (Fig. 3B and C). Body length tended to be significantly shorter in the most contaminated rearing tanks (p-value < 0.001; Fig. 3C). The arms were significantly longer from the 10th day post fertilization in the larvae in the contaminated rearing tanks compared to the control (p-value < 0.001; Fig. 3D). In the control, no significant increase of the arm length was observed in contrast to those in contaminated tanks (Fig. 3D). There was a timeframe during which the length variabilities are high (*e.g.* between 12th day to 18th). This was probably due to greater stage variability during this period (Fig. 4) but also to variability of samples.

The larvae were all in the 4-arm stage at 4 days post-fertilization (Fig. 4). The larvae reached the 6-arm stage from the 9th day post-fertilization and 8-arm stage from the 11th day post-fertilization in the control tanks (Fig. 4A). Under metallic exposure, only a fraction of the larvae could complete their development until the 8-arm stage, and that delays were observed for both 4- to 6-arms and 6- to 8-arms transitions (Fig. 4). Whatever the metallic concentration, a similar delay was noted to observe the 6-arms larvae (2 to 3 days). By contrast, the delays observed for the appearance of 8-arms stage is a function of the metallic concentrations, varying from 6 days to 10 days (Fig. 4). Moreover, the metallic concentration has an influence on the proportion of larvae that could effectively develop. The higher the concentration, the lower the proportion of larvae at both 6- and 8-arms stages (Fig. 4).

3.2. *Effects of contamination resulting from mining activity*

The aim of the second and third experiments, with respectively spawners from reference and contaminated sites, was to determine the effects of asbestos mine contamination on the development of *Paracentrotus lividus* larvae. No malformation was observed under the stereomicroscope using a 100-fold magnification. The same trends were observed for the measurements of the larvae according to the different treatments (Figs. 5 and 6). Control larvae exhibited significantly shorter total and arm lengths compared to contaminated larvae (p-value $<$ 0.001; Fig. 5A, D, 6A, D). Body width tended to be significantly greater in larvae contaminated with treatment 1 (average contamination) and significantly lower in larvae contaminated with the most contaminated treatment (p-value < 0.001, Figs. 5C, 6C). The body length differed between experiments 2 and 3 (Figs. 5B, 6B). In experiment 2, larvae tended to have significantly higher body length in treatment 1 while in experiment 3, all treatments resulted in significantly higher body length than control (p-value < 0.001). Overall, larvae contaminated with average contamination (treatment 1) tended to be significantly wider than the others.

Larvae obtained from sea urchins from Albo (experiment 3) tended to be significantly larger compared to larvae from sea urchins collected at Calvi (experiment 2). There were also some differences between the two experiments regarding the stage of development (Figs. 7 and 8). All larvae had four arms at 4 days post-fertilization. In experiment 3, the 6-arm stage appeared earlier (7 days post-fertilization; Fig. 8B, C) in

