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Influence of Ploidy and Metal–Metal Interactions on the Accumulation of Ag, Cd, and Cu in Oysters Crassostrea Gigas Thunberg

J.-C. Amiard[1*], H. Perrein-Ettajani[2], A. Gérard[3], J. P. Baud[4] and C. Amiard-Triquet[1]

[1]ISOMer, GDR 1117 du CNRS, SMAB, Two Rue de la Houssinière, Service drsquoEcotoxicologie, BP 92208, Nantes Cedex 3, France
[2]IRFA, CEREA, Angers, France
[3]Laboratoire de Génétique et Pathologie, IFREMER, La Tremblade, France
[4]Laboratoire Conchylicole des Pays de Loire, IFREMER, Bouin, France

*: Corresponding author : jean-claude.amiard@isomer.univ-nantes.fr

Abstract: The present study was designed to compare the response to contaminants in diploid with triploid specimens of the oyster Crassostrea gigas. The reproduction investment in bivalve molluscs has priority on somatic growth. Thus, genetic sterilization by triploidy induction enables the energy flux to be directed toward somatic growth and glucide storage. Bioaccumulation was examined for Ag (10 mgrg/L), Cd (10 mgrg/L), and Cu (30 mgrg/L) to determine if the response to metals follows similar patterns in diploid (2n) and triploid (3n) groups. The effect of ploidy was also evaluated as a function of dry weight of soft tissue and condition index. Moreover, the reciprocal influence of these metals on their incorporation was studied. The results showed that the major factor governing the degree of metal bioaccumulation by oysters is the nature of the metal introduced in the experimental medium. Thus, the uptake of Cd is proportionally more important than in the case of Ag and even more in Cu. For Cu-treated samples, the influence of ploidy on weight and metal body burden (and Cu concentration) was not significant, whereas for Ag and Cd, significant differences according to genetic type were evidenced by higher tissue weight and lower concentrations in triploid than diploid specimens. Metal–metal interactions study especially showed a reciprocal antagonism between Ag and Cu.

Keywords: Polyploidy / Metal bioaccaumulation / Oyster culture

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J.C. Amiard [1], H. Perrein-Ettajani [2], A. Gérard [3], J.P. Baud [4] and C. Amiard-Triquet [1]

1- *ISOMer*, GDR 1117 du CNRS, SMAB, Service d'Ecotoxicologie, 2, rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France

2- IRFA, CEREA, 3 place André-Leroy, BP 808 – 44008 Angers Cedex 01, France

3- IFREMER, Laboratoire de Génétique et Pathologie, BP 133, Ronce-les-Bains, 17390 La Tremblade, France

4- IFREMER, Laboratoire Conchylicole des Pays de Loire, Polder des Champs, 85230 Bouin, France

Corresponding author: Amiard J-C, *ISOMer*, SMAB, Service d'Ecotoxicologie, 2, rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France.

Tel/FAX (33) 2 51 12 56 77; E-mail : Jean-Claude.Amiard@isomer.univ-nantes.fr

Abstract

The present study has been designed to compare the response to contaminants in diploid and triploid specimens of the Oyster *Crassostrea gigas*. The reproduction investment in bivalve molluscs has priority on somatic growth. Thus, genetic sterilization by triploidy induction enables the energy flux to be directed towards somatic growth and glucide storage. Bioaccumulation has been examined for silver (10 µg/l), cadmium (10 µg/l), and copper (30 µg/l), to determine if the response to metals follows similar pattern in diploid (2n) and triploid (3n) groups. The effect of ploidy was also evaluated as a function of dry weight of soft tissue and condition index. Moreover, the reciprocal influence of these metals on their incorporation has been studied. The results show that the major factor which governs the

degree of metal bioaccumulation by oysters is the nature of the metal introduced in the experimental medium. Thus, the uptake of Cd is proportionally more important than in the case of Ag and more over Cu. For copper treated samples, the influence of ploidy on weight and metal body burden (and Cu concentration) was not significant, whereas for Ag and Cd, significant differences according to genetic type have been evidenced with higher weight of tissues and lower concentrations in triploid specimens than in diploid individuals. Metal-metal interactions study shows especially a reciprocal antagonism between silver and copper.

Introduction

Since sites at risk for metal contamination such as coastal areas are also often devoted to shellfish culture, bioaccumulation has given rise to an extensive literature (cited in: Cossa and Lassus, 1989; Sanders and Abbe, 1989; Berthet *et al.*, 1992; Amiard and Berthet, 1996). Moreover, mussels and oysters are widely used as biological indicators in national and international monitoring programs (Golberg *et al.*, 1983; Goldberg, 1986; Bayne, 1989; Claisse *et al.*, 1992; RNO, 1995; Garcia-Rico *et al*., 2001).

