Cadmium accumulation in the barnacle biomonitor *Balanus amphitrite*: Combining field and laboratory observations with modelling

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Abstract

The present study addressed the need to understand how short-term variations in metal concentrations in the environment determine its concentrations in a biomonitor, and how this information affects the use of the biomonitor in environmental monitoring programs. As a case study, the barnacle biomonitor *Balanus amphitrite* present in Ross Creek (Townsville, Queensland, AU) and the heavy metal Cd were used. The research methodology for this study comprised three integrated approaches: field measurements; the performance of laboratory experiments, and the development of an ecotoxicological simulation model, in order to understand the processes controlling Cd accumulation in *Balanus amphitrite* in the field. Two sampling programs were carried out along Ross Creek, in the dry season of 2002 and the wet season of 2004, in which barnacles, their food sources (two class sizes of suspended particulate material, SPM, and microzooplankton) and water (dissolved phase) were sampled weekly for Cd concentrations and mass abundances. Sampling periods were selected to test whether the concentration of Cd in the biomonitor responded to any variation in the dissolved and particulate phase Cd concentrations in Ross Creek, as caused by rainfall variation.

In both sampling periods, the Cd concentration in the dissolved phase increased upstream, ranging from 1.6 to 283 ngL^{-1}. The Cd concentration in the barnacle's food sources exhibited the same pattern – ranging from <0.01 to 2.10 mg kg^{-1} for the small size class of SPM (0.45–50 μm), from 0.07 to 1.62 mg kg^{-1} for large SPM (50–200 μm), and from 0.03 to 0.80 mg kg^{-1} for microzooplankton (50–200 μm). The Cd concentration in two populations of *Balanus amphitrite* increased upstream between two sites 2.20 km apart and ranged from 2.15 to 6.40 mg kg^{-1} and from 5.22 to 12.8 mg kg^{-1}. Even though no significant temporal variation was

detected for the Cd sources to the barnacles, the biomonitor Cd concentrations varied over the three sampling months, within each sampling period, exhibiting specific patterns for this variation. These observations suggest that changes in the Cd concentrations in the food sources and the relative mass abundance of these sources may result in a specific Cd concentration in *Balanus amphitrite.*

Similar Cd concentrations, within sites, were observed for the particles between the dry and wet seasons. Only the most contaminated site exhibited significant differences in the dissolved Cd concentration between seasons. Because more than 95% of the total Cd in the Ross Creek water (<200 μm) was in the dissolved phase (<0.45 μm), the differences in the dissolved Cd concentration resulted in the barnacles from the most Cd-contaminated site being exposed to a total Cd concentration in the wet season (45.8 ng L^{-1}) that was a half of that in the dry season (91.6 ng L^{-1}). Such Cd differences were not indicated by the biomonitor whose Cd concentration did not vary significantly between dry (8.4 mg kg^{-1}) and wet (7.4 mg kg^{-1}) seasons. A budget analysis based on Thomann's bioenergetic kinetic model, indicated that Cd flux from food contributes >80% of the Cd concentration in *Balanus amphitrite.* Thus, because no significant variation was identified for the Cd concentration in the food, no variation in the Cd concentration in the biomonitor was observed at the most contaminated site between seasons. A sensitivity analysis on the model showed that physiological characteristics of the biomonitor are the key parameters controlling Cd accumulation in *Balanus amphitrite*, rather than the metal concentration in the dissolved or particulate phases. This, coupled with the fact that the Cd flux from food is the major source of Cd to *Balanus amphitrite* suggests no tight coupling between Cd in the biomonitor and its availability in the environment.

A simulation model was developed based on Thomann's bioenergetic kinetic model. The daily-simulated Cd concentration in *Balanus amphitrite* produced by the model reproduced the general trend observed in the field. However, even though high and low patterns of Cd concentration in this organism could be reproduced by the model, it could not reproduce the short-term temporal variations accurately. A model investigation suggested that variations in the mean weight of the sampled barnacles might mask the real pattern of temporal variation of the barnacles Cd concentration; even though no size effect has been identified in the field data.

Two simulation exercises indicated that *Balanus amphitrite* may present some weakness in indicating temporal variations in Cd concentrations in the environment. The model results suggested that this organism could not indicate a 6-month Cd-pulse in the environment that increased the Cd concentration in its main source (small SPM, 0.45–50 μm) by a factor of 2.8 using a realistic sampling effort. In addition, this organism took more than a year to reach equilibrium for its Cd concentration in a simulated relocation experiment. These problems may be critical for the use of *Balanus amphitrite* as a biomonitor, and suggest that this organism can only provides a poor measure of current bioavailability of the metal in the environment. However, if a long-term mean Cd availability in the particulate fraction (sized <200 μm) is required, *Balanus amphitrite* can provide such an information.

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Chapter 1. *General introduction*

1.1. Introduction

In the last decades, the rapid development of coastal areas has led to a large increase in human impact on aquatic systems. The study of contaminant[1] effects on rivers and estuaries has received special attention due to the potential risk that they may present to fishing, water catchments and recreation (Timbrell 1989). Urban effluent is one of the most important sources of contaminants to the environment, being responsible for introducing to coastal ecosystems a considerable amount of organic matter, oils and greases, detergents, metals and industrial residues (Häni 1991, Novotny 1995, Clark 1997). Among the contaminants, heavy metals are important because of their high toxicity, bioaccumulation capacity in food webs (Timbrell 1989, Hapke 1991, Clark 1997, Kookana et al. 1998).

Ecological surveys focused on environmental monitoring for heavy metals have been used to provide information about long-term developments in ecosystems, and to drive management actions on the control of anthropogenic activities (Morris et al. 1988, Hertz 1991, Lam et al. 2000).Three components have been used to assess metal contaminations in the marine environment: metal concentration in the water, sediment and biota. The measures of metal concentration in the water have presented some problems. Metals in the dissolved phase are

[1] Following the recommendations by such international advisory bodies as the United Nations Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) and the International Commission for the Exploration of the Sea (ICES), the terms 'input' or 'effluent', 'contamination' and 'pollution' will be used herein to identify waste, occurrence of them in the sea, and the damaging effects they have, respectively (Clark, 1997).

usually close to the detection limits of the techniques available and to discriminate between their toxic and non-toxic forms can be quite challenging (Rainbow 1993). Moreover, they may exhibit irregular inputs and natural temporal variability that are difficult for the normal time frame of monitoring programs to detect (Morris et al. 1988). Metal concentrations in sediment, conversely, are far higher than in the dissolved phase and represent a time integration that overcomes the effect of temporal variability (Rainbow 1993, Turner et al. 2004), However, metal concentration in sediments represents the total metal concentration and characteristics of the sediment, especially organic content and grain size, that may affect metal concentrations independently of changes to metal concentration in the environment, thus confounding interpretation (Rainbow 1993). Organisms, conversely, accumulate the bioavailable metal in the environment, and concentrations in the organism's tissues are usually high enough to not present analytical problems. Additionally, organisms time-integrate the metal signal in the environment over weeks, months or years according to the species (Rainbow 1993). Therefore, measuring metal concentration in organisms offers significant advantages over measurements of water or sediments and consequently have been used in several monitoring programs in USA and Europe (Morris et al. 1988).

However, some problems still need special attention, for example, there is an enormous gap between the information obtained from the organism (i.e., its metal concentration), and any subsequent conclusions about environmental health (Poethke et al. 1994). Different metal concentrations in a set of biomonitor[2] organisms may not be a function of distinct metal

[2] The term biomonitor in this study refers to species used to provide information about the presence of a contaminant in an environment based on the concentration of this contaminant in their tissues. Even though this definition is actually applied for sentinel species (Beeby, 2001), the term biomonitor became much more popular in pollution ecology, so it was adopted in this study.

inputs, but a consequence of the particular environment or organism physiology. This occurs because both geochemical and physiological processes are involved in controlling metal accumulation in organisms. Metal speciation by precipitation, adsorption, complexation or hydrolysis (Morgan & Stumm 1991, Bourg 1995), variations in the composition and relative abundance of the food items ingested by the biomonitor (Wang et al. 1999a, Wang et al. 1999b, Wang & Rainbow 2000, Beeby 2001, Ke & Wang 2002, Wang & Wong 2003), differences in the organism's growth and efflux rates (Wang et al. 1999a, Wang et al. 1999b), duration and/or level of exposure to the metal (Beeby 2001) are some examples of parameters that may vary metal accumulation in the organisms independent of changes in the total metal concentration and/or its bioavailability in the environment.

Therefore, if the monitoring of physical and chemical aquatic parameters, including sediment, does not provide adequate quality standards for the protection of the marine environment (Morris et al. 1988), the use of organisms may also fail. Few studies have produced reliable sets of geochemical and biomonitor data to validate metal concentrations in organisms against environmental contamination (Luoma & Rainbow 2005). Several steady state models using physiological and geochemical approaches have predicted metal concentrations in marine bivalves, aquatic insects, crustaceans, phytoplankton and zooplankton with reasonable success (Thomann et al. 1974, Karez et al. 1988, Hare & Tessier 1996, Wang & Fisher 1998, Warren et al. 1998, Connell & Sanders 1999, Wang et al. 1999a, Wang et al. 1999b, Luoma & Rainbow 2005). However, these models have presented some differences between their results and reality. In most of the cases, such differences have been attributed to environmental changes, which in steady state models cannot be taken into account (Lee & Luoma 1998, Wang & Fisher 1998, Wang et al. 1999a, Wang et al. 1999b). Another important aspect regarding the use of biomonitors is the extent to which these organisms

integrate the temporal and spatial signal of metal concentration in the marine environment. This information is crucial to set up the standards for the use of an organism as a biomonitor, but it is rarely measured. For example, in monitoring programs it is usually assumed that the time-lag for a biomonitor to reflect changes in metal concentrations in its tissue is much longer than the environmental rates of changes, which results in a very poor measure of the current bioavailability of metals in the environment (Beeby 2001).

The present study addressed the need to understand how short-term variations in metal concentrations in an environment would affect its concentrations in a biomonitor, and how this information could affect the use of the biomonitor in environmental monitoring programs. The research methodology for this study comprised three integrated approaches: field measurements, the performance of laboratory experiments and the development of an ecotoxicological simulation model.

1.2. Aims of the Study

This study aimed to verify to what extent a biomonitor reflects variation in the metal concentration in the environment. As a case study, the main processes controlling the Cd accumulation in the barnacle biomonitor *Balanus amphitrite* present in Ross Creek (Queensland, AU) were investigated.

The specific objectives of this study were:

(i) To quantify the Cd concentration in the barnacle's soft tissue, in the water, and in its food items;

(ii) To quantify some processes of incorporation and elimination of Cd by the barnacle with radiochemical experiments in the laboratory;

(iii) To develop a steady-state model and an ecotoxicological simulation model to study the processes of Cd accumulation in the barnacle;

(iv) To identify the main controls driving the Cd accumulation in the barnacle, and

(v) To produce simulation scenarios in order to investigate the performance of using *Balanus amphitrite* as a biomonitor for Cd contamination in the marine environment.

1.3. Justification

1.3.1. The organism

Balanus amphitrite has been successfully used as a biomonitor for heavy metals especially in the Indo-Pacific region (Rainbow & Smith 1992, Rainbow 1993, Rainbow et al. 1993, Blackmore et al. 1998, Blackmore 1999, Wang et al. 1999a, Blackmore & Rainbow 2001, Rainbow & Blackmore 2001, Rainbow et al. 2003). Laboratory studies have shown that *Balanus amphitrite* is a useful biomonitor for several metals, including Cd, because it exhibits a low depuration rate that leads to high metal concentrations (Wang et al. 1999a, Wang et al. 1999b, Rainbow et al. 2003). Moreover, as microphagous feeders, barnacles may ingest potentially metal-rich particles and actively pass large volumes of water across the permeable surface of the cirri, facilitating further high rates of metal uptake (Rainbow & White 1989, Hunt & Alexander 1991, Rainbow & Blackmore 2001), which makes them very good biomonitors for heavy metals.

Even though some work applying steady-state models to understand metal incorporation from food in *Balanus amphitrite* (e.g., Wang et al. 1999a, Wang et al. 1999b, Luoma & Rainbow 2005) has shown that it is possible to predict metal concentrations in this species, some questions remain unclear. For example, Wang and co-workers (1999b) pointed out that "whether there is a tight coupling between the Cd concentration in *Balanus amphitrite* and the bioavailable Cd concentration in the environment is unclear". Moreover, the inclusion of environmental variability, such as those associated with the metal sources, would represent an important advance in understanding metal concentration in *Balanus amphitrite* and the relative contribution of each one of its sources of metal (Wang et al. 1999a, Wang et al. 1999b).

1.3.2. The environment

A survey carried out in several rivers in North Queensland showed that Ross Creek is the most polluted by heavy metals and hydrocarbons (Inglis & Kross 1999, 2000). In a recent study carried out in the winter of 2002 (da Silva et al. 2004), Ross Creek exhibited dissolved Cd concentrations up to 280 ng L^{-1}, which accounted for >90% of the total Cd in the creek (sized <200 μm). High values of Cd concentrations, approaching the screening levels recommended by the Australian and New Zealand Environment and Conservation Council, were also found in its bottom sediments (Doherty et al. 2000). Notwithstanding the total maximum Cd concentration in the Ross Creek water is below 0.3 μg L^{-1}, which is less than half of the value (0.7 μg L^{-1}) recommended as the trigger value to protect 99% of marine species (ANZECC 2000), some Cd contamination issues have been identified. For example, high levels of Cd, potentially capable of inducing health risks by human consumption, were identified in some of the commercially important marine species present in the Townsville

region (Clarke 1998). *Saccostrea amassa* collected at the Townsville Harbour presented Cd concentrations higher than the limits recommended by the Australian National Health and Medical Research Council (Jones 1981, 1992).

1.3.3. The importance of this study

Because the maximum total Cd concentration in the Ross Creek water column are below the trigger value, Cd may not be the subject of environmental concern. However, because of the bioaccumulation and biomagnification capacity that Cd exhibits through food chains, its concentration in biota may be of concern as it relates to human consumption. Despite the fact that pollution, including that from Cd, has been the subject of concern in Ross Creek and vicinities for more than two decades, except for two papers arising from this study[3], there are no other published data on Cd concentrations in the Ross Creek water and in its planktic[4] components, nor studies comparing metal accumulation in organisms to its availability in the creek.

The marine environment in North Queensland is of great relevance since it supports the important tourism, fishing, nautical and navigation industries (Queensland Transport1998). As rivers and estuaries close to urban areas are under the influence of multiple uses (e.g.

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[3] da Silva ET, Ridd M, Klumpp D, Ridd P (2004) Relative contribution of food and water to the Cd burden in *Balanus amphitrite* in an urban tidal creek discharging into the Great Barrier Reef lagoon. Estuarine, Coastal and Shelf Science 60:313-324.

da Silva ET, Ridd M, Klumpp D (2005) Can body burden in the barnacle *Balanus amphitrite* indicate seasonal variation in cadmium concentrations? Estuarine, Coastal and Shelf Science 65:159-171.

[4] Term as defined in Harris and co-workers (2000).

shipping, small industries and municipal waste), they may suffer several contaminant discharges, including heavy metals, which may compromise their uses. The Queensland Department of Premier and Cabinet support a policy formulation to protect the GBR from riverine runoff based on the development of modelling tools (Intergovernmental Steering Committee 2003, http://www.premiers.qld.gov.au/about/ reefwater.pdf.). Thus, the understanding of processes of metals accumulation in biomonitors matches with their recommendations.

In addition, the understanding of processes of the Cd accumulation in *Balanus amphitrite* and the information that will be generated will provide a better picture of the behaviour of Cd in Ross Creek, in both temporal and spatial scale, and its potential contribution to the GBR lagoon. Moreover, the study by combining field and laboratory observations with a simulation model will provide a framework to investigate the use of biomonitors in an environmental perspective.

1.4. Thesis Outline

The following chapters present the study by combining field and laboratory observations with a modelling to understand Cd accumulation in the barnacle biomonitor *Balanus amphitrite*. Chapter 2 of this thesis examines the current information available in the literature regarding Cd in the environment and in aquatic organisms. Some critical issues and recent alternatives that have been applied to deal with heavy metal in the marine environment and its accumulation in aquatic organisms, especially for Cd are discussed. Several new approaches, such as techniques of ultra-filtration and chemical analysis, the use of radioisotope techniques to determine rates and parameters in the metal accumulation processes in aquatic organisms,

and the use of mathematical models as predictive tools for environmental risk assessment that have opened new perspectives on marine pollution issues are also presented in this chapter.

Chapter 3 presents the methodology applied for the data obtained in the field and analysis that were used in Chapters 4, 5 and 6. The sampling strategy and analysis for the Cd determination in the soft tissue of *Balanus amphitrite*, and in its food items (i.e., two size-classes of suspended particulate matter and microzooplankton) are presented. A general characterization of the Ross Creek area and the sampling sites are also presented.

Chapter 4 presents and discusses the data sampled in the first sampling program (dry season 2002). Non-parametric statistics and biological concentration and magnification factors are applied to analyse the spatial variations of Cd concentrations in *Balanus amphitrite*. A simple diffusion coefficient model was also applied to estimate the Cd export during the dry season from Ross Creek into the GBR lagoon.

Chapter 5 presents the results of the second sampling program (wet season 2004) and compares them with those from the first sampling program. Using a bioenergetic kinetic model, a budget analysis is performed to determine the importance of each Cd source to *Balanus amphitrite*. The model is also used in a sensitivity analysis to identify the main controls on the Cd accumulation in the barnacle. The barnacle's growth rate and clearance rate, determined in the field and laboratory, respectively, were used to support the model development.

In Chapter 6 an ecotoxicological simulation model was developed to study on a daily basis the processes controlling Cd accumulation in *Balanus amphitrite*. This model was validated

based on the data from the two sampling programs. To support the model development, some radiochemical experiments were carried out to determine some processes controlling Cd accumulation in *Balanus amphitrite*. The model was used in a series of simulation exercise to analyse the performance of *Balanus amphitrite* as a biomonitor for Cd contamination in the environment. To conclude the thesis, Chapter 7 presents a summary of the main outcomes of this study, the general conclusions about the use of *Balanus amphitrite* as a biomonitor for Cd, and some suggestions for further research that may improve the use of this organism in biomonitoring programs.

This work generated two publications and a third that was submitted to Marine Ecology Progress Series; their titles and sources are listed bellow:

da Silva ET, Ridd M, Klumpp D, Ridd P (2004) Relative contribution of food and water to the Cd burden in Balanus amphitrite in an urban tidal creek discharging into the Great Barrier Reef lagoon. *Estuarine, Coastal and Shelf Science* 60: 313-324.

da Silva ET, Ridd M, Klumpp D (2005) Can body burden in the barnacle Balanus amphitrite indicate seasonal variation in cadmium concentrations? *Estuarine, Coastal and Shelf Science* 65: 159-171.

da Silva ET, Ridd M, Klumpp D (submitted) Exploring the potential of Balanus amphitrite as a biomonitor for temporal and spatial variations in Cd contamination in a simulation model. *Marine Ecology and Progress Series*.

Chapter 2. *Cadmium accumulation in aquatic organisms*

2.1. Environmental Chemistry of Cd

Cadmium is a ubiquitous heavy metal with an estimated global emission into the ocean of around 15,000 tonnes a year of which 60% come from anthropogenic sources (Nriagu 1980, Stoeppler 1991, Laws 2000). It is present in sewage sludge, dust from zinc and phosphate industries, municipal waste, pesticides, fertilisers, pigments and batteries (Fassett 1980, Nriagu 1980, Stoeppler 1991, Llewellyn 1994, Clark 1997). Toxicity tests have shown that a dissolved concentration of Cd of 0.2 μg L^{-1} is able to produce harmful effects to biota, and it is the second most toxic element in the marine environment, after mercury (Ketchum et al. 1975, Phillips 1980, Raspor 1980, Wong et al. 1980, Timbrell 1989, Clark 1997). Cadmium was included on the "Black List"; produced by the international agencies of environmental control, because it may present a potential risk to the public health (Novotny 1995). For example, consumption of Cd-contaminated organisms by man can cause many health problems, such as irritation of the digestive tract, fragility of bones, kidney disease and even death (Stoeppler 1991, ATSDR 1993, Novotny 1995).

The principal routes for Cd entry into the marine environment are through urban wastes (municipal and industrial), illegal dumps and aerial deposition (Raspor 1980, Garman & Sutherland 1983, Häni 1991, Stoeppler 1991, Novotny 1995). Once Cd reaches the water it is under influence of four groups of interacting processes that affect its fate: transport; transformation; speciation and bioaccumulation (Kremiling 1988, Novotny 1995). Because this study seeks to understand metal accumulation in biomonitors, only literature related to

metal availability in the environment, measuring techniques, and mechanisms of metal incorporation into organisms will be discussed herein.

2.1.1. Cadmium in the marine environment

Cadmium can reach high concentrations in food chains because of its strong capacity for bioaccumulation (Thomann et al. 1974, Raspor 1980, Wang 1985, Hapke 1991, Hertz 1991, Stoeppler 1991, Kookana et al. 1998, Warren et al. 1998, Wang et al. 1999b). Typical Cd concentrations in the dissolved phase in estuaries range from 0.04 to 2 μg L^{-1} (Phillips 1980, Stoeppler 1991). However, organisms may accumulate Cd concentrations that are 10^4 times or greater than those in water due to bioaccumulation through the food chain (Phillips 1980, Raspor 1980, Stoeppler 1991, Warren et al. 1998).

The understanding of metal contamination in aquatic organisms requires more sophisticated chemical measurements than mere measurement of the total amount of a metal in the environment, since metals in different forms present different toxicities (Khalid 1980, Raspor 1980, Wang 1985, Kremiling 1988, Morgan & Stumm 1991). Some processes, such as speciation, and transformation or biomodification, result in changes in the metal toxicity in aquatic environments (Raspor 1980, Kremiling 1988, Morgan & Stumm 1991, Novotny 1995). Speciation, which includes acid-base equilibria, complexation and sorption, affects the ionic form of metals and their complexation with ligands present in the medium. Precipitation/dissolution equilibria, oxidation/reduction reactions, dilution and adsorption are the main controls of speciation (Morgan & Stumm 1991, Bourg 1995, Novotny 1995). Transformation refers to those processes carried out by microbial metabolism that may change the original toxicity of a metal (Morgan & Stumm 1991, Novotny 1995).

In aquatic environments, Cd can be present in either the solid or the dissolved phase, which results in completely different chemical and biological behaviour as well as different fluxes through the environment (Morgan & Stumm 1991, Bourg 1995). These two forms (solid and dissolved) are exchangeable between each other and can be sub-divided into four groups as shown in Figure 2.1.

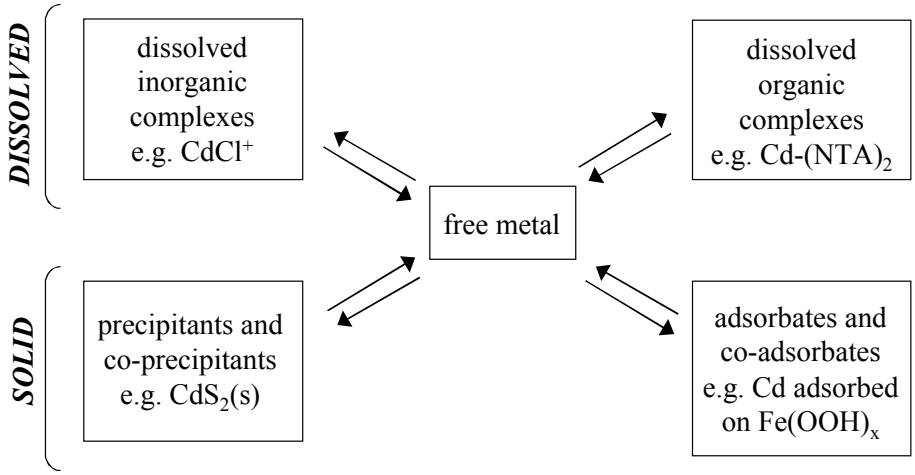

Figure 2.1: Geochemical speciation of Cd (from Bourg 1995).

Four processes drive the exchange among these forms: precipitation, adsorption, complexation and hydrolysis (Khalid 1980, Bourg 1995). As claimed by these authors, on precipitation, Cd is removed from suspension mainly through reactions to form carbonates, oxides, phosphates, silicates and sulfides. On adsorption, Cd is sorbed to the surface of solids and thus removed from suspension. Both processes result in a decrease in the metal availability in the water column (Bourg 1995). Conversely, complex formation with dissolved organics (e.g. dissolved organic carbon and synthetic ligands such as EDTA and NTA), inorganic ligands (e.g. Cl), and hydrolysis are responsible for decreasing Cd availability in the

water column. Therefore, the understanding of the Cd transference in food chains means that one must have the capability to distinguish among its several forms.

2.1.2. Dealing with Cd speciation

A particular challenge in modelling ecotoxicological issues is the selection of the right level of complexity required to deal with the several forms of toxic substances in the environment (Jørgensen 1994). On the one hand, the complex behaviour of metals described above, and the spatial and temporal variability of the environmental properties (mainly pH, Eh, and salinity), make speciation prediction difficult using geochemical models (Khalid 1980, Morgan & Stumm 1991, Bourg 1995). On the other hand, isolating and studying each one of the constituents separately with the available methods is practically impossible (Bourg 1995). An alternative is to work with size differentiation obtained through sequential filtration processes (e.g., Morgan & Stumm 1991, Bourg 1995, Novotny 1995, Muller 1996, Wen et al. 1996).

In natural waters, Cd is present either bound to particles (in or adsorbed to them) or in the dissolved phase (Khalid 1980, Nriagu 1980). The most common approach is to use a 0.45-μm filter to operationally separate the particulate from the 'dissolved' phase (Coombs 1979). However, the 'dissolved' phase contains Cd that is truly dissolved as well as Cd associated with colloidal material (Morgan & Stumm 1991, Bourg 1995, Novotny 1995, Muller 1996, Wen et al. 1996). The technique of cross-flow ultrafiltration (CFF) is a method to recover the colloidal phase. This technique has been recently applied, allowing good mass balance between truly dissolved and colloidal phases when compared with the total Cd in the dissolved phase (<0.45 μm) (Wen et al. 1996).

In the truly dissolved phase metals are present in a wide range of chemical forms, including the free ions and inorganic and organic complexes (Muller 1996). Some attempts have been made to deal with the presence of Cd complexes in the truly dissolved and colloidal phases. Differential Pulse Anodic Stripping Voltammetry with Mercury Film Electrodes (DPASV-MFE) has been applied as the best technique to find out the total soluble metal ion concentration; after filtration followed by acidification and UV-digestion (Aylett 1979, Raspor 1980, Wang 1985, Morgan & Stumm 1991, Stoeppler 1991). For example, from the truly dissolved phase, as separated by CFF, the free ion of Cd and hydrated ones (very labile forms) are assayed by DPASV-MFE at pH 2 (Raspor 1980, Wang 1985). If UV digestion is applied before the DPASV-MFE analysis, Cd complexed by organic material, such as amino, fulvic and nuclide acids, proteins, thionines, and chelators of anthropogenic origin (e.g. EDTA and NTA) are assayed as well. From the colloidal phase, as separated by CFF, at pH 2 and UV digested, Cd chelated in rather stable and in inert complexes with organic ligands, such as humic acids, and inorganic compounds like clays, are assayed (Raspor 1980).

Therefore, a mechanical differentiation of the several size forms attained through CFF after a 0.45-μm filtration, followed by DPASV-MFE, is a feasible (although experimentally challenging) approach to measure Cd speciation in natural waters. It is worth mentioning that ultra-clean techniques are essential during all stages of sample collection, transporting, handling, processing and analysis, in order to produce reliable data (Wen et al. 1996), especially when the environment presents a low Cd concentration.

Regarding the fraction retained on the 0.45-μm filter, i.e. suspended particulate material, two approaches have been applied: DPASV on samples UV digested and acidified or some atomic

absorption technique after an acid digestion of the sample (Raspor 1980, Wang 1985, Holland & Tanner 1999). These techniques have given comparable results (Wang 1985).

2.1.3. Cadmium accumulation in aquatic organisms

The metal bioavailability and the level of toxicity of a metal are intrinsically related, since the metal uptake by biota is dependent on its chemical form (Khalid 1980, Wang 1985, Morgan & Stumm 1991, Novotny 1995). The size and charge of the chemical form of a metal provide information on the ability of it to penetrate membranes and enter food webs (Phillips 1980, Raspor 1980). For example, Cd can be taken up from water by adsorption to-, and/or by absorption across the body surface (Phillips 1980) and obtained from food, including suspended sediment and biota (Blackmore et al. 1998, Qiu et al. 2001, Wang 2002).

Several studies on bioavailability indicated that metals in the dissolved phase are more likely to be incorporated by biota (Sunda et al. 1978, Phillips 1980, Wong et al. 1980, Salomons et al. 1988, Rainbow & White 1989, Morgan & Stumm 1991, Hare & Tessier 1996, Warren et al. 1998). However, some recent publications have shown that the main route of metal accumulation in aquatic organisms is through food rather than water. For example, in a study for Cd, selenium and zinc uptake in copepods, <3% originated from the dissolved phase, indicating that food was the principal source of these elements for these organisms (Wang & Fisher 1998). Connell & Sanders (1999) found that the main route of Cd taken up by zooplankton was from food (phytoplankton) and not from water. Several works have shown that the greater the presence of living particles on suspended particulate matter (SPM) carrying Cd, the greater the assimilation efficiency of Cd by organisms that feed on them (e.g., Lee & Luoma 1998, Ke & Wang 2002, Wang & Wong 2003). Warren and co-workers

(1998) studying metal accumulation in various benthic taxa exposed to an artificial sedimentary Cd gradient observed that: (i) the organisms respond to the metal in the sediment rather than the dissolved phase, and (ii) the organisms respond differently to the sedimentary Cd gradient as a function of their feeding behaviour. A kinetic model predicted that 87% of the Cd burden in the slipper limpet *Crepidula onyx* originated from the dietary phase (phytoplankton and bacteria) rather than the dissolved phase (Qiu et al. 2001).

These studies show that feeding behaviour is very important in determining the organisms exposure to Cd. Not only the free and soluble forms of Cd can be incorporated by organisms but also Cd associated with sediment and food. This fact leads to two issues in the understanding of metal accumulation in aquatic organisms: (i) the quality of food may change the metal accumulation in an organism as a function of different assimilation efficiencies that each source of food may present (Lee & Luoma 1998, Wang et al. 1999a, Wang et al. 1999b, Ke & Wang 2002), and (ii) food selection may account for changes in the overall metal balance in organisms (Wang & Fisher 1996, Connell & Sanders 1999). Therefore, determining uptake pathways for metals in organisms is important to understanding metal bioavailability in the environment, and it is also critical for the setting of water quality criteria (Wang et al. 1999b).

2.1.4. Understanding metal transference using radioisotopes

A critical aspect when modelling ecotoxicological issues is to obtain reliable estimates of the parameters and rates that control the metal accumulation in the organisms. Manipulative experiments using radioisotopes are among the best methods to attain this (Wang & Fisher 1999), and there is an abundant literature of studies determining assimilation efficiency (AE),

influx and efflux rates[5] for Cd and other metals by organisms (e.g., Lee & Luoma 1998, Wang & Fisher 1998, 1999, Wang et al. 1999a, Wang et al. 1999b, Qiu et al. 2001, Rainbow & Wang 2001, Ke & Wang 2002, Rainbow et al. 2003).

Independently of which parameter is under study (i.e. assimilation efficiency, influx or efflux rate), the experiments that may be undertaken are very similar. For the determination of the metal uptake rate constant from the dissolved phase, organisms are exposed to the radioisotope (e.g., ^{109}Cd) present in solution in a 0.45-μm filtered water for a certain period of time (e.g., Rouleau et al. 2001, Blackmore & Wang 2002, Rainbow et al. 2003, Blackmore & Wang 2004, Rainbow et al. 2004a). After the exposure time, the organisms are removed from the medium and assayed for the radioisotope activity with a gamma counter. The activity in the organism is then transformed to mass of radioisotope taken up from solution by a calibration curve relating radioisotope's activity and mass.