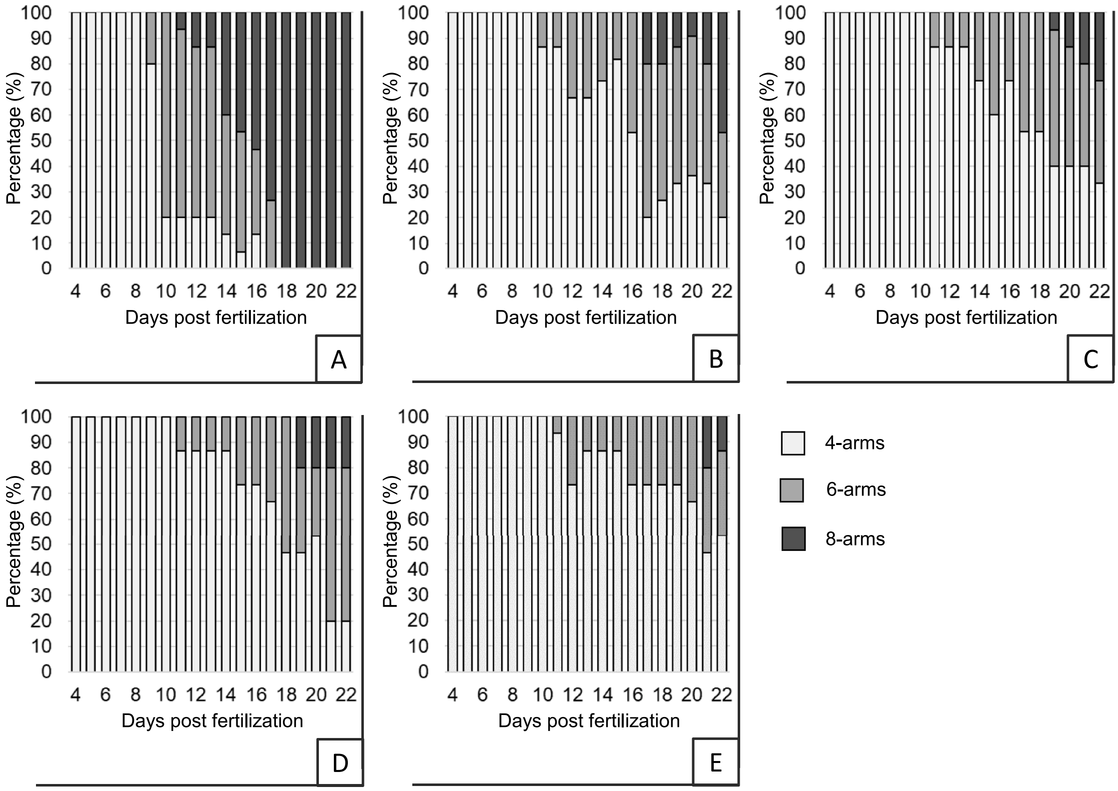

Fig. 4. Effects of chronic exposure to a mixture of trace elements on stage development of *Paracentrotus lividus* larvae produced by sea urchins from Calvi (experiment 1; A: control; B: treatment 1; C: treatment 2; D: treatment 3 and E: treatment 4).