It has been shown that oysters exposed to silver under similar experimental conditions exhibited highly variable silver levels in their soft tissues. This variability was independent on soft tissues weight, sexual maturity and seasonal factors, although these oysters were sampled from distinct populations. Therefore, their genetic characteristics may have induced different responses (Berthet *et al.*, 1992).

More recently polyploid oysters have become available at the commercial level, therefore rising questions concerning their bioaccumulation capacity compared to diploid oysters. The high performance of triploid shellfish is known (Allen and Downing 1991; Hand and Nell, 1999; Ruiz-Verdugo *et al*., 2001) and it is in weight that the advantages of triploids over diploids are most obvious. Thus, Hand *et al*. (1998a) found that triploids of the rock oyster

Saccostrea commercialis were on average 30.7 % heavier than sibling diploids. Triploid organisms are totally or partially sterile, so that a change in allocation of energy from reproduction enables them to have a better growth (Stanley *et al*., 1984).

Literature on disease resistance of triploid oysters is available and shows that, in general Pacific oyster seems to be resistant to disease and associated mortality caused by parasites (in the review of Nell, 2002). Whereas, considering pollution stress, there is no study comparing heavy metal bioaccumulation in diploid and triploid oysters. Thus, the present study was designed to compare the response to contaminants in diploid and triploid specimens. Bioaccumulation has been examined for silver, cadmium and copper to determine if the response to metals follows similar pattern in diploids (2n) and triploids (3n).

Moreover, the phenomenon of mutual influence of heavy metal ions on the kinetics of each individual element was studied. This is of importance in the light of the use of bivalve molluscs in monitoring heavy metals in seawater. In addition, when pollution in aquatic environments occurs, it is often a matter of pollutants mixture.

Materials and methods

Gametes handling and fertilization

During 1992, oysters were conditioned i.e. brought to sexual maturation, in an open circulating system at 20°C. Sperm and oocytes were stripped from the gonads. Four males and four females were used for fertilization. The stripped gametes were filtered on a 45 µm sieve for males and on a 75 µm sieve for females and then resuspended in 3 µm filtered sea water (FSW). The oocyte number was adjusted to a concentration of 100/ml and fertilization was achieved with a ratio of 50 spermatozoa per ovum.

Triploidy Induction

Oyster embryos were concentrated in one liter of FSW at 25°C and treated with 300 µM/l (or 49 mg/l) of 6-dimethylaminopurine (6-DMAP) (Desrosiers *et al.* 1993). Induction was achieved 15 min after fertilization over a period of 15 min. After treatment, the 6-DMAP was thoroughly washed off the embryos by filtering the samples on a 10 µm sieve. An untreated batch was kept as diploid control.

Larval and spat culture

Following treatment, embryos were incubated at a density of about 50 larvae per ml for 24 h in 30 liter tanks at 24°C. The D-shaped larvae were then collected, counted and diluted to 5 larvae per ml. Every other day, the seawater was changed. Larvae were fed with a mixed diet of *Isochrysis sp., Pavlova lutheri* and *Chaetoceros calcitrans* at a rate of 20 cells/µl/day for each algae species. A measurement of size and percentage survival was made every 2 or 3 days until metamorphosis. The eyed larvae (with pigmentation spot) were then transferred into 150 µm sieves with a flow through system of unfiltered seawater (salinity: 35). Oyster shell cultch was used for larval settlement. The seawater was enriched every day with a mixture of 3 algae species out of the 6 species routinely produced in the hatchery (*Isochrysis sp., Isochrysis* (Tahiti clone), *Platymonas suecica, Pavlova lutheri, Chaetoceros* (Pumilum clone) and *Chaetoceros calcitrans*). The sieves were washed daily and changed regularly, following the spat growth.

Ploidy determinations

The ploidy levels were determined by image analysis on spat. This analysis provides the optical density of Feulgen-stained nuclei (Gérard *et al.* 1994 a,b). By comparison with the diploid control, it is determined whether the spat is diploid or triploid. Image analysis was conducted on 5 month old spat, and triploid percentage was up to 90% in the treated sample.

Procedures of rearing and exposure

After 5 months of nursing (Baud and Bacher, 1990; Bacher and Baud, 1992), diploid and triploid specimens were selected among each experimental population by using a sieve with a mesh-size of 18 mm to obtain individuals with identical size. Then young oysters were exposed for 15 d to metals introduced in seawater as nitrates diluted in HNO_3 0.5 mol/l: 10 µgAg/l, 10 µgCd/l and 30 µgCu/l. These concentrations were chosen to induce measurable bioaccumulation and to be realistic with concentrations encountered in polluted environments (Table 1). The concentrations of stock solutions and the subsequent dilutions were chosen to retain the normal pH of sea water. Thirty individuals belonging to each genetic type were submitted to these different conditions of exposure and similar groups were reared concomitantly as controls. Each experimental group was distributed in three containers leading to a density of one specimen per liter. They were fed with lyophilized *Spirulina* once a day, 1 h before renewing water and contaminants.

