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L'importance relative de l'eau et de la nourriture comme vecteurs d'accumulation du cadmium chez le bivalve d'eau douce *Pyganodon grandis*

Par

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FOREWORD / AVANT-PROPOS

In recognition of the university in which this project was conducted, this thesis has an extended abstract written in French for francophone readers. Chapters 4, 5, 6, 7 and 8 have been written in the style of complete manuscripts. Thus, some repetition of concepts may be detected in the introductions and methodologies of these chapters.

En reconnaissance à l'égard de l'université où cette étude a été réalisée, cette thèse comprend un résumé étendu en français pour les lecteurs francophones. De plus, les titres de tableaux et de figures sont présentés dans les deux langues. Les chapitres 4, 5, 6, 7 et 8 sont écrits sous forme d'articles complets. Ceci crée inévitablement une certaine redondance dans la thèse puisque plusieurs idées et concepts seront répétés dans les introductions et méthodologies des chapitres.

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RÉSUMÉ

Résumé français

La dégradation des écosystèmes aquatiques marins et d'eaux douces par les contaminants, tels que les métaux, est étudiée depuis plusieurs décennies. Dans ce domaine, l'utilisation de mollusques comme biomoniteurs se fait de plus en plus commune. En effet, les mollusques sont utilisés pour évaluer l'étendue et l'impact de la pollution métallique dans une région. Leur durée de vie relativement longue, leur tolérance à des concentrations internes élevées de métaux et leur taux de dépuration lent permettent l'utilisation de mollusques comme biomoniteurs à long terme. Cependant, la très grande majorité des études consacrées à l'accumulation des métaux chez les mollusques se sont portées sur des espèces marines. Quelques efforts récents ont été faits ces dernières années pour ajouter des espèces d'eau douce à la liste de biomoniteurs; un candidat potentiel serait le bivalve *Pyganodon grandis*.

De nombreuses études récentes, qui ont suivi les changements spatiaux et temporaux des concentrations en Cd chez ce bivalve, ont démontré qu'il y a une bonne relation entre les concentrations en Cd chez *P. grandis* et les concentrations de Cd dans son environnement. Cependant, ces études n'ont pu déterminer par quel(s) vecteur(s), l'eau ou la nourriture, provenait le Cd. Avant que *P. grandis* ne puisse être pleinement exploité comme biomoniteur, il serait important de savoir si ce mollusque accumule les métaux par ingestion ou via l'eau ambiante (ou par les deux vecteurs).

L'objectif global de cette étude était donc de déterminer l'importance relative de l'eau et de la nourriture comme vecteurs d'accumulation de Cd chez le bivalve d'eau douce *Pyganodon grandis*. Afin d'évaluer l'importance relative de chaque vecteur comme source de Cd, le bivalve devait être exposé à une source de Cd à la fois, c'est-à-dire des expositions séparées au Cd aqueux et alimentaire. Les résultats de ces expériences ont été intégrés dans un modèle de bioaccumulation qui a permis de prédire l'importance de chaque vecteur pour divers scénarios d'exposition.

Des spécimens adultes de *P. grandis* ont été recueillis dans un lac peu contaminé de la région de Rouyn-Noranda et transportés au laboratoire à Québec afin d'établir une culture mère. Dans une première série d'expériences, les bivalves ont été exposés à une gamme de concentrations de Cd dissous (0,1; 0,5; 5 et 20 nM) pour une période de 96 h. Afin de faciliter l'alimentation des animaux, les bivalves ont été enlevés de l'aquarium d'exposition et mis dans une chambre expérimentale, remplie d'eau non contaminée, et nourris avec des algues non contaminées. Les taux de filtration des bivalves ont été mesurés durant ces périodes d'alimentation. Dans des expériences subséquentes, les bivalves ont été nourris d'algues vertes (*Pseudokirchneriella subcapitata*) contaminées en Cd pendant 4 périodes d'alimentation de 4 h. Après la dernière exposition, les bivalves ont subi une période de dépuration de 8 j. Les taux de filtration des bivalves ont

été mesurés durant l'expérience d'alimentation afin de calculer les taux d'ingestion des bivalves et l'efficacité d'assimilation du Cd ingéré. Dans une dernière expérience, la répartition subcellulaire du Cd a été déterminée chez des bivalves exposés au Cd aqueux ou alimentaire dans le but d'observer si l'accumulation du Cd dans les fractions subcellulaires changeait selon la provenance du métal.

Nos résultats ont montré que le devenir interne à court terme du Cd chez *P. grandis* dépend de la provenance du métal. Lorsque le Cd provient de la phase aqueuse, la majorité du Cd s'accumule dans les branchies des bivalves, notamment dans les granules, tandis que lorsque le Cd provient d'une source alimentaire, le Cd se dirige surtout vers la glande digestive où il s'associe à la fraction cytosolique de cet organe. Nos expériences ont aussi démontré que le taux de filtration a une influence significative sur l'accumulation du Cd dans les branchies de *P. grandis*, les concentrations de Cd branchial étant plus élevées chez les animaux ayant un taux de filtration élevé. D'autres expériences seraient cependant nécessaires afin d'explorer l'influence du taux de filtration sur l'accumulation du Cd chez *P. grandis*. D'autre part, les expériences avec le Cd alimentaire ont démontré que le taux d'ingestion influait sur l'efficacité d'assimilation du Cd alimentaire, l'efficacité d'assimilation étant plus élevée chez les animaux ayant un taux d'ingestion faible.

Les résultats de cette thèse, obtenus dans des conditions de laboratoire, suggèrent que l'eau est la source majeure de Cd chez *P. grandis* dans son habitat naturel. Cette conclusion aidera dans l'interprétation des variations spatiales et temporelles des concentrations en Cd chez *P. grandis*, puisque nous pourrons dorénavant présumer que ces variations reflètent des changements qui ont eu lieu dans les concentrations de Cd dissous. Cependant, le modèle de bioaccumulation du Cd utilisé durant cette étude n'a pu prédire de manière satisfaisante la concentration de Cd branchiale mesurée chez les bivalves recueillis de lacs de la région de Rouyn-Noranda, les concentrations prédites étant jusqu'à 10 fois inférieures aux concentrations mesurées. Nos expositions au Cd aqueux et alimentaire menées au laboratoire n'ont pas été conçues pour simuler les conditions du terrain. Par exemple, la concentration en Ca dissous retrouvée dans l'eau synthétique utilisée pour l'exposition des bivalves au Cd aqueux et alimentaire était nettement plus élevée que celles observées dans les lacs de la région de Rouyn-Noranda. Il est possible que ces concentrations élevées en Ca aient diminué l'accumulation du Cd chez *P. grandis* durant nos expériences. Par ailleurs, les concentrations de Cd calculées pour la glande digestive se conformaient plutôt bien aux valeurs observées, le modèle ayant pu prédire adéquatement la concentration en Cd dans la glande digestive chez les bivalves recueillis dans la majorité des lacs. Nos résultats démontrent aussi le besoin d'autres expériences, portant notamment sur l'influence de la densité algale et du type de nourriture sur l'accumulation du Cd par la voie alimentaire, ainsi que sur les concentrations en Cd retrouvées chez les populations d'algues naturelles.

English abstract

Contamination of marine and freshwater environments by metals has become a global problem. Several attempts have been made in recent years to use aquatic species as sentinel organisms, as they can reveal spatial and temporal variations in bioavailable metal concentrations and overall water quality. The use of bivalves as sentinels has become common practice as their relative long life-spans, their tolerance of high internal metal concentrations and their slow depuration rates allow them to be used as long-term biomonitors. However, the vast majority of past research on metal accumulation in molluscs has been focused on marine species. There has been some interest in recent years for adding freshwater bivalves to the list of sentinel organisms; one potential candidate is the freshwater bivalve, *Pyganodon grandis*.

Recent spatial and temporal studies have shown a strong relationship between Cd concentrations in *P. grandis* and Cd concentrations in its environment. However, these studies were unable to determine whether *P. grandis* accumulated Cd from the dissolved or particulate phase (or both). If this bivalve is to be used as a Cd biomonitor, it is important to complement the field studies with laboratory studies that determine the filtration, ingestion and efflux rates, as well as Cd assimilation efficiencies from water and food, for this organism. The results from these laboratory studies could then be used in a bioaccumulation model to estimate the relative importance of waterborne and diet-borne Cd as sources of Cd for this bivalve.

The main objective of the present study was thus to determine the relative importance of water and food as sources of cadmium for the freshwater bivalve, *Pyganodon grandis*. In order to better evaluate cadmium uptake from either food or water, the animals were exposed to one source at a time, meaning separate aqueous and dietary exposures. The results of these experiments were then used in a bioaccumulation model to predict the relative importance of each pathway as a source of Cd for *P. grandis*.

Adult specimens of *P. grandis* were collected from a lake having low trace-metal concentrations in the mining region of Rouyn-Noranda, and were transported to Quebec City to establish a stock culture in the laboratory. In a first series of experiments, bivalves were exposed to several different concentrations of dissolved Cd (0.1, 0.5, 5.0 and 20 nM) during short-term experiments (96 h). During these experiments, the bivalves were removed from their exposure aquaria and allowed to feed on non-contaminated food in a Cd-free medium for 4 h, during which time bivalve filtration rates were measured; these filtration rates were then used to calculate Cd absorption efficiencies from the inhaled water. In second series of experiments, bivalves were fed Cd-contaminated algae (*Pseudokirchneriella subcapitata*) during 4 x 4-h feeding periods and allowed to depurate for 8 days follow the exposure to dietary Cd. Bivalve filtration rates were measured during the feeding experiments in order to calculate the bivalve ingestion rates and the assimilation efficiency of ingested Cd. In a third series of experiments, the subcellular partitioning of Cd was determined in bivalves exposed to

either aqueous or dietary Cd. The goal was to see whether the subcellular partitioning of Cd in the gills and digestive gland of bivalves differed based on route of uptake.

The results from the present study illustrate that the short-term fate of cadmium within *P. grandis* is dependent on its uptake source. When the bivalve is exposed to aqueous Cd, a large proportion of the accumulated Cd is associated with the gills, notably in the calcium-rich granules. Under these conditions, less Cd accumulates in the digestive gland than in the gills, and most of it accumulates in the cytosolic fraction of the digestive gland cells. In contrast, after exposure to diet-borne Cd, the metal is predominantly associated with the digestive gland, although some of the Cd accumulated by the digestive gland is subsequently transferred to the gills (where it again is largely bound to the granule fraction). Bivalve filtration rates were shown to have a significant influence on Cd accumulation from the dissolved phase as $[Cd]_{gills}$ generally increased as bivalve filtration rates increased. These results illustrate the need to take into account filtration and ventilatory activity in dissolved metal accumulation studies. For the dietary Cd exposure, the assimilation efficiency of Cd in bivalves was inversely related to their ingestion rates; AE values decreased as ingestion rates increased, suggesting that lower ingestion rates allow more complete digestion of the algal food and higher assimilation of the associated Cd.

The results from simulations with the bioaccumulation model suggest that water is the major source of Cd for *P. grandis*. These results should facilitate the interpretation of spatial and temporal variations in Cd concentrations accumulated in the whole body or in individual target organs: it can now be presumed that such changes in accumulated Cd reflect changes in bioavailable waterborne Cd. For a final study, we used field data from earlier studies on Cd accumulation in the gills and digestive gland of native bivalves, collected from lakes in the Rouyn-Noranda region, to test our kinetic bioaccumulation model for Cd in *P. grandis*. Agreement between the gill Cd concentrations predicted with the model and those observed in native bivalves was poor; the model consistently underestimated Cd accumulation in *P. grandis* gills, even after food was added as a Cd source. This tendency of the biodynamic model to under-predict gill Cd concentrations in wild specimens of *P. grandis* may be the result of the relatively high dissolved Ca concentrations that were used during the laboratory experiments. On the other hand, the model successfully predicted Cd concentrations in the digestive gland in many of the lakes, notably for bivalves collected from the moderately and highly contaminated lakes. The results of the present study also highlighted the need for additional studies regarding dietary Cd uptake in *P. grandis* and how algal density and algal species can influence assimilation efficiencies and Cd uptake from food.

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INTRODUCTION

Métaux dans l'environnement

La dégradation des écosystèmes aquatiques par les contaminants, tels que les métaux, est étudiée depuis plusieurs décennies. En effet, la possibilité que les métaux exercent des effets toxiques sur les animaux aquatiques devient préoccupante, principalement à cause de l'accroissement des rejets domestiques et industriels (Nriagu et Pacyna 1988; Olendrzynski *et al.* 1996). Les métaux ne se dégradent pas et peuvent représenter un danger pour les organismes puisque, lorsque présents à des concentrations intracellulaires élevées, ils sont associés à divers dysfonctionnements cellulaires, dont les altérations de la structure des protéines et l'inhibition d'activités enzymatiques. Les métaux peuvent donc provoquer, de façon directe ou indirecte, de conséquences physiologiques susceptibles d'affecter la survie, la croissance et la reproduction d'organismes aquatiques (Campbell *et al.* 2006; Chapman et Wang 2000; Mason et Jenkins 1995).

Dans le cadre de cette recherche, nous nous sommes attardés seulement à un métal, soit le cadmium. Le cadmium est un métal que l'on retrouve naturellement dans les sols, les sédiments et les milieux aquatiques. Ses concentrations sont habituellement inférieures à 1 nM dans les eaux douces (Hoffman 1995). Ses principales sources naturelles sont l'activité volcanique, l'érosion et les feux de forêts (Nriagu 1989). Les apports anthropiques du cadmium environnemental proviennent principalement des fonderies de mines de cuivre-nickel-zinc, du raffinage et de l'usage industriel du cadmium, et de la combustion d'essence (Wren *et al.* 1995). La contamination des écosystèmes aquatiques peut provenir des eaux de drainage minier, des eaux de ruissellement des zones

minéralisées et des émissions atmosphériques provenant des fonderies (Nriagu et Pacyna 1988; Pacyna *et al.* 1995).

D'un point de vue écotoxicologique, le cadmium se trouve dans la catégorie des éléments très toxiques (Campbell et Couillard 2004). Ce métal est aussi considéré comme non essentiel et intervient très rarement dans les processus biologiques chez les organismes. Une seule exception a été observée chez la diatomée marine *Thalassiosira weissflogii*, où Lee *et al*. (1995) ont constaté que le cadmium pouvait remplacer partiellement le zinc comme nutriment et ainsi intervenir dans la croissance de cet organisme. En dehors de ce cas, le cadmium demeure un élément toxique qui se trouve sur la liste des substances prioritaires de l'USEPA, Agence environnementale des États-Unis (Adhiya *et al.* 2002) et sur la liste des substances d'intérêt prioritaire d'Environnement Canada (1994).

La spéciation du cadmium dans le milieu aquatique varie selon les conditions ambiantes. En milieu marin, la majorité du cadmium est complexé par les chlorures, donnant principalement les complexes solubles $CdCl^+$ et $CdCl_2$ (Byrne *et al.* 1988; Millero et Hawke 1992), ce qui implique une prise en charge inférieure par les organismes marins, en comparaison avec les organismes d'eaux douces (Langston et Bebianno 1990). En eaux douces, la spéciation du cadmium varie selon le pH, la concentration de Cl^-, et la concentration de la matière organique dissoute (Lum 1987; Stumm et Morgan 1996; Xue et Sigg 1998). Cependant, dans les eaux du Bouclier canadien où le pH varie entre 4 et 7, la spéciation du cadmium varie relativement peu et l'ion libre (Cd^{2+}) est l'espèce qui domine (Nelson et Campbell 1991). À un pH $\geq$ 7,4, le cadmium se trouve majoritairement sous forme de complexes et s'associe avec la matière particulaire. Par exemple, Xue et Sigg (1998) ont démontré qu'en milieu lacustre eutrophe (Suisse), et avec des concentrations totales dissoutes en Cd plutôt faibles, la presque totalité du cadmium dissous était complexée à la matière organique. La spéciation du cadmium aura des conséquences importantes sur son accumulation chez les animaux aquatiques, notamment les mollusques.

La prise en charge du cadmium chez les mollusques

Dans notre étude nous nous concentrons sur l'accumulation du cadmium chez les bivalves d'eaux douces. Dans le domaine de l'écotoxicologie aquatique, l'utilisation des mollusques comme biomoniteurs se fait de plus en plus commune (*cf*. le programme "Mussel Watch" en milieu marin; O'Connor (2002)). Dans ce cadre, les mollusques sont utilisés pour évaluer l'étendue et l'impact de la pollution métallique dans une région grâce à leur capacité de bioconcentrer les métaux de leur environnement, même si ceux-ci sont présents en faibles concentrations (Beckvar *et al.* 2000). De plus, grâce à leur capacité de reconnaître un changement brusque dans la qualité de l'eau, les mollusques ont déjà été employés comme biosentinelles en utilisant des techniques de valvométrie (Tran *et al.* 2003). Par exemple, la vitesse de réaction des bivalves exposés aux contaminants dissous peut être utilisée pour determiner le degré de contamination. La présence d'une concentration élevée en contaminants provoque une réponse rapide chez les bivalves, qui ferment brusquement leurs valves, tandis qu'une concentration moins élevée provoquera une réponse plus lente (Tran *et al.* 2007).

Leur durée de vie relativement longue, leur tolérance à des concentrations internes élevées de métaux, comme le cadmium, et leur taux de dépuration lent permettent l'utilisation des mollusques comme biomoniteurs à long terme (Beckvar *et a*l. 2000). Leur tolérance à des concentrations internes élevées de métaux a été attribuée à l'existence de mécanismes de détoxication impliquant la capture des espèces métalliques diffusant dans le cytosol par des ligands, tels que les métallothionéines, et par des granules insolubles (Phillips et Rainbow 1989; Viarengo 1989). Ces deux mécanismes de séquestration protègent les mollusques des effets toxiques causés par les métaux traces en réduisant leur disponibilité biochimique interne (George et Langston 1994).

L'accumulation du cadmium chez les mollusques peut se produire par l'une ou l'autre de deux voies : l'ingestion du métal par sa nourriture ou l'absorption du métal dissous de l'eau ambiante (Fisher *et al.* 1996). L'accumulation du cadmium dissous chez les

bivalves se déroule généralement par les branchies, mais le métal ainsi absorbé peut être transporté aux autres organes de l'animal tels que la glande digestive et le tractus digestif (Wren *et a*l. 1995). Cependant, malgré cette translocation, la majorité de la charge totale en Cd se trouvera souvent dans les branchies, suivi par le manteau et les viscères (Wang et Fisher 1996a).

L'accumulation du cadmium dissous par les bivalves dépend grandement de sa spéciation. De nombreuses expériences montrent que la biodisponibilité d'un métal dissous est mieux prédite par la concentration de l'ion métallique libre que par la concentration totale du métal (Campbell and Couillard 2004). La prise en charge de l'ion métallique libre a lieu grâce à la présence de sites de transport à la surface de la membrane cellulaire (Zamuda et Sunda 1982). Suite à de nombreuses études montrant l'importance de l'ion libre dans l'accumulation des métaux chez les organismes aquatiques (Campbell 1995; Hudson 1998; Morel 1983), le «Modèle du ligand biotique» (Biotic Ligand Model, ou BLM) a été formulé afin d'expliquer la réponse biologique d'un organisme exposé aux métaux traces dissous. Les hypothèses de base du modèle sont les suivantes (Campbell *et al.* 2002; Paquin *et al.* 2002b) :

- la membrane épithéliale est le site primaire pour l'interaction d'un métal M avec un organisme vivant, et ce, par le biais d'un site de liaison membranaire, X-membrane (où «X-membrane» peut représenter un site de transport transmembranaire ou un site physiologiquement sensible);
- l'interaction avec la membrane biologique peut être représentée par la formation d'un complexe de surface «M-X-membrane»;
- la réaction de complexation à la surface biologique se déroule rapidement (plus vite que la réponse biologique); un pseudoéquilibre s'établit entre les espèces en solution et celles à la surface biologique;
- la réponse biologique (prise en charge, micronutrition, toxicité) est proportionnelle à la concentration du complexe «M-X-membrane»;

- dans la gamme pertinente de concentrations du métal, les variations de «M-X-membrane» suivent celles de [M^{z+}].

La complexation du métal ionique avec des substances naturelles (ex. matière organique dissoute) et des substances synthétiques (ex. EDTA, éthylènediamine tetra-acétate) peut aussi affecter l'accumulation du cadmium dissous chez les bivalves. Plusieurs chercheurs ont démontré que la complexation du cadmium avec des ligands synthétiques peut diminuer l'accumulation de celui-ci chez les mollusques (Holwerda *et al.* 1988; Mcleese et Ray 1984) tandis que d'autres ont observé une augmentation dans l'accumulation de ce métal chez ces animaux lorsque le cadmium était associé à la matière organique naturelle (Roditi *et al.* 2000b).

L'importance de la nourriture comme source de cadmium pour les bivalves varie grandement entre études. Selon Decho et Luoma (1991), une des raisons pour ce désaccord est que les mécanismes de prise en charge des métaux par la nourriture sont peu connus chez les bivalves. En général, lorsque le métal est assimilé par la nourriture, la majeure partie s'accumule dans les viscères. Le comportement nutritionnel de l'organisme, la digestion de particules ingérées et l'efficacité d'assimilation auront une grande influence sur l'accumulation du cadmium par la voie alimentaire chez les bivalves (Borchardt 1985).

Il y a désaccord dans la littérature scientifique sur l'importance relative de l'eau et de la nourriture comme sources de Cd chez les bivalves. D'après les données recueillies par la NOAA (National Oceanic and Atmospheric Administration, É-U) pour le programme « Mussel Watch » (O'Connor 2002), Thomann *et al.* (1995) ont calculé, en utilisant leur modèle cinétique, l'importance relative de l'eau et de la nourriture comme sources de Cd chez *Mytilus edulis*. D'après leurs calculs, >90% du Cd accumulé par *M. edulis* provenait de la nourriture. Pour leur part, Wang *et al.* (1996) suggèrent un partage 50/50 entre l'eau et la nourriture comme sources de Cd chez des spécimens indigènes de *M. edulis* recueillis de la baie de San Francisco. D'autre part, Borchardt (1983), d'après des

résultats obtenus en laboratoire, déclare que <10 % du Cd accumulé par *M. edulis* provenait d'une nourriture contaminée.

Ce désaccord illustre bien la difficulté d'extrapoler des études de laboratoire (tableau S1) aux conditions réelles sur le terrain, pour les raisons énoncées par Munger *et al.* (1999) :

(1) les concentrations des métaux traces dans le milieu d'exposition excèdent souvent celles observées dans l'environnement, même aux sites les plus contaminés;
(2) la nourriture et le consommateur sont souvent exposés à des concentrations différentes de métaux dissous;
(3) il n'y a aucun contrôle ou suivi sur la spéciation du métal;
(4) l'exposition de la nourriture aux métaux dissous n'est pas assez longue pour assurer que les concentrations métalliques internes atteignent un état stationnaire;
(5) les consommateurs sont stressés par des conditions expérimentales non naturelles;
(6) un mélange naturel de nourriture n'est pas offert au consommateur.

Des conditions expérimentales réalistes sont nécessaires afin d'assurer que les résultats obtenus au laboratoire reflètent ceux observés dans l'environnement. Ceci a encore plus d'importance lorsqu'il s'agit d'un organisme biomoniteur potentiel.

Il faut noter que la très grande majorité des études consacrées à l'accumulation des métaux chez les mollusques ont porté sur les espèces marines (Reinfelder *et al.* 1997). De plus, dans les programmes de surveillance, les mollusques employés comme biosentinelles sont surtout des espèces marines. En accord avec ces programmes de surveillance, de nombreuses études ont été consacrées au développement d'un modèle cinétique qui permet d'estimer l'importance relative de l'eau et de la nourriture comme sources de contaminants chez les bivalves marins (Decho et Luoma 1991; Luoma *et al.*

1992; Reinfelder et Fisher 1994; Reinfelder *et a*l. 1997; Thomann *et al.* 1995; Wang *et al.* 1996; Wang et Fisher 1997).

Tableau S 1: Classification des études sur l'importance relative de l'eau et de la nourriture comme sources de métaux chez les bivalves par rapport à leur accord (O) ou non (N) aux critères méthodologiques discutés dans le texte.

Animal	Critères						Source du métal	Référence
Marin	1	2	3	4	5	6		
Macoma balthica[1]	N	N	?	N	N	N	E, N	(Harvey et Luoma 1985)
Mytilus edulis	O	O	O	O	O	O	N	(Wang *et a*l. 1996)
Mytilus edulis	N	N	?	?	N	N	E, N	(Borchardt 1983)
M. galloprovincialis	O	N	?	O	N	N	E, N	(Fisher *et a*l. 1996)
Perna viridus	N	N	?	N	O	N	E	(Chong et Wang 2001)
Eau douce								
Anodonta cygnea	N	N	N	N	N	N	E	(Cassini *et al.* 1986)
Corbicula fluminea	O	O	O	O	O	N	E, N	(Tran *et al.* 2002)

(1) concentrations de métaux dissous réalistes; (2) la nourriture et le consommateur sont exposés à des concentrations semblables de métaux dissous; (3) la spéciation du métal est contrôlée; (4) biodisponibilité réaliste du métal dans la nourriture; (5) conditions expérimentales réalistes; (6) mélange naturel de nourriture; ? information non présente dans l'article; E, eau; N, nourriture.

Ce n'est que récemment qu'il y a plus d'études consacrés à l'accumulation des métaux chez les bivalves d'eau douce. La moule zébrée, *Dreissena polymorpha*, et la moule asiatique, *Corbicula fluminae*, sont parmi les espèces les plus étudiées en raison de leur statut d'espèces envahissantes. Récemment, la moule zébrée à été ajoutée au programme « Mussel Watch » en Amérique du Nord. Cependant, malgré le développement d'un modèle cinétique qui estime l'accumulation des métaux chez chacune de ces espèces (Fournier *et al.* 2005; Roditi *et al.* 2000a; Tran *et a*l. 2002), l'utilisation de la moule zébrée et de la moule asiatique comme biomoniteur est limitée aux Grands Lacs (Roditi *et a*l. 2000a). Il faudrait donc instaurer l'utilisation d'une espèce sentinelle pour les

[1] En Amérique du Nord, *Macoma balthica* a récemment changé de nom, et il est maintenant connu sous le nom de *Macoma petalum* (Luoma and Rainbow 2008).

plans d'eaux douces à l'intérieur de l'Amérique du Nord. Un candidat potentiel serait le bivalve *Pyganodon grandis*, un organisme sentinel prometteur.

PYGANODON GRANDIS

Pyganodon grandis est un bivalve filtreur distribué à travers les bassins versants du Missouri-Mississippi, du fleuve St-Laurent et de la baie d'Hudson. Il constitue l'espèce de bivalve la plus fréquemment retrouvée dans les lacs et rivières du Bouclier canadien (Green 1980). La durée de vie maximale de cet animal est estimée à 15 ans (Huebner *et al.* 1990). Nous avons choisi *P. grandis* pour nos études et comme organisme biomoniteur potentiel parce qu'il répond bien aux critères énoncés par Phillips et Rainbow (1993) :

(1) *P. grandis* est très **répandu** à travers les lacs et rivières du Bouclier canadien et **d'abondantes populations** de ce bivalve prospèrent dans la région de nos études, Rouyn-Noranda, Québec (Couillard *et al.* 1993; Clarke 1981).
(2) Des études récentes suggèrent qu'il est **tolérant aux métaux traces** et possède la faculté de fortement **bioconcentrer les métaux**, tout en effectuant une **détoxication efficace** (Couillard *et a*l. 1993; Couillard *et al.* 1995b; 1995a; Wang *et al.* 1999).
(3) Des études ont démontré qu'il a une **relation entre les concentrations du Cd chez *P. grandis* et les concentrations de Cd dans son environnement** (Tessier *et al.* 1993; Wang *et a*l. 1999; Giguère *et al.* 2003).
(4) La **grande taille** de *P. grandis* assure qu'il aura assez de tissu pour faciliter l'analyse des métaux traces.
(5) Son **cycle de vie relativement long**, ses **habitudes sédentaires** et sa **facilité de récolte** en font un animal biomoniteur potentiel à long terme (Giguère *et a*l. 2003).

En plus d'avoir tous ces caractéristiques, il est préférable que l'animal sujet à l'étude soit **facile à entretenir au laboratoire**. Plusieurs chercheurs ont réussi à maintenir des « cultures mères » de bivalves marins tels que *Mytilus galloprovincialis* (Fisher *et a*l. 1996), et *Mytilus edulis* (Wang and Fisher 1996a), ainsi que des bivalves d'eau douce,

tel que *Corbicula fluminea* (Tran *et a*l. 2002; Tran *et al.* 2001), pour de longues périodes (> 4 mois) en laboratoire.

Plusieurs études écotoxicologiques ont été réalisées au sujet de *P. grandis*, notamment à l'INRS-ETE. Dans une des premières études, Tessier *et al.* (1993) ont noté qu'il y avait une forte relation entre $[Cd]_{organismes}$ et $[Cd^{2+}]$, cette dernière ayant été calculée à l'aide d'un modèle d'équilibres chimiques, suggérant que le cadmium dissous dans l'eau ambiante est important pour l'accumulation de ce métal chez *P. grandis.* En parallèle à ces études, Couillard *et al.* (1993) et Wang *et al.* (1999) ont étudié les mécanismes de détoxication de Cd, Cu et Zn chez *P. grandis*. Ils ont démontré que les métallothionéines, des protéines cytosoliques reconnues pour leur capacité de séquestrer des métaux intracellulaires, jouaient un rôle important dans la détoxication du cadmium chez *P. grandis*. Les concentrations tissulaires en métallothionéine, [MT], augmentaient le long du gradient de concentrations en Cd, et en général les concentrations en MT dans les branchies, les viscères et l'organisme total étaient significativement corrélées avec les concentrations de Cd dans les organes correspondants. De plus, ces concentrations de MT étaient associées à $[Cd^{2+}]$ à l'interface sédiment-eau. Ces observations suggèrent que l'activité du Cd^{2+} dans l'environnement influe sur la production des métallothionéines chez ce bivalve (Couillard *et a*l. 1993).

Cependant, Giguère *et al.* (2003) ont démontré que la détoxication du Cd par les MTs n'est pas parfaite dans des conditions où les bivalves sont exposés à des concentrations chroniques faibles, puisque le Cd s'accumulait chez des fractions intracellulaires considérées comme sensibles aux métaux (ex. : protéines). Des études subséquentes, entreprises par Bonneris *et al.* (2005a; 2005b), ont démontré que les granules insolubles dans les branchies jouent elles aussi un rôle important dans la détoxication du Cd chez *P. grandis* recueillis le long d'un gradient de concentrations métalliques. Bonneris *et al.* (2005a) ont noté, d'après les calculs de bilan massique, qu'une faible proportion du Cd retrouvé dans les branchies de *P. grandis* était associée aux métallothionéines (~10 %). La majorité du Cd était associé aux granules retrouvés dans les branchies (58 ± 13 % du

Cd total des branchies). Des granules ont aussi été retrouvés dans la glande digestive, mais elles étaient moins abondants que dans les branchies (Bonneris *et al.* 2005b).