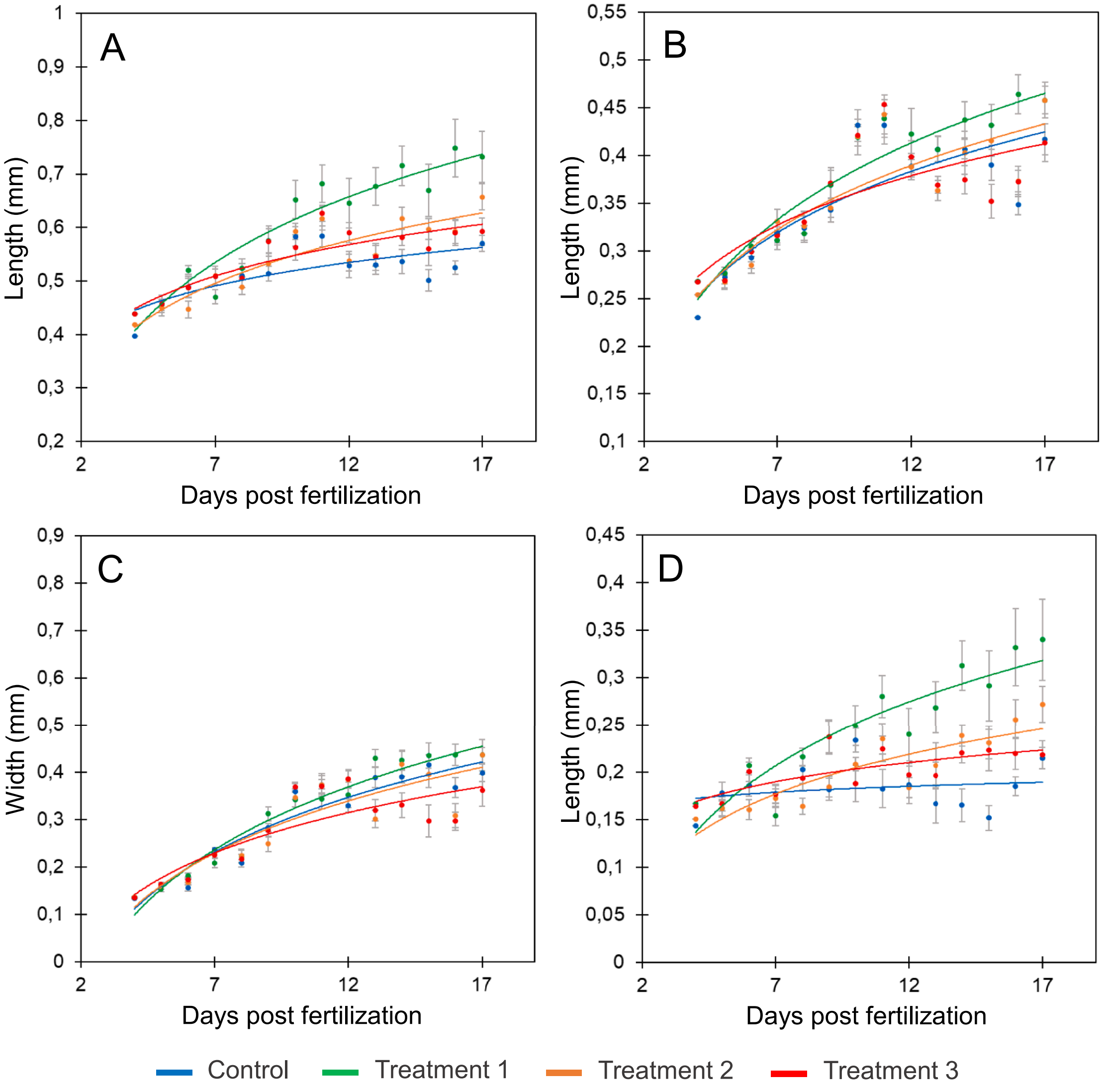

Fig. 5. Effects of chronic exposure to a mixture of trace elements on development of larvae of *Paracentrotus lividus* (A: total length; B: body length; C: body width and D: arm length) produced by sea urchins from Calvi (reference site) for concentrations measured in an old asbestos mine at Canari (Corsica. France) and 5- to 50-fold higher concentrations (experiment 2).

treatments 1 and 2 (average and 5-fold concentration, respectively). The larvae acquired the 8-arm stage from the 11th or 12th days post-fertilization in controls and treatments 1 and 2 for both experiments. In treatment 3, the 8-arm stage appeared from the 13th or 14th day post-fertilization (Figs. 7D, 8D). Despite the similarities between the two experiments, the number of larvae at the 8-arm stage was higher in experiment 3 (over than 70% at the 15th day post-fertilization; Fig. 8), notably in treatment 1 where all the larvae had eight arms from the 13th day post-fertilization (Fig. 8B).

3.3. *Comparison of the effects of different contaminations*

Experiments 1 and 2 were carried out with larvae from adult sea urchins from Calvi (reference site). The first experiment was performed in order to study the effects of world ocean seawater average concentration while the second experiment was carried out to understand the effects of contaminations resulting from mining on the larval development of *Paracentrotus lividus*. Three treatments at different concentrations were considered: the average concentrations (treatment 1), and this same concentration multiplied by 5 (treatment 2) and 10 (treatment 3; Table 1 and Table 2). The arm and total length of the larvae tended to be significantly greater in experiment 1 (p-value < 0.001; Fig. 3A, 3D, 5A, 5D). Conversely, body length and width tended to be greater in experiment 2 (Figs. 3B, C, 5B, C). The 6-arm stage appears in all treatments between the 9th and 11th day post-fertilization of both experiments (Figs. 4 and 7). However, the acquisition of 8-arms stage appeared later for experiment 1, depending on the trace elements concentration (17th-21st day; Fig. 4).

4. Discussion

The chronic toxicity of trace elements on the larval cycle of *Paracentrotus lividus* remains largely unknown. To our knowledge, no study has focused on the last larval stages of sea urchins (6- and 8-arms). The toxic properties of some trace elements have been demonstrated for several years (*e.g.* Dermeche et al., 2012; Radenac et al., 2001). However, their effects on larval development of sea urchin have often been studied in the context of acute exposure to one or a combination of two trace elements and at levels higher than those recorded in the environment (*e.g.* Bielmyer et al., 2005; Dermeche et al., 2012). This methodology is important to determine the median effective concentrations (EC_{50}), *i.e.* the metal concentrations reducing embryogenesis success to 50% of the control values, and the lowest observed effect concentrations (LOEC), *i.e.* the lowest concentrations significantly inhibiting growth

