Environmental Pollution
January 2008, Volume 151, Issue 1, Pages 148-157
http://dx.doi.org/10.1016/j.envpol.2007.02.017

Impact of chronic cadmium exposure at environmental dose on escape behaviour in sea bass (Dicentrarchus labrax L.; Teleostei, Moronidae)

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Abstract:

The effect of chronic exposure to a low concentration (0.5 μg l−1) of cadmium ions was investigated on escape behaviour of sea bass, Dicentrarchus labrax, using video analysis. Observations were also performed on the microanatomy of lateral system neuromasts. When fish were exposed for 4 h per day over 8 days to the cadmium ions, most of both types of neuromasts observed remained intact. However, some of them presented damaged sensory maculae. Whereas before cadmium exposure, fish responded positively to nearly all the lateral system stimulations, after exposure they decreased by about 10% their positive responses to stimulations. From the 15th day after the beginning of cadmium exposure, neuromasts presented progressively less damage, cadmium accumulation in gills and scales decreased significantly and fish escape behaviour had recovered. This study presents a new concept in ecotoxicology: using behavioural change to reveal the effects of pollution levels, scarcely detectable by currently used techniques (physiological responses).

Cadmium exposure involved a significant bioaccumulation in fish scales, slight damage to the lateral line system and a significant decrease in fish escape behaviour.

Keywords: Fish lateral system; Neuromast; Chronic cadmium exposure; Escape behaviour; Bioaccumulation

1. Introduction

In French coastal ecosystems, a significant number of contaminants are present, particularly metal ions (Chiffoleau et al., 1999; Boutier et al., 2000). Yet, many such affected coastal areas constitute nurseries, essential habitats for juvenile fish. Originating from the discharge of waste, specialized industries or mining activities, these pollutants are thought to be responsible for the reduction of fish resources in estuaries and coastal waters (Cole, 1979; Waldichuk, 1979). In order to protect aquatic wildlife, it is therefore necessary to determine contamination levels. In this way, water quality criteria have been established, based primarily on bioaccumulation in mollusc and crustacean organs (Cole, 1979; Waldichuk, 1979 for review) or on acute lethality tests (Eisler and Hennekey, 1977; Voyer et al., 1979; Hollis et al., 1999, 2000). However, one aim of aquatic toxicology is to reveal the subtler and more insidious changes induced by pollutants on aquatic organisms and their environment (Larsson et al., 1985). According to Atchinson et al. (1987), tests based on standard acute-toxicity assays (LC_{50}: concentration of the toxic substance that is lethal to 50 % of individuals after a specific exposure time) and chronic-toxicity tests based on either full or partial life cycles, or on early life stages (LOEC: lower observed effect concentration), are less sensitive than behavioural studies. Behaviour is obviously a very important organism-level response that is the result of molecular, physiological and ecological processes (Scott and Sloman, 2004; Weis, 2004). According to Scott and Sloman (2004), behaviour may hence be useful for studying environmental-pollutant effects because it can provide a bioassay to determine an “ecological death” that may occur after much lower exposures to the toxicant. These authors also argued that fish are an excellent model in this regard, since many ecologically relevant fish behaviours are easily observed and quantified in a controlled setting. Even if fish are not overtly harmed by a

contaminant, they may be unable to function in an ecological context if their normal behaviour is altered (Bruslé and Quignard, 2004). Indeed, behavioural reactions may occur at concentrations significantly less than those producing gross physiological effects or death (Jensen and Bro-Rasmussen, 1992; Baker and Montgomery, 2001). Behavioural consequences may include: 1) impaired predatory behaviour resulting in poor diet, which can cause reduced growth and longevity; 2) altered predator-avoidance behaviour; or 3) impaired schooling leading to increased mortality and / or altered reproductive function (Weis, 2004). All these behavioural events form an important part of a successful adaptive life history strategy. Altered behaviours caused by exposure to pollutants may hence cause serious risks to the success of fish populations and disrupt aquatic communities (Scott and Sloman, 2004). These authors underline that more research is required concerning the impact of chronic exposure to low toxicant concentrations on fish behaviours.

In fish, the lateral line system is involved in many behavioural events such as predator and prey detection (Hoekstra and Janssen, 1986; Montgomery, 1989; Janssen et al., 1999), rheotaxis (Montgomery et al., 1997; Northcutt, 1997; Baker and Montgomery, 1999a, b; Coombs et al., 2001), obstacle avoidance (Blaxter and Batty, 1985) and intraspecific interactions (Partridge and Pitcher, 1980). The functional units of this lateral line system are mechanoreceptors, the neuromasts, distributed on the head, trunk and tail of the fish (Coombs et al., 1989). The morphological and functional integrity of these mechanoreceptors thus appears indispensable for the existence and the survival of a fish species in an ecosystem. Among metal ions, cadmium is considered as the most toxic ion after mercury because concentrations leading to death are much lower than for other metal ions (Eisler and Hennekey, 1977). Also, in contrast to several

98 metal ions (cobalt, copper, iron, zinc, etc.), the cadmium ion has no known metabolic
99 role and does not seem to be biologically essential or beneficial to metabolism (Friberg
100 et al., 1974; Bryan, 1979). Given that cadmium is a calcium antagonist at the level of
101 the gills (Verbost et al., 1987, 1988), and that calcium ions play a preponderant role in
102 signal transduction mechanisms in neuromast hair cells in the fish lateral line system
103 (Sand, 1975; Hudspeth and Corey, 1977; Jørgensen, 1984), cadmium ions might affect
104 mechanoreception and thereby alter the behaviour of fish exposed to them. Several
105 studies carried out mainly in freshwater, reported the impact of metal ions on the fish
106 sensory system and the consequences for behaviour (see Atchinson et al., 1987 for
107 review). For example, Baker and Montgomery (2001) showed that cadmium ions were
108 responsible for impaired olfactory function and altered rheotaxis behaviour associated
109 with damage to the lateral line system in freshwater fish. Very few studies, to date, have
110 been performed on the effect of cadmium exposure on marine fish behaviour. A
111 previous study (Faucher et al., 2006) showed that when sea bass were submitted to
112 acute cadmium exposure at low concentration (4 hours at 0.5 $\mu g.l^{-1}$, which represents
113 the maximal cadmium concentration encountered in contaminated French estuaries),
114 neither alteration in neuromast tissue, nor any behavioural modification could be
115 detected. In contrast, after an acute cadmium exposure at 10-fold higher concentration,
116 severe neuromast tissue damage was observed, contributing to a decrease in their escape
117 behaviour by about 56 %. This escape behaviour is induced by the detection of
118 hydrodynamic stimuli from predator displacements that act on fish lateral line system in
119 association with their inner ear (Coombs et al., 1989).
120 The aim of this study has been to determine the impact of chronic low-
121 concentration cadmium exposure on the fish escape response. A major innovation in the

present study is the determination of cadmium effects over a long time span (chronic exposure) at a concentration close to that measured in the fish's more polluted habitats (0.5 $\mu g.l^{-1}$) on the lateral line system of sea bass *Dicentrarchus labrax*. In addition, this work combines for the first time data concerning accumulation of cadmium in tissues, sensory tissue damage on both types of neuromasts of the lateral line and consequences on fish-escape behaviour.

2. Material and methods

2.1. Experimental fishes

Experimental sea bass were obtained from the Ferme des Baleines, Ile de Ré, France. They were placed in 400-l seawater tanks at constant temperature (18 °C) for three months with a natural photoperiod. They were fed twice a week with commercial pellets. The experiments took place between June and September 2005. They were carried out in two identical sets realized simultaneously, each one consisting of twelve fish (about 6 g and 7 cm standard length).

