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1 The barnacle *Balanus amphitrite* as a biomonitor for Cd: Radiolabelled experiments.

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20

1 **Abstract**

2

3 Radiolabelled experiments were carried out to measure necessary parameters in the
4 development of a biodynamic ecotoxicological simulation model of Cd accumulation in the
5 barnacle biomonitor *Balanus amphitrite*. The Cd uptake rate constant from the dissolved
6 phase, the Cd Assimilation Efficiency (AE) from suspended particulate matter (SPM) and the
7 efflux rate constant were obtained using ¹⁰⁹Cd. A Cd uptake rate constant from the dissolved
8 phase (k_u) of $0.0072 \text{ L g}^{-1} \text{ h}^{-1}$ was determined for the barnacle under environmentally realistic
9 dissolved Cd concentrations (maximum of 400 ng L^{-1}). Cd AE from SPM was determined
10 from the barnacle feeding on SPM with low and high chl *a* concentrations, resulting in AEs of
11 39.0% and 48.7%, respectively, and an efflux rate of 0.0072 d^{-1} . The difference between the
12 AEs resulted from differences in chl *a*:SPM ratios suggesting a general tendency of higher AE
13 when SPM is enriched with chl *a*. These results reinforce that the accuracy of
14 ecotoxicological models for metal accumulation in organisms depends on how representative
15 the selected food items are of the organism's natural diet.

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21 **Keywords:** *Balanus amphitrite*, biomonitor, Cadmium-109, uptake rate, assimilation
22 efficiency.

23

1 1. Introduction

2
3 A critical aspect when modelling ecotoxicological processes involving heavy metals is to
4 obtain reliable estimates of the parameters that control metal uptake and accumulation in
5 organisms. Manipulative experiments using radioisotopes are among the best methods to
6 obtain these (Wang and Fisher, 1999), and there have been many studies determining
7 assimilation efficiency (AE), influx and efflux rates¹ for Cd and other metals by organisms
8 (Lee and Luoma, 1998; Wang and Fisher, 1999; Wang, Qiu and Qian, 1999a; Wang, Qiu and
9 Qian, 1999b; Qiu, Qian and Wang, 2001; Rainbow and Wang, 2001; Ke and Wang, 2002;
10 Rainbow, Blackmore and Wang, 2003).

11
12 Current work on marine pollution has pointed out the importance of a systemic approach with
13 a predictive capability to support environmental risk assessments for contaminants, especially
14 for heavy metals (Jørgensen, 1994; Poethke, Oertel and Seitz, 1994; Bradbury, 1995; Brock,
15 1998; Luoma, van Geen, Lee and Cloern, 1998; Wang et al., 1999b). With this in mind, we
16 carried out research addressing the need to understand how short-term variations in Cd
17 concentrations in the environment determine its concentrations in the barnacle biomonitor
18 *Balanus amphitrite* (da Silva et al., 2004, 2005).

19
20 Following on from these previous researches, a series of radiolabelled experiments was
21 carried out in the present study, in order to ultimately develop a biodynamic ecotoxicological
22 simulation model to study Cd accumulation in the barnacle biomonitor *Balanus amphitrite* in
23 the natural environment (da Silva et al., in press). The Cd uptake rate constant from the
24 dissolved phase, Cd AE from suspended particulate matter (SPM) and the efflux rate constant
25 were obtained for *Balanus amphitrite* with experiments using ¹⁰⁹Cd. Even though some of
26 these parameters have been published for *Balanus amphitrite* (e.g., Wang et al., 1999b;
27 Rainbow et al., 2003; Rainbow, Ng, Shi and Wang, 2004), in the present study the Cd
28 concentrations used in the dissolved phase were more realistic for the environment being
29 studied, where maximum dissolved Cd concentration is in the order of 400 ng L⁻¹. Moreover,
30 quality of food may change the metal accumulation in an organism if there are different

¹ Assimilation efficiency is the ingested material (or the influx of material into the organisms) less faeces, and it can be calculated as: $[\text{influx} - \text{faeces}] / \text{influx}$. Faeces elimination represents a rapid loss of some unassimilated metals that are purged from the gut (extracellular digestion). Efflux represents metabolic loss of assimilated metals (Wang and Fisher, 1999).

1 assimilation efficiencies between sources of food (Lee and Luoma, 1998; Wang et al., 1999a;
2 Wang et al., 1999b; Ke and Wang, 2002). For example, bivalves have exhibited higher metal
3 AE when suspended particles are enriched with phytoplankton (e.g., Lee and Luoma, 1998;
4 Ke and Wang, 2002; Wang and Wong, 2003). Therefore, in the present study the Cd AE from
5 SPM for *Balanus amphitrite* was determined working with the whole assemblage of diverse
6 particles that comprise the natural environment of suspended particulate matter.

8 **2. Methodology**

10 *2.1. Organism Collection and Acclimatization*

12 *Balanus amphitrite* were collected, during the Austral Spring, from concrete bridge piers in
13 Ross Creek, a tidal creek surrounded by the city of Townsville (19°15'S, 146°50'E,
14 Queensland, Australia). During the collection the water temperature and salinity were 26.2°C
15 and 37.3, respectively. A complete description of the sampling area can be found in da Silva
16 et al. (2004; 2005). The barnacles were removed from the piers with stainless steel chisel.
17 Several of them were attached to dead barnacles or oysters. After collection, the organisms
18 were directly transferred to a constant temperature room for preparation and the
19 acclimatization period before the experiments. The barnacles were individualized when
20 possible, cleaned and brushed to remove any other live organism. About 30 – 40 barnacles, as
21 individuals or groups of 2 – 4 barnacles, were placed into 300-mL HDPE plastic chambers for
22 acclimatization, without any substrate for attachment other than the walls and the bottom of
23 the chambers, and fed with natural particles in seawater pumped from Ross Creek. A
24 manually operated diaphragm pump was used to reduce any damage to the plankton
25 community, thus keeping the food supply as close as possible to the one found in Ross Creek.

27 During the 17-day acclimatization period, the water temperature was $24 \pm 1^\circ\text{C}$ (mean \pm range
28 of variation), salinity was 37 ± 2 (mean \pm range of variation), and barnacles were inspected
29 daily for any epibiota and brushed if necessary. When salinity acclimatization was also
30 needed, another 11-day acclimatization period was used. In this period, the organisms were
31 acclimatized, with gradual changes of less than four units per day, to three salinities, 27.3,
32 32.3 and 37.3 (all ranging ± 2), which is in the range observed in Ross Creek. The lower
33 salinities were obtained by mixing tap water with Ross Creek water in the appropriate
34 proportion in a flow-through system, in order to achieve a required salinity and constant food
35 supply.