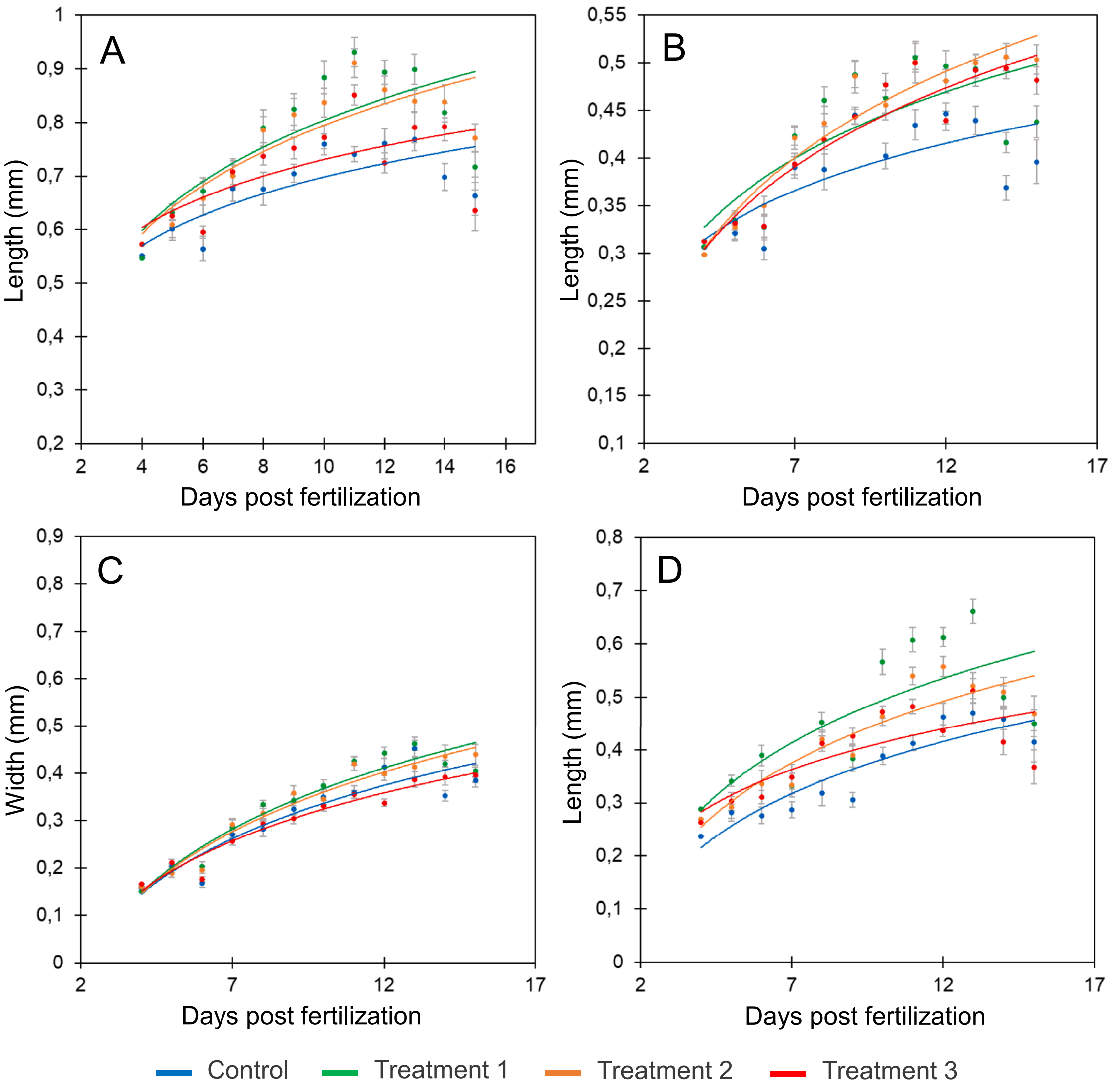

Fig. 6. Effects of chronic exposure to a mixture of trace elements on development of larvae of *Paracentrotus lividus* (A: total length; B: body length; C: body width and D: arm length) produced by sea urchins from Albo (contaminated site) for concentrations measured in an old asbestos mine at Canari (Corsica. France) and 5- to 50-fold higher concentrations (experiment 3).

(Fernandez and Beiras, 2001). In order to determine the effects of trace elements on marine organisms, it is necessary to consider both low concentrations and a mixture of several contaminants. This approach is difficult due to the large number of possible combinations and interactions in the mixture. The toxicity of a chemical can be increased (synergy), reduced (antagonism) or unaffected (no interaction) by the presence of another toxicant (Amiard, 2011; Fernandez and Beiras, 2001). According to Fernandez & Beiras (2001), from the environmental standpoint, the main purpose is to predict the toxicity of mixtures of pollutants, rather than to classify the combinations of toxicants by their types of interaction. Therefore, the main aim of this study was to evaluate the impact of trace element mixtures measured in the environment. Mixtures of seven and fifteen classic and emerging trace elements were tested to better understand the impact on sea urchins considering contamination levels found in the environment.