2.2. Experimental set up

Experiments took place in two 40 l-tanks (100 x 40 x 10 cm) of seawater at constant temperature (18 °C). The photoperiod was controlled (14-L: 10-D) and an automatic feeder delivered food each day, about thirty minutes after the beginning of the

light phase. Fish were placed for one week in the tanks before the beginning of the experiment.

In order to test the function or the dysfunction of their lateral line system, the same set up was used as that previously employed (Faucher et al., 2006) to study the impact of acute cadmium exposure on the trunk lateral line neuromasts and consequences on the fish behaviour. A pipette connected to a hand-operated syringe was used to inject a water jet between the water surface and the base of the tank when fish swam in the vicinity (about 5 cm) of the pipette. Each day, three stimulations (injection of a water jet) were performed and the fish responses were recorded with an analog video camera (SONY CCD-VX1E Handicam Pro, 25-frames.s^{-1}) positioned at a height of ~ 1 m above the water surface. The lateral line system of the fish was considered as functional when the water jet stimulation provoked a sudden escape reaction, characterised by the bending of the fish's body into a C-like shape, followed by an abrupt swimming acceleration away from the initial location (see Faucher et al., 2006). Such a response was counted as a positive response and noted as 1. Immobility or a constant swimming velocity was noted as null response and noted as 0 (see Faucher et al., 2006 for illustrations). Each day, the number of positive responses out of the three expected was calculated. In this way, sea bass were recorded each day under control conditions for three weeks.

2.3. Cadmium exposure

To reveal the impact of chronic cadmium exposure on the lateral line system through its consequences on escape responses, two sets of experiments with two

separate groups of fish were performed simultaneously. First, fish response was recorded under control conditions every day for 3 weeks. Then, each day for 8 days, sea bass were collected and placed for 4 h (the time needed for cadmium adsorption onto a particle, Chiffoleau et al., 1999) in a 10 l-tank of seawater to which 0.5 $\mu g.l^{-1}$ cadmium ($Cd(NO_3)_2$, Merck, cadmium standard solution 1000 $mg.l^{-1}$ in nitric acid 0.5 M) had been added. This intermittent exposure for 4 h per day during 8 consecutive days in another tank was chosen instead of 8 continuous days in the experimental tank to avoid the risk that cadmium ions might destroy the biological filtration system in the experimental tank. The concentration tested represents the maximal cadmium concentration encountered in highly polluted estuaries such as the Gironde, Scheldte and Hudson estuaries (Klinkhammer and Bender, 1981; Elbaz-Poulichet et al., 1987; Jouanneau et al., 1990). Such an intermittent cadmium exposure may occur in natural estuaries when fish cross through maximum turbidity zone where sediments are known to adsorb contaminated particles as metal ions (Jouanneau et al., 1990; Chiffoleau et al., 1999). Fish were then placed back in their experimental tank. Their swimming behaviour was normal. After allowing several hours for recovery, the time required for the entire disappearance of the stress caused by cadmium exposure, the sea bass responses to the three daily stimulations by the water jet were recorded, every day until the restoration of a normal behaviour.

To evaluate the stress caused by daily manipulations, two placebo treatments (4-hour baths in seawater without cadmium) were performed one week apart during the three weeks of recording under control conditions.

192 *2.4. Water contamination analyses*

193

194 To determine the cadmium concentration to which the fish were really exposed
195 each day during the 4 hours of exposure, sample water was collected at 0, 2 and 4 hours
196 after the addition of 0.5 $\mu g.l^{-1}$ cadmium, on the 6^{th}, 7^{th} and 8^{th} day of exposure. Samples
197 collected at each time (0, 2 and 4h) from the 6^{th}, 7^{th} and 8^{th} day were taken to constitute
198 a "0h" sample, a "2h" sample and a "4h" sample. Analyses of cadmium concentration in
199 these three water samples were performed in the Institut Pasteur of Lille. Water samples
200 were filtered through 0.45 µm, and cadmium concentration measurements were realized
201 using ion-adsorption onto resin.

202

203 *2.5. Metal analyses*

204

205 To evaluate metal tissue contamination, gills and scales covered by mucus
206 were collected from three fish sampled simultaneously: in control conditions, and on the
207 3^{rd} day, the 8^{th} day, the 15^{th} day (8 days of exposure followed by 7 days of depuration)
208 and the 21^{st} day (8 days of exposure followed by 13 days of depuration) after the
209 beginning of cadmium exposure. Fish gills and scales were chosen because they are in
210 direct contact with pollutants and thus might represent short-term biomarkers of
211 contamination compared to the long-term contamination biomarkers (liver, kidney)
212 usually used. Tissue samples were dried at 50°C for 2 days. Dry samples were weighed
213 and digested for 2 days in 5 ml concentrated (14 N) nitric acid at 80°C until the
214 digestion was completed, then heated to dryness. 2 ml 0.3 N nitric acid was then added.
215 Three analytical blanks were prepared in a similar manner without samples to check for

possible contamination. Corrections were applied wherever necessary. The digestion procedure was also applied to standards (200 mg of DORM-1: dogfish muscle powder) of Cd concentrations to check the spectrophotometer calibration. Metal concentrations were then measured with an atomic absorption spectrophotometer (HITACHI, Polarized Zeeman Atomic Absorption Spectrophotometer Z-5000). The results for the standard reference materials were in good agreement with the certified values reported (2.56 % deviation). All glassware was carefully decontaminated with acid (3.5 % nitric acid, Merck + 5 % fuming hydrochloric acid, Merck) for 24 hours and was then copiously rinsed with distilled water before any new use. The experiment was conducted in triplicate and the reported values are an average of the three values measured in tissue of the three fish collected in control conditions, on the 3rd day, the 8th day, the 15th day and the 21st day after the beginning of cadmium exposure. Results are expressed as the metal concentration in µg reported on a dry weight basis.

2.6. Observation of lateral line system tissue status

For each set of experiments, to verify the tissue status of both superficial and canal lateral line system neuromasts in sea bass after cadmium exposure, four batches of twelve fish (three taken on each date) were collected. The first batch, sampled on the 3rd day after cadmium exposure, the second at the end of the period of chronic cadmium exposure (the 8th day), the third batch on the 15th day, and the last batch sampled on the 21st day after the beginning of chronic cadmium exposure. The neuromast tissue status of cadmium-exposed fish was compared with that of two control fish collected from each experimental tank after three weeks of recording under control conditions. Prior to

sacrifice, collected fish were anaesthetised with 75 mg.l^{-1} MS-222 (3-aminobenzoic acid ethyl, Sigma) for about 15 minutes. The whole of trunk lateral line mechanoreceptors was then sampled. Tissue samples were prepared for scanning electron microscope observations following the same set up as in Faucher et al. (2003).

2.7. Statistical analyses

To estimate damage caused by cadmium to both types of neuromasts, the average number of superficial and canal neuromasts damaged per scale was counted. Data obtained were then compared between fish exposed to cadmium and control fish using χ^2-tests.

Cadmium accumulation data in fish gills and scales were examined with a two-factor analysis of variance (ANOVA) with organ (gills and scales) and exposure time (control, 3, 8, 15 and 21 days) as dependent variables, after finding homogeneity of variances ($p > 0.05$). Significant main effects were followed up with Student-Newman-Keuls post-hoc tests.

Behavioural responses to water jet stimulations were analysed following the same data treatment as in Faucher et al. (2006). Data obtained are expressed as the average percentage of positive responses $\overline{P_k} \pm$ SD (standard deviation of the mean). The number of data points obtained each day with the two sets of experiments is indicated between brackets. The percentages obtained before and after cadmium exposure were compared using χ^2-test.