Afin d'évaluer la réponse de *P. grandis* à une brusque augmentation de métaux en milieu naturel, Tessier *et al*. (1987), Couillard *et al.* (1995a; 1995b) et Perceval *et al*. (2004) ont transplanté plusieurs bivalves d'un lac relativement peu contaminé vers un lac très contaminé. Ils ont noté une augmentation de la concentration totale en Cd chez les animaux transplantés, mais cette augmentation était plutôt lente. Couillard *et al*. (1995b) et Perceval *et al*. (2004) ont noté que même après ~400 j (deux étés de croissance), les concentrations en cadmium atteintes dans les bivalves transplantés étaient seulement le tiers de celles observées chez les animaux indigènes du lac contaminé. Des études récentes ont démontré que, même après 860 j d'exposition, les concentrations de cadmium dans les bivalves transplantés étaient la moitié de celles observées chez les animaux indigènes du lac contaminé, démontrant que ces animaux sont des accumulateurs lents (Cooper 2008). L'étude de Cooper (2008) a aussi démontré l'importance des granules (pour les branchies) et de la métallothionéine (pour la glande digestive) dans la séquestration du Cd chez *P. grandis*.

Malgré le nombre important d'études précédentes, nous ne connaissons pas l'importance relative de l'eau et de la nourriture comme sources de cadmium pour *P. grandis*. Avant que *P. grandis* ne puisse être exploité comme biomoniteur valide pour la contamination métallique d'un milieu naturel, il serait important de savoir si ce mollusque accumule les métaux par ingestion ou via l'eau ambiante (ou par les deux vecteurs).

OBJECTIFS

L'objectif global de cette étude était de déterminer l'importance relative de l'eau et de la nourriture comme vecteurs d'accumulation de cadmium chez le bivalve d'eau douce *Pyganodon grandis*. Par cette étude, nous cherchions à établir un modèle qui nous permettrait de prédire l'importance de chaque vecteur pour divers scénarios

d'exposition. On pouvait s'attendre à ce que l'importance relative de l'eau et de la nourriture comme vecteurs d'accumulation varie d'un milieu d'exposition à un autre, et il importait alors que notre modèle puisse tenir compte de ces variations.
Afin d'étudier l'importance relative de l'eau et la nourriture comme sources de cadmium, *P. grandis* devait être exposé à une source de cadmium à la fois, c'est à dire :

(1) une exposition des bivalves à une gamme de concentrations réalistes de Cd aqueux, en absence de nourriture contaminée (mais avec de la nourriture non contaminée);
(2) une exposition des bivalves à la nourriture (des algues) préalablement contaminée en Cd (et ceci en absence de Cd aqueux).

Afin d'assurer que les deux séries d'expériences soient comparables, les algues devaient idéalement être exposées à un milieu de culture qui contenait une concentration semblable en Cd dissous à celle déjà utilisée pour l'exposition des bivalves à l'eau ambiante contaminée. Ces études devaient aussi nous permettre de déterminer lequel des organes (branchies, glande digestive, manteau, etc.) est important dans l'accumulation du Cd et si leurs concentrations en Cd changent selon la provenance / source du métal.

L'importance relative de l'eau et de la nourriture comme sources de Cd chez *P. grandis* devait être vérifiée à l'aide d'un modèle cinétique de bioaccumulation. Le projet global a donc impliqué des études sur :

(1) l'accumulation du cadmium à partir de l'eau ambiante et l'influence du taux de filtration sur cette accumulation;
(2) l'efficacité d'assimilation du cadmium algal ingéré par les bivalves;
(3) le comportement nutritionnel de *P. grandis* au laboratoire.

Une méthodologie soignée a été établie afin d'observer la ventilation branchiale des bivalves et de mesurer le taux de filtration de ces animaux. Un suivi de ces paramètres a permis aussi de mieux estimer l'efficacité d'absorption du Cd dissous chez *P. grandis*. Il

fallait aussi établir le taux d'ingestion chez *P. grandis* afin d'évaluer l'assimilation des métaux chez ce bivalve.

MÉTHODOLOGIE GÉNÉRALE[2]

Échantillonnage et maintien de bivalves en laboratoire

Des spécimens adultes de *P. grandis* ont été recueillis dans le lac Opasatica, un lac peu contaminé de la région minière de Rouyn-Noranda. Des plongeurs ont recueilli les bivalves à une profondeur de ~ 4 m. À la surface, les bivalves ont été triés par espèce et taille (puisque les plongeurs ne peuvent pas distinguer entre les différentes espèces de bivalves sous l'eau). Seuls les animaux qui avaient une longueur entre 50 mm et 90 mm ont été conservés pour cette étude. Les bivalves ont été placés dans des glacières remplies de l'eau du lac et transportés au laboratoire. À Québec, une «culture mère » a été établie dans une chambre environnementale à 15°C. Cette température correspond à celle mesurée dans le lac lors de la récolte des bivalves. Les bivalves ont été placés dans 8 aquariums (entre 30 et 40 bivalves par aquarium) qui contenaient chacun 5 cm de sédiments superficiels recueillis du lac Opasatica et recouverts de 50 L d'eau du robinet. Les sédiments ont été renouvelés une fois par année. L'eau du robinet provenait du fleuve St-Laurent et a été traitée par bullage avec l'air environnant afin d'éliminer le chlore. Les bivalves ont eu droit à une période d'acclimatation d'un mois avant le début des expériences. Ceci avait pour but de réduire le niveau de stress éprouvé par ces animaux et d'assurer que leur comportement au laboratoire serait le plus similaire possible à celui dans leur lac d'origine. Durant leur séjour au laboratoire, les bivalves étaient nourris tous les jours d'un mélange 50/50 (cellules/cellules) de *Pseudokirchneriella subcapitata* (anciennement *Selenastrum capricornutum*), une algue verte unicellulaire cultivée au laboratoire, et une nourriture commerciale d'algues marines (Phytoplex, Kent Marine). Une fois par semaine la moitié de l'eau a été changée afin d'éliminer les déchets métaboliques produits par les bivalves.

[2] Des détails sur les méthodes spécifiques sont donnés dans la section suivante, « Bilan des travaux réalisés ».

Mesures des taux de filtration

Les taux de filtration ont été estimés à partir de la vitesse de disparition de cellules algales introduites dans une chambre expérimentale de 2-L. Des mesures de densité algale ont été faites avec un compteur de particules; l'appareil, qui compte électriquement le nombre de particules en suspension, a été ajusté pour détecter les particules ayant une taille de 1,4 µm à 10 µm. Nous avons appliqué la formule Jorgensen (Coughlan 1969; Tran *et al.* 2000) pour calculer le taux de filtration :

$$FR = \frac{V \cdot \left\{ \left(\ln(d_o) - \ln(d_f) \right) - \left(\ln(d_o') - \ln(d_f') \right) \right\}}{(t \cdot M)}$$

où FR est le taux de filtration ($mL \cdot h^{-1} \cdot g^{-1}$ poids frais)
V est le volume de chaque chambre expérimentale (mL)
ln(do) est la densité algale à t_0 ($algues \cdot mL^{-1}$)
ln(dt) est la densité algale au temps final ($algues \cdot mL^{-1}$)
ln(do') est la densité algale à t_0 dans le témoin, sans bivalve ($algues \cdot mL^{-1}$)
ln(dt') est la densité algale au temps final dans le témoin, sans bivalve ($algues \cdot mL^{-1}$)
t est le temps de la mesure (h)
m est la masse de chair fraîche (g).

Répartition subcellulaire

Des échantillons de branchies et de glandes digestives recueillis des bivalves contrôles (non exposés au Cd) et des bivalves expérimentaux (exposés au Cd) ont été homogénéisés manuellement dans un homogénéisateur en verre placé dans la glace. Le nombre de tours réalisé par l'expérimentateur a été compté à chaque fois, permettant l'homogénéisation complète des échantillons ; ainsi, les échantillons contenant les branchies ont été homogénéisés en 20 tours de pilon et ceux détenant les glandes

digestives, en 12 tours. Pour les deux tissus, l'homogénéisation a été réalisée dans un tampon Tris (Omnipur) ajusté à un pH de 7,2 à 4°C ; un ratio tissu : tampon de 1 : 2 (poids frais : volume de tampon) a été choisi. Un sous-échantillon (0,5 mL) d'homogénat à été systématiquement prélevé, à ce stade du protocole, pour la détermination de la concentration totale en métaux dans le tissu.

La procédure de centrifugation différentielle a été adaptée de celle décrite par Giguère *et al*. (2006). Pour chaque échantillon traité (branchies ou glandes digestives), six fractions subcellulaires ont été recueillies : quatre fractions dites « particulaires », représentant les différentes organelles cellulaires susceptibles de séquestrer les métaux, et deux fractions dites « cytosoliques » (Figure S1).

A l'issue de l'obtention des concentrations en métaux dans les échantillons, un bilan de masse a été réalisé. La quantité d'un métal obtenue après analyse de la portion de l'homogénat retenue au départ (concentration totale en métaux) devait être la plus proche de la somme des quantités du même métal contenu dans chacune des six fractions subcellulaires analysées (culots et surnageants). Dans le cadre de l'étude, les résultats relatifs à ce contrôle étaient acceptables (branchies contrôles: Cd: 99 ± 5 %; Cu 100 ± 7 %; Zn 93 ± 3 %; glandes digestives contrôles: Cd: 102 ± 7 %; Cu 99 ± 6 %; Zn 103 ± 10 %).

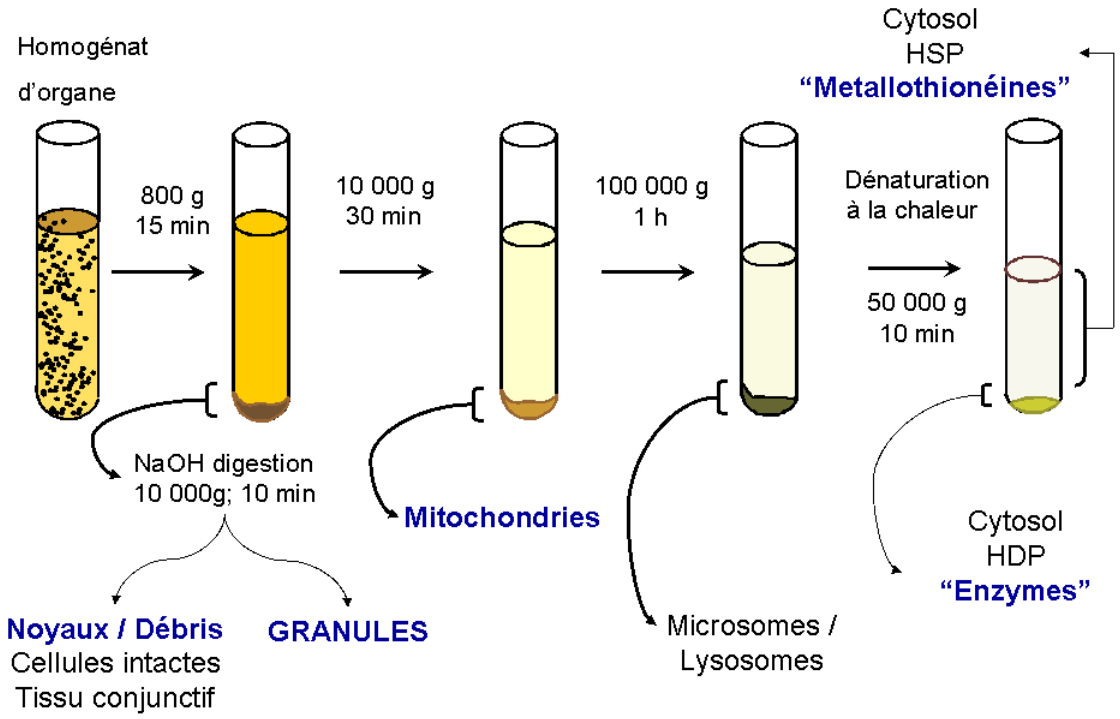


Figure S 1: Protocole de la répartition subcellulaire par centrifugation différentielle.

BILAN DES TRAVAUX RÉALISÉS

Filtration branchiale

Pour déterminer les taux de filtration, nous avons suivi la vitesse à laquelle les bivalves réussissaient à enlever le phytoplancton suspendu dans leur milieu d'exposition. En déterminant la diminution du nombre de cellules algales, sachant la densité algale (nombre moyen de cellules par mL) et supposant que les bivalves captent toutes les algues filtrées (100 % d'efficacité; cf. Tran *et al.* (2001)), on peut calculer le volume total filtré par chaque bivalve. D'après les résultats de ces expériences, réalisées en absence du Cd, nous avons pu conclure que :

(1) les taux de filtration varient entre bivalves (ex. : de < 2 à ~ 60 $mL \cdot h^{-1} \cdot g^{-1}$ (poids frais); moyenne = 26 ± 13 $mL \cdot h^{-1} \cdot g^{-1}$ (poids frais)) (N =10);
(2) le taux de filtration d'un bivalve individuel peut varier de jour en jour.

La variabilité interindividuelle observée dans le taux de filtration des bivalves ne nous a pas beaucoup surpris; d'ailleurs, elle a été documentée dans plusieurs études (Bayne *et al.* 1993; Widdows 1985). Cependant, nous ne nous attendions pas à obtenir une si grande variabilité journalière chez le même individu. De tels comportements ne sont pas décrits dans la littérature. Afin de mieux tenir compte de l'influence de la filtration branchiale durant les expériences de prise en charge de Cd, nous avons décidé de regrouper les bivalves et bien identifier les animaux qui avaient un taux de filtration élevé, moyen et faible, afin que la bioaccumulation globale du Cd dans les trois aquariums d'exposition soit semblable. De plus, il fallait augmenter la période d'exposition au Cd alimentaire de 72 h à 96 h. Ces modifications devaient donner des résultats plus exacts en réduisant l'influence de la variabilité journalière des taux de filtration des bivalves individuels. Cependant, même avec des périodes d'expérimentation plus longues, la variabilité journalière du taux de filtration demeurait appréciable.

Exposition au cadmium aqueux

Méthodes

Ces expériences comprenaient l'exposition des bivalves à une gamme de concentrations de Cd dissous (0,1; 0,5; 5 et 20 nM). Pour chaque concentration nominale, quatre aquariums (3 expérimentaux avec bivalves et 1 témoin sans bivalves) de 6-L ont été remplis d'eau artificielle qui avait été contaminée avec du ^{109}Cd radioactif. Les bivalves ont été exposés au Cd dissous pendant 20 h et nourris pendant 4 h. Afin de faciliter l'alimentation des animaux, chaque bivalve a été enlevé de son aquarium d'exposition après 20 h et mis à part dans une chambre expérimentale de 2-L, remplie d'eau non contaminée, et nourri avec des algues non contaminées. Les taux de filtration des bivalves ont été mesurés durant chaque période d'alimentation. Après la période d'alimentation (4 h), les bivalves ont été remis dans leurs aquariums d'exposition, où la concentration en Cd dissous avait été remontée à sa concentration initiale, pour une autre période d'exposition de 20 h. Après 72 h, les bivalves ont été dépurés pendant 12 h et sacrifiés. Les organes (branchies, glande digestive, manteau, pied, et les tissus restants) ont été séparés et rincés avec un agent complexant, l'éthylènediamine tétra-acétate (EDTA, 10^{-3} M), pendant 20 min afin d'enlever le Cd adsorbé à leur surface. Les organes ont été pesés séparément et placés au compteur gamma afin d'analyser la concentration de Cd accumulé.

Résultats et discussion

Les résultats des expériences de prise charge suggèrent qu'après 3 jours d'exposition ~50 ± 3 % de la charge totale en Cd s'accumule dans les branchies, le reste étant localisé dans le manteau (20 ± 1,5 %), les tissus divers (18 ± 1,2 %), la glande digestive (10 ± 1,1 %), et le pied (2 ± 0,9 %). Cette contribution des branchies à la prise en charge totale du Cd chez un bivalve est légèrement plus élevée que la contribution moyenne de 40 % observée par Tessier *et al.* (1993) chez les bivalves indigènes de lacs contaminés. Le

manteau, la glande digestive et les tissus divers contribuaient respectivement 21%, 11 % et 28 % de la charge totale du Cd chez les bivalves recueillis par Tessier *et al.* (1993). Notre valeur est aussi plus élevée que celle de Tran *et al.* (2001), qui ont rapporté une contribution de 30 % dans les branchies chez *C. fluminea* en condition laboratoire (15 j d'exposition au Cd dissous).

Une analyse des concentrations moyennes en Cd branchial pour chaque régime d'exposition au Cd^{2+} a démontré que les bivalves exposés à 20 nM Cd^{2+} accumulaient plus de Cd dans les branchies que ceux exposés à 5 nM, à 0,5 nM, ou à 0,1 nM Cd^{2+}. Cette relation observée entre la concentration moyenne de Cd branchial et la concentration de Cd dissous dans le milieu d'exposition n'est pas surprenante. En effet, Tessier *et al.* (1984; 1993) ont échantillonné des bivalves le long d'un gradient de contamination en métaux (étude spatiale de divers lacs) et ont noté une augmentation de $[Cd]_{branchies}$ chez les bivalves récoltés dans des lacs les plus contaminés en cadmium.

Selon une analyse de régression, l'accumulation du Cd chez les branchies de *P. grandis* était faiblement reliée aux taux de filtration chez les bivalves exposés à 0,1, 0,5 et 5 nM de $[Cd^{2+}]$ – les animaux ayant des taux de filtration élevés accumulaient des concentrations plus élevées de cadmium dans leurs branchies. L'explication de cette relation apparente entre la concentration de Cd branchiale et la filtration branchiale n'est pas évidente. Selon les principes du modèle du ligand biotique (BLM), une augmentation de la ventilation branchiale ne devrait pas augmenter la prise en charge d'un métal chez un organisme aquatique. D'après le BLM, c'est l'interaction du métal avec les transporteurs membranaires, ainsi que le nombre de sites de transporteur libres, qui devraient contrôler le taux d'accumulation d'un métal dans une branchie (Gorsuch *et al.* 2002). Le transport du métal au travers de la membrane branchiale est normalement l'étape lente dans le processus de prise en charge d'un métal, et dans de telles conditions la surface des branchies se trouve en équilibre avec le milieu d'exposition et la ventilation branchiale n'a pas d'influence sur la vitesse de prise en charge.

Nos résultats ne concordent pas avec le BLM, mais ils ne sont pas sans précédent – quelques autres études ont démontré l'influence de la ventilation branchiale sur l'accumulation des métaux chez les animaux aquatiques. Par exemple, Tran *et al.* (2000; 2001) ont noté que la prise en charge du Cd chez le bivalve *Corbicula fluminea* augmentait dans des conditions hypoxiques. Une réduction de la concentration en oxygène entraînait une augmentation dans les taux de filtration des animaux, ce qui augmentait l'accumulation du Cd dans les branchies. De plus, les conditions hypoxiques changeaient la distribution et la charge de Cd dans les branchies et les viscères.

L'ensemble de ces observations suggère que la ventilation branchiale influence l'accumulation du Cd dissous chez *P. grandis*. Sans nécessairement rejeter le modèle du ligand biotique, il faudrait néanmoins y incorporer des éléments de la physiologie respiratoire. Par exemple, il est possible que l'influence apparente de la ventilation branchiale corresponde non pas à un phénomène hydrodynamique (couche limite plus mince à la surface branchiale), mais plutôt à l'irrigation d'une plus grande surface branchiale. Si les bivalves exploitaient une plus grande proportion de leur surface branchiale, on pourrait s'attendre à une prise en charge plus importante des métaux. De même, dans le cas d'un bivalve, une diminution du taux global de ventilation (tel que mesuré par la disparition de particules en suspension dans le milieu d'exposition) peut refléter une baisse du taux de filtration moyen, ou une augmentation de la proportion du temps pendant lequel le bivalve est fermé. Dans ce second cas, les concentrations en Cd^{2+} présentes dans la cavité branchiale pourraient diminuer beaucoup pendant la période de fermeture, donnant lieu à une exposition moindre au métal et une accumulation moins importante du métal.

Pour compléter l'étude de la prise en charge du Cd aqueux, nous avons également déterminé la répartition subcellulaire du Cd chez *P. grandis* après une exposition de courte durée au Cd aqueux. Les résultats démontrent le rôle important des granules dans la séquestration du Cd, notamment dans les branchies. Cependant, une concentration importante de Cd a été mesurée dans les fractions considérées comme sensibles aux

métaux. L'association du Cd aux mitochondries et aux protéines dénaturées à la chaleur (HDP) démontre que la détoxication du Cd par les granules et les métallothionéines n'est pas parfaite après une exposition de courte durée au Cd aqueux. Des résultats semblables ont été observés chez des bivalves recueillis de lacs contaminés de la région de Rouyn-Noranda. Cependant, malgré le fait que les métaux s'accumulent dans les mitochondries et lysosomes de ces bivalves, les métaux n'étaient pas associés à la fraction « HDP » comme au laboratoire, suggérant que les granules et les MTs procurent une forme de protection aux bivalves exposés aux métaux de façon chronique (Bonneris *et a*l. 2005b; Cooper 2008; 2005a).

Exposition au cadmium par la voie alimentaire

Méthodes

Cette expérience a été divisée en deux étapes : la contamination des algues *Pseudokirchneriella subcapitata* et l'alimentation des bivalves avec les algues contaminées. *P. subcapitata* a été choisie pour cette expérience, car elle est facile à cultiver et acceptable comme nourriture pour le bivalve. Les algues ont été cultivées dans un milieu de culture Bristol pendant 72 h dans un incubateur à 20°C ± 1°C, sous une lumière continue fournie par de tubes à fluorescence blanche (57 µmol·photons·m^{-2}·s^{-1}) et une agitation par bullage avec de l'air comprimé. Une fois que la densité algale a atteint ~ 20 x 10^6 cellules·mL^{-1}, les algues ont été rincées trois fois par centrifugation (7 000 rpm) dans un milieu de culture simplifié. Ce milieu de culture ne contenait pas de métaux traces, d'EDTA ou de PO_4. Le rinçage complété, les algues ont été mises dans une fiole erlenmeyer de 1 L remplie de milieu de culture simplifié (sans métaux, EDTA, PO_4) pour assurer une prise en charge maximale du Cd dissous par les algues. Le pH du milieu de culture simplifié a été préalablement remonté et stabilisé à 7,5 avec un tampon HEPES (10 mM; acide 1-piperazine-4-(2-hydroxyéthyl)- éthanesulfonique). Le pH du lac Opasatica, d'où proviennent les mollusques, est d'environ 7,6 (Bonneris *et al.* 2005).

Le milieu a été contaminé pour atteindre 4,6 nM de ^{109}Cd radioactif dissous et les algues ont été exposées au milieu contaminé pendant 24 h. Les algues étaient légèrement agitées durant la période d'exposition pour assurer qu'elles ne s'établissent pas au fond de la fiole erlenmeyer. Après 24 h, les algues ont été rincées avec du EDTA (10^{-4} M) pendant 20 min afin d'éliminer le Cd qui s'était adsorbé sur leur surface. Les algues étaient rincées trois fois avec le milieu de culture simplifié pour éliminer toute trace d'EDTA et de Cd adsorbé. Trois réplicats de 5 mL ont été prélevés du milieu de culture final et filtrés sur deux membranes de polycarbonate (2 μm, Nuclepore), la première servant à récolter les algues, la seconde à mesurer le bruit de fond de la radioactivité. Chaque filtre était introduit dans une bouteille de 20 mL et placé dans le compteur gamma; de même, une portion de 5 mL du filtrat a été placée dans une bouteille de 20 mL et comptée dans le compteur gamma. Les concentrations de Cd-algal sont exprimées en $nmol \cdot m^{-2}$ et en $nmol \cdot g^{-1}$, la surface algale sur le filtre ayant été calculée à partir du nombre total de cellules algales filtrées et la surface spécifique des cellules algales. La densité algale ainsi que les dimensions de ces cellules (surface, diamètre, superficie cellulaire) étaient obtenues grâce au compteur de particules.

Pour l'alimentation des bivalves avec les algues contaminées, trois bivalves ont été placés dans des enceintes expérimentales individuelles de 2-L remplies d'eau artificielle, non contaminée. Une densité de 500 000 $cellules \cdot mL^{-1}$ de *P. subcapitata* a été introduite dans chaque enceinte expérimentale. De l'EDTA (10^{-4} M) a été ajouté dans l'eau de chaque enceinte expérimentale pour capter le Cd^{2+} qui aurait pu être relargué par les algues. Les bivalves ont été exposés aux algues contaminées pendant 4 h et leur taux de filtration était mesuré. Après 4 h, les bivalves étaient regroupés dans un aquarium de 6-L rempli d'eau artificielle, non contaminée, contenant de l'EDTA (10^{-4} M) et sans présence d'algues, pendant une période de 20 h. Cette séquence de 4 h d'alimentation et de 20 h d'exposition à l'eau non contaminée a été répétée 4 fois. En tout, l'expérience a duré 96 h, avec 16 h d'alimentation aux algues contaminées.

Une fois l'exposition terminée, les bivalves ont été dépurés pendant 8 j dans leurs enceintes expérimentales avec des algues non contaminées, afin de purger les algues contaminées de leur système. Les bivalves ont été sacrifiés après 0, 2, 4, 6 et 8 jours et les organes (branchies, manteau, glande digestive, pied, tissu divers) étaient rincés avec de l'EDTA, introduits dans des bouteilles de 20 mL et placés dans le compteur gamma.

Résultats et discussion

La concentration moyenne de cadmium chez *P. subcapitata* avait atteint 2,19 nmol·m^{-2} ou 76 nmol·g^{-1} après une exposition de 24 h à 4,6 nM de Cd dissous. Après 4 j d'exposition à une nourriture contaminée, ~40 % de la charge totale du Cd se trouvait dans la glande digestive, ~30 % dans les branchies, ~20 % dans les organes divers, ~10 % dans le manteau et <1 % dans le pied. Les taux d'ingestion des bivalves varient de 9 à 54 μg algues poids sec·h^{-1}·g^{-1} bivalve (poids frais) pour une moyenne de 22 ± 3 μg algues poids sec·h^{-1}·g^{-1} bivalve (poids frais ± erreur type de la moyenne, $\sigma\sqrt{n}$). L'efficacité d'assimilation du Cd a aussi varié entre les bivalves (de 5 à >100 %) pour une moyenne de 57 ± 9 % (± erreur type). Cette variabilité dans le taux d'ingestion et l'efficacité d'assimilation du Cd à été démontrée par d'autres chercheurs, qui ont noté que les taux d'ingestion et les efficacités d'assimilation varient selon l'espèce d'algue, le type de mélange (ex. algues + sédiments) et le comportement nutritionnel de l'animal (Reinfelder *et a*l. 1997; Lee et Luoma 1998; Wang and Fisher 1996a).

D'après ces résultats, il semblerait que le Cd ingéré et absorbé dans la glande digestive soit transporté vers les autres organes du bivalve, notamment les branchies. En effet, les résultats de l'étude de la répartition subcellulaire du Cd ont démontré qu'après une exposition au Cd alimentaire, une portion du Cd se déplace vers la glande digestive pour s'accumuler dans les granules des branchies durant la période de dépuration. L'étude a aussi démontré que, de la même manière qu'après une exposition de courte durée au Cd dissous, la séquestration du Cd par les granules et la métallothionéine n'est pas parfaite puisqu'une partie du Cd s'accumule dans les fractions potentiellement sensibles aux métaux, tels que les mitochondries et les protéines dénaturées à la chaleur (HDP). Ces

résultats diffèrent de ceux observés sur le terrain, où les métaux ne s'accumulent pas dans les HDP des bivalves exposés au Cd de façon chronique (Bonneris *et a*l. 2005b; Cooper 2008; 2005a).

Importance relative de l'eau et de la nourriture comme sources de cadmium chez Pyganodon grandis

Un modèle cinétique de bioaccumulation du Cd a été établi afin de déterminer l'importance relative de l'eau et de la nourriture comme sources de Cd chez *P. grandis*. Ce modèle dynamique tient compte de l'accumulation du Cd à partir de l'eau et de la nourriture selon l'équation :

$$[Cd]_{bivalve}^{SS} = \left(\frac{\alpha_w \cdot FR \cdot [Cd]_w}{k_{ew} + k_g} \right) + \left(\frac{AE \cdot IR \cdot [Cd]_f}{k_{ef} + k_g} \right)$$

où $[Cd]_{bivalve}^{SS}$ = la concentration du Cd chez le bivalve en état stationnaire ($nmol \cdot g^{-1}$ poids frais); α_w = l'efficacité d'absorption du Cd dissous (sans unité); FR = le taux de filtration de l'animal ($L \cdot j^{-1} \cdot g^{-1}$ poids frais); $[Cd]_w$ = la concentration du Cd dissous dans l'eau ambiante ($nmol \cdot L^{-1}$); k_{ew} = la constante de taux de perte du Cd accumulé préalablement à partir de l'eau ambiante (j^{-1}); AE = l'efficacité d'assimilation du Cd obtenu par la nourriture ingérée (sans unité); IR = le taux d'ingestion (g poids sec$\cdot j^{-1} \cdot g^{-1}$ poids frais de l'animal); $[Cd]_f$ = la concentration du Cd dans la nourriture ($nmol \cdot g^{-1}$ poids sec); k_{ef} = la constante de taux de perte du Cd accumulé préalablement à partir de la nourriture (j^{-1}); k_g = la constante du taux de croissance (j^{-1}). Les paramètres du modèle ont été obtenus dans le cadre d'expériences menées au laboratoire où les bivalves ont été exposés séparément au Cd aqueux et alimentaire. Les valeurs des constantes de croissance et de perte ont été retrouvées dans la littérature (Tessier *et al*. 1987; Perceval *et al.* 2006).

Les résultats de notre étude suggèrent que, selon les conditions établies au laboratoire, la voie aqueuse joue un rôle prépondérant dans l'accumulation du Cd chez *P. grandis*. Même après la manipulation de certains paramètres du modèle (ex. : augmentation du taux de filtration, du taux d'ingestion, ou de l'efficacité d'assimilation du Cd), l'eau

demeurait la source dominante du Cd chez ce bivalve (entre 59 et 100%), sauf dans les cas où les bivalves étaient exposés à 0,1 ou 0,5 nM Cd dissous. Dans ces cas, la nourriture devenait la source dominante du Cd chez *P. grandis* (entre 60 et 91 %). Nos résultats sont en accord avec ceux décrits par Tessier *et al*. (1993), qui ont observé une relation entre $[Cd]_{bivalve}$ et $[Cd^{2+}]_{aqueux}$. Cependant, ils ne pouvaient pas éliminer la possibilité que la nourriture soit aussi une source de Cd chez *P. grandis*, puisque les concentrations en Cd chez la nourriture (ex. : phytoplancton), seraient elles-aussi, corrélées à $[Cd^{2+}]_{aqueux}$.