Larvae contaminated with the mixture of fifteen trace elements have a higher total length associated with the significantly longer arms likely resulting from morpho-anatomical abnormalities. Numerous larval malformations have already been observed in the literature, skeletal anomalies being the main consequences (*e.g.* Kobayashi and Okamura, 2004; Roccheri et al., 2004). The sensitivity of skeletogenesis related to specific contaminants has been widely demonstrated (e.g. Moureaux et al., 2011; Radenac et al., 2001). Malformations and skeletal developmental delays in pluteus are mainly caused by Zn, Cd and Co (*e.g.* Kobayashi and Okamura, 2004; Mannaerts, 2007). Longer arms have likely been caused by skeletal elongation of the larvae even if no evidence of malformations was detected during stereomicroscopic observations, as described at Carbaillera *et al.* (2012). Nickel may also play a role because its presence is frequently associated with decreased Ca^{2+} uptake (Blewett et al., 2016) which is essential for the calcification of the internal skeleton (Tellis et al., 2013). Several authors have reported a decreased skeletal formation at higher Ni concentrations (Hardin et al., 1992; Kobayashi and Okamura, 2004). Larvae with significantly reduced body length and width observed in the contaminated rearing tanks seem to confirm this hypothesis. Recently, skeletal integrity has been reported as a more sensitive criterion compared to description of malformations or EC_{50} determination, ranking abnormalities according to the severity of skeletal alteration (Carballeira et al., 2012). However, Buttino et al. (2016) suggest that morphological abnormalities are not always determined by incorrect skeletal structures. In this experiment, the differences reported are not necessarily due to skeletal structures, but may also lead to decreased mobility making feeding, predator avoidance and settlement more difficult (O'Donnell *et al.*, 2010). Besides the measurement data, no signs of malformation were visible under