All statistical analyses were performed with the statistical softwares XlSTAT-Pro 6.0 and Minitab 13.0. The level of significance was set at $p < 0.05$.

3. Results

Water analyses showed that sea bass were exposed to an average concentration of dissolved cadmium ions in seawater of 0.48 ± 0.18 µg.l^{-1} (n = 3) during the four hours of exposure. For all the duration of the experiment, fish mortality was null.

3.1. Effects of cadmium exposure on sea bass lateral line system

Compared to control fish (Fig. 1A and B), the majority of superficial (Fig. 1C) and canal (Fig. 1D) neuromasts of sea bass exposed to 0.5 µg.l^{-1} for three days, did not present any apparent tissue damage. The majority of them possessed intact sensory maculae: hair bundles of subjacent sensory hair cells were well developed. However, some seemed to be slightly damaged (Fig. 1E and F): their hair cell bundles were sparse, shortened, and sometimes not visible. Although the percentage of superficial neuromasts damaged (8.6 %, n = 35) was not significantly different from that in control fish for which all neuromasts were intact (0 % destroyed, n = 19, $\chi^2 = 2.515$, p = 0.113), the percentage of canal neuromasts damaged (27.8 %, n = 18) after three days of cadmium exposure was significantly greater than in control fish (0 %, n = 12, $\chi^2 = 4.623$, p = 0.032). At the end of the period of chronic cadmium exposure (8 days), fish presented mainly intact superficial (Fig. 2A) and canal neuromasts (Fig. 2B). However, some of them were nevertheless damaged as illustrated by the figure 2C and D. The percentage of superficial neuromasts altered (6.3 %, n = 24) was not significantly different from that in control fish (0 %, n = 19, $\chi^2 = 1.709$, p = 0.191). In contrast, the

288 percentage of canal neuromasts damaged (30.8 %, n = 13) remained higher than that
289 observed in control fish (0 %, n = 12, $\chi^2 = 4.719$, p = 0.030). Then, 15 days after the
290 beginning of cadmium exposure, superficial (Fig. 3A) and canal (Fig. 3B) neuromasts
291 were still mainly intact. A small percentage of superficial (4.8 %, n = 21) and canal
292 (22.2 %, n = 9) neuromasts were once again damaged (Fig. 3C and D) but less markedly
293 than previously. The percentage of neuromasts altered by cadmium exposure was not
294 significant compared to that observed in control fish, whether in the case of superficial
295 ($\chi^2 = 1.267$, p = 0.260) or canal neuromasts ($\chi^2 = 3.159$, p = 0.076). As before, at the
296 end of the experiment (21 days after the beginning of cadmium exposure), nearly all the
297 neuromasts of each type were intact (Fig. 4A and B). Nevertheless, some superficial
298 (5.6 %, n = 18) and canal (16.7 %, n = 6) neuromasts remained slightly altered (Fig. 4C
299 and D): their hair bundles seemed to be a little sparse or shortened. However, the
300 percentage of damaged neuromasts was not significantly greater than in control fish (χ^2
301 = 1.413, p = 0.235 for superficial and $\chi^2 = 2.094$, p = 0.148 for canal neuromasts). It is
302 relevant to note that the percentages of damaged neuromasts and their tissue alteration
303 (hair bundles almost non visible) were maximal between 3 and 8 days after exposure.
304 Indeed, no significant difference was observed in the case of the percentage of damaged
305 superficial ($\chi^2 = 0.096$, p = 0.757, n = 59) and canal neuromasts ($\chi^2 = 0.018$, p =
306 0.894, n = 31) between 3 and 8 days after the beginning of cadmium exposure.
307
308 *3.2. Cadmium bioaccumulation*
309
310 Figure 5 shows the Cd accumulation as a function of exposure time. For gills
311 (Fig. 5A) as for scales (Fig. 5B), average cadmium concentrations measured in control

312 fish were relatively high: 0.054 ± 0.032 µg.g^{-1} of dry weight (n = 3) in gills and 0.052 ±
313 0.005 µg.g^{-1} of dry weight (n = 3) for scales. In fish gills, cadmium ions did not seem to
314 accumulate significantly ($F_{4,15}$ = 0.760, p = 0.574, n = 15). In contrast, for the whole
315 duration of experiment, the average concentration of cadmium in fish scales was
316 significantly greater than that in gills ($F_{2,30}$ = 13.811, p = 0.001, n = 30). The maximal
317 concentration in cadmium was observed in scales after 3 days of exposure and was
318 0.147 ± 0.015 µg.g^{-1} dry weight (n = 3). This concentration of cadmium in scales after 3
319 days of exposure was significantly higher than that measured in control fish (t = 5.856,
320 p < 0.0001, n = 6), and after 8 days (t = 4.874, p = 0.001, n = 6), 15 days (t = 4.434, p =
321 0.001, n = 6) and 21 days (t = 6.329, p < 0.0001, n = 6). Then, cadmium concentration
322 in scales tended to decrease to be not significantly different from that measured in
323 control fish ($F_{3,12}$ = 1.273, p = 0.348, n = 12).
324
325 *3.3. Consequences of cadmium exposure on fish responses to the water jet*
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327 During the three weeks of recording under control conditions, sea bass
328 responded positively at 94.05 ± 8.88 % (n = 42): they swam away after stimulation by
329 the water jet (Fig. 6). The two placebo treatments realised did not generate any
330 significant behavioural modification in fish. The day of cadmium exposure, the average
331 positive response percentage fell significantly (χ^2 = 6.290, p = 0.012): sea bass
332 responded positively in only 66.67 ± 0.00 % (n = 2) of stimulations. From day 1 and
333 during all the cadmium exposure period (8 days), this average positive response
334 percentage progressively decreased from 100.00 ± 0.00 % (n = 2) to 66.67 ± 0.00 % (n
335 = 2). Until the 15th day (8 days of exposure followed by 8 days of depuration),

cadmium-treated fish went on being significantly less reactive to stimulation by the water jet than control fish: they presented an average response percentage of 84.17 ± 15.22 % (n = 30, $\chi^2 = 5.284$, p = 0.022). Then, from the 15th day, fish started to positively respond again to stimulations in 95.56 ± 7.63 % (n = 15) of cases. From this day, the average percentage positive response was no longer significantly different from that recorded in control conditions (94.05 ± 8.88 %, n = 42, $\chi^2 = 0.168$, p = 0.682).

All results obtained in this study are summed up in the figure 7. In summary, cadmium exposure involved: 1) a significant cadmium bioaccumulation in scales, 2) slight damages to both types of neuromasts, canal neuromasts being the more altered, and 3) a significant decrease in fish escape behaviour during the time of exposure. After this time, fish tended to restore their escape behaviour in association with a regeneration of neuromasts tissue and a cadmium depuration in gills and scales.

4. Discussion

Many researchers have proposed using behavioural indicators in fish for ecologically relevant monitoring of environmental contamination (reviewed by Atchinson et al., 1987). We have now done this for chronic cadmium exposure in sea bass, *Dicentrarchus labrax*, at the concentration occurring *in situ* in polluted French estuaries.

In a previous study, Faucher et al. (2006) showed that 48h after 4 hours 0.5 $\mu g.l^{-1}$ acute cadmium exposure, neither type of neuromasts presented any apparent tissue damage. In the present study, after 3 days of similar exposure, some neuromasts

of both types (at most 8.6 % for superficial and 30.8 % for canal neuromasts) were damaged by cadmium ions. Three days of intermittent 0.5 $\mu g.l^{-1}$ cadmium exposure might thus be the threshold period needed to affect sea bass lateral line system tissues. This hypothesis is reinforced by our other results: the maximum cadmium bioaccumulation in fish scales and major behavioural consequences were also measured after three days of exposure.