1

2 *2.2. Radioisotope and Measurements*

3

4 The radioisotope ^{109}Cd (half-life: $t_{1/2} = 462$ d) was obtained from Amersham Biosciences UK
5 Limited (Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, England) as CdCl_2
6 in 0.1 M hydrochloric acid with a total activity of 37.3 MBq in a volume of 1.26 mL
7 containing 2.14 μg of ^{109}Cd . The activity of ^{109}Cd in the organisms was measured by a COBRA
8 II – Auto-Gamma (Packard, Model D5002) gamma detector. The counting time was 2
9 minutes at an energy level of 15–30 keV, in order to maximize the number of counts per
10 minute (CPM), produce propagated counting errors <5%, and keep counting time short
11 enough to manage counting for the AE experiment. The measured radioactivity was
12 transformed to ^{109}Cd mass in the organisms by comparison with standards of known ^{109}Cd
13 mass and corrected for decay.

14

15 *2.3. Cadmium Uptake Rate from the Dissolved Phase*

16

17 The Cd uptake rate from the dissolved phase for *Balanus amphitrite* was determined under
18 different Cd concentrations (referred to as the 'Cd concentration experiment') and salinities
19 (referred to as the 'salinity experiment'). In the 'Cd concentration experiment' three acid
20 leached 1-L HDPE Nalgene containing 500 mL of 0.45- μm filtered Ross Creek water (salinity
21 37.3) were prepared with final Cd concentrations of 106, 268 and 407 ng L^{-1} . These Cd
22 concentrations were determined by Anodic Stripping Voltammetry (method presented
23 elsewhere, da Silva et al., 2004; 2005), 1 day after the medium preparation, to allow
24 saturation of all adsorption sites in the bottles. These Cd concentrations were obtained by
25 spiking the filtered Ross Creek water with 5 ng of ^{109}Cd (86.37 kBq). As the initial Cd
26 concentration in the filtered Ross Creek water was 16 ng L^{-1} , the necessary amount of natural
27 Cd was added to it to obtain the desired concentrations. Seven organisms were placed into
28 each bottle and kept for 24 hours, under 12:12 h light/dark cycle, at a temperature of 24 ± 1
29 $^{\circ}\text{C}$ (mean \pm range of variation), salinity 37.3, and constant aeration. Rainbow et al. (2003;
30 2004) have shown that the Cd uptake rate from the dissolved phase by *Balanus amphitrite* is
31 constant over time; therefore, a single exposure of 24 h was used in all experiments. At the
32 end of the exposure period, the organisms were rinsed with non-labelled seawater, **and the**
33 **barnacle's soft tissue was removed from shells (i.e., all soft parts less the mantle –**
34 **'bodies' in previous studies)**, rinsed with distilled water, dried to a constant weight in a fume
35 cupboard, and assayed for the ^{109}Cd activity.

1
2 The hourly Cd uptake rate from the dissolved phase ($\text{ng g}^{-1} \text{h}^{-1}$) for each barnacle exposed to
3 each tested dissolved Cd concentration was calculated as indicated below:

$$4 \quad \text{Uptake rate} = \frac{{}^{109}\text{Cd}_b (C_w + {}^{109}\text{C}_w)}{24W_b \cdot {}^{109}\text{C}_w}, \quad (1)$$

5 where ${}^{109}\text{Cd}_b$ is the mass of ${}^{109}\text{Cd}$ in the barnacle's soft tissue (ng), C_w is the natural Cd
6 concentration in the dissolved phase (ng L^{-1}), ${}^{109}\text{C}_w$ is the ${}^{109}\text{Cd}$ concentration in the dissolved
7 phase at the end of the 24-h exposure (ng L^{-1}), and W_b is the barnacle's soft tissue dry weight
8 (g). Loss of Cd from the dissolved phase due to the organism's uptake, or due to adsorption to
9 the container wall or organisms' shell, was insignificant ($<2\%$ between the beginning and the
10 end of the experiment), ensuring an effectively constant Cd concentration during the
11 experiment course.

12
13 In the 'salinity experiment' a similar procedure as described for the 'Cd concentration
14 experiment' was adopted, except that three acid leached 1-L Nalgene bottles were produced
15 with 500 mL of salt water with salinities 27.3, 32.3 and 37.3 obtained by dilution with
16 distilled water. Each batch presented a total Cd concentration of $369 \pm 11 \text{ ng L}^{-1}$ (mean \pm
17 range), which was obtained by adding 5 ng of ${}^{109}\text{Cd}$ (86.37 kBq) plus the necessary amount of
18 natural Cd. Seven organisms were placed into each bottle and kept for 24 hours, under 12:12
19 h light/dark cycle, at a temperature of $24 \pm 1 \text{ }^\circ\text{C}$ (mean \pm range of variation), and constant
20 aeration. The Cd uptake rate was calculated as indicated by Eq. 1.

21 22 2.4. Cadmium AE from SPM

23
24 In the determination of the AE of Cd in *Balanus amphitrite* natural suspended particulate
25 matter (SPM, size-class of 0.45–50 μm , as defined in da Silva et al., 2004; da Silva et al.,
26 2005) was used as ${}^{109}\text{Cd}$ -labeled food for *Balanus amphitrite*. Water for SPM collection was
27 sampled from Ross Creek from two different locations, at which different phytoplankton
28 abundances in SPM have been identified during previous sampling programs. SPM was
29 obtained by passing $\bullet 4 \text{ L}$ of 50- μm pre-filtered water through 0.45- μm Millipore filters and
30 the retained material was resuspended in 500 mL of 0.45- μm filtered Ross Creek water.