At the end of the experiment, several factors were determined in each individual to calculate condition index: Dry weight of soft tissues / Total weight minus shell weight x 1000 (Lawrence and Scott, 1982). Then the soft tissues were prepared to trace element analysis by atomic absorption spectrometry (AAS).

Metal determination by atomic absorption spectrophotometry

Soft tissues of individual oysters were dried to constant weight (85°C) then digested at 95°C with HNO_3 and the total volume was adjusted to 4 ml with deionised water. Trace elements were analyzed in these solutions by using a polarized Zeeman atomic absorption spectrophotometer (Hitachi Z 8100). These analyses were achieved according to the method described by Amiard *et al.* (1987) and validated through internal quality control (USNBS,

standard reference material SRM 1566, oyster tissue) and by international intercalibration exercises (Coquery and Horvat, 1996).

Statistics

Analyses were carried out using the microcomputer statistical package Stat View II. Differences in soft tissues weight and condition index were compared using ANOVA Post Hoc Multiple Comparisons between all groups. This analysis was also carried out to compare Ag levels (body burdens and concentrations) in diploid and triploid oysters exposed to Cd or Cu or used as controls. Ag levels in diploid and triploid oysters exposed to Ag were compared using t-test. The same procedure was used for Cd and Cu.

Results

Biological factors' variability

Probability data are shown in table 2, mean values in table 3.

Dry weight of soft tissues. Ploidy had a significant influence on this factor ($p = 0.0240$). Except in oysters exposed to Cu, triploid specimens showed mean dry weight higher than those registered in diploid oysters. On the other hand, the conditions of exposure have not induced significant changes ($p = 0.5899$).

Condition index. Neither the genetic type nor the conditions of exposure have induced significant changes of the condition index ($p = 0.9491$ and $p = 0.2909$, respectively). However, the combination of these factors had a significant influence ($p = 0.0255$) and the highest index was observed in diploid oysters exposed to copper.

Metal bioaccumulation in soft tissues

Metal body burdens (Fig. 1) and concentrations (Table 4) were increased approximately tenfold in Ag-contaminated oysters (Fig. 1A) compared to controls (C), whereas this ratio was about 150 for cadmium (Fig. 1B), and only 5 for copper (Fig. 1C).

Metal-metal interactions

Probability data are shown in table 2, mean values in Fig. 1.

Silver body burden (Fig. 1A). In oysters exposed to Cd or Cu, the background level of silver was significantly modified ($p = 0.0001$) by the presence of these contaminants but opposite effects were observed : Cu induced Ag depletion in both diploid and triploid specimens in which Ag levels represented respectively 71 and 54 % of controls. Ag enhancement due to cadmium was patent only in the diploid group (140% compared to control).

Cadmium body burden (Fig. 1B). It was significantly enhanced ($p = 0.0005$) in oysters exposed to other contaminants. Cd body burden of oysters exposed to Ag and Cu represented respectively 110 and 115% of controls in diploid specimens, 131 and 132% in triploid specimens.

Copper body burden (Fig. 1C). In oysters, not being exposed to Cu, the presence of other contaminants in the experimental environment induced significant changes ($p = 0.0001$). Cu body burden of oysters exposed to Ag represented 73 to 80% of controls. The effect of Cd was negligible.

The balance of metal quantities taken up or released is presented in Table 5.

Influence of ploidy on metal levels in soft tissues.

Probability data are shown in table 2, mean values in Fig. 1.

Silver body burden (Fig. 1A). In oysters, not being exposed to Ag (C = controls and exposed to Cd or Cu), the body burden was influenced significantly ($p = 0.0001$) by the genetic type. It was lower in diploid specimens, corresponding to 64, 85 and 83% of values in triploid groups respectively in controls and specimens exposed to Cd or Cu. In oysters exposed to 10 µg Ag/l, the influence of ploidy was reversed with the highest Ag body burden observed in diploid individuals (t-test, $p < 0.001$).

Cadmium body burden (Fig. 1B). In oysters, not being exposed to Cd (C = controls and exposed to Ag or Cu), the level of this metal was influenced by the genetic type ($p = 0.0006$). The most important difference between diploid and triploid specimens has been registered among controls: the Cd body burden in triploid oysters was 77% of the body burden in diploid individuals whereas this percentage was about 90% in oysters exposed to Ag or Cu. In oysters exposed to 10 µg Cd/l, Cd body burden was also slightly higher in diploid specimens than in triploid individuals (t-test, $p < 0.05$).

Copper body burden (Fig. 1C). T-test applied to oysters exposed to 30 µg Cu/l and ANOVA applied to controls and specimens exposed to other contaminants have not evidenced significant differences in copper accumulation according to the genetic type.