Nos résultats suggèrent que, même si le taux de filtration avait peu d'influence sur l'importance relative de l'eau comme source de Cd chez *P. grandis*, il demeure néanmoins un paramètre important pour estimer l'efficacité d'absorption du Cd dissous. En général, des taux de filtration élevés entraînent des valeurs de α_w plus faibles que la valeur constante de 7 % utilisée lors de nos simulations, et il est possible que l'efficacité d'absorption à partir de l'eau soit plus faible sur le terrain. Cependant, la gamme de valeurs de α_w mesurées durant nos expériences est semblable à celle observée pour *Corbicula fluminea* par Tran *et al*. (2002), qui ont noté que α_w variait entre 2 et 12 % selon le taux de filtration de *C. fluminea*.

Les résultats d'autres simulations démontrent que dans certains cas, par exemple lorsque la concentration de matière en suspension ou le taux d'ingestion sont augmentés, la nourriture pourrait devenir une source non négligeable de Cd chez *P. grandis*. Comme pour la phase aqueuse, le taux d'ingestion et l'efficacité d'assimilation auront des conséquences importantes sur l'accumulation du Cd chez *P. grandis*. Plusieurs facteurs auront une influence sur l'efficacité d'assimilation du Cd alimentaire chez les bivalves, tels que l'espèce d'algue ingérée, la composition de la matière en suspension et la durée de la digestion (Lee and Luoma 1998; Reinfelder *et a*l. 1997; Chong et Wang 2000b). Puisque nos bivalves ont été nourris d'une seule espèce algale, il est possible que l'efficacité d'assimilation mesurée au laboratoire soit différente de celles qui prévalent sur le terrain. D'autre part, des calculs suggèrent que nos bivalves ont été exposés à une

densité algale similaire à celle observée sur le terrain (400 000 cellules·mL^{-1} ou ~730 µg·L^{-1} (dry wt). Il est possible, cependant, qu'une augmentation du taux de filtration entraîne une augmentation du taux d'ingestion, notamment si le taux de filtration des bivalves monte à 140 $mL \cdot h^{-1} \cdot g^{-1}$ (poids frais). Comme conséquence, le taux d'ingestion chez les bivalves a été augmenté à un maximum de 80 µg poids $sec \cdot h^{-1} \cdot g^{-1}$ bivalve (poids frais).

Nous avons testé notre modèle cinétique de bioaccumulation, en utilisant les données recueillies durant cette étude au laboratoire, afin de voir s'il pourrait prédire les concentrations de Cd mesurées dans les branchies et la glande digestive de *P. grandis* analysés dans le cadre d'expériences antérieures menées sur le terrain (Bonneris *et al*. 2005a). La simulation pour le Cd branchial se conformait mal aux valeurs observées, les valeurs prédites étant nettement inférieures à celles observées (Figure S2). Pour la glande digestive, cependant, les concentrations de Cd calculées par le modèle se conformaient relativement bien aux valeurs observées, notamment chez les bivalves recueillis des lacs modérement et hautement contaminés en Cd (Figure S3).

Nos expositions au Cd dissous et alimentaire menées au laboratoire n'ont pas été conçues pour simuler les conditions du terrain. Des paramètres physico-chimiques dont on n'a pas tenu compte ont peut-être contribué à la dissemblance des valeurs prédites et observées de concentration de Cd dans les branchies. Par exemple, la concentration en Ca dissous (625 µM) retrouvée dans l'eau synthétique utilisée pour l'exposition des bivalves au Cd dissous et alimentaire était nettement plus élevée que celles observées dans les lacs de la région de Rouyn-Noranda. Il est possible que ces concentrations en Ca dissous élevées aient diminué l'accumulation du Cd chez *P. grandis* durant nos expériences. D'autres facteurs, tels que la spéciation du Cd, la concentration de matière organique dissoute et le pH, auraient eux aussi une influence sur l'accumulation du Cd chez *P. grandis*.

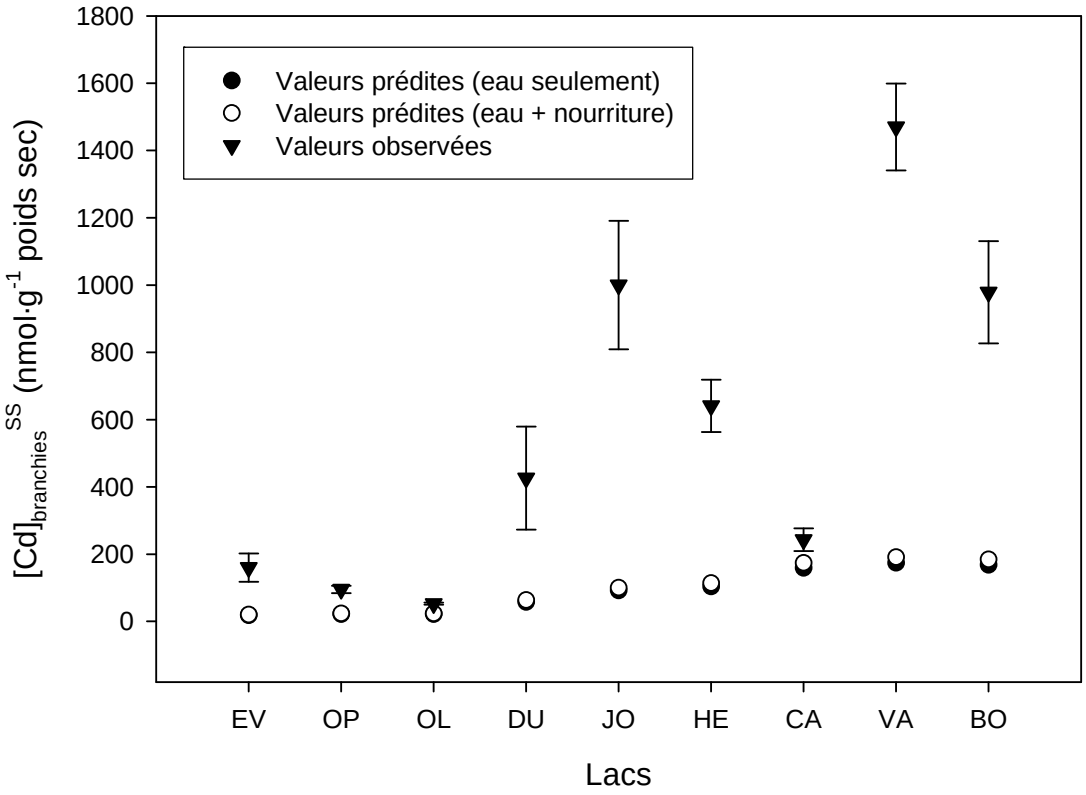


Figure S 2: Comparaison entre les concentrations de Cd chez les branchies de *P. grandis*, telles que calculées pour un état stationnaire et en tenant compte de la prise en charge à partir de l'eau seulement et à partir de l'eau et de la nourriture et ajustée pour un taux d'ingestion maximum, et les concentrations de Cd mesurées (± écart-type) dans les branchies de bivalves recueillis dans les lacs de la région de Rouyn-Noranda (Bonneris *et al.* 2005a).

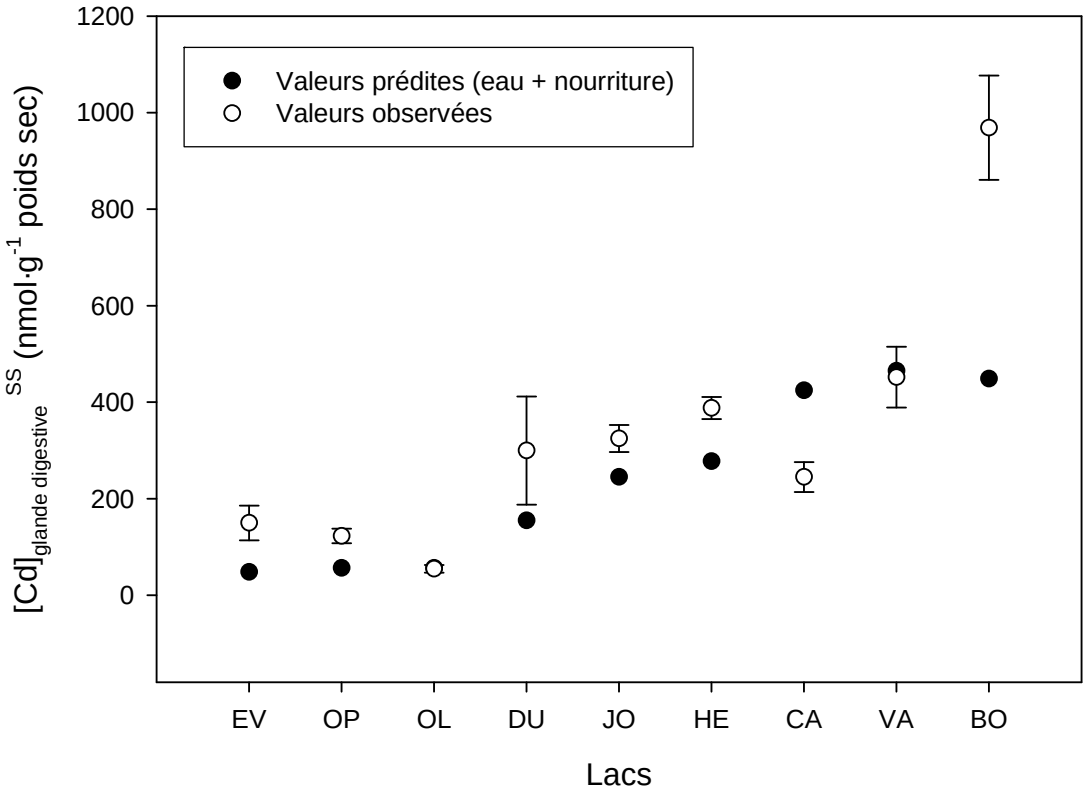


Figure S 3: Comparaison entre les concentrations de Cd chez les glandes digestives de *P. grandis*, telles que calculées pour un état stationnaire et en tenant compte de la prise en charge à partir de l'eau et de la nourriture et ajustée pour un taux d'ingestion maximum, et les concentrations de Cd mesurées (± écart-type) dans les glandes digestives de bivalves recueillis dans les lacs de la région de Rouyn-Noranda (Bonneris *et al.* 2005a).

CONCLUSIONS

Le bivalve *Pyganodon grandis* montre un grand potentiel comme biomoniteur de métaux, étant donné que ce bivalve est généralement retrouvé dans les lacs de référence et dans des lacs contaminés en métaux. *P. grandis* possède d'autres atouts recherchés chez un biomoniteur, tels que la facilité de récolte, la capacité de concentrer des métaux dans ses tissus et la capacité de survivre une transplantation dans un milieu autre que son habitat d'origine. Sa sédentarité facilite son utilisation comme biomoniteur ainsi que l'utilisation de ces organismes pour des études de transplantation. Cependant, avant que *P. grandis* ne soit exploité comme biomoniteur, il importe de savoir si ce mollusque accumule les métaux par ingestion ou via l'eau ambiante (ou par les deux vecteurs). Les résultats de cette thèse, obtenus dans des conditions au laboratoire, suggèrent que l'eau est la source majeure de Cd chez *P. grandis*. Cette conclusion aidera dans l'interprétation des études spatiales et temporelles sur l'accumulation du Cd chez *P. grandis* puisque nous pouvons maintenant présumer ces changements dans les concentrations de Cd accumulé dans le bivalve seront le reflet des changements qui ont eu lieu dans les concentrations de Cd dissous. Cependant, puisque *P. grandis* n'accumule le cadmium que très lentement, il pourra seulement être utilisé comme biomoniteur à long terme.

Nos résultats ont montré que le devenir interne à court terme du Cd chez *P. grandis* dépend de la provenance du métal. Lorsque le Cd provient de la phase aqueuse, la majorité du Cd s'accumule dans les branchies des bivalves, notamment dans les granules, tandis que lorsque le Cd provient d'une source alimentaire, le Cd se dirige surtout vers la glande digestive où il s'associe à la fraction cytosolique de cet organe. Contrairement à ce que l'on observe sur le terrain, le Cd s'accumule aussi dans les fractions dites « sensibles aux métaux », telle que la fraction HDP (« heat-denaturable proteins ») durant nos expositions de courte durée au Cd dissous et alimentaire. Ce résultat suggère que les mécanismes de détoxication étaient moins efficaces au

laboratoire, lors d'une augmentation subite de la concentration ambiante et interne de Cd, que sur le terrain.

Nos expériences ont aussi démontré que le taux de filtration a une influence significative sur l'accumulation du Cd aqueux dans les branchies de *P. grandis*, les concentrations de Cd branchial étant plus élevées chez les animaux ayant un taux de filtration élevé. Cependant, il n'est pas évident si cette augmentation dans l'accumulation du Cd est reliée à une augmentation de la quantité d'eau qui passe dans la cavité branchiale ou à une augmentation de l'efficacité d'absorption du métal à cause d'une augmentation dans l'irrigation sanguine des branchies.

Le modèle de bioaccumulation du Cd utilisé durant cette étude n'a pu prédire la concentration de Cd branchial mesurée dans des bivalves recueillis de 9 lacs de la région de Rouyn-Noranda, les concentrations prédites étant jusqu'à 10 fois inférieures aux concentrations mesurées. Pour la glande digestive, les concentrations de Cd calculées par le modèle se conformaient relativement bien aux valeurs observées, le modèle ayant pu prédire adéquatement la concentration de Cd chez les bivalves recueillis dans la majorité des lacs. Nos résultats contrastent avec ceux obtenus par Kraemer *et al.* (2008) pour la perchaude, *Perca flavescens*. Comme pour *P. grandis*, Kraemer *et al.* (2008) avaient démontré que la phase aqueuse était la source prédominante de Cd chez la perchaude et ils ont donc utilisé un modèle de bioaccumulation qui prenait en compte seulement l'accumulation du Cd à partir de la phase aqueuse. Cependant, le modèle établi par Kraemer *et al.* (2008) surestimait grandement les concentrations de Cd branchial chez les poissons sauvages, chroniquement exposés à ce métal. Les chercheurs ont suggéré que la perchaude était capable de changer sa capacité de prendre en charge le Cd, une habilité non retrouvée chez *P. grandis* puisque le bivalve accumule le Cd de façon très lente, mais continue.

Bien que les résultats de cette thèse aient avancé la compréhension de l'accumulation en Cd chez le bivalve *P. grandis*, certains aspects demeurent néanmoins ambigus. D'autres

expériences sont nécessaires afin d'explorer l'influence du taux de filtration sur l'accumulation du Cd dissous chez *P. grandis*. Pour ce faire, il sera nécessaire d'établir un protocole de recherche qui permettrait de mesurer les taux de filtration de ces animaux sans les exposer à une source alimentaire de métaux. Des expériences sur l'influence de la densité algale et le type de nourriture sur l'accumulation du Cd par la voie alimentaire seront aussi nécessaires, ainsi que des études sur la concentration en Cd retrouvée chez les populations d'algues naturelles.

1. INTRODUCTION

1.1 Metals in the environment

Metals are naturally present in the environment (Reimann et Garrett 2005), and thus living organisms have learned to cope with, and indeed utilize, many of them. Metals became important in early human civilisation as man identified uses for metals in jewellery, currency, tools and weapons. The Industrial Revolution (between 1780 and 1830) brought about an unprecedented demand for metallic items for industrial equipment and machinery. In the 20th Century, the demand for metals continued to increase as they were used for household items (e.g., appliances), transportation, and human health (e.g., surgical tools and medication). While the benefits of metals are indisputable, the potential ecological price of their utilisation is great as human activities have led to increased metal concentrations in certain environmental compartments (Nriagu 1989; Nriagu and Pacyna 1988; Olendrzynski *et al.* 1996). Although it has recently been reported that metal concentrations in the environment are decreasing overall in North America (Mahler *et al.* 2006), their concentrations are increasing in other countries such as Russia and China.

The accumulation of metals in aquatic organisms has been widely studied in the past 30-40 years. Metals do not breakdown in the environment and can represent a danger to aquatic organisms if their internal concentrations become too elevated. Some metals, such as Co, Cu, Fe, Mn, and Zn, play important roles in the enzymatic and metabolic reactions that take place within an organism. However, these essential metals can become cytotoxic when present at concentrations that exceed certain thresholds (Depledge *et al.* 1994). Other metals, such as Ag, Cd, Hg and Pb, are normally considered non-essential since they have no known biological function and can be toxic to organisms at very low concentrations (Hoffman 1995). These metals may cause severe cellular dysfunctions by altering protein structures and inhibiting enzyme activity within an organism. Metals can, therefore, directly or indirectly hamper the survival,

growth or reproduction of aquatic organisms (Campbell *et al.* 2006; Mason et Jenkins 1995; Chapman et Wang 2000).

1.2 Study metal: Cadmium

Our study will focus on cadmium, a metal that is generally considered to be non-essential and toxic to organisms. Cadmium is found naturally in the earth's crust at concentrations varying from 0.1 to 0.5 $\mu g \cdot g^{-1}$ (0.9 to 4.4 $nmol \cdot g^{-1}$) (Wren *et a*l. 1995). It is also present naturally in freshwater lakes and rivers at concentrations varying from 0.01 to 0.05 $\mu g \cdot L^{-1}$ (0.09 to 0.4 nM) (Hoffman 1995). Principal natural sources of Cd include soil erosion, forest fires and volcanic emissions (Nriagu 1989).

The major anthropogenic sources of cadmium include the emissions from copper-nickel-zinc mines, the combustion of fossil fuels, and the refining and industrial use of cadmium (Wren *et a*l. 1995). Cadmium has five main applications: nickel-cadmium batteries, coatings, pigments for glass and plastics, stabilizers in plastics and synthetic material, and alloys as a corrosion protector (Wilson 1988). Small amounts of Cd are also found in television picture tubes, telephone wires, automobile parts, motor oils and in curing agents for rubber (Hoskin 1991). Contamination of aquatic ecosystems occurs from mine drainage systems, as run-off from tailings and mined areas, and from atmospheric emissions from base metal refineries (Nriagu and Pacyna 1988; Pacyna *et a*l. 1995; Block et Wicklund Glynn 1992).

As a non-essential metal, Cd generally does not have any biological functions. The only known exception was reported by Lee *et al*. (1995) who demonstrated that Cd could partially replace Zn as nutrient in the marine diatom *Thalassiosira weissflogii*. Cadmium may accumulate in other aquatic organisms by replacing other divalent metals in metabolic processes and, in doing so, may provoke harmful results (Cassini *et a*l. 1986). In common with other non-essential metals, cadmium is generally not regulated by organisms and may accumulate to toxic levels. Cadmium has also been shown to have

long retention time within some aquatic organisms (Ray *et al.* 1980; Friberg *et al.* 1974; Bias et Karbe 1985). Although biota may bioaccumulate Cd, most evidence suggests that little or no biomagnification occurs in aquatic ecosystems (Kay 1985; Wren *et al.* 1983). However, a recent study by Croteau *et al.* (2005) did find evidence for Cd biomagnification in discrete epiphyte-based food webs in the delta of San Francisco Bay. Due to its toxicity, Cd is found on the United States Environmental Protection Agency's (USEPA) Priority Substances List (Adhiya *et a*l. 2002) as well as Environment Canada's (1994) First Priority Substances List. Cadmium uptake in aquatic organisms, including bivalves, is greatly dependent on its speciation in the environment which, in turn, varies as a function of the physico-chemical characteristics observed in the aqueous environment (e.g., pH, [Cl^-], [dissolved organic matter]).

1.3 Speciation of cadmium

In nature, two oxidation states of cadmium (0 and +2) are possible; however, Cd(0) or the metallic state is rare (NRCC 1979). Cadmium speciation, mobility and bioavailability in freshwater ecosystems are affected by pH, hardness, salinity, the concentration of natural organic matter and the concentration of major and minor cations and anions (Lum 1987; Stumm and Morgan 1996; Xue and Sigg 1998). In marine systems, the speciation and bioavailability of Cd are primarily influenced by suspended matter content and salinity. As salinity increases from estuarine to marine environments, the proportion of soluble cadmium chloride species increases (e.g., $CdCl^+$, $CdCl_2$, $CdCl_3^-$) (Byrne *et a*l. 1988; Millero and Hawke 1992), as a result, for a given Cd concentration, uptake of the metal tends to be slower in marine organisms compared to freshwater animals (Langston and Bebianno 1990).

In freshwater systems, cadmium exists in its free ion form (Cd^{+2}) (Stumm and Morgan 1996), but it may also bind to simple ligands such as chloride (Cl^-), sulfate (SO_4^{-2}) and carbonate (CO_3^{-2}). In addition, in aquatic environments contaminated by an anthropogenic source of thiosulfate ($S_2O_3^{2-}$), this inorganic ligand can form a series of stable complexes with Cd (CdS_2O_3, $Cd(S_2O_3)_2^{-2}$, $Cd(S_2O_3)_3^{-4}$, $Cd(S_2O_3)_3^{-6}$) (Martell et

Smith 2004). However, for the concentration of these complexes to be significant, the concentration of thiosulfate in the medium must be elevated. For example, for a Cd concentration of 1 nM, it would take a concentration of ~0.6 mM of thiosulfate to bind 80% of the dissolved Cd, a concentration that would not occur naturally in aquatic ecosystems (Tremblay 2008).

In general, cadmium tends to be more mobile in the environment than other trace metals. Studies have indicated that aqueous concentrations of Cd increase along a spatial gradient of diminishing lake pH (Almer *et al.* 1978). Thus, Cd concentrations in acidic lakes (pH 5.0 to 6.5) are generally higher than those in more neutral lakes as increased acidity inhibits the sorption of Cd to particles, both in the lake and in the soils of the watershed (Stephenson et Mackie 1988). In contrast to this sensitivity of Cd mobility to acidification, the speciation of Cd seems to be relatively insensitive to pH changes as it does not change greatly over a pH range of 4 to 7 (Nelson and Campbell 1991; Campbell et Tessier 1987).

At a pH ≥ 7.4, Cd will tend to associate with natural organic matter. For example, Xue and Sigg (1998) noted that in eutrophic lakes with low dissolved Cd concentrations, the majority of Cd was associated with suspended organic matter. In Canadian freshwater systems like the St-Lawrence River and Lake Erie, 60 to 90% of total Cd may occur in the dissolved phase, although at high concentrations of suspended particulate matter (e.g., > 200 $mg \cdot L^{-1}$) the particulate phase predominates as a result of particle scavenging (Lum 1987). Cadmium bound to or incorporated in organic matter will be constantly removed from surface waters through settling of organic particles and detritus. Through decomposition and bioturbation in sediments, some of the Cd associated with organic matter can be released to overlying waters and recirculated into the water column (Wilson et Chung 2000; Bewers *et al.* 1987).

1.4 Metal uptake in aquatic organisms and bivalves

1.4.1 Dissolved metal uptake in aquatic organisms

Physico-chemical factors influence the bioavailability of dissolved metals for aquatic organisms, in large measure because they affect the metal's speciation in the exposure environment. Many studies have shown that the bioavailability of a given cationic metal is better predicted by the concentration of the free metal ion than by the total concentration of the dissolved metal (Morel 1983; Morel et Hering 1993). The majority of these studies have looked at phytoplankton, macro-invertebrates and fish (Campbell 1995; Campbell and Couillard 2004), but in some cases bivalves have been the test organisms (see Section 1.4.2). The relationships between metal bioavailability and the free-metal ion concentration have been interpreted to mean that metal uptake involves the initial binding of the metal to a metal transporter found on the cell surface (Figure 1.1).

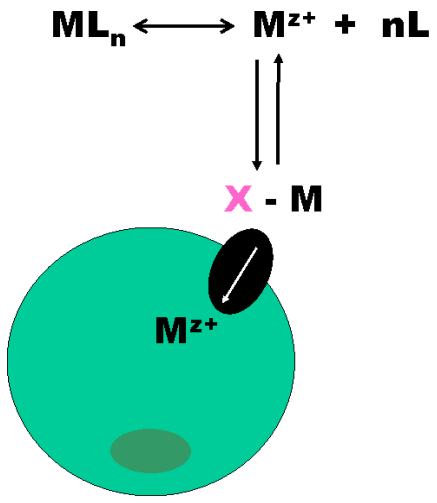


Figure 1.1: Uptake of the free metal ion (M) into a cell via a transport site X. / Prise en charge de l'ion libre métallique grâce à la présence d'un site de transport (X) à la surface d'une membrane épithéliale.

This concept was first developed in the "Free-Ion Activity Model", and it remains the basis of the "Biotic Ligand Model" or BLM (the most recent formulation of the prevailing paradigm for metal-organism interactions) (Paquin *et al.* 2002a; Campbell *et a*l. 2002; Hassler *et al.* 2004; Slaveykova et Wilkinson 2005). The basic assumptions of the BLM are the following (Campbell 1995; Di Toro *et al.* 2001; van Leeuwen 1999):

- metal transport in solution, towards the membrane, and the subsequent surface complexation reaction occur *rapidly*, such that an equilibrium is established between metal species in the bulk solution and those at the biological surface ('rapid'= faster than metal uptake, faster than the expression of the biological response);
- the plasma membrane is the primary site for metal interactions with living organisms (i.e., the transport sites are embedded in the plasma membrane), and this interaction occurs via a ligand exchange reaction, yielding M–X-cell;
- the biological response, whether it be metal uptake, nutrition or toxicity, is dependent on the concentration of the M–X-cell surface complex, «M–X-cell»[3]; in those cases where $^{-}$X-cell corresponds to a membrane transport site, metal internalization involves cation transport;
- variations of «M–X-cell» as a function of $[M^{z+}]$ in solution follow a Langmuir-type adsorption isotherm; provided the concentration of free sites, «$^{-}$X-cell», remains relatively constant in the range of metal concentrations of interest, variations in «M–X-cell» will follow those of $[M^{z+}]$ in solution;
- during exposure to the metal of interest, the nature of the biological surface remains constant (i.e., the metal does not induce any changes in the nature of the plasma membrane or its ion transporters).

[3] Note that the «…» brackets refer to concentration of surface complexes, whereas the normal square brackets, […], refer to the concentration of species in solution.

According to the BLM, complexation of the metal ion (M^{z+}) with ligands in solution (L), whether they be natural ligands such as humic acids or synthetic ones like ethylenediaminetetra-acetate (EDTA) or nitrilotriacetate (NTA), should lead to a decrease in the concentration of the free-metal ion and thus a decrease in the metal's bioavailability. Indeed, this is almost always the case (Campbell 1995), but there are some exceptions to this general rule. For example, certain metal complexes with assimilable ligands, such as amino acids or citric acid, have been shown to contribute to a metal bioaccumulation. In such cases, metal uptake can be higher than predicted by the BLM, but normally the assimilable complex remains less bioavailable than the free metal ion (Errécalde *et al.* 1998; Errécalde et Campbell 2000). In contrast, in the presence of organic ligands such as diethyldithiocarbamate, xanthates or oxine, which all form lipophilic ML_n^0 complexes, the complexes are more bioavailable than the free-metal ion, and metal uptake by organisms such as diatoms, amphipods, and fish increases dramatically (Phinney et Bruland 1994; Ahsanullah et Florence 1984; Block and Wicklund Glynn 1992; Poldoski 1979).

1.4.2 Dissolved metal uptake in bivalves

As has been observed for fish, accumulation of dissolved metals in bivalves can occur in the gills, but metals absorbed into the gills can then be transported to other organs such as the digestive gland and the digestive tract (Wren *et a*l. 1995). However, despite this possibility of translocation, most of the total Cd burden in free-living and laboratory-exposed bivalves is normally found in the gills, followed by the mantle and miscellaneous organs (Mason and Jenkins 1995; Wang *et a*l. 1996).

As with algae, zooplankton and fish, dissolved metal accumulation in bivalves depends on metal speciation. For marine bivalves, salinity plays an important role in metal speciation. The susceptibility of marine molluscs to the toxic effects of certain metals is reduced with increasing salinity due to the formation of complexes between the metal and the anions present in the water (McLusky *et al.* 1986). For example, Cd is present in

seawater largely as chloro-complexes ($CdCl^+$, $CdCl_2$, $CdCl_3^-$, $CdCl_4^{2-}$), and the concentration of the free Cd^{2+} ion increases as salinity decreases (Mackey 1983). The effect of such an increase in Cd^{2+} was demonstrated by Bjerregaard and Depledge (1994) who measured an increase in Cd accumulation by the mussel *Mytilus edulis* when salinity decreased.

The complexation of the dissolved metal ion with natural substances (e.g., dissolved organic matter) or synthetic substances (e.g., EDTA, NTA) can also influence metal accumulation in bivalves. Similarly to Cd, accumulation of Cu by bivalves is greatly dependent on its speciation (Zamuda and Sunda 1982). Zamuda *et al.* (1985) noted reduced Cu accumulation in the oyster *Crassostrea virginica* when Cu was bound to organic compounds (e.g., chitin). Contrary to the case for Cu, some researchers have reported that Zn accumulation in the mussel *Mytilus edulis* was either greater or unchanged when Zn was bound to an organic or a synthetic ligand (Jansen *et al.* 2002; Vercauteren et Blust 1996). For Cd, Holwerda *et al.* (1988) noted a decrease in Cd accumulation in the gills of the freshwater bivalve *Anodonta anatina* if the metal was present as the Cd-EDTA complex. A reduction in the Cd bioconcentration factor on addition of EDTA was also observed in oysters (Hung 1982) and in the marine bivalve *Macoma balthica*[4] (Mcleese and Ray 1984). However, a small number of studies have shown that the complexation of metals with natural ligands may facilitate their accumulation. For example, Roditi *et al.* (2000b) reported that the complexation of dissolved metal (Cd, Ag and Hg) with dissolved organic matter increased the accumulation of these metals in the zebra mussel *Dreissena polymorpha*. They attributed this increase to the absorption of colloidal forms of the metals (colloids involving the organic matter and the metals). Voets *et al.* (2004) noted that Cd-humic acid complexes were partly available to the zebra mussel and, although Cd accumulation did decrease by

[4] In temperate North America, *Macoma balthica* has been reassigned to the species *Macoma petalum* (Luoma and Rainbow 2008).

a factor of 1.6 when Cd was bound to humic acid, uptake rates of the complexed Cd were higher than predicted by the free-ion activity model (FIAM).