Before chronic cadmium exposure, gills and scales from control fish already presented a relatively high amount of cadmium, as previously found in the black goby *Gobius niger* (Migliarini et al., 2005) and in the juvenile olive flounder *Paralichthys olivaceus* (Kim et al., 2004). This may be due to previous cadmium exposures of the fish during the course of their life. Sea bass used in this study were obtained from a commercial source located in Ile de Ré, an island located on the west coast of France and known for its relatively high concentration of dissolved cadmium ions in seawater (Boutier et al., 2000). Cadmium concentrations measured in gills and scales of control fish corresponded thus to the background. Nevertheless, bioaccumulation data obtained showed that after exposure, cadmium accumulated much more in fish scales than in gills. It is hence relevant to note that fish scales might be a pollutant marker more sensitive than gills which have been commonly used until now. This suggestion is supported by the observation of the adverse effect of cadmium on the morphology (Yoshitomi et al., 1998) and the structural aspect (Rishi and Jain, 1998) of freshwater fish scales. Rishi and Jain (1998) argued that fish scales could thus be used as a biomarker of pollution, particularly as these can be used without sacrificing the animal. In vitro, it has been also demonstrated that cadmium influenced osteoclastic activities after acute exposure and inhibited osteoblastic activities under long-term exposure

384 (Suzuki et al., 2004). Moreover, our results showed that after a maximum at three days,
385 cadmium accumulation tended to decrease. The fact that fish accumulate less cadmium
386 in their scales after 8 days of exposure could be explained by the action mechanism of
387 cadmium ions on cells. Cadmium and calcium ions are known to be mutually
388 antagonistic in their fixation on sites located at the gills (Verbost et al., 1987, 1988).
389 Given that the lateral line system functions through calcium ion flux, we suggest that
390 cadmium may block the Ca^{2+}-ATPase pump of the baso-lateral membrane of neuromast
391 hair cells. The result would be a blocking of calcium transport in cells associated with
392 their clearly observed degeneration. One could hypothesise that, as the cells degenerate,
393 fixation sites become less available to cadmium ions and the consequence could be less
394 measured cadmium accumulation in the tissue. This hypothesis corroborates Migliarini
395 et al.'s (2005) speculation that cadmium ions fix to binding sites on the gills until they
396 are totally occupied. Yet, fish gills and scales are thought to be usually covered by
397 mucus that protects for the skin and the sensitive gill epithelium against xenobiotics
398 such as metal ions (Pawert et al., 1998; Bruslé and Quignard, 2004). However, at least
399 in the present work, mucus appeared not to protect much against metal ions such as
400 cadmium, since at least a few of both types of neuromasts were damaged by cadmium.
401 Cadmium ions must have passed across the mucus layer and damaged sensory hair cell
402 bundles of the fish lateral line system, in spite of the low-concentration of cadmium
403 applied. This result, combined with those obtained with fish lateral line systems
404 exposed to acute high concentration of cadmium (Faucher et al., 2006), refutes the
405 proposal of Døving (1991) that the lateral line organs are shielded from direct pollutant
406 exposure by a set of supporting cells and by their gelatinous cupulae. Our results show
407 that, in contrast to Hudspeth's (1983) and Døving's (1991) hypotheses,

mechanoreceptors of the lateral line system are not only accessible by pollutants *via* the internal path (blood) but also by direct external exposure.

In our experiments, the behavioural consequence of alteration of the lateral line system by cadmium ions was a decrease in fish escape responses by about 10 %. This result is supported by some studies realized on freshwater fish showing that cadmium ions can disrupt reproductive behaviour (Jones and Reynolds, 1977), agonistic behaviour (Sloman et al., 2003), spawning site selection and natal homing (Baker and Montgomery, 2001), predator avoidance and prey capture (Scott et al., 2003) and also electroreception (Neuman et al., 1991). Our previous study had shown that when fish were exposed to acute high-concentration cadmium, the sea bass lateral line system regenerated itself after about twenty-one days (Faucher et al., 2006). In this study, about fifteen days were necessary after the beginning of the chronic low-concentration cadmium exposure for fish to show a progressive restoration of their escape behaviour. In parallel, at this time, the tissues of both types of neuromasts presented progressively less damage compared to observations realized during high-cadmium exposure. We can thus conclude that, after such a chronic low-concentration cadmium exposure, the lateral line system needs about 15 days to regenerate itself at a sufficient rate to allow full detection of hydrodynamic stimuli. After chronic exposure (for 8 days) to cadmium, regeneration of both types of neuromasts proved to be quicker than Faucher et al. (2006) found after acute exposure (for only 1 day) to 10-fold higher concentration cadmium. This shorter time needed for neuromasts to regenerate is likely because, in the present study, few neuromasts of both types were entirely destroyed whereas after the acute exposure to high-concentration cadmium, all superficial canal neuromasts were totally destroyed.

To test the sea bass lateral line system function, the stimuli applied in this study were relatively strong, probably more intense than signals received by fish when a predator or a prey approaches. In this way, if sea bass exposed to this low concentration of cadmium responded less frequently (reduction by 10 %) to strong stimuli, it is likely that reaction to the weaker stimuli characteristic of their natural environment would be reduced relatively even more markedly. Even if the tissues of their neuromasts remained normal in aspect, lower efficiency is likely in the detection of potential predators or prey in natural environments after exposure to cadmium. To confirm or refute this hypothesis, the lateral line system response to variable intensity stimuli would have to be tested by varying the current velocity of stimulations, attempting to approach as far as possible the range of stimuli generated by moving prey. In addition, in French estuaries, fish are permanently exposed to a mixture of metal ions (Ag, Cd, Co, Cu, Hg, Ni, Pb, Zn, etc.), it would hence be relevant to examine whether there exists any synergy or antagonism among these different metal ions on fish lateral line systems inducing consequences for fish behaviour and for the survival of different fish species *in situ*.

5. Conclusions

This study has produced new data and understanding about the vulnerability of the sea bass lateral line system to cadmium, and it also illustrates a new concept in ecotoxicology. Although after chronic low-concentration cadmium exposure, accumulation and sensory tissue damage were both relatively slight, we have clearly demonstrated that such exposure leads to behavioural consequences. More behavioural studies in ecotoxicology are now needed. Behaviour is an important organism trait

457 response that may represent a pollution marker more sensitive and more relevant than
458 observations of changes in physiology or microanatomy alone (Doving, 1991; Scott and
459 Sloman, 2004; Weis, 2004). Behaviour is furthermore integrated with other levels of
460 biological organization (Scott and Sloman, 2004), so it needs to be considered as a
461 predictor and a result of other internal and external biological processes such as
462 ecological and physiological indicators of toxicity.
463

464 **Acknowledgements**

465

466 We wish to thank Ian Jenkinson for improving our English writing. Thanks to
467 the Ferme des Baleines for providing the animals. We would also like to thank the
468 Centre Commun d'Analyses (CCA), Université de La Rochelle, for allowing the use of
469 a scanning electron microscope.
470

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Figure 1: Scanning electron micrographs showing the effect of low-concentration cadmium exposure (4 hours per day at 0.5 µg.l^{-1}) for 3 days on tissue status of both types of neuromasts from the trunk lateral line of sea bass, *Dicentrarchus labrax* (L.). Intact superficial (A) and canal (B) neuromasts observed in a control fish. Superficial neuromast is still covered by its cupula (A) whereas its absence on canal neuromast reveals hair bundles (insert in B). The crushed appearance of superficial neuromast cupula is due to a manipulation artefact. C, D. Three days after chronic cadmium exposure, the majority of superficial (C) and canal (D) neuromasts appeared still intact. Hair bundles present within sensory maculae were normal (insert in D). However, some superficial (E) and canal (F) neuromasts were damaged: their sensory maculae presented hair bundles shortened, sparse (black arrow in F) or not visible (E).