31 32 2.4.1. Food Particles Radiolabelling

33

1 Each SPM batch was radiolabelled in 500 mL of 0.45- μm filtered Ross Creek water (1-L
2 Nalgene bottle) spiked with 25.5 ng of ^{109}Cd (4.4 kBq), under 14:10 h light/dark cycle under a
3 light flux of the order of $100 \mu\text{E m}^{-2} \text{s}^{-1}$, at a temperature of $27 \pm 1 \text{ }^\circ\text{C}$ (mean \pm range of
4 variation), salinity 36.6, and constant stirring. The radiolabelling procedure lasted five days, a
5 time that the literature has identified as being enough to allow uniform radiolabelling of the
6 particles (see, e.g., Wang et al., 1999b; Ke and Wang, 2002; Wang and Wong, 2003; Rainbow
7 et al., 2004). During this period, SPM was removed daily from the medium by filtration (0.45-
8 μm Millipore filter), resuspended in fresh 500 mL of 0.45- μm filtered Ross Creek water and
9 spiked with 25.5 ng of ^{109}Cd (4.4 kBq). This procedure was adopted instead of using culture
10 mediums such as f/2 (e.g., Guillard, 1975) to minimize any change in the particles
11 assemblage, since the objective was to work with the natural SPM from the barnacle's
12 environment.

13
14 After the radiolabelling procedure, each batch of radiolabelled SPM was filtered and
15 transferred into 500 mL of fresh 0.45- μm filtered Ross Creek water, before being offered to
16 the barnacles. This procedure was done twice, for each SPM batch, with 12-h intervals
17 between them. This was done in order to guarantee that most of ^{109}Cd was in SPM and not in
18 solution. Low ^{109}Cd activities of <40 counts per minute (CPM) in 500 μL of 0.45- μm filtered
19 medium (i.e., $<1 \text{ ng } ^{109}\text{Cd L}^{-1}$) were determined for each SPM batch just before being offered
20 to the barnacles. The ^{109}Cd concentrations in each SPM batch were 5.3 and 5.5 $\text{ng}(^{109}\text{Cd}) \text{ g dry}$
21 weight (SPM)^{-1} . These ^{109}Cd concentrations were obtained from a small amount of SPM of
22 each batch retained on 0.45- μm filters and assayed for their activity.

23 24 2.4.2. The 'labelled' phase

25
26 In the determination of the AE of Cd in *Balanus amphitrite* from SPM, was determined using
27 the pulse-chase feeding technique (see, e.g., Wang and Fisher, 1999; Wang et al., 1999b;
28 Rice, Eaton and Clesceri, 2005). During the 'labelled' phase, 28 organisms were placed in two
29 acid leached 500-mL HDPE plastic chambers, with each chamber receiving radiolabelled
30 SPM in a recirculating system from each SPM batch (total volume 4 L in each), under light, at
31 a temperature of $24 \pm 1 \text{ }^\circ\text{C}$ (mean \pm range of variation), salinity 36.6, and constant aeration.
32 Recirculation was enough to keep particles in suspension. At the end of the radiolabelling
33 procedure, each SPM batch, originally obtained from site 1 and 2, presented a SPM mass
34 concentration of 15.6 and 20.3 mg L^{-1} , and a chl *a* concentration of 9.2 and 9.4 $\mu\text{g L}^{-1}$,
35 respectively. Chlorophyll-a was collected in duplicate from each SPM batch onto 25-mm

1 Whatman GF/F filters, using a Sartorius mini filter holder adapted to a 60-ml plastic syringe.
2 Pigment determinations from acetone extracts of filters were done with a Turner Design AU-
3 10 fluorometer following Arar & Collins (1992). The organisms were kept starved for 2 days
4 before the 'labelled' phase to maximize the feeding activity. After 30–45 min feeding, before
5 egestion of radioactive faeces, individual barnacles were rinsed with non-radiolabelled
6 seawater and their radioactivity counted. The seven most radioactive organisms from each
7 feeding batch were selected to take part in the 'non-labelled' phase.

8 9 2.4.3. The 'non-labelled' phase

10
11 In the 'non-labelled' phase, the organisms were placed into new plastic chambers receiving
12 water with SPM (0.45–50 μm) obtained from Ross Creek to promote depuration of the
13 ingested radiolabelled SPM. The 'non-labelled' phase was run in a flow-through system, under
14 12:12 h light/dark cycle, at a temperature of 24 ± 1 °C (mean \pm range of variation), salinity 36
15 ± 1 (mean \pm range), and constant aeration. The 'non-labelled' phase for both batches lasted
16 68.2 h, in which the organisms were periodically removed from the depuration chambers,
17 rinsed with non-radiolabelled seawater, their radioactivity counted, and placed back into the
18 chambers. In addition, both depuration chambers were periodically rinsed with non-labeled
19 seawater to remove any faeces thus avoiding any re-ingestion of egested labeled material.
20 Counts for water from the depuration chambers presented an undetectable ^{109}Cd activity, thus
21 guaranteeing no ^{109}Cd uptake from the dissolved phase. It was not possible to identify
22 individual barnacles that had moulted and data for all moulters (<5% of the individuals for all
23 experiments carried out) have been included in analyses.

24 25 2.5. Cadmium Efflux Rate

26
27 In the determination of the efflux rate constant, a similar procedure as for the determination of
28 the AE was adopted. At the end of the course of the experiment for the AE determination, the
29 four organisms that exhibited the highest radioactivity in the last counting (i.e., at time 68.2 h)
30 from both SPM batches were selected to be kept in the depuration chamber allowing further
31 depuration up to 144 h. The Cd efflux rate constant was determined by applying an
32 exponential decay model to the temporal variation of the percentage of remained radioactivity
33 in each organism from 22h onwards, which is the time when all labelled food has passed
34 through the gut of *Balanus amphitrite* (Rainbow et al., 2004; Rice et al., 2005). No moulting
35 was observed at this stage of the experiment.

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3. Results

3.1. Cadmium Uptake Rate from the Dissolved Phase

The Cd uptake rates ($\text{ng g}^{-1} \text{h}^{-1}$, Fig. 1) at three different dissolved Cd concentrations were determined for 21 individual barnacles. A linear model has been used to represent variations in the uptake rate as a function of the dissolved Cd concentrations (e.g., Rainbow et al., 2003; Rainbow et al., 2004). The best-fit least squares linear regression model passing through zero was applied to Figure 1, and it yielded a Cd uptake rate constant (k_u) of $0.0072 \text{ L g}^{-1} \text{ h}^{-1}$, which is the slope of this fitting.

In the 'salinity experiment' (tested salinities were 27.3, 32.3 and 37.3) from the Cd uptake rates ($\text{ng g}^{-1} \text{h}^{-1}$) for a dissolved Cd concentration of $369 \pm 11 \text{ ng L}^{-1}$ (C_w , mean \pm range) did not vary significantly over the three salinities tested (linear regression, $r^2 = 0.01$ and $p = 0.90$, data not presented).