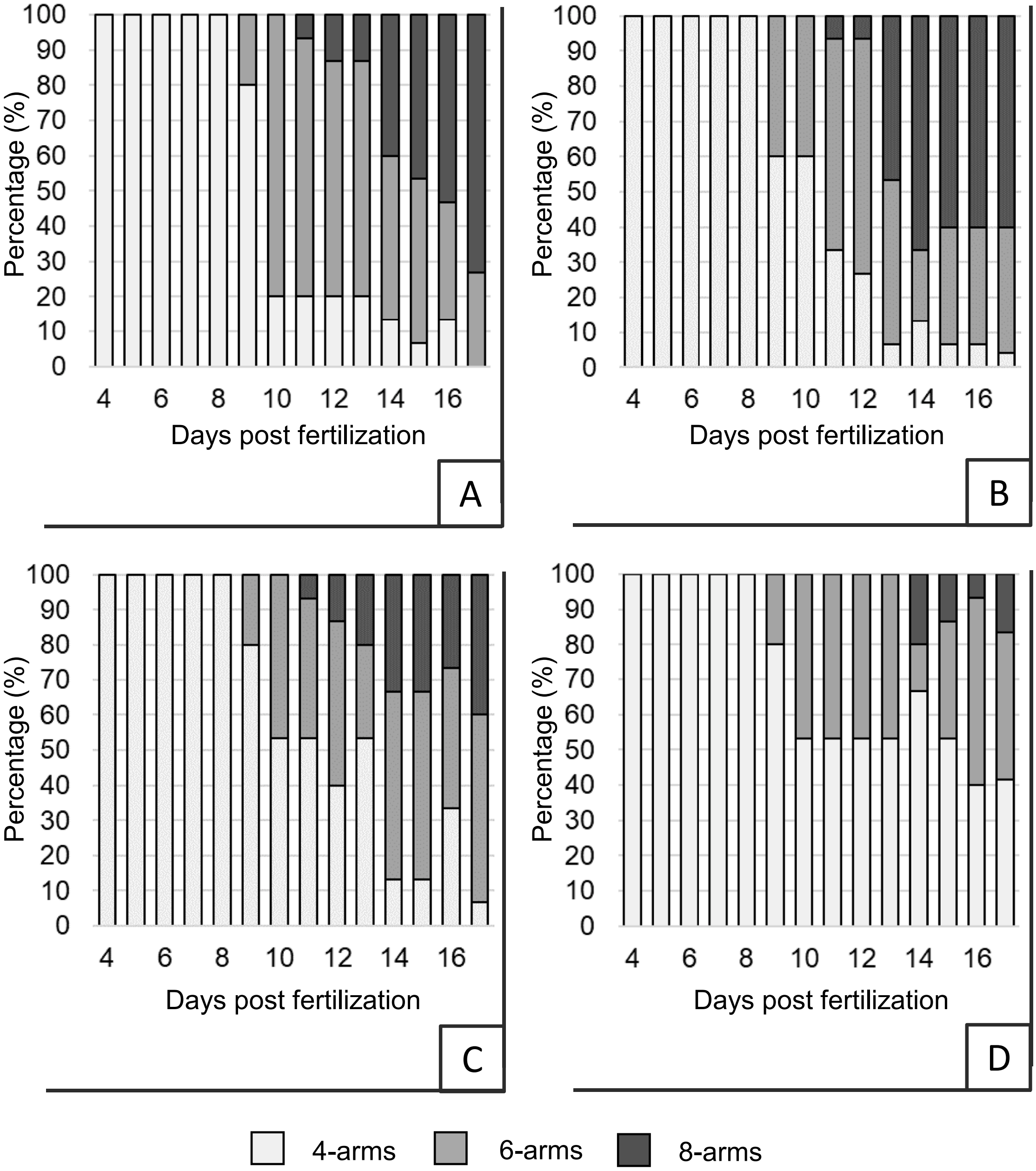

Fig. 7. Effects of chronic exposure to a mixture of trace elements on stage development of *Paracentrotus lividus* larvae produced by sea urchins from Calvi (experiment 2; A: control; B: treatment 1; C: treatment 2 and D: treatment 3).

stereomicroscopy. Thus, despite a broad mixture of trace elements, the reduced concentrations may be insufficient to cause malformations, confirming that larval measurements are essential to clarify the effects of low contaminant levels on larval development. Fernandez & Beiras (2001) indicate that larval length reflects a more gradual response to trace elements concentration allowing a better sensitivity of the bioassay with low levels of contamination.

Higher number of trace elements could induce more synergistic effects causing the arm elongation observed in experiment 1. Indeed, arm and total length tended to be greater when larvae were contaminated with fifteen trace elements (experiment 1) compared to larvae contamined with seven trace elements (experiment 1). Synergistic effects are known for mixtures of trace elements such as Cu-Zn (MacInnes, 1981), Hg-Pb (Fernandez and Beiras, 2001), Cu-Ag (Coglianese and Martin, 1981), Mn-Mo (Morgan et al., 1986). However, since the notion of synergy or antagonism depends on the organism studied, it will be necessary to be vigilant. For example, the Zn-Cd mixture has been reported as synergistic for white shrimp, *Litopenaeus setiferus* (Vanegas et al., 1997), and antagonistic for bivalve embryos (Pavicic, 1980). Furthermore, the toxicity of trace elements depends on abiotic (*e.g.* pH, salinity) or biotic (*e.g.* organic ligands) factors (Fernandez and Beiras, 2001). The high number of trace elements probably also increases competitive interactions with essential ions due to the chemical similarity (Nogueira et al., 2021). This could explain a body length and width tending to be smaller in larvae contaminated with the mixture of 15 trace elements.Overall, the results obtained in experiment 1 on the development of the larval stages showed few differences for the 4- and 6-arm stages. However, there was a significant delay in the development of 8-arms stage in contaminated larvae. This delay is even greater as the number and concentration of trace elements increases. This may be related to morpho-anatomical abnormalities causing elongation of the arms. Therefore, it is likely that mixing trace elements induces slowing