Figure 2: Scanning electron micrographs showing the effect of low-concentration cadmium exposure (0.5 µg.l^{-1} for 4 hours per day) for 8 days on tissue status of both types of neuromasts of sea bass trunk lateral line at the end of exposure. The majority of superficial (A) and canal (B) neuromasts presented normal morphology (insert in B): their sensory maculae were similar to those observed in control fish. Nevertheless, some superficial (C) and canal (D) neuromasts were damaged: their sensory maculae presented hair bundles shortened, sparse (insert in D) or even not visible (C).

Figure 3: Scanning electron micrographs showing the effect of chronic low-concentration cadmium exposure (0.5 µg.l^{-1} for 4 hours per day), 15 days after the beginning of the exposure, on tissue status of both types of neuromasts of sea bass trunk lateral line. Superficial (A) and canal (B) neuromasts were usually intact. Inserts in A

and B illustrate details of sensory maculae with normal hair bundles. However, some superficial (C) and canal (D) neuromasts did present altered morphology. Their hair cell bundles seemed to be damaged: they were shortened, sparse (insert in D) or even not visible.

Figure 4: Scanning electron micrographs showing the effect of chronic low-concentration cadmium exposure (0.5 $\mu g.l^{-1}$ for 4 hours per day) on tissue status of both types of neuromasts of sea bass trunk lateral line at the end of the experiment, 21 days after the beginning of exposure. Superficial (A) and canal (B) neuromasts were generally intact. Inserts in A and B illustrate details of sensory maculae with normal hair bundles. Nevertheless, some superficial (C) and canal (D) neuromasts did appear slightly altered.

Figure 5: Average cadmium concentrations (in $\mu g.g^{-1}$ of dry weight) in fish gills (A) and scales (B) of sea bass, *Dicentrarchus labrax*, exposed chronically to cadmium ions (4 hours per day at 0.5 $\mu g.l^{-1}$) for 8 days. Vertical bars represent the standard deviation.

Figure 6: Average percentages of positive C-start escape responses caused by lateral line system stimulations over consecutive days. Day zero on the x-axis corresponds to the day from when fish were exposed to 0.5 $\mu g.l^{-1}$ cadmium. Before cadmium exposure, the majority of sea bass positively reacted to water jet. In contrast, as soon as their lateral line system was exposed to low-concentration cadmium, the average positive response percentage fell significantly. This average percentage positive response declined during the period of cadmium exposure (8 days). Then, a recovery to baseline

643 escape behaviour percentages in response to jet stimulation was observed from the 15th
644 day after the beginning of cadmium exposure. Vertical bars represent the standard
645 deviation.
646
647 Figure 7: Summary of all results obtained in this study: cumulated percentage positive
648 escape responses of fish (continuous black line) obtained before, at 3, 8, 15 and 21 days
649 after the beginning of cadmium chronic exposure (grey box), cumulated percentages of
650 superficial (NS, white histogram) and canal neuromasts (NC, black histogram) damaged
651 and average concentrations of cadmium in fish gills (wide black dashed line) and scales
652 (narrow black dashed line). Vertical bars represent the standard deviation of average
653 cadmium concentration.
654

Figure 1

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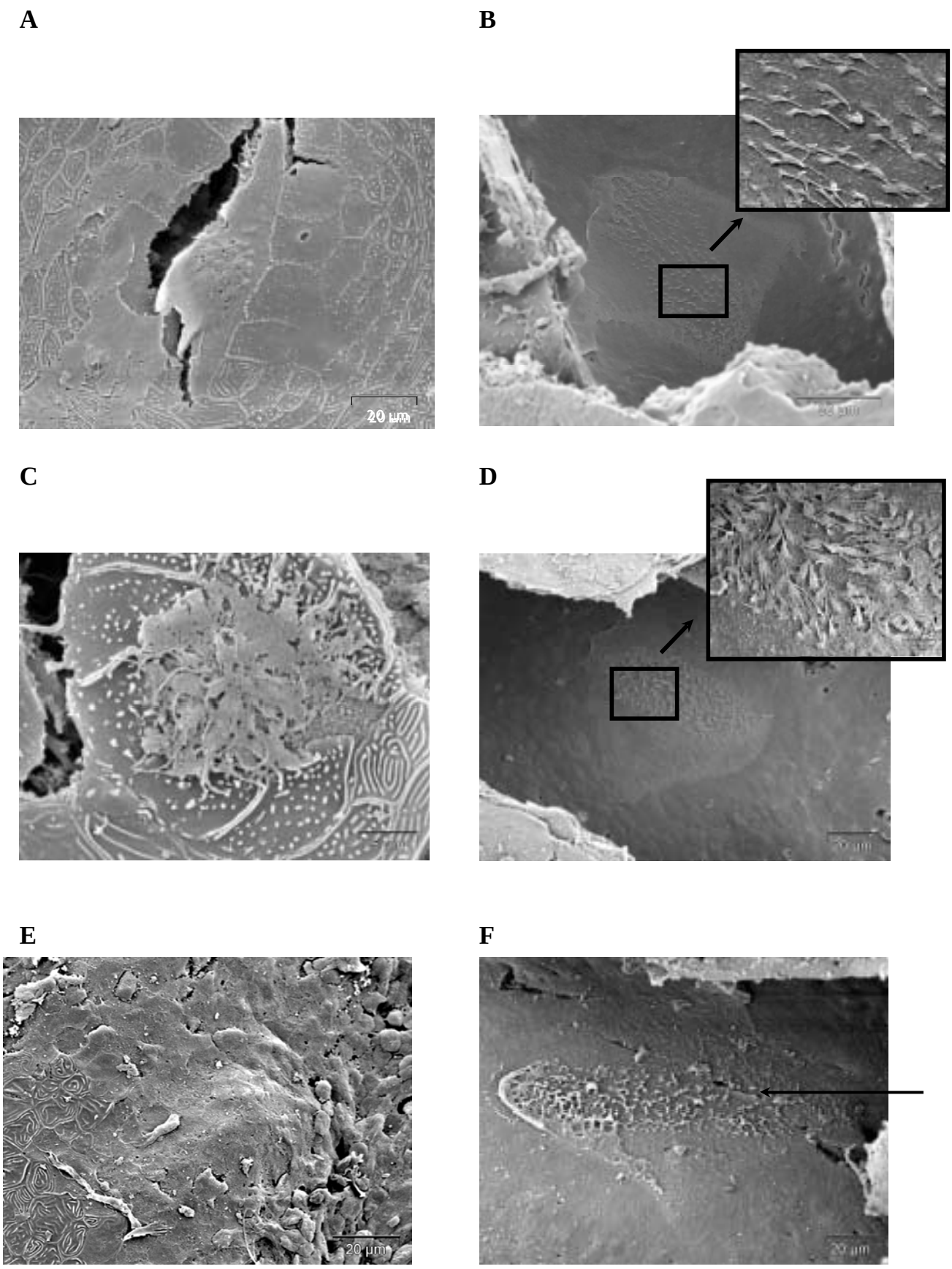