Water hardness and pH can also influence dissolved metal accumulation in aquatic organisms. The hardness cations (Ca^{2+}; Mg^{2+}) and the proton itself (H^{+}) may compete with dissolved metals for the transport site X found on the cell surface and, as a result, metal uptake would be reduced as fewer transport sites would be available to the metal (Croteau *et al.* 2002) (Figure 1.2). Calcium has been shown to reduce Cd bioaccumulation in freshwater water bivalves in the laboratory (*Hyridella depressa* and *Velesunio ambiguous*) (Markich et Jeffree 1994) and in the field (Perceval *et al.* 2002). Using a multiple regression analysis, Perceval *et al.* (2002) demonstrated that increasing dissolved Ca concentrations had a negative effect on accumulated Cd concentrations in the freshwater bivalve, *Pyganodon grandis*. However, these researchers were unable to determine if the reduction in Cd accumulation in the bivalve was the result of the two ions competing with each other for the transport sites found on the gill epithelial membrane or the result of a reduction in Cd concentrations in the food due to Cd^{2+} - Ca^{2+} competition on the algal surface. The same tendency was observed with H^{+} ions (Perceval *et a*l. 2002). It is known that Cd can pass through some animal membranes via channels used for Ca (Simkiss et Taylor 1989) and the presence of Ca channel blockers has been shown to inhibit Cd entry into the gills of both freshwater (Holwerda *et al.* 1989) and marine bivalve species (Roesijadi et Unger 1993; Vercauteren et Blust 1999). The competition between Ca and Cd ions may be more pronounced in bivalves compared to other aquatic organisms because these organisms have a high metabolic requirement for Ca, notably for shell growth (Silverman *et al.* 1983).

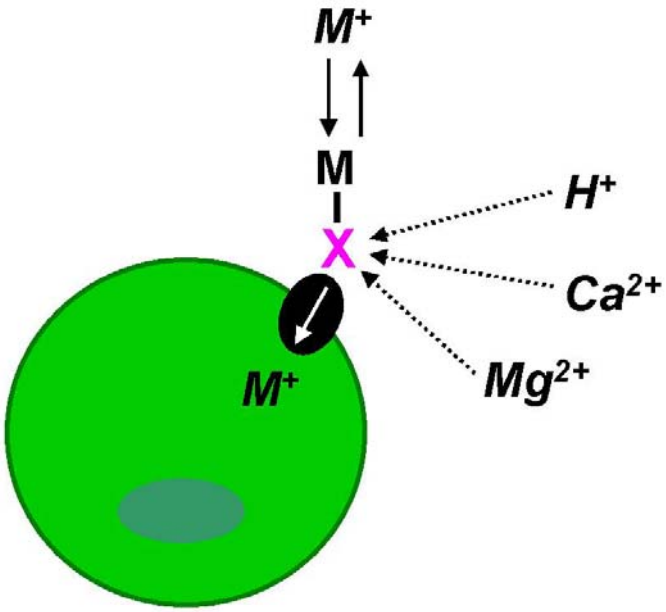


Figure 1.2: Competition between the free metal ion (M^{z+}) and H^+, Ca^{2+} et Mg^{2+} cations for the transport site X. / Compétition entre l'ion libre métallique et les cations H^+, Ca^{2+} et Mg^{2+} pour les sites de transport sur la surface épithéliale d'un animal aquatique.

The presence of other dissolved metals (e.g., Zn or Cu) in the exposure medium can also reduce the availability and uptake of Cd in bivalves. Hemelraad *et al.* (1987) noted a 50% reduction in Cd accumulation in excised gills of the freshwater bivalve, *Anodonta cygnea*, when gills were exposed to a medium containing 100x more dissolved Zn than dissolved Cd. A similar reduction in Cd accumulation in the presence of Zn was also reported for the excised gills of *A. anatina* and for intact adult specimens of *M. edulis* under laboratory conditions (Holwerda *et a*l. 1989; Jackim *et al.* 1977; Elliott *et al.* 1986). However, the environmental relevance of the studies conducted by Hemelraad *et al.* (1987) and Holwerda *et al.* (1989) is debatable because the Cd concentrations during these exposures were not environmentally realistic. In a natural setting, Stewart (1999) demonstrated that the presence of a mixture of metals (Zn, Cu, Pb, and Ni) significantly reduced total Cd accumulation in *Pyganodon grandis*. As with the H^+ and Ca^{2+} ions,

those metals were possibly in competition with Cd for the transport sites found on the gill surface (Stewart 1999).

Biological factors such as age and gender may also affect metal accumulation in bivalves. Metal accumulation rates are regularly higher in younger marine and freshwater bivalves (Cossa *et al.* 1980; Lobel *et al.* 1991; Lobel *et a*l. 1991; Metcalfe-Smith *et al.* 1996). For example, Hemelraad *et al.* (1986) noted that younger specimens of *A. cygnea* had higher Cd accumulation rates compared to older bivalves. The authors suggested that the higher accumulation rate was due to a higher (gill surface/gill weight) ratio observed in smaller animals, or to a higher filtration rate. However, Ardisson (1985) noted that age explained very little of the variability observed in Cd concentrations for specimens of *P. grandis* collected along a metal concentration gradient. The author suggested that the variability in Cd concentration was seasonal. In a study conducted on the effects of biological factors on metal accumulation in the bivalves *Elliptio complanata* and *Lampsilis radiata radiata* collected from the St-Lawrence River, Metcalfe-Smith *et al.* (1996) observed that As, Cd, Mn, Zn, Hg and Fe were higher in older-larger individuals of both species whereas the concentrations of Al, Cu, Cr, Ni, and Se were higher in younger-smaller specimens of *L. r. radiata*, but only Cu showed this trend in *E. complanata*.

The effect of gender on metal accumulation varies from study to study and among species. Metcalfe-Smith *et al.* (1996) noted higher Cu concentrations in female *E. complanata* whereas Cd, Fe and Zn concentrations were higher in male *L. r. radiata*. Lobel *et al.* (1991) observed higher Cu, Mn, and Zn concentrations in female *M. edulis*. Several studies on marine and freshwater bivalves have noted that some metal concentrations increase in both genders before the breeding season (Lobel *et al.* 1989; Baudrimont *et al.* 1997b; Klumpp et Burdon-Jones 1982; Metcalfe-Smith 1994). Variations in metal concentration due to gender and season have been studied in the bivalve marine *Donax trunculus* (Marina et Enzo 1983). The authors observed that females of this species had higher concentrations of Mn and Zn compared to males.

Moreover, the concentrations of these metals fluctuated seasonally and rose once the animals reached sexual maturity.

1.4.3 The relative importance of food as a source of metals in bivalves

Bivalves can accumulate metals both from the dissolved phase and from the ingestion of suspended particles, whether they be organic (e.g., algae) or inorganic (e.g., sediment) (Luoma 1989; Reinfelder *et a*l. 1997). Several studies have indicated that dietary accumulation of metal is at least as important as metal uptake from the aqueous phase, and in some cases may be the main source of metal accumulation by bivalves (Chong and Wang 2001; Wang *et a*l. 1996; Ke et Wang 2002). Metal influx into bivalves from the dietary phase is a function of metal assimilation efficiency from ingested food particles, metal concentration in ingested particles, and the ingestion rate of the animal. Considerable efforts have been made over the past few years to study trace metal assimilation by different species of marine bivalves (Decho and Luoma 1991; Lee and Luoma 1998; Reinfelder *et a*l. 1997; Wang and Fisher 1996a; 1996b) and freshwater bivalves (Roditi et Fisher 1999; Tran *et a*l. 2002).

The relative importance of food as a source of metals in bivalves varies greatly among studies for a given metal and bivalve species. The reasons for these differences are not fully understood, but it is clear that species-specific biological factors (e.g., gut residence time, particle selection, and digestive mechanisms) influence at least some aspects of metal bioavailability from food (Decho and Luoma 1991; Arifin et Bendell-Young 2000; Reinfelder *et a*l. 1997; Bayne *et a*l. 1993). In general, bivalves selectively feed on small (< 10 μm), organic or organic-coated particles. By selecting particles that are richer in organic carbon, animals may be selecting particles with the highest bioavailable concentration of metals. For example, Lee and Luoma (1998) showed that the bivalves *Macoma balthica* and *Potamocorbula amurensis* assimilated Cd and Zn from seston more efficiently as the proportion of phytoplankton within the seston increased. The mechanisms leading to the absorption of diet-borne metals will also vary among bivalve species and include (1) reducing gut pH to solubilise essential

metals and nutrients from food; (2) producing digestive fluids that contain amino acids to extract metals and nutrients from food; and (3) using surfactants to solubilise metals from ingested food (Schlekat *et al.* 2001).

Researchers have worked extensively on developing metal bioaccumulation models to determine the relative importance of food in the uptake of metals such as Cd (Reinfelder *et a*l. 1997), Cr (Decho and Luoma 1991; Wang *et al.* 1997), and Se (Luoma *et a*l. 1992; Wang *et a*l. 1996; Reinfelder and Fisher 1994) for various marine mussel species. Kinetic models of metal bioaccumulation are based on a simple conceptual model in which the concentration of a metal in an organism is controlled by the balance between uptake, elimination, and growth (eq. 1.1). These models require information on metal assimilation efficiencies from food and water, efflux rates of metals from the animals, and filtration, ingestion, and growth rates. The models can be solved for steady-state tissue concentrations under any set of environmental conditions.

$$[Cd]_{bivalve}^{SS} = \left(\frac{\alpha_w \cdot FR \cdot [Cd]_w}{k_{ew} + g} \right) + \left(\frac{AE \cdot IR \cdot [Cd]_f}{k_{ef} + g} \right) \qquad (1.1)$$

where $[Cd]_{bivalve}^{ss}$ = metal concentration in bivalve at steady state ($nmol \cdot g^{-1}$), α_w = metal assimilation efficiency from the dissolved phase (%), FR = filtration rate ($L \cdot d^{-1} \cdot g^{-1}$), $[Cd]_w$ = dissolved metal concentration ($nmol \cdot L^{-1}$), AE = metal assimilation efficiency from ingested food (%), IR = ingestion rate (g algae$\cdot d^{-1} \cdot g^{-1}$), $[Cd]_f$ = metal concentration in ingested particles ($nmol \cdot g^{-1}$), k_{ew} = efflux rate constant following dissolved uptake (d^{-1}), k_{ef} = efflux rate constant following food uptake (d^{-1}), g = growth rate constant (d^{-1}).

The above parameters are usually determined under laboratory conditions and these values are then used to extrapolate and predict values measured under natural conditions. However, as noted by Munger *et al.* (1999), results obtained through laboratory studies do not necessarily reflect those prevailing in the field and should be accepted with caution (Table 1.1) because:

(1) metal concentrations in artificial exposure media often exceed those observed at even the most contaminated sites;

(2) bivalves and food are often exposed to different metal concentrations;

(3) metal speciation in water is usually not controlled or monitored;

(4) food is usually not exposed to metals for a sufficient length of time to reach internal steady state;

(5) animals are stressed by unnatural experimental conditions; and

(6) a natural mixture of food is not always offered to the animals.

Table 1.1: Classification of marine and freshwater bivalve studies on the relative importance of water and food as metal sources according to their adherence (Y) or not (N) to six key methodological criteria. / Classification des études sur l'importance relative de l'eau et de la nourriture comme sources de métaux chez les bivalves par rapport à leur accord (Y) ou non (N) aux critères méthodologiques.

Animal	Criterion[a]						Metal source	Reference
Marine	1	2	3	4	5	6		
Macoma balthica	N	N	?	N	N	N	W, F	(Harvey and Luoma 1985)
Mytilus edulis	Y	Y	Y	Y	Y	Y	F	(Wang *et a*l. 1996)
Mytilus edulis	N	N	?	?	N	N	W, F	(Borchardt 1983)
M. galloprovincialis	Y	N	?	Y	N	N	W, F	(Fisher *et a*l. 1996)
Perna viridus	N	N	?	N	Y	N	W	(Chong and Wang 2001)
Freshwater								
Anodonta cygnea	N	N	N	N	N	N	W	(Cassini *et a*l. 1986)
Corbicula fluminea	Y	Y	Y	Y	Y	N	W, F	(Tran *et a*l. 2002)

(1) environmentally realistic metal exposure concentrations; (2) animals and food exposed to similar dissolved metal concentrations; (3) speciation of dissolved metal was controlled; (4) realistic metal bioavailability in food; (5) realistic experimental conditions; (6) natural mixture of food; ? information not available in publication; W, water; F, food.

Parameters for the metal bioaccumulation model must be obtained under realistic experimental conditions to ensure that results obtained under a laboratory setting reflect those observed in the field. This is all the more important if bivalves are to be used as biomonitors or sentinel organisms.

1.5 Using bivalves as sentinels for aquatic pollution

Bivalves have been widely used as bioindicators, biomonitors or sentinel organisms for aquatic pollution. Bioindicator species are used to monitor the health of an environment or ecosystem. Because bioindicators are usually sensitive species, the absence of a bioindicator species may indicate a problem within the ecosystem (Phillips et Rainbow 1993). For example, freshwater bivalves are general found in lakes whose pH is above 5.5; the absence of freshwater bivalves in a lake may thus reflect that the water pH is below that threshold (Perceval *et a*l. 2002). A biomonitor or sentinel organism is defined as an organism that provides quantitative information on the quality of its environment or ecosystem. They are generally used for regular surveillance of environmental health and may provide a historical record of pollution in the area. Biomonitor or sentinel organisms are typically metal- or contaminant- tolerant species (Phillips and Rainbow 1993).

In ecotoxicological studies, the use of bivalves as biomonitors has become common practice. Molluscs have been used to evaluate the spread and impact of metal pollution in affected areas in part due to their ability to bioconcentrate metals from their environment, even if these metals are present at low concentrations (Beckvar *et a*l. 2000). Furthermore, molluscs have been used as biosentinels due to their ability to detect small changes in water quality (such as the sudden presence of a toxic metal) and to respond to such changes by altering their filtration rates (Kraak *et al.* 1992) or by closing their valves (valvometry) (Tran *et a*l. 2003; Kraak *et al.* 1993). For example, the response time for bivalves to close their valves can be used to determine the level of contamination in an area. A fast response indicates a high contaminant concentration

whereas a lower contaminant concentration requires a much longer exposure period to elicit a response from the animal (Tran *et a*l. 2007).

Their relatively long lifespan, their tolerance of high internal metal concentrations and their slow depuration rates allow molluscs to be used as long-term biomonitors or sentinels (Beckvar *et a*l. 2000). Their tolerance of high internal metal levels has been attributed to the existence of detoxification mechanisms that sequester metals entering the cytosol with metal-binding ligands such as metallothioneins, or with insoluble granules (Viarengo 1989; Phillips and Rainbow 1989). These two mechanisms help protect the animals from toxic effects that the metal might cause, by reducing the metal's intracellular bioavailability (George and Langston 1994).

Since the mid-1970's, scientists in many countries have been using filter-feeding bivalves to monitor selected contaminants in coastal marine waters. This approach to marine monitoring has been successfully applied in several national and regional programs in Europe (United Nations Environment Program – UNEP), the United States (National Oceanographic and Atmospheric Administration - NOAA) as well as Asia, Africa, the Caribbean and South America (regional branches of the International Oceanographic Commission – IOC). An extensive scientific literature has been generated from the International Mussel Watch program and the mussel watch approach has been adopted as one of several coastal environmental quality monitoring strategies (Jernelov 1996).

The major part of past research has been focused on marine bivalves, by virtue of the economic importance of marine ecosystems and the existence of numerous institutes for marine fisheries research. Much marine pollution, however, derives from inland industrial activities and reaches coastal waters by rivers and streams. Metals are often discharged via waste water that will burden the freshwater ecosystem before reaching the coastal waters (Hemelraad *et al.* 1986). Moreover, metals such as Cd may be less

bioavailable to marine organisms than to their freshwater counterparts, due to the formation of charged chloro-complexes (Bjerregaard et Depledge 1994).

It is only recently that more studies focusing on metal accumulation in freshwater bivalves have been conducted. Two species in particular, the zebra mussel, *Dreissena polymorpha*, and the Asiatic clam, *Corbicula fluminae*, have been the subject of many such studies and have been used as biosentinels with researchers developing kinetic models to estimate metal accumulation in these species (Fournier *et a*l. 2005; Roditi *et a*l. 2000a; Tran *et a*l. 2002). Recently, the zebra mussel was added to the NOAA Mussel Watch program (Roditi *et a*l. 2000a). However, because these animals are considered invasive species, their use as biosentinels is confined to the Great Lakes region. There is interest in enhancing the value of the Mussel Watch program by adding other freshwater bivalves as monitoring organisms for inland lakes and rivers. One such potential sentinel species is the freshwater bivalve, *Pyganodon grandis*.

1.6 The bivalve Pyganodon grandis

1.6.1 Taxonomy and identification of *Pyganodon grandis*

There are 10 species of *Pyganodon* species in North America, but only 2 are commonly found in the province of Québec, with 3 varieties found for one of the species: *Pyganodon grandis*, *Pyganodon grandis* var. *grandis*, *Pyganodon grandis* var. *corpulenta*, *Pyganodon grandis* var. *simpsoniana*, and *Pyganodon cataracta*. Distinguishing among species is difficult as the only outer physical difference between species is either a larger hinge line, an extra ridge or two on the beak or a slightly different colour (Clarke 1981).

1.6.2 Biology and life cycle

Pyganodon grandis is a suspension-feeding bivalve found throughout North America. Its range extends from the Missouri-Mississippi watersheds to the St-Lawrence River region and as far north as Hudson Bay. It is the most frequently observed bivalve in

arctic and sub-arctic lakes on the Canadian Shield (Green 1980). It is generally found in neutral to alkaline lakes (pH > 6; alkalinity > 20 mg $CaCO_3 \cdot L^{-1}$) with soft, non consolidated and non rocky substrata, at a depth of 1.5 to 4 m (Clarke 1981). The maximum lifespan of this bivalve is estimated to be 15 years (Huebner *et a*l. 1990).

Fertilization and incubation of young bivalves occur in the marsupial pockets of the gills of the female parent. After a variable incubation period (5-10 months), the females release larvae into the water so that they may attach themselves to fish (preferably perch), either on the gills or fins. After a couple of weeks on their host, the larvae fall into the sediments where they become young endobenthic bivalves (Jansen 1991; Jansen et Hanson 1991). *Pyganodon grandis* can either have a tachytictic reproduction (fertilization and incubation of young during the spring or early summer, followed by release of the larvae in the late summer or autumn) or a bradytictic reproduction (fertilization in the early summer, incubation of young through the following winter and release of the larvae during the following spring), depending on the environmental conditions (Lewis 1985). Bradytictic reproduction is more common in northern habitats.

1.6.3 *Pyganodon grandis* as a biomonitor for metals

Pyganodon grandis has been identified as a potential biomonitor on the basis of the criteria compiled by Phillips and Rainbow (1993):

(1) *P. grandis* is widely found in lakes and rivers located on the Canadian Shield and large populations of this bivalve can be found in our study area, near Rouyn-Noranda, Québec (Couillard *et a*l. 1993; Clarke 1981).
(2) Recent studies have suggested that *P. grandis* tolerates a wide range of environmental metal concentrations and is capable of bioconcentrating metals and detoxifying them effectively (Couillard *et a*l. 1993; Couillard *et a*l. 1995b; Couillard *et al.* 1995a; Wang *et a*l. 1999).

(3) Studies have shown a strong relationship between Cd concentrations in *P. grandis* and Cd concentrations in its environment (Tessier *et a*l. 1993; Perceval *et a*l. 2002; Giguère *et a*l. 2003; Wang *et a*l. 1999).
(4) The large size of *P. grandis* facilitates tissue/organ analyses.
(5) The relatively long life span of *P. grandis*, its sedentary habits and the fact that it is easily collected make it a potential long-term biomonitor.

As well as having these characteristics, the study animal should ideally be able to survive under laboratory conditions for long periods of time. Several studies have reported the successful culturing of bivalves that were collected in the field, transferred to the laboratory and maintained there over appreciable periods (> 4 months), e.g., the marine bivalves *Mytilus galloprovincialis* (Fisher *et a*l. 1996) and *M. edulis* (Wang and Fisher 1996a), and the freshwater bivalve *C. fluminea* (2002; Tran *et a*l. 2001).

1.6.4 Previous studies regarding *Pyganodon grandis* and metal accumulation

Spatial studies

A number of ecotoxicological studies have been performed on *P. grandis*, especially by researchers at INRS-ETE. The first bivalve studies at INRS involved a comparison of two unionids, *Elliptio complanata* and *P. grandis*, collected in lakes located along a metal concentration gradient (Tessier *et al.* 1984). In general, bivalves collected from the most contaminated lakes had the highest metal concentrations. For *E. complanata*, elevated concentrations of Cu, Pb and Zn were observed in the gills and the mantle whereas lower values were observed in the digestive gland and the gonads. A regression analysis indicated that Cu, Pb and Zn levels in the organs of *E. complanata* were correlated with various metal fractions extracted from the host sediments. Tessier *et al.* (1984) suggested that the accumulation of Cu, Pb and Zn in *E. complanata* was indirectly influenced by the presence in the sediments of iron oxyhydroxides and, to a lesser degree, organic matter. For example, for a given concentration of Cd in the sediment, the Cd concentration in the molluscs decreased as the concentrations of extractable Fe increased in the sediment.

After these early studies, the INRS researchers focused their studies on *P. grandis* because in lakes where the two species co-existed, the concentration of Cd was generally higher in this species compared to *E. complanata*. For *P. grandis*, the gills always contained the highest proportion of the total Cd burden (40 ± 13%), followed by the mantle, the digestive gland and gonads which contained respectively 21 ± 7, 11 ± 8, and 28 ± 11% of the total Cd burden (Tessier *et a*l. 1993). Contrary to the earlier results with *E. complanata*, for *P. grandis* no relationship could be established between Cd concentrations in the whole organism ($[Cd]_{organism}$) and total Cd concentrations in the sediments. Tessier *et al.* (1993) did observe a strong relationship between $[Cd]_{organism}$ and the concentration of dissolved $[Cd^{2+}]$ at the sediment-water interface, which was calculated using a chemical equilibrium model, but as the researchers point out, they could not determine if dissolved Cd was the main source of Cd for *P. grandis* (a similar relationship might well have been observed if phytoplankton were the major source, since the Cd concentrations in the phytoplankton would reflect those in the water column).

In a subset of the lakes studied by Tessier *et al.* (1993), Couillard *et al.* (1993) measured metallothionein (MT) concentrations in *P. grandis*. Metallothioneins are low weight proteins that play an important role in the response of an organism when it is exposed to an oxidative, metallic or immunitary stress (Jenny *et al.* 2004). A change in hormone concentrations or exposure to a physical stress may also provoke the production of metallothioneins in an organism (George and Langston 1994). However, among these various potential stimuli, metal exposure is generally considered to be the strongest factor influencing metallothionein concentrations, with MT synthesis being linked to the detoxification of non essential metals such as Cd and Ag (Roesijadi 1992). As in the previous studies, [Cd] were considerably higher in bivalves collected in contaminated lakes and metallothionein concentrations also increased along the metallic gradient. Metallothionein concentrations in the gills, gonads and total organism were significantly correlated with the Cd concentrations of these organs, and with the dissolved $[Cd^{2+}]$

calculated for the conditions prevailing at the water-sediment interface. These observations suggest that Cd present in the environment influences metallothionein production in bivalves (Couillard *et a*l. 1993).

As a follow-up to the demonstration of metallothionein induction in response to chronic metal exposure, several subsequent studies focused on the subcellular partitioning of Cd in *P. grandis*, i.e., its binding to subcellular ligands other than MT. In the first studies, which considered only the dissolved fractions (cytosolic Cd), the subcellular partitioning was determined by size-exclusion high performance liquid chromatography (SE-HPLC) (Wang *et a*l. 1999; Giguère *et a*l. 2003). Both studies confirmed that most of the cytosolic Cd was bound to metallothioneins, but some binding also occurred to ligands with molecular weights higher than or lower than MT (HMW and LMW fractions respectively). For example, Giguère *et al.* (2003) reported that 80% of the cytosolic Cd was associated with MT, 7% with the LMW fraction and 13% with the HMW fraction. The presence of Cd in the low and high molecular weight fractions suggests that the detoxification process is incomplete under conditions where the bivalves are chronically exposed to sublethal Cd concentrations. Consistent with the idea of incomplete metal detoxification, the concentration of malondialdehyde (MDA), an indicator of oxidative stress, increased as the Cd concentration in the gill cytosol increased in bivalves collected along a metal gradient (Giguère *et a*l. 2003).

Similar spatial results were obtained by Bonneris *et al*. (2005b; 2005a), who applied a more complete subcellular fractionation scheme that included both dissolved (cytosolic) and particulate metals. Differential centrifugation was used to separate fractions corresponding to cellular debris, granules, mitochondria, lysosomes + microsomes, and the cytosol; the cytosol was then further separated into heat-stable and heat-denaturable fractions. The major contribution of these studies was to demonstrate that insoluble granules in the bivalve gills play a very important role in binding Cd. Bonneris *et al.* (2005a) calculated a mass balance for the gills and showed that only a small proportion of the gill Cd was associated with metallothioneins (10%); the majority of the Cd was

associated with the calcium-rich granules found in the gills (58 ± 13% of the total Cd burden). Granules were also found in the digestive gland, but they were much less abundant than in the gills (Bonneris *et a*l. 2005b) and their contribution to Cd sequestration was correspondingly much lower (10 ± 10%). According to Phillips and Rainbow (1989), granules can play an important role in the digestion, excretion and storage of metals in many organs such as the digestive gland, the intestines, the kidneys and the gills. In the present case, it would appear that the primary role of the granules is to serve as a calcium reserve for the bivalves, to be mobilized for shell growth and especially during the development of the glochidea (Silverman *et al.* 1985; Silverman *et a*l. 1983). The abundance of the granules did not increase along the metal contamination gradient, indicating that *P. grandis* does not increase its production of granules in response to Cd exposure. Bonneris *et al.* (2005a) concluded that these constitutive elements of unionid gills should be considered as non-specific metal sinks at the cellular level.

Transplant studies

To evaluate how *P. grandis* responds to a sudden increase in metal concentrations in a natural setting, Tessier *et al.* (1987) transplanted specimens of *P. grandis* from a metal-contaminated lake (Lake Joannès, Rouyn-Noranda) to a non-contaminated lake (Lake Brompton, Eastern Townships). A similar transplant experiment was done in the reverse direction, from the non-contaminated site to the contaminated lake. Bivalves were collected at different times (t = 0, 8, 21, 71 and 133 d) to follow changes in metal concentrations (Cd, Cu, Pb and Zn). The study reported (i) an increase in metal concentrations in bivalves transplanted from the non-contaminated lake to the contaminated lake, attributed to net metal uptake due to an increase in environmental metal concentrations; and (ii) a decrease in metal concentrations in bivalves transplanted in the opposite direction, attributed to the net elimination of the metal due to a reduction in environmental metal concentrations. Changes in bivalve metal concentrations were, however, very slow as the total metal concentrations in the bivalves transplanted from the non-contaminated site did not reach those observed in indigenous bivalves from the

contaminated site after 133 days of exposure. The results from this experiment demonstrated that *P. grandis* was able to tolerate a sudden change in ambient metal concentrations and that these animals had potential as indicators of contaminated environments.

In a subsequent study Couillard *et al.* (1995a) transplanted specimens of *P. grandis* from a reference lake to a contaminated lake and followed metal accumulation (Cd, Cu Zn) and metallothionein concentrations over time. They noted an increase in total [Cd] in the transplanted animals, but the rate of accumulation was rather slow; after 400 d, Cd concentrations in the transplanted bivalves were only about one-third of those observed in native bivalves collected in the contaminated lake. After 90 d, the transplanted bivalves showed a detectable increase in [MT] in the gills, digestive gland and whole organism; detectable increases were also seen in the mantle and other organs at the next sampling date (400 d). The increase in [MT] over time was correlated with an increase in Cd concentrations in the same organs.

In the same study, using size-exclusion HPLC, Couillard *et al.* (1995b) determined the subcellular partitioning of Cd in the cytosol of the gills of transplanted *P. grandis*. After 14 d, Cd was largely bound to the high molecular weight fraction. After 90 d, Cd had moved to the intermediate molecular weight fraction, including MT. After 400 d, transplanted bivalves began to show signs of toxic effects as the Cd was associated with the low molecular weight fraction. Couillard *et al.* (1995b) also noted that the condition indices for the transplanted bivalves had decreased over time.

A subsequent transplant experiment, conducted by Perceval *et al.* (2006), consisted of transferring *P. grandis* specimens from a reference site (Lake Opasatica) to various experimental sites situated along a Cd exposure gradient (Lakes Joannès, Vaudray, Dasserat and Dufault). *P. grandis* specimens were exposed for a period of 400 days (from July 1999 to September 2000) at the various sites. The objectives of the study were to follow metal accumulation and MT synthesis in bivalves subjected to a sudden

change in environmental metal concentrations, to observe the subcellular distribution of metal at the end of the 400 d experiment, and lastly to evaluate whether the changes in subcellular distribution could be linked to changes in bivalve growth and survival. Perceval *et al.* (2006) hypothesized that bivalves transplanted to sites along the Cd exposure gradient would exhibit increased concentrations of Cd in the non-thionein ligand pool, and that this condition would coincide with a decrease in organism growth and an increase in mortality rates.

Accumulation of Cd by the bivalves depended upon transplant destination and generally increased with water and sediment Cd exposure. Total gill MT concentrations in bivalves after a 400-day exposure were also significantly different among transplant sites and were directly related to Cd concentrations in bivalves. *P. grandis* specimens transplanted to Lakes Joannès and Vaudray did not reach the Cd or MT concentrations of the indigenous populations even after 400 days, suggesting that steady-state between internal and external media had not been reached. Determinations of subcellular Cd partitioning by size-exclusion chromatography showed that the majority of Cd in the gill cytosol of transplanted bivalves was always associated with MT-like proteins. Cadmium was never detected in the low molecular weight (LMW) ligand pool, but the concentration of Cd found associated with the high molecular weight (HMW) fraction varied significantly among sites, and systematically increased as the environmental Cd concentration increased.

These results contrast markedly with those observed by Couillard *et al*. (1995a) – unlike these earlier results, Perceval *et al*. (2006) did not observe a radical shift in the subcellular partitioning of Cd in the gill cytosol at the end of the experiment. Although the amount of Cd bound to the MT-like and HMW fractions increased along the Cd exposure gradient, there was no apparent shift from one subcellular compartment to another. However, Perceval *et al*. (2006) only determined the distribution of Cd at the end of the metal exposure period; the interpretation of their results is thus limited by this

lack of information about metal distribution changes that might have occurred during the experiment.

In a recent transplant study reported by Bonneris (2004) and by Cooper (2008), in which *P. grandis* specimens from a reference site (Lake Opasatica) were transplanted to a highly contaminated lake (Lake Vaudray) for 860 d, metal detoxification strategies were shown to differ between target organs; for instance, metals accumulated in the gills were mostly located in the granules, whereas in the digestive gland the same metals accumulated in the cytosol, in particular in the metallothionein-like protein fraction. Although Cd did increase in certain metal-sensitive fractions (i.e. mitochondria and lysosomes) of the digestive gland, metal concentrations did not increase in the HDP fraction of the gills or digestive gland. These results suggest that cadmium detoxification is rather efficient in *P. grandis*, unlike some other aquatic species (e.g., the yellow perch, *Perca flavescens*) (Giguère *et al*. 2006). Interestingly, although the transplant experiment lasted 860 d, Cd concentrations in the gills of transplanted bivalves were only half of those measured in indigenous bivalves collected from the contaminated lake (Cooper 2008).