3.2. Cadmium AE from SPM with Different Chlorophyll Concentrations

The Cd AEs showed a bi-phasic depuration pattern for both low and high chl *a*-containing SPM (Fig. 3), the ratios chl *a* ($\mu\text{g L}^{-1}$):SPM (mg L^{-1}) being equal to 0.48 and 0.59, respectively. The results show initial rapid loss of ingested ^{109}Cd labeled SPM within the first 4 h and then a slower loss up to 68.2 h (Fig. 2). Cadmium AEs were obtained separately for both low and high chl *a*-containing SPM. Each AE was obtained by fitting a single exponential decay curve to the second stage of depuration (i.e. considering only the data points within 4 - 68.2 h of experiment, as in Wang, 1999), for all data from all barnacles. The fittings yielded two AEs of 39.0 and 48.7% for low and high chl *a* containing SPM, respectively. In each curve fitting, the 14 barnacles used in each experiment were considered together.

3.3. Cadmium Efflux Rate Constant

An efflux rate constant of 0.0072 d^{-1} was obtained by multiplying the x-coefficient of the exponential decay model (Fig. 3) by 24, thus transforming the x-coefficient from an hour basis to daily basis. The curve fitting was obtained as a single best fit line considering the data

1 points, from 22 h onwards, for four of the most ¹⁰⁹Cd active barnacles after 68.2 h of the
2 beginning of the AE.

4 **4. Discussion**

6 *4.1. Cadmium Uptake Rate Constant from the Dissolved Phase*

8 The calculated Cd uptake rate constant (k_w) for *Balanus amphitrite* of 0.0072 L g⁻¹ h⁻¹
9 compares well with literature values of 0.012 and 0.008 L g⁻¹ h⁻¹ (Rainbow et al., 2003; 2004,
10 respectively) despite their much higher dissolved Cd concentrations of between 500–50000
11 ng L⁻¹ compared to 100–400 ng L⁻¹ of this study, and the fact that barnacles were dissected
12 before the analysis (therefore mantle was not included). This fact suggests that Cd uptake
13 from the dissolved phase is a linear process for *Balanus amphitrite* and its Cd absorption sites
14 are not saturated even by very high Cd concentrations, such as 50000 ng L⁻¹. Even though the
15 Cd uptake rate may vary between populations of *Balanus amphitrite* (Rainbow et al., 2003),
16 which justify repeating this experiments for the barnacles in Ross Creek, these authors did not
17 observe correlation between uptake rate from solution and accumulated metal concentration, a
18 surrogate measure of previous contaminant history. Thus, the rates determined in this work
19 are suitable for *Balanus amphitrite* living along the total Cd gradient in Ross Creek.

21 Variations in salinity may affect accumulation of Cd by complex organisms such as barnacles
22 (Phillips, 1980). Salinity acts on chemical speciation, thus changing the metal bioavailability,
23 and making metals more or less available for uptake from solution (Sunda, Engel and Thuotte,
24 1978). For example, Rainbow et al. (2003) found a significantly higher Cd uptake for *Balanus*
25 *amphitrite* exposed to salinity 15 compared to 33. Similar results were found by Lee et al.
26 (1998) for bivalves (*Potamocorbula amurensis* and *Macoma balthica*) when salinity ranged
27 from 5 to 30, but minor changes were observed in Cd uptake from 20 to 30. In fact, small
28 changes in activity of the free Cd ion are expected in such high salinities, when a relative
29 complete complexation of dissolved Cd (Phillips, 1980) is expected. Therefore, the similar
30 range of salinity in this study (from 27.3 to 37.3), which is in the range that *Balanus*
31 *amphitrite* experiences in Ross Creek, had no-significant effect on the Cd uptake rate from
32 solution.

34 The organism's size and its filtration rate, have been shown to affect the metal uptake rate
35 constant from the dissolved phase in bivalves (e.g., Wang and Fisher, 1997; Lee et al., 1998).

1 We performed a comparison between uptake rates and individual barnacles' dry weight to test
2 whether the barnacles' size affects its Cd uptake rate from the dissolved phase. Such a
3 comparison was based on the fact that metal uptake rates for a species can be compared after
4 allowance for the dissolved metal concentration (Rainbow et al., 2003). Comparing the
5 individual barnacles dry weight (ranging from 1.1 to 5.8 mg) against the Cd uptake rates
6 obtained for them in both the 'Cd concentration experiment' and the 'salinity experiment'
7 revealed a non-significant correlation (linear regression, $r^2 = 0.01$ and $p = 0.81$, data not
8 presented). These results suggest that the barnacles' size does not affect the Cd uptake rate
9 from the dissolved phase. Thus, the Cd uptake rate from solution obtained here can be used
10 for a wider size range of *Balanus amphitrite*.

11

12 4.2. Cadmium AE from SPM with Different Chlorophyll Concentrations

13

14 The AE determined in this study are within the range of other studies of *Balanus amphitrite*
15 feeding on different types of living particles (phytoplankton and zooplankton, Table 1).
16 However, on average, lower AEs were found in our study when the barnacles were fed on
17 SPM poor in chl *a*. This suggests that non-living particles in SPM may reduce the Cd AE, or
18 conversely that AE increases with higher proportions of phytoplankton (chl *a*) in SPM. In a
19 previous study, da Silva et al. (2005) comparing Cd accumulation in barnacles from two sites
20 with a steady-state model found that the highest phytoplankton abundance in SPM resulted in
21 a higher Cd AE. Similar patterns have been identified in bivalves that exhibited higher metal
22 AE when suspended particles are enriched with phytoplankton (e.g., Lee and Luoma, 1998;
23 Ke and Wang, 2002; Wang and Wong, 2003).

24

25 The ratio between chl *a* mass concentration ($\mu\text{g L}^{-1}$) and SPM mass abundance (mg L^{-1}) used
26 in the AE experiments were calculated for both SPM batches and compared to the AE
27 determined in the experiments. The same procedure was applied to the data presented in da
28 Silva et al. (2005). These two set of data presented the same general tendency of higher AE
29 was observed when SPM is enriched with chl *a* (Fig. 4). The best-fit least squares linear
30 regression model was applied to Figure 4 yielding the following equation ($r^2 = 0.77$, $p < 0.01$,
31 $n = 6$):

32

$$y = 49.18 \cdot x + 23.97, \quad (2)$$

33

34 where the y -coefficient represents the Cd AE from SPM for *Balanus amphitrite* (in %), and x -
35 coefficient represents the chl *a*:SPM ratio (in $\mu\text{g chl } a \text{ L}^{-1} : \text{mg SPM L}^{-1}$).