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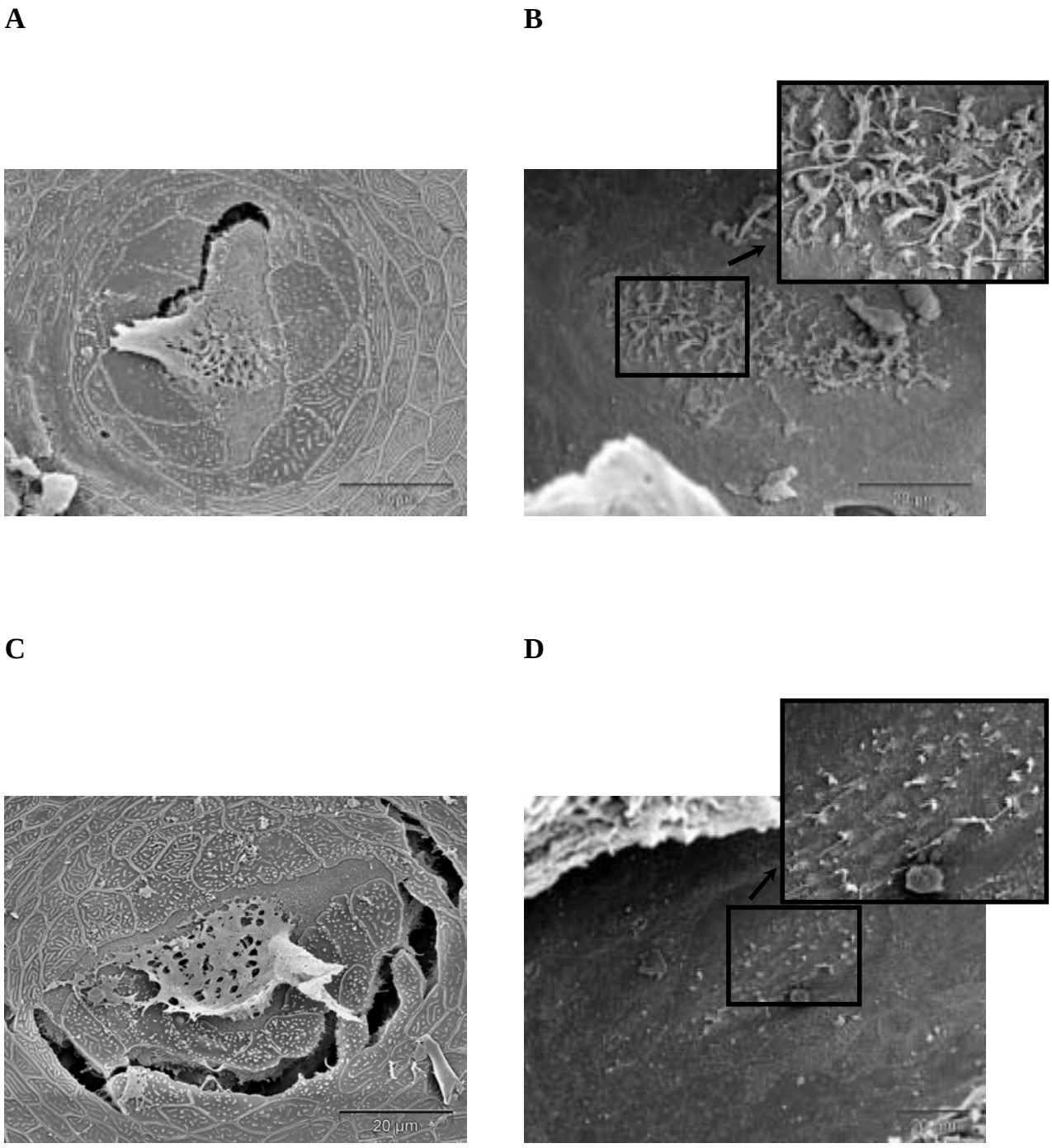

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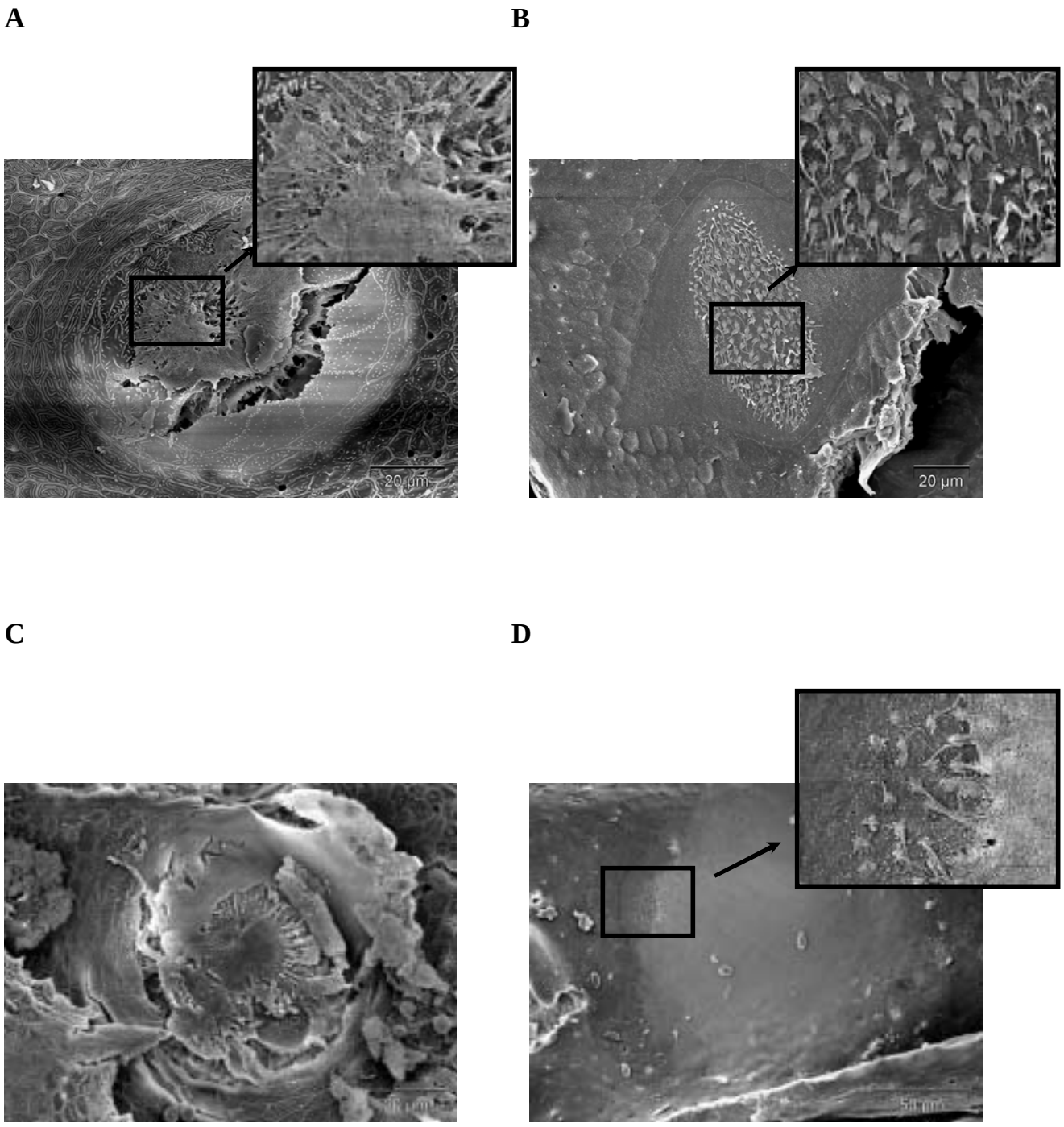