1.6.5 Unknowns concerning *Pyganodon grandis*

Despite the important findings of the above-mentioned studies, there are still some unanswered questions regarding the dynamics of metal accumulation in *P. grandis*, in particular with respect to the relative importance of food and water in Cd accumulation in this bivalve. Moreover, if *P. grandis* is to be used as a Cd biomonitor, and if we are to be able to interpret spatial and temporal changes in its Cd concentrations, it will be important to complement the field studies with laboratory studies that determine the filtration, ingestion and efflux rates, as well as Cd assimilation efficiencies from water and food, of this organism. The results from these laboratory studies could then be used in a bioaccumulation model to estimate the relative importance of waterborne and diet-borne Cd in the field.

2. OBJECTIVES

The main objective of the present study was to determine the relative importance of water and food as sources of cadmium for the freshwater bivalve, *Pyganodon grandis*. In order to better evaluate cadmium uptake from either food or water, the animals were exposed to one source at a time, that is:

(1) bivalves were exposed to environmentally relevant concentrations of dissolved Cd in simple aqueous media, in the absence of contaminated food;
(2) bivalves were exposed to Cd-contaminated food (a green algae, *Pseudokirchneriella subcapitata*) in the absence of aqueous Cd (the algae were pre-exposed to a concentration of dissolved Cd that fell within the range of those used in the waterborne exposures).

Bivalve filtration rates were measured periodically during the dissolved Cd exposures and the absorption efficiency of dissolved Cd was calculated after the experiments. Similarly, bivalve filtration and ingestion rates were measured during the diet-borne exposures and the assimilation efficiency of Cd from the ingested food was calculated for each bivalve.

The results of these experiments were used in a bioaccumulation model to predict the relative importance of each pathway as a source of Cd for *P. grandis*. A biodynamic model was selected for this purpose as it allows for multiple uptake pathways. An important assumption of such models is that metal accumulation in an organism is an additive function of dissolved and dietary uptake pathways meaning that each pathway can be calculated independently, as has been done for this study. Biodynamic models have been successfully used to evaluate the bioaccumulation of metals and other contaminants in aquatic animals, including mussels, and have been shown to be very

flexible and applicable to diverse environments with varying physico-chemical conditions.

Along with determining the relative importance of food and water as sources of Cd to *P. grandis*, the study will also address other questions regarding Cd accumulation in this species: (1) Does the target organ (i.e., the main organ where Cd accumulates) change with the source of contamination? (2) Does bivalve filtration rate influence Cd accumulation in *P. grandis*, as has been observed in other bivalve species?

In the next chapters, we present the following facets of the project:

- general methodology (Chapters 3 and 4)
- waterborne exposures to Cd (Chapter 5)
- diet-borne exposures to Cd (Chapter 6)
- subcellular fate of Cd (Chapter 7)
- relative importance of water and food as sources of Cd (Chapter 8)
- conclusions (Chapter 9).

3. GENERAL METHODS

3.1 Sampling Area

Bivalves were sampled near the mining city of Rouyn-Noranda, located in the Abitibi-Témiscamingue region of western Québec. The city lies on geological terrain that contains both major and minor base metal and gold deposits. The principal productions from these deposits included Cu, Zn, Au, Ag with lesser quantities of Pb, Cd and pyrite (Lavergne 1985). The largest of these deposits, the Horne copper deposit, was the catalyst for the development of a mining industry in the region. From its beginning, the Horne smelter treated mainly local ore concentrates, producing Cu anodes and extracting other metals such as Zn, Ag, and Au. After the closure of the local Horne mine in 1976, the smelter started treating imported ore concentrates and metal-rich recyclable materials. Since 1989, improvements in the processing of metals have made the Horne smelter complex an important copper and precious metal producer, while significantly reducing atmospheric emissions of metals and SO_2 in recent years (Kliza et Telmer 2001).

Most of the lakes in the area were formed by glacial activity, and those located downwind from the smelter (especially in the northeast and southeast directions) have been contaminated by atmospheric fallout. For example, a survey of lake sediments in the vicinity of the Horne smelter indicated that metal concentrations are elevated in surface sediments proximal to the point source and decrease with distance in the path of the prevailing wind direction as a function of anthropogenic metal inputs and distance of transport (Kliza et Telmer 2001).

3.2 Bivalve sampling

Pyganodon grandis were collected from a lake having low trace-metal concentrations (Lake Opasatica) and located upwind from the Horne smelter in Rouyn-Noranda. During each field campaign (8 in total between May 2002 and October 2006), divers collected bivalves at a depth of 4 m. *Pyganodon grandis* measuring between 5 and 9 cm were

transported to the laboratory, in Quebec City, in coolers filled with aerated lake water. Lake sediments were also collected during each field campaign and transported to the laboratory for use in establishing a stock culture.

3.3 Laboratory set-up and bivalve maintenance

A stock culture was set-up in a cold room maintained at 15 ^{o}C. Thirty to 40 bivalves were placed in each of the eight 55-L plastic containers filled with 50-L of dechlorinated tap water and 5 cm of unsieved lake sediments. Tap water, originating from the St-Lawrence River, was dechlorinated by aerating it for 6 days in a large container. Although a fine-grained sand or till would have sufficed for our stock culture, we opted to use lake sediments believing that the bivalves would be more "comfortable" in their sediments of origin. Florescent lamps and timers were used to maintain a 12-h day/12-h night photoperiod. The water in the containers was renewed weekly by removing 2/3 of the water and refilling the containers with fresh dechlorinated tap water. The sediments were replaced every year to reduce the accumulation of waste products. Once the stock culture had been established, bivalves were allowed to acclimate for one month before being used in experiments. Bivalve mortality rates and condition indexes were monitored throughout their captivity (Appendix A).

Bivalves were fed daily with a 50/50 (cells:cells) mixture of the green alga *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) grown under laboratory conditions and a commercial food composed of marine algae (Phytoplex, Kent Marine). According to the supplier, Phytoplex includes *Nanochloropsis sp.*, *Tetraselmis sp.* and *Isochrysis sp.*; these species range in size from 2 to 4 µm. *Pseudokirchneriella subcapitata* cultures were grown in a 20-L container filled with Bristol nutrient medium (Table 3.1) and held in an incubator at 20 ± 1 °C for 72-h. Continuous light was provided by white fluorescent lights (57 µmol·photons·m^{-2}·s^{-2}) and the medium was agitated using an air stone attached to an air pump. Once algal densities reached ~ 20 x 10^{6} cells·mL^{-1}, aliquots of the algal suspension (unrinsed algae + accompanying medium) were given to the bivalves.

Table 3.1: Chemical composition of Bristol nutrient medium used to grow *Pseudokirchneriella subcapitata* under laboratory conditions. / Composition chimique du milieu de culture Bristol utilisé pour cultiver *Pseudokirchneriella subcapitata* au laboratoire.

Element	Concentration ($g \cdot L^{-1}$)	Volume ($mL \cdot L^{-1}$)
$NaNO_3$	25	5
$CaCl_2 \cdot 2H_2O$	2.5	5
$MgSO_4 \cdot 7H_2O$	7.5	5
K_2HPO_4	7.5	5
KH_2PO_4	17.5	5
NaCl	2.5	5
Na_2EDTA	50	0.5
KOH	31	0.5
$FeCl_3 \cdot 6H_2O$ + 1-mL H_2SO_4	4.84	0.5
H_3BO_3	11.42	0.5
$MnCl_2 \cdot 4H_2O$	1.44	0.5
$ZnSO_4 \cdot 7H_2O$	8.82	0.5
MoO_3	0.71	0.5
$CuSO_4 \cdot 5H_2O$	1.57	0.5
$Co(NO_3)_2 \cdot 6H_2O$	0.49	0.5

3.4 Preparing bivalves for experiments

Because the presence of sediments could have influenced metal accumulation by bivalves, experiments were not conducted in the stock culture. Individual bivalves were transferred into separate 2-L plastic beakers filled with dechlorinated tap water that was aerated with an air stone (Figure 3.1). Bivalves were placed inside a 60-mL plastic cup, weighed down by glass marbles, containing a plastic egg holder that allowed the bivalves to remain upright so that they could feed (Figure 3.1). Beakers, cups, marbles and egg holders were acid washed prior to experiments. The beakers were placed on padded shelves to minimize vibrations that might alter bivalve behaviour. Bivalves were fed daily during the acclimation period and the water was changed every 2 days.

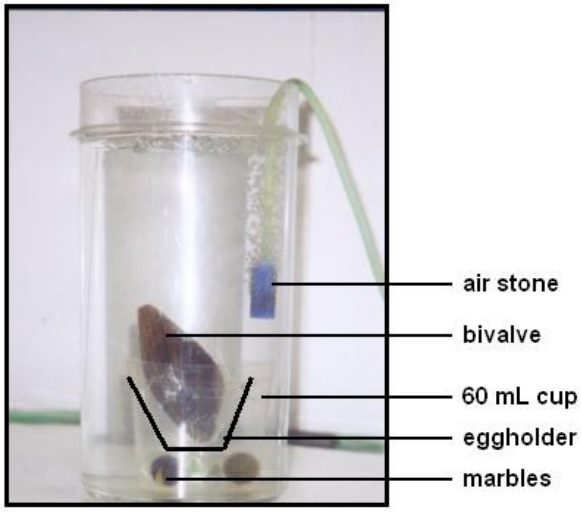


Figure 3.1: Bivalve in experimental beaker. / Bivalve dans un bécher expérimental.

Before an experiment, bivalves were allowed to acclimate for 4-5 days in the experimental set-up. To promote bivalve filtration during the experiment, bivalves were starved 24-h prior to an experiment and the tap water was replaced with a fresh synthetic medium, the chemical properties of which resembled those of tap water (Table 3.2). The purpose of the synthetic medium was to minimize the concentration of organic matter that might bind dissolved Cd in the medium and thereby reduce its uptake by the bivalves. The synthetic medium was prepared a week in advance and its pH was adjusted (average pH 7.1) prior to use.

Table 3.2: Chemical composition of the synthetic medium used during experimental studies on *Pyganodon grandis* and solutions used to prepare the synthetic medium. / Composition chimique du milieu artificiel utilisé durant les études sur *Pyganodon grandis* et liste des solutions utilisées pour préparer le milieu artificiel.

		Solutions used		
Element	**Final concentrations in synthetic medium ($mg \cdot L^{-1}$)**	**Compound**	**Concentration ($g \cdot L^{-1}$)**	**Volume ($mL \cdot L^{-1}$)**
Ca	26	$Ca(OH)_2$	2.6	60
Mg	3	$MgSO_4$	3	6
K	2	NaCl	2	6
Na	3	Na_2CO_3	5	6
SO_4	28	$NaNO_3$	0.17	6
NO_3	0.17	KCl	2	6
CO_3	5			
Cl	2			

3.5 Radiochemistry

Radioactive cadmium-109 was used to measure Cd accumulation in bivalves from either dissolved or dietary sources. A Gamma counter (Wallac 1420 Wizard^{TM} Automatic Gamma Counter) was used to measure ^{109}Cd in bivalves after experimental exposures. The counting window for ^{109}Cd was between 16 and 32 keV with a peak maximum at 22 keV. The counting period was fixed at 2,000 s (33 min) which insured a minimum of 80,000 counts (average error of 1%). Cadmium concentrations in samples were calculated as follows:

- CPM values (counts per minute) given by the Gamma counter for the blank samples (background noise) were subtracted from those given for the bivalve samples (i.e., organs)
- CPM values were then divided by the counting efficiency of the Gamma counter (35.2 ± 3.5%) to obtain the DPM values (disintegrations per minute). Counting efficiency was determined by analyzing a standard having a known concentration of ^{109}Cd and verifying (in percentage) how much of the ^{109}Cd was effectively detected by the Gamma counter.
- DPM values were transformed into DPS (disintegrations per second) by dividing the DPM values by 60.
- The DPS values were then divided by 1.777×10^{-8} (radioactivity constant for Cd obtained by dividing ln 2 by the half-life, in seconds, of ^{109}Cd) to obtain the number of ^{109}Cd atoms.
- These results were then divided by Avogadro's number (6.023×10^{23}) to give the number of moles of ^{109}Cd.
- These results were divided by the adjusted isotopic ratio "A" (eq. 3.1) to obtain the total number of moles of Cd.

$$A = (^{109}Cd)/(\text{Total Cd}) \qquad \text{(eq. 3.1)}$$

where (^{109}Cd) is the number of moles of ^{109}Cd, and (Total Cd) is the total number of moles of Cd (^{109}Cd + ^{112}Cd)

- The results were then divided by the weight of the sample to get a value in $mol \cdot g^{-1}$ (fresh weight).

The isotopic ratio "A" takes into account the decreasing radioactivity of ^{109}Cd (disintegration), the description of the Cd stock solution provided by the manufacturer (specific activity, volume, certification date), as well as the date of the experiment (i.e., the number of days between the certification date and the date of analysis). The following equation is used to calculate the disintegrations of ^{109}Cd:

$$N = N_0 \cdot e^{-\ln 2 \cdot t / 462.3} \quad (3.2)$$

where t is the number of days elapsed between the certification date and the day that the samples were analyzed, N_0 is the specific activity of the stock solution on the certification date, N is the specific activity of the solution on the experiment date and 462.3 represents the half-life in days for ^{109}Cd.

3.6 Statistical analyses

All statistical results were calculated using the software SYSTAT (version 10, SPSS Science Marketing Department, Chicago, IL, USA) and SigmaPlot (version 8.0). Values are given as means ± standard errors unless otherwise stated. The standard error is calculated by dividing the standard deviation (σ) by the square root of the sample size ($\sqrt{n}$). Relationships are presented by either a linear regression or a non-linear regression using the least squares approach. The goodness-of-fit of each regression was assessed using standard statistics (coefficient of determination, r^2, and probability level). Differences between control and test groups were determined using either the Student's t-test or a one-way analysis of variance (ANOVA). If a significant difference was found, *a posteriori* tests were performed using the Tukey method. Differences were considered significant at $p<0.05$.

4. BIVALVE CLEARANCE AND FILTRATION RATES

4.1 Introduction

In aquatic ecotoxicology, using bivalves as sentinel organisms to monitor aquatic contaminants has become increasingly common (see Section 1). Not only are these organisms exposed to dissolved contaminants but also to a wide variety of particles that may also be a source of contaminants. Bivalves, and other molluscs, are capable of concentrating metals from their environment, even when the metals are present at low concentrations (Beckvar *et a*l. 2000; Beckvar *et a*l. 2000). Bivalve molluscs have also been used as biosentinels, using valvometer techniques, due to their capacity to respond to a sudden change in water quality including the presence of contaminants (Tran *et a*l. 2003; Tran *et a*l. 2003).

In some studies, aqueous metal uptake by bivalves has been quantified without taking into account the animal's ventilation-control mechanisms or by starving the animals for a prolonged period (> 2 weeks) (Cassini *et a*l. 1986; Chong and Wang 2001; Borchardt 1985). Such studies are not very realistic because the metal influx rate measured without food particles is not likely to be representative of metal influx rates in a natural environment where food particles are present in varying quantity and quality (Fournier *et a*l. 2005). A compromise would be to allow a short feeding period in a separate tank where the animals would be fed in a non-contaminated medium with non-contaminated food. This would help promote natural filtration rates during the exposure period without exposing the animals to any dietary metal exposure.

The reason that some researchers ignore bivalve ventilatory activity during their studies is that they believe that bivalves were unable to adjust their filtration rates (Jorgensen 1990). On the contrary, many studies have indicated that bivalve ventilation activity and filtration is greatly dependent on environmental factors such as pH, temperature, food availability and food quality (Hornbach *et al.* 1984; Way *et al.* 1990; Hornbach *et a*l. 1984; Foe et Knight 1986; Way *et a*l. 1990; Lei *et al.* 1996). Moreover, several

studies have shown that metal availability to bivalves is not only linked to aqueous metal speciation, but also to the bivalve's respiratory physiology (Waitling et Waitling 1982; Tran *et a*l. 2002; Fournier *et a*l. 2005). These studies emphasize the importance of taking into account the ventilatory activity of filter-feeding bivalves in studies regarding the bioavailability of contaminants.

Another environmental factor that has been shown to influence filtration rates in bivalves, and hence, metal uptake, is the concentration of dissolved oxygen in the water. In a given aquatic environment, oxygen concentrations can vary widely, with low values often being noted near the sediment surface where bivalves generally live (Horne et Goldman 1994). Bivalves are known to tolerate extended periods of hypoxia and anoxia (Sobral et Widdows 1997; Sobral and Widdows 1997; Widdows *et al.* 1989; Wang et Widdows 1993). Surprisingly, although numerous studies of respiratory physiology have demonstrated the major role of O_2 as a ventilatory driver in water-breathers, few studies have taken into account this factor in studying metal uptake in these animals. Tran *et al.* (2001), however, showed that cadmium uptake in *Corbicula fluminea* was enhanced when the bivalves were exposed to hypoxic conditions, presumably due to increased ventilatory activity, again illustrating the importance of considering bivalve filtration rates when studying metal uptake.

The purpose of the present study was to measure both the clearance and filtration rates of *P. grandis* under laboratory conditions. For this study, clearance rate ($mL \cdot h^{-1}$) refers to the volume of water that is cleared of suspended particles per unit time, whereas filtration rate ($mL \cdot h^{-1} \cdot g^{-1}$) refers to the volume of water that passes through a bivalve per unit time (normalized for bivalve soft tissue weight). To our knowledge, such studies have not been previously performed on this bivalve species. Because O_2 levels can affect metal uptake in bivalves, *P. grandis* were exposed to hypoxic conditions to determine whether low dissolved oxygen levels have a significant effect on their filtration rates. The observations and conclusions obtained from this preliminary study were used to

establish an optimum laboratory protocol for subsequent experiments involving the exposure of *P. grandis* to aqueous and dietary cadmium.

4.2 Methods

4.2.1 Measuring bivalve clearance and filtration rates

A first study was performed on 10 animals held separately in individual 2-L plastic beakers filled with synthetic medium (Figure 3.1). Bivalves were placed in the beakers 4-5 days before the experiment and initially fed daily with *Pseudokirchneriella subcapitata* but were starved for 24-h prior to the experiment. Filtration rates were measured on 3 separate days between 10h00 and 17h00. Bivalves were given *P. subcapitata* at an initial density of ~7 x 10^5 cell·mL^{-1}, the cells being mixed into the medium using bubbling air stones. Each air stone was checked prior to the experiment to ensure that all were bubbling at the same intensity. The medium was allowed to stabilize for 30 min before taking an initial sample and subsequent samples were taken every hour for a minimum of 4-h. The 1-mL samples were diluted in 9-mL of Isoton III (balanced electrolyte solution, Beckman Coulter), for a 1/10 dilution, and cell density was determined using a particle counter (Beckman Coulter Counter, Multisizer 3). A control beaker, with no bivalve, was set up to see if there was any change in algal density due to growth or sedimentation. Filtration rates were measured by determining the clearance rate (i.e., volume of water cleared of algae per unit of time in the experimental beakers). Coughlan's equation (Coughlan 1969; Tran *et a*l. 2000) was used to calculate *FR*, the bivalve's filtration rate:

$$FR = \frac{V \cdot \left\{ \left(\ln(d_o) - \ln(d_f) \right) - \left(\ln(d_o') - \ln(d_f') \right) \right\}}{(t \cdot M)} \qquad (4.1)$$

where *FR* = the filtration rate (ml·h^{-1}·g^{-1} fresh weight); *V* = the volume of water in each beaker (mL); ln(d_o) and ln(d_f) = the natural logarithm of algal densities (algae·mL^{-1}) at the beginning and end of the measurement period, respectively; ln(d_o') and ln(d_f') =

algal densities (algae·mL^{-1}) in the control chamber without a bivalve, at the beginning and end of the measurement period, respectively; t = the duration of each exposure period (h); and M = body mass (fresh weight, g). It is important to note that four assumptions are associated with the use of eq. 4.1, that is: the concentration of algae in the water was homogeneous; the decrease in algal density was due only to animal filtration, as confirmed by the lack of significant change within the control beaker; all the algae passing over the gills were retained; and the filtration rate was constant throughout the measurement period.

Bivalve appearance was also noted throughout the experiment. This included noting (1) if the inhalant valve was fully open (i.e., mantle can be seen between the shells and the siphons are open wide enough that one can see inside the bivalve), partially open (i.e., mantle cannot be seen and siphons are open but one cannot see inside the bivalve), or closed; and (2) the presence of pseudofeces (clumps or strings of uneaten algae found near the exhalant valve). These observations were done without the aid of a video camera. Bivalve appearance/behaviour was observed and noted every 30 min during the 4 h experiment.

4.2.2 Measuring filtration rates under hypoxic conditions

To measure the effects of hypoxia on the ventilation rate of *P. grandis*, the filtration rates of 11 bivalves were first measured under normoxic conditions (~10 mg·L^{-1}) as described above (Section 4.2.1) with the exception that: i) beakers were placed on magnetic plates and the medium was mixed by a magnetic stirrer rather than an air stone; ii) the cups in which the bivalves were held inside the beaker (Figure 3.1) were raised on platforms to allow better water circulation; iii) bivalves were split into 4 groups of 2 or 3 bivalves for these experiments.

For experiments under hypoxic conditions, dissolved oxygen concentrations in the experimental beakers were lowered to 2 mg·L^{-1} (measured with a portable dissolved oxygen meter; YSI Inc., Model 50B) by bubbling nitrogen into the synthetic water. Once

the desired O_2 level (2 mg·L^{-1}) was reached, the bivalves were allowed to acclimate overnight in the experimental beakers, which were sealed and left on the magnetic plates without any stirring. The next morning, O_2 levels, which had risen during the night, were decreased from ~5 mg·L^{-1} to 3 mg·L^{-1} by additional bubbling with N_2 and algae were added to the medium. The whole set-up was allowed to stabilize for 1-h before taking an initial sample. There was very little risk that the bivalves would consume any algae during this period as they tended to be closed due to the stress of being handled and the sudden movement of the magnetic stirrer. Algal density was measured every hour for a minimum of 4-h as described above (Section 4.2.1), and the filtration rates were calculated (eq. 4.1). The filtration rates obtained during hypoxic conditions were compared to those obtained under normoxia and any significant differences were noted.

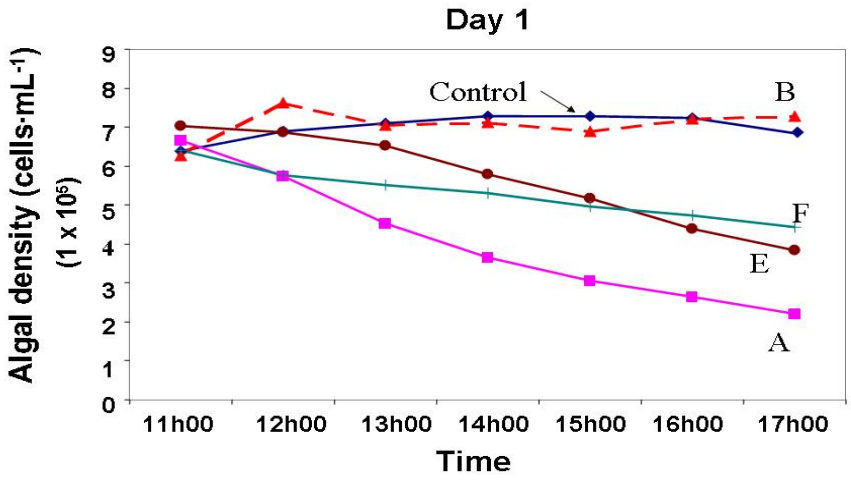
In a third experiment, 6 bivalves were exposed to dissolved oxygen concentrations of < 1 mg·L^{-1}. As with the previous experiment, bivalve filtration rates were initially obtained under normoxic conditions. Later, the same bivalves were exposed to hypoxic conditions. As before, dissolved oxygen concentrations in the experimental beakers were lowered to 2 mg·L^{-1} and the bivalves were allowed to acclimate overnight in the experimental beakers. The next morning, O_2 levels, which had risen in the interim, were decreased from ~5 mg·L^{-1} to < 1 mg·L^{-1} and algae were added to the medium. Oxygen levels were measured every hour and adjusted as needed. Following the same protocol as described above, filtration rates obtained during hypoxic conditions were compared with those obtained under normoxia and any significant differences were noted.

4.3 Results

Consumption of algae varied not only among individual bivalves, but also from day to day for each individual bivalve (Figure 4.1). Algal density (cells·mL^{-1}) varied little in the control beaker (Figure 4.1). Whereas the clearance rate for some bivalves was relatively constant among days (e.g., bivalves A, F, G. H and I), clearance rates varied from day to day for other bivalves (e.g., bivalves B, C, D, E and J) (Table 4.1). On a given day, some bivalves had similar clearance rates (e.g., bivalves A and E on day 1; Figure 4.1).

Overall, bivalve clearance rates varied from < 1 to 550 $mL \cdot h^{-1}$. The variability observed in feeding was reflected in the wide range of filtration rates calculated for *P. grandis*, which varied from < 2 to 62 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt) for an average of 26 ± 4 $mL \cdot h^{-1} \cdot g^{-1}$ (mean ± SE; fresh wt) for the 10 individuals over the 3 days (Table 4.2). A Kruskal-Wallis one-way analysis of variance determined that bivalve size, which varied from 4.6 to 10.3 g, had no significant influence of bivalve clearance or filtration rates.

Bivalve behaviour, as noted by valve opening and production of pseudofeces, also varied daily among bivalves. In general, filtration rates seem to be related to the width of the opening of the inhalant valve; bivalves whose inhalant valves were fully open had higher filtration rates as opposed to those whose inhalant valves were partially opened or closed (Figure 4.2). Few pseudofeces were produced throughout the experiment, but some pseudofeces were present on bivalves that had fed for 3 consecutive days (e.g., bivalves A, F, and G).



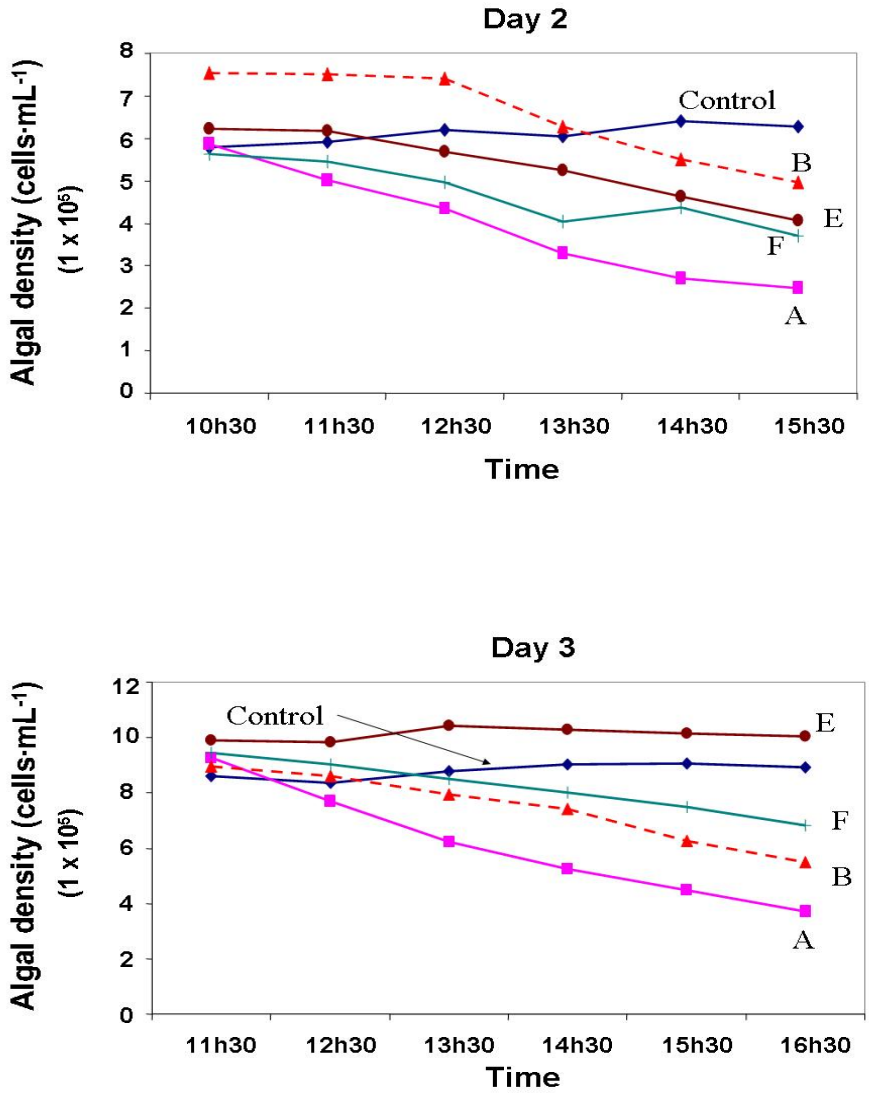


Figure 4.1: **Decreasing algal concentrations in feeding beaker (cells·mL^{-1}), over three consecutive days. Values for the empty control beaker are also shown (solid line with diamonds). Each letter (A, B, …) corresponds to one bivalve. / Diminution de la concentration de cellules algales dans la chambre expérimentale (cellules·mL^{-1}). Les résultats pour le témoin (chambre expérimentale sans bivalves) sont aussi présentés (ligne avec diamant). Les expériences ont été menées pendant trois jours successifs. Chaque lettre (A, B, …) correspond à un bivalve particulier.**

Table 4.1: Daily mean clearance rates for *Pyganodon grandis* (mL·h^{-1} ± SE). / Voumes d'eau moyens filtrés quotidiennement par *Pyganodon grandis* (mL·h^{-1} ± erreur type).

Bivalve	Day 1	Day 2	Day 3
A	453 ± 30	376 ± 62	382 ± 20
B	36 ± 6	199 ± 27	209 ± 47
C	61 ± 30	191 ± 51	209 ± 43
D	60 ± 20	65 ± 15	307 ± 20
E	285 ± 43	200 ± 46	9 ± 5
F	207 ± 22	200 ± 40	145 ± 15
G	126 ± 60	143 ± 50	122 ± 30
H	238 ± 21	205 ± 11	219 ± 66
I	65 ± 15	70 ± 20	32 ± 15
J	272 ± 25	481 ± 57	0

Table 4.2: Daily and mean filtration rates (mL·h^{-1}·g^{-1} fresh wt) of *Pyganodon grandis*. / Taux de filtration journaliers et moyens (mL·h^{-1}·g^{-1} poids frais) observés chez *Pyganodon grandis*.