35

1 As pointed out by Lee and Luoma (1998), the radiolabelling procedure can be critical in the
2 interpretation of pulse-chase feeding experiments using mixed-particles assemblage, because
3 a short equilibrium time may result in preferential partitioning of isotopes to easily
4 exchangeable pools. Distribution coefficients as a function of time for Cd have exhibited
5 rapid removal from solution within the first few hours, followed by a slower removal over a
6 period of a few days (Turner and Millward, 1994; Ciffroy, Garnier and Benyahya, 2003). The
7 similarity between the AE determined in the radiolabelled experiments and those suggested
8 by the steady-state model, presented in da Silva et al. (2005), indicates that a five-day labeling
9 was enough time to produce Cd-labeled particles that behaved similarly to those found in the
10 environment (same trend for the points in Fig. 4).

11

12 4.3. Cadmium Efflux Rate Constant

13

14 The efflux rate constant determined in this study (ca. 0.0072 d^{-1}) agrees with efflux rate
15 constants reported in the literature for *Balanus amphitrite* (Wang et al., 1999b; Rainbow et al.,
16 2003), except for an unusual high value presented in Rainbow et al. (2004). The low efflux
17 rate constant confirms that *Balanus amphitrite* has potential as a biomonitor for Cd, since this
18 barnacle retains most of the metal that is assimilated. This fact makes the barnacle a more
19 appropriate biomonitor for registering long term trends in Cd concentration in the
20 environment rather than short term variations.

21

22

23 5. Community vs Single Species Approach

24

25 The quality of food may change the metal accumulation in an organism if there are different
26 assimilation efficiencies between sources of food as seen in this study and elsewhere (Lee and
27 Luoma, 1998; Wang et al., 1999a; Wang et al., 1999b; Ke and Wang, 2002). From our AE
28 experiment, variations in the composition of the food particles (e.g., abundance of chl *a*)
29 affected the Cd accumulation in *Balanus amphitrite*, and it is therefore important to take this
30 into account in kinetic models. In addition, when applying a systemic approach to understand
31 Cd accumulation in aquatic organisms, it must be considered that: (i) different food particles
32 may exhibit temporal and spatial variability that can change the relative Cd contribution to the
33 organism's burden, and (ii) organisms can selectively eat different sources of food, which can
34 present not only a specific Cd concentration but also different assimilation efficiencies. Thus,

1 the variation in the quantity and quality of the ingested food may result in changes in Cd
2 concentrations in organisms.

3
4 Therefore, the accuracy of ecotoxicological models for metal accumulation in organisms
5 depends on how representative the selected food items are of the organism's natural diet (Qiu
6 et al., 2001), because each item may present a specific AE. Usually, two feasible options can
7 be adopted on the determination of AE from laboratory experiments: (i) to select a small
8 number of species (Wang et al., 1999b; Wang and Rainbow, 2000; Rainbow and Wang, 2001)
9 or (ii) to work with the 'in situ' food (Lee and Luoma, 1998; Luoma et al., 1998; Ke and
10 Wang, 2002). This paper presents the results of experiments designed to improve the
11 parameterisation of an ecotoxicological model (da Silva et al., in press). Accordingly we
12 decided to use a natural food source, including the whole array of particles usually present in
13 SPM, which is expected to have a Cd AE similar to that for SPM in Ross Creek.

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16
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22 Pessoal de Nível Superior (CAPES, Brazil).

23 24 **7. References**

- 25
26 Arar, E.S. and Collins, G.B. (1992) Using the Turner Designs 10 AU fluorometer with EPA
27 method 445.0: "In Vitro Determination of Chlorophyll-a and Phaeophytin-a in Marine
28 and Freshwater Phytoplankton by Fluorescence." Version 1.1., Cincinnati, OH 45268
- 29 Bradbury, S.P. (1995) Ecological risk assessment for chemical stressors: Challenges in
30 predictive ecotoxicology research. *Australian Journal of Ecotoxicology*, 1, 3-9
- 31 Brock, T.C.M. (1998) Assessing chemical stress in aquatic ecosystems: remarks on the need
32 of an integrative approach. *Aquatic Ecology*, 32, 107-111

- 1 Ciffroy, P., Garnier, J.M. and Benyahya, L. (2003) Kinetic partitioning of Co, Mn, Cs, Fe,
2 Ag, Zn and Cd in fresh waters (Loire) mixed with brackish waters (Loire estuary):
3 experimental and modelling approaches. *Marine Pollution Bulletin*, 46, 626-641
- 4 da Silva, E.T. (2005) Cadmium accumulation in the barnacle biomonitor *Balanus amphitrite*:
5 Combining field and laboratory observations with modelling. PhD, James Cook
6 University
- 7 da Silva, E.T., Ridd, M. and Klumpp, D. (2005) Can body burden in the barnacle *Balanus*
8 *amphitrite* indicate seasonal variation in cadmium concentrations? *Estuarine, Coastal*
9 *and Shelf Science*, 65, 159-171
- 10 da Silva, E.T., Ridd, M., Klumpp, D. and Ridd, P. (2004) Relative contribution of food and
11 water to the Cd burden in *Balanus amphitrite* in an urban tidal creek discharging into
12 the Great Barrier Reef lagoon. *Estuarine, Coastal and Shelf Science*, 60, 313-324
- 13 da Silva, E.T., Klumpp, D. and Ridd, M. (in press) The barnacle *Balanus amphitrite* as a
14 bioindicator for Cd: Development and application of a simulation model. *Estuarine,*
15 *Coastal and Shelf Science* (DOI 10.1016/j.ecss.2008.11.026)
- 16 Guillard, R.R.L. (1975) Culture of phytoplankton for feeding marine invertebrates. In W.L.
17 Smith and M.H. Chanley, *Culture of marine invertebrate animals*. (pp 26-60), New
18 York: Plenum Press
- 19 Jørgensen, S.E. (1994) *Fundamentals of ecological modelling*. Elsevier, Amsterdam
- 20 Ke, C. and Wang, W.-X. (2002) Trace metal ingestion and assimilation by the green mussel
21 *Perna viridis* in a phytoplankton and sediment mixture. *Marine Biology*, 140, 327-335
- 22 Lee, B.-G. and Luoma, S.N. (1998) Influence of microalgal biomass on absorption efficiency
23 of Cd, Cr and Zn by two bivalves from San Francisco Bay. *Limnology and*
24 *Oceanography*, 43, 1455-1466
- 25 Lee, B.-G., Wallace, W.G. and Luoma, S.N. (1998) Uptake and loss kinetics of Cd, Cr, and
26 Zn in the bivalve *Potamocorbula amurensis* and *Macoma balthica*: effects of size and
27 salinity. *Marine Ecology Progress Series*, 175, 177-189