To determine the AE and efflux rate constant, the pulse-chase feeding technique is usually applied (e.g., Wang & Fisher 1999, Wang et al. 1999a, Wang et al. 1999b). Basically, this technique is undertaken in two phases, named 'hot' and 'cold' phases. During the 'hot' phase starved organisms are placed into a chamber in which the only source of the radiochemical is in the food. This phase lasts for 30–45 min, a period of time short enough to avoid egestion of the ingested material. After the 'hot' phase, the organisms are removed from the medium and assayed for the radioisotope activity with a gamma counter. The organisms are then placed

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

into a new chamber with the same quality and quantity of food used in the 'hot' phase, but without radioisotope. This is the 'cold' phase, in which the organisms depurate the radiochemical ingested, and may last for 3–4 days for the AE determination, and longer for the efflux rate constant determination. In this phase, the organisms are assayed time by time for the radioisotope activity in the organism body.

In the calculation of either AE or the efflux rate constant, the percentage of variation in the radioisotope activity in the organisms is plotted against the time. Figure 2.2 presents a typical bi-phasic pattern for the radioactive decay in *Balanus amphitrite* over time in the depuration phase of a pulse-chase feeding experiment after the 'hot' phase. The fast decay, which lasts 4–6 h, represents the passage of most of the labelled food by the gut of the organisms, during which some unassimilated materials are purged from the gut. The second and slow stage represents the excretion of metal that has been assimilated into the gut cells (Wang & Fisher 1999). When an exponential-decay model as presented in Equation 2.1 is applied for the second stage of depuration (II, in Figure 2.2), the coefficient A is determined as the assimilation efficiency. The determination of the efflux rate constant requires that the organisms be kept on depuration for a longer time than for the AE determination. For example, in 22 h *Balanus amphitrite* empties completely its gut (Rainbow et al. 2004a) and subsequent losses after that can be attributed to the efflux process. The same model Equation 2.1 is then adjusted and the coefficient B is determined as the efflux rate constant (Wang & Fisher 1999).

$$y = A \cdot e^{-B \cdot x}. \qquad \text{Equation 2.1}$$

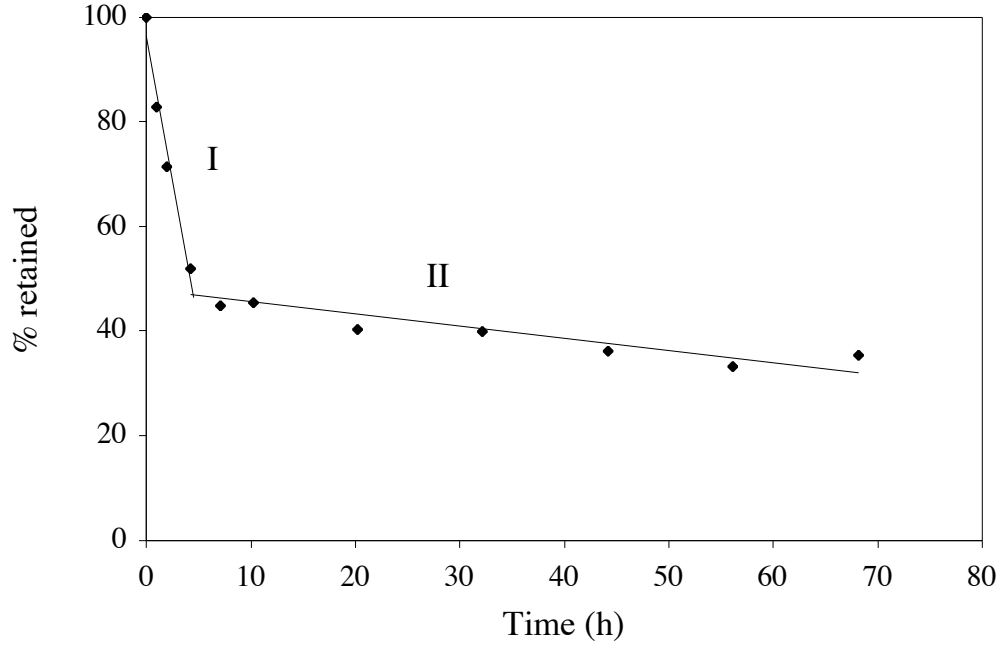

Figure 2.2: Typical bi-phasic pattern for the variation in the percentage of ^{109}Cd activity in *Balanus amphitrite* following the 'hot' phase in the pulse-chase feeding experiment carried out in this study (see complete series of data in section 6.3.2.2).

An important issue must be taken into account when using AE results from the laboratory experiments to understand metal accumulation in organisms in the environment. Because AE is dependent on the source, each source of metal (e.g. sediment, food items) must be considered independently (Wang & Fisher 1999). This fact has two implications that must be taken into account when planing a radiochemical experiment. The first one is conceptual; one must distinguish which group of organisms (e.g. phytoplankton, zooplankton, bacteria) and non-living material in the water (e.g. truly dissolved phase, colloids, suspended particular material) must be included as 'sources' of metal for the organisms. The second one is operational. It is important to distinguish during the course of the experiment the exact route by which the organism is taking up metal. For example, adventitious consumption of faeces

with labelled material during the depuration phase would result in an incorrect estimation of AE. This may affect the overall metal balance in the organisms because microbial degradation of pellets and faeces may quickly release metal to the dissolved phase, which would add an extra source of metal to the organism (Lee & Luoma 1998). Two strategies suggested by Lee and Luoma (1998) may be adopted to deal with such questions. First, to distinguish between the uptakes from dissolved and particulate phases a correction, subtracting the uptake from the dissolved phase experiment from the uptake from the particulate phase experiment, can be applied. Second, to avoid any overestimation of the metal uptake from sources other than the radiolabelled food, a mass balance taking into account the tissue activity after depuration plus the radioactivity in the egested material during the depuration period, may be applied to provide the total ingested radioactivity. Thus, any re-ingestion of the metal lost by the organism, as a function of egestion and/or faeces liberation, would be corrected. Alternatively, the egested material may be removed by frequent filtration of the culture medium, avoiding any extra source of metal in the culture medium (Wang & Fisher 1998).

Obtaining a good picture of heavy metal accumulation in aquatic organisms is not a trivial task. As seen throughout this chapter, knowledge about the chemical species of the metal in the environment, the routes that the metal follows until it reaches the organism and the speed of the processes that control the accumulation and elimination of the metal by the organism are required to understand metal accumulation in aquatic organisms. In addition, all this information must be integrated in a concise picture and include in some way any environmental changes. One way of dealing with these data is to develop an ecotoxicological model that integrates all the information from a systemic perspective (Jørgensen 1994, Brock 1998).

2.2. Modelling Metal Accumulation in Aquatic Organisms

Models represent a powerful tool to assess the metal accumulation in aquatic organisms (Jørgensen 1994), and they are strongly recommended for the interpretation of metal concentrations in biomonitors (Luoma & Rainbow 2005) including barnacles (Wang et al. 1999a, Wang et al. 1999b). Two basic modelling approaches may be applied to understand systems in a holistic viewpoint: steady state and simulation models (Jørgensen 1994). Several steady state models using physiological and geochemical approaches have predicted metal concentrations in marine bivalves, aquatic insects, crustaceans, phytoplankton and zooplankton with reasonable success (Thomann et al. 1974, Karez et al. 1988, Hare & Tessier 1996, Wang & Fisher 1998, Warren et al. 1998, Connell & Sanders 1999, Wang et al. 1999a, Wang et al. 1999b, Griscom et al. 2002, Luoma & Rainbow 2005). However, these models have presented some differences between their results and reality. In most of the cases, such differences have been attributed to environmental changes, which in steady state models cannot be taken into account (Lee & Luoma 1998, Wang & Fisher 1998, Wang et al. 1999a, Wang et al. 1999b).

Simulation models offer the capability of simulating metal concentrations in biomonitors under conditions of environmental changes. A literature search failed to find an example of a simulation of metal accumulation in *Balanus amphitrite* or any other marine filter feeder. In this study, a simulation model of Cd accumulation in *Balanus amphitrite* will be developed, and accordingly an introduction to simulation modelling is now presented.

2.2.1. Simulation modelling: Theoretical background

Models are simplifications of reality, in which the main characteristics of the system being studied are represented (Wiegert 1975). Four steps are involved in the use of a modelling approach, and they were extensively discussed elsewhere (e.g., Wiegert 1975, Hall & Day Jr 1977, Jørgensen 1994, Hannon & Ruth 1997, Grant et al. 2000). Therefore, a brief explanation of each one of these steps is presented herein.

In the first step, a detailed examination of the environment and a literature search are undertaken. This aims to find out the limits of the system and its main interactive components that are relevant to the understanding of the problem. In this step, three elements of the model are defined: state variables, processes and forcing functions. State variables are stocks of material and/or energy that represent the structure of the system (i.e. the model components). Processes control inflows and outflows of material and/or energy in each state variable. They may represent interactions among the state variables, or among the state variables to beyond the system limits. Forcing functions are external influences on the system that provide information on it. They can change the system dynamics, changing the speed of the processes, but they are not disturbed by the system.

In the second step, a conceptual model is drawn as a simplification of the reality to be studied. In this step, the structure of the system (state variables), its main processes, and the controllers of these processes (forcing functions) are represented. If a lake, as presented in Figure 2.3, is taken as an example, the bottom grass and the herbivorous fish would be two state variables, which would represent the quantities of each of them over time. The grass production and the fish feeding on grass would be two processes, which would control

variations in the quantities of the state variables (i.e., grass and fish). Light, nutrients, and temperature would be forcing functions of the lake system, controlling the processes of production and grazing, as indicated in Figure 2.3. Usually, an appropriate diagrammatic language used to conceptualise the model (see Appendix I).

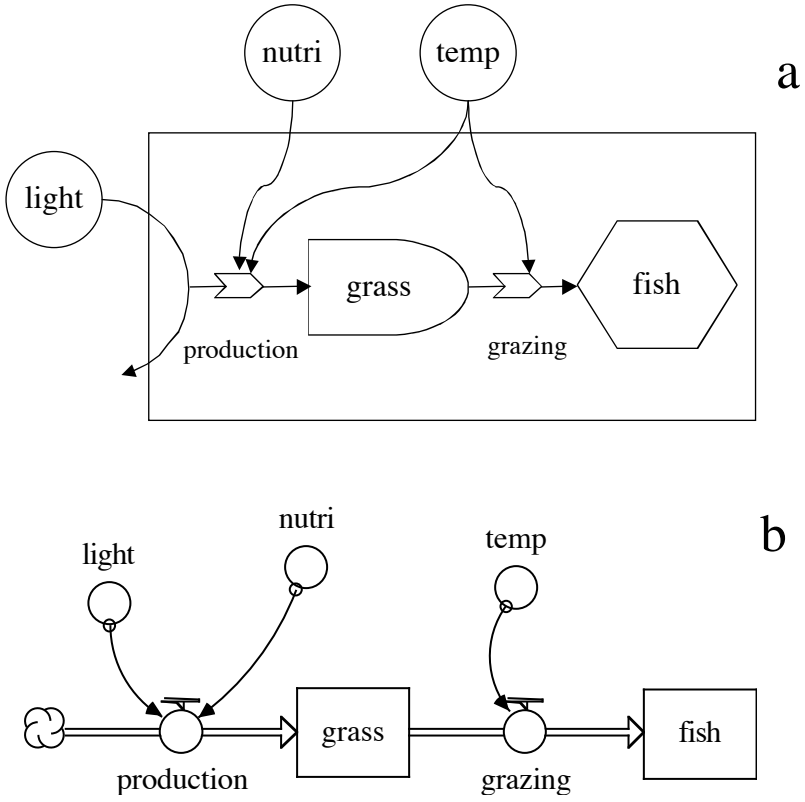

Figure 2.3: Conceptual model of the lake system. Diagram follows the symbolic language (a) as proposed by Odum (1983), and (b) as used in the Stella software. It represents two state variables (i.e. grass and fish), two processes (production and grazing) and three forcing functions (light, nutrient and temperature). The box in upper diagram represents the limit of the system.

In the third step, equations and their parameters, used to describe the model's structure and processes, are defined and written out in a computational environment. These equations describe: (i) the behaviour of each state variable, which are written as differential equations

(see, e.g., Swartzman & Kaluzny 1987); (ii) the processes, which are defined by ecological parameters and coefficients (see, e.g., Jørgensen 1994), and (iii) the forcing functions, which are represented by either equations or environmental data collected in the field (see, e.g., Hall & Day Jr 1977).

In the fourth step, an analysis of the model's result based on the theoretical and empirical knowledge about the system under study is undertaken. This analysis involves four processes: model calibration; model validation; sensitivity analysis and simulation exercises. In the model calibration, the equations and parameters included in the model are adjusted to better reproduce the patterns observed in the field. In another words, the quantities simulated in each state variable of the model are compared with the quantities of the components observed in the field, and adjustments in the model's parameters are performed to match them. In the model validation, a different data set is compared with the model's result to find out the capacity of the model to reproduce the studied environment. Sensitivity analysis and exercises of simulation represent powerful tools in the investigation of the processes in the model. Not only do they test the integrity and stability of the model, but they facilitate model exploration, which is responsible for bringing new insights about the system dynamics (Jørgensen 1994, Grant et al. 2000). In the sensitivity analysis, variations are imposed on the forcing functions and/or on the processes, and the behaviour of the components (state variables) is evaluated. It is very common, for example, to increase temperature (a forcing function used in the example of the lake, Figure 2.3) by 10, 20 and 30% of its nominal value and to verify which state variable is more sensitive to such variations. This process is repeated for every parameter in the model individually, allowing each forcing function or process in the model to be hierarchically ordered as a function of their importance to the system dynamics. The simulation exercises are similar to the sensitivity analysis. However, changes are imposed on

the model in order to answer specific questions. For example, it may be interesting to know if an artificial increase in the nutrient supply of 30% would bring some benefit to the fishing catch in the case of the lake shown in Figure 2.3.

2.2.2. Thomann's bioenergetic kinetic model

Thomann's bioenergetic kinetic model (see, e.g., Thomann 1981, Wang & Fisher 1998, Wang et al. 1999b, Luoma & Rainbow 2005) is a generic relationship to explain metal variation in any aquatic organism, and it can be applied for both simulation and steady-state models. The model formulation varies from author to author, but it is always assumed that water and food are the main routes of metal intake by an organism, and excretion and growth dilution represent a reduction in the metal concentration. The model can be represented as:

$$\frac{dM_o}{dt} = \left(k_w \cdot M_w\right) + \left(AE \cdot IR \cdot M_f\right) - \left(k_e + g\right) \cdot M_o, \qquad \text{Equation 2.2}$$

where dM_o/dt is the variation in time of the metal concentration in an organism (M_o), M_w is the metal concentration in the water, M_f is the metal concentration in the food, AE is the assimilation efficiency, IR is the food ingestion rate, k_w is the metal uptake rate constant from water, k_e is the metal efflux rate constant and g is the organism growth rate.

In this model, when metal is obtained from water, the metal flux into the organism is only dependent on the metal concentration in the water and on a specific rate in which the organism uptakes the metal from the medium. When a metal is ingested together with food, the metal flux into the organism is dependent on the metal concentration in the food, and on the amount of food ingested. After uptake of the metal by the organism, three processes of reduction in the metal concentration in the organism take place. Faeces elimination is applied

only for the metal obtained from the particulate phase, and determines the AE of the metal from the particles, resulting in the amount of metal that is incorporated in the tissue of the organism. Depuration, which is controlled by the efflux rate, represents a low rate of metal loss due to some metabolic loss of the assimilated metal, and it is a process also applied to metal obtained from the water (Wang & Fisher 1999). Dilution represents a reduction in the organism metal concentration as a function of the organism growth.

2.2.3. "Matching model and the real world"

When applying a systemic approach to understand Cd accumulation in aquatic organisms, some aspects must be taken into account: (i) aquatic organisms are exposed to a range of Cd sources that include the dissolved and particulate forms, including food; (ii) these sources may exhibit temporal and spatial variability that can change the relative Cd contribution to the organism's burden, and (iii) organisms can selectively eat different sources of food, which can present not only a specific Cd concentration but also different assimilation efficiencies. Thus, the variation in the quantity and quality of the ingested food may result in changes in Cd concentrations in organisms. For example, Wang and Fisher (1996) observed that molluscs could selectively eat some phytoplankton species, which may present different affinities for Cd, and consequently, to affect the overall Cd balance in the organisms. Wang & Fisher (1998), studying metal transference in copepods, observed that food quantity (i.e. phytoplankton availability) affected Cd uptake from the particulate phase relative to the dissolved phase. Lee & Luoma (1998) observed that the greater the presence of living particles on suspended particulate matter (SPM) carrying Cd, the greater the assimilation efficiency for Cd by organisms that feed on them. Therefore, in order to represent all metal sources from food to the organism, Equation 2.2 can be better written as:

$$\frac{dM_o}{dt} = \left(k_u \cdot M_w\right) + \sum_{i=1}^{n}\left(AE_i \cdot IR_i \cdot M_{f\,i}\right) - \left(k_e + g\right) \cdot M_o, \qquad \text{Equation 2.3}$$

where *i* represent each one of the n-different food items eaten by the organism, and all other symbols are as in Equation 2.2.

The accuracy of kinetic models for metal accumulation in organisms depends on how representative the selected food items are of the organism's natural diet (Qiu et al. 2001). Ideally, each food item should be individually treated to represent the sum of metal obtained from their ingestion. Thus each species or other particle taken as food should be individually considered when under investigation in the laboratory experiments. This is because each one of them may present specific AE, and therefore they should be included separately into a model. However, this is virtually impossible to do in practice due to the number of species and other particles that would have to be considered as food items. Therefore, two options remain feasible: to select a small number of species (Wang et al. 1999b, Wang & Rainbow 2000, Rainbow & Wang 2001) or to work with the whole community (Lee & Luoma 1998, Luoma et al. 1998, Ke & Wang 2002).

When selecting a small number of species to represent the particulate metal source to an organism, the results may reflect more the relationship between these species and the organism than the organism and the whole community. For example, the selected species may present distinct metal uptake rates, growth rates, and/or AE that may confer characteristics to the model that are not realistic for the whole community. When undertaking the laboratory experiments using the whole community, and including this information into a model, one avoids such problems. However, this results in a stronger link between the model results and the modelled environment, which may restrict the model's applicability for other environments.

Chapter 3. *Site description and general methodology of sampling and analyses*

3.1. Study Site

Fieldwork for this study was carried out in Ross Creek (Figure 3.1), a tidal creek surrounded by the city of Townsville (19º15'S, 146º50'E), the biggest city in North Queensland (population ≈ 130,000). The creek is 4.9 km long, and is on average 92 m wide and 7.4 m deep, including the Harbour area, and has a water volume of 5.6×10^6 m^3 at the Lowest Astronomical Tide (LAT) and a superficial area of 4.5×10^5 m^2. Ross Creek's catchment (2.05×10^7 m^2) is totally urbanized, including commercial and residential centres and light and heavy industries, and its hydrology has been completely altered. The creek is situated in the dry-tropics of North Queensland, a region characterized by distinct wet (Nov–Apr) and dry (May–Oct) seasons. Except for episodic rainfall events during the wet season, Ross Creek does not receive any significant fresh water input, resulting in its hydrodynamics being controlled by tide variation.

The samples were collected at four sites (Figure 3.1, denoted A, B, C and D), three of which along Ross Creek and the fourth, site A, was on the coast off the mouth of Ross Creek. These sites were chosen to be distributed along a dissolved-Cd gradient that had been identified in a pilot study. The water column depth at sites A, B, C and D at the Lowest Astronomic Tide are 5, 2.1, 0.9 and 2.3 m respectively. Another two sites in the Ross Creek catchments were irregularly sampled for dissolved Cd determinations and measurements of temperature and

salinity. One of these sites was located in the most southward portion of the creek, which receives Ross Creek water only during the high tides (named 'Pool'). The second site was located at the Lakes, an artificial water reservoir 2 km westward from site D (Figure 3.1), which connects to Ross Creek through a channel regulated by a gate. Thus, site D represents the most upstream site in Ross Creek with free connection to Cleveland Bay.

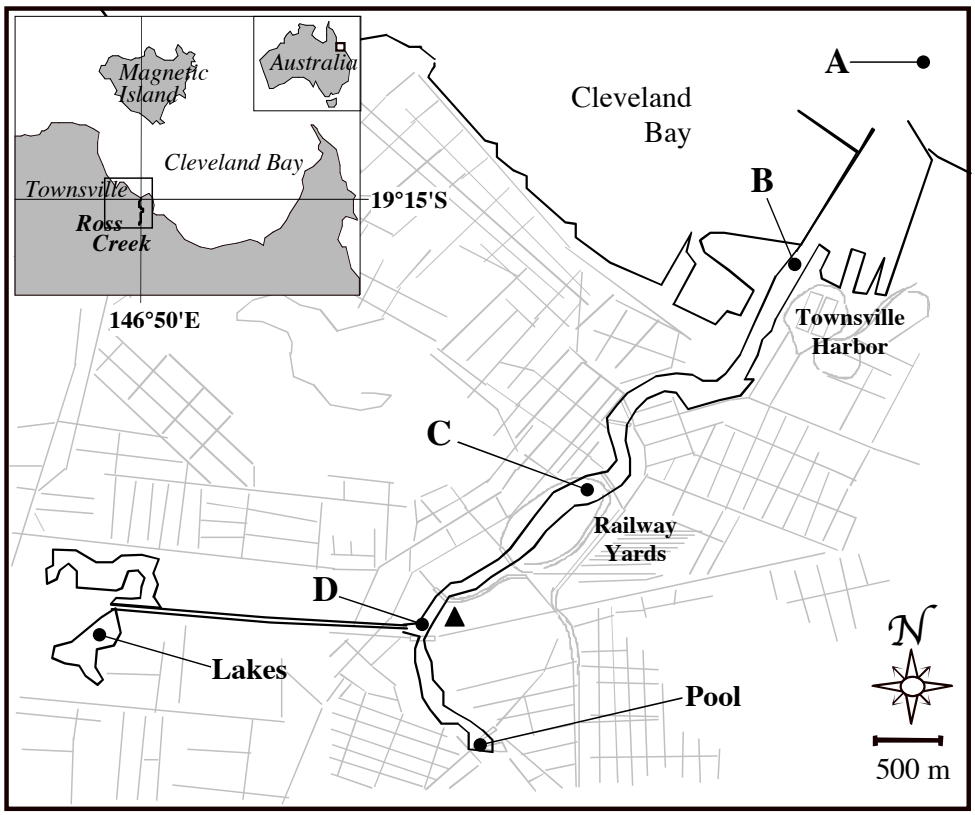

Figure 3.1: Sampling area in Ross Creek (Townsville, Queensland), its urbanized vicinity and the location of the sampling sites (A, B, C and D). The locations of another two non-regular sampling sites (Lakes and Pool) and the revegetated municipal dump (black triangle) are also represented.

3.2. Sampling Program

Two three-month sampling programs measuring Cd in the environment and the biomonitor (*Balanus amphitrite*) were undertaken in the dry season of 2002 and wet season of 2004 in Ross Creek. The dry season sampling program was carried out from 6 August to 30 October 2002 at sites A, B and C (Figure 3.1). Sites B and C were visited weekly at the peaks of the high tides and at the trough of the low and, and site A only at the peak of the high tides. During all site visits water and plankton were collected for the determination of the Cd concentration and mass abundance. Barnacles were sampled only at sites B and C at the trough of the low tide. CTDs from Sea-Bird Electronics-SBE and Aanderaa were moored at sites B and C, where barnacles were sampled, to record water temperature and salinity at a 20-minutes sampling rate.

The wet season sampling was carried out from 21 January to 14 April 2004 at sites A, B, C and D (Figure 3.1). The sampling strategy was as in the dry season sampling program, except that water was sampled only at the high tides and the plankton samplings were only carried out during the high tides, and only at sites B and C, from where barnacles were collected. As this study is mainly aimed at the understanding of the Cd concentration in *Balanus amphitrite*, and based on previous field observations about the low immersion time for these organisms during the dry season sampling program, the aim was to collect the potential Cd sources that were available to the barnacles. Site D was included as a sampling site to get a better characterization of the Cd distribution along the creek. Site D was located close to the revegetated municipal dump, where the highest Cd concentration in the dissolved phase was identified in the dry season sampling program. During all site visits, salinity (measured in psu) and temperature were recorded with a portable S-T probe calibrated weekly and cross-

calibrated with a CTD from Sea-Bird Electronics. On some occasions the sites were also visited at the trough of the low tides for water sampling.

Additional environmental data such as daily rainfall and evaporation were obtained from the Bureau of Meteorology, from a station located at the Townsville Airport. The Ross Creek water level, obtained from the Environmental Protection Agency, was sourced from a recorder at the Port of Townsville at a sampling rate of 20 min. Water temperature at site A, for the dry season sampling period, was obtained from an oceanographic buoy moored in Cleveland Bay (19°12'S, 146°45'W) maintained by the Great Barrier Reef Marine Park Authority and the Australian Institute of Marine Science.

3.2.1. Sample collection

Water was collected from 40 cm below the water surface in 1-L acid-washed Nalgene bottles, stored on ice and transported to the laboratory for processing. Plankton was collected by passing 300–1000 L of water through a Hydrobios plankton net (50-μm mesh with 200-μm pre-sieving mesh) equipped with a General Oceanics flowmeter, towed 30 cm below the water surface. Plankton samples were placed in clean 250-ml Nalgene bottles, preserved with 2% M&B Proanalys formaldehyde (40% w/v), and transported to the laboratory for processing. The plankton sampling targeted the small species <200 μm, which are usually abundant enough to be properly quantified by sampling a discrete volume of water (McKinnon & Klumpp 1998, Harris et al. 2000). A plankton size fraction up to 200 μm encompasses the majority of particles that are eaten by *Balanus amphitrite* in Ross Creek (Bigelow 1997).

Barnacles were randomly removed from pillars at low tide, always from the same area of 30 × 150 cm, using a stainless steel chisel, placed into clean polyethylene vials, stored on ice and transported to the laboratory for processing. The barnacle sampling area was selected in order to guarantee that enough organisms could be collected over the three-month sampling period. Barnacle sampling sites in the dry season sampling program were 2.3 and 2.0 m above the LAT on site B and C respectively, resulting in corresponding average immersion times during the sampling period of 6.5 and 10.8 hours day^{-1}. In the wet season sampling program, the sampling spot at site B was 40 cm below that of the dry season's sampling spot in order to better approximate the barnacles' immersion time between sites B and C. This resulted in the barnacle sampling sites being 1.9 and 2.0 m above the LAT at site B and C respectively, resulting in corresponding average immersion times during the sampling period of 12.9 and 12.5 h d^{-1}. Barnacles were stored frozen and water samples were stored under refrigeration until analysis.

3.2.2. Sample treatment

Within 24 h of collection the water samples (approx. 1 L) were passed through a 50-μm acid-washed mesh and filtered on pre-weighted 0.45-μm Millipore filters for gravimetric determination of suspended particulate material (defined as small SPM, SSPM). After collection of SSPM, filters were rinsed with deionized distilled water (DI-DW) to remove salt. These operations were carried out in a laminar flow hood (Clyde-Apac, HWS Series). The water filtrate (dissolved phase, <0.45 μm) was acidified to pH 2 with 30% Merck Suprapur HCl (20 μl per 10-ml sample) and stored in 125-ml Nalgene acid-washed bottles at room temperature for subsequent Cd determination. Because no plankton samples were collected at sites A and D during the wet season campaign, the water samples from these sites

were passed through a 200-μm acid-washed mesh before being filtered on 0.45-μm filters. Thus, the fraction of suspended particulate matter retained on these filters represents the sum of all particles sized <200 μm. This allowed comparing the total suspended particles (Cd and mass concentrations) sampled at sites A and D with those sampled at site B and C, when SSPM and LSPM are taken into account combined.

Plankton samples (50–200 μm) were rinsed with DI-DW to remove formaldehyde and then gravity separated using a funnel into live microzooplankton (top fraction) and large SPM (LSPM, bottom fraction) in a solution of 20% Aldrich LUDOX TM-50 colloidal silica in DI-DW (Robertson et al. 1988). Under magnification a separation efficiency of >90% was estimated. Fractions were rinsed with DI-DW to remove LUDOX and retained on pre-weighed 8.0-μm Millipore filters for dry weight and Cd determination. All filters were dried to constant weight in the laminar flow hood. Filters were not acid-leached prior to use since there was no difference in the Cd blanks for acid-washed and non-acid washed filters (Student t-test, $p = 0.45$, $n = 20$). The effect of weight reduction resulting from formaldehyde preservation (Harris et al. 2000), was corrected by multiplying the microzooplankton and LSPM dried masses by 2.35 ± 0.02 (mean ± 1 SD, $n = 6$) and 1.17 ± 0.03 (mean ± 1 SD, $n = 6$), respectively. These factors were obtained comparing samples of microzooplankton and LSPM, separated using light attraction, before and after 24-h formaldehyde exposure. Weight reductions of 57 and 15% for microzooplankton and LSPM respectively were obtained, which are in the range of those presented in the literature (Beers 1976, Steedman 1976).

Barnacle bodies, consisting of thorax with six pairs of thoracic limbs, the abdomen and part of the head that was not left behind attached to the mantle, were removed from shells with stainless steel forceps, rinsed with DI-DW, grouped by size class (4–10 organisms within 6–8

size classes) and freeze-dried to constant weight in clean polyethylene vials. Gut contents were not purged because contamination from food particles would be low given that the Cd concentrations in barnacles were on average 10 times higher than the Cd concentrations in their food sources.

3.2.3. Cadmium determination

Filtered and acidified water was analysed by Differential Pulse Anodic Stripping Voltammetry with a Mercury Film Electrode (DPASV-MFE), using an accumulation potential of -1100 mV *vs*. the Ag/AgCl reference electrode, and a deposition time of 120 s (Metrohm Application Note V-68: Determination of Cd and Pb in sea water, http://www.metrohm.ch/). A Metrohm 746 VA Trace Analyser with carbon rotating disk electrode was used, and a detection limit 2.03 ng L^{-1} was determined by analysis of blank samples (n = 8). Samples were not UV-digested prior to DPASV-MFE since Cd concentrations measured in typical Ross Creek samples were no different to UV-digested samples (Student t-test, p = 0.90, n = 28).

Material on filters and barnacles were digested with 2 ml of 69% Merck Suprapur HNO_3 and 200 μl of 70% Normatom Ultrapur $HClO_4$ in 10-ml Pyrex open test tubes in two consecutive steps (3 h at 120°C and another 3 h at 180°C), within a computer-regulated heating block. After digestion, the samples were cooled to room temperature and the final volume was made up to 2–3 ml with reverse osmosis distilled water (RO-DW). Digested filters were analysed with a Varian Spectra 400 Zeeman Atomic Absorption Spectrometer (ZGF-AAS) and barnacles with ZGF-AAS or a Varian Liberty 200 Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), depending on the Cd concentration in the digest. Both

techniques were calibrated by standard additions. Exposure of samples to formaldehyde did not significantly affect Cd concentration of these samples, after adjustment for weight change on preservation (Student t-test: $p = 0.32$ for microzooplankton ($n = 12$), $p = 0.39$ for LSPM ($n = 8$), and $p = 0.36$ for microzooplankton and LSPM combined ($n = 40$)).

3.2.4. Data analyses and quality control

Analysis of the depth-averaged salinity variation along Ross Creek, obtained from vertical profiles done with a CTD (Sea-Bird Electronics-SBE), showed that conservative properties travel 1,200 m between the low and high tides (Figure 3.2). Thus, the four samples collected at sites B and C, at the low and high tides in at each day in both sampling periods, plus the samples collected at site D at both tides in the wet season of 2004, were considered as six spatially different samples. This allowed obtaining a better picture of the spatial distribution of the sampled parameters along Ross Creek, thus maximizing the data comparison between the two sampling periods.

Non-parametric statistical tests as presented in Sokal & Rohlf (1987) and Helsel & Hirsch (1992) were applied for data analyses since most of the sampled variables did not satisfy all the conditions required to apply parametric tests, even after data transformation. All data are presented as mean ± 1 SD unless otherwise stated. Isopleth graphics, as presented in Figure 4.1 and Figure 4.2, were used to represent the spatial and temporal variation in abundance and Cd concentration of the sampled components.