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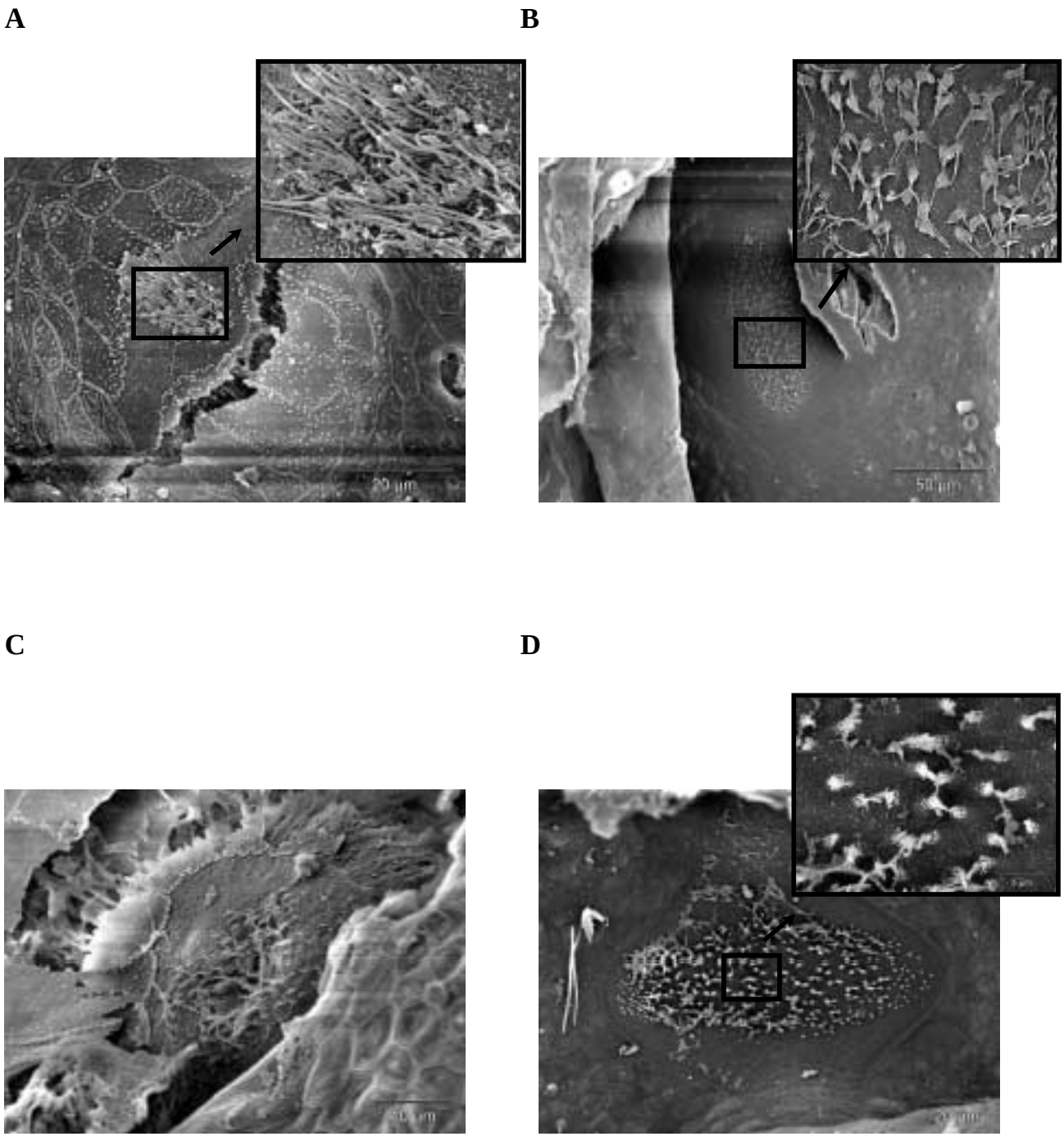

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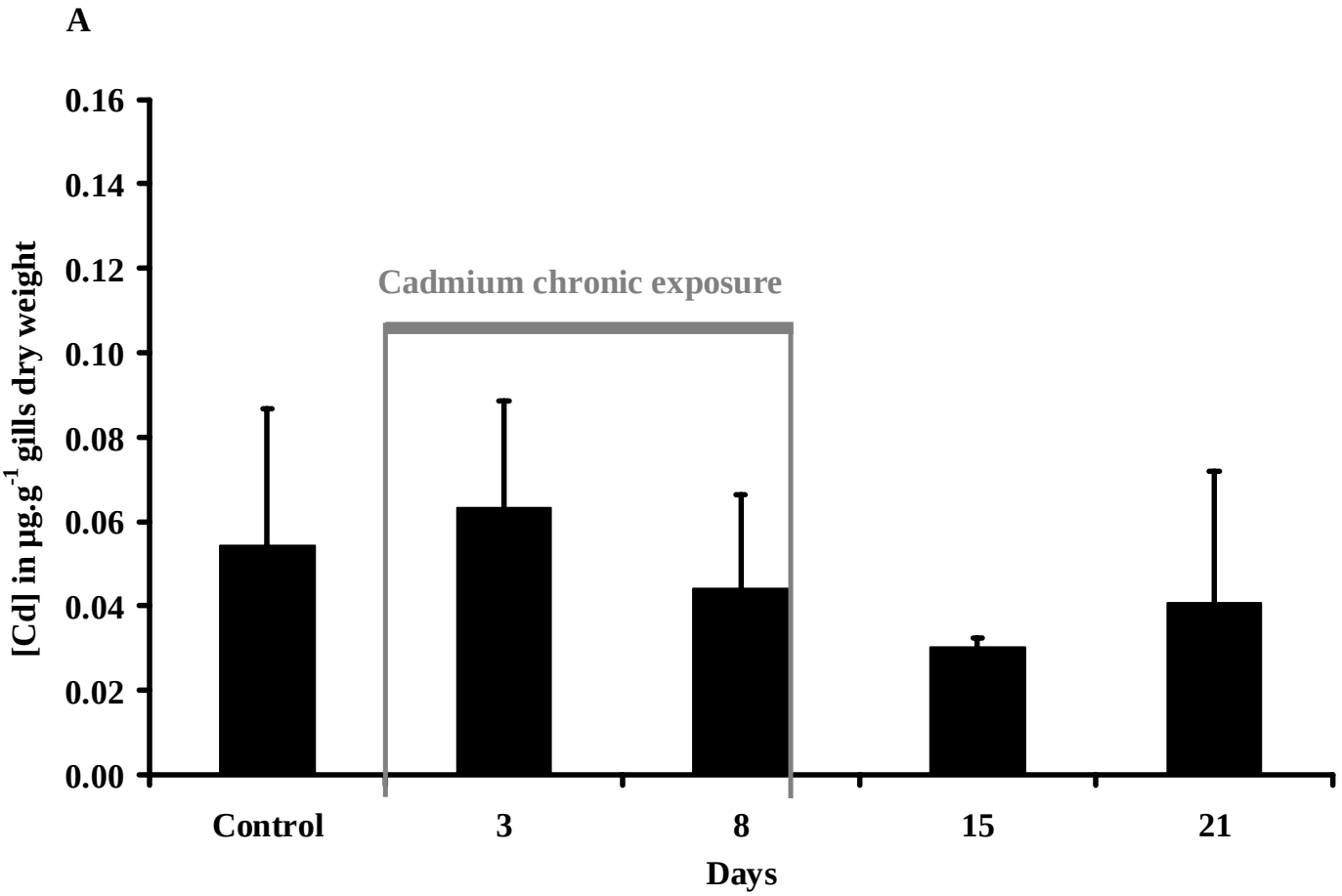