- 1 Luoma, S.N., van Geen, A., Lee, B.-G. and Cloern, J.E. (1998) Metal uptake by
2 phytoplankton during a bloom in South San Francisco Bay: Implications for metal
3 cycling in estuaries. *Limnology and Oceanography*, 43, 1007-1016
- 4 Phillips, D.J.H. (1980) Toxicity and accumulation of cadmium in marine and estuarine biota.
5 In J.O. Nriagu, *Cadmium in the environment. Part I: Ecological cycling.* (pp 425-585),
6 New York: John Wiley & Sons, Inc.
- 7 Poethke, H.J., Oertel, D. and Seitz, A. (1994) Modelling effects of toxicants on pelagic food
8 webs: Many problems - some solutions. *Ecological Modelling*, 75/76, 511-522
- 9 Qiu, J.-W., Qian, P.-Y. and Wang, W.-X. (2001) Contribution of dietary bacteria to metal
10 accumulation in the slipper limpet. *Aquatic Microbial Ecology*, 25, 151-161
- 11 Rainbow, P.S., Blackmore, G. and Wang, W.-X. (2003) Effects of previous field-exposure
12 history on the uptake of trace metals from water and food by the barnacle *Balanus*
13 *amphitrite*. *Marine Ecology Progress Series*, 259, 201-213
- 14 Rainbow, P.S., Ng, T.Y.-T., Shi, D.L. and Wang, W.-X. (2004) Acute dietary pre-exposure
15 and trace metal bioavailability to the barnacle *Balanus amphitrite*. *Journal of*
16 *Experimental Marine Biology and Ecology*, 311, 315-337
- 17 Rainbow, P.S. and Wang, W.-X. (2001) Comparative assimilation of Cd, Cr, Se, and Zn by
18 the barnacle *Elminus modestus* from phytoplankton and zooplankton diets. *Marine*
19 *Ecology Progress Series*, 218, 239-248
- 20 Rice, E.W., Eaton, A.D. and Clesceri, L.S. (2005) *Standard Methods for the Examination of*
21 *Water and Wastewater.* American Public Health Association, American Water Works
22 Association & Water Environment Federation, New York
- 23 Sunda, W.G., Engel, D.W. and Thuotte, R.M. (1978) Effect of chemical speciation on toxicity
24 of cadmium to grass shrimp, *Palaemonetes pugio*: Importance of free cadmium ion.
25 *Environmental Science and Technology*, 12, 409-413

- 1 Turner, A. and Millward, G.E. (1994) Partitioning of trace metals in a macrotidal estuary.
2 Implications for contaminant transport models. *Estuarine, Coastal and Shelf Science*,
3 39, 45-58
- 4 Wang, W.-X. and Fisher, N.S. (1997) Modeling the influence of body size on trace element
5 accumulation in the mussel *Mytilus edulis*. *Marine Ecology Progress Series*, 161, 103-
6 115
- 7 Wang, W.-X. and Fisher, N.S. (1999) Assimilation efficiencies of chemical contaminants in
8 aquatic invertebrates: A synthesis. *Environmental Toxicology and Chemistry*, 18,
9 2034-2045
- 10 Wang, W.-X., Qiu, J.-W. and Qian, P.-Y. (1999a) Significance of trophic transfer in
11 predicting the high concentration of zinc in barnacles. *Marine Biology*, 33, 2905-2909
- 12 Wang, W.-X., Qiu, J.-W. and Qian, P.-Y. (1999b) The trophic transfer of Cd, Cr, and Se in
13 the barnacle *Balanus amphitrite* from planktonic food. *Marine Ecology Progress*
14 *Series*, 187, 191-201
- 15 Wang, W.-X. and Rainbow, P.S. (2000) Dietary uptake of Cd, Cr, and Zn by the barnacle
16 *Balanus trionus*: Influence of diet composition. *Marine Ecology Progress Series*, 204,
17 159-168
- 18 Wang, W.-X. and Wong, R.C.K. (2003) Combined effects of food quantity and quality on Cd,
19 Cr, and Zn assimilation to the green mussel, *Perna viridis*. *Journal of Experimental*
20 *Marine Biology and Ecology*, 290, 49-69
21

1 7. Figure Captions

2

3 Figure 1: The best-fit least squares linear regression model, passing through zero, between Cd
4 uptake rates and dissolved Cd concentration obtained from *Balanus amphitrite* exposed to
5 ^{109}Cd for 24 h. The fitting yielded a Cd uptake rate constant (k_u) of $0.0072 \text{ L g}^{-1} \text{ h}^{-1}$.