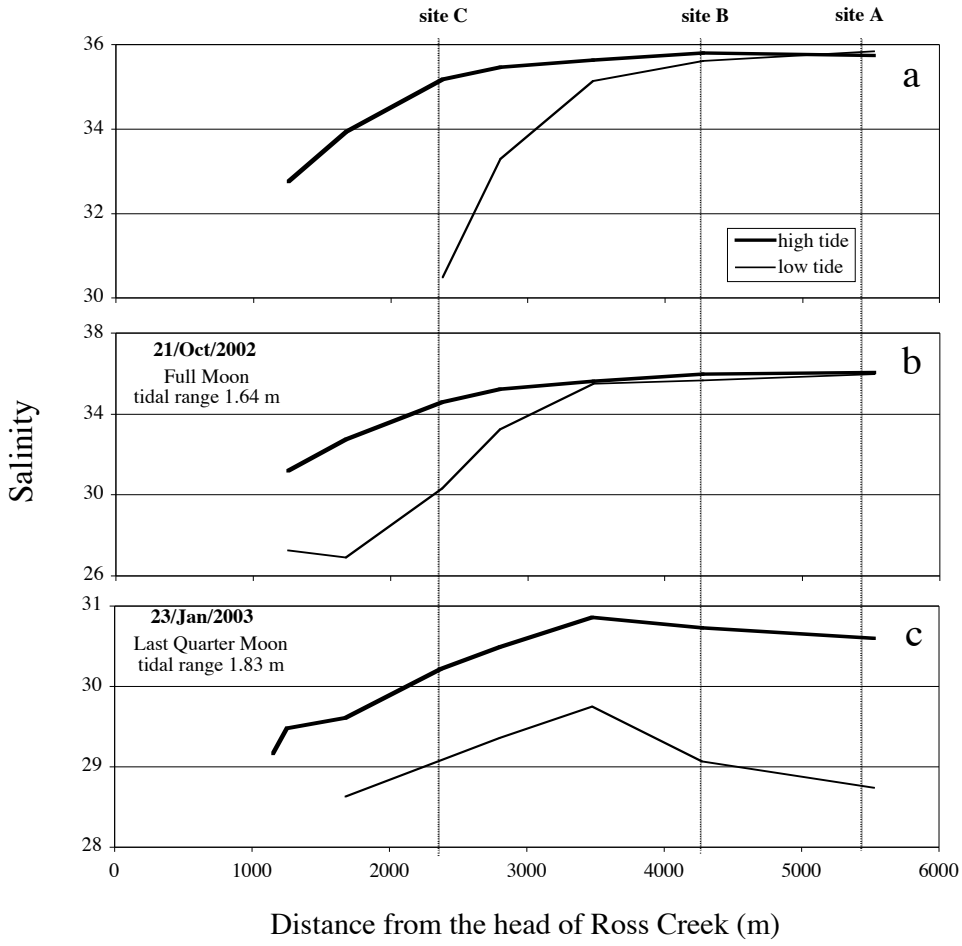

Figure 3.2: Horizontal superficial salinity profile in Ross Creek, comparing for three tide situations: (a) a syzygy, (b) a quadrature and (c) an intermediary situation.

Procedural blanks for Cd concentrations in the 2-mL digested 0.45-μm and 8.0-μm filters were 0.08 ± 0.20 μg L^{-1} (n = 27) and 0.15 ± 0.22 μg L^{-1} (n = 23) respectively, and the blank for the Cd concentration in the DI-DW water was 2.23 ± 1.79 ng L^{-1} (n = 26). Analyses of National Research Council (Canada) certified materials generally show good agreement with our measured values (Table 3.1). The low Cd recovery for PACS-2 (marine sediment) may be because the digestion did not include HF, and thus the digest was not total. Therefore, the total Cd concentration in SSPM and LSPM, which may contain some inorganic material, may

be underestimated. However, since understanding Cd transference from food and water to a biomonitor is the aim of this research, the method used is appropriate to quantify the maximum concentration of Cd in both SPM fractions that is available to the organisms that feed on them. The %RSD (standard deviation / average × 100) for replicate sampling and analyses (n = 10, undertaken on two occasions) of water and plankton from Ross Creek were: for mass abundance, SSPM (33%), microzooplankton (20%) and LSPM (14%), and for the Cd concentrations in the dissolved phase (9%), SSPM (13%), microzooplankton (22%) and LSPM (26%).

Table 3.1: Analyses of National Research Council (Canada) certified materials for quality control purpose (mean ± 1 SD).

Material	*Measured*	*Certified Value*
SLEW-3[‡]	0.048 ± 0.003 $\mu g.l^{-1}$ (n = 27)	0.048 ± 0.004 $\mu g.l^{-1}$
CASS-4[¢]	0.025 ± 0.002 $\mu g.l^{-1}$ (n = 16)	0.026 ± 0.003 $\mu g.l^{-1}$
DORM-2[§]	0.041 ± 0.011 $mg.kg^{-1}$ (n = 27)	0.043 ± 0.008 $mg.kg^{-1}$
PACS-2[€]	1.792 ± 0.325 $mg.kg^{-1}$ (n = 19)	2.11 ± 0.15 $mg.kg^{-1}$

[‡]estuarine water, [¢]costal water, [§]dogfish muscle and liver and [€]marine sediment

3.3. Seasonal Environmental Differences Between the Two Sampling Periods

Rainfall marked a clear seasonal differentiation between the two sampling periods, which was also identified for other parameters sampled in Ross Creek. During the three-month sampling period carried out in the dry season of 2002 (6/Aug-30/Oct) the total rainfall was 11.8 mm, which was 38 times smaller than that registered in the wet season of 2004 (ca., 445.8 mm, from 21 January to 14 April). No significant difference was detected for the mean daily evaporation (Mann-Whitney test, $p = 0.50$, $n = 170$), being 7.10 ± 2.19 mm and 7.17 ± 1.37 mm d^{-1} for the dry and wet seasons, respectively. The mean water level was higher in the wet season (2.11 m) compared to the dry season (1.95 m), and the mean low and high tides for the dry and wet seasons ranged between 0.84 and 3.05 m, and between 1.02 and 3.21 m, respectively. These data are presented on Figure 3.3.

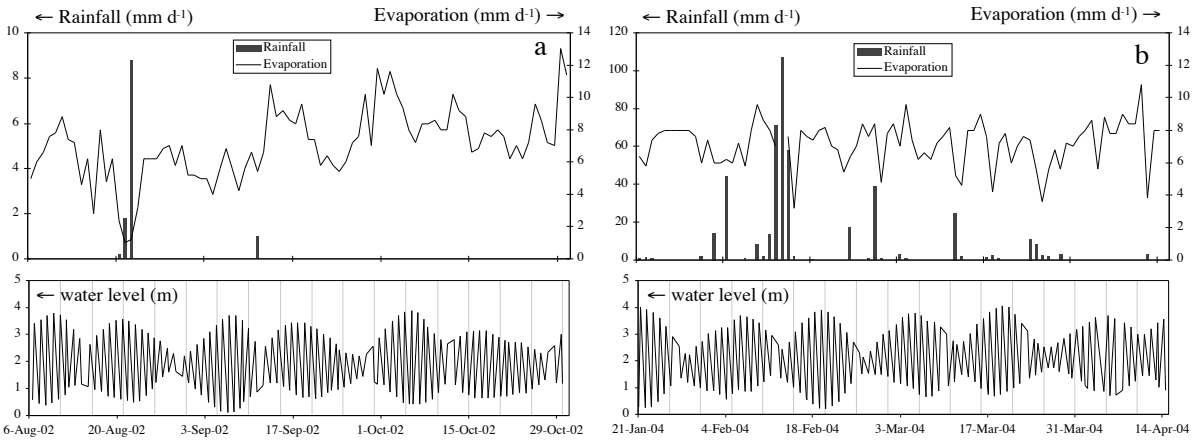

Figure 3.3: Temporal variation of the rainfall and evaporation (upper graphic) and the water level (lower graphic) for the dry season of 2002 (a) and the wet season of 2004 (b). Rainfall scales differ on graphics.

The higher rainfall index in the wet season resulted in a higher fresh water input in Ross Creek compared to the dry season, which was confirmed by the salinity data. The salinity measured at sites B and C at low and high tides (Figure 3.4a), which have corresponding data for both sampling periods, exhibited a significant drop from 34.3 ± 1.9 in the dry season to 32.5 ± 2.6 in the wet season (Mann-Whitney test, $p < 0.01$, $n = 94$). The Kruskal-Wallis test identified for both seasons wet ($p < 0.01$, $n = 42$) and dry ($p < 0.01$, $n = 78$) a significant decreasing salinity gradient upstream. The water temperature also presented a seasonal variation (Figure 3.4b), being lower in the dry season of 2002, which was carried out in the austral winter, compared to the wet season of 2004 (austral summer). For example, the mean water temperature calculated for sites B and C at the low and high tides varied from 25.4 ± 2.6 °C in the dry season to 30.0 ± 1.9 °C in the wet season (Mann-Whitney test, $p < 0.01$, $n = 94$). Therefore, the environmental data suggest that Ross Creek experienced some seasonal variation between the dry season of 2002 and the wet season of 2004.

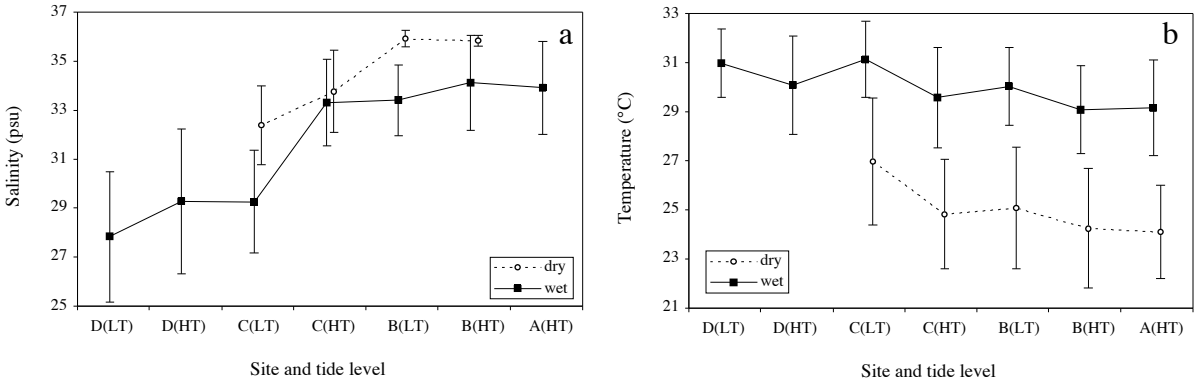

Figure 3.4: Mean water salinity (a) and temperature (b) measured at the low tide (LT) and at the high tide (HT) at sites A, B, C and D along Ross Creek in the dry season of 2002 and the wet season of 2004. Bars stand for ±1 SD.

Chapter 4. *Relative contribution of food and water to the Cd concentration in Balanus amphitrite*[6]

4.1. Introduction

This chapter discusses the relative importance of the Cd sources in the environment (i.e., water and food) to the metal concentration in *Balanus amphitrite*, and provides a general characterisation of the particles (sized <200 μm) distribution and their Cd concentration along Ross Creek. Several studies on bioavailability indicated that metals in the dissolved phase are more likely to be incorporated by biota (Sunda et al. 1978, Phillips 1980, Wong et al. 1980, Salomons et al. 1988, Rainbow & White 1989, Morgan & Stumm 1991, Hare & Tessier 1996, Warren et al. 1998). However, some recent publications have shown that the main route of metal accumulation in aquatic organisms is through food rather than water (Lee & Luoma 1998, Wang & Fisher 1998, Warren et al. 1998, Connell & Sanders 1999, Wang et al. 1999a, Wang et al. 1999b, Qiu et al. 2001, Ke & Wang 2002). Therefore, determining uptake pathways for metals in food chains is important to understand metal bioavailability in the environment, and it is critical for the setting of water quality criteria (Wang et al. 1999b).

The results of the first three-month small-scale monitoring program on Cd concentrations in *Balanus amphitrite*, its food sources and water are presented in this chapter. The sampling

[6] Data presented in this chapter was published in da Silva ET, Ridd M, Klumpp D, Ridd P (2004) Relative contribution of food and water to the Cd burden in *Balanus amphitrite* in an urban tidal creek discharging into the Great Barrier Reef lagoon. Estuarine, Coastal and Shelf Science 60:313-324

program was carried out in the dry season of 2002, and the methodology of sampling and data analysis were presented in Chapter 3. All data used in this chapter is presented in Appendix II. Two approaches were applied in the data interpretation: statistical analysis using non-parametric tests, and the calculation and interpretation of the concentration factor models (the biological concentration and biological magnification factors).

4.2. Results and Discussion

4.2.1. Temporal and spatial variation in abundance of the particles sized <200 μm

Over the three sampling months in the dry season of 2002, SSPM was by far the most abundant component (sized <200 μm) in the Ross Creek water column (7.96 ± 4.38 mg L^{-1}), being on average an order of magnitude higher than LSPM (0.87 ± 1.60 mg L^{-1}) or microzooplankton (0.68 ± 0.59 mg L^{-1}). SSPM ranged from 3.03 to 23.4 mg L^{-1}, with the highest concentrations at the shallowest site, C (Figure 4.1a). On average, SSPM was significantly higher (Mann-Whitney test, $p < 0.01$, $n = 65$) at low tides (10.93 ± 4.33 mg L^{-1}) than at high tides (5.97 ± 3.15 mg L^{-1}), suggesting that the reduced depth of the water column at the low tide (2.21 m lower on average) promoted conditions that enhanced the concentration of SSPM compared to the conditions at the high tides.

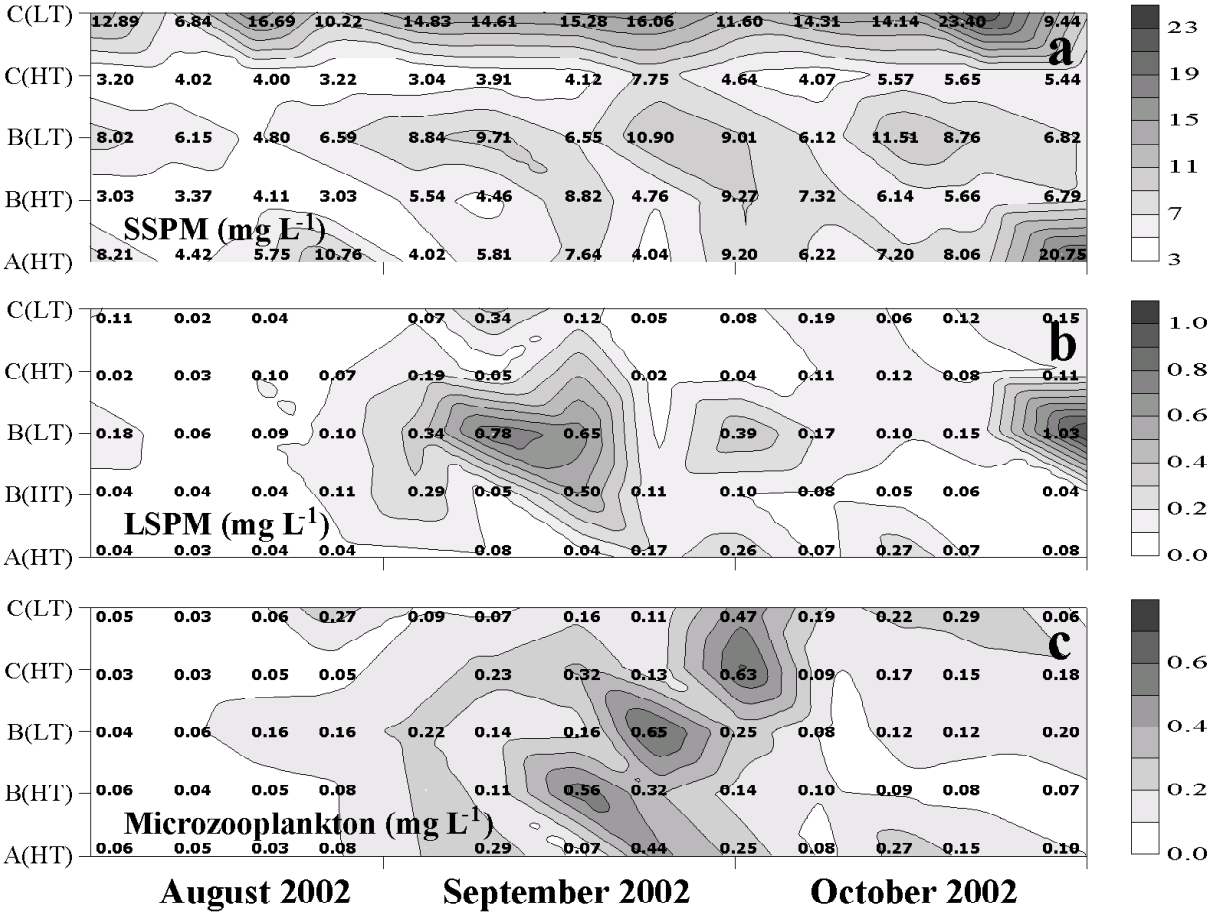

Figure 4.1: Mass concentration (mg L^{-1}) of (a) SSPM (0.45–50 μm), (b) LSPM (50–200 μm) and (c) microzooplankton (50–200 μm), sampled from 6 August to 30 October 2002 in Ross Creek at site A during the high tide, A(HT), site B at high tide, B(HT) and at low tide, B(LT), and at site C at high tide, C(HT) and at low tide, C(LT).

No consistent pattern in abundance was observed for LSPM and microzooplankton at any of the sites (Figure 4.1b, c). They presented very low concentrations compared to SSPM during the three sampling months, except during the second-half of September, when higher concentrations occurred at all sampling sites. This peak event was responsible for raising the mean concentrations from 0.08 and 0.11 mg L^{-1}, prior to and after the peak, to 0.97 and 0.29 mg L^{-1} during the peak for LSPM and microzooplankton, respectively. The data set collected

did not explain these peak events observed for LSPM and microzooplankton. Phytoplankton abundance (as chlorophyll-a, data presented Appendix II and in Chapter 6, Figure 6.3) did not present any peak that could explain the high abundance of microzooplankton; but a sharp drop in phytoplankton abundance was observed during the high microzooplankton abundance, as should be expected taken into account the classical Loktica-Volterra relationship (Valiela 1984).

In a Kruskal-Wallis test, both SPM fractions showed significant spatial differences and no temporal ones, while conversely microzooplankton varied significantly with time but not spatially (Table 4.1). This difference in patterns may be a result of the microzooplankton self-mobility, allowing more homogeneous distribution along Ross Creek, while both SPMs would be more dependent on the Ross Creek hydrodynamics. Furthermore, the Pearson's correlation coefficient (pairwise deletion) showed that LSPM was more related with microzooplankton ($r = 0.51$, $n = 61$) than with SSPM ($r = 0.03$, $n = 63$). The correlation coefficient between microzooplankton and SSPM was 0.09 ($n = 62$). Under magnification, the LSPM samples presented a small amount of sediment, some organic material such as large phytoplankton cells, filaments of macro algae, mangroves roots, and a large abundance of dead microzooplankton, which supports the correlation between microzooplankton and LSPM.

To conclude, both SPM fractions are driven by similar physical controls (i.e. hydrodynamics, as demonstrated by the Kruskal-Wallis test results), but they have different mechanisms of formation. While variation in SSPM abundance was related to physical processes, associated with the water column height (as showed by the Mann-Whitney test results), LSPM was

related to biological processes (i.e. secondary production, as demonstrated by the Pearson's correlation coefficients).

Table 4.1: P-values (p), number of samples (n) and number of groups (ng, i.e., either number of sites or dates) for the Kruskal-Wallis test applied to the concentrations in mass and Cd of SSPM (0.45–50 μm), LSPM (50–200 μm), microzooplankton (50–200 μm) and Cd in the dissolved phase (<0.45 μm) sampled in Ross Creek from 6 August to 30 October 2002.

Mass concentration	Site (ng = 5)	Date (ng = 13)
SSPM (n = 65)	$p < 0.01$	$p = 0.78$
LSPM (n = 63)	$p = 0.01$	$p = 0.11$
Microzooplankton (n = 62)	$p = 0.76$	$p < 0.01$
Cd concentration		
SSPM (n = 65)	$p < 0.01$	$p = 0.22$
LSPM (n = 63)	$p < 0.01$	$p = 0.36$
Microzooplankton (n = 62)	$p = 0.09$	$p = 0.08$
Dissolved phase (n = 65)	$p < 0.01$	$p > 0.99$

4.2.2. Temporal and spatial variation of Cd concentrations in water and particles sized <200 μm

Over the three sampling months in the dry season of 2002, the Cd concentration in the dissolved phase (Figure 4.2a) increased consistently upstream. The average Cd concentration

at site A ranged from 1.72 to 10.2 ng L^{-1} for the high and low tide respectively, the corresponding range at site B was from 4.29 to 55.2 ng L^{-1} and at site C from 52.5 to 283 ng L^{-1}. The Cd concentration in SSPM (Figure 4.2b) exhibited a less clear pattern than the dissolved phase, and ranged from below detection limit (<0.01 mg kg^{-1}) up to 1.14 mg kg^{-1}, with an average of 0.24 ± 0.25 mg kg^{-1}. However, in general the Cd concentration in SSPM at site C was greater than that observed lower down the creek. LSPM and microzooplankton (Figure 4.2c, d) presented a very similar pattern of Cd concentration, but they differed in magnitude, the mean value for LSPM (0.52 ± 0.36 mg kg^{-1}) being twice that of microzooplankton (0.21 ± 0.15 mg kg^{-1}). This difference was quite constant even during some events of higher Cd concentrations such as those observed in October, when LSPM reached 1.62 mg kg^{-1} and microzooplankton reached 0.80 mg kg^{-1}. Based on the data set collected no explanation for these peak events in the Cd concentration for LSPM and microzooplankton is apparent.

Cadmium concentrations in all the sampled components increased significantly upstream (Kruskal-Wallis test, $p < 0.01$, Table 4.1) with the exception of microzooplankton ($p = 0.09$). This suggests that with exception of microzooplankton, which has self-mobility, the Cd concentration in both SPMs, which have their mass abundances controlled by the Ross Creek hydrodynamics (section 4.2.1), had similar patterns to the Cd concentration in the dissolved phase. From the same analysis, none of the components showed any significant difference in Cd concentration at each site, over the three sampling months (Table 4.1). No significant rainfall was observed in the Ross Creek catchments during the dry season sampling period (as seen in Figure 3.3), which may explain the remarkable temporal stability of Cd concentrations in the dissolved phase and thus in SSPM, LSPM, and microzooplankton at each site.

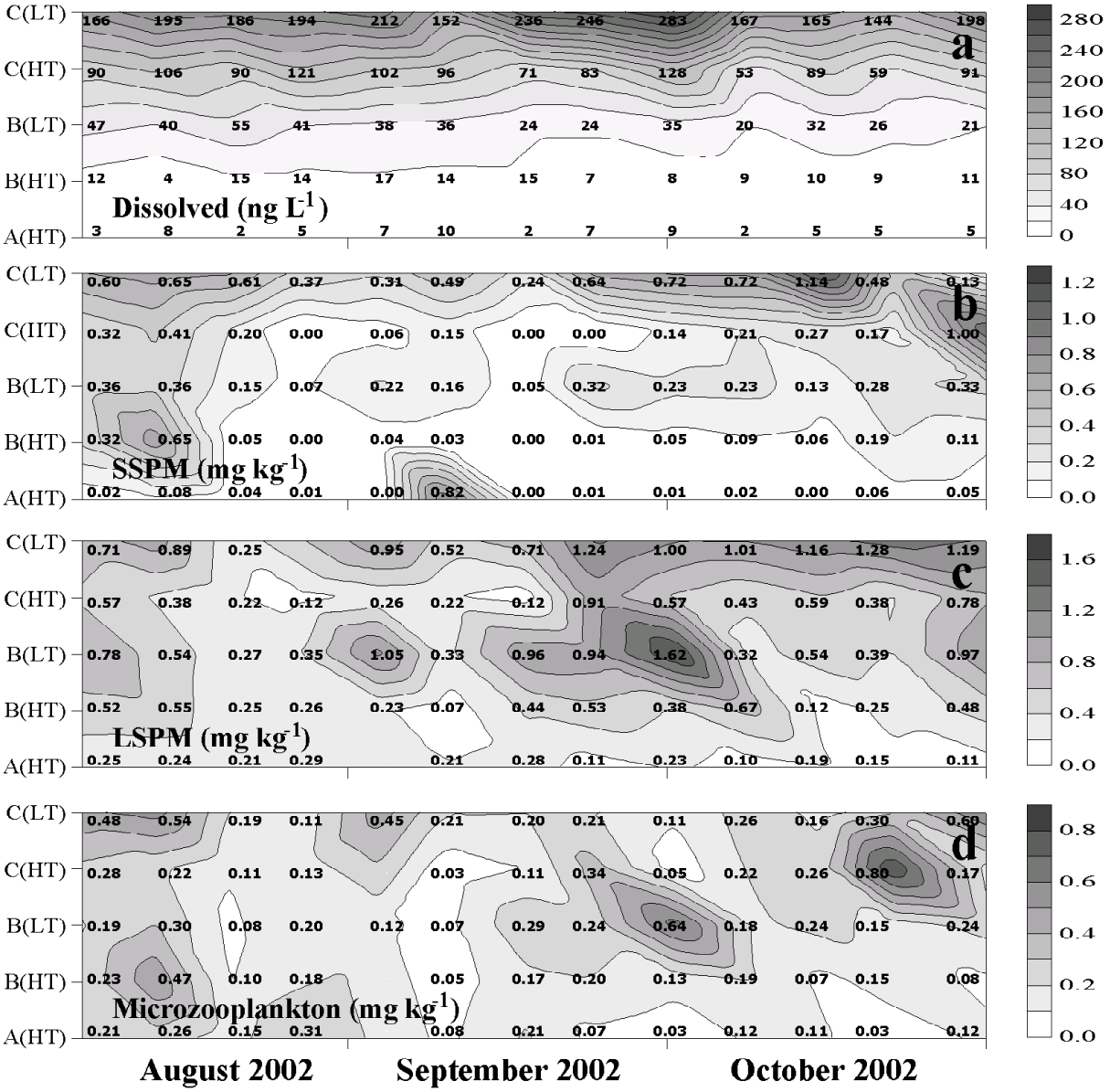

Figure 4.2: Cd concentration in (a) the dissolved phase (<0.45 μm, ng L^{-1}), (b) SSPM (0.45–50 μm, mg L^{-1}), (c) LSPM (50–200 μm, mg L^{-1}) and (d) microzooplankton (50–200 μm, mg L^{-1}), measured from 6 August to 30 October 2002 in Ross Creek at site A during the high tide, A(HT), site B at high tide, B(HT) and at low tide, B(LT), and at site C at high tide, C(HT) and at low tide, C(LT).

A further survey carried out in Ross Creek (unpublished data), in which seven sites along the creek were sampled for dissolved Cd determination, indicated that the highest Cd concentration occurs close to the site of an old revegetated municipal dump (indicated by a black triangle in Figure 3.1). This pattern was confirmed by the samples carried out in the wet season of 2004, in which site D was located close to the old revegetated municipal dump. Sites at the head of the creek exhibited Cd concentrations <10 ng L^{-1}, suggesting that the Cd source is located close to the dump and the sediments in the creek associated with it. Therefore, the remarkable stability exhibited for Cd in the dissolved phase seems to be controlled by an inflow of Cd sourced from the sediment (pore water) and an outflow controlled by a tide-dependent diffusive process (see below). Similar behaviour for Cd was observed in Los Angeles/Long Beach harbours, California, USA, based on field measurements of water-sediment flux with benthic chambers (Colbert et al. 2001).

4.2.3. Partitioning of Cd in the Ross Creek water and its export into the GBR Lagoon

The partitioning of the total Cd concentration between the four components (i.e. dissolved phase, SSPM, LSPM and microzooplankton) was obtained by multiplying the mass concentration of each component (Figure 4.1) by its Cd concentration (Figure 4.2). This calculation indicated that Cd in the dissolved phase represents more than 95% of the total Cd in the Ross Creek water column (<200 μm), with a total average of Cd concentration of all samples of 67.3 ng L^{-1}. For SSPM, LSPM and microzooplankton values of 2.3 ng L^{-1}, 0.5 ng L^{-1} and 0.1 ng L^{-1} were found, respectively. Since most of the Cd is in the dissolved phase, and therefore subject to the hydrodynamics of Ross Creek, the potential flux of Cd from Ross Creek into Cleveland Bay was calculated applying a simple diffusion coefficient model (Fisher et al. 1979), as indicated below:

$$J = k\frac{\Delta C}{\Delta x}, \qquad \text{Equation 4.1}$$

where ΔC is the mean difference in Cd concentration in the dissolved phase between sites C and B at the low tide (162 μg m^{-3}), Δx is the distance between them (2,250 m) and k is the diffusion coefficient. Since the freshwater discharge was negligible for the study period, the diffusion coefficient was derived from a mass balance equation for salt as presented in Ridd and co-workers (1988). For this calculation it was assumed that a condition of equilibrium for the salt concentration in Ross Creek existed, and only the water that does not exchange directly with Cleveland Bay was considered, i.e. the volume of water that remains in the estuary during the low tide. A similar approach was adopted in Ridd and co-workers (1990) for determination of the salt fluxes in Dickson's inlet. Therefore, the mass balance equation (Ridd et al. 1988) could be written as:

$$EL\left(\frac{\overline{S}_B + \overline{S}_C}{2}\right) + kH\frac{\left(\overline{S}_B - \overline{S}_C\right)}{\Delta x} = 0, \qquad \text{Equation 4.2}$$

where $\overline{S}_B$ and $\overline{S}_C$ are the mean salinity for site B (35.9) and C (32.9) at the low tide, respectively, E is the mean free surface evaporation rate (0.007 m d^{-1}), L is the creek length (4,900 m), H is the mean depth (7.4 m) Δx in the distance between sites B and C (2,250 m) and k is the diffusion coefficient, which can be estimated by Equation 4.2 as 1.4 m^2 s^{-1}. Therefore, applying a k of 1.4 m^2 s^{-1} in Equation 4.1, a flux of 0.10 μg Cd m^{-2} s^{-1} was found. When the flux is multiplied by the mean area of the cross-section of the creek (7.4 m × 92 m = 681 m^2) a Cd export rate of about 6 g d^{-1} into Cleveland Bay was determined.

In order to describe the Cd reactivity in the Ross Creek water column a simple partition coefficient (K_d) was calculated as the weighted Cd concentration in the particles (mg kg^{-1}) divided by the Cd concentration in the dissolved phase (mg L^{-1}). The weighted Cd concentration in the particles (wCd_p, mg kg^{-1}) was calculated as indicated below:

$$wCd_p = \frac{Cd_{SSPM} \cdot M_{SSPM} + Cd_{LSPM} \cdot M_{LSPM} + Cd_{MZ} \cdot M_{MZ}}{M_{SSPM} + M_{LSPM} + M_{MZ}}, \qquad \text{Equation 4.3}$$

where Cd_x is the Cd concentration in x (mg kg^{-1}), M_x is the mass concentration of x (mg L^{-1}), and *MZ* stands for microzooplankton. Despite the fact that the Cd concentration in the particles increased upstream, K_d decreased because the Cd concentration in the dissolved phase increased faster upstream than the Cd concentration in the particles did (Figure 4.3). Wang & Fisher (1998), modelling Cd uptake in marine copepods, found that the higher the value of K_d, the lower the importance of Cd uptake from the dissolved phase. Therefore, Cd in the dissolved phase might be expected to contribute more to the Cd concentration in organisms living upstream in Ross Creek than those downstream.