If concentrations in soft tissues rather than body burdens were taken into account, the changes with genetic types and conditions of exposure showed the same trends (Table 2). In the case of cadmium concentrations the higher weight of soft tissues of triploid specimens (Table 4) emphasized the difference yet observed considering body burdens.

Discussion and conclusion

This study shows that the major factor which governs the degree of metal bioaccumulation is the nature of the metal introduced in the experimental medium. Thus, the uptake of Cd by oysters is more important than for Ag and more over Cu. The ability of bivalves to concentrate Cd to a greater extent than organisms of other zoological groups has been evidenced in a comparative laboratory study by Eisler and Gardner (1973). Comparisons between several bivalve species collected from the same area (Frazier, 1975): Chesapeake Bay, USA; Berthet *et al*., 1986: Bay of Bourgneuf, France) indicate that under conditions of equivalent environmental Cd (and Zn) exposure, oysters reach the highest tissue concentrations. In control diploid and triploid oysters metal concentrations (Table 4) are mostly very similar to those reported in RNO data where oysters from Manche – Atlantic sites accumulated 6.11 ± 4.1 mg/kg for silver (RNO, 2001), 2.25 ± 1.20 mg/kg for Cd and 138 ± 102 mg/kg for copper (RNO, 1995).

With regard to ploidy level, bioaccumulation processes were variable and function of metal characteristic and concentration. While no difference was observed for diploid and triploid oyster exposed to Cu, bioaccumulation was limited for triploids exposed to Ag and Cd. This cannot be explained by a biological dilution of these metals in the significant high dry tissue weight of triploid (except for the copper group) as the same trends were observed when considering either metal body burden or concentration.

The oysters used in the present study were from the same size and from nearly six month of age. Triploids were, in general, heavier than diploids. This finding agrees with several observations in Pacific oysters reviewed by Nell (2002). Wang *et al*. (1999) however, reported that for *C. gigas* sampled at one year of age, there was no significant difference in body weight between triploid and diploid groups. Whereas, body size measurement at 1 year of age showed that triploids were significantly bigger than normal diploids (14%) although

there were considerable variation among replicates (Wang *et al*., 2002). Some parameters as the size (Hand *et al*., 1999) or food may have a significant effect on subsequent growth of triploids. Thus, in an environment where food supplies are limited, triploids may not be larger than diploids before sexual maturation (Guo and Allen, 1994).

Diploid oysters were in an early stage of gametogenesis, greater metal bioaccumulation in diploids may be related to physiological changes associated with the reproduction state with gonads more developed than in triploids. Gould *et al*. (1988) showed that Cd and Cu increased in the gonad tissue of scallops exposed to these metals, indicating that the gonad is not so efficient in riding itself of toxic metals as the kidney (Fowler and Megginson, 1986 *in* Gould *et al*., 1988). Moreover, the gonads of *Ostrea edulis*, exposed to Cu and Zn, were the organs producing the greatest quantity of proteins such as proteins metallothioneins (MTs), followed by the mantle, gut and by muscle and plasma (Alonso and Martin-Mateo, 1996). The particular behavior of Cu in this study (no differences in weight and metal body burden with the ploidy level) compared to Ag and Cd may be due to the fact that this essential element exhibits a strong inhibitory effect on gamete production and maturation (Gould *et al.*, 1988) so that no significative differences were observed between diploids and triploids. In the contrast, Cd appeared to stimulate early gamete maturation and spawning (Gould *et al*., 1985 *in* Gould *et al*., 1988).

In addition, triploids and diploids show different pattern of energy storage and utilization (Allen and Downing, 1986). Energy stored in the form of glycogen prior to gametogenesis is utilized during periods of high metabolic demand such as during the production of gametes. The sterility of triploids is associated with lower metabolic energy costs, thus more energy is available for growth or survival under stressful conditions (Hawkins and Day, 1996) which may be parasite infestation (Hand *et al*., 1998b) or heavy metals exposure. Some studies revealed the role of the heterozygosity of triploids in the performance (Garnier-Géré *et al*., 2002; Stanley *et al*., 1984). Thus, the higher expected heterozygosity of triploids supports the

hypothesis of a positive influence of this parameter on metal detoxification and excretion processes being more efficient in triploids as the transcription is faster with three copies of each gene (Garnier-Géré *et al*., 2002).

The positive role of heterozygosity upon feeding rate and absorption efficiency (Hawkins *et al.*, 2000) has also been emphasized. This suggests a higher metal level in triploids relative to diploids which is in contrast with our finding. Kersacodi-Watson *et al*. (2001) however reported that no major differences in feeding and energetic between the ploidy in either juvenile or adult *S. commercialis* and the only significant differences between ploidy conditions were in pseudofeces and excretion in adult oysters. In this experimental study, the food regime was at the standard level for the two groups in a way to prevent pseudofeces production.