6

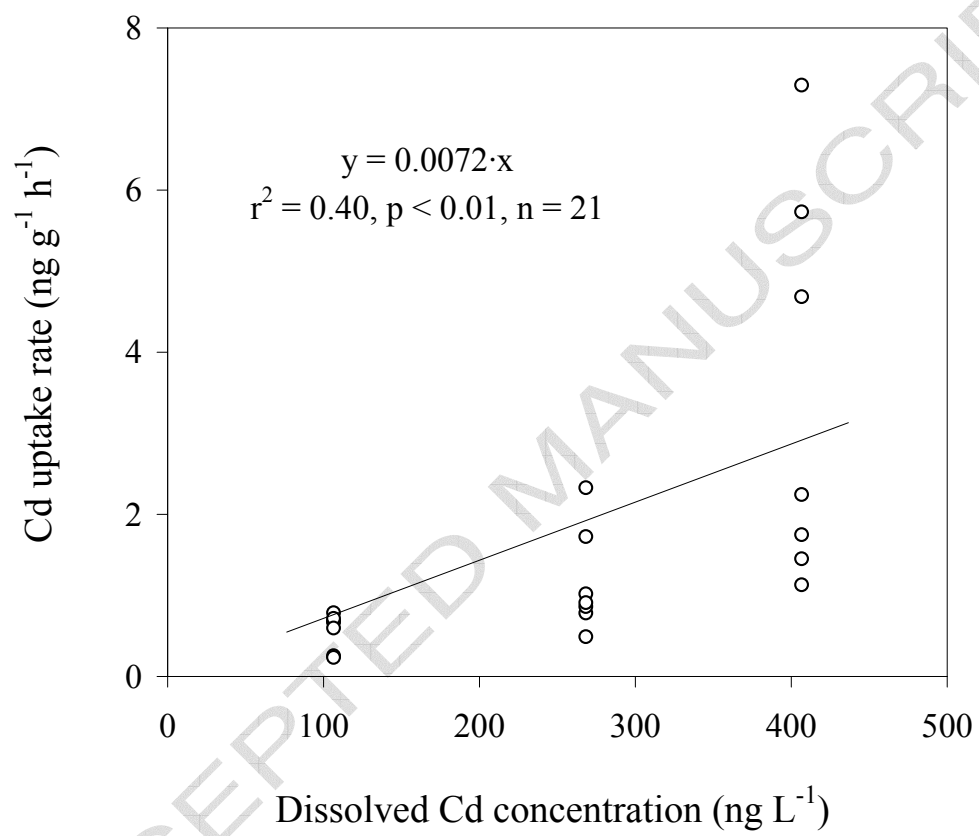
7 Figure 2: The relationship of the percentage of retention of ^{109}Cd as a function of time for 14
8 barnacles after seven of them been fed with ^{109}Cd -labelled SPM poor in chl *a*, chl *a*:SPM ratio
9 of 0.46 (a), and the other seven been fed with ^{109}Cd -labelled SPM rich in chl *a*, chl *a*:SPM
10 ratio of 0.59 (b). Numbers in brackets stand for the standard error of the parameter in the
11 adjusted model. Different symbols denote each of the seven barnacles fed with each batch of
12 SPM.

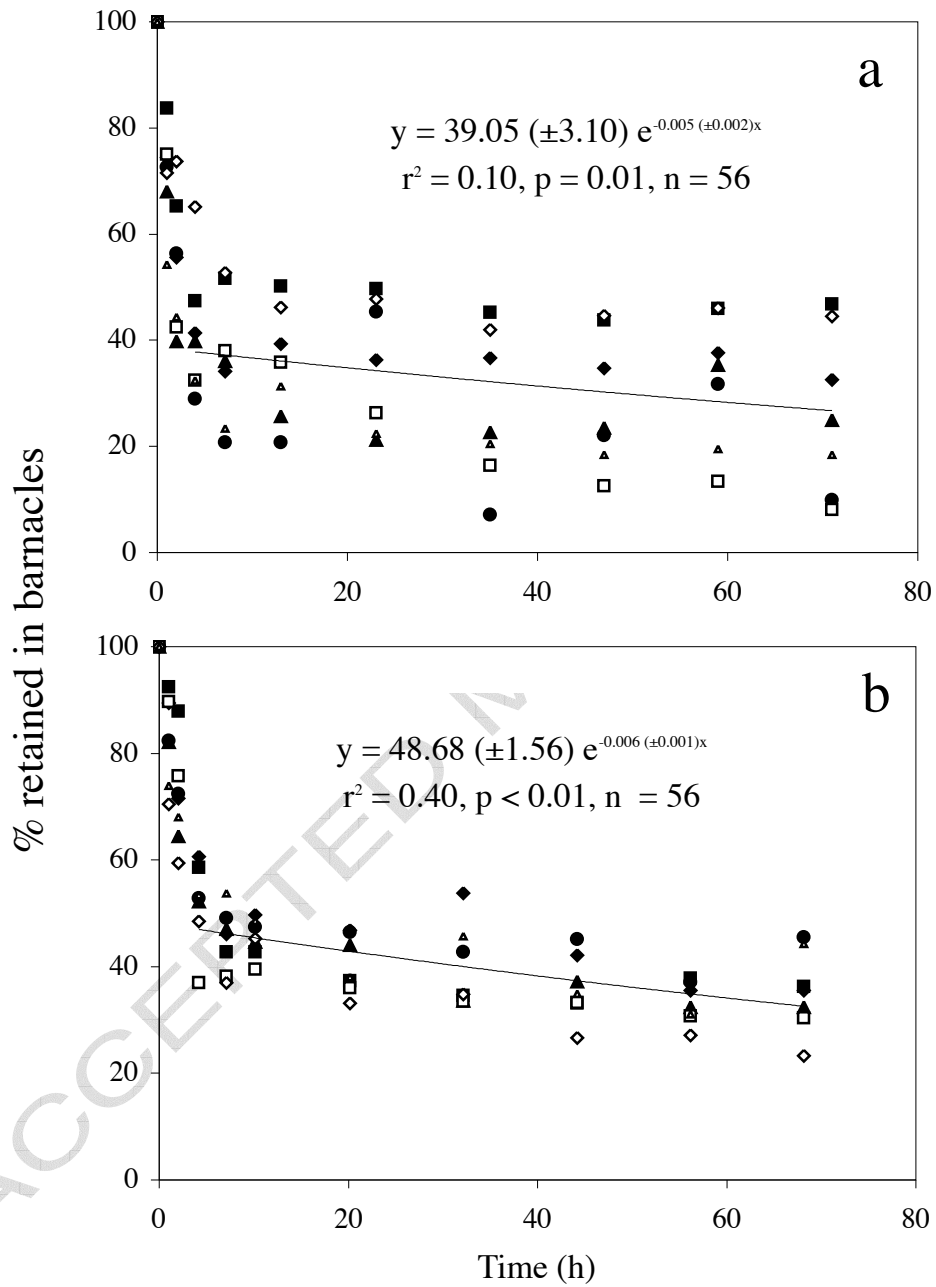
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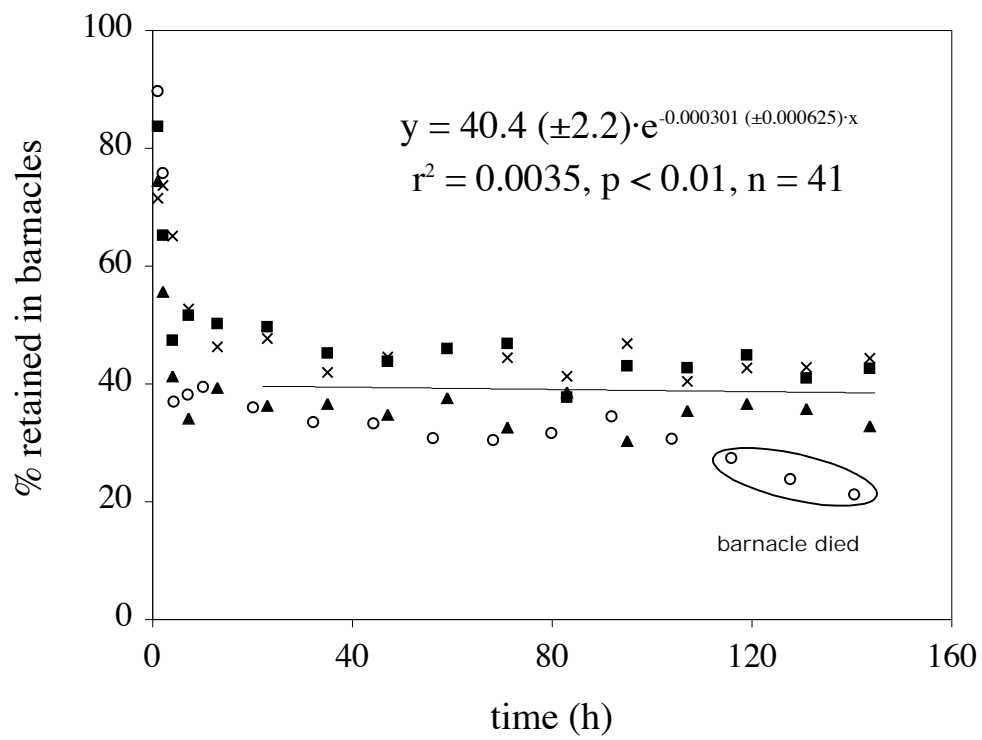
14 Figure 3: The relationship between the percentage of retention of ^{109}Cd as a function of time
15 for the four most ^{109}Cd active barnacles after they were used in the experiment for the AE
16 determination. Numbers in brackets denote the standard error of the parameter in the adjusted
17 model. Different symbols denote each of the four barnacles. Marked white circles were not
18 considered in the analysis because the barnacle was dead.