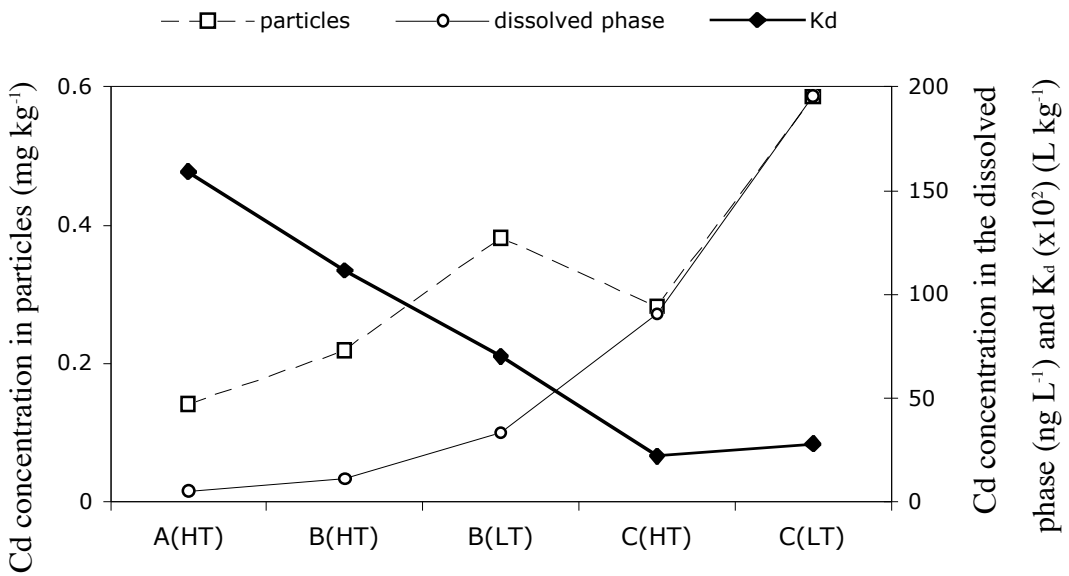

Figure 4.3: Mean temporal variation of K_d, Cd concentration in the dissolved phase and Cd concentration in the particles over the sampling sites: at site A during the high tide, A(HT), site B at high tide, B(HT) and at low tide, B(LT), and at site C at high tide, C(HT) and at low tide, C(LT).

4.2.4. Temporal and spatial variation of Cd in *Balanus amphitrite*

No correlation between Cd concentration and barnacle body size was found in 826 animals collected on 11 occasions at sites B and C over three months, grouped in 6–8 size classes, with 3–6 individuals in each. Therefore, the Cd concentration in all the barnacles collected at each day at each of the sites B and C were considered as replicates for further data analysis. Metal concentration in the barnacles is dependent on the rates of accumulation, excretion and dilution due to body growth, which may produce a size effect (Blackmore et al. 1998). Such size effects have been reported in some studies of *Balanus amphitrite* (Rainbow & Smith 1992, Rainbow et al. 1993), but not in others (Blackmore et al. 1998). A discussion about potential causes of a size effect in *Balanus amphitrite* based on a simulation exercise in a steady-state model is presented in section 5.4.4.

The barnacle Cd concentrations at site C, with an average of 8.40 ± 3.35 mg kg^{-1} were significantly higher (Mann-Whitney test, $p < 0.01$, $n = 170$) than at site B (2.98 ± 1.26 mg kg^{-1}) (Figure 4.4), following the trend exhibited for the Cd concentrations in the dissolved and solid phases. Even though no significant temporal variation was found for the majority of the water column components, both in terms of Cd concentrations and masses (excluding microzooplankton, Table 4.1), *Balanus amphitrite* showed temporal variation at site C (Kruskal-Wallis test, $p < 0.01$, $n = 83$) but not at site B ($p = 0.07$, $n = 87$). Moreover, a significant difference was found for the pattern of temporal variation for the mean Cd concentration at each sampling day between sites B and C (Wilcoxon Signed Rank test, $p < 0.01$, $n = 11$). This indicates that each population had a specific temporal variation pattern for Cd concentration. For example, over the first four sampling days (each separated by a week) the Cd concentration in barnacles at sites B and C showed a divergent, near mirror-image

pattern. There are no data in the literature presenting short-term temporal variation of Cd in *Balanus amphitrite*, however such variations are likely to be a function of the season of the year and, therefore, differences in size, growth rate, reproductive conditions, food availability, and other factors (Blackmore et al. 1998).

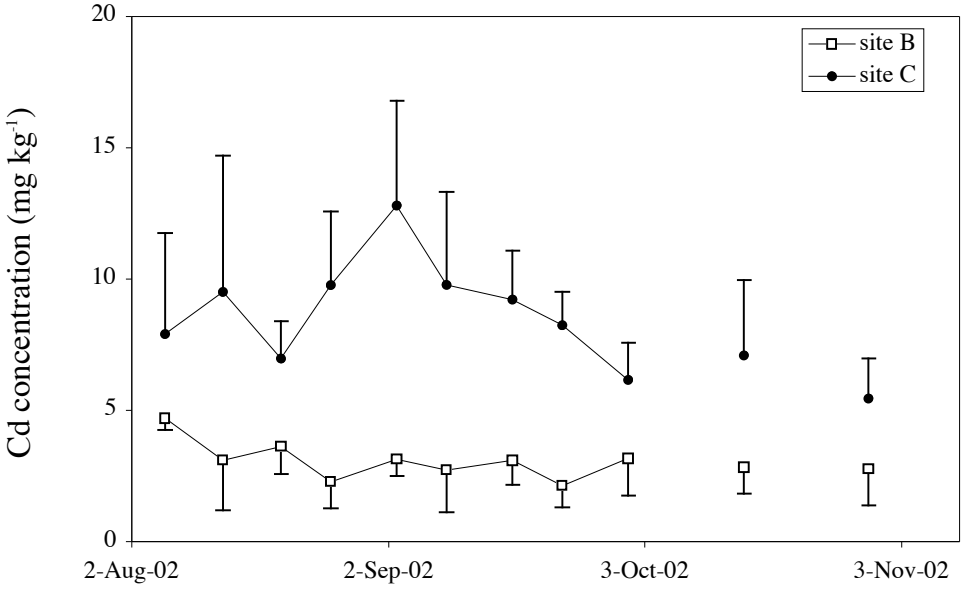

Figure 4.4: Cd concentration in *Balanus amphitrite* (mg kg^{-1}) sampled in Ross Creek at sites B and C, from 6 August to 30 October 2002. Data are means for 6–8 size class with 3–6 individuals in each, and error bars represent 1 SD.

4.2.5. Biological concentration and magnification factors applied to the spatial variations of Cd concentrations in *Balanus amphitrite*

As a first approach to understand the relative contribution of the Cd sources (i.e. water and three categories of particles sampled) to the Cd concentration in *Balanus amphitrite*, a biological concentration factor (BCF) and a biological magnification factor (BMF) were

determined. BCF was calculated as the mean barnacle Cd concentration divided by the mean Cd concentration in the dissolved phase measured at the high tide over the three sampling months. BMF was calculated as the mean barnacle Cd concentration divided by the mean weighted Cd concentration of its food sources (i.e. SSPM, LSPM and microzooplankton), measured at the high tide over the three sampling months, as presented in Equation 4.3. For these calculations and for the further ones, we assumed that the Cd concentrations in *Balanus amphitrite* were mainly a reflection of the Cd availability at the high tide, since during the three sampling months, *Balanus amphitrite* had an immersion time of 6.5 h d^{-1} at site B and 10.8 h d^{-1} at site C.

Balanus amphitrite showed a strong capacity for Cd accumulation, resulting in BCF of 313,000 and 96,000 at sites B and C respectively, and corresponding BMF of 22 and 37 (Table 4.2). It can be seen that the site C/B ratio for BCF was <1 while the site C/B ratio for BMF was >1 (Table 4.2). This can be understood by comparing the difference in Cd concentrations between barnacles, their food sources, and water for sites B and C. The mean Cd concentration in barnacles at C was approx. 2.8 times of that at site B, such a difference could not be explained by the 0.7 times higher mean weighted Cd concentration in the food sources at site C compared to B. Therefore, the 9 times higher Cd concentration in the dissolved phase at site C than at site B suggests that water had a greater contribution to the Cd concentration in barnacles at site C compared to site B, in which higher K_d were determined (Figure 4.3). This agrees with the findings of Wang & Fisher (1998) that the lower the K_d the higher the importance of the trace element uptake from the dissolved phase.

Table 4.2: Mean Cd concentration in the dissolved phase (<0.45 μm), SSPM (0.45–50 μm), LSPM (50–200 μm), microzooplankton (50–200 μm) and *Balanus amphitrite*. The biological concentration factor between *Balanus amphitrite* and water (BCF) and the biological magnification factor between *Balanus amphitrite* and all its Cd sources (BMF) are also presented (see text for full description).

Mean Cd concentration	Site B	Site C	C/B ratio
Dissolved phase (μg L^{-1})	0.01	0.09	9
SSPM (μg kg^{-1})	123	197	1.6
Microzooplankton (μg kg^{-1})	169	226	1.3
LSPM (μg kg^{-1})	365	426	1.2
Food sources (μg kg^{-1})*	121	202	1.7
Balanus amphitrite (μg kg^{-1})	3130	8630	2.8
BCF	313,000	96,000	0.3
BMF	22	37	1.7

* Food source (μg kg^{-1}) is the mean weighted Cd concentrations in SSPM, LSPM and microzooplankton.

4.3. Final Remarks

Metal concentrations in organisms depend on a range of physico-chemical (e.g. temperature, salinity, pH, metal concentration) and biological (e.g. growth rate, food availability, population age structure) parameters that may result in a specific pattern of metal concentration in the organism (Blackmore et al. 1998, Brock 1998, Wang et al. 1999a, Wang et al. 1999b). In Ross Creek, *Balanus amphitrite* from two sites less than 2.2 km apart showed

three distinct patterns of Cd concentrations: (a) the average Cd concentrations in each population were different, since one was located in an area with higher Cd availability; (b) barnacles showed some temporal Cd variation over three sampling months while most of their Cd 'food' sources did not, and (c) each 'population' presented a specific pattern for the temporal Cd variation. These patterns suggest that variations in the Cd levels in the food sources as well as the relative abundance of these sources may result in specific patterns for Cd concentrations in *Balanus amphitrite*.

The results presented in this chapter highlighted the importance of determining the metal pathways to a biomonitor in order to understand the meaning of a metal concentration in it. In addition, some questions remain unanswered, such as whether seasonal changes in the Cd availability in the environment would affect the Cd concentration in the biomonitor, or which Cd source would contribute more to the Cd concentration in the organism. In order to answer these questions, another three-month sampling program was carried out in Ross Creek, and a steady-state model was developed. In this model, data collected in the field and some processes investigated in the field and in the laboratory experiments were taken into account. This study and its results are presented in the next chapter.

Chapter 5. *Does Balanus amphitrite indicate seasonal variation in Cd concentrations in the environment?*[7]

5.1. Introduction

Findings of the dry season sampling program (Chapter 4) showed an increasing total Cd gradient going upstream in Ross Creek and a remarkable temporal stability for the Cd concentration in the dissolved and particulate phases. In spite of this temporal stability, barnacles living in different sites 2.2 km apart exhibited distinct temporal variations in their Cd concentrations for each site, which confirms that Cd accumulation in *Balanus amphitrite* is not simply related to the metal concentrations in the environment.

In the wet season of 2004 the sampling program was repeated to test whether the Cd concentration in the biomonitor responded to any variation in the availability of the dissolved and particulate phases of Cd in Ross Creek, caused by rainfall variation. In this chapter, the findings of this second sampling program are discussed and compared with the results of the first sampling program. The results of both sampling programs are summarized in budget models that describe Cd influxes from food and water to *Balanus amphitrite*. These models

[7] Data presented in this chapter is in da Silva ET, Ridd M, Klumpp D (2005) Can body burden in the barnacle *Balanus amphitrite* indicate seasonal variation in cadmium concentrations? Estuarine, Coastal and Shelf Science 65: 159-171.

are then used in a sensitivity analysis to estimate key parameters controlling Cd accumulation in *Balanus amphitrite* in Ross Creek. They are also used in a simulation exercise to investigate the potential influence of variation in growth rate on a size effect in the Cd concentration in *Balanus amphitrite*, which has previously been observed by some workers (Rainbow & Smith 1992, Rainbow et al. 1993, Blackmore & Rainbow 2001, Rainbow & Blackmore 2001) but not by others (Blackmore et al. 1998, this study in Chapter 4).

5.2. Material and Methods

The methodology for sampling and analyses were described in Chapter 3. Therefore, only additional processes investigated to develop the budget model, and how the model was used to investigate Cd accumulation in *Balanus amphitrite* in the field will be reported herein. Parameters relating to food ingestion and the barnacles' growth were investigated in the laboratory and in the field, respectively. Food ingestion was determined as a function of the clearance rate, which represents the water volume cleared of particulate matter by an organism per time (see, e.g., Kesarcodi-Watson et al. 2001), and growth rate was determined based on photographic census. The methodology for the determination of these processes is fully described in the following sections.

5.2.1. Determination of the clearance rate for *Balanus amphitrite* in the laboratory

The clearance rate for *Balanus amphitrite* was experimentally determined in the laboratory. Barnacles were collected from Ross Creek on two occasions, separated, cleaned and brushed to remove any epibiota. One batch of barnacles was divided into five size-classes with 12–15

organisms in each, placed into 300-mL plastic chambers, and acclimatized for 17 days. During acclimatization and clearance rate determination the barnacles were fed with natural particles (sized <200 μm, 4,000–7,000 particles mL^{-1}) in seawater pumped daily from Ross Creek. A manually operated diaphragm pump was used to reduce any damage to the plankton community, thus keeping the food supply as close as possible to the one found in Ross Creek. Particle concentrations were determined with a Coulter Counter MULTISIZER II. The experiment was carried out in a controlled temperature room, under a 12:12 h light/dark cycle, at a temperature of 25 ± 1 °C (mean ± range of variation), salinity 37 ± 1 (mean ± range of variation), using a flow-through system under constant aeration. The flow rate in chambers varied between 30 and 60 mL min^{-1}, resulting in total water exchange in the experimental chambers in 5–10 minutes. The other batch of barnacles was divided in three size-classes with 15–20 organisms in each, and nourished exclusively with *Chaetoceros muelleri* (10,000–15,000 cells mL^{-1}) in filtered seawater. The environmental conditions and experimental design for this second batch were as for the first batch, except that acclimatization lasted for 9 days.

The clearance rate (*CR*, in L mg^{-1} h^{-1}) was determined by measuring the reduction in the number of particles in a sub-sample of 1 mL (measured in quadruplicate) between each experimental chamber (P_{CH}) and the control (P_C, with no barnacles), taking into account the number of barnacles filtering actively (*N*), the mean dry weight of each size-class (*W*, in mg, determined at the end of the experiment), and the water flow rate in each experimental chamber (*F*, in mL min^{-1}) as indicated below:

$$CR = \frac{0.06F\left(P_C - P_{CH}\right)}{P_C NW}. \qquad \text{Equation 5.1}$$

Sub-samples were withdrawn for the determination of the number of particles after a constant number of barnacles (between 4 to 6) filtering actively could be identified for a period not less than 5–10 min depending on the chamber flow rate.

5.2.2. Determination of the growth for *Balanus amphitrite* in the field

The barnacles' growth rate was determined by measuring variations in the barnacles' orifice size from photos taken in the field in both sampling periods following methodology presented elsewhere (Calcagno et al. 1997, 1998, Jeffery & Underwood 2001). The orifice size was defined by the product of the two biggest internal distances measured digitally from the photos. The barnacles' orifice size was transformed to soft tissue dry weight with a relationship determined from 60 barnacles collected at sites B and C, in which the orifice distances were determined with a digital calliper (Figure 5.1). The growth rate of each 160-photographed barnacles was then determined as the slope of the temporal variation of the natural logarithm of its dry weight obtained from a sequence of 7–9 photos.

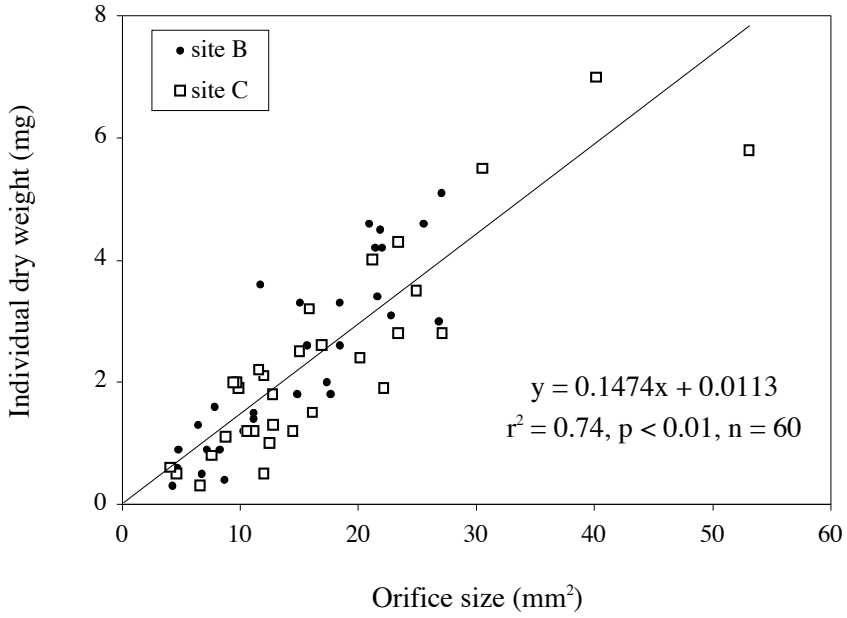

Figure 5.1: Relationship between *Balanus amphitrite*'s orifice size (mm^2) and its individual dry weight (mg) determined from the barnacles sampled at sites B and C on April 20, 2004.

5.2.3. Budget analysis

For the comparison and interpretation of the Cd concentrations in the barnacles sampled at sites B and C in winter 2002 and summer 2004, four budget models were built, for each site (B and C), in each sampling period. They were based on the Thomann's bioenergetic kinetic model (see, e.g., Thomann 1981, Wang & Fisher 1998, Wang et al. 1999b), in which the daily variation in the Cd concentration in *Balanus amphitrite* (dC_b/dt, in ng mg^{-1} d^{-1}) can be described as a function of the Cd obtained from the dissolved and particulate phases, and reduction of Cd concentration as follows:

$$\frac{dC_b}{dt} = k_w C_w T + CR_b T \sum_{f=1}^{3} \left(M_f C_f AE_f \right) - C_b (g + k_e), \qquad \text{Equation 5.2}$$

where k_w is the Cd uptake rate constant from water, ca. 1.18×10^{-5} L mg^{-1} h^{-1} (Rainbow et al. 2003, Rainbow et al. 2004a), C_w is the dissolved Cd concentration (ng L^{-1}, values in Table 5.1), and T is the time of exposure to the Cd source, i.e., the period in which barnacles were underwater, which is 12.9 and 12.5 h d^{-1} in the summer and 6.5 and 10.8 h d^{-1} in the winter, for sites B and C, respectively. CR_b is the clearance rate for *Balanus amphitrite* (L mg^{-1} h^{-1}, Equation 5.4), and it was determined in the laboratory as a function of its individual mean dry weight (W, in mg, values in Table 5.1) The $\sum$ symbol indicates that three food items are considered in this model (f = 1 to 3, for SSPM, LSPM and microzooplankton). Thus, M_f, C_f and AE_f stand for mass abundance (mg L^{-1}, values in Table 5.1), Cd concentration (mg kg^{-1}, values in Table 5.1), and the Cd assimilation efficiency from each food item (see below), respectively. Cadmium detoxification is considered in the last term of the equation and it is dependent on the dilution as a function of the dry weight-based growth rate (g, in d^{-1}) and the depuration as a function of the efflux rate constant, k_e = 0.007 d^{-1} (Wang et al. 1999b, Rainbow et al. 2003). The growth rates for barnacles at both sites in both sampling periods were estimated based on the growth rate determined in the field (see section 5.3.5). No food preference was assumed in this model, i.e., barnacles ingest each food source in proportion to that in the environment, and a maximum daily food ingestion of 40% of the barnacles' individual dry weight (Wang et al. 1999b) was used in the model. The contributions of the Cd sources in the model were considered to be additive (see, e.g., Qiu et al. 2001, Griscom et al. 2002).

Cd AE from microzooplankton was assumed to be 80% (Wang & Fisher 1998, Wang et al. 1999b). The Cd AE from LSPM, which is mainly composed of dead microzooplankton (see Chapter 4), was also assumed to be 80%. Cd AE from SSPM was estimated for each site in each sampling period using Equation 5.2. Assimilation efficiencies were calculated as the

values that would be required to keep a constant Cd concentration in *Balanus amphitrite*, assuming a steady-state (i.e., $dC_b/dt = 0$) and that all the other parameters in the model were as defined above. Cadmium AEs from SSPM were numerically calculated applying finite difference equations solved by a Runge-Kutta 4 integration method (Wallis et al. 2002), in which variations from 0 to 1 (1 stands for 100% efficiency) were applied with an increment of 0.01. The Cd fluxes for the budget models were then determined including the Cd AEs from SSPM estimated by Equation 5.2, plus all other parameters previously defined.

5.2.4. Sensitivity analysis

A sensitivity analysis based on Equation 5.2 was performed to estimate the relative importance of each model's parameter to the Cd accumulation in *Balanus amphitrite*. An average value for each parameter was calculated taking into account the values of the parameters for site B and C in winter and summer. In the sensitivity analysis variations of ±25% and ±50% were individually applied to each averaged parameter, and the alteration produced in the barnacles Cd concentration was used to quantify the model sensitivity. The range of variations applied was chosen to be consistent with the range of observational data in the field. Each alteration was numerically performed as for the determination of the Cd AE from SSPM, but using a simulation time of 900 days. The length of the simulation time was chosen, based on previous simulations, to guarantee enough time for the model to reach another steady-state condition after the imposed alteration. The model sensitivity to each parameter was calculated as the percentage variation in the Cd concentration in *Balanus amphitrite* (V, in %) caused by the imposed variation in the parameter as indicated below:

$$V = \left(100\frac{C_m}{C_s}\right) - 100, \qquad \text{Equation 5.3}$$

where C_m is the end value (i.e., time = 900) of the modified Cd concentration in the barnacles as a function of the variation applied, and C_s is the end value of the standard Cd concentration in the barnacles (i.e., no variation is applied).

5.3. Results

An environmental characterization comparing the two sampling periods was presented in Chapter 3 (section 3.3), in which distinct patterns were observed between these periods for rainfall, salinity, and tidal ranges that in general presented higher values in the wet season compared to the dry season, except for salinity that exhibited an opposite pattern. Table 5.1 summarizes all components sampled at sites B and C in the wet season of 2004 and compares their values with those sampled in the dry season of 2002. Because no significant temporal variation was observed for either Cd or mass concentrations in summer and winter for the majority of the sampled components in the Ross Creek water column, they were represented as mean values ± 1 SD.

No consistent relationship between barnacles' dry weight and Cd concentration was identified for 1159 animals grouped by 4–7 organisms within 8 size-classes collected each day at sites B and C in wet season 2004. Therefore, the Cd concentration in each size-class sampled each day were considered as replicates, in order to compare them with the barnacles sampled in the dry season of 2002.

Table 5.1: Cadmium concentrations, mass abundances, total Cd concentration in the Ross Creek water (sized <200 μm), Cd concentration in *Balanus amphitrite* and individual dry weights, sampled at sites B and C, in the wet and dry seasons (mean ± 1 SD). The Mann-Whitney test (p-value), comparing dry and wet seasons' data for each site, and the number of samples (n) are also presented.

Material		Site	Wet season	Dry season	p-value	n
Cd concentration	Dissolved (ng L^{-1})	B	13.73 ± 8.15	11.23 ± 3.66	0.70	26
		C	43.79 ± 12.82	90.64 ± 21.56	< 0.01	26
	SSPM (mg kg^{-1})	B	0.17 ± 0.23	0.12 ± 0.18	0.14	26
		C	0.22 ± 0.05	0.22 ± 0.26	0.29	26
	LSPM (mg kg^{-1})	B	0.31 ± 0.20	0.36 ± 0.18	0.36	25
		C	0.64 ± 0.18	0.43 ± 0.25	0.03	25
	Microzooplankton (mg kg^{-1})	B	0.08 ± 0.03	0.17 ± 0.11	< 0.01	24
		C	0.16 ± 0.07	0.23 ± 0.20	0.49	24
Mass abundance	SSPM (mg L^{-1})	B	11.82 ± 4.28	5.56 ± 2.06	< 0.01	26
		C	8.54 ± 1.83	4.51 ± 1.31	< 0.01	26
	LSPM (mg L^{-1})	B	0.03 ± 0.03	0.12 ± 0.13	< 0.01	25
		C	0.02 ± 0.01	0.08 ± 0.05	< 0.01	24
	Microzooplankton (mg L^{-1})	B	0.13 ± 0.06	0.14 ± 0.15	0.22	24
		C	0.12 ± 0.06	0.17 ± 0.17	0.69	24
Total Cd load (ng L^{-1})		B	15.1 ± 8.77	10.8 ± 2.93	0.20	21
		C	45.8 ± 13.3	93.6 ± 23.3	< 0.01	22
Cd in *Balanus amphitrite* (mg kg^{-1})		B	3.49 ± 2.15	2.98 ± 1.26	0.33	188
		C	7.36 ± 3.41	8.40 ± 3.35	0.06	175
Balanus amphitrite weight (mg)		B	2.45 ± 1.89	3.31 ± 1.96	< 0.01	188
		C	2.35 ± 1.70	2.99 ± 2.06	0.02	175

5.3.1. Dissolved Cd concentration

More than 90% of the total Cd in Ross Creek water sized <200 μm was in the dissolved phase in the wet season of 2004, as observed in the dry season of 2002. This is in accordance with Cd inorganic aqueous speciation, where at particle concentrations of a few mg L^{-1} (the case for Ross Creek), the Cd is predominantly found in the hydrophilic aqueous form (Stumm 1992, Turner et al. 2004). In the wet season, the Cd concentration in the dissolved phase was significantly higher upstream (Mann-Whitney test, $p < 0.01$, $n = 26$), as observed in the dry season. In the seasonal comparison, site C in wet season 2004 exhibited half of the dissolved Cd concentration observed in dry season 2002, while no significant difference was identified for site B (Table 5.1).

5.3.2. Particulate Cd concentration and mass abundance

In the wet season of 2004, Cd in SSPM corresponded to >97% of the particulate Cd sized <200 μm at sites B and C. The same pattern was observed in 2002 (section 4.2.3). The dominance of the Cd-laden SSPM occurs because on average the SSPM mass abundance (10.2 mg L^{-1}) is up to 38 times larger than LSPM (0.03 mg L^{-1}) and microzooplankton (0.13 mg L^{-1}) combined, while their Cd concentrations are of the same order of magnitude (0.20, 0.47 and 0.12 mg kg^{-1} for SSPM, LSPM and microzooplankton, respectively). Spatially, the Cd concentration in SSPM, LSPM and microzooplankton followed the dissolved Cd concentrations, being significantly higher at site C than B (Mann-Whitney test, $p < 0.01$ for all three parameters, and $n = 26$, 24 and 24, respectively). On the temporal scale, no significant difference was in general identified comparing Cd concentration in the particulate

phase between wet and dry seasons, except for microzooplankton at site B that was higher in the dry season, and for LSPM at site C that was higher in the wet season (Table 5.1).

The mass abundance of SSPM, LSPM and microzooplankton showed no significant difference between sites B and C in the wet season (Mann-Whitney test, $p = 0.06$, 0.73 and 0.56 respectively, and $n = 26$, 24 and 24 respectively). However, some differences were identified on the temporal scale between both sites: While SSPM was higher in wet season 2004 and LSPM was higher in dry season 2002, microzooplankton exhibited no difference (Table 5.1).

5.3.3. Cadmium concentration in *Balanus amphitrite*

In the wet season of 2004, the Cd concentration in barnacles at site C (7.36 ± 3.41 mg kg^{-1}) was significantly higher than at site B (3.49 ± 2.15 mg kg^{-1}, Mann-Whitney test, $p < 0.01$, $n = 193$). At both sites, the barnacles exhibited a significant temporal variation (Kruskal-Wallis test: site B: $p = 0.03$, $n = 101$ and site C: $p = 0.04$, $n = 92$) (Figure 5.2), even though no temporal variation was detected for the Cd concentrations in the dissolved and particulate phases. This pattern was also observed in the dry season sampling program (see section 4.2.1 and 4.2.2). Comparing the dry and wet sampling periods, no significant difference was identified for the mean Cd concentration in barnacles for both sites B and C (Table 5.1).

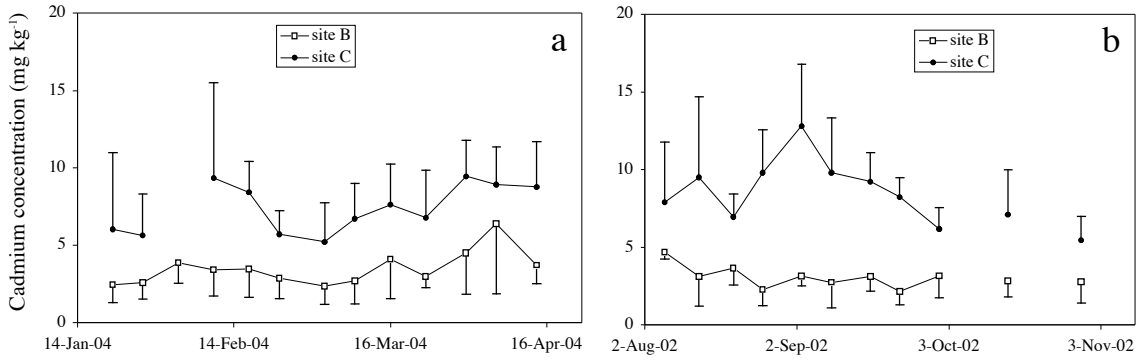

Figure 5.2: Temporal variation of the mean Cd concentration in *Balanus amphitrite* (mg kg^{-1}) sampled at sites B and C during the wet season of 2004 (a) and the dry season of 2002 (b) in Ross Creek. Error bars stand for 1 SD.

In monitoring programs, bigger organisms are usually targeted to avoid bias on the Cd determination caused by any size effect (Rainbow & Smith 1992, Rainbow et al. 1993). The barnacles' population sampled in the wet season did not differ significantly in size (as mean individual dry weight) between sites B and C (Mann-Whitney test, $p = 0.75$, $n = 193$), which allows the comparison between their Cd concentrations. Comparing the barnacles' size between the wet and dry seasons for each site, a significant difference was identified for both sites (Table 5.1). However, because no size effect was identified in either of the sampling periods (i.e., no consistent relationship between barnacles' dry weight and Cd concentration was identified), the difference in the barnacles' size has no effect on the interpretation of their Cd concentrations.

5.3.4. Clearance rate for *Balanus amphitrite* in the laboratory

The clearance rates and corresponding mean dry weight of each size-class of barnacles are presented in Figure 5.3. The two sets of experiments, in which barnacles were fed on either natural particles or only *Chaetoceros muelleri* cells, were combined to determine the best fit between clearance rate (CR_b, L mg^{-1} h^{-1}) and the barnacles' individual dry weight (W, mg) as indicated below:

$$CR_b = \frac{4.0435 \times 10^{-2}}{W} + 0.6658 \times 10^{-2}. \qquad \text{Equation 5.4}$$

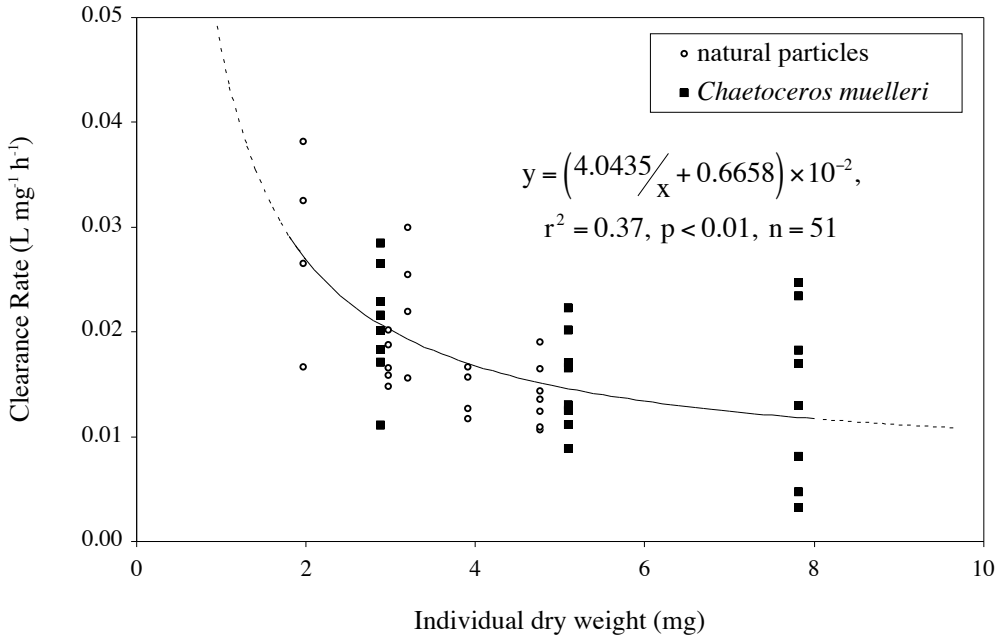

Figure 5.3: Clearance rate (L h^{-1} mg^{-1}) as a function of the *Balanus amphitrite* individual dry weight (mg) determined in the laboratory for organisms feeding on two types of food (natural suspended particular matter from Ross Creek and the algae *Chaetoceros muelleri*).