Bivalve	Day 1	Day 2	Day 3	Mean ± SE (n = 3)
A	52.4	43.6	44.6	46 ± 3
B	5.5	30.5	32.1	23 ± 8
C	8.1	25.3	27.8	20 ± 6
D	5.7	6.2	29.8	14 ± 8
E	58.9	41.4	1.9	34 ± 17
F	47.9	46.1	33.4	42 ± 4
G	15.1	17.3	14.7	16 ± 1
H	30.3	26.1	27.8	27 ± 1
I	6.8	7.2	3.4	6 ± 1
J	35.1	61.9	0.0	32 ± 18
Mean ± SE (n = 10)	27 ± 7	31 ± 6	21 ± 5	

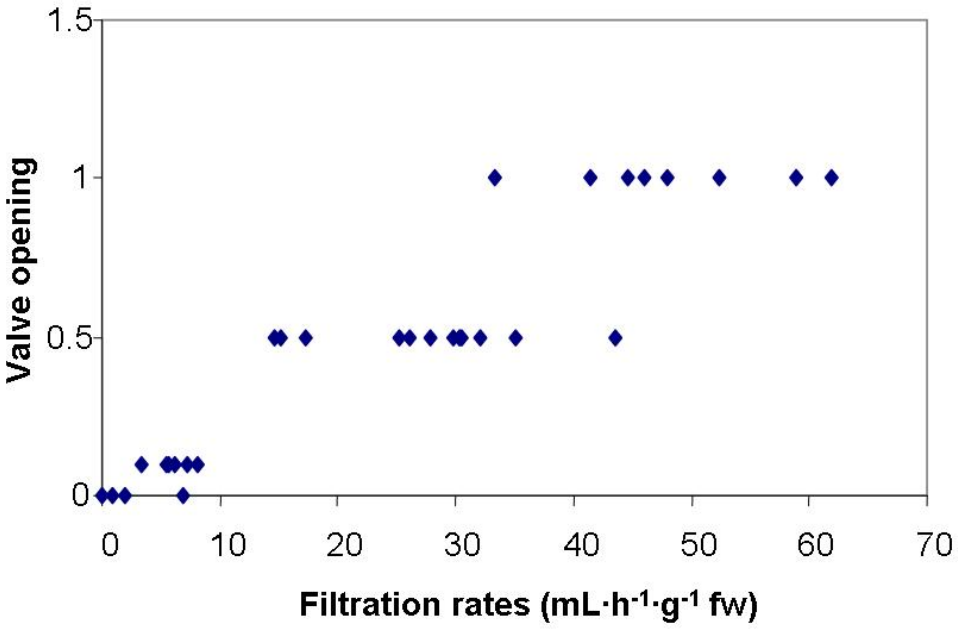


Figure 4.2: Relationship between width of inhalant valve opening and filtration rates (mL·h^{-1}·g^{-1} fresh wt) of *Pyganodon grandis*. A valve opening of 1 signifies that inhalant valve was fully open; a value of 0.5 signifies that inhalant valve was partially opened; and a value of 0.1 or 0 signifies that the inhalant valve was nearly closed or completely closed. / Relation entre la largeur de la valve inhalante et les taux de filtration (mL·h^{-1}·g^{-1} poids frais) de *Pyganodon grandis*. Une valeur de 1 pour l'ouverture de la valve inhalante signifie que la valve inhalante était ouverte au maximum; une valeur de 0,5 signifie que la valve inhalante était partiellement ouverte; une valeur de 0,1 ou de 0 signifie que la valve inhalante était presque fermée ou complètement fermée.

In general, bivalve filtration rates did not differ significantly when bivalves were exposed to hypoxic conditions. Out of 11 bivalves, only two (A and G) had significantly different filtration rates under hypoxic conditions from those measured under normoxia, and their responses were different (Figure 4.3): filtration rates for bivalve A were higher under normoxia whereas higher filtration rates were measured for bivalve G under hypoxic conditions. Filtration rates for the bivalves under hypoxic conditions varied from < 2 to 45 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt) and had a mean of 13 ± 3 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt). This range and mean are similar to those observed for the same bivalves previously exposed to normoxia, that is, < 2 to 50 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt) with a mean of 11 ± 2 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt).

In spite of this apparent similarity in filtration rates, other evidence suggests that bivalve behaviour is influenced by the hypoxic conditions. Thus the inhalant valves of 7 bivalves out of 11 were open at their maximum width more often under hypoxic conditions than when the same bivalves were under normoxic conditions (Figure 4.4). Bivalves also produced more pseudofeces under hypoxia, as clumps or strings of pseudofeces were present near the exhalant valve of every bivalve exposed to hypoxic conditions. Such clumps or strings were not present when bivalves were exposed to normoxic conditions.

No filtration rates could be obtained from bivalves exposed to extreme hypoxia (dissolved oxygen levels < 1 $mg \cdot L^{-1}$), as bivalves closed their valves and shells within 1 h of exposure. However, prior to valve closure, bivalves were completely open; shells and inhalant valves were opened to their fullest, but no detectible decrease in algal densities was observed. Once the bivalves had closed, they would not reopen for 3-4 days.

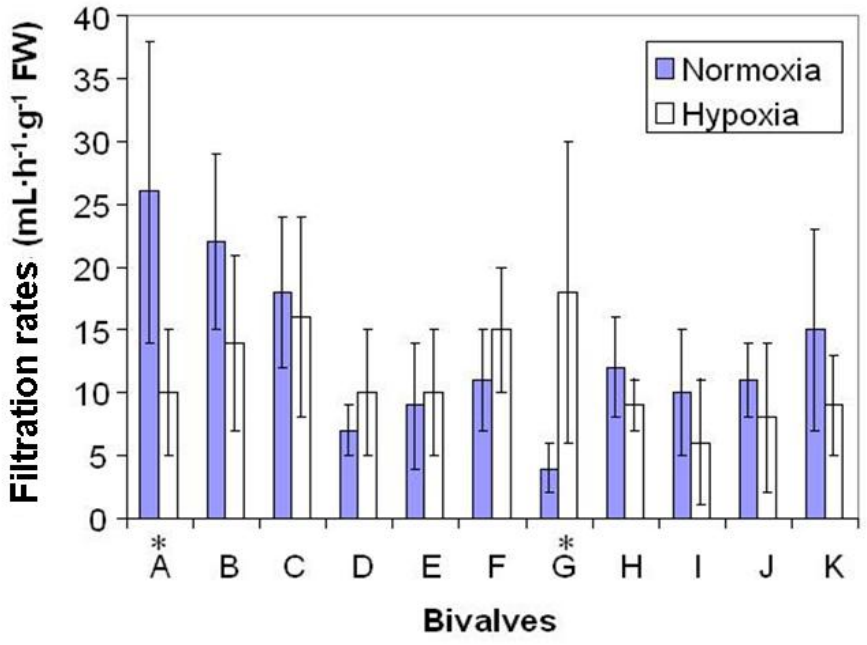


Figure 4.3: Mean filtration rates (± SE) of *P. grandis* (mL·h^{-1}·g^{-1} fresh wt) exposed to normoxia ([O_2] 10 mg·L^{-1}) and hypoxia ([O_2] 3 mg·L^{-1}). An asterisk denotes that a significant difference between filtration rates was observed. / Taux de filtration moyens (± erreur type de la moyenne) des bivalves exposés en normoxie (O_2 : 10 mg·L^{-1}) et en hypoxie (O_2 : 3 mg·L^{-1}). Un astérisque signifie qu'une différence significative a été observée entre les taux de filtration.

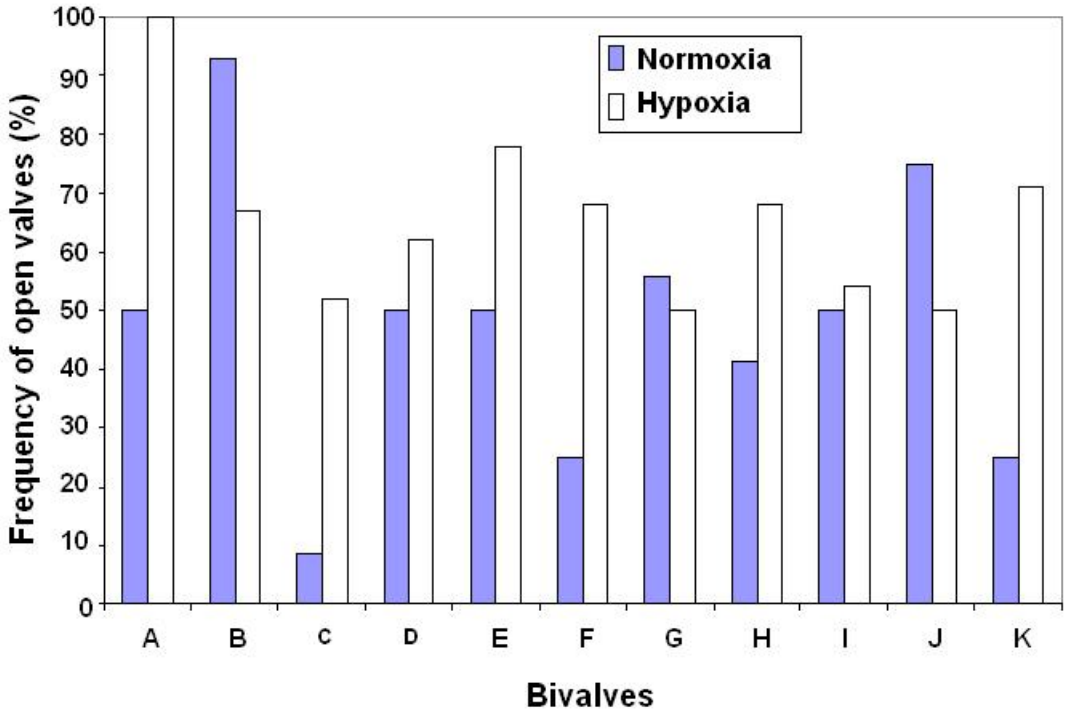


Figure 4.4: Percentage of times (n = 6) that inhalant valves were seen to be fully open in bivalves initially exposed to normoxia (10 mg·L^{-1}) and then to hypoxia (3 mg·L^{-1}). / Pourcentage de fois que les valves inhalantes des bivalves étaient ouvertes au maximum en normoxie (10 mg·L^{-1}) et, ensuite, en hypoxie (3 mg·L^{-1}).

4.4 Discussion

Interest in modelling the feeding behaviour of suspension-feeding bivalves has grown in recent years in response to two important demands. First, the cultivation of oysters and mussels has led to a demand for models of growth that can be used to evaluate the production potential of shellfish growing areas (Bayne 1998; Bayne 1998). Second, in order to properly use bivalves for environmental monitoring purposes, researchers need to understand the behavioural, physiological and environmental factors that influence the availability of contaminants to bivalves (Tran *et a*l. 2003).

According to Bayne (1998), bivalve suspension feeding is a complex synergy between behavioural and physiological traits, which are responsive to a variety of environmental factors. Although an extensive literature deals with water transport and particle retention in suspension-feeding bivalves, our knowledge of the mechanisms involved is still incomplete. This lack of knowledge stems in part from the difficulty of creating a natural feeding regime under laboratory conditions.

MacGinitie (1941) was the first to stress the importance of studying undisturbed animals because, according to him, even slightly disturbed bivalves stop feeding normally but may continue to transport water and retain particles. He, therefore, dismissed all studies made on disturbed animals. Jorgensen (1975b; Jorgensen 1975b) noted that bivalves are also subjected to environmental factors that disturb or enhance their feeding behaviour in the wild. He concluded that, although a detailed analysis of the mechanisms of feeding in bivalves does imply some disruptions, the variability in bivalve feeding behaviour obtained under laboratory conditions may be similar to that observed in nature.

The clearance and filtration rates for *P. grandis* fall within the range of those observed for the freshwater bivalves *C. fluminea*, *Anodonta sp. and Unio sp.* (Table 4.3). It was not surprising to observe a large inter-individual variability in bivalve filtration rates for our study, as this phenomenon has been well documented in other studies (Bayne 1998;

Bayne *et a*l. 1993; Widdows 1985). What was surprising was the large variability observed for each individual bivalve. Nowhere is it described in the scientific literature that a bivalve's filtration rate can vary so greatly from one day to another. Knowledge of individual variability should be taken into consideration when conducting ecological and toxicological studies on these animals (Bayne 1998). For example, the uptake rate of dissolved metal and the assimilation rate of metal in food ingested by a bivalve have been reported to be highly dependent on bivalve filtration rates (Wang 2001; Tran *et a*l. 2002).

Food quantity and quality have been shown to strongly influence bivalve feeding behaviour. Tran *et al.* (2002) and Fournier *et al*. (2005) noted that filtration rates for *C. fluminea* were strongly dependent on algal concentrations, as low algal numbers induced higher filtration rates in *C. fluminea*. As shown in Table 4.2, Fournier *et al.* (2005) measured higher filtration rates for *C. fluminea* than did Tran *et al.* (2002). Fournier *et al.* (2005) attributed this discrepancy to the bivalves having been fed different algal species, which may have had different nutritional qualities. Thus caution must be used when comparing results among studies.

Another environmental factor that can influence filtration rates in bivalves is dissolved oxygen concentrations. Low oxygen concentrations can be found in eutrophic lakes subject to stratification during the summer months, in lakes in northern climates during late winter (minimal re-oxygenation under ice cover), and in freshly flooded reservoirs where the biodegradation of terrestrial organic matter leads to an increased benthic oxygen demand (Horne and Goldman 1994). In some slow-moving tropical rivers, dissolved oxygen concentrations can drop below 3 $mg \cdot L^{-1}$ during the summer months, generally as a result of plant respiration and decomposition (Wilcock *et al.* 1998; Wilcock *et a*l. 1998).

Table 4.3: Comparison of filtration and clearance rates among bivalve species. / Comparaison entre les taux de filtration et les volumes d'eau filtrés pour d'autres espèces de mollusques.

Bivalve species	*Rates*	Length of exposure	Reference
Filtration rates			
Marine	**($L \cdot h^{-1} \cdot g^{-1}$ DW)[a]**		
Crassostrea gigas	10 ± 4.5	1 h	(Jorgensen 1975a; Jorgensen 1975a)
Perna perna	3.9 ± 1.6	0.5 h	(Anandraj *et al.* 2002; Anandraj *et a*l. 2002)
Perna perna	1.04 to 2.5	1 h	(Pessatti *et al.* 2002; Pessatti *et a*l. 2002)
Freshwater	**($mL \cdot h^{-1} \cdot g^{-1}$ FW)[b]**		
Anodonta piscinalis	14 ± 6	?	(Lewandowski et Stanczykowska 1975)
Corbicula fluminea	20 to 150	1 h	(Prokopovich 1969)
Corbicula fluminea	10 to 70	1 h	(Tran *et a*l. 2000; Tran *et a*l. 2001)
Corbicula fluminea	13 ± 1 to 285 ± 23	1 h	(Tran *et a*l. 2002)
Corbicula fluminea	40 to 800	0.25 - 0.5 h	(Fournier *et a*l. 2005)
Pyganodon grandis	26 ± 12 (2 to 61)	4 -5 h	Present study
	($L \cdot h^{-1} \cdot g^{-1}$ DW)		
Pyganodon grandis	0.5 ± 0.2 (0.05 to 1)	4 -5 h	Present study
Clearance rates			
Marine	**($L \cdot h^{-1}$, per animal)**		
Mytilus trossulus	1 to 4	4 h	(Widmeyer *et al.* 2004)
Freshwater	**($L \cdot h^{-1}$, per animal)**		
Andonta anatina	0.2 to 1.2	3 h	(Mills et Reynolds 2002)
Anodonta cygnea	0.3 to 2.0	3 h	(Mills and Reynolds 2002)
Unio pictorum	0.15 to 0.7	3 h	(Mills and Reynolds 2002)
Unio tumidus	0.2 to 1.0	3 h	(Mills and Reynolds 2002)
Pyganodon grandis	< 0.1 to 0.5	4 h	Present study

a,b: mean values (± SD) or ranges in clearance and filtration rates, as given in the literature.

In bivalves, hypoxia may be caused by shell valve closure and/or depletion of oxygen in the surrounding water; physiological responses to hypoxia vary among bivalve species. Physiological compensatory mechanisms induced by environmental hypoxia can include an increase in water pumping/filtration and/or blood circulation (Herreid 1980), or the suppression of aerobic metabolism and an increase in anaerobic metabolism (De Zwann *et al.* 1991; Shick *et al.* 1986; Widdows *et a*l. 1989; Wang and Widdows 1993). Suppression of aerobic metabolism serves to conserve energy, and enables bivalves to survive hypoxia and anoxia for limited periods of time.

In our study, dissolved oxygen concentrations under hypoxic conditions (3 $mg \cdot L^{-1}$) were not sufficiently low to induce a significant change in filtration rates for *P. grandis* (Figure 4.3). These results contrast with those noted by Tran *et al.* (2001), who observed a significant increase (by a factor of 4) in filtration rates for *C. fluminea* exposed to oxygen levels as low as 3 $mg \cdot L^{-1}$. Our results do, however, agree with those obtained by Sobral and Widdows (1997), who observed that filtration rates of the tropical marine bivalve *Ruditapes decussatus* were unaffected by dissolved oxygen concentrations of 3 $mg \cdot L^{-1}$. Sorbal and Widdows (1997) did, however, observe a significant decrease in filtration rates when *R. decussatus* was exposed to extreme hypoxic conditions ($[O_2] < 1$ $mg \cdot L^{-1}$).

We were unable to measure filtration rates for *P. grandis* when dissolved oxygen levels were decreased to < 1 $mg \cdot L^{-1}$. The bivalves' behaviour was strongly affected by the decrease in oxygen levels. Few bivalves remained open long enough for us to attempt to measure filtration rates, and those that did remain open did not consume any algae. After further consideration, this result is perhaps not surprising, given that *P. grandis* is a freshwater bivalve that is able to survive several months of hibernation buried in sediments. It is possible that by exposing *P. grandis* to hypoxic conditions, we may have induced a hibernation-like response. It is possible that *P. grandis* survives hypoxia by lowering its metabolic rate or by utilizing anaerobic processes. Metabolic arrest

processes have been shown to increase hypoxia tolerance in *Mytilus* by a factor of 20, in diving turtles by a factor of 60, and in brine shrimp embryos by several orders of magnitude (Hochachka 1986). By arresting metabolism at an early stage in response to external stresses, animals can limit or prevent metabolic and cellular damage.

4.5 Conclusions

The objective was to observe *P. grandis* in a laboratory setting in order to adapt the experimental design of any subsequent experiments to this bivalve. We observed that clearance and filtration rates for *P. grandis* vary not only among bivalves, but also daily in individual bivalves. Despite the large variability observed in bivalve feeding behaviour, clearance and filtration rates for *P. grandis* were similar to those observed for other freshwater bivalve species under laboratory conditions. Based on our results and an examination of the literature, the following points need to be considered when establishing the protocols for future experiments.

4.6 Implications for subsequent experiments

- Temperature (15 °C) and dissolved oxygen concentrations (10 $mg \cdot L^{-1}$) should remain constant throughout the duration of the experiment as any changes in these parameters can affect bivalve behaviour.
- *P. grandis* should be fed the green alga *P. subcapitata* alone, not in a mixture, during both the acclimation period and the experimental exposure to ensure that feeding behaviour will be similar for both sections. Because algal density has been shown to greatly affect filtration rates in other bivalve species, the initial algal density for our experiment should be no higher than 9×10^5 $cells \cdot mL^{-1}$ and should also not be allowed to drop below 2×10^5 $cells \cdot mL^{-1}$. This should ensure that bivalves will filter within the ranges observed during this study.
- Bivalves should be starved 24 h before an experiment to promote feeding and to reduce the production of pseudofeces which could affect the concentrations of algae in the feeding beaker and make it difficult to measure filtration rates.

- For dietary cadmium exposures, the duration of feeding experiments should be at least 4 d to allow for 4 feeding periods so as to reducing the influence of variations in filtration rate over time. This will also increase the chance of obtaining data in case a bivalve does not eat for a day or two.
- Bivalve behaviour (e.g., valve opening) should be monitored throughout future experiments so as to ensure that the experimental conditions (i.e., pH, temperature, photoperiod) are not inhibiting bivalve feeding behaviour and filtration rates.

5. AQUEOUS CADMIUM EXPOSURES

5.1 Introduction

Contamination of marine and freshwater environments by metals has become a global problem. In the field of aquatic ecotoxicology, several attempts have been made to use aquatic species as sentinel organisms, as they can reveal spatial and temporal variations in bioavailable metal concentrations and overall water quality. The use of bivalves as sentinels is becoming more and more common because of their capacity to concentrate metals from their environment, even when these metals are present in very low concentrations (Beckvar *et a*l. 2000). Moreover, these animals are exposed to both dissolved and particulate contaminants due to the large amount of water and suspended material that they pump through their mantle cavities, increasing their exposure to contaminants.

The accumulation of dissolved metals, such as Cd, Cu and Zn, in natural populations of the freshwater bivalve *Pyganodon grandis* has been studied by INRS researchers for more than 20 years (Tessier *et a*l. 1993; Couillard *et a*l. 1993; Couillard *et a*l. 1995b; Couillard *et a*l. 1995a; Wang *et a*l. 1999; Giguère *et a*l. 2003; Perceval *et a*l. 2002; Bonneris *et a*l. 2005a). The results compiled from these field studies suggest that *P. grandis* may be used as a sentinel species for metallic contamination because it is capable of concentrating metals, notably Cd, from its environment, and because it appears to tolerate a wide range of metal contaminants (suggesting the existence of effective detoxification mechanisms). However, despite these studies, it is still unclear whether water or food is the main source of Cd for this bivalve. If *P. grandis* is to be used as a Cd biomonitor, and if we are to be able to interpret spatial or temporal changes in its Cd burdens, it will be important to complement previous field studies with laboratory studies designed to quantify the uptake of waterborne and diet-borne Cd for this animal under controlled conditions, and then use these results to estimate the relative importance of waterborne and diet-borne Cd in the field.

The majority of laboratory and field studies focusing on Cd accumulation in bivalves have been conducted on marine species – mussels, oysters, scallops, etc. – and many field monitoring programs (e.g., "Mussel Watch") rely on measurements of contaminant accumulation in marine species. However, more studies focusing on metal uptake in freshwater bivalves are needed for several reasons. Firstly, for a given dissolved Cd concentration, marine species are less likely to suffer a toxic effect due to Cd because of the Cl^{-1} ions present in high concentrations in seawater, which tend to reduce cadmium accumulation and its toxicity (McLusky *et a*l. 1986; Sunda *et al.* 1978). Secondly, many of the laboratory studies conducted on marine and freshwater species have exposed animals to very high concentrations of dissolved cadmium (up to 500 $\mu g \cdot L^{-1}$), making it difficult to extrapolate the results to natural situations (Table 5.1). Thirdly, metal accumulation can differ greatly among different bivalve species. For example, studies have shown that filter-feeding mussels, such as *Mytilus edulis*, have different "metal accumulation strategies" (Rainbow 2002) than deposit-feeding mussels, such as *Scrobicularia plana* (Southgate *et al.* 1983; Bryan et Hummerstone 1978). Also, studies focusing on metal accumulation in freshwater bivalve species are needed as these organisms can be used to monitor the impact that mining and smelting activities have on life in lakes and rivers. Overall, more studies exposing freshwater bivalves to environmentally relevant concentrations of metals are needed if these organisms are to be used as sentinel species.

A factor that was typically neglected in earlier laboratory studies, and that can potentially influence dissolved Cd accumulation in bivalves, is the animal's filtration rate. Several studies have reported that metal accumulation is influenced by filtration rates (Waitling and Waitling 1982; Tran *et a*l. 2002; Tran *et a*l. 2001; Fournier *et a*l. 2005). Due to the findings in these studies, the present study will take into account the bivalve filtration rate and its influence on dissolved metal accumulation in the bivalve *P. grandis*.

Table 5.1: Examples of laboratory studies conducted on marine and freshwater bivalves exposed to high concentrations of dissolved cadmium. / Exemples d'études au laboratoire sur des espèces de bivalves marins et d'eaux douces exposées à des concentrations de cadmium dissous élevées.

Species	Exposure concentration		Exposure length	Reference
Marine	**(µg Cd·L^{-1})**	**(nM Cd)**		
Mercenaria mercenaria	100	900	1 h to 31 d	(Robinson et Ryan 1986)
Mytilus edulis	100	900	3 to 4 months	(Frazier 1986)
Macoma balthica	100	900	29 d	(Langston et Zhou 1987)
Crassostrea virginica	200	1800	21 d	(Roesijadi *et al.* 1989)
Mytilus edulis	400	3600	65 d	(Bebianno et Langston 1991)
Mytilus edulis	500	4400	5 to 12 d	(Ferrarello *et al.* 2000)
Freshwater				
Anodonta grandis grandis	5 to 50	45 to 445	22 d	(Malley *et al.* 1993)
Dreissena polymorpha	3 to 50	25 to 445	40 to 90 d	(Bias and Karbe 1985)
Dreissena polymorpha	0.1 to 12	0.9 to 110	2 d	(Stuijfzand *et al.* 1999)

To our knowledge, very few experiments have been devoted to measuring Cd accumulation in *P. grandis* under laboratory conditions and none have studied the influence of filtration rates on metal accumulation in *P. grandis*. Malley *et al.* (1993) exposed *P. grandis* to a range of dissolved Cd concentrations (0, 45, 90, 180 and 450 nM) for 22 d to determine (a) whether *P. grandis* could produce metallothioneins in response to Cd exposure, (b) which organs were the main site of MT production, and (c) whether there was a dose-response relationship between Cd concentrations in various organs and their MT concentrations. Malley *et al*. (1993) reported that Cd concentrations in the whole organism increased with increasing dissolved Cd concentrations and that MT concentrations in the gills were the only organ to show a statistically significant relationship with dissolved Cd concentrations. However, metal accumulation and MT production were not studied in the digestive gland.

The efflux rate of Cd is often slow in bivalves, with calculated biological half-lives varying from 14 to >1250 days for some species (Cossa 1989). The extremely low elimination rates observed for certain mollusc species are related to the bivalves'

detoxification mechanisms, which involve sequestering metals either by binding them to metallothioneins, or by forming granules that keep Cd inside the cell but in a non-toxic form (Amiard *et al.* 2006; Viarengo 1989) An earlier study that followed Cd efflux in *P. grandis* transplanted from a contaminated lake into a lesser contaminated one showed that metal elimination rates for this species were indeed very slow (half-life for Cd: $t_{½}$ = 315 d) (Tessier *et al*. 1987). However, the animals in this study had been subjected to prolonged chronic metal exposures, i.e., years, and efflux rates for *P. grandis* may be faster in animals subjected to short-term (h) exposures of dissolved Cd under laboratory settings.

The purpose of the present study was to observe the accumulation of dissolved Cd by *P. grandis* in a laboratory setting, and then to follow its subsequent loss when the bivalves were placed in a Cd-free environment. Bivalve filtration rates were also monitored throughout the experiment to determine how bivalve filtration influences dissolved Cd accumulation in *P. grandis*. The results from this study will later be used as inputs to a Cd bioaccumulation model that takes into account accumulation of Cd from water and food. These experiments were not meant to simulate the complexities of nature but merely to measure Cd uptake in *P. grandis* when exposure is to waterborne Cd alone.

5.2 Methods

5.2.1 Uptake of aqueous cadmium

For the first series of experiments, a total of 54 bivalves were exposed to several different concentrations of total dissolved Cd (radioactive ^{109}Cd + cold ^{112}Cd): 0.1, 0.5, 5 and 20 nM (specific activity of ^{109}Cd: 0.5 to 4 μCi·μg Cd^{-1}). There were 9 bivalves for each individual experiment; exposures at 0.5 and 5 nM were replicated (i.e. 2 x 9 bivalves). Before an experiment, bivalves were removed from their "stock culture" and placed separately in 2-L beakers filled with uncontaminated synthetic medium (described in Section 3.3). Bivalves were allowed to acclimate in the experimental set-up for 4-5 days and were fed daily with the green alga *Pseudokirchneriella subcapitata*.

Bivalve filtration rates were also measured during the acclimation period (Section 3.2). To reduce variability in our data caused by unequal decreases in dissolved Cd concentrations during the exposure periods, we sought to have similar average filtration rates in each exposure chamber (similar filtration rates should result in similar losses of Cd from solution). Three bivalves were grouped together in each chamber, such that each group contained a bivalve that had been identified as high filterer, an average filterer and a low filterer during the acclimation period. This approach ensured that similar amounts of dissolved Cd would be consumed by each group during the experiments. To minimize Cd binding by organic matter in the exposure aquaria, bivalves were starved during the last 24-h of acclimation to minimize the production of pseudofeces.

For every Cd concentration, four 6-L aquaria (3 experimental aquaria containing 1 group of bivalves each + 1 control aquarium without bivalves) were filled with synthetic medium that had been contaminated with dissolved Cd. Bivalves were exposed to the dissolved Cd for 3 consecutive days and fed daily with *P. subcapitata*. After 20-h each bivalve was transferred from its experimental aquarium into a separate 2-L beaker, filled with uncontaminated synthetic medium, and fed with uncontaminated algae (Figure 5.1). This procedure ensured that algae would not become contaminated during feeding. The filtration rate for each bivalve was measured (described in Section 4.2.1) during this feeding period. After 4-h, the bivalves were transferred back into their experimental aquaria for another 20-h exposure period. During the feeding period, the concentration of dissolved Cd in the exposure aquaria was returned to its initial concentration prior to the bivalves being placed back into the aquaria (Figure 5.1). After 72-h, the bivalves were placed in separate beakers to depurate overnight before dissection. In total, the bivalves were exposed to the dissolved Cd for 60-h during the 3-d exposure.

During dissection, organs (gills, digestive gland, mantle, foot and miscellaneous organs (including the gonads and intestines)) were separated and rinsed with EDTA (10^{-3} M) for 20 min to remove any Cd adsorbed to their surface. The organs were weighed

individually, placed in a scintillation vial and analyzed for ^{109}Cd using a Gamma counter. The water in the mantle cavity and the EDTA rinse water were also collected in scintillation vials and analyzed for ^{109}Cd. Cadmium concentrations in the organs were calculated according to the description provided in Section 3.4.

5.2.2 Cadmium efflux following exposure to aqueous cadmium

This second experiment consisted of two phases: (i) a waterborne Cd exposure; (ii) a depuration period. Fifteen bivalves were exposed to dissolved Cd (5 nM; ^{109}Cd + ^{112}Cd; specific activity: 0.5 mCi $^{109}Cd \cdot \mu g^{-1}$) for 3 consecutive days. As before (Figure 5.1), bivalves were fed daily by transferring them from the exposure aquaria into separate 2-L feeding beakers where their filtration rates were measured (described in Section 4.2.1). After the exposure period, three bivalves were sacrificed, after being allowed to depurate overnight, and their organs were analyzed for Cd to obtain Cd concentrations at t = 0 d of depuration.