This investigation has provided also evidences of metal-metal interactions in the oyster *Crassostrea gigas* with a reciprocal antagonism between silver and copper. This reciprocal influence was demonstrated in *Crassostrea gigas* (Ettajani *et al.*, 1992). Literature considering different metal co-exposure and levels is available, but less uniformity exists about the influence of the accumulation of each other (Table 6). Metallothioneins (MTs) are low molecular weight proteins with a high content of certain trace metals. It is generally assumed that they play a central role in essential metal (Cu, Zn) homeostasis but their role in metal detoxification has been frequently evoked (Carpene, 1993). The presence of MT has been demonstrated in numerous species of invertebrates including oysters (Roesijadi *et al*., 1989). Competition for MT binding sites has been proposed as an explanation for metal-metal interactions (Ray *et al*., 1980; Skwarzec *et al*., 1984; Weiss *et al*., 1986; Holwerda, 1991; Pelgrom *et al*., 1994). Toxic elements such as Ag and Cd show a greater affinity for MT than essential metals (Otvos *et al*., 1993) but the ratio between the quantities of different metal ions interfere with competitive affinities. The concentrations of toxic elements are several

orders of magnitude lower than those determined for essential metals as shown in tables 1 and 4.

The high performance, in terms of survival (Hand *et al*., 1998b), growth (Allen and Downing, 1986), meat condition (Hand and Nell, 1999), flavor and texture (Allen and Downing, 1991) of triploid oysters compared to diploids had improve the profitability of farming triploid molluscs. In addition, the less metal bioaccumulation by triploids shown in the present study might be of interest for commercial purposes in several French rearing areas considering new EU directives reducing to halve legal thresholds.

Additional experiments have to be undertaken as *in situ* contamination, biochemical composition, and MT(s) characterization, both for diploid and triploid oysters, to understand better factors underlying these differences.

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Table 1 - Exposure of oysters to metals : comparison of experimental environmental concentrations.

	Ag	Cd	Cu
Typical nearshore seawater (ng/l)	0.1-5[a]	19-26[b]	291-675[b]
Polluted nearshore seawater (µg/l)	max. 2.7[c]	0.3-15[d]	2.5-126[d]
Experimental overloads (µg/l)	10	10	30

(a) In : Sanders and Abbe, 1989 ; (b) NRC Canada Reference Materials CASS-1, CASS-2 ; (c) Bowen, 1985 ; (d) In: Bryan, 1984.

Table 2 - P. values associated with the influence of the ploidy (A), conditions of exposure (B) and combined effect of both factors (AB) on biological and contamination factors (ANOVA Post Hoc Multiple Comparisons).

		A	B	AB
Dry weight		0.0240	0.5899	0.5180
Condition index		0.9491	0.2909	0.0255
Body burden	Ag	0.0001	0.0001	0.0506
	Cd	0.0006	0.0005	0.4165
	Cu	0.4913	0.0001	0.6421
Concentration	Ag	0.0343	0.0001	0.4100
	Cd	0.0001	0.0091	0.1456
	Cu	0.1132	0.0001	0.5698

Table 3 - Biological characteristics of experimental oysters (mean, standard error)

Genetic type	Conditions of exposure			
	Control	Ag(10µg/l)	Cd (10µg/l)	Cu (30µg/l)
		Dry weight of soft tissues (mg)		
2n	118(13)	119(10)	114(12)	133(15)
3n	133(14)	133(13)	131(15)	131(17)
		Condition index *		
2n	57.0 (1.0)	58.5 (2.1)	56.6 (2.1)	68.9 (4.6)
3n	61.0 (2.1)	60.5 (2.6)	61.6 (3.0)	58.4 (3.3)

* Dry weight of soft tissues/Total weight minus shell weight x 1000 (Lawrence and Scott, 1982).

Table 4 - Crassostrea gigas. Metal concentrations in soft tissues in µg/g dry weight (mean, standard deviation).

Genetic type	Conditions of exposure			
	Control	Ag	Cd	Cu
		Ag concentrations in soft tissues		
2n	0.99 (0.08)	10.0 (1.0)	1.47 (0.13)	0.61 (0.06)
3n	1.39 (0.13)	7.6 (0.6)	1.58 (0.20)	0.74 (0.09)
		Cd concentrations in soft tissues		
2n	1.08 (0.04)	1.14 (0.07)	150 (9)	1.12 (0.05)
3n	0.74 (0.05)	0.96 (0.06)	127 (8)	0.98 (0.04)
		Cu concentrations in soft tissues		
2n	368 (13)	294 (14)	405 (18)	1 698 (64)
3n	366 (25)	268 (15)	365 (17)	1 703 (117)

Table 5 - Balance of metal quantities (nmol/individual) taken up or released over two weeks of exposure to Ag, Cd or Cu.