The fitted curve ($r^2 = 0.37$, $p < 0.01$, $n = 51$) suggests that large organisms are less active than small ones per unit mass and that CR_b behaves similarly independent of the particle type. Furthermore, CR_b was independent of the number of particles offered to the barnacles. The CR_b variability in each size-class was attributed to individual variability, since the barnacles filtering actively were not the same individuals for each CR_b determination, and to a minor degree to differences in weight among the organisms within each size-class.

5.3.5. Growth rate for *Balanus amphitrite* in the field

The growth rate and the corresponding initial individual dry weights for the barnacles photographed at sites B and C in the dry season of 2002 and the wet season of 2004 are presented in Figure 5.4. Because no distinguishable pattern was identified among sites regardless of season, all data were combined, resulting in the following adjustment equation:

$$GR = \frac{-1.285 \times 10^{-2} + 5.210 \times 10^{-2} W^{-1.110}}{4.354 + W^{-1.110}}, \qquad \text{Equation 5.5}$$

where GR stands for growth rate (d^{-1}) and W for the barnacles' individual dry weight (mg). The fitted curve ($r^2 = 0.83$, $p < 0.01$, $n = 160$) demonstrates a higher growth rate for small organisms, and that *Balanus amphitrite* in Ross Creek stops growing around 3.5 mg of dry weight. Similar patterns have been observed for barnacles including *Balanus amphitrite* (Anderson 1994, Calcagno et al. 1997, 1998, Jeffery & Underwood 2001). Equation 5.5 was used to estimate a mean growth rate for the barnacles sampled at each site in each sampling period. Thus, taking into account the individual mean dry weight of the barnacles, the following mean growth rates were determined: zero for barnacles at site B in the dry season, 0.001 d^{-1} at site B in the wet season and site C in the dry season, and 0.002 d^{-1} for barnacles at site C in the wet season.

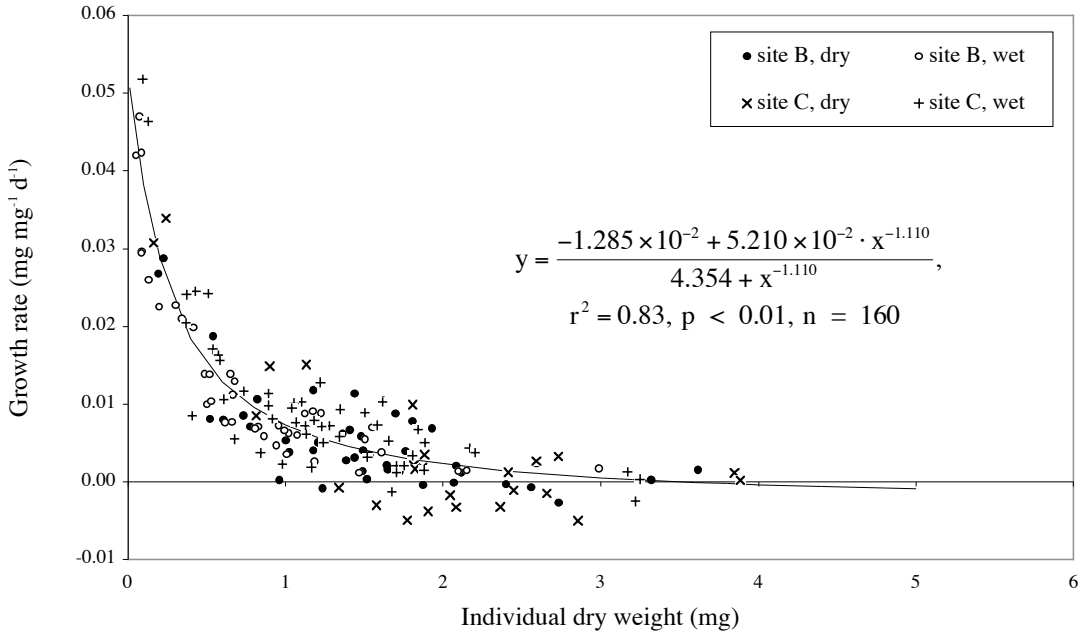

Figure 5.4: Growth rate (dry weight-based, in d^{-1}) as a function of the *Balanus amphitrite* individual dry weight (mg) determined from photos taken in the field at both sites in the dry and wet seasons.

5.3.6. Budget analysis

The budget analysis requires an estimate of Cd AE from SSPM. This was done by assuming a steady-state for Equation 5.2, from which the following Cd AE from SSPM were determined: 0.39 and 0.37 for the barnacles at site B, and 0.64 and 0.56 for the barnacles at site C, in the wet and dry seasons, respectively (the differences in AE are discussed in section 5.4.2). Based on the AEs, the fluxes of Cd in and out of *Balanus amphitrite* were calculated for sites B and C in the wet and dry seasons. These fluxes were then used to produce four budget models presented in Figure 5.5. On average for all four situations, the Cd influx from food accounts for >80% of the total Cd influx to *Balanus amphitrite*, even though >90% of the total Cd in

Ross Creek water column (sized <200 μm) was in the dissolved phase. This pattern agrees with previous observations that the main route of heavy metals to aquatic organisms is often from food rather than from the water (Wang et al. 1999a, Wang et al. 1999b, Wang 2002, Luoma & Rainbow 2005).

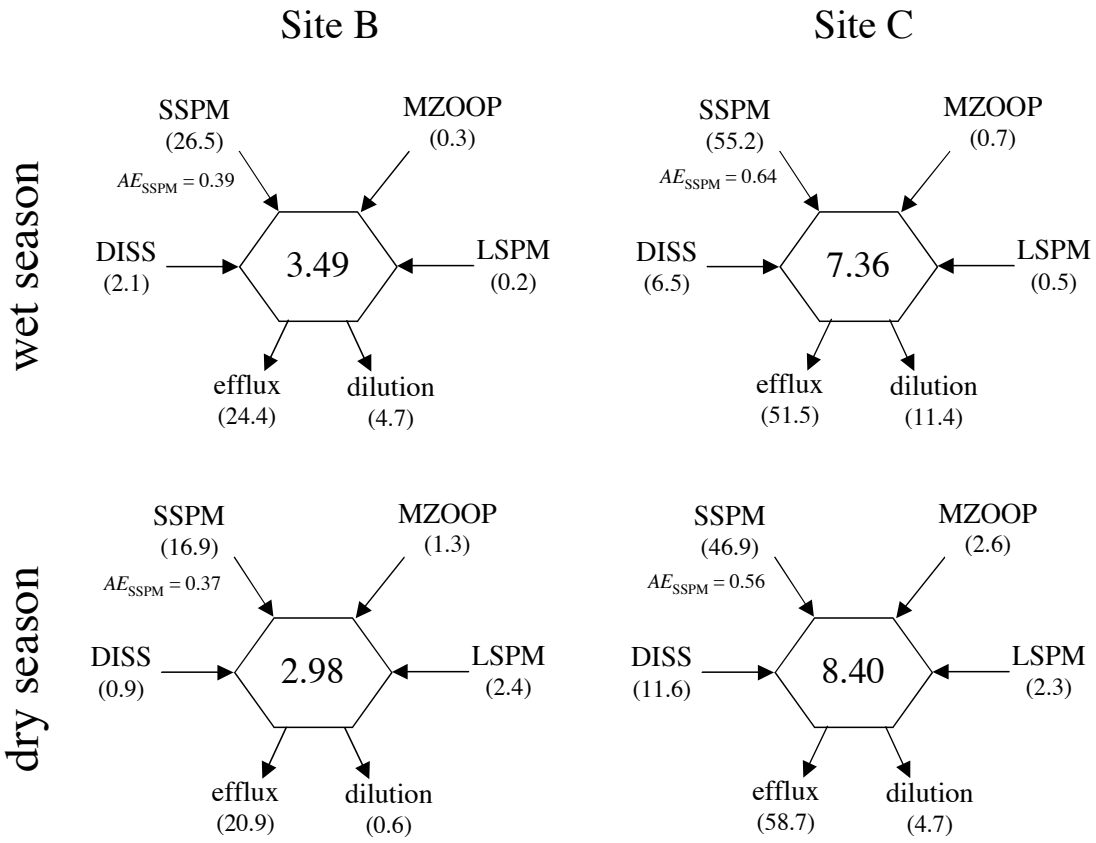

Figure 5.5: Budget models for site B and C in the wet season of 2004 and dry season of 2002. The Cd fluxes from the dissolved phase (DISS), SSPM, microzooplankton (MZOOP) and LSPM to *Balanus amphitrite* and the decrease in Cd concentration (efflux and dilution) are represented. Cadmium fluxes (in brackets) are in μg kg^{-1} d^{-1}, and Cd concentrations in barnacles (within hexagons) are in mg kg^{-1}.

5.3.7. Sensitivity analysis

The sensitivity analysis allowed ranking the model's parameters in order of decreasing importance on the control of the Cd concentration in *Balanus amphitrite* (Figure 5.6). In general, the parameter's sequence agrees with the fluxes calculated in the models presented in Figure 5.5; the bigger the flux, the bigger the influence on the Cd concentration in the barnacles. Efflux rate, maximum food ingestion rate, SSPM Cd concentration, Cd AE from SSPM and the barnacles' individual mean weights were identified as the most sensitive parameters in the model. Similar results were found by Wang and co-workers (1999b), in a sensitivity analysis for *Balanus amphitrite*, in which assimilation efficiency, maximum food ingestion and barnacles' growth rate were identified as the most important parameters.

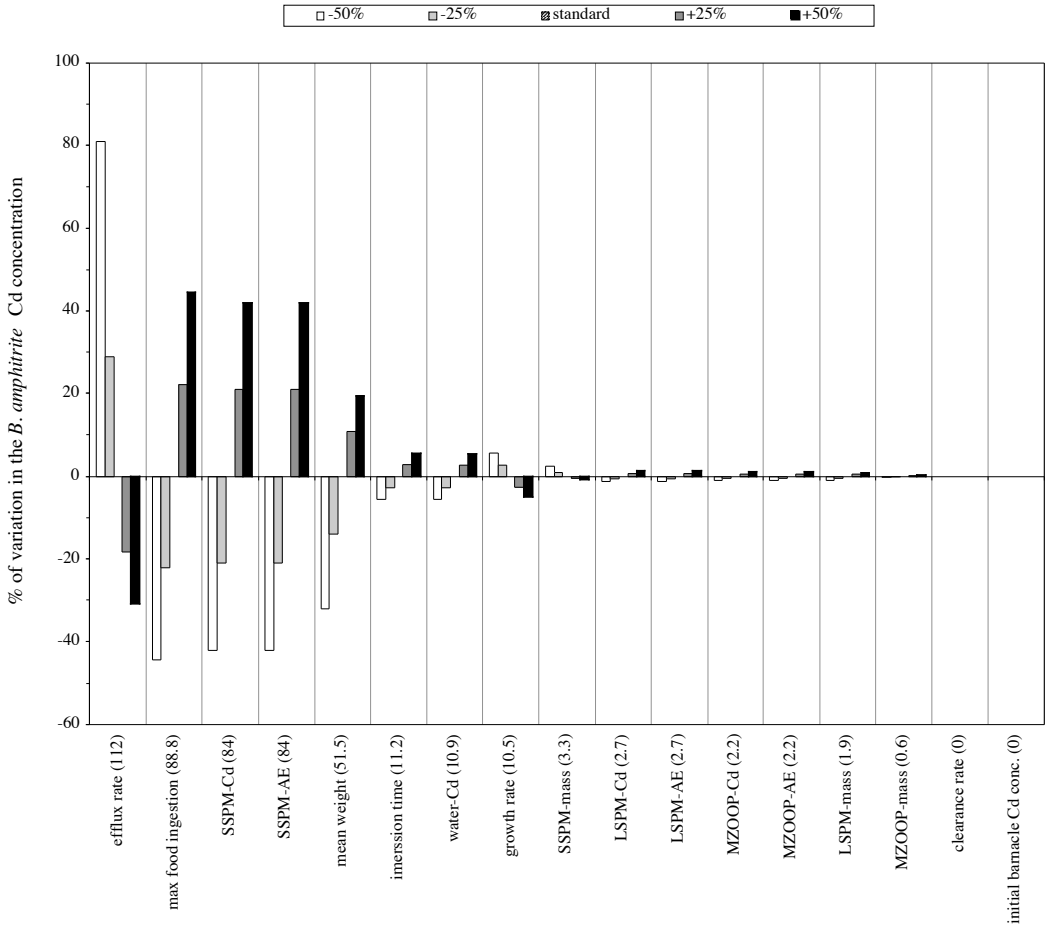

Figure 5.6: The result of the sensitivity analysis performed on all the parameters included in Equation 5.2. Each bar represents the % variation in the *Balanus amphitrite* Cd concentration when variations of ±25% and ±50% are applied. Numbers in brackets represent the range of variation between the two extreme bars for each parameter.

5.4. Discussion

The objective of this chapter is to test whether variations in the availability of dissolved and particulate Cd affect the Cd concentration in *Balanus amphitrite*. Therefore, Cd

concentrations in the dissolved and particulate phases and mass abundance of the particles (i.e., SSPM, LSPM and microzooplankton) will be addressed herein only to understand their effect on the Cd concentration in the barnacles.

5.4.1. Spatial and seasonal variation of the Cd concentration in *Balanus amphitrite*

Comparing the spatial pattern of the Cd concentration in the *Balanus amphitrite* sources (i.e. in the dissolved phase, SSPM, LSPM and microzooplankton) in the wet season of 2004, it is not unexpected to find the highest Cd concentrations in barnacles living upstream, since site C exhibited the highest Cd concentrations in both the dissolved and particulate phases (Table 5.1). The same pattern was observed in the dry season of 2002; the higher the dissolved Cd concentration, the higher the Cd concentration in particles and in the barnacles as well. This pattern has been observed elsewhere for phytoplankton, zooplankton, and barnacles (Rainbow & White 1989, Blackmore et al. 1998, Luoma et al. 1998, Rainbow 1998, Connell & Sanders 1999, Strom et al. 2001), and is the rationale for using such organisms as biomonitors.

The seasonal variation of the Cd concentration in the barnacles at each site did not present a simple pattern as observed spatially, in which particles and barnacles for both sampling periods followed the dissolved Cd concentration. For example, no significant difference between seasons was identified for the total Cd load in the water column at site B, as occurred for the barnacles at that site (Table 5.1). Conversely, the total Cd at site C was significantly higher in the dry season, but no significant difference between seasons was identified for the Cd concentration in the barnacles at that site. These field observations suggest that *Balanus amphitrite* may record differences in Cd concentrations in the environment on a spatial scale (i.e., between sites), but it fails to do so in the temporal scale (i.e., between seasons). This

apparent contradictory behaviour was explained by the budget analysis performed in this work (next section).

5.4.2. Budget analysis of the seasonal Cd variation in *Balanus amphitrite*

When a steady-state was assumed for the determination of the Cd AE from SSPM using Equation 5.2, it was found that AE is higher at site C than site B. The relative contribution of phytoplankton to suspended particles has been shown to have an important effect on the Cd accumulation in bivalves; the assimilation efficiency is higher when phytoplankton is present in suspended particles (Lee & Luoma 1998, Ke & Wang 2002, Wang & Wong 2003). A higher phytoplankton abundance measured as chlorophyll-a (see section 6.3.1 and Appendix II) was identified at site C (4.33 ± 2.85 $\mu g\ L^{-1}$, mean for both sampling periods) compared to site B (2.09 ± 1.15 $\mu g\ L^{-1}$, mean for both sampling periods) (Mann-Whitney test, $p < 0.01$, $n = 50$), whereas higher SSPM abundances were observed at site B compared to site C (Table 5.1). This suggests that barnacles may behave similarly to bivalves regarding the effect of phytoplankton enrichment in SSPM. Thus, the higher phytoplankton abundance in SSPM at site C increased the Cd assimilation efficiency from SSPM for the barnacles at that site.

The relative importance of metal uptake from the dissolved and particulate phases by aquatic organisms is dependent on the relative magnitude of metal influx from each source (Wang & Fisher 1998). The budget analysis indicated that there is a dominance of the importance of Cd in food over Cd in the dissolved phase as the source of Cd to the barnacles, even though the dissolved Cd accounts for >90% of the total Cd in the environment. This pattern explains the contradiction previously pointed out, in which *Balanus amphitrite* records spatial variation in the Cd concentration in the environment, but fails to record temporal variability. The lower

total Cd concentration in the environment observed in the wet season at site C was driven by variations in the dissolved phase (Table 5.1). The Cd concentrations in the particulate phase did not vary significantly between the wet and dry seasons for both sites (Table 5.1). Thus, variations in the Cd concentration in the barnacles were not observed at sites B and C between those periods. Conversely, on the spatial scale, the Cd concentration in the food was always higher at site C, resulting in higher Cd accumulation in the barnacles at that site.

5.4.3. Sensitivity analysis of the controls on Cd concentrations in *Balanus amphitrite*

In the sensitivity analysis performed in this study as well as the sensitivity analysis presented in Wang and co-workers (1999b), the most important parameters controlling the Cd concentration in *Balanus amphitrite* are not directly related to the Cd sources in the environment. Efflux rate, maximum food ingestion rate, growth rate, and size are parameters intrinsically related to the physiology of organism. Therefore, independently of whether or not *Balanus amphitrite* is a good biomonitor of variations in the Cd concentrations in the environment, there is also a strong physiological control on the Cd concentration in this organism. The combination of these physiological controls and the dominance of particulate Cd on the biomonitor Cd load may produce a gap between the information obtained from the biomonitor and any subsequent conclusion about contamination in the environment, as already pointed out by Poethke and co-workers (1994).

The maximum food ingestion rate is an example of a physiological characteristic on the Cd accumulation in *Balanus amphitrite* that overcomes the Cd concentration in the particulate phase, which the budget analysis showed to be the major Cd flux into the biomonitor (Figure 5.5). A maximum food ingestion rate of 40% of the organism dry weight per day was

assumed in the model (Equation 5.2). For the mean individual weight of the organisms considered in the model (ca. 2.71 mg dry weight), food availability in Ross Creek is on average much higher than the maximum barnacles' food requirement. This fact was also verified in the sensitivity analysis for clearance rate, which was shown to be insensitive to the change imposed in the model (Figure 5.6). Thus, any change that increased or decreased the maximum food ingestion rate would result in a corresponding change in the uptake of Cd by the biomonitor. Because the maximum food ingestion acts simultaneously on the three food sources considered in the model, its influence on the Cd accumulation in the biomonitor is bigger than the influence of any food source individually.

To avoid bias in the Cd concentration caused by differences in the immersion time it is recommended to sample barnacles from the same horizon (Blackmore et al. 1998). However, the result of the sensitivity analysis suggests that it may not necessarily be a critical requirement for *Balanus amphitrite*. Immersion time controls the Cd uptake from water and food in Equation 5.2 – the greater the immersion time, the greater the chance of Cd uptake from these sources. Cadmium uptake from food does not vary as a function of the variations imposed by immersion time because the barnacles can match their nutritional requirements in a short time period, as discussed previously. Thus, immersion time exerts control only on the Cd obtained from the dissolved phase. Since dissolved Cd does not represent a major source of Cd to the barnacles, immersion time has a slight influence on the Cd concentration in the biomonitor. This model's prediction is supported by the observations that no significant difference was identified for the Cd concentration in barnacles sampled on the same day at site B separable by a vertical distance of 50 cm (Mann-Whitney test, $p = 0.12$, $n = 16$), even though such a vertical difference represents a reduction in the immersion time from 12.9 to 7 h d^{-1}.

In general, for those parameters related to Cd outflow from barnacles, such as efflux and growth rates, increasing values of the multiplicative factor resulted in decreased Cd loads (Figure 5.6). SSPM mass abundance exhibited the same decreasing behaviour despite it being a Cd inflow to the barnacles. This occurs because an increase in SSPM mass abundance represents a dilution in the overall Cd burden in the food sources. For example, SSPM exhibits a lower Cd concentration compared to LSPM. Ke & Wang (2002) pointed out that the pre-ingestion particle selection affects the metal accumulation in filter feeders, such as mussels. In our model, no food selection was assumed, but if *Balanus amphitrite* can select the food intake, this may change the overall Cd influx from food to the barnacles.

The sensitivity analysis for the barnacles' weight exhibited a non-linear pattern for the imposed alterations, being more sensitive to reductions in their values than increases (Figure 5.6). Smaller organisms exhibit higher clearance rates per unit of mass than bigger ones (Figure 5.3), which leads to higher proportional Cd intake from food. Conversely, smaller organisms exhibit higher growth rate (Simpson & Hurlbert 1998, Jeffery & Underwood 2001, and this study Figure 5.4), which leads to higher proportional Cd dilution. The results of the sensitivity analysis suggest that the dilution effect is stronger than accumulation for smaller organisms because of their high growth rate. Therefore, variations in growth rate are expected to increase or decrease the dilution effect in relation to the Cd accumulation, which may be one of the causes of the size effect sometimes observed for the Cd concentration in *Balanus amphitrite* in the field (see next section).

5.4.4. Effect of the growth rate on the Cd concentration in *Balanus amphitrite*

The metal concentration in barnacles is dependent on the rates of accumulation, excretion and dilution due to body growth, which may produce a size effect (Blackmore et al. 1998, Blackmore & Rainbow 2001). Such size effects have been reported in some studies of *Balanus amphitrite* (Rainbow & Smith 1992, Rainbow et al. 1993, Blackmore & Rainbow 2001, Rainbow & Blackmore 2001), but not in others (Blackmore et al. 1998, this study). A simulation exercise was performed using the model as in the sensitivity analysis to test whether different growth rates would cause size effects in *Balanus amphitrite*. Six different growth rates were considered in the model, while all other parameters were kept constant. This resulted in six curves of Cd concentration in *Balanus amphitrite* as a function of its individual dry weight. The Cd concentrations were then made relative by dividing each Cd concentration within each growth rate curve by the smallest Cd concentration in that curve. The relative Cd concentration in the barnacles was then plotted against the barnacles' weight (Figure 5.7). It should be noted that, as shown in Figure 5.4, the barnacles effectively cease to grow for individuals with a mean dry weight above 3.5 mg. This results in the relative Cd concentration in the barnacles reaching a plateau as shown in Figure 5.7.

The result of the simulation exercise suggests that the higher the growth rate, the higher the possibility of a size effect for *Balanus amphitrite*. This agrees with the findings of Luoma and Rainbow (2005) about the importance of considering growth rate on the Cd accumulation in organisms, particularly when organisms grow rapidly. For mean growth rates $<0.003\ d^{-1}$, such as were determined for *Balanus amphitrite* in Ross Creek, it is difficult to identify any size effect. For rates $>0.01\ d^{-1}$, which are more often determined for *Balanus amphitrite* in the

field (Calcagno et al. 1997, Simpson & Hurlbert 1998, Thiyagarajan et al. 2003), there is a clear size effect for the Cd concentration in *Balanus amphitrite*.

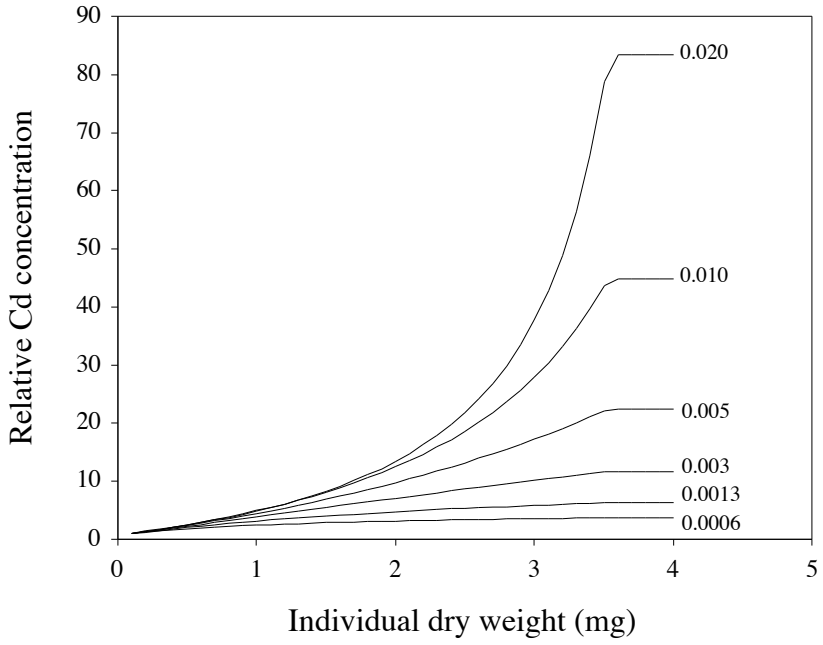

Figure 5.7: Simulation of the relative Cd concentration in *Balanus amphitrite* as a function of its individual dry weight (mg) for different growth rates (values close to the curves, in mg mg^{-1} d^{-1}).

5.5. Final Remarks

In this chapter, the barnacle biomonitor *Balanus amphitrite* showed some weaknesses in indicating variations in the Cd load in an estuarine environment. Even though seasonal and temporal variations were observed for the total Cd concentration in the Ross Creek water (including particles sized <200 μm), the biomonitor failed to indicate seasonal variation,

being able to detect variation in the spatial scale only. The budget analysis indicated that Cd influx from food to *Balanus amphitrite* accounted for >80% of the total Cd influx, so although >90% of the total Cd was in the dissolved phase in the environment, variations in this form of Cd were not reflected in barnacles. Because the particulate phase did not exhibit significant seasonal variation in the Cd concentration, no variation was observed for the Cd concentration in the barnacles. However, the particles exhibited differences in their Cd concentration spatially, which was indicated by the biomonitor.

Wang and co-workers (1999b) pointed out that it is unclear whether there is a tight coupling between the Cd concentration in *Balanus amphitrite* and the bioavailable Cd concentration in the environment. The sensitivity analysis performed in this study identified a strong physiological control on the Cd accumulation in *Balanus amphitrite*. The strong dependence of Cd accumulation in the biomonitor to the maximum food ingestion rate and to the efflux rate, the likely variation in the overall Cd uptake as a function of pre-ingestion particle selection, and the influence of growth rate that may cause a size effect in the Cd concentration in *Balanus amphitrite* are some examples identified in this study. These results lead to the conclusion that physiological characteristics of the biomonitor may mask a direct relation and hence a tight coupling between Cd availability in the environment and in the biomonitor.

The extent to which a biomonitor integrates the temporal variation in pollutant levels is rarely measured (Beeby 2001). A few studies have demonstrated that metal concentrations in barnacles show temporal variations of between months and years (e.g., Rainbow & Smith 1992, Rainbow et al. 2000, Blackmore & Rainbow 2001, Rainbow & Blackmore 2001, Rainbow et al. 2004b). However, to what extent a barnacle biomonitor such as *Balanus amphitrite* would respond to short-term temporal variations in its metal sources in the

environment were unknown. To provide some insights on this matter, a simulation model that reproduces the Cd fluxes on a daily basis for *Balanus amphitrite* was built. The model was based on the bioenergetic kinetic model implemented in this study, to which were added information regarding some processes investigated in the laboratory using radiochemical techniques. This study and its results are presented in the next chapter.

Chapter 6. *Exploring the potential of Balanus amphitrite as a biomonitor for temporal and spatial variations in Cd contamination in a simulation model*

6.1. Introduction

Models have been used to integrate information from several sources (e.g. field, laboratory and literature), in order to present them in a concise way and to quantitatively test several hypotheses simultaneously (Wiegert 1975, Hall & Day Jr 1977, Kremer & Nixon 1978, Jørgensen 1994, Hannon & Ruth 1997, Grant et al. 2000). For this reason, models represent an important contribution to the understanding of processes and mechanisms that drive complex natural systems (Wiegert 1975, Lomnicki 1988). Additionally, models have allowed the identification of gaps in knowledge and suggested further research (Valentin 1987). In particular, when models are used together with monitoring programs, a better knowledge of the environmental processes is attained. Thus, new hypotheses about the environmental dynamics, suggested by the model and supported by the field data, may support management actions (de Jonge & de Groodt 1989).

As discussed in section 2.2, steady-state models are unable to cope with temporal variability whereas simulation models can. This chapter describes an ecotoxicological simulation model that was developed to simulate on a daily basis the processes controlling Cd accumulation in

Balanus amphitrite in the field. Field data on Cd sources (water and food) to *Balanus amphitrite*, mass abundance of food items (i.e., SSPM, LSPM and microzooplankton) and some environmental parameters that may affect the Cd accumulation in the barnacles (e.g., immersion time, chlorophyll abundance in SSPM) were used in the model. In addition, radiochemical experiments aimed at the determination of Cd uptake rate constant from the dissolved phase and Cd assimilation efficiency from SSPM for *Balanus amphitrite* were carried out in the laboratory to support the model development.

6.2. Methodology

6.2.1. Simulation model

In common with the steady-state model, presented in section 5.2.3 (Equation 5.2), the simulation model was based on Thomann's bioenergetic kinetic model (see, e.g., Thomann 1981, Wang & Fisher 1998, Wang et al. 1999b). The basic difference between these two models is that the former (presented in section 5.2.3) represents the Cd concentration in *Balanus amphitrite* on a steady-state condition, and therefore its parameters are constants. Because the simulation model represents the temporal variation in the Cd concentration of *Balanus amphitrite*, most of the model's parameters in the steady-state version (Equation 5.2) were altered to cope with temporal variations. These alterations are explained in the next section.

The simulation model reproduced on a daily basis (dt = 1 d) four series of variations in the Cd concentration in *Balanus amphitrite*. In the dry season, the simulation period was from 6 August to 31 October 2002 (86 days) and in the wet season from 21 January to 14 April 2004

(87 days), for each site (B and C) in each season. The model was coded using the Stella software (Wallis et al. 2002), and its diagrammatic representation is presented in Appendix III.

The state variable, Cd mass (ng) in an individual barnacle, was simulated using differential equations (Hall & Day Jr 1977) applying the Runge-Kutta 4 method of numeric integration (Swartzman & Kaluzny 1987), and the Cd flows were expressed in ng organism^{-1} d^{-1}. The model validation was performed comparing the Cd concentration in *Balanus amphitrite* obtained in the field at each sampling day to the corresponding calculated Cd concentrations in *Balanus amphitrite*, which was obtained dividing the Cd mass (model's result) by the mean individual barnacles dry weight (forcing function, data obtained from the field). Two approaches were used in the model validation: A bi-lateral paired Student t-test, and the model efficiency coefficient, *EF* (Mayer & Butler 1993), which is calculated as:

$$EF = 1 - \frac{\sum_{i=1}^{n}\left(Obs_i - Sim_i\right)^2}{\sum_{i=1}^{n}\left(Obs_i - \overline{Obs}\right)^2}, \qquad \text{Equation 6.1}$$

where Obs_i and Sim_i stand for the Cd concentration in the barnacles on day i observed in the field and produced by the model, respectively, and $\overline{Obs}$ is the mean value of the observational data. For this test a value equal to 1 means that the model reproduces reality accurately, and negative values indicate that the model requires further improvements.

6.2.2. Parameters for the simulation model

Three strategies were used to determine the model's parameters: (i) some of the parameters were obtained from the literature; (ii) some were obtained in the field, and (iii) others in the laboratory experiments.

Mass abundances and Cd concentrations of the food items, and the abundance of phytoplankton were obtained in the field as well as the mean individual barnacles dry weight, and their immersion time. Phytoplankton abundance is a new parameter included in this model, compared to the one described by Equation 5.2, which controls the Cd AE from SSPM (explanation is presented in section 6.3.2.2). The methodology for sampling and determination of these parameters was presented in Chapter 3, except for phytoplankton that was determined as chlorophyll *a* concentrations. Chlorophyll *a* was collected in duplicate onto 25-mm Whatman GF/F filters, using a Sartorius mini filter holder adapted to a 60-ml plastic syringe. Pigment determinations from acetone extracts of filters were done with a Turner Designs AU-10 fluorometer following the method of Arar & Collins (1992). All these data obtained in the field were used in the model as forcing functions to control the intensity of the processes driving Cd concentrations in *Balanus amphitrite*. In order to compare the model's result, i.e., the Cd concentration in *Balanus amphitrite*, barnacles were also collected in the field and their Cd concentrations determined as described in Chapter 3. All data are presented in Appendix II.