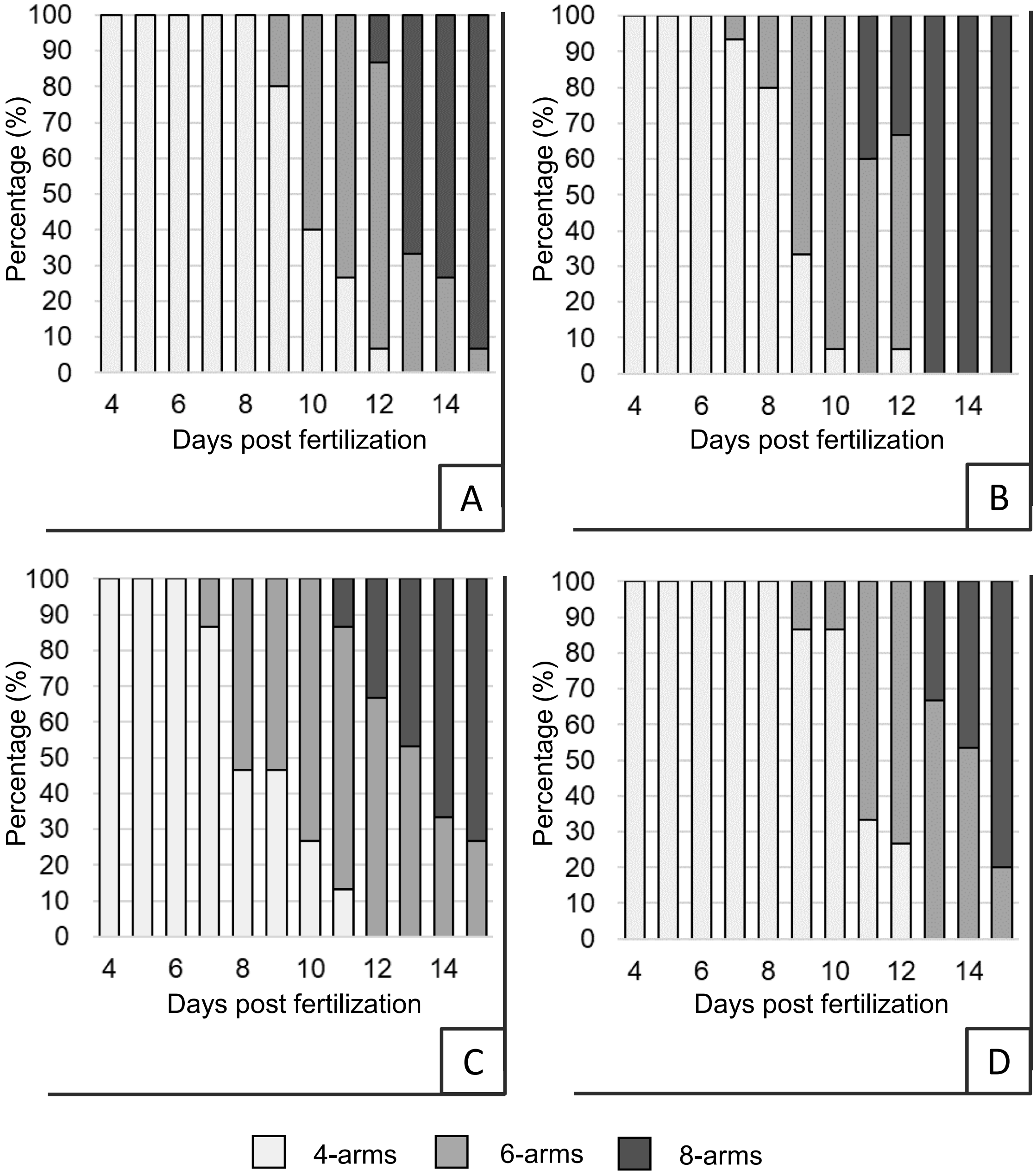

Fig. 8. Effects of chronic exposure to a mixture of trace elements on stage development of *Paracentrotus lividus* larvae produced by sea urchins from Albo (experiment 3; A: control; B: treatment 1; C: treatment 2 and D: treatment 3).

or inhibition of larval development as concentrations increase. Several authors highlighted growth inhibition linked to the concentration of trace elements (Chiarelli et al., 2014; Filosto et al., 2008; Roccheri et al., 2004). According to MacInnes (1981), the degree of synergy is greater as the concentrations in mixtures increase. This confirms the hypothesis of an inhibition of larval development when number and concentrations of trace elements increase. Furthermore, exposure time appears to be a factor in the occurrence of abnormalities and developmental delays (Filosto et al., 2008) confirming the results observed in experiment 1 where the larvae were contaminated for 22 days. According to many studies, first developmental stages of marine invertebrates were recommended for performing bioassays due to their sensitivity to contaminants (*e.g.*, Paredes and Bellas, 2015; Pétinay et al., 2009). This high sensitivity may be due to the important processes and vital events that occur during the early hours of development (Paredes and Bellas 2015). In this study, the stage at which the differences between control and contaminated larvae tended to be greatest was near the end of the larval cycle. Therefore, it appears of interest to study the entire larval cycle when the aim is to understand the real effects of contamination on the organisms.