For the second phase, the remaining 12 bivalves were allowed to depurate in separate 2-L beakers filled with uncontaminated synthetic medium that contained EDTA (10^{-3} M) to bind any inorganic Cd lost from the bivalves. The bivalves were fed daily with uncontaminated *P. subcapitata*, but their filtration rates were not measured during this period. The medium was changed daily and samples of the medium were also taken daily to measure aqueous ^{109}Cd concentrations in order to follow Cd elimination from each bivalve.

Six bivalves were sacrificed after 8-d of depuration whereas the remaining 5 animals (one bivalve died during the experiment) were sacrificed after 16 days. During dissection, organs were separated, rinsed with EDTA, weighed and placed in a scintillation vial, as before, and analyzed for ^{109}Cd using a Gamma counter. Cadmium concentrations in the organs were compared among groups in order to determine if there was any significant Cd loss over time. A mass balance was also calculated by adding the total Cd remaining in the depurated bivalves and the total amount of Cd eliminated (i.e.,

loss) by the bivalves during depuration. These values were then compared with the total accumulated Cd calculated for the reference bivalves.

5.2.3 Calculations

The absorption efficiency of Cd from the dissolved phase (α_w) was calculated for each Cd treatment for both the gills and the whole organism. The absorption efficiency represents the proportion of dissolved cadmium that the bivalve accumulated with respect to the total amount of aqueous Cd that it filtered over a given time period:

$$\alpha_w = \left[\Delta[Cd]_{bivalve} / \left(FR \cdot t \cdot [Cd]_w \right) \right] \quad (5.1)$$

where α_w = metal assimilation efficiency from dissolved phase (unitless, values range from 0.01 to 1), $\Delta[Cd]_{bivalve}$ = increase in Cd concentration in the bivalve after Cd-exposure (nmol·g^{-1} fresh wt), FR = filtration rate (L·h^{-1}·g^{-1} fresh wt), t = exposure duration (60 h), and $[Cd]_w$ = dissolved Cd concentration (0.1, 0.5, 5.0 and 20 nmol·L^{-1}). The calculated value is multiplied by 100 to be expressed as a percentage (as seen in the figures and tables).

5.2.4 Data analysis

The total Cd burden, as percentages, in bivalve organs obtained for each Cd treatment was transformed (arcsine) and compared using a one-way analysis of variance (ANOVA, $p < 0.05$). When the general variance model was significant, multiple comparisons were conducted using the Tukey test. A Pearson correlation was performed to determine if there was a relationship between Cd concentrations in the gills and bivalve ventilation rates. Differences in metal concentrations in the organs of depurated bivalves were compared using an ANOVA ($p < 0.05$). Statistical analyses were performed using SYSTAT and Sigma Plot software.

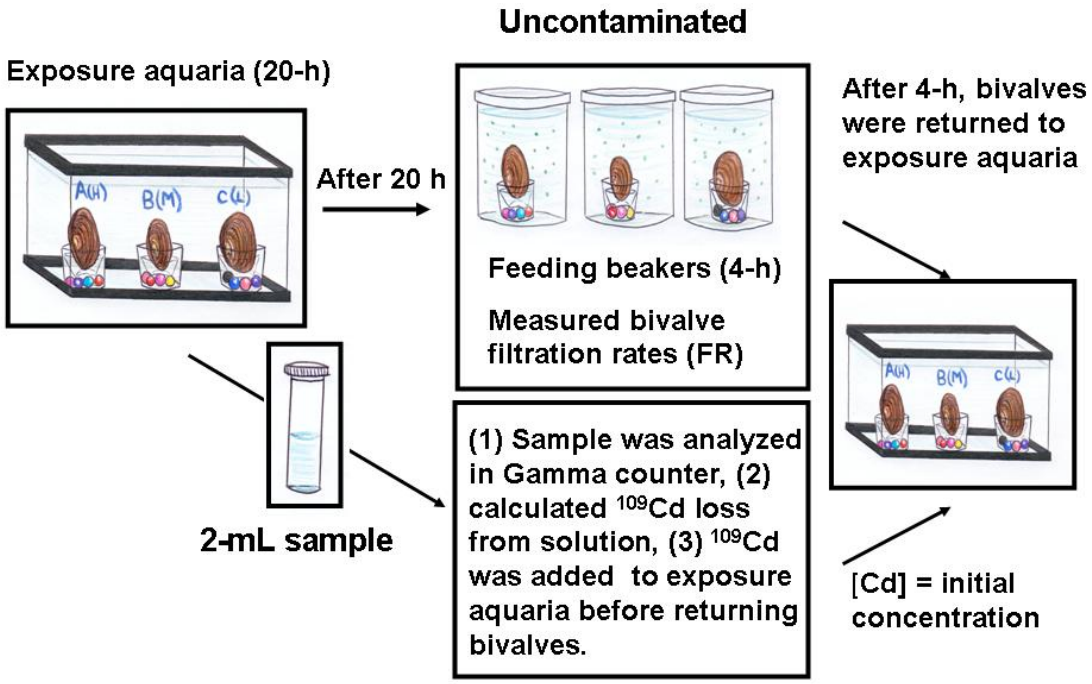


Figure 5.1: Diagram illustrating the steps taken to expose bivalves to aqueous Cd and to feed them without contaminating their food (algae). / Schéma des étapes d'exposition au Cd aqueux et d'alimentation durant les expériences de prise en charge à partir de l'eau.

5.3 Results

5.3.1 Accumulation of aqueous cadmium

Cadmium concentrations in bivalves increased as the dissolved Cd concentrations in the medium increased (Table 5.2). The highest Cd concentrations were generally observed in the gills, followed by the digestive gland, mantle, miscellaneous organs and the foot, respectively. Whereas Cd concentrations in gills and mantle continually increased as dissolved [Cd] increased, Cd concentrations in the other organs collected from bivalves exposed to the two higher concentrations of dissolved Cd (5 and 20 nM) were very similar.

Table 5.2: Mean cadmium concentrations (nmol·g^{-1} fresh wt ± SE) for *Pyganodon grandis* exposed to dissolved cadmium (nM) for 60 h. / Concentrations moyennes de cadmium (nmol·g^{-1} poids frais ± erreur type de la moyenne) observées chez *Pyganodon grandis* exposé au cadmium dissous (nM) pendant 60 h.

Dissolved [Cd] (nM)	Gills	Digestive Gland	Mantle	Miscellaneous Organs	Foot
0.1 (n=9)	0.01 ± 0.002^{a}	0.006 ± 0.001^{a}	0.004 ± 0.001^{a}	0.005 ± 0.001^{a}	0.002 ± 0.001^{a}
0.5 (n=9 x 2)	0.06 ± 0.006^{b}	0.02 ± 0.002^{b}	0.02 ± 0.003^{b}	0.02 ± 0.003^{b}	0.005 ± 0.001^{a}
5.0 (n=9 x 2)	0.51 ± 0.06^{c}	0.29 ± 0.09^{c}	0.22 ± 0.02^{c}	0.19 ± 0.03^{c}	0.09 ± 0.04^{b}
20 (n=9)	0.69 ± 0.08^{d}	0.31 ± 0.09^{c}	0.27 ± 0.05^{c}	0.20 ± 0.02^{c}	0.09 ± 0.01^{b}

Superscript a,b,c,d denote significant difference between groups of n = 9 as determined by ANOVA ($p<0.05$) and compared using a Tukey test.

Approximately 50% of the total Cd burden of *P. grandis* was found in the gills, followed by the mantle (~20%), the miscellaneous organs (~20%), the digestive gland (~10%) and the foot (3%) (Table 5.3). An ANOVA performed on the arcsine-transformed percentages showed that the proportion of Cd in each organ was relatively insensitive to the dissolved Cd concentrations in the exposure medium. For example, the relative contributions of the gills and mantle to the total Cd burden did not change significantly as the dissolved Cd concentration increased. For the digestive gland, foot and miscellaneous organs, the proportions were somewhat more variable (Table 5.3), but this variability was not related to the exposure regime.

Absorption efficiency (α_w) of dissolved Cd in bivalve gills ranged from 1 to 45% for an overall average of 9 ± 2% (± SE) whereas α_w for the whole organism ranged from 4 to 65% for an overall average of 25 ± 3% (± SE). For bivalves exposed to 0.1, 0.5 and 5 nM of dissolved Cd, cadmium concentrations in *P. grandis* gills were positively correlated with the bivalve filtration rates that had been determined during the daily 4-h feeding periods – animals with the higher filtration rates accumulated higher concentrations of Cd in their gills (Figure 5.2). No such relationship was observed in bivalves exposed to the highest dissolved Cd concentration (20 nM). Moreover, filtration rates for bivalves exposed to 20 nM of dissolved Cd were lower than the filtration rates measured for bivalves exposed to the other Cd treatments.

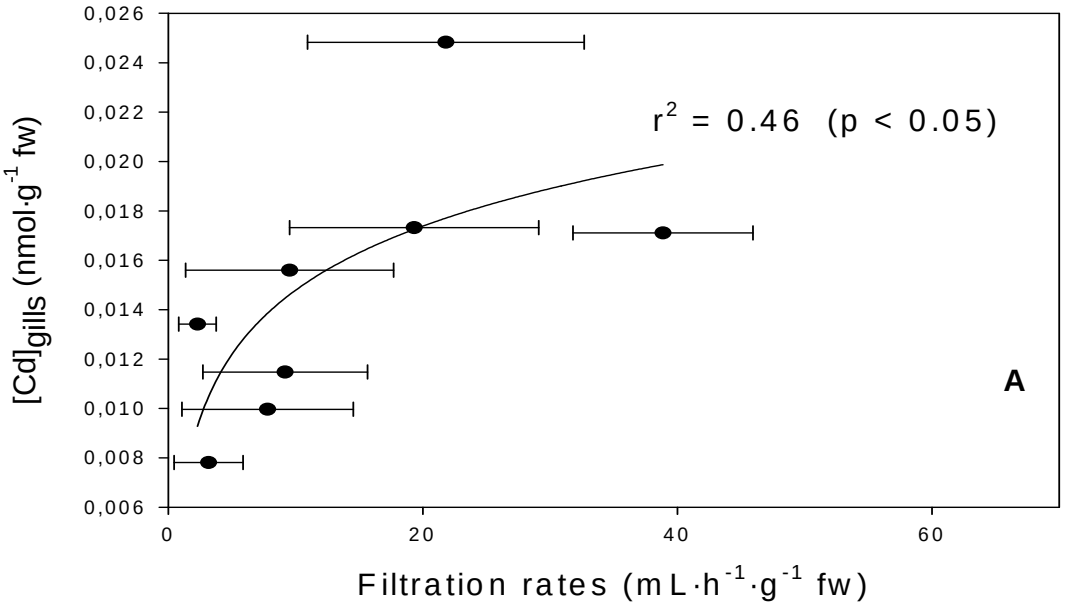
Table 5.3: Relative Cd burdens (% ± S.E.) observed in *Pyganodon grandis* organs exposed to dissolved Cd (nM) for 60 h. / Pourcentage de la charge totale en cadmium (avec l'erreur type de la moyenne) observé dans les différents organes des bivalves exposés au cadmium dissous (nM) pendant 60 h.

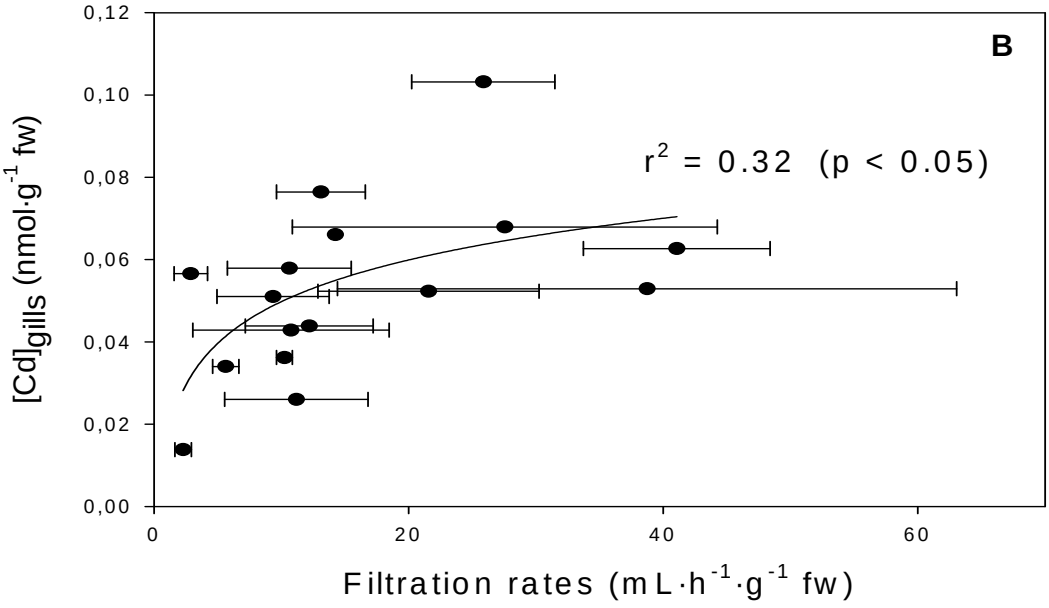
[Cd] (nM)	Gills	Digestive Gland	Mantle	Miscellaneous Organs	Foot
0.1 (n=9)	52 ± 3^{a}	9 ± 1^{a}	17 ± 1^{a}	20 ± 2^{a}	1 ± 0.1^{a}
0.5a (n=9)	47 ± 3^{ab}	16 ± 1^{b}	17 ± 1^{ab}	14 ± 1^{b}	5 ± 0.9^{b}
0.5b (n=9)	47 ± 3^{ab}	9 ± 3^{a}	23 ± 1^{b}	18 ± 2^{ab}	3 ± 0.8^{c}
5.0a (n=9)	48 ± 3^{ab}	8 ± 1^{a}	20 ± 2^{ab}	20 ± 1^{a}	3 ± 0.5^{c}
5.0b (n=9)	50 ± 2^{a}	10 ± 1^{a}	20 ± 1^{ab}	18 ± 1^{a}	2 ± 0.5^{a}
20 (n=9)	44 ± 1^{b}	16 ± 2^{b}	20 ± 1^{ab}	15 ± 1^{b}	5 ± 0.5^{b}
Mean (n=54)	**49 ± 2**	**11 ± 2**	**19 ± 1**	**18 ± 1**	**3 ± 0.5**

Superscript a,b,c denote significant differences between groups of n = 9 as determined by ANOVA ($p<0.05$) and compared using a Tukey test.

5.3.2 Depuration test

No significant difference (ANOVA; significant difference: $p < 0.05$) was observed between the mean Cd concentrations in *P. grandis* organs for bivalves of the reference group (day 0) compared to those that had depurated for 8 d (Table 5.4). There was, however, a significant difference between Cd concentrations in the organs of bivalves that had depurated for 16 d (except for the foot) and those of the reference group. Only Cd concentrations for the digestive gland of bivalves that had depurated for 16 d were significantly lower than those observed for the other two groups (Table 5.4).


0,026
0,024
0,022
0,020
0,018
0,016
0,014
0,012
0,010
0,008
0,006
[Cd]gills (nmol·g-1 fw)
r2 = 0.46 (p < 0.05)
A
0
20
40
60
Filtration rates (mL·h-1·g-1 fw)


0,12
0,10
0,08
0,06
0,04
0,02
0,00
[Cd]gills (nmol·g-1 fw)
B
r2 = 0.32 (p < 0.05)
0
20
40
60
Filtration rates (mL·h-1·g-1 fw)

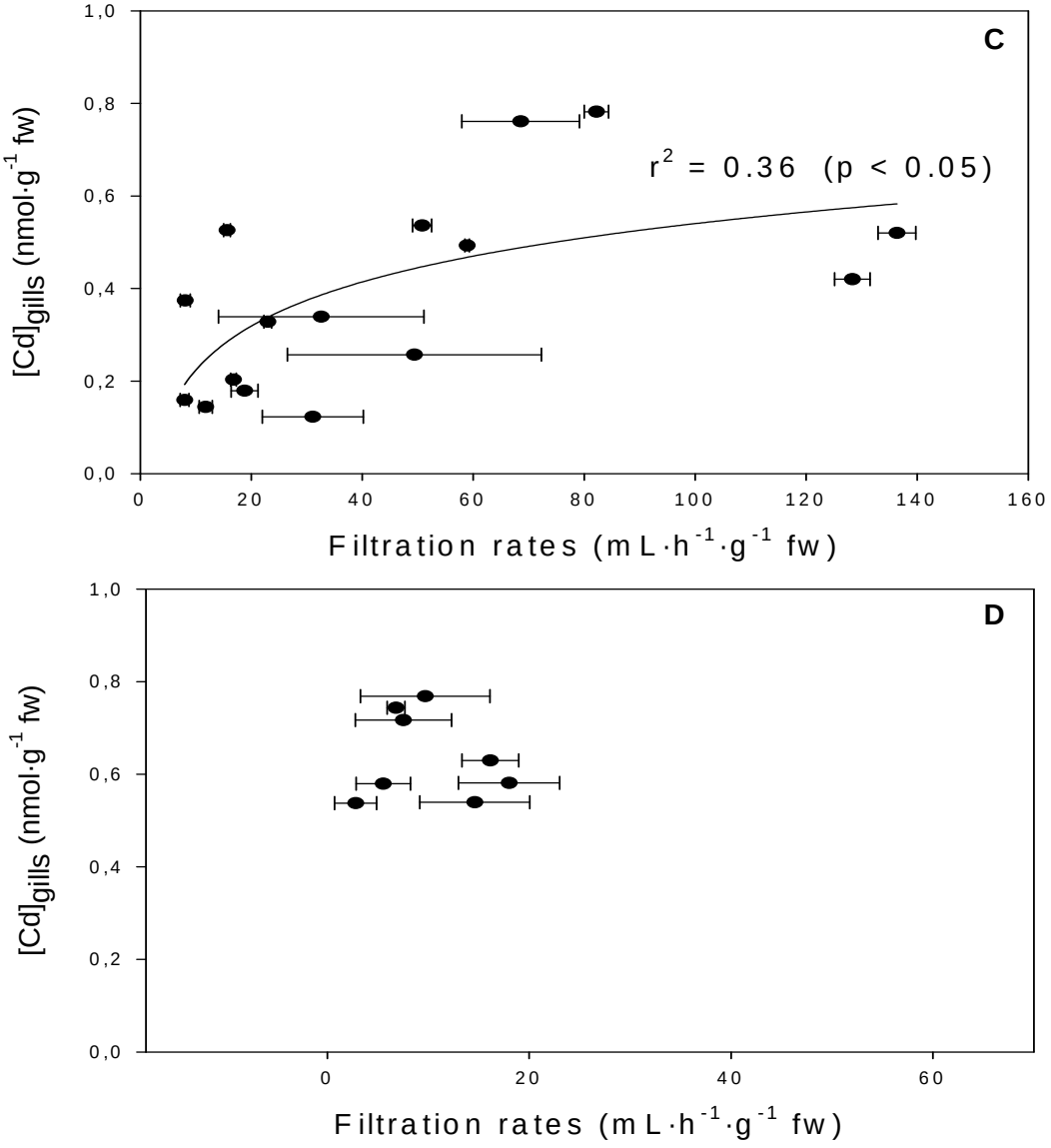


Figure 5.2: Relationship between $[Cd]_{gills}$ and bivalve filtration rates for *Pyganodon grandis* exposed to dissolved Cd: (a) 0.1 nM; (b) 0.5 nM; (c) 5 nM; and (d) 20 nM. Each dot represents the mean filtration rate (± SE; line) for one bivalve. The curves were obtained by non-linear regression. / Relation entre $[Cd]_{branchies}$ et les taux de filtration des bivalves exposés au Cd dissous: (a) 0,1 nM; (b) 0,5 nM; (c) 5 nM; et (d) 20 nM. Chaque point représente le taux de filtration (± erreur type de la moyenne; ligne) d'un bivalve. Les courbes ont été obtenues par régression non-linéaire.

Table 5.4: Mean Cd concentrations (nmol·g^{-1} fresh wt ± SE) in *Pyganodon grandis* organs during depuration after pre-exposure to 5 nM dissolved Cd. / Concentrations moyennes (nmol·g^{-1} poids frais ± erreur type de la moyenne) des organes de *Pyganodon grandis* durant la dépuration après une pré-exposition à 5 nM Cd dissous.

Organ	Day 0 (n = 3)	Day 8 (n = 6)	Day 16 (n = 5)
Gills	0.69 ± 0.08^{a}	0.64 ± 0.15^{ab}	0.45 ± 0.03^{b}
Digestive gland	0.28 ± 0.03^{a}	0.33 ± 0.08^{a}	0.12 ± 0.03^{b}
Mantle	0.21 ± 0.03^{a}	0.19 ± 0.04^{ab}	0.14 ± 0.01^{b}
Miscellaneous organs	0.23 ± 0.03^{a}	0.14 ± 0.04^{ab}	0.10 ± 0.01^{b}
Foot	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01

Superscript a,b denote significant difference between organs as determined by ANOVA ($p<0.05$) and compared using a Tukey test.

Table 5.5: Calculated Cd mass balance (nmol) for bivalves before and after depuration. The bivalves were pre-exposed to 5 nM dissolved Cd. Values in bold are the mean accumulated Cd concentrations (± SE) for each bivalve group. / Bilan massique (nmol) du cadmium calculé pour les bivalves avant et après dépuration. Les bivalves étaient exposés préalablement à 5 nM Cd dissous. Les valeurs en gras représentent les moyennes (± erreur type de la moyenne) des concentrations en Cd accumulées par les bivalves.

Contaminated bivalves (0 d)	**Depurated bivalves (8 d)**			**Depurated bivalves (16 d)**		
Calculated accumulated Cd (nmol)	Total remaining Cd (nmol)	Loss (nmol)	Calculated accumulated Cd (nmol)	Total remaining Cd (nmol)	Loss (nmol)	Calculated accumulated Cd (nmol)
10.9, 9.0, 8.4	2.2, 1.6, 2.9, 1.3, 2.4, 1.0	6.8, 5.6, 4.6, 5.6, 8.1, 6.3	9.0, 7.2, 7.5, 6.9, 10.5, 7.3	1.2, 2.5, 1.3, 1.6, 0.9	5.6, 8.9, 7.7, 7.0, 2.1	6.8, 11.4, 9.0, 8.6, 3.0
Mean: 9.4 SE: 0.7	**Mean: 1.9 SE: 0.3**	**Mean: 6.2 SE: 0.5**	**Mean: 8.1 SE: 0.6**	**Mean: 1.5 SE: 0.3**	**Mean: 6.3 SE: 1.2**	**Mean: 7.8 SE: 1.4**

Mean dissolved Cd concentrations in the samples taken daily from the depuration beakers suggest a rapid initial release of Cd from the bivalves (Figure 5.4). Between day 0 and day 5, dissolved Cd concentrations decreased sharply from 1.0 ± 0.2 to 0.10 ± 0.01 nmol·L^{-1}, but after day 6, mean dissolved Cd concentrations remained constant at 0.13 nmol·L^{-1}, implying that bivalves were eliminating Cd at a slower, constant rate. The calculated mass balance for Cd shows that the total amount of Cd accumulated in the bivalves at t = 0 was not significantly different from the sum of the Cd remaining in the depurated bivalves plus the Cd lost to the aqueous phase (Table 5.5), which suggests that a route of Cd efflux was not measured.

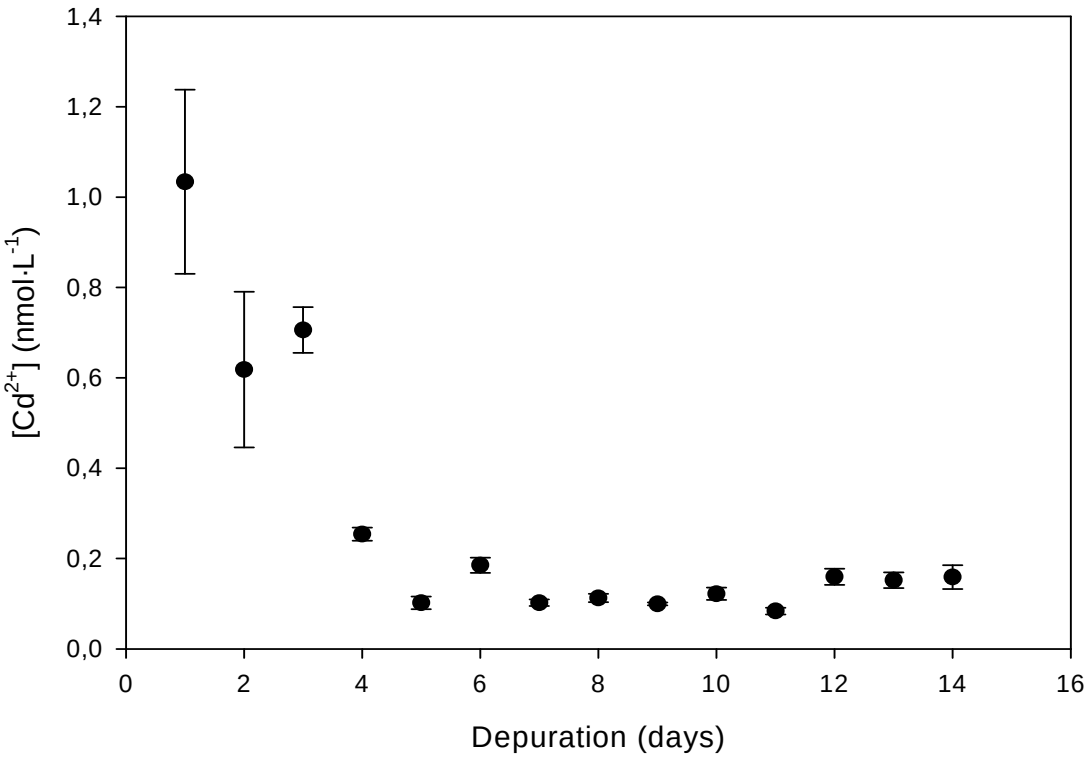


Figure 5.3: Mean dissolved Cd concentrations (± SE; nmol·L^{-1}) in water samples collected from the depuration beakers. / Concentrations en cadmium dissous moyennes (± erreur-type; nmol·L^{-1}) provenant des bacs de dépuration.

5.4 Discussion

Historically, dissolved Cd concentrations in lakes studied in Rouyn-Noranda have ranged from < 0.1 nM to 19.5 nM (Croteau *et al.* 1998); however, recent data suggest that the range in dissolved Cd concentrations in Rouyn-Noranda lakes has decreased (< 0.1 nM to ~10 nM) (Croteau *et a*l. 2002). In any case, *P. grandis* is not present in lakes whose dissolved Cd concentrations are > 5 nM, due to the low pH values (< 5.0) usually associated with these lakes (Perceval *et al.* 2002). Therefore, with the exception of the 20 nM exposure, the exposure concentrations used for this study fall within the range of those observed in lakes from the Rouyn-Noranda region.

5.4.1 Cadmium uptake by *Pyganodon grandis*

The results from the present study suggest that the gills are the main target organ when *P. grandis* is exposed to dissolved Cd. Regardless of the exposure concentration; Cd concentrations in the gills were consistently higher than those obtained for the other organs. Despite their relatively modest contribution to the total weight of the organism (23%), the gills account for about 50% of the total Cd burden in *P. grandis*. This contribution of the gill to the total Cd burden in *P. grandis* is slightly higher than the average of 40% that was observed by Tessier *et al.* (1993) in native bivalves collected from contaminated lakes; but falls within the 58 ± 13% (SD) range reported by Bonneris *et al.* (2005b). The mantle, digestive gland and miscellaneous organs contributed 21%, 11% and 28% to the total Cd burden, respectively, in the bivalves collected by Tessier *et al.* (1993). Our results are also higher than those reported by Inza *et al.* (1997) and Tran *et al.* (2001) who noted that the gills represented 30% of the total Cd burden for *Corbicula fluminea* (15 d exposure to dissolved Cd), as well as those observed by Roesijiadi and Klerks (1989) who reported that the gills represented 37% of the total Cd burden for the oyster, *Crassostrea virginica*.

Gill Cd concentrations increased as dissolved Cd concentrations increased (Table 5.2). This positive relationship between Cd concentrations in the bivalve and the dissolved Cd concentration in the medium is not unexpected, given that Cd is a non essential element;

indeed, such trends have been observed in several laboratory studies (Table 5.6). A similar relationship was observed in the field for *P. grandis*. Bonneris *et al.* (2005b; 2005a), Giguère *et al.* (2003) and Tessier *et al.* (1984) collected bivalves along a metal gradient and noted an increase in $[Cd]_{gills}$ in *P. grandis* collected from the more contaminated lakes.

Table 5.6: Examples of laboratory studies conducted on bivalves where a relationship between dissolved Cd concentrations and $[Cd]_{gills}$ was observed. / Exemples d'études au laboratoire qui ont démontré une relation entre la concentration en Cd dissous et $[Cd]_{branchies}$ chez les bivalves.

Species	Dissolved [Cd] (nM)	Exposure duration (d)	$[Cd]_{gills}$ ($nmol \cdot g^{-1}$)	Relationship	Reference
Marine					
Mytilus edulis	8 to 445	8 to 16	8 to 124[fw]	curvilinear	(Roesijadi et Fellingham 1987)
Mytilus edulis	4 to 445	21	0.04 to 15[fw]	linear*	(Erk *et al.* 2005)
Crassostrea virginica	90 to 1780	14	13 to 2000[fw]	linear	(Roesijadi et Klerks 1989)
Freshwater					
Anodonta cygnea	45 to 225	14 to 46	17 to 1500[dw]	curvilinear	(Hemelraad *et a*l. 1986)
Corbicula fluminea	9000 to 90000	14	0.8 to 31[fw]	linear	(Inza *et al.* 1997)
Corbicula fluminea	45 to 310	15 to 45	17 to 205[fw]	curvilinear	(Baudrimont *et a*l. 1997b)

* log transformed data; fw: fresh weight; dw: dry weight

In our experiments, the rate of Cd uptake in bivalves reached a plateau when the animals were exposed to dissolved Cd concentrations greater than 5 nM, as illustrated in Figure 5.4. Such a relationship would be expected if Cd were accumulated by facilitated transport. The results were transformed using the Lineweaver-Burke equation (equation 5.2):

$$1/v = K_m / V_{max} \cdot 1/[Cd] + 1/V_{max} \qquad (5.2)$$

where v = the uptake rate in the gills ($nmol \cdot g^{-1}$ of bivalve $gill \cdot h^{-1}$), V_{max} = the maximum uptake rate (corresponding to the saturation of all the uptake sites involved in Cd transport; $nmol \cdot g^{-1}$ of bivalve $gill \cdot h^{-1}$) and K_m = the dissolved Cd concentration (nM) needed to give an uptake rate corresponding to 50% of V_{max}. A calculated value of 8.8 nmol $Cd \cdot g^{-1}$ of bivalve $gill \cdot h^{-1}$ was obtained for V_{max} and a value of 3.4 nM was obtained

for K_m (Figure 5.5). When comparing the observed uptake (solid line; Figure 5.4) with the calculated uptake rate (dotted line; Figure 5.4), we see that there is an appreciable difference between the calculated and observed values at the higher Cd concentrations. This discrepancy between the observed and calculated values at higher Cd concentrations may be the result of Cd entering the cell by processes other than carrier-mediated transport, processes that are not taken into consideration in the Lineweaver-Burke equation. Note, however, that the 95% confidence intervals for the observed and calculated values overlap, suggesting that the apparent difference between observed and calculated values may simply reflect the relatively small number of experimental points (ideally we would have more than four experimental points to plot; this would give a more accurate estimate of V_{max}). Note too that the agreement between the two curves is acceptable in the lower Cd range (< 2 nM), i.e., at concentrations that are typically observed in a natural setting.