The barnacles' clearance rate and growth rate were determined in the laboratory experiment and in the field, respectively, and the methodology for each was presented in Chapter 5. The Cd AE from LSPM and microzooplankton for *Balanus amphitrite* were obtained from the

literature, because the sensitivity analysis presented in Chapter 5 showed that they are of minor importance driving Cd accumulation in *Balanus amphitrite* in Ross Creek, and therefore, they are as those presented in section 5.2.3. The Cd uptake rate constant from the dissolved phase, Cd AE from SSPM for *Balanus amphitrite* and the efflux rate constant were determined with radiochemical experiments carried out in the laboratory, and they are presented in the next section.

6.2.3. Radiochemical experiments using ^{109}Cd

The Cd uptake rate constant from the dissolved phase, Cd AE from SSPM for *Balanus amphitrite* and the efflux rate constant were obtained with radiochemical experiments using ^{109}Cd. Even though some of these parameters can be found elsewhere (e.g., Wang et al. 1999b, Rainbow et al. 2003, Rainbow et al. 2004a) in the present study the Cd concentrations used in the dissolved phase were more realistic for the environment being studied, in which the maximum dissolved Cd concentration was approx. 0.3 μg L^{-1}. The Cd AE from SSPM was determined working with the whole assemblage of diverse particles common for suspended particular matter in Ross Creek. Thus, a better match between the results of the model, based on these experimental data, and the environment was achieved.

For the radiochemical experiments, *Balanus amphitrite* was collected from Ross Creek, separated, cleaned and brushed to remove any epibiota. Barnacles were placed into 300-mL plastic chambers for acclimatization, and fed with natural particles in seawater pumped from Ross Creek, as in the experiment for the determination of the clearance rate (see section 5.2.1). During the acclimatization period, barnacles were daily inspected for any epibiota and brushed if necessary. The radioisotope ^{109}Cd (half-life: $t_{1/2}$ = 462 d) was obtained from

Amersham Biosciences UK Limited (Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, England) as $CdCl_2$ in 0.1 M hydrochloric acid with a total activity of 37.3 MBq in a volume of 1.26 mL containing 2.14 μg of ^{109}Cd.

6.2.3.1. Cadmium uptake rate constant from the dissolved phase

The Cd uptake rate constant from the dissolved phase for *Balanus amphitrite* was determined under different Cd concentrations (referred to as the 'Cd concentration experiment') and salinities (referred to as the 'salinity experiment'). The organisms were acclimatized to three salinities, 27.3, 32.3 and 37.3 (all ranging ± 2), for a period of 11 days prior to the experiment. The environment from which the barnacles were collected had salinity 37.3, thus those transferred to the lower salinities were acclimatized over 3 days with gradual changes of less than four units per day. The salinities were in the range of those observed in Ross Creek. The lower salinities were obtained by mixing tap water with Ross Creek water in the appropriate proportion in a flow-through system, in order to achieve a required salinity and constant food supply.

In the 'Cd concentration experiment' three 1-L Nalgene bottles containing 500 mL of 0.45-μm filtered Ross Creek water (salinity 37.3) were prepared with final Cd concentration of 106, 268 and 407 ng L^{-1}, which were determined with ASV as described in Chapter 3. These Cd concentrations were obtained by adding to the filtered Ross Creek water (initial Cd concentration 16 ng L^{-1}) 5 ng of ^{109}Cd (86.37 kBq) plus the necessary amount of natural Cd to obtain the desired concentrations. Seven organisms were placed into each bottle and kept for 24 hours, under 12:12 h light/dark cycle, at a temperature of 24 ± 1 °C (mean ± range of variation), salinity 37.3, and constant aeration. Rainbow and co-workers (2003, 2004a) have

shown that the Cd uptake rate from the dissolved phase by *Balanus amphitrite* is constant over time; therefore, a single exposure of 24 h was used in all experiments. At the end of the exposure period, the organisms were rinsed with non-labelled seawater and frozen. On the same day, barnacles were removed from shells, rinsed with distilled water and dried to a constant weight in a fume cupboard. The activity of ^{109}Cd in the organisms was measured by a COBRA II – Auto-Gamma (Packard, Model D5002) gamma detector. The counting time was 2 minutes at an energy level of 15–30 keV, in order to maximise the number of counts per minute (CPM). The measured radioactivity was transformed to ^{109}Cd mass in the organisms using a calibration curve (Figure 6.1).

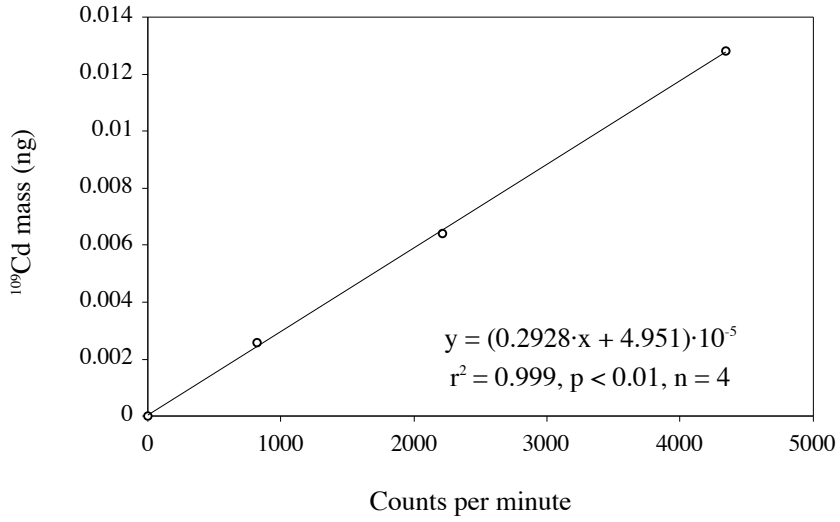

Figure 6.1: Calibration curve produced by measuring the ^{109}Cd activity in a blank (500 μL of distilled water) and three standards with 0.0026, 0.0064 and 0.0128 ng of ^{109}Cd (i.e., activity of 44.7, 110 and 220 Bq, respectively) in 500 μL of distilled water. The corresponding %RSD for blank and standards were 77.1, 2.47, 1.50 and 1.07.

The Cd uptake rate from the dissolved phase (ν, ng g^{-1} h^{-1}) for each barnacle exposed to each tested dissolved Cd concentration was calculated as indicated below:

$$\nu = \frac{^{109}Cd_b\left(C_w + ^{109}C_w\right)}{W_b \cdot ^{109}C_w}, \qquad \text{Equation 6.2}$$

where $^{109}Cd_b$ is the mass of ^{109}Cd in the barnacle's soft tissue (ng), C_w is the natural Cd concentration in the dissolved phase (ng L^{-1}), $^{109}C_w$ is the ^{109}Cd concentration in the dissolved phase at the end of the 24-h exposure (ng L^{-1}), and W_b is the barnacle's soft tissue dry weight (mg). Loss of Cd from the dissolved phase due to the organism's uptake was insignificant for the calculations, since variations <2% in the dissolved ^{109}Cd mass was observed between the beginning and the end of the experiment.

In the 'salinity experiment' a similar procedure as described for the 'Cd concentration experiment' was adopted, except that three 1-L Nalgene bottles were produced with 500 mL of salt water with salinities 27.3, 32.3 and 37.3, each one containing a total Cd concentration of 369 ± 11 ng L^{-1} (mean ± range), including 5 ng of ^{109}Cd (86.37 kBq). Seven organisms were placed into each bottle and kept for 24 hours, under 12:12 h light/dark cycle, at a temperature of 24 ± 1 °C (mean ± range of variation), and constant aeration. The Cd uptake rate was calculated as described in the previous experiment.

6.2.3.2. Cadmium AE from SSPM with different chlorophyll concentrations

The assimilation efficiency of Cd was determined in *Balanus amphitrite* feeding on natural suspended particulate matter (0.45–50 μm, SSPM, as defined in previous chapters) obtained from Ross Creek. The relative abundance of chl *a* in SSPM affects the Cd AE in bivalves (Lee & Luoma 1998, Luoma et al. 1998), and similar behaviour seems to occur for *Balanus*

amphitrite (this study, section 5.4.2). Therefore, water for SSPM collection was sampled at sites B and D (Figure 3.1) in which different phytoplankton abundances in SSPM have been identified during the sampling programs. SSPM was obtained by passing ≈ 4 L of 50-μm pre-filtered water through 0.45-μm Millipore filters and the retained material was resuspended in 500 mL 0.45-μm filtered Ross Creek water.

Each SSPM batch (from sites B and D) were radiolabelled in a 500-mL 0.45-μm filtered Ross Creek water (1-L Nalgene bottle) spiked with 25.5 ng of ^{109}Cd (4.4 kBq), under 14:10 h light/dark cycle provided by a fluorescent light (25 W) and two incandescent lights (75 W each) (a light flux of the order of 100 μE m^{-2} s^{-1}), at a temperature of 27 ± 1 °C (mean ± range of variation), salinity 36.6, and constant stirring. The radiolabelling procedure lasted five days, which allowed uniform radiolabelling of the particles (see, e.g., Wang et al. 1999b, Ke & Wang 2002, Wang & Wong 2003, Rainbow et al. 2004a). During this period, SSPM was daily removed from their medium by filtration (0.45-μm Millipore filter), resuspended in fresh 500-mL 0.45-μm filtered Ross Creek water and spiked with 25.5 ng of ^{109}Cd (4.4 kBq). This procedure was adopted instead of using culture mediums such as f/2 (e.g., Guillard & Ryther 1962, Guillard 1975) to minimise any change in the particles assemblage, since the objective was to work with the natural SSPM that *Balanus amphitrite* found in its environment.

Each batch of radiolabelled SSPM was transferred into new 500-mL 0.45-μm filtered Ross Creek water, before being offered to the barnacles. This procedure was done twice, for each SSPM batch, with 12-h intervals between them. This was done in order to guarantee that most of ^{109}Cd was in SSPM and not in solution. Low ^{109}Cd activities as <40 counts per minute in 500 μL of 0.45-μm filtered medium (i.e., <1 ng ^{109}Cd L^{-1}) were determined for each SSPM

batch just before being offered to the barnacles. The ^{109}Cd activity in SSPM were 12,248 and 10,431 CPM in 6.8 and 5.6 mg, respectively, which correspond to ^{109}Cd concentrations of 5.3 and 5.5 ng g^{-1}, respectively.

The AE of Cd in *Balanus amphitrite* from SSPM was determined using the pulse-chase feeding technique (see, e.g., Wang & Fisher 1999, Wang et al. 1999b). During the 'hot' phase, 28 organisms were placed in two 500-mL plastic chambers, with each chamber receiving radiolabelled SSPM in recirculating system from each SSPM batch (total volume 4 L in each), under light, at a temperature of 24 ± 1 °C (mean ± range of variation), salinity 36.6, and constant aeration. Each SSPM batch presented a SSPM mass concentration of 20.3 and 15.6 mg L^{-1}, and a chl *a* concentration of 9.7 and 9.2 μg L^{-1}, respectively. The organisms were kept starved for 2 days before the 'hot' phase to maximise the feeding activity. After 30–45 min feeding, before egestion of radioactive faeces, individual barnacles were rinsed with non-radiolabelled seawater and their radioactivity counted. The seven most radioactive organisms from each feeding batch were selected to take part in the 'cold' phase.

In the 'cold' phase, the organisms were placed into new plastic chambers receiving water with SSPM (0.45–50 μm) obtained from Ross Creek to promote depuration of the ingested radiolabelled SSPM. The 'cold' phase was run in a flow-through system, under 12:12 h light/dark cycle, at a temperature of 24 ± 1 °C (mean ± range of variation), salinity 36 ± 1 (mean ± range), and constant aeration. 'Cold' phase for both batches lasted 68.2 h, in which the organisms were periodically removed from the depuration chambers, rinsed with non-radiolabelled seawater and their radioactivity counted. In addition, both depuration chambers were periodically rinsed with non-labelled seawater to remove any faeces thus avoiding any re-ingestion of egested labelled material. Counts for water from the depuration chambers

presented an undetectable ^{109}Cd activity, thus guaranteeing no ^{109}Cd uptake from the dissolved phase. It was not possible to identify individual barnacles that had moulted and data for all moulters (<5% of the individuals for all experiments carried out) have been included in analyses.

6.2.3.3. Cadmium efflux rate constant

In the determination of the efflux rate constant, a similar procedure as for the determination of the AE was adopted. At the end of the course of the experiment for the AE determination, the four organisms that exhibited the highest radioactivity in the last counting (i.e., at time 68.2 h) from both SSPM batches were selected to be kept in the depuration chamber allowing further depuration up to 144 h. No moulting was observed at this stage of the experiment.

6.3. Results and Discussion

6.3.1. Field data used as the model's forcing functions

A general description of the Cd concentrations (Figure 6.2) and mass abundances (Figure 6.3) of the components sampled in both sampling periods has already been presented in Chapters 4 and 5. These data are presented here again to show their temporal variation, which were considered in the simulation mode, and to present chl *a* data, which has not been presented before. Chlorophyll *a* at both sites exhibited lower values in the dry season of 2002 than in the wet season of 2004 (Mann Whitney test, $p < 0.01$, $n = 25$ for both sites). In the dry season chl *a* concentrations at site B (1.44 ± 0.82 μg L^{-1}) were significantly lower (Mann Whitney test, $p = 0.02$, $n = 24$) than at site C (2.58 ± 1.68 μg L^{-1}). In the wet season the same pattern

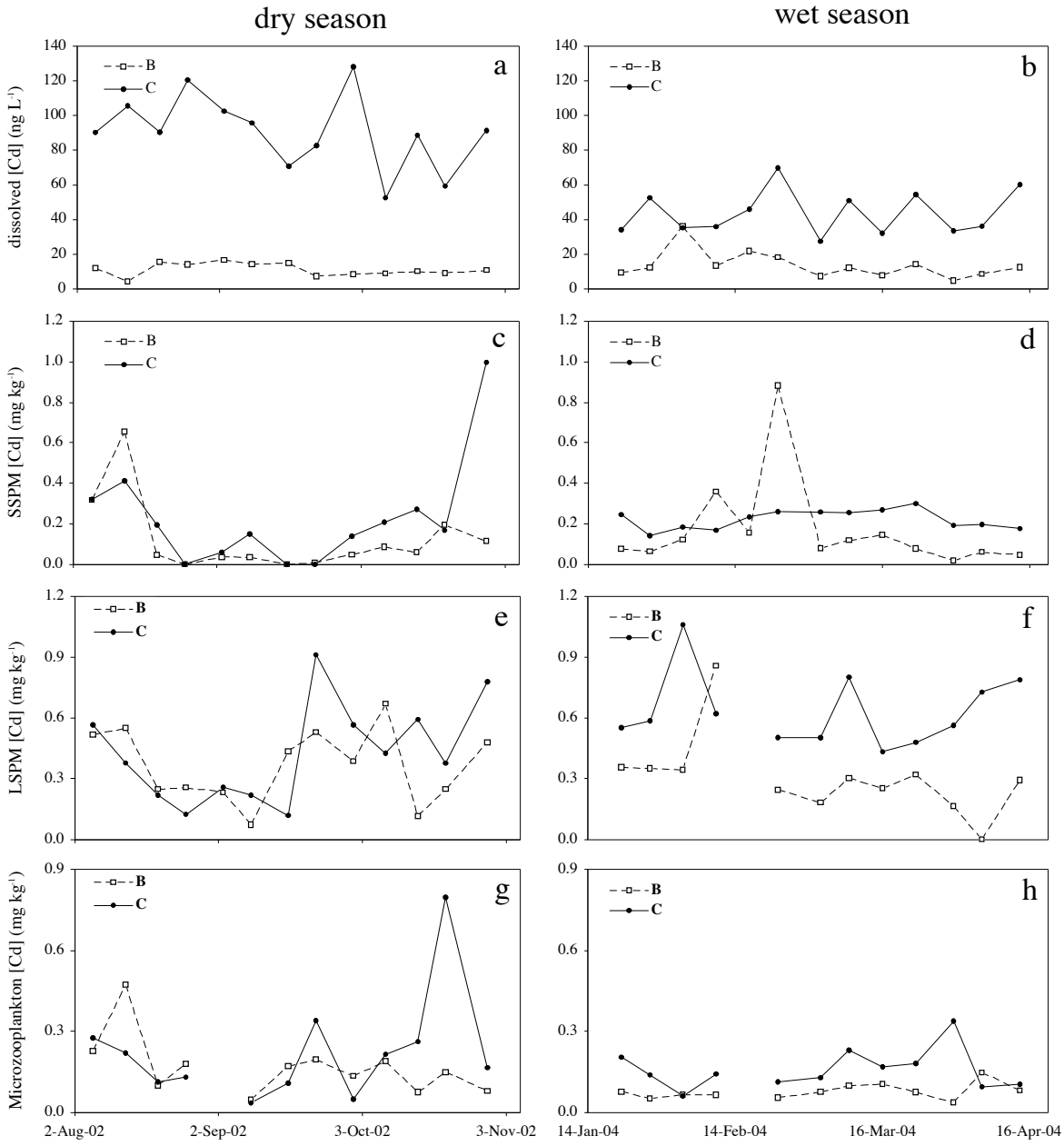

Figure 6.2: Temporal variation of the Cd concentration in the dissolved phase (a and b, <0.45 μm), in ng L^{-1}, and in SSPM (c and d, 0.45–50 μm), LSPM (e and f, 50–200 μm) and microzooplankton (g and h, 50–200 μm), all in mg kg^{-1}, sampled at sites B and C during the dry season of 2002 (graphics on left) and the wet season of 2004 (graphics on right) in Ross Creek.

was observed, chl *a* concentrations at site B (2.69 ± 1.76 μg L^{-1}) were significantly lower (Mann Whitney test, $p < 0.01$, $n = 26$) than at site C (5.94 ± 2.77 μg L^{-1}).

A possible explanation for the higher chl *a* concentrations in the wet season may be the higher temperature observed for this period (Chapter 3). Moreover, because the wet season campaign was carried out in the austral summer while the dry season campaign was in the austral winter, sunlight was also more available in the second sampling campaign. The spatial difference in chl *a* concentrations between sites B and C could not be explained based on the data sampled. No significant pairwise correlation was identified between chl *a* concentrations and nutrients (data in Appendix II) sampled at both sites in both sampling periods, and the Mann Whitney test did not detect differences for nutrients between sites B and C ($p = 0.20$, 0.73, 0.83 and 0.39 for ammonium, nitrite, nitrate and dissolved-P, respectively). However, two aspects may explain the high phytoplankton abundance at sites upstream: (i) a higher primary production in Ross Creek compared to Cleveland Bay associated with a minimal advective transport (specially in the dry season), and (ii) a more intense resuspension facilitated by lower depth at site C compared to B. The higher mass abundance of SSPM at both sites B and C during the low tides than high tides (see section 4.2.1), suggests that resuspension, and consequent input of benthic diatoms into the water column, would be more intense in shallow areas, and site C is shallower than site B. Thus, the higher chl *a* concentration at site C compared to B seems to be a result of the effect of resuspension, primer production and advection working in combination.

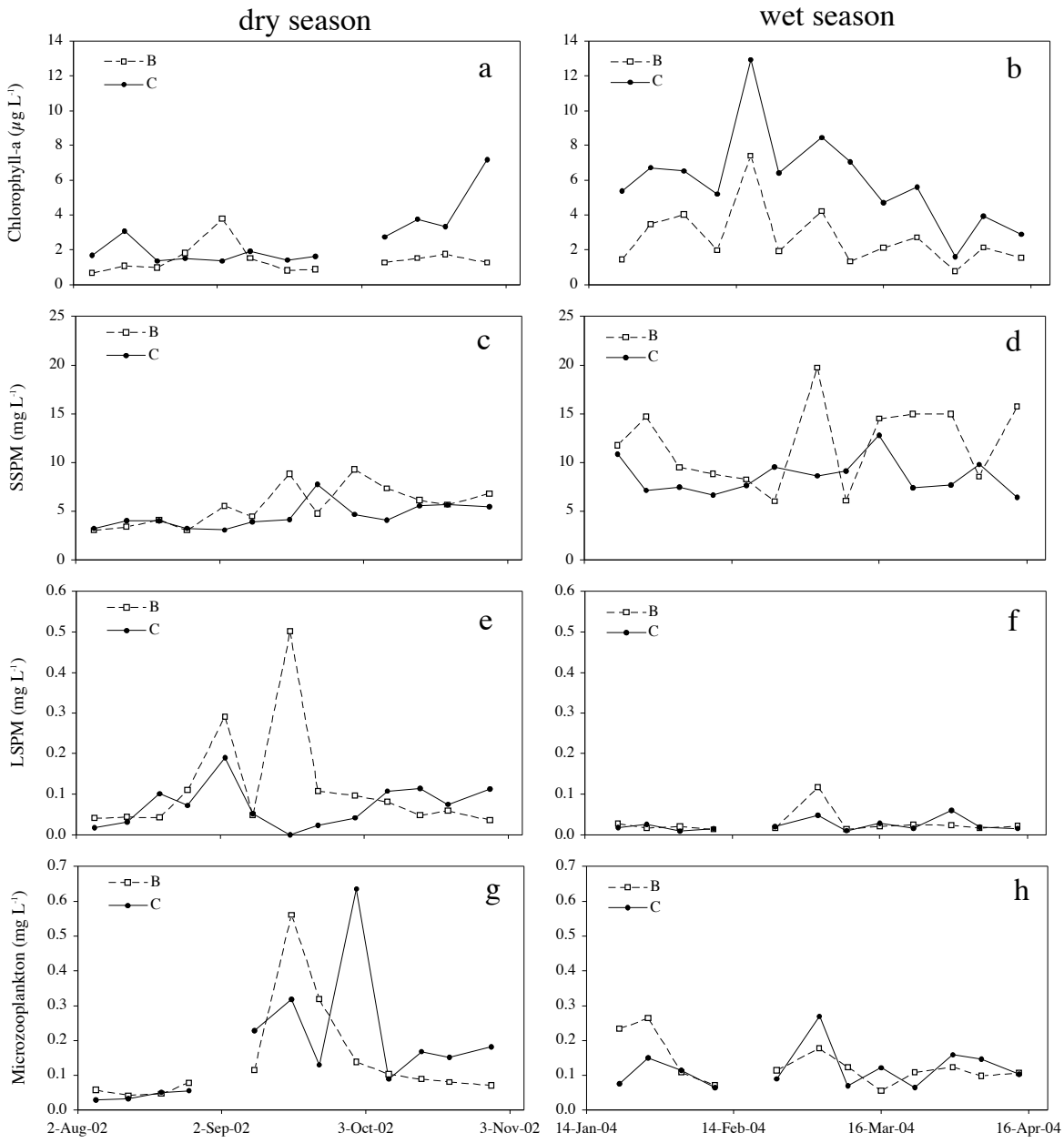

Figure 6.3: Temporal variation of the mass abundance of chl *a* (a and b, in μg L^{-1}), and SSPM (c and d, 0.45–50 μm), LSPM (e and f, 50–200 μm) and microzooplankton (g and h, 50–200 μm), all in mg L^{-1}, sampled at sites B and C during the dry season of 2002 (graphics on left) and the wet season of 2004 (graphics on right) in Ross Creek.

The temporal variation of the Cd concentration in *Balanus amphitrite* sampled at sites B and C in both sampling period, and their individual dry weights were fully presented in the previous chapter (see sections 4.2.4 and 5.3.3). In order to include the temporal variation of the data presented above into the model, as forcing functions, a linear variation between two consecutive sampling days separated approximately by a week was assumed, thus generating one data point for each day for each parameter. For the Cd concentrations in *Balanus amphitrite* such an approach was not applied, because these data are the output of the model.

6.3.2. Radiochemical experiments with ^{109}Cd

6.3.2.1. Cadmium uptake rate constant from the dissolved phase

The Cd uptake rates for 21 individual barnacles were studied at three different dissolved Cd concentrations. The Cd uptake rates from each one of them (ν, ng g^{-1} h^{-1}) from the dissolved phase as a function of different dissolved Cd concentrations (C_w, ng L^{-1}) are presented in Figure 6.4. A linear model has been used to represent variations in ν as a function of the dissolved Cd concentrations (e.g., Rainbow et al. 2003, Rainbow et al. 2004a) and other metals (Luoma & Rainbow 2005); therefore, the best-fit least squares linear regression to describe ν is presented below:

$$\nu = 0.0095 \cdot C_w - 0.746. \qquad \text{Equation 6.3}$$

Based on the fitted curve ($r^2 = 0.40$, $p < 0.01$, $n = 21$), the Cd uptake rate constant from the dissolved phase (k_w) is defined as the slope of this line, i.e., 0.0095 ng g^{-1} h^{-1} per ng L^{-1} or L g^{-1} h^{-1}, as suggested by Rainbow and co-workers (2003, 2004a). Even though there is an uncertainty in the k_w determination (standard error ±0.0027 L g^{-1} h^{-1}), this value is in the range of others presented in the literature. For example, Rainbow and co-workers (2003) determined

a k_w of 0.013 L g^{-1} h^{-1} and in a further study a k_w of 0.0080 L g^{-1} h^{-1} was determined for the Cd uptake rate constant from the dissolved phase for *Balanus amphitrite* (Rainbow et al. 2004a).

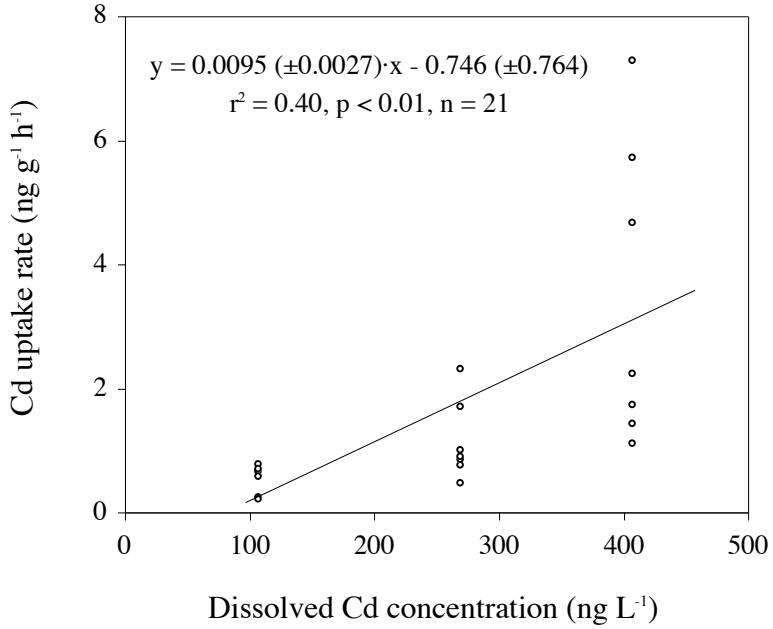

Figure 6.4: Cadmium uptake rates as a function of different dissolved Cd concentrations. Numbers in brackets stand for the standard error of the parameter in the adjusted model.

In the 'salinity experiment', the Cd uptake rates (v, ng g^{-1} h^{-1}) for a dissolved Cd concentration of 369 ± 11 ng L^{-1} (mean ± range) did not vary significantly over the three salinities tested (Figure 6.5). It has been observed for complex organisms such as barnacles, that variations in salinity may affect Cd accumulation in such organisms (Phillips 1980). Salinity acts on chemical speciation changing the metal bioavailability, making them more or less available for uptake from solution (Sunda et al. 1978). For example, Rainbow et al. (2003) found a significantly higher Cd uptake for *Balanus amphitrite* exposed to salinity 15 compared to 33. Similar results were found by Lee and co-workers (1998) for bivalves (*Potamocorbula*

amurensis and *Macoma balthica*) when salinity ranged from 5 to 30, but minor changes were observed in Cd uptake from 20 to 30. For the range of salinities tested in this study from 27.3 to 37.3, which is in the range those *Balanus amphitrite* experiences in Ross Creek, no-significant variation in the Cd uptake rate from solution was identified (linear regression, r^2 = 0.0008 and p = 0.9029).

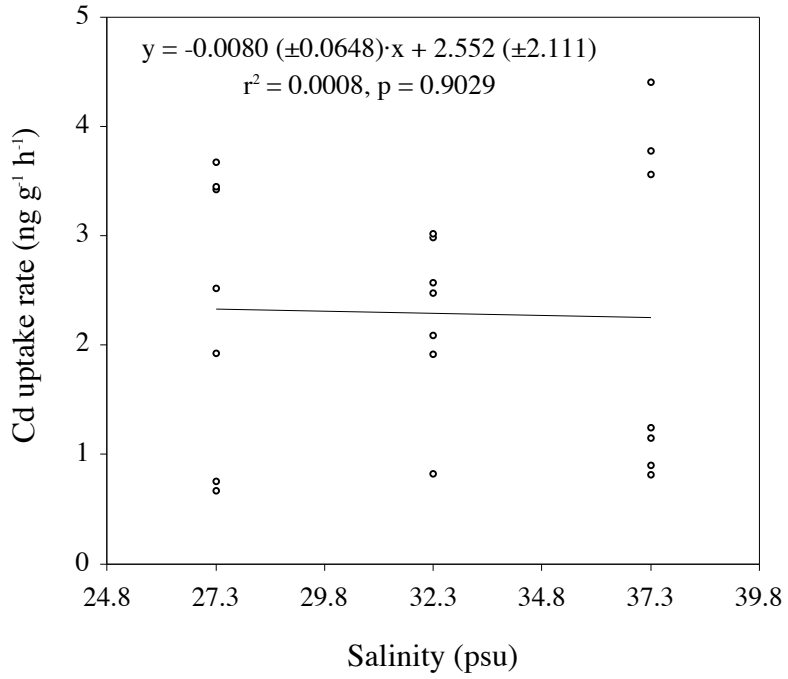

Figure 6.5: Cadmium uptake rates as a function of three different salinities at a dissolved Cd concentration of 369 ± 11 ng L^{-1} (mean ± range). Numbers in brackets stand for the standard error of the parameter in the adjusted model.

The organism's size and its filtration rate, which is dependent on size (see Figure 5.3), have been shown to affect the metal uptake rate constant from the dissolved phase (e.g., Wang & Fisher 1997, Lee et al. 1998). The Cd uptake rate from the dissolved phase is the rate of metal uptake from solution per unit of body weight of barnacle per unit concentration of dissolved metal. Therefore, each Cd uptake rate obtained for each organism can be compared after

allowance for the dissolved metal concentration (Rainbow et al. 2003). Accordingly, a comparison between uptake rates and individual barnacles' dry weight was done to test whether the barnacles' size affects its Cd uptake rate from the dissolved phase. The results are presented graphically in Figure 6.6.

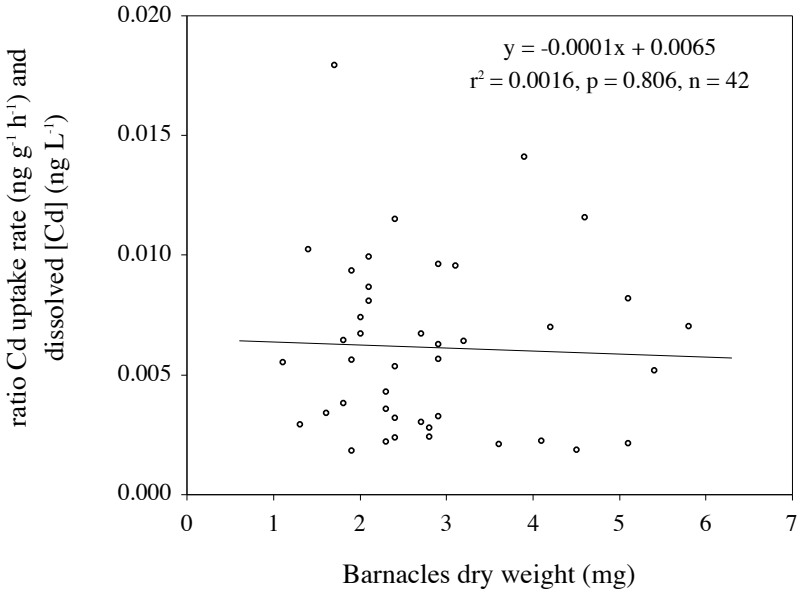

Figure 6.6: Dependence of the individual barnacles' dry weight on the ratio between the Cd uptake rate from the dissolved phase and the corresponding dissolved Cd concentration.