19

20 Figure 4: The relationship between the Cd AE from SPM for *Balanus amphitrite* as a function
21 of the ratio between chl *a* abundance ($\mu\text{g L}^{-1}$) and SPM mass abundance (mg L^{-1}) obtained
22 from two independent measurements: 'estimated' from the steady-state model and field data
23 (white circle), and 'measured' in the laboratory with ^{109}Cd and pulse chase feeding technique
24 (black circle). See text for explanation.







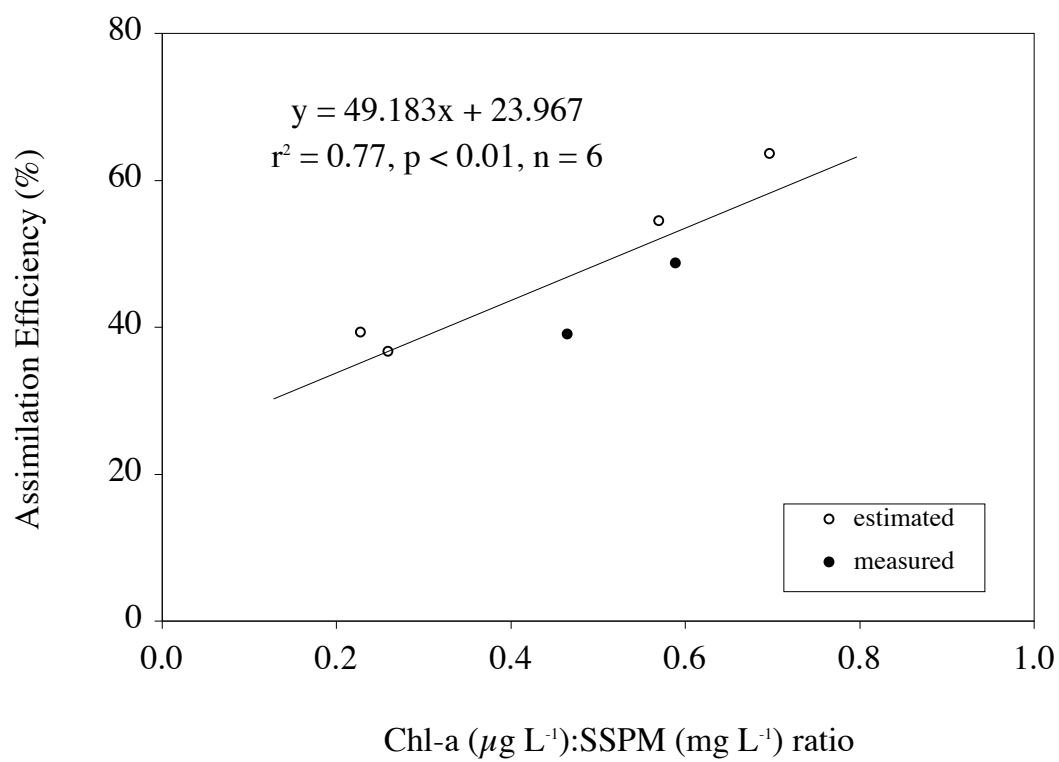


Table 1: The Cd AE from different food types for *Balanus amphitrite*.

Food type	Time interval for AE determination	Mean AE \pm 1 SD (%)	AE range (%)	Source
Phytoplankton				
<i>Thalassiosira weissflogii</i>	24 h ^a	35.2 \pm 5.8	26.8–41.5	c
<i>Thalassiosira weissflogii</i>	22 h ^a	59.6 \pm 10.1	40.2–69.9	d
<i>Chaetoceros Muelleri</i>	18–68 h ^b	34.8 \pm 5.1		e
<i>Skeletonema costatum</i>	18–68 h ^b	86.2 \pm 8.2		e
Zooplankton				
<i>Artemia salina</i> larvae	18–68 h ^b	63.4 \pm 9.0	52.6–71.5	e
<i>Canthocalanus pauper</i>	18–68 h ^b	71.6 \pm 17.4		e
<i>Temora turbinata</i>	18–68 h ^b	87.9 \pm 8.9		e
Natural SPM (rich in chl <i>a</i>)	4–68 h ^b	48.7 \pm 1.6		f
Natural SPM (poor in chl <i>a</i>)	4–68 h ^b	39.0 \pm 3.1		f

^a Activity remaining in the organism after a specific time had elapsed, compare to the initial activity.

^b Exponential decay model adjusted to the data of the temporal variation of the remained activity in the organism in a specific time interval.

^c Rainbow et al. (2003).

^d Rainbow et al. (2004).

^e Wang et al. (1999b).

^f This study.