Genetic type	Contaminant	Uptake of contaminant	Uptake (+) or release (-) of other element	
2 n	Ag	9.5	Cd + 0 .1	Cu - 133
3 n		7.2	+ 0.3	- 202
2 n	Cd	154	Ag + 0.4	Cu + 52
3 n		144	+ 0.1	- 9
2 n	Cu	2917	Ag - 0.3	Cd + 0.2
3 n		2907	- 0.7	+ 0.3

Table 6 - Literature sources dealing with metal-metal interactions in marine bivalves.

Pair of metals*	Conditions of exposure	Biological model	Effect of bioaccumulation
	Effect of a toxic on bioaccumulation of an essential element		
Ag/Cu	10 µg Ag/l	*Crassostrea gigas*	Antagonism[a,b]
Cd/Cu	10 µg Cd/l	*Crassostrea gigas*	No noticeable effect[a]
	10 µg Cd/l + 10 µg Cu/l	*Mytilus edulis planulatus*	No effect[c]
	+ 50 µg Pb/l + 100 µg Zn/l		Antagonism[d]
	0.5 µg Cd/1	*Mizuhopecten yessoensis*	Antagonism[e]
	2 µg Cd/µl + 0.5 µg Cu/ml	*Anadara granosa*	
	Reciprocal effects of toxic elements		
Ag/Cd	10 µg Ag/l	*Crassostrea gigas*	Synergy[a]
Cd/Ag	10 µg Cd/l	*Crassostrea gigas*	Synergy in diploid specimens only[a]
	Effect of an essential element on bioaccumulation of toxic elements		
Cu/Ag	30 µg Cu/l	*Crassostrea gigas*	Antagonism[a]
	30 µg Cu/l	*Crassostrea gigas*	Synergy (significant only after 21 d exposure)[b]
Cu/Cd	30 µg Cu/l	*Crassostrea gigas*	Synergy[a]
	10 µg Cu/l	*Mytilus edulis planulatus*	No interaction[f]
	20 µg Cu/l	*Mytilus edulis planulatus*	Antagonism[f]
	0.01-1 mg Cu/l	*Mytilus edulis*	
		visceral mass	No effect[g]
		gills	Synergy at low doses, no effect at the highest dose[g]
		remainder	Antagonism[g]
	Cu 0.5 µg/ml	*Mytilus edulis* gills	No interaction[h]
	+ Cd 0.01 - 60 µg/ml		

* The studied effect is the one of metal M1 on accumulation of metal M2

a) present study ; b) Ettajani *et al*., 1992 ; c) Ritz *et al*., 1982 ; d) Evtushenko *et al*., 1986 ; e) Patel and Anthony, 1991 ; f) Elliot *et al*., 1986 ; g) Berthet *et al*., 1985 ; h) Carpene and George, 1981.