In this comparison, the Cd uptake rates and corresponding individuals barnacles' dry weights obtained from both the 'Cd concentration experiment' and the 'salinity experiment' were considered. The low r-squared and non-significant linear regression between individual barnacles' dry weight and Cd uptake rates divided by the corresponding dissolved Cd concentration ($r^2 = 0.0016$ and $p = 0.806$, Figure 6.6), indicate that the barnacles' size does not affect the Cd uptake rate constant from the dissolved phase. Thus, the Cd uptake rate constant

determined in this study can be used to reproduce the metal uptake rate from solution for a wider size range of barnacles, ranging from 1 up to 6 mg dry weight.

6.3.2.2. Cadmium AE from SSPM with different chlorophyll concentrations

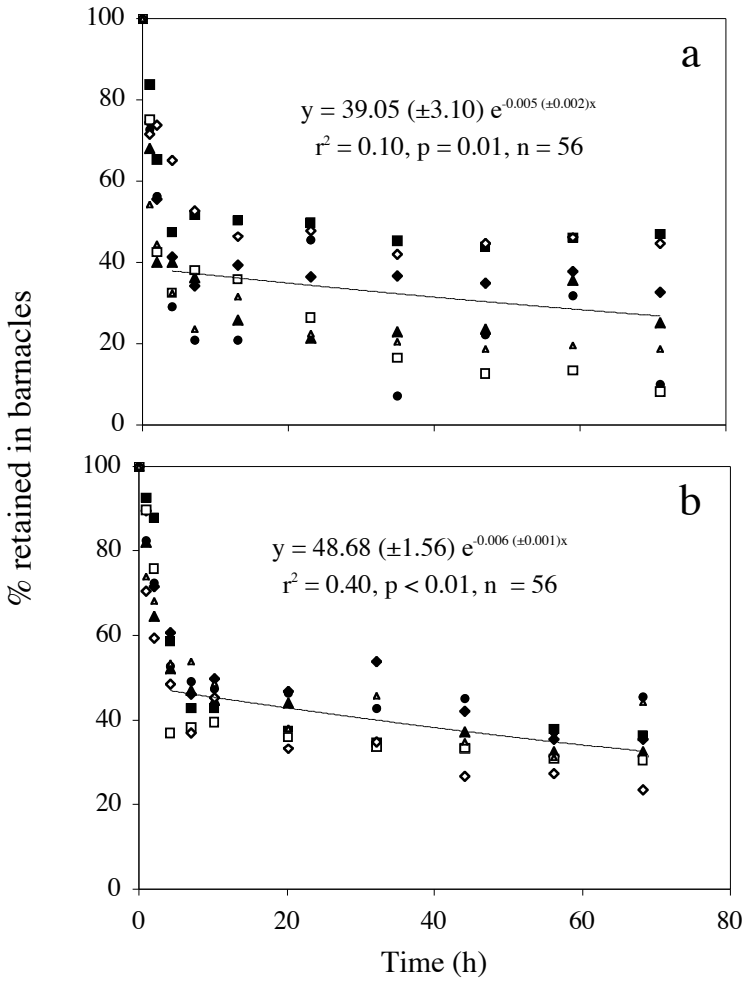

Figure 6.7: Retention of ^{109}Cd in 14 barnacles after seven of them been fed with SSPM poor in chl *a* (a) and the other seven been fed with SSPM rich in chl *a* (b). Numbers in brackets stand for the standard error of the parameter in the adjusted model. Different symbols stand for each of the seven barnacles fed with each batch of SSPM.

For the determination of the Cd AE from SSPM for *Balanus amphitrite* two batches of SSPM with low and high chl *a* concentrations were used. For both SSPM treatments, a bi-phasic depuration pattern, including an initial rapid loss of ingested ^{109}Cd labelled SSPM within the first 4 h and then a slower loss up to 68.2 h, was observed (Figure 6.7). Considering separately the depuration for each SSPM batch, two Cd AE from SSPM, i.e., 39.0% when SSPM was poor in chl *a* (Figure 6.7a) and 48.7% when SSPM was rich in chl *a* (Figure 6.7b), were determined. The AEs were obtained by adjusting an exponential decay model to the second stage of depuration (Wang & Fisher 1999), i.e., from the 4th ^{109}Cd assay, i.e., 4 h after depuration has started. The AE determined in this study are within the range of other studies of *Balanus amphitrite* feeding on different types of particles (Table 6.1).

The ratio between chl *a* mass concentration (μg L^{-1}) and SSPM mass abundance (mg L^{-1}) used in the AE experiments were calculated for both SSPM batches and compared to the AE determined in the experiments. The lowest chl *a*:SSPM ratio (ca. 0.46) correlated with the lowest Cd AE (i.e., 39.0%), and the highest chl *a*:SSPM ratio (ca. 0.59) correlated with the highest Cd AE (i.e., 48.7%). Even though there is an uncertainty in both AE determinations (standard error ±3.10 and ±1.56% for SSPM poor and rich in chl *a*, respectively), and the difference between the chl *a*:SSPM ratios between both SSPM batch is ≈ 20%, a general tendency of higher AE was observed when SSPM is enriched with chl *a*. A linear regression analysis between AE and the chl *a*:SSPM ratio described the variation in Cd AE from SSPM for *Balanus amphitrite* as a function of chl *a* abundances in SSPM, as indicated below:

$$y = 77.535 \cdot x + 3.031, \qquad \text{Equation 6.4}$$

where y is the Cd AE from SSPM for *Balanus amphitrite* and x is the ratio between chl *a* abundance (μg L^{-1}) and SSPM mass abundance (mg L^{-1}).

Table 6.1: The Cd AE from different food types for *Balanus amphitrite*.

Food type	Time interval for AE determination	Mean AE ± 1 SD (%)	AE range (%)	Source
Phytolankton				
Thalassiosira weissflogii	24 h[a]	35.2 ± 5.8	26.8–41.5	Rainbow and co-workers (2003)
Thalassiosira weissflogii	22 h[a]	59.6 ± 10.1	40.2–69.9	Rainbow and co-workers (2004a)
Chaetoceros muelleri	18–68 h[b]	34.8 ± 5.1		Wang and co-workers (1999b)
Skeletonema costatum	18–68 h[b]	86.2 ± 8.2		Wang and co-workers (1999b)
Zooplankton				
Artemia salina larvae	18–68 h[b]	63.4 ± 9.0	52.6–71.5	Wang and co-workers (1999b)
Canthocalanus pauper	18–68 h[b]	71.6 ± 17.4		Wang and co-workers (1999b)
Temora turbinata	18–68 h[b]	87.9 ± 8.9		Wang and co-workers (1999b)

[a] Activity remaining in the organism after a specific time had elapsed, compared 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.

Similar patterns have been identified in bivalves that exhibited higher metal AE when suspended particles are enriched with phytoplankton (e.g., Lee & Luoma 1998, Ke & Wang 2002, Wang & Wong 2003). Moreover, mean chl *a* concentrations and SSPM abundances measured at sites B and C at both sampling periods and the corresponding Cd AE from SSPM determined with a steady-state model (see section 5.2.1) indicated that the highest phytoplankton abundance in SSPM at site C resulted in a higher Cd AE from SSPM for barnacles when compared to site B. Thus, the previous cited works and the steady-state model's results compared to field observations support the general pattern of an increase in

AE as chl *a* abundance in SSPM increases. The same trends were found in Equation 6.4 and the plotting of the Cd AE from SSPM resulted from the steady-state model and its corresponding chl *a*:SSPM ratio observed in the field (Figure 6.8), which reinforces that the higher the chl *a*:SSPM ratio, the higher the Cd AE from SSPM.

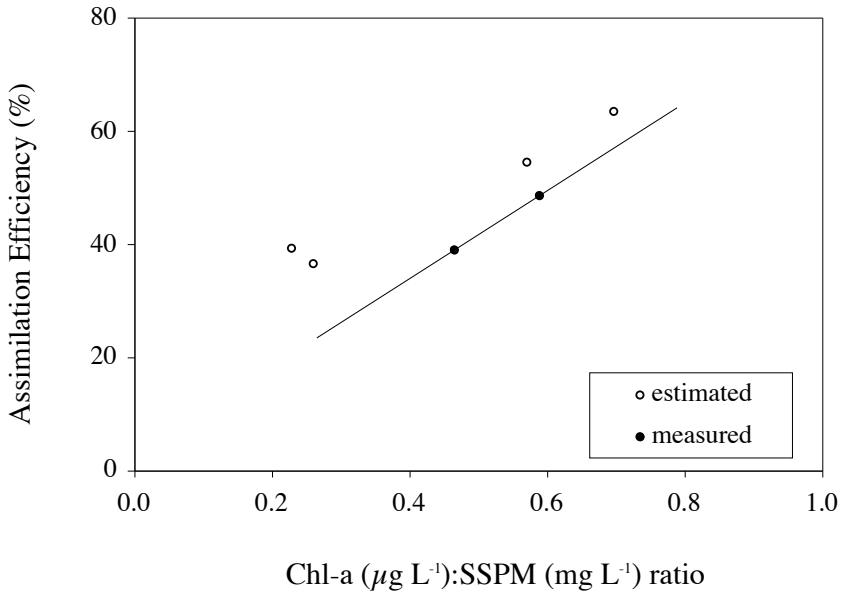

Figure 6.8: The Cd AE from SSPM for *Balanus amphitrite* as a function of the ratio between chl *a* abundance (μg L^{-1}) and SSPM mass abundance (mg L^{-1}) obtained from two independent measurements: 'estimated' from the steady-state model and field data, and 'measured' in the laboratory with ^{109}Cd and pulse chase feeding technique. See text for explanation.

As pointed out by Lee and Luoma (1998), the radiolabelling can be critical in the interpretation of pulse-chase feeding experiments using mixed-particles assemblage, because a short equilibrium time may result in preferential partitioning of isotopes to easily exchangeable pools. The similarity between the AE determined in the radiochemical experiments and those suggested by the steady-state model, presented in section 5.2.1,

indicates that a five-day labelling was enough time to produce Cd-labelled particles that behaved similarly to those found in the environment.

6.3.2.3. Cadmium efflux rate constant

The Cd efflux rate constant was determined by applying an exponential decay model to the temporal variation of the percentage of remained radioactivity in each organism, considering data from the 22nd hour (Figure 6.9), which is the time when all labelled food has passed through the gut of *Balanus amphitrite* (Rainbow et al. 2004a). The efflux rate constant (0.0072 d^{-1}) was obtained by multiplying the *x*-coefficient by 24, thus transforming the *x*-coefficient from an hour basis to daily basis. Even though there is an uncertainty in the determination of the *x*-coefficient (standard error ±0.000625), and consequently on the Cd efflux rate constant, the value presented in this study agrees with efflux rates constant reported in the literature for *Balanus amphitrite* (Wang et al. 1999b, Rainbow et al. 2003), except for an unusual high one presented in Rainbow and co-workers (2004a). The low efflux rate constant determined in the present study confirms that *Balanus amphitrite* has potential as a biomonitor for Cd, since most of the metal that is assimilated in its tissue remains in it.

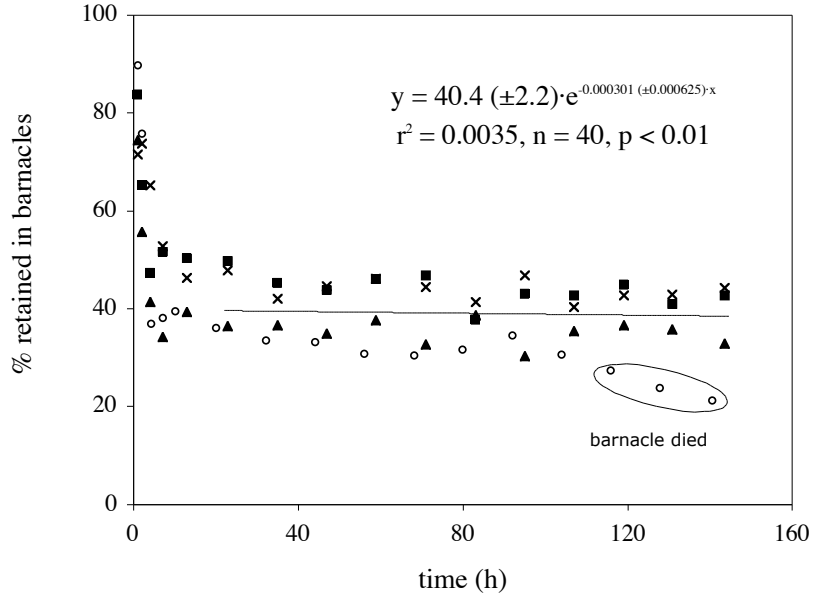

Figure 6.9: Retention of ^{109}Cd in the four most ^{109}Cd active barnacles after they have been used in the experiment for the AE determination. Numbers in brackets stand for the standard error of the parameter in the adjusted model. Different symbols stand for each of the four barnacles. Marked white circles were not considered in the analysis because the barnacle was dead.

6.3.3. Summary of the model's parameters and baseline simulations

In order to reproduce the daily temporal variation of the Cd mass in a barnacle (Cd_b, ng), Equation 5.2 was written in a differential form as:

$$dCd_{b(t)} = Cd_{b_{(t-dt)}} + \left[k_w C_w TW_b + \sum_{f=1}^{3} \left(MI_f C_f AE_f \right) - Cd_b (g + k_e) \right] \cdot dt , \qquad \text{Equation 6.5}$$

where parameters are as defined in Equation 5.2, except MI_f (mg) that represents the mass ingested of each food item per day (f = 1 to 3, for SSPM, LSPM and microzooplankton, respectively), and t is time in days. MI_f calculations take into account two model assumptions

that have been adopted in the steady-state model presented in section 5.2.3: (i) no feed preference, i.e., barnacles ingest each food item in proportion to that in the environment, and (ii) a maximum food ingestion of 40% of the barnacles' individual dry weight per day (Wang et al. 1999a, Wang et al. 1999b). Thus, MI_f was defined as:

$$MI_f = M_f \cdot CR_b \cdot T \cdot MI_{40\%}, \qquad \text{Equation 6.6}$$

where $MI_{40\%}$ (unitless) is a logic function that defines a limit to the ingested food based on a maximum food ingestion rate of 40% of the barnacle soft tissue dry weight. $MI_{40\%}$ is equal to 1 if $\frac{MT}{W_b}$ is smaller then 0.4, otherwise $MI_{40\%}$ is equal to $0.4 \cdot \frac{W_b}{MT}$, and MT (mg) is the total amount of food that could potentially be ingested by a barnacle per day if there was not the $MI_{40\%}$ control, and it is calculated as $CR \cdot T \cdot \sum_{f=1}^{3} M_f$. Table 6.2 summarises all parameters used in the simulation model. The Stella computational code of the simulated model is presented in Appendixes IV.

For the radiochemical experiments, barnacles were collected in Ross Creek from a location situated halfway between sites B and C. This was done to minimize any differences in the determination of the parameters in the laboratory as a function of previous dissolved and particulate Cd field-exposure history. However, in recent studies Rainbow and co-workers (2003, 2004a) observed that Cd AE from food and Cd uptake rate constant from solution were not affected by *Balanus amphitrite* pre-exposure to food or to solutions with high Cd concentrations. Thus, both parameters determined in this study can be used to represent the Cd uptake rate constant from the dissolved phase and Cd AE from SSPM for *Balanus amphitrite* living at both sites B and C, even though these sites present distinct dissolved and particulate Cd concentrations.

Table 6.2: Simulation model's parameters, their description/equation, units and the source of information.

Parameter	*Description/Equation (unit)*	*Source*
Cd_b	Mass of Cd in the soft tissue of a barnacle: Initial values: 10.7[a], 22.6[b], 5.8[c] and 18.4[d] (all in ng)	Field data
k_w	Cd uptake rate constant from solution: 0.0095 (L g^{-1} h^{-1})	Laboratory experiment
C_w	Dissolved Cd concentration: Figure 6.2a,b (ng L^{-1})	Field data
T	Immersion time: Calculated based on the water level presented in Appendix II (h day^{-1})	Field data
W_b	Barnacle soft tissue dry weight: Appendix II (mg)	Field data
MI_f	Ingested mass of each food item: Equation 6.6 (mg)	This chapter
M_f	Mass abundances of each food item: Figure 6.3c-h (mg L^{-1})	Field data
CR_b	Clearance rate: $\frac{4.0435\times10^{-2}}{W}+0.6658\times10^{-2}$ (L mg^{-1} h^{-1})	Laboratory experiment
$MI_{40\%}$	Maximum food ingestion rate control: $MI_{40\%}=\text{if } \frac{MT}{W_b}<0.4 \text{ then 1 else } 0.4\cdot\frac{W_b}{MT}$	Wang et al. (1999a)
C_f	Cd concentrations in each food type Figure 6.2c-h (mg kg^{-1})	Field data
AE_1	Cd AE from SSPM: $77.535\cdot\frac{\text{chl } a}{M_1}+3.031$	Laboratory experiment
chl *a*	Mass concentration of chl *a*: Figure 6.3a,b (μg L^{-1})	Field data
AE_3	Cd AE from microzooplankton: Assumed as 80%	Wang and Fisher (1998) and Wang et al. (1999b)
AE_2	Cd AE from LSPM: As for microzooplankton (i.e., 80%). LSPM is mainly composed of dead microzooplankton.	Observational data
g	Dry weight-based growth rate: $\frac{-1.285\times10^{-2}+5.210\times10^{-2}W^{-1.110}}{4.354+W^{-1.110}}$ (d^{-1})	Field data
k_e	Efflux rate constant: 0.0072 (d^{-1})	Laboratory experiment

[a] Barnacles sampled at site B on 13 August 2002.
[b] Barnacles sampled at site C on 6 August 2002.
[c] Barnacles sampled at site B on 21 January 2004.
[d] Barnacles sampled at site C on 21 January 2004.

Figure 6.10 presents the temporal variation of the Cd concentrations in *Balanus amphitrite* comparing the values obtained from the simulation model to the values obtained in the field at sites B and C during the dry season of 2002 and wet season 2004. The first value of the Cd concentration in *Balanus amphitrite* collected at site B in the dry season of 2002 was excluded from the simulation. Previous simulations indicated that this Cd concentration is an outlier. When this value is included in the simulation, it produces exactly the same simulated pattern for the Cd concentration in the barnacles as seen in Figure 6.10a, but displaced upwards. Because all the other series simulated were in the same range of Cd concentrations observed in the field, and if that value is not included in the simulation, the results fall in the

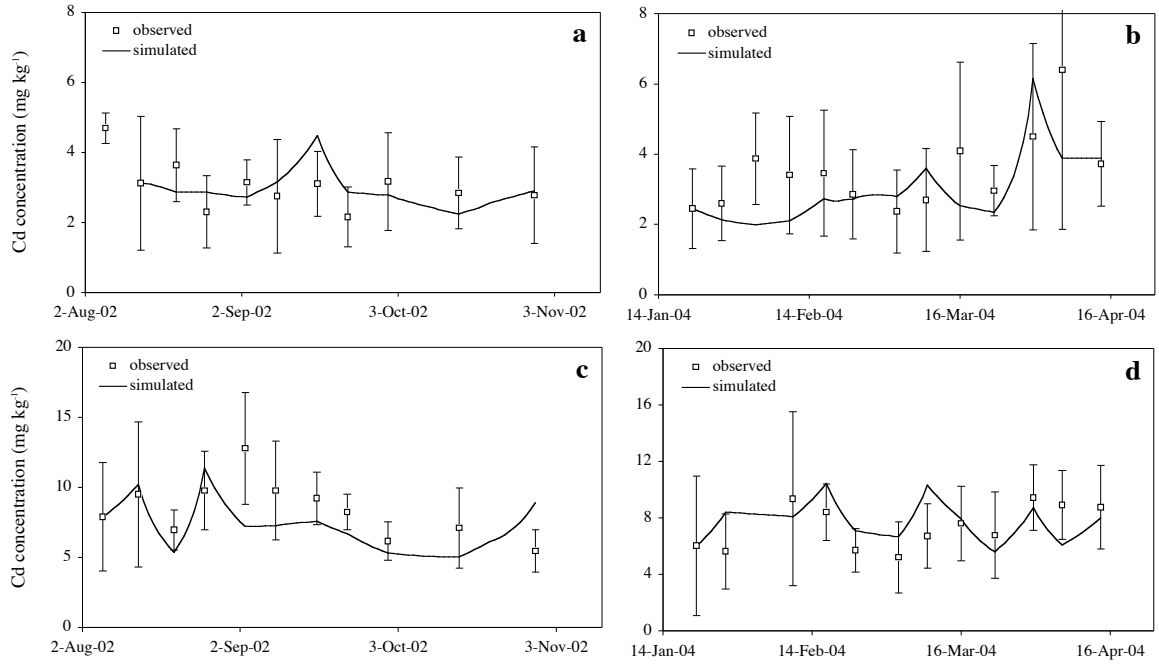

Figure 6.10: Model's baseline simulation. Comparison between Cd concentrations in *Balanus amphitrite* simulated (line) and observed in the field (white square) at site B in the dry season of 2002 (a) and wet season 2004 (b), and at site C in the dry season of 2002 (c) and wet season 2004 (d). Error bars stand for ±1 SD.

same range of the data observed in the field (as shown in Figure 6.10a), the Cd concentration in the barnacles obtained at site B on 6 August 2002 was not included in the simulation.

The model reproduced the general pattern of temporal variation of the Cd concentrations observed in the field, and reproduced the magnitude of the values observed at both sites in both seasons. The model also reproduced some short-term variations in the Cd concentrations observed in the field, especially at site C in both sampling periods (Figure 6.10c,d). Even though the model underestimated the observational data in the dry season 2002 and overestimated in the wet season 2004, the general trend of temporal variation of the Cd concentration in the barnacles was reproduced. Regarding site B, the model reproduced the increase in Cd concentrations observed in the field at the end of the wet season (Figure 6.10b) and also reproduced the low temporal variation in the dry season, as observed in the field (Figure 6.10a). The lack of temporal variability in the Cd concentration in the barnacles at site B (dry season) was confirmed by the Kruskal-Wallis test ($p = 0.07$, section 4.2.4) whereas for all the other series a significant temporal variation was identified ($p = 0.01$, 0.03 and 0.04 for sites C, dry season, B wet season and C wet season, respectively, sections 4.2.4 and 5.3.3).

The model validation based on the t-test and model efficiency (*EF*) is presented on Table 6.3. The p-values >0.05 from the t-tests suggest that the simulated Cd concentrations in *Balanus amphitrite* were in the same range as those observed in the field, showing the model reproduced the low and high patterns of Cd concentrations at sites B and C, respectively, in both sampling periods. Conversely, *EF* presented negative values for all the simulated series, suggesting a bad fit between simulated and observed data. Even though significant temporal variations were identified for three of the field data series (as seen in the Kruskal-Wallis test results referred before), the set of data in each series did not vary much from its average. This

resulted in a low variance for the field data, which makes *EF* very sensitive to any slight difference between each simulated and observed pair, resulting in negative values. In summary, the results of both tests combined suggest that the model predicted the general trend of Cd concentrations in *Balanus amphitrite* in the field, distinguishing high and low patterns of Cd concentrations in *Balanus amphitrite*, but it could not predict their short-term temporal variations accurately.

Table 6.3: Model validation. Paired t-test (p-value) and the model efficiency coefficient, *EF* (Mayer & Butler 1993) comparing model results and field data of Cd concentrations in *Balanus amphitrite*. The number of pair compared (n) is also represented.

Series simulated	p-value	*EF*	n
Site B, dry season 2002	0.66	-1.43	10
Site C, dry season 2002	0.23	-0.53	11
Site B, wet season 2004	0.17	-0.31	13
Site C, wet season 2004	0.46	-0.54	12

6.3.4. Model investigation

In order to investigate what caused the model failure in reproducing short-term temporal variations of the Cd concentration in *Balanus amphitrite* in the field, a simulation exercise was performed. In this exercise, the forcing function 'individual barnacle's dry weight', which was included in the model as the data obtained in the field (W_b presented in Equation 6.5),

was replaced by a state variable to reproduce the temporal variation of the soft tissue dry weight of an individual barnacle. To do this, the dry weight-based growth rate determined in the field (g in d^{-1}, Table 6.2) was used to control the temporal variation of this individual barnacle's weight, as indicated below:

$$dW_{b_{(t)}} = W_{b_{(t-dt)}} + g \cdot dt . \qquad \text{Equation 6.7}$$

The variable barnacle's soft tissue dry weight ($W_{b_{(t)}}$, mg) was used in the model replacing all W_b in Equation 6.5. Dilution as a function of the growth rate was made inactive in this model version. This was done because the variations in the barnacle's weight, which are now represented in the model, already incorporate the dilution effect when the variable barnacle's weight is used to determine the barnacle Cd concentration (see model diagram in Appendix V). The initial barnacle's weight used to simulate each series of Cd concentrations in *Balanus amphitrite* was that obtained in the field on the first sampling day for each sampling period at each site (data in Appendix II). An exception occurred for the barnacles sampled at site B in the dry season 2002. Because the data for the first sampling day for that series was considered an outlier (as discussed before), the data for the second sampling day was used instead.

The barnacle's weight was selected for the simulation exercise because of three major factors: (i) barnacle's weight had been identified as one of the five most important parameters controlling Cd accumulation in *Balanus amphitrite* in the steady-state model presented in section 5.3.7; (ii) when Cd concentration in barnacles is determined from the field data, initially the mass of Cd in the sampled barnacles is determined and then the Cd concentration is obtained by dividing this Cd mass by the weight of the sampled organisms, and (iii) including barnacle's weight as a state variable permits simulation of Cd concentrations in what could be called as 'ideal biomonitor', i.e., an organism that could have its Cd

concentration determined over the time without killing it. The simulation results are in Figure 6.11.

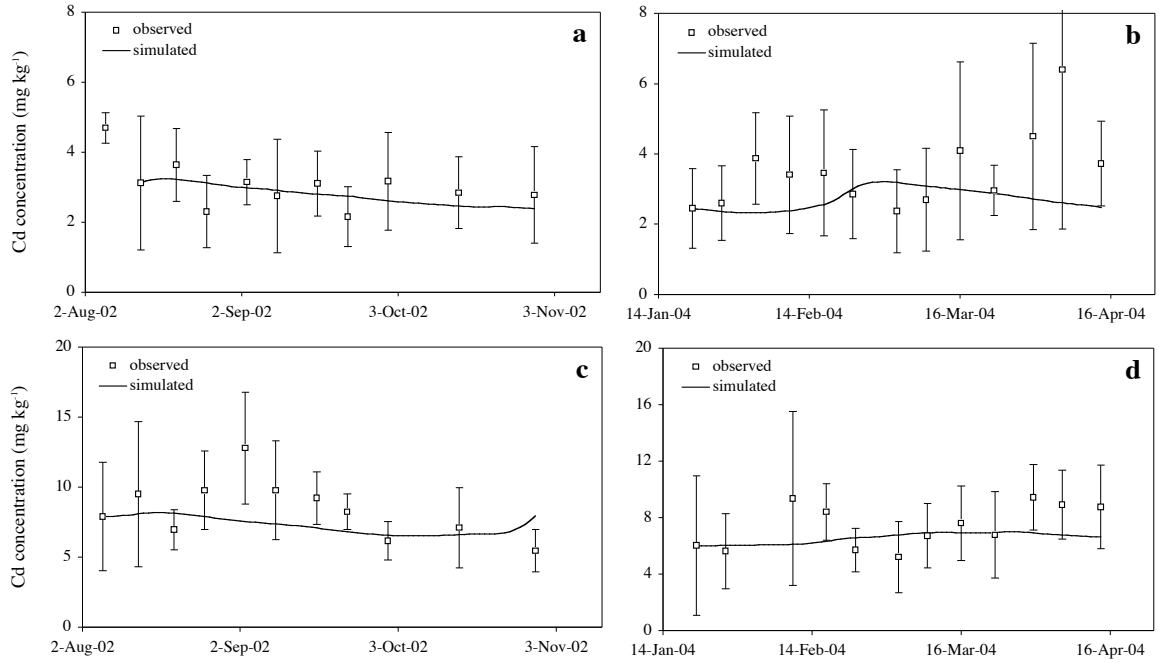

Figure 6.11: Model's results of the simulation exercise in which barnacles' weight was considered as a state variable in the model. Comparison between Cd concentrations in *Balanus amphitrite* simulated (line) and observed in the field (white square) at site B in the dry season of 2002 (a) and wet season 2004 (b), and at site C in the dry season of 2002 (c) and wet season 2004 (d). Error bars stand for ±1 SD.

When the forcing function 'barnacle weight' (W_b) was replaced by a state variable 'barnacle weight' ($W_{b_{(t)}}$,Equation 6.7), the temporal variation of the Cd concentration in the barnacle became independent of the sample size. As can be seen from Figure 6.11, Cd concentrations in a barnacle, over the three-month simulation time, exhibited a more constant temporal pattern of variation than observed in the field data at both sites and periods. It is not a surprise

that Cd concentrations in *Balanus amphitrite* do not vary much over three-month period. Metal concentrations in organisms represent a time-average integration of the bioavailable

Several factors may imply specific patterns of temporal variation in Cd concentrations in organisms, such as metal speciation (Morgan & Stumm 1991, Bourg 1995), variations in the composition and relative abundance of the food items ingested by the biomonitor (Wang et al. 1999a, Wang et al. 1999b, Wang & Rainbow 2000, Beeby 2001, Ke & Wang 2002), differences in the organism's growth rate and efflux rate constant (Wang et al. 1999a, Wang et al. 1999b), duration and/or level of exposure to the metal and population age/size (Rainbow & Smith 1992, Beeby 2001, Blackmore & Rainbow 2001). Most of these factors have been considered in the simulation model developed in this study, even though the simulation exercise's results presented a constant temporal pattern for the Cd concentration in the barnacles for all sites and periods. Only changes in the metal availability in the dissolved phase, as a function of metal speciation, and potential variations in the LSPM and microzooplankton composition, which may affect the Cd AE from these sources, were not considered in the model. However, these factors have been shown to be of minor importance controlling Cd accumulation in *Balanus amphitrite* as seen in the sensitivity analysis presented in section 5.3.7.

Regarding variations in Cd concentrations as a function of size differences within the sampled organism, it is worth mentioning that no size effect was observed for the barnacles sampled in the field (see sections 4.2.4 and 5.3.3). Therefore, assessing Cd concentrations in the barnacles from the field no allowance was made for differences in their mean individual weights. The lack of size effect was also confirmed by the results of a simulation exercise presented in Section 5.4.4. This exercise indicated that *Balanus amphitrite* growth rates

<0.003 d^{-1}, which are in the range of those determined in the field in this study, do not result in size effect.

It might be argued that the temporal variability that is observed in this study reflects an inadequate sampling intensity. However, the percentage error of the 95% upper limit of confidence compared to the mean for the determination of the Cd concentrations in *Balanus amphitrite* in the field (i.e., [mean - CL95%up] / mean × 100) obtained in this work (ca. 40%) was lower than values presented elsewhere (e.g., Rainbow & Smith 1992, Rainbow et al. 1993, Blackmore et al. 1998, Blackmore & Rainbow 2001). This suggests that the sampling effort used in this study is comparable to previous studies of Cd concentrations in *Balanus amphitrite*.

The results of the simulation exercise (Figure 6.11) suggest that the temporal variability observed in the field data may be an artefact of variations in the mean weight of the sampled barnacles, which may mask the real pattern of temporal variation of the barnacles Cd concentration in the field. However, the reason for this remains unknown, especially since no size effect was detectable for the field data (see sections 4.2.4 and 5.3.3). This fact may cause problems in any interpretation of temporal pattern of Cd concentrations variation in the environment, when using *Balanus amphitrite* as a biomonitor. For example, as observed for barnacles sampled in Ross Creek, three of the four series of Cd determinations in the barnacles indicated a significant temporal variation; whereas the model suggested that a such temporal variation was not real.