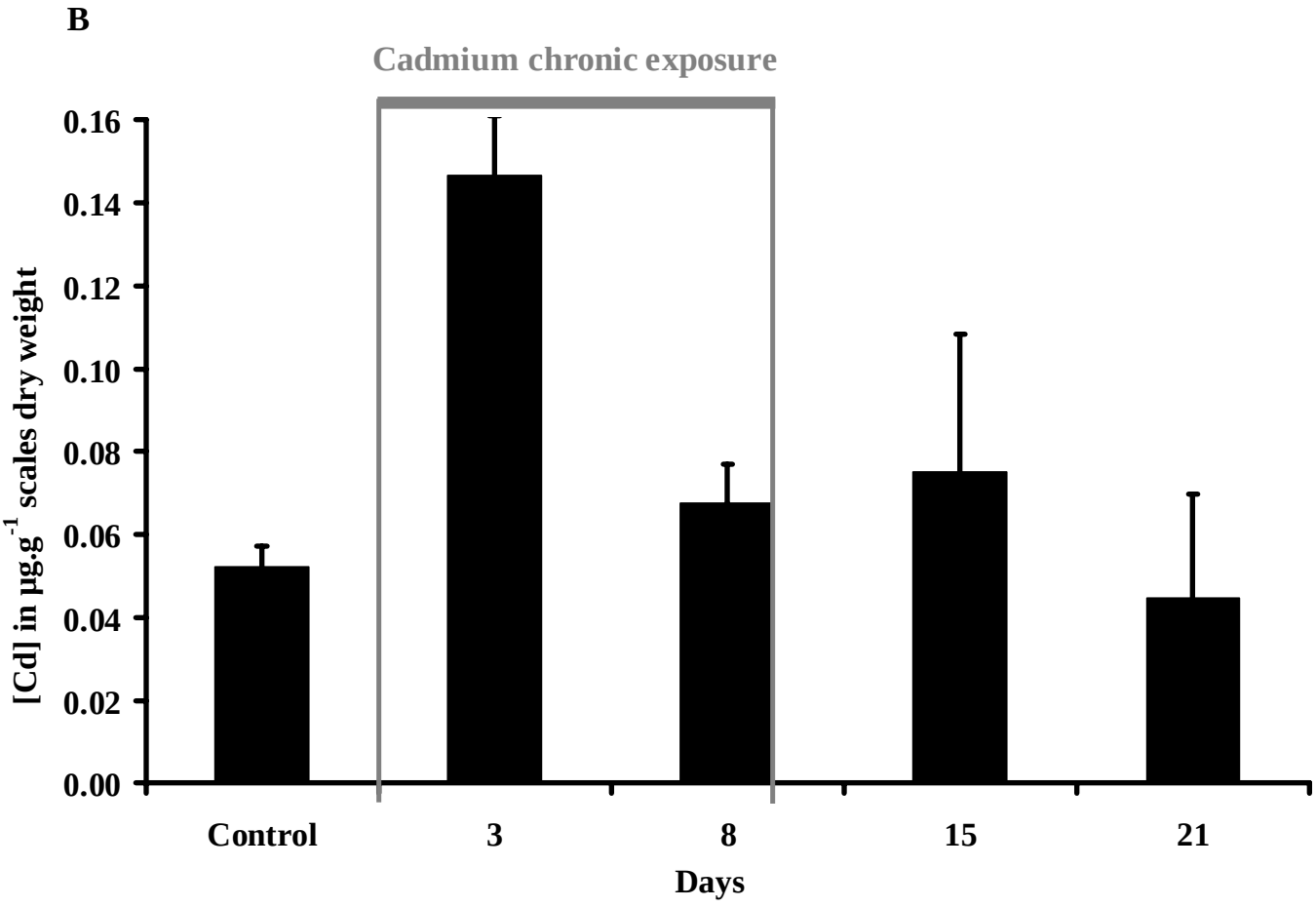

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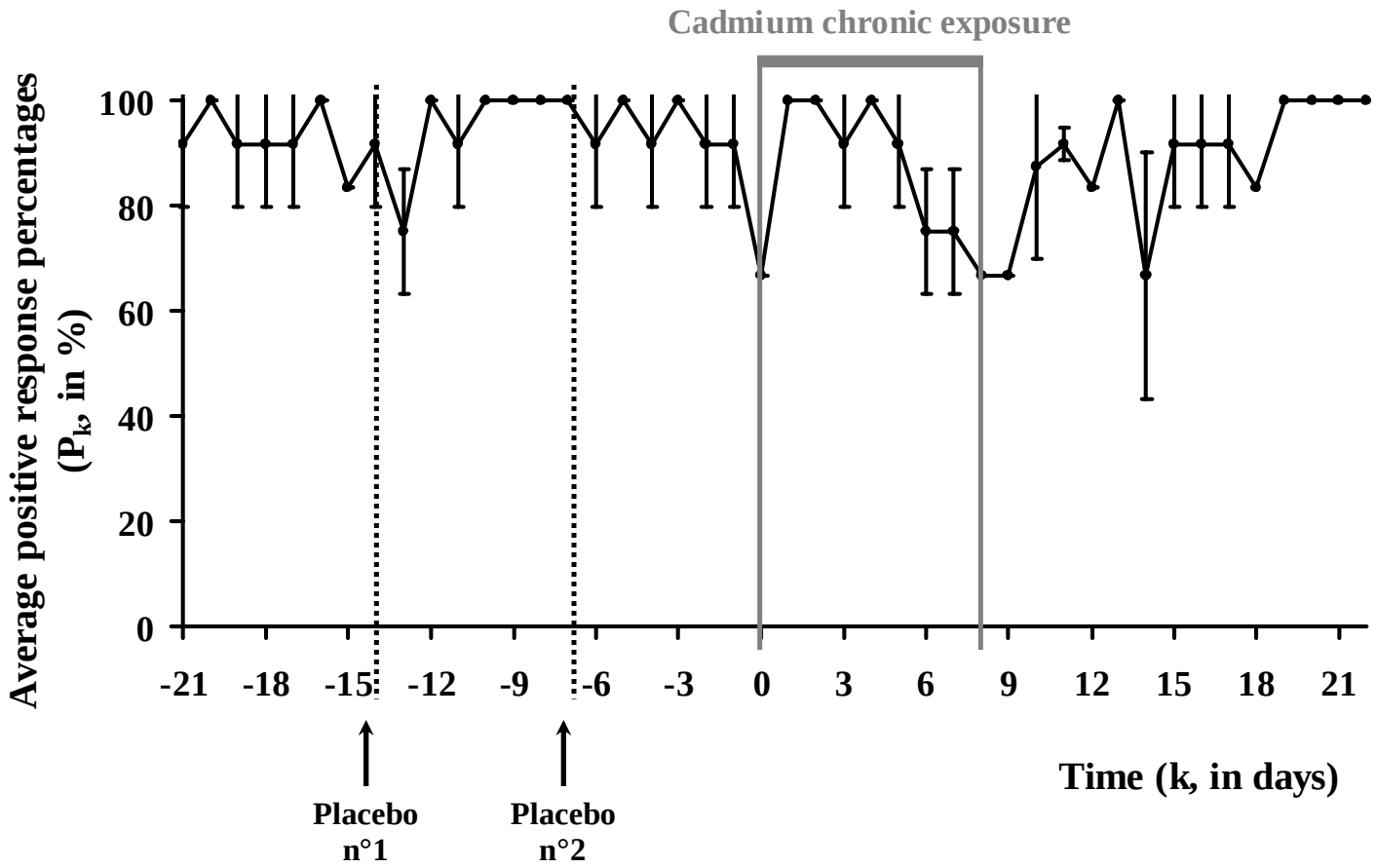

Figure 7

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