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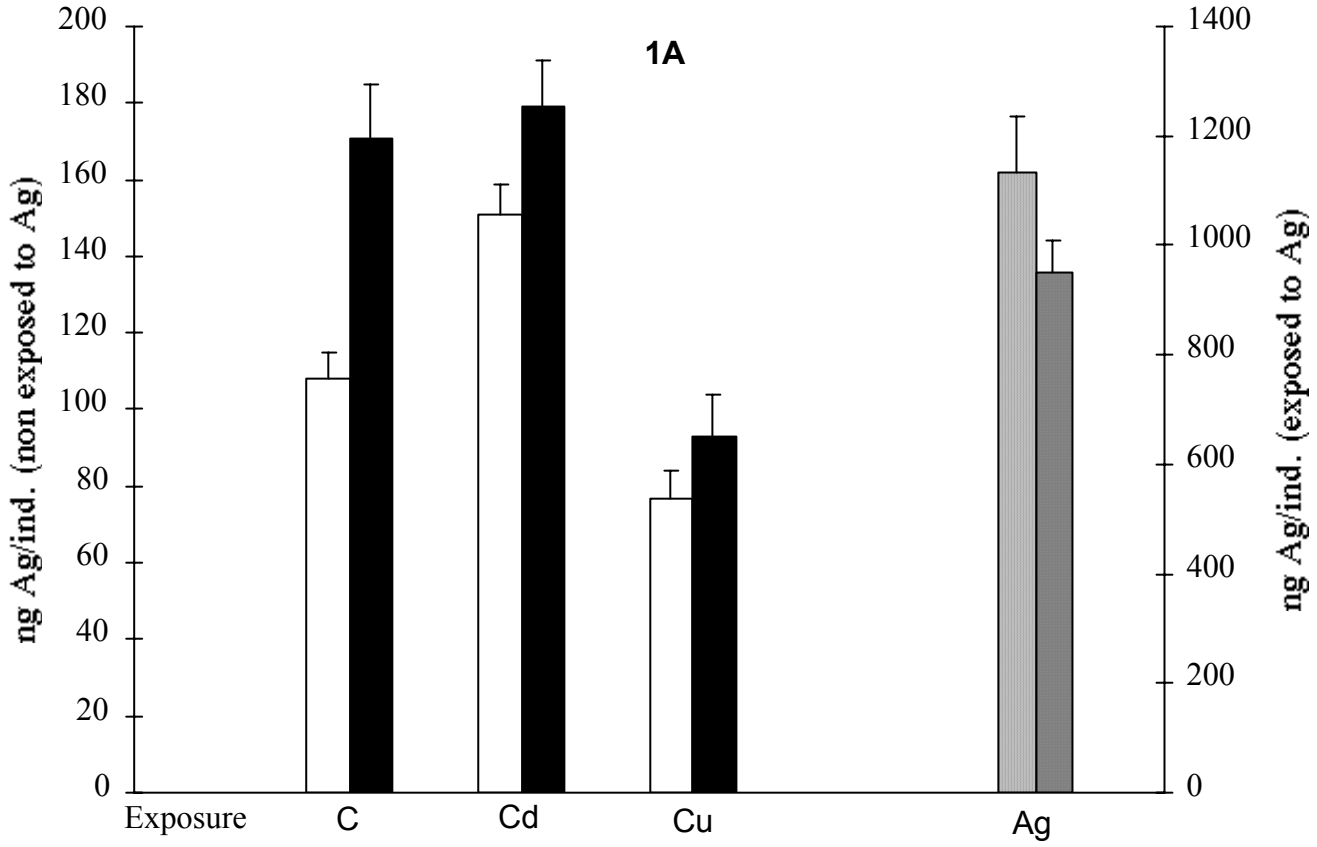
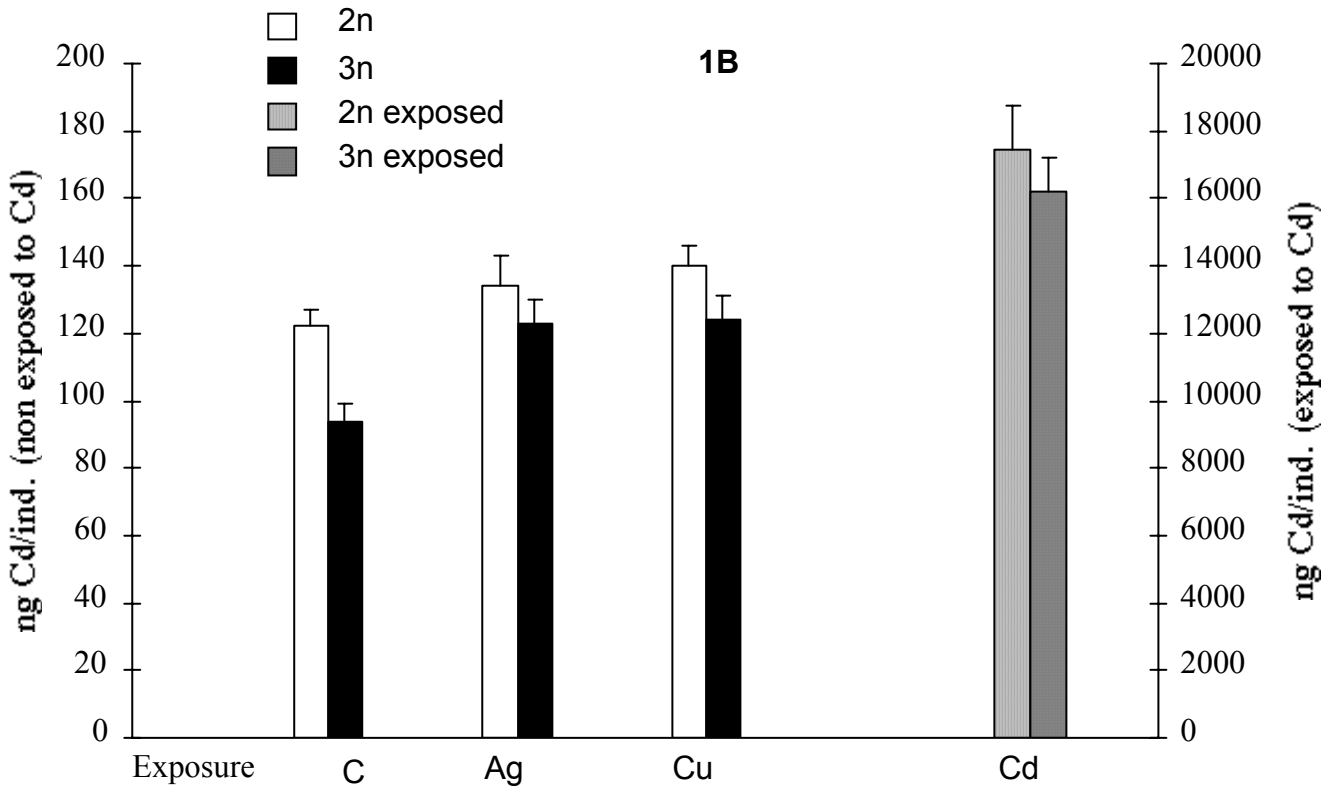
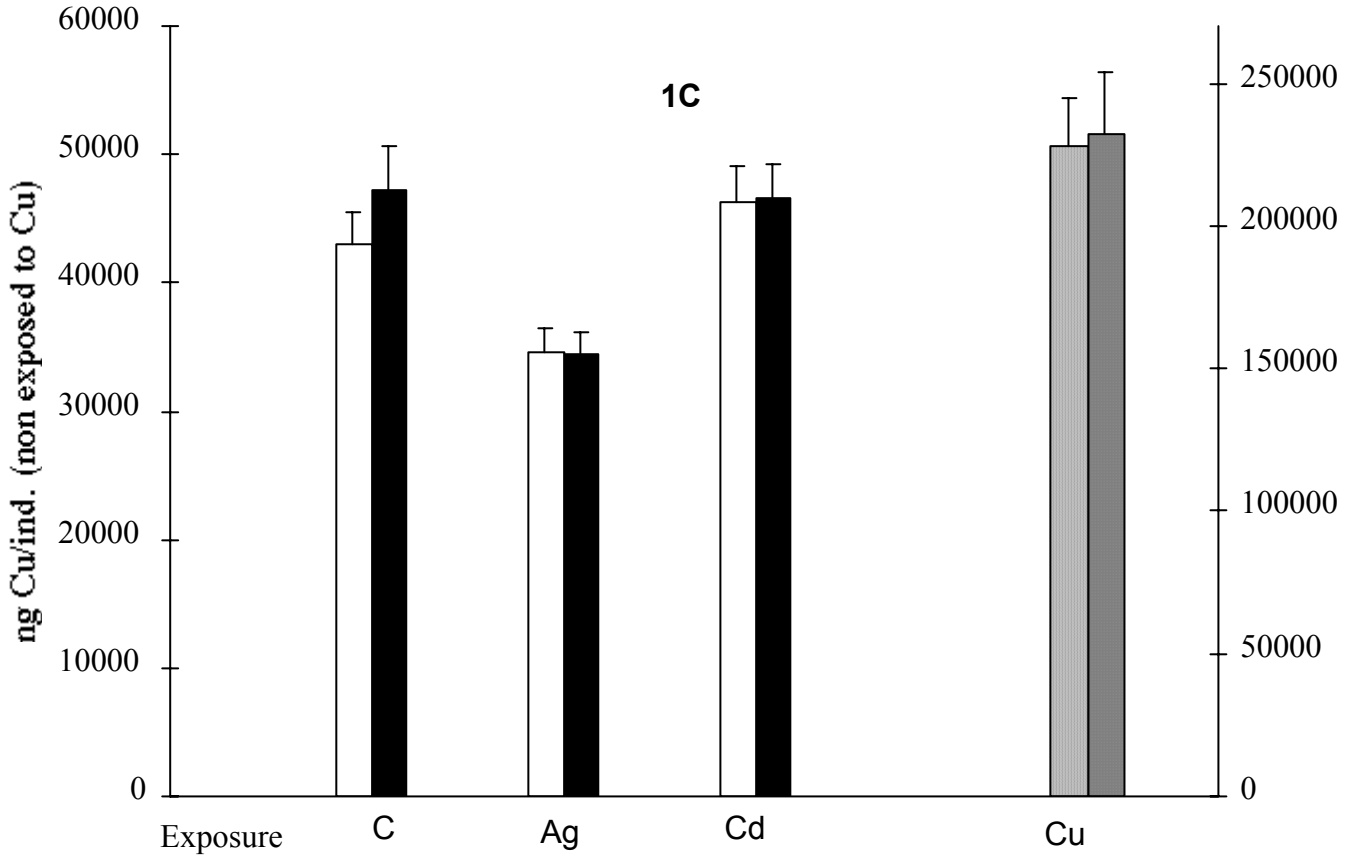
Fig. 1. *Crassostrea gigas*. Body burden (silver : 1A ; cadmium : 1B ; copper : 1C) in diploïd (2n) and triploïd (3n) specimens from control groups (C) or exposed to Ag (10 µl/l), Cd (10 µg/l) or Cu (30 µg/l).




2n
3n
2n exposed
3n exposed
1B
ng Cd/ind. (non exposed to Cd)
ng Cd/ind. (exposed to Cd)
200
180
160
140
120
100
80
60
40
20
0
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Exposure
C
Ag
Cu
Cd


1C
ng Cu/ind. (non exposed to Cu)
60000
50000
40000
30000
20000
10000
0
250000
200000
150000
100000
50000
0
Exposure
C
Ag
Cd
Cu