6.3.5. Exploring the potential for *Balanus amphitrite* as a biomonitor for Cd environmental contamination in a simulation model

Two simulation exercises were performed: (i) to test the efficiency of *Balanus amphitrite* in responding to changes in Cd contamination in the environment, and (ii) to determine the time lag for the organism to respond to changes in Cd contamination in its sources. In the first exercise, a six-month dredging operation scenario was simulated. In dredging operations, bottom sediment is resuspended and they usually represent an input of contaminants to the dissolved and particulate phases (Caetano et al. 2003, Audry et al. 2004). Thus, in this exercise, an increase in the SSPM Cd concentration was simulated. No alteration was applied to the mass abundance of SSPM because the sensitivity analysis performed in the steady-state model (section 5.3.7) indicated that it is of minor importance to the Cd accumulation in *Balanus amphitrite*. The aim was to test if *Balanus amphitrite* would register an increase in Cd availability in a component that this study identified as the major source of Cd to *Balanus amphitrite* (i.e., SSPM, see section 5.4.2). Even though the dissolved Cd concentration may increase more than the particulate Cd concentration as a result of a dredging operation (Caetano et al. 2003), this simulation exercise focused on the SSPM impact since the dissolved phase represents a minor source of Cd to *Balanus amphitrite* (see section 5.3.6).

In the second simulation exercise, a relocation experiment was reproduced. In relocation experiments, organisms are moved from a low-level contaminated location (or from culture) to a high-level one or *vice versa*, and changes in the contaminant concentration in the organisms are assayed. This facilitates identification, for example, of the time lag for an organism to respond to changes in metal concentrations in the environment (see, e.g., Klumpp & Burdon-Jones 1982, Roesijadi et al. 1984, Regoli & Orlando 1994). Even though the extent

to which a biomonitor integrates the spatial and temporal variations in pollutant levels is essential to quantify its exposure, it is rarely measured (Beeby 2001). Thus, the aim of this second simulation exercise was to estimate what time period of Cd bioavailability is reflected in the integrated accumulated Cd concentration of *Balanus amphitrite*.

In both simulation exercises, the model presented in section 6.3.4, which simulates the daily variations of the Cd concentration in a single barnacle, was used. This model was chosen because it reproduces the case of an 'ideal' biomonitor, and thus any bias as a function of changes in the organism's size, as discussed in section 6.3.4, could be avoided in the interpretation of the results of these simulation exercises. Three consecutive years (i.e., a period of 1095 days) were simulated in each exercise to allow the Cd concentration in the organism to approach equilibrium after the model perturbation (i.e., after the dredging or relocation operations)[8]. Because no significant difference was identified for the majority of the data sampled in Ross Creek (see sections 4.2.1, 4.2.2, 5.3.1 and 5.3.2), which are used as the model's forcing functions, they were included in the simulation exercises as mean values calculated for each sampling site (B and C), taking into account both sampling periods (dry and wet seasons).

6.3.5.1. Modelling effects of localized dredging operation on Cd accumulation in Balanus amphitrite

To reproduce the dredging operation scenario in the model, a 180-day pulse of increased Cd concentration in SSPM was simulated starting on Julian Day 90. The model was set up to

[8] *Balanus amphitrite* presents a maximal longevity of 5.5 years (Calcagno et al. 1997, 1998).

reproduce the environmental conditions at site B, which is the closest barnacle-sampling site to the Townsville Harbour, where a dredging operation would be more likely to happen. The simulated Cd pulse in SSPM was generated by multiplying the mean Cd concentration in SSPM by 2.8 for the period correspondent to the dredging operation. This resulted in a realistic increase in the SSPM Cd concentration equal to two times the standard deviation of the mean Cd concentration in SSPM measured at site B. Increases in the particulate Cd concentration of 2–3 fold as a function of dredging operations have been observed elsewhere (Caetano et al. 2003). As initial conditions for this simulation, an adult barnacle of 4 mg dry weight soft tissue, which has stopped growing in Ross Creek (see Figure 5.4), with an initial Cd tissue concentration of 1.81 mg kg^{-1} were assumed. Thus, the simulations started from steady-state conditions, making it easy to identify any effect that the increased Cd concentration in SSPM would have to the Cd concentration in the barnacle. All the other model's parameters were kept as in the standard simulation. The result of this simulation exercise is presented in Figure 6.12.

An increase in the Cd concentration in the barnacle's tissue up to 1.91 mg kg^{-1} above the background will occur resulting from the 2.8 fold increase in the Cd concentration in SSPM for a time-period of 180 days. In order to verify if such an increase in the Cd concentration in the barnacle would be detected in a sampling program, a sample size and power test to compare two sample means was performed. This test estimates, for example, if the difference between two group means is significant at a specific α level, based on the number of samples and the within-group variance. Alternatively, it can be also used to determine how powerful the test would be to detect the difference between the means, i.e., what would be the probability that for a fixed α level the test will reject H_o when a particular alternative value of the parameter is true (Frank & Althoen 1994, Moore & McCabe 1998, Castelloe 2004). In

this test three assumptions were made: (i) a mean standard deviation of 1.63 mg kg^{-1}, calculated for the barnacles Cd concentration sampled at site B during both sampling periods; (ii) a power test of 0.999 to detect the difference between the background and the peak of Cd concentration, and (iii) α equal to 0.05. Under such conditions, the sample size and power test indicates that 38 samples for each group would be needed to detect a 1.91 mg kg^{-1} increase in the barnacles Cd concentration between each group. If the same test is applied to determine the smallest difference that could be detected assuming the same SD of 1.63, a sample size for each group equal to 7 (i.e., total sample equals to 14) and α of 0.05, a curve describing the relationship between the difference and the power is obtained (Figure 6.13).

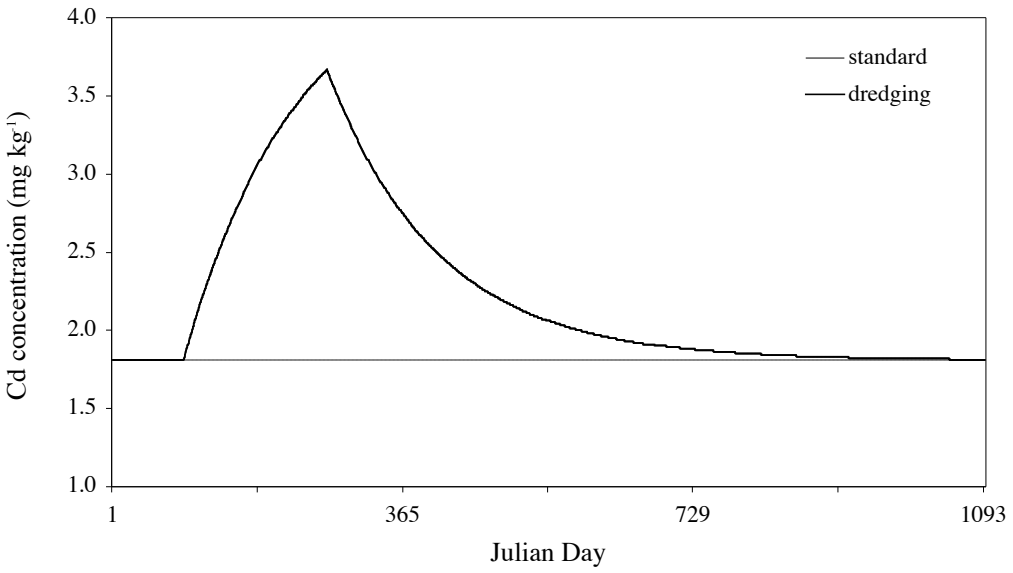

Figure 6.12: Results of the dredging operation's simulation exercise. The 'standard' scenario, in which SSPM presents its normal Cd concentration observed in the field, and the 'dredging' scenario, in which the SSPM Cd concentration was increased by a factor of 2.8 from Julian Day 90 to 270, are represented.

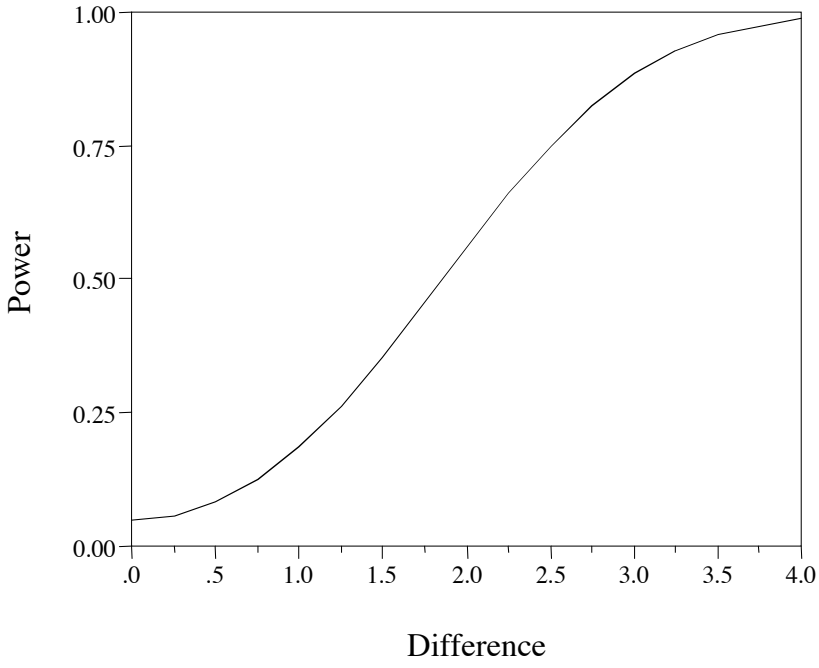

Figure 6.13: Simulation of the power test to detect difference between two sets of data (in this case: Cd concentrations in barnacles) as a function of the difference between them, assuming that data has a SD of 1.63 mg kg^{-1} and $\alpha = 0.05$ for a total number of samples for both groups of 14.

Figure 6.13 suggests that to get a power approaching 1 in the test, the difference between two means should be bigger then 4 mg kg^{-1}. Comparing this result to what was observed in the field, regarding Cd concentrations in the barnacles at site B, the Kruskal-Wallis test detected significant temporal variation only for the wet season, but not for the dry season (see sections 4.2.4 and 5.3.3). In the wet season, the difference between the lowest and highest Cd concentrations was just 2.5 mg kg^{-1}, whereas in the wet season this difference was >4 mg kg^{-1}. Therefore, the lack of temporal variability for the Cd concentrations in the barnacles at site B in the dry season, in which a difference of just 2.5 mg kg^{-1} was observed, is consistent with the sample size and power test since a difference approaching 4 mg kg^{-1} is required for a

statistical test to detect significant difference at the sampling intensity used in this study (4–7 individuals within 6–8 samples per site per sampling day).

In conclusion, the results of the simulation exercise, combined with the sample size and power test, suggest that the use of *Balanus amphitrite* as a biomonitor at a realistic sampling rate would not indicate the increase in the Cd concentration as a result of the dredging operation. Even though the main source of Cd to *Balanus amphitrite* (i.e., SSPM) was realistically contaminated with Cd, a sampling effort, five-fold greater that applied in this study, increasing the sample number from 6–8 to 38, would be required for someone to detect a significant difference in the Cd concentration in organisms collected prior to and after the dredging operation.

6.3.5.2. Modelling effects of a relocation experiment on the Cd concentration in Balanus amphitrite

In the relocation experiment simulated in the model, two scenarios were produced: (i) barnacles from site B were relocated to site C, and (ii) *vice versa*. To do this, the model was set up to represent the temporal variation of the Cd concentration in two barnacles, one living at site B and one at site C. To represent the relocation of the barnacles between the two sites, on Julian Day 90, all forcing functions that were for site B were replaced with those for site C and *vice versa*, as if a barnacle was relocated from B to C and the other one from C to B, respectively. The same initial conditions as those in the dredging operation simulation were adopted in this simulation, and for the barnacle at site C, an initial Cd concentration of 7.49 mg kg^{-1} was also assumed. Thus, the simulated Cd concentration in both barnacles started from steady-state conditions, making it easy to identify the effect that the relocation would

have on their Cd concentrations. All the other model's parameters were kept as in the standard simulation. The results of this simulation exercise are presented in Figure 6.14.

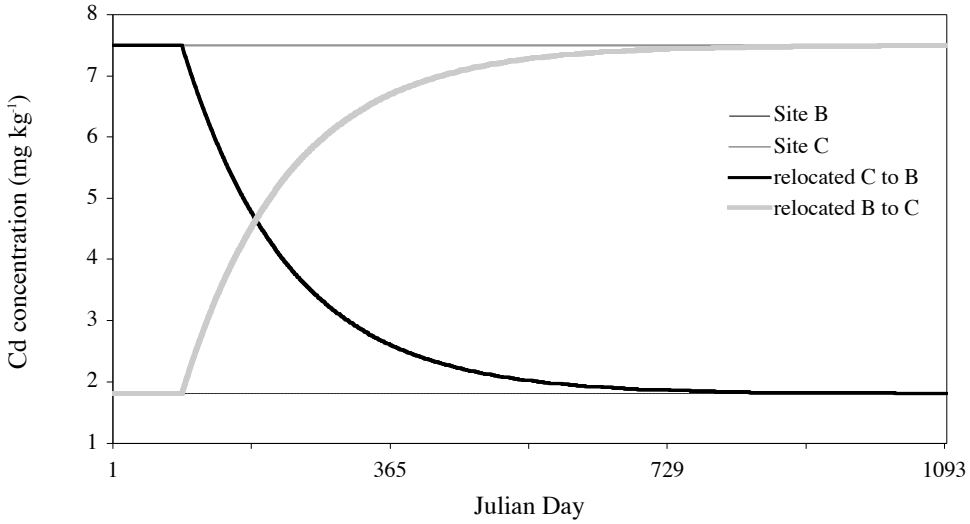

Figure 6.14: Results of the relocation simulation exercise. 'Site B' and 'Site C' curves stand for the standard simulation of the Cd concentrations in *Balanus amphitrite* living at sites B and C, respectively. The curve 'relocated' represents the Cd concentrations in a barnacle that was moved from site B to site C and *vice versa* on Julian Day 90.

The Cd concentration in the barnacles shifted from the original Cd concentration to a new one, reflecting the Cd availability in the relocated site. The simulation results suggest that the organisms would experience a rapid increase (or decrease) in their Cd concentrations, corresponding to a doubling (or halving) over a period of a month, and that more than a year would be required for the organisms to reach a Cd concentration within 20% of the level that would be expected at the relocated environment. This long time-lag before reaching equilibrium supports the pattern for the Cd concentration in *Balanus amphitrite* reproduced in

Figure 6.11, in which no temporal variation was observed in the three-month long simulation. Therefore, a long time-lag would be expected for this organism in response to changes in the Cd availability in its sources.

Relocation experiments carried out using mussels indicated that, in general, their metal concentrations vary in a relatively short period. For example, *Mytilus galloprovincialis* relocated to a polluted environment reached equilibrium for Fe, Mn, and Pb in only two weeks (Regoli & Orlando 1994). *Mytilus californianus* exhibited daily variations in its Cd concentrations, following changes of the dissolved Cd concentrations as result of pulses of this metal during an upwelling event (Lares & Orians 1997). *Trichomya hirsuta* relocated to a high Cd-contaminated environment also varied its metal concentration proportionally to the environment in two-month period (Klumpp & Burdon-Jones 1982). *Mytilus edulis* also varied its metal concentrations (i.e., Cu, Zn, Hg, Ag, and Cd) when relocated from pristine to polluted areas and *vice versa*, reaching equilibrium between 4 and 24 weeks, depending on the metal (Roesijadi et al. 1984). These studies indicate that mussels can pick up short-term temporal variations in metal concentrations in the environment, whereas barnacles apparently cannot.

Comparing the rate of accumulation and elimination of Cd between barnacles and mussels, some differences can be identified. For example, the Cd uptake rate constant from the dissolved phase and efflux rate constant for the mussel *Mytilus edulis* are about twice that for *Balanus amphitrite*: Uptake rate constants are 0.36 and 0.114 L g^{-1} d^{-1} and efflux rates constant are 0.014 and 0.0072 d^{-1} for the mussel (Luoma & Rainbow 2005), and the barnacle (this study, Table 6.2), respectively. These values suggest that Cd accumulation and elimination is more rapid in the mussel than in the barnacle, resulting in more rapid changes

in Cd concentrations in the former. Therefore, based on the simulation of a relocation experiment, *Balanus amphitrite* seems to be a weak biomonitor for identifying *short-term* temporal variations in Cd concentrations in the environment.

6.4. Final Remarks

Balanus amphitrite has been recognized as a good biomonitor for heavy metals, especially Cd (Rainbow & Smith 1992, Rainbow 1993, Rainbow et al. 1993, Blackmore et al. 1998, Blackmore 1999, Wang et al. 1999a, Blackmore & Rainbow 2001, Rainbow & Blackmore 2001, Rainbow et al. 2003), because it exhibits a low depuration rate that leads to high metal concentrations (Wang et al. 1999a, Wang et al. 1999b, Rainbow et al. 2003). The present study confirmed a low Cd efflux rate constant for *Balanus amphitrite*, and therefore, that most of the assimilated Cd remains in its tissue. Moreover, the Cd uptake rate constant from solution was independent of the organism's size, and of salinity variations, at least for the range observed in Ross Creek (ranged about 10).

The model results, supported by the validation tests applied to the baseline simulations, indicated that the model predicts the general trend of Cd concentrations in *Balanus amphitrite* in the field but it cannot predict their short-term temporal variations accurately. This problem was clarified in a model investigation, in which it was shown that variations of the individual weight of the sampled population of barnacles in the field masked real patterns of Cd concentrations in the organism. Another problem was identified in a simulation exercise, in which a pulse of Cd in one of the main sources to the organism could not be detected if a normal sampling effort was applied. These two problems may be critical for the use of this organism as a biomonitor, which also responds slowly to changes in the Cd level in its

sources in the environment. Regarding the later problem, a further simulation, reproducing a relocation experiment, indicated that a long time would be required for the organism to approach a new equilibrium reflecting the Cd level in the relocated environment. Beeby (2001) pointed out that, if the biomonitor's tissue is slow to match large-scale shifts in its sources, a poor measure of current bioavailability will be obtained. Therefore, based on the relocation simulation exercises, *Balanus amphitrite* provide a poor measurement of the current Cd availability in the environment.

Chapter 7. *Summary, conclusions and recommendations for further research*

7.1. General Thesis Overview and its Major Conclusions

The present study addressed the need to understand how short-term variations in Cd concentrations in an environment would determine the Cd concentration in the barnacle biomonitor *Balanus amphitrite*. This organism has successfully been used as a biomonitor for Cd, but whether its Cd concentration is tightly coupled with those in its sources is unclear. To understand the processes controlling the Cd accumulation in *Balanus amphitrite*, two three-months sampling programs were carried out in Ross Creek, a tidal creek discharging into the Great Barrier Reef lagoon (Townsville, Queensland, AU). A dry and wet seasons were selected to test whether the concentration of Cd in the biomonitor responded to any variation in the dissolved and particulate phase Cd concentrations in Ross Creek, as caused by rainfall variation. The barnacle, its food sources (two class sizes of suspended particulate matter and microzooplankton) and water (dissolved phase) were sampled for Cd concentrations and particle mass abundances. Three strategies were adopted on the data analysis: (i) non-parametric statistical tests, (ii) the determination of biological concentration and magnification factors, and (iii) the development of steady-state and simulation models based on Thomann's bioenergetic kinetic model.

The Cd concentration in *Balanus amphitrite* from two sites 2.2 km apart exhibited significant spatial differences in both sampling periods. The barnacles located upstream, in an area with

higher Cd availability, in both the dissolved and particulate phases, exhibited higher Cd concentrations. Within each sampling period, no significant temporal variation was found for the majority of the water column components, in terms of both Cd concentrations and mass abundances, even though the Cd concentration in *Balanus amphitrite* showed temporal variation for three of the four series of barnacles sampled over three-month period. On a seasonal scale, the Cd concentration in the barnacles upstream did not vary significantly, even though the Cd availability in the environment was reduced in the wet season to a half of that in the dry season, driven by changes in the dissolved Cd concentration. These field observations supported by the non-parametric statistical tests suggest that variations in the Cd levels in the water and food as well as the relative abundance of the food sources may result in specific patterns for Cd concentrations in *Balanus amphitrite*. This finding was confirmed by comparing the biological concentration and magnification factors, determined for the components sampled in the dry season 2002, at each site. These comparisons suggested a higher contribution of the dissolved Cd to the barnacles' Cd concentration living upstream, where the lowest partitioning coefficient (K_d) was determined. However, the lack of capability of *Balanus amphitrite* to register differences in Cd availability between season could not be explained by the two approaches used in the data analysis.

A budget and a sensitivity analysis using a steady-state Cd accumulation model developed for *Balanus amphitrite* provided a better understanding of the patterns of the Cd concentration in this organism observed in the field. The budget analysis indicated that food is the main source of Cd to the barnacles, even though the dissolved Cd accounts for >90% of the total Cd in the environment. Thus, seasonal variations in the total Cd availability in the environment, driven by the dissolved Cd, were not recorded in the *Balanus amphitrite*'s tissue. These outcomes highlighted the importance of determining metal pathways to *Balanus amphitrite* in order to

understand the meaning of Cd concentrations in this biomonitor, and that a gap between its Cd concentration and Cd in its sources may occur. The reasons for a such gap became clear when a sensitivity analysis was performed using the steady-state model. This analysis indicated that the most important parameters controlling Cd accumulation in the barnacles are not directly related to the Cd sources in the environment, but intrinsically related to the physiology of organism. Maximum food ingestion rate and efflux rate, the likely variation in the overall Cd uptake as a function of pre-ingestion particle selection, and the influence of growth rate that may cause a size effect in the Cd concentration in *Balanus amphitrite* are some examples identified by the sensitivity analysis. The combination of these physiological controls and the dominance of particulate Cd on the biomonitor Cd load masked a direct relation between Cd availability in the environment and in the biomonitor.

The processes of Cd accumulation in *Balanus amphitrite* were then studied using a simulation model. Based on the steady-state model, the simulation model reproduced the daily variation of the Cd concentrations in *Balanus amphitrite* observed in the field. The model predicted the general trend of Cd concentrations in *Balanus amphitrite* in the field, distinguishing high and low patterns of Cd concentrations in the biomonitor. However, the short-term temporal variations in its Cd concentration were not accurately reproduced in the model. This fact was attributed to variations in the mean weight of the sampled barnacles, which masked the real pattern of temporal variation of the barnacles Cd concentration in the field. Two other problems were identified in simulation exercises that also may compromise the use of *Balanus amphitrite* as a biomonitor for Cd contamination in the environment. In a simulation exercise that reproduced a dredging operation, a five-fold higher sampling effort than that applied in this study was indicated to be required to detect a 2.8-fold increase in the Cd concentration in the main biomonitor's source, occurring for a 6-month period in the

environment. Another simulation exercise, reproducing a relocation experiment, suggested that if *Balanus amphitrite* were relocated from the upstream site to the downstream site or *vice versa*, more than a year would be required for the organisms to reach a Cd concentration within 20% of the level that would be expected at the relocated environment. These three problems identified by the simulation model: (i) that variations in the mean weight of the sampled population may affect the barnacles Cd concentration determination; (ii) that an increased and impractical sampling effort would be required to detect a realistic increase in the main source of Cd to *Balanus amphitrite*, and (iii) that barnacle's tissue requires a long time to respond to changes in the Cd level in its sources, may be critical for the use of this organism as biomonitor and indicate that *Balanus amphitrite* can only provide a poor measure of current bioavailability of Cd in the environment. However, if long-term mean Cd availability in the particulate Cd sized <200 μm is required, *Balanus amphitrite* can provide this information.

The three approaches used in this study allowed an understanding at increasing levels of complexity, the Cd accumulation in *Balanus amphitrite* in the field. The statistical approach and the biological concentration and magnification factors identified a gap between the metal concentration in the barnacle's sources and its tissue, but they could not explain the origin of a such gap. When a steady-state and a simulation models were developed based on Thomann's bioenergetic kinetic model, a better understanding of the meaning of the metal concentration in the biomonitor was achieved. This indicated that a systemic approach, which incorporates some of the complexity of the multitudinous interaction in nature, is strongly recommended for the study of metal accumulation in organisms. In particular, when monitoring programs are been carried out, the development of models to study processes on short-term temporal basis, can provide a clear picture of the meaning of the metal concentration in a biomonitor.

Thus, a better use of the biomonitor may be achieved in order to protect the environment against pollution.

7.2. Environmental Management Applications

Two aspects are noteworthy regarding the use of *Balanus amphitrite* as a biomonitor for Cd in Ross Creek. The Australian and New Zealand Environment and Conservation Council (ANZECC 2000) nominates 0.7 μg L^{-1} as the trigger value for the total Cd concentration in marine water to protect 99% of species. Therefore, the maximum total Cd concentration found in the Ross Creek water column (i.e. about 0.3 μg L^{-1}) probably does not represent a risk to biota. However, the Cd concentrations found in *Balanus amphitrite* (2.2 to 12.8 mg kg^{-1}) are comparable to those found for *Balanus amphitrite* from a relatively polluted area in Hong Kong coastal waters (9.5 mg kg-1, Rainbow & Blackmore 2001). Previous work undertaken in Ross Creek and vicinities found high levels of Cd potentially capable of inducing health risks by human consumption in the commercially important marine species present in the Townsville region (Clarke 1998), and *Saccostrea amassa* from the Townsville Harbour had Cd concentrations higher than the limits recommended by the Australian National Health and Medical Research Council (Jones 1992). Therefore, although the total Cd concentration in Ross Creek water column may not be the subject of environmental concern, because of the bioaccumulation and biomagnification that Cd exhibits through food chains, its concentration in the biota may be of concern as it relates to human consumption.

The other noteworthy aspect is regarding the fact that metal concentrations in suspended particles (organic and inorganic fractions) usually follow the metal concentration in the dissolved phase (see, e.g., Rainbow & White 1989, Blackmore et al. 1998, Luoma et al. 1998,

Rainbow 1998, Connell & Sanders 1999, Strom et al. 2001). Therefore, it should be expected that once the main Cd flux into *Balanus amphitrite* is from food, the biomonitor should reflect the Cd concentration in the dissolved phase and ultimately in the total Cd concentration in the environment. In Ross Creek, the Cd concentration in suspended particles, especially in SSPM (the main Cd flux to the biomonitor, budget analysis' result, section 5.3.6) did not follow the seasonal variation observed for the dissolved Cd. Because Ross Creek does not receive any significant river discharge that could bring suspended particles to the creek, most SSPM are generated locally by resuspension of the bottom sediment. Bottom sediments usually represent a time-averaged characteristic of the bulk particles of a system (Turner et al. 2004), which may explain the lack of variation in the Cd concentration between the dry and wet seasons. Usually, a particular species stands as biomonitor for a particular pollutant type and source (Beeby 2001). *Balanus amphitrite* as a biomonitor in Ross Creek responds mainly to the Cd concentration of SSPM. However, since SSPM does not closely correlate with the total Cd concentration in the environment, a gap between the information obtained from the organism (i.e., its metal concentration) and any subsequent conclusions about environmental health is generated.

7.3. Suggestions for Further Research

In this study, some processes controlling the Cd accumulation in *Balanus amphitrite* were identified as critical either for their potential importance in determining the Cd concentration in the organism or because of a lack of knowledge. The maximum ingestion food rate was identified in the sensitivity analysis as a very important parameter because it affects the amount of food ingested by an organism per day and ultimately, its Cd intake. Thus, any change that increased or decreased the maximum food ingestion rate would result in a

corresponding change in the uptake of Cd by the biomonitor. Because the maximum food ingestion acts simultaneously on the three food sources considered in the model (i.e., two size classes of SPM and microzooplankton), its influence on the Cd accumulation in the biomonitor is bigger than the influence of any food source individually. In the models developed in this study, the maximum food ingestion rate was assumed to be 40% of the dry weight of the organism per day. However, variations in this parameter as a function of differences in the food quantity and quality may be expected (Wang 2002). Thus, experiments designed to find out a relationship between the maximum food ingestion rate and food quantity and quality would improve the bioenergetic kinetic models for Cd accumulation in *Balanus amphitrite*.

Another critical process that may affect the Cd accumulation in *Balanus amphitrite* refers to the pre-ingestion particle selection. Ke & Wang (2002) pointed out that such a process affects the metal accumulation in filter feeders, such as mussels. In the models developed in this study, no food selection was assumed, which means that *Balanus amphitrite* ingests each food source in proportion to that in the environment. However, if *Balanus amphitrite* can select the food intake, this may change the overall Cd influx from food to the barnacles. Thus, experiments designed to find out if there is a food preference in *Balanus amphitrite* and to what extent it occurs should be carried out, and such an information would also improve the understanding of Cd accumulation in this organism.

A third aspect that would be worthwhile to investigate is to what extent the simulation model developed in this study would predict Cd concentrations in *Balanus amphitrite* from a population presenting growth rates >0.01 d^{-1}. The result of the simulation exercise performed in this study suggested that the higher the growth rate, the higher the possibility of a size

effect for *Balanus amphitrite*. For growth rates >0.01 d^{-1}, which are more often found for *Balanus amphitrite* in the field (Calcagno et al. 1997, Simpson & Hurlbert 1998, Thiyagarajan et al. 2003), the simulation results suggested that there is a clear size effect for the Cd concentration in this organism. In this case, the Cd concentration in *Balanus amphitrite* could be corrected for variations in the organism's weight, and thus the model would be independent of the sampled barnacles' weight, as the case of the model run on section 6.3.4. This would give an opportunity to test if a population of *Balanus amphitrite* presenting a size effect, as a function of growth rates >0.01 d^{-1}, would present temporal variation for its Cd concentration at weekly periods in the field and if they will be reproduced in the model.

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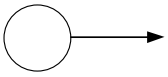
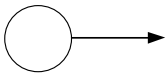
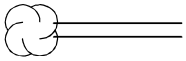
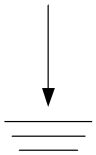
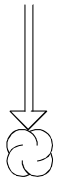
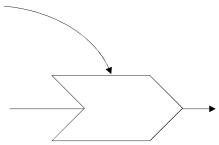
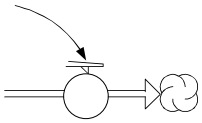
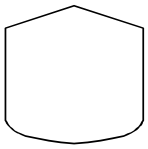
Appendix I: Energetic language as proposed by Odum (1983) and its comparison with the language used on the Stella software (Wallis et al. 2002). This is not a complete list of all the symbols; only those used on the models are presented.

Odum	*description*	*Stella*
 forcing function or energy source indistinctly	Energy source or forcing functions. As an energetic subsidy, it controls the development of a system as a function of its quality and quantity (e.g. light or the undetermined Cd source to the dissolved Cd). As forcing function, it controls the system processes (e.g. temperature or immersion time).	 forcing function  energy source
	Energy or material flow. The arrow indicates the pathway that the flow is travelling (e.g. the pathway followed by the grass taken up by the fish or the Cd taken up from water by the barnacle).	
	Sink energy. It represents an energetic dissipation, in agreement with the second thermodynamic law. It occurs in any process where an energy transformation is involved (e.g. respiration, Cd elimination).	
	Process or interaction. It combines two energy flows resulting in work (another outflow). It is mathematically represented by an equation that describes the control of a forcing function in a process (e.g. temperature controlling the grass production or the dissolved Cd controlling the Cd taken up from water by the barnacle).	
	State variable or system component. It is a quantity of material or energy storage in a system component, and informs at any time the system conditions (e.g. the grass biomass per area or the Cd mass in a barnacle). Alternatively, in the Odum energetic language, a bullet may be used to represents autotrophic organisms and a hexagon for heterotrophic ones. Both were used on Figure 2.3.	

Appendix II: All data sampled in the field in the two sampling programs, from 6 August 2002 to 30 October 2002 and from 21 January 2004 to 14 April 2004, in Ross Creek (Townsville, Queensland, AU).

Symbols in the tables: * HT and LT stand for high tide and low tide, respectively. # stands for the tide height (data supplied by the Townsville Port) at the sampling time. @ stands for the water temperature. ¥ stands for the water salinity measured in practical salinity unit (p.s.u.). © stands for the water volume passed through the plankton net. SSPM, LSPM and MZOOP stand for small suspended particulate matter (0.45–50 μm), large SPM (50–200 μm) and microzooplankton (50–200 μm), respectively, and [Cd] stands for the Cd concentration. Data in grey was considered outlier and therefore excluded from any analysis.

Nutrient analyses were carried out at the Advanced Analytical Centre (James Cook University). Total nitrogen and phosphorus were determined by an alkaline persulfate digestion procedure and filtrable reactive phosphorus and nitrate, nitrite and ammonium were done by standard colorimetric techniques (Clesceri et al 1998). Spikes recoveries fell within 95 to 104% for total nitrogen, and 99 to 109% for total phosphorus.