In experiments 2 and 3, dealing with contamination near the old asbestos mine, larvae contaminated at an average concentration (treatment 1) tended to be significantly larger in size and to develop more quickly. This may be due to the many essential trace elements present in the contaminant mixture, which are of nutritional interest and play a role in the enzyme systems (Chiarelli and Roccheri, 2014). A phenomenon of hormesis has already been described in the literature (*e.g.,* Nogueira et al., 2020; Pétinay et al., 2009), which is a biphasic dose-response relationship characterized by improvement of the biological aptitude at low doses and inhibitory or toxic effects at high doses (Mattson, 2008). Pétinay et al. (2009) observed a relative increase in the size of larvae with a concentration of 10 µg L^{-1} of Cu which they describe as the result of this phenomenon. Thus, the biological response to organic or inorganic stress does not necessarily lead to a negative effect on short-term growth. Therefore, each effect, positive or negative, must be interpreted with some caution.

Larvae from contaminated sea urchins tend to be wider than larvae from reference sea urchins in contaminated rearing tanks. Larvae contaminated at the average concentration tend also to develop faster when the spawners are from Albo. These results are surprising because the most common assumption would have been that larvae respond less well to contamination when they are from contaminated sea urchins. Indeed, according to several authors, the gametes of lower quality are more likely to be sensitive to a toxic agent (*e.g.* Bougis et al., 1979; Pétinay et al., 2009). Bougis et al. (1979) suggest that the effect of Cu is variable depending on the quality of the spawners. The effects of trace elements could be variable depending on the biochemical composition of the eggs, the genetic quality of the spawners or the water temperature (Bougis et al., 1979). This would also explain the different results obtained between the various authors (Pétinay et al., 2009). Here, the temperature in rearing tanks was controlled to remain at 20 °C. Then, the first hypothesis would assume that the sea urchins from Calvi were subject to other sources of pressure; therefore, the spawners would have gametes of less good quality. However, the site has been described as a reference site in the literature (El Idrissi *et al.*, 2020; Richir et al., 2013). The second hypothesis would be that the hormesis phenomenon is greater in larvae from contaminated spawners. These larvae would be more sensitive to toxic agents and the stress induced by contaminants allowing a higher growth rate. A last hypothesis is that prior exposure of sea urchins from Albo to contaminants may make their gametes more resistant (Crean and Immler, 2021; Wells et al., 1998). It would be interesting to investigate this hypothesis by performing biochemical analyses in order to understand if there is an oxidative stress or a higher expression of certain genes in larvae related to spawner contamination. These hypotheses demonstrate the importance of considering spawner quality in ecotoxicological studies. Pétinay et al. (2009) propose to use standardization of spawner conditioning to achieve the same quality and avoid bias. Cryopreservation of gametes or fertilized eggs may simplify the technique and make it available to all laboratories (Paredes and Bellas, 2015). However, to assess the impact of contaminants at site-scale, it would be more appropriate to perform the tests onorganisms from the same study site in order to have a more reliable response and better visibility regarding the consequences in the environment.

5. Conclusion

This study has contributed to better understanding of the effects of chronic exposure to trace element contamination on the entire larval stage of *Paracentrotus lividus*. No visible malformations were observed at the concentrations studied. However, the increase in the number and concentration of trace elements caused an elongation of the arms and a delay in the larval development which increased with time. Therefore, this work highlights the need to consider the entire larval cycle of the sea urchin. In contrast, the larvae contaminated by 7 trace elements (Co, Cu, Cr, Fe, Hg, Ni and Zn) developed more quickly. This may be due to the several essential trace elements present in the mixture but also to a phenomenon of hormesis already observed in the literature for certain trace elements. The origin of the spawners also plays a role in the results observed in this study. It is therefore necessary to include this factor in the ecotoxicology bioassays.

CRediT authorship contribution statement

O. El Idrissi: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **S. Gobert:** Writing – review & editing, Funding acquisition, Resources. **A. Delmas:** Investigation. **M. Demolliens:** Investigation. **A. Aiello:** Funding acquisition, Resources. **V. Pasqualini:** Writing – review & editing, Funding acquisition, Resources. **S. Ternengo:** Writing – review & editing, Funding acquisition, Resources.

Declaration of Competing Interest

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

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Supplementary materials

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