Our results suggest that bivalve ventilation activity plays an important role in Cd accumulation from solution. A relationship between $[Cd]_{gills}$ and bivalve filtration rates was observed when bivalves were exposed to dissolved Cd concentrations less than or equal to 5 nM – i.e. $[Cd]_{gills}$ increased as bivalve filtration rate increased (Figure 5.2 a,b,c). For bivalves exposed to the highest dissolved Cd concentration (20 nM), there was no relationship between $[Cd]_{gills}$ and filtration rates, suggesting that the membrane transport sites involved in Cd uptake at the gill surface were saturated, regardless of bivalve filtration rate (Figure 5.2 d).

The relationship between $[Cd]_{gills}$ and bivalve filtration rates deserves further consideration. According to the principles of the Biotic Ligand Model (BLM), an increase in gill ventilation should <u>not</u> increase the accumulation of dissolved metals in aquatic organisms. Within the BLM construct, metal uptake involves three steps: diffusion of the metal from the bulk solution to the biological interface (gill surface), reaction of the metal with a transport site «X» at the gill surface, and movement of the metal across the gill membrane, via the transport site (Gorsuch *et a*l. 2002).

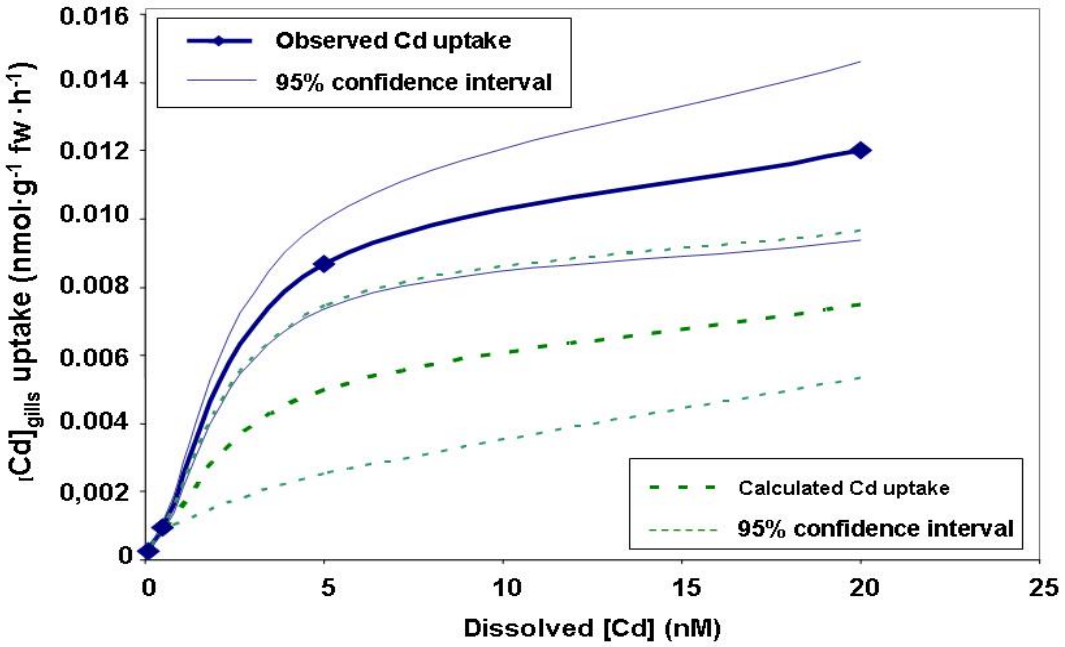


Figure 5.4: Relationship between the uptake rate of $[Cd]_{gills}$ and dissolved Cd concentrations. Observed values (solid line) and calculated values (dotted line) for bivalves exposed to Cd under laboratory conditions. / Relation entre la prise en charge $[Cd]_{branchies}$ et la concentration en Cd dissous. Valeurs observées (ligne solide) et calculées (ligne pointillée) chez les bivalves exposés en laboratoire.

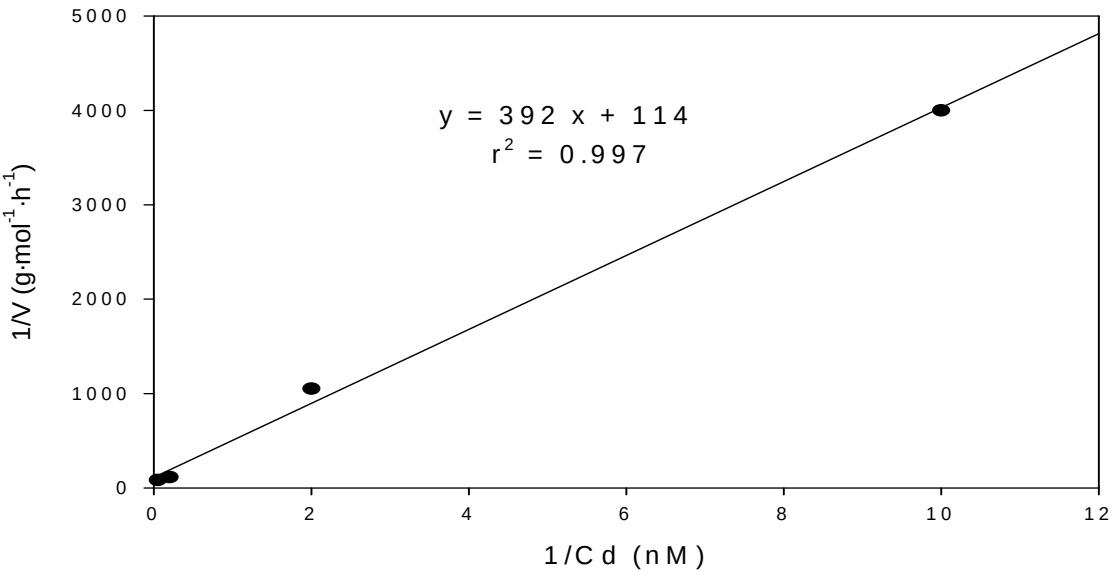


Figure 5.5: Transformation of the results from the accumulation study using the Lineweaver-Burke equation. / Transformation des données de prise en charge selon l'équation Lineweaver-Burke.

The transport of the metal across the apical gill membrane and into the gill cell is postulated to be the slow step in metal uptake. Under such conditions, the gill surface is in equilibrium with the external medium and, therefore, the filtration rate should not influence how much metal is bound to the transport site «X» or how quickly the metal is accumulated into the cell.

Although our results do not agree with the BLM, they are not unprecedented – other studies have shown that gill ventilation may influence metal uptake in aquatic animals. Lloyd and Hebert (1962) observed an increase in Zn uptake in rainbow trout when these animals were exposed to hypoxic conditions. According to these authors, a reduction in dissolved oxygen concentrations caused an increase in gill ventilation rates, increasing the amount of water passing over the gills. However, these authors did not offer a convincing mechanistic explanation for their results. Hughes and Flos (1978) demonstrated that physiological and morphological changes occur in fish gills when

these animals are exposed to hypoxic or anoxic conditions, and these changes can have important consequences in metal uptake. It has been hypothesised that an increase in gill filtration rates, which causes a higher water flow over the gills, may explain an increase in metal accumulation. This idea was supported by a mechanistic gill model showing that diffusion rates of hydrophobic micro-pollutants in the gill and gill epithelium were faster than the water flow over the gills (Sijm *et al.* 1994). However, this hypothesis has been rejected for metals as Pilgaard *et al*. (1994) did not observe any significant differences in the degree of tissue copper accumulation between copper/normoxia- and copper/hypoxia-exposed rainbow trout. Therefore, it was suggested that higher water flow over the gills does not result in higher metal uptake rates in fish (Hayton et Barron 1990).

Similar studies investigating the relationship between gill ventilation and metal uptake have been performed on the freshwater bivalve *Corbicula fluminea*. Tran *et al.* (2000; 2001) noted an increase in Cd uptake in *C. fluminea* when the animals were exposed to hypoxic conditions. A reduction in dissolved oxygen concentrations caused an increase in filtration rates leading to an increase in Cd accumulation in gills. Moreover, hypoxia induced a change in the burden of Cd in the gills. During a 15 d exposure to dissolved Cd (2 $\mu g \cdot L^{-1}$), Tran *et al.* (2001) observed that the proportion of the total Cd burden in the gills increased from 13.1 ± 2.8% (SE) under normoxia to 31.4 ± 3.7% (SE) under hypoxic conditions.

Our results suggest that gill ventilation may influence dissolved metal accumulation in *P. grandis*. Without completely rejecting the BLM, these observations on respiration and filtration rates do suggest that some modifications to the model may be needed. For example, changes in filtration rates might not be explained by a hydrodynamic phenomenon (thinner unstirred layer at the gill surface) but rather by an increase in the gill surface area that is irrigated by either increased blood flow within the gills or increased water flow over the gill surface. If the bivalves are exploiting a larger surface area of the gills, then we could expect a higher rate of metal uptake. Also, a decrease in

filtration rates could be a consequence of the bivalve's behaviour, (e.g., the bivalve could be closed for lengthier periods of time). In this case, Cd concentrations in the mantle cavity would decrease while the bivalve is closed, reducing the Cd-exposure concentration and Cd uptake in the bivalve.

It must be noted that the filtration rates used to illustrate the relationship between bivalve filtration rates and $[Cd]_{gills}$ were measured during the 4 h feeding period and not during the 20 h exposure to dissolved cadmium. Because bivalve filtration rates can vary greatly during a 24 h period (see Section 4), it is possible that the bivalves were filtering at a different rate during the dissolved Cd exposure. A subsequent study was conducted to observe whether bivalve filtration rates could be measured using latex beads, which would have allowed us to measure bivalve filtration rates without creating a dietary source of cadmium. However, the experiment was unsuccessful as the bivalves would not filter the latex beads (Appendix B). Therefore, the results from this chapter must be regarded as preliminary until a method can be devised that would allow one to measure bivalve filtration rates during a dissolved metal exposure without contaminating the bivalves through the diet.

5.4.2 Cadmium efflux by *Pyganodon grandis*

Measurements of Cd elimination from aquatic organisms can be difficult to obtain under field conditions and are often questioned when obtained under laboratory conditions. Laboratory studies on the mussel, *Mytilus edulis*, conducted by Borchardt (1983), indicated that Cd elimination rates are strongly influenced by food quantity and that elimination is slowest when food quantity is low. It has been observed that mussels reduce their filtration rates, and hence, their water turnover is reduced, when no food or only small quantities below maintenance level are available (Tran *et a*l. 2001; Fournier *et a*l. 2005).

In the present study, specimens of *P. grandis* were fed throughout the depuration period in hopes that the efflux rates observed in this study would approach those that prevail in

the field. Rapid Cd elimination occurred in the first 3 days of depuration followed by a slower, constant release after day 5. This in agreement with what has been previously noted by Fisher *et al*. (1996) and Lares and Orians (2001), while studying Cd elimination rates in *Mytilus* spp. Using a radiotracer methodology, both researchers found that ^{109}Cd release followed a two-compartment exponential model, the first part of which consisted of a very rapid loss and that this was followed by a slower release. The initial rapid loss of Cd from *P. grandis* observed between Day 0 and Day 5 of the depuration period is likely the result of Cd that was adsorbed onto the organs and shell surface being released into the medium, whereas the second compartment represents what may actually be lost from bivalve cells.

The value of the Cd efflux rate constant (k_e; 2.1 d^{-1}) measured during the first five days of this study is ~1000 times faster than the value of the efflux rate constant (k_e = 0.0022 d^{-1}) estimated from data provided in Tessier *et al.* (1987) who transplanted *P. grandis* from a contaminated lake into an uncontaminated lake. This contrast in efflux rate constant values may be the result of the difference between newly acquired Cd and Cd that has been accumulated over the lifetime of the animal. The newly acquired metal would be expected to be more labile and more easily lost than the metal accumulated in chronically exposed bivalves.

The rapid Cd loss observed between days 1 and 5 indicates that a large proportion of the accumulated Cd was bound in a labile, easily lost form. The slower efflux rate observed after day 5 suggests that the remaining accumulated Cd had been sequestered in a much less labile (detoxified) subcellular fraction such as the granules. Cadmium may have also been bound to metallothioneins, metal-binding proteins that also play an important role in the detoxification of metals in organisms (Viarengo 1989). The biosynthesis of these proteins is generally induced after an organism has been exposed to metals but their biosynthesis is not instantaneous (Langston *et al*. 1998). This delay in synthesis may partly explain why a majority of the accumulated Cd was lost at the beginning of the depuration period (Figure 5.3).

The results from the mass balance calculation of Cd hint that an additional route of Cd elimination may not have been taken into account during the experiment. The total Cd accumulated in the bivalves at the beginning of the depuration period was somewhat higher than the sum of the Cd remaining in the depurated bivalves after 16 d plus the Cd that had been lost to the aqueous medium over the 16-d period (Table 5.5). It is possible that the bivalves may have lost Cd through defecation, as bivalves were fed during depuration, but their fecal matter was not collected and analyzed. This may account for the slight discrepancy in the mass balance. However, other researchers have found that the feces are not a major pathway to eliminate Cd, especially in marine species. Borchardt (1983) found that Cd elimination via the feces following a dissolved Cd exposure was negligible (0.7-1.6%) in *M. edulis*. Wang *et al.* (1996) also concluded that the Cd elimination via the feces did not significantly influence metal efflux rates in *M. edulis*. However, many researchers (Lares et Orians 2001; Griffin *et al.* 1980; Ritz *et al.* 1982) have noted that the kinetics of metal uptake and release in bivalves vary greatly among species. Further investigation on Cd efflux rates for *P. grandis* is needed if this species is to be used as a sentinel organism for monitoring spatial and temporal changes in environmental concentrations of Cd and other metals.

5.5 Conclusions

The present study with *P. grandis* demonstrates that metal accumulation from the dissolved phase may be related both to the aqueous Cd concentration and to the animal's filtration rate. The present study is the first to establish such a relationship between $[Cd]_{gills}$ and *P. grandis* filtration rates and further illustrates the need to take ventilatory activity into account in metal accumulation studies. The results obtained from the Cd accumulation section of this study will later be used to develop a biodynamic model that will take into account Cd accumulation from water and food. Because Cd efflux rate constants for *P. grandis* measured under a laboratory setting were much faster than those observed in the field, efflux rate constants observed in the laboratory will not be used in the biodynamic model.

6. DIETARY CADMIUM UPTAKE

6.1 Introduction

Interest in the mechanisms of metal accumulation in marine and freshwater bivalves has increased due to their potential as sentinel organisms or biomonitors of metal contamination. Several studies have demonstrated that bivalves can accumulate metals from both the dissolved phase and from ingested phytoplankton food as well as various suspended organic and inorganic particulates (Decho et Luoma 1994; Wang *et a*l. 1997; Tran *et a*l. 2002). However, determining the contribution of each phase, dissolved or dietary, to metal accumulation in these organisms is crucial if bivalves are to be used successfully as biomonitors.

The importance of food as a source of cadmium to bivalves varies widely among studies. According to some researchers, one reason for such variability is that the mechanisms of dietary metal uptake are not well known for bivalves (Widmeyer et Bendell-Young 2007; Amiard *et al.* 2007; Decho and Luoma 1991). Bivalve feeding behaviour (e.g., selectivity), the digestion rate of the ingested particles and assimilation efficiency have an impact on their accumulation of dietary Cd (Borchardt 1985). For filter-feeding bivalves, feeding behaviour is influenced by the quality and quantity of suspended particulate matter in the surrounding medium. Some bivalves can adjust to changes in the concentration of food in the environment by using a selective feeding strategy (Widmeyer and Bendell-Young 2007). The goal of this strategy appears to be to maximize the portion of ingested organic matter found in suspension, so that the amount of carbon assimilated remains constant despite a large variability in the quality and quantity of the food in the environment (Borchardt 1985) .

This selective feeding strategy occurs throughout the digestive process. In general, the suspended particulate matter enters the bivalve through the inhalant siphon into the mantle cavity. A first physical sorting is performed by the gills, which act as a sieve, to

concentrate particles rich in organic matter and push them towards the mouth (Bayne *et al.* 1987). Large particles or inorganic matter tend to be rejected by the bivalve as pseudofeces (Bayne et Newell 1983). A first digestion takes place in the stomach and intestines of the animal. Digestive enzymes are released from the crystalline stylet to breakdown the organic matter. This extracellular digestion is generally fast and the absorption efficiency of this type of digestion is relatively low (Widdows *et al*. 1979). A portion of the partially digested food will then undergo a glandular digestion, a slow intracellular digestion that has a higher absorption efficiency than the intestinal digestion (Widdows *et a*l. 1979).

The selective feeding strategy of bivalves has important implications if these animals are to be used as biomonitors. According to Decho and Luoma (1991), dietary metal exposure can be independent of the metal concentration found in suspended particulate matter and may be more dependent on what is actually consumed and digested by the bivalve. The length of time attributed to both intestinal or glandular digestion, and the amount of food digested during each phase of digestion, will influence metal uptake from the diet (Decho and Luoma 1991). The distribution of food between both digestion phases affects metal absorption because metals are not necessarily absorbed or accumulated during intestinal digestion but rather during glandular digestion. Studies have shown that bivalves that have a higher assimilation efficiency during the glandular digestion phase are more vulnerable to metal toxicity, especially in contaminated ecosystems that contain less food (Decho and Luoma 1991).

The purpose of the present study was to measure the accumulation of dietary Cd by *Pyganodon grandis* in a laboratory setting. Several field studies have concluded that *P. grandis* is a promising sentinel species for metallic pollution. However, it is still unclear whether water or food is the main source of Cd for this bivalve. To our knowledge, no prior experiments have focused on the uptake of diet-borne Cd by *P. grandis*. The results from this study will be used later as inputs to a Cd bioaccumulation model that takes into account accumulation of Cd from the aqueous and dietary phases.

6.2 Methods

6.2.1 Cadmium exposure

This experiment consisted of two phases: (a) contamination of the green alga *Pseudokirchneriella subcapitata* and (b) feeding contaminated algae to *P. grandis*. *Pseudokirchneriella subcapitata* was selected for this experiment because it is easy to grow under laboratory conditions and has been shown to be palatable to *P. grandis*. *Pseudokirchneriella subcapitata* was grown as described in Section 3.2[5]. Once cell densities had reached 20 x 10^6 cells·mL^{-1} (usually after 72 h), the algal cells were separated from the culture medium and rinsed three times by centrifugation (7,000 rpm) with a simplified culture medium. This simplified culture medium did not contain any metals, EDTA or PO_4 (Table 6.1). After this initial rinsing, the algal cells were transferred into two 1-L Erlenmeyer flasks filled with simplified culture medium that was contaminated with dissolved Cd (^{109}Cd + ^{112}Cd; 4.6 nM; specific activity 0.7 mCi $^{109}Cd \cdot \mu g^{-1}$). The exposure medium was buffered with HEPES (10 mM; 1-piperazine-4-(2-hydroxyethyl)-ethanesulfonic acid) to ensure that the pH would remain at 7.5.

Table 6.1: Chemical composition of the simplified culture medium used to rinse *Pseudokirchneriella subcapitata* after exposure to dissolved Cd. / Composition chimique du milieu de culture simplifié utilisé pour rincer *Pseudokirchneriella subcapitata* après son exposition au Cd dissous.

Component	Stock solution		Final Solution
	Concentration ($g \cdot L^{-1}$)	Volume ($mL \cdot L^{-1}$)	($mg \cdot L^{-1}$)
$NaNO_3$	25	5	125
$CaCl_2 \bullet 2H_2O$	2.5	5	12.5
$MgSO_4 \bullet 7H_2O$	7.5	5	37.5
NaCl	2.5	5	12.5

[5] Growing *P. subcapitata* in a Cd-contaminated medium was not feasible as the necessary algal density could not be obtained.

The algal cells remained in the Cd-contaminated medium for 24 h[6]. To reduce the sedimentation of algal cells during the exposure period, the medium was stirred using a magnetic stirrer. After 24 h, three 5-mL replicate samples of the exposure medium were collected from both Erlenmeyer flasks for analysis, and the remaining algal cells in the 1-L Erlenmeyer flasks were separated from the contaminated medium by centrifugation as described above. After the initial rinsing, the algal cells were washed with Na_2EDTA (10^{-4} M) for 20 min to eliminate any Cd that had adsorbed onto their surface. Afterwards, the algal cells were again rinsed three times with the simplified culture medium to remove any remaining EDTA and Cd.

The three replicate samples of the exposure medium that were collected prior to rinsing were filtered through two polycarbonate membranes (2 μm, Nuclepore). The first membrane served to collect the algae, whereas the second membrane was exposed only to dissolved ^{109}Cd and thus served as a filtration blank (accounting for any passive sorption of ^{109}Cd to the filter). Each filter was placed in a separate scintillation vial and analyzed in a Gamma counter. The filtered medium and a 1-mL sample of the EDTA rinse medium were also placed in counting vials and analyzed for dissolved ^{109}Cd. Cadmium concentrations in the algae were expressed both in nmol $Cd \cdot g^{-1}$ and nmol $Cd \cdot m^{-2}$. The surface area of the algae on the filter was calculated based on the number of total algal cells filtered and the mean surface area of an individual algal cell. Algal density and cell dimensions (i.e., surface area and diameter) were obtained using a particle counter (Beckman Multisizer^{TM} 3 Coulter Counter).

The next step was to feed the contaminated algae to the bivalves. Nine bivalves were placed in separate 2-L feeding beakers filled with synthetic medium (described in Section 3.3) that contained Na_2EDTA (10^{-4} M) to capture any Cd released by the algae during the experiment. Each bivalve was given Cd-contaminated *P. subcapitata* at a

[6] Preliminary studies showed that a 4-h exposure period was too short for Cd concentrations in *P. subcapitata* to reach the desired Cd concentration of ca. 1.7 nmol $Cd \cdot m^{-2}$.

density of 500,000 cells·mL^{-1}. The bivalves were allowed to feed on the contaminated algae for 4 h and their filtration rates (FR) were measured as described in Section 4.2.1. After 4 h, the bivalves were transferred into an algae-free 6-L holding tank filled with synthetic medium spiked with Na_2EDTA (10^{-4} M) to complex any dissolved Cd released by the bivalve's digestion processes (Figure 6.1). The bivalves remained in the tanks for 20 h after which they were transferred back into a feeding beaker that contained fresh medium and algae. This sequence of 4 h feeding and 20 h starvation was repeated 4 times. In all, the experiment lasted 96 h, including a total of 16 h of exposure to contaminated algae. The experiment was repeated once more for a total of 18 bivalves (n = 9 x 2)[7].

Once the exposure period was completed, the bivalves were transferred into feeding beakers that contained fresh medium spiked with Na_2EDTA (10^{-4} M) and allowed to depurate for 8 d[8]. During the depuration period, bivalves were fed continuously with uncontaminated *P. subcapitata* to encourage them to purge any contaminated algae from their system. As previously described in Section 5.2.1, the bivalves were dissected and their organs (gills, digestive gland, mantle, foot and miscellaneous tissues) were rinsed with Na_2EDTA (10^{-3} M) and analyzed using a Gamma counter.

6.2.2 Calculations

Hourly bivalve ingestion rates were calculated for each individual bivalve using the amount of algae consumed by the bivalve during the 4-h feeding period, and taking into account algal growth in the control beakers without bivalves (equation 6.1):

$$IR = \left[V \times \frac{\left((d_0 - d_f) + \left(d_f' - d_0' \right) \right)}{t} \times 18.3 \times 10^{-13} \right] \times {}^{1}\!/_{M} \qquad (6.1)$$

[7] One bivalve from the first test died during the depuration period.

[8] Preliminary studies showed that a depuration period of 4 or 6 days was not sufficiently long for the bivalves to purge the contaminated algae from their gut.

where IR = the ingestion rate (g dry wt of algae·h^{-1}·g^{-1} bivalve fresh wt); V = the volume of water in each beaker (mL); d_o = the algal density at the beginning of the feeding period (cells·mL^{-1}); d_f = the algal density at the end of the feeding period (cells·mL^{-1}); d_o' = algal densities (cells·mL^{-1}) in the control beaker without a bivalve, at the beginning of the feeding period; d_f' = algal densities (cells·mL^{-1}) in the control beaker without a bivalve, at the end of the feeding period; t = the duration of each exposure period (h); M = bivalve body mass (fresh weight, g) and 18.3 x 10^{-13} is the average weight of one algal cell (g dry wt·$cell^{-1}$)[9]. Although it is possible that the average weight of an individual cell will vary during the lifetime of a cell, cells in our culture are not dividing synchronously and the average cell weight for the whole culture should vary little.

Assimilation efficiencies were calculated by dividing the total amount of Cd accumulated in the gills, in the digestive gland or in the whole bivalve by the amount of Cd consumed by each bivalve in a 16 h period:

$$AE = \left[\left(Cd_{bivalve} \right) / \left(IR \cdot \left[Cd \right]_f \cdot t \right) \right] \qquad (6.2)$$

where AE = the assimilation efficiency of Cd per bivalve (unitless; values range from 0.01 to 1); IR = the mean ingestion rate measured for each bivalve during four 4-h feeding periods (g dry wt of algae·h^{-1}·g^{-1} bivalve fresh wt); $[Cd]_f$ = the mean Cd concentration in the algae (nmol·g^{-1} dry wt); t = the length of the feeding experiment (4 x 4 = 16 h); and $Cd_{bivalve}$ = the final amount of Cd in the bivalve (gills, digestive gland or whole organism soft tissues; nmoles Cd) accumulated after the feeding experiment which includes the four separate 4-h feeding periods and the 8-d depuration period. The calculated values are multiplied by 100 to be expressed as a percentage (as seen in figures and tables).

[9] Personal communication from Amiel Boullemant. In the literature, values of 2.5 x 10^{-12} and 1.4 x 10^{-12} g·$cell^{-1}$ dry weight have been reported (Braddock et Brown 1994; Price *et al.* 1990).

6.2.3 Data analysis

The Cd burdens in bivalve organs, presented as percentages of burdens in whole bivalves, were calculated for each Cd treatment, transformed (arcsine) and compared using a one-way analysis of variance (ANOVA, $p < 0.05$). When the general variance model was significant, multiple comparisons were conducted using the Tukey test. A Pearson correlation was performed to determine if there was a relationship between Cd concentrations in the digestive gland and bivalve ingestion rates. Differences in Cd concentrations in the algal cultures were analyzed using a Student's t-test ($p < 0.05$). Statistical analyses were performed using SYSTAT and SigmaPlot software.

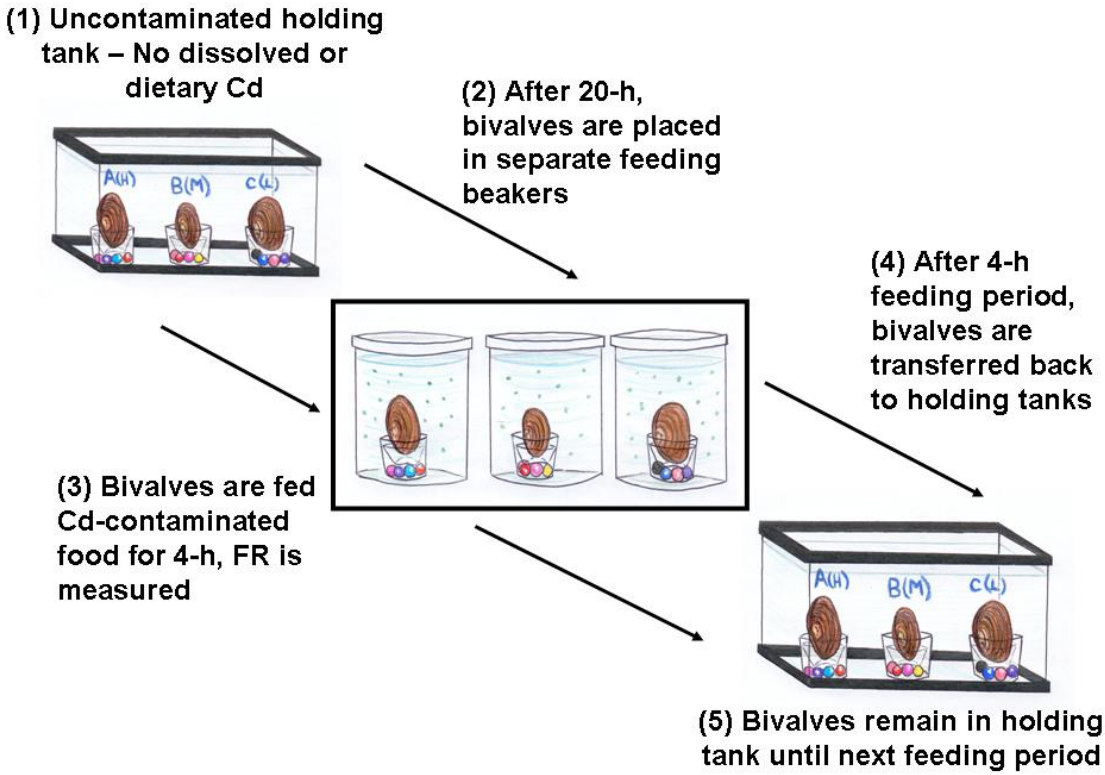


Figure 6.1: Diagram illustrating the steps taken to expose bivalves to dietary Cd during the experiments with radiolabelled algae. / Schéma des étapes d'exposition au Cd alimentaire durant les expériences de prise en charge à partir d'algues radiomarquées.

6.3 Results

6.3.1 Cadmium uptake by *Pseudokirchneriella subcapitata*

Based on the amount of Cd accumulated in the bivalves from the dissolved phase, it was determined that an accumulation of ~1.7 nmol $Cd \cdot m^{-2}$ or ~60 nmol $Cd \cdot g^{-1}$ (dry wt) in *P. subcapitata* was needed in order to detect ^{109}Cd in the bivalves after the dietary exposure. After 24 h, ~65% of the dissolved Cd in the algal exposure medium was associated with *P. subcapitata*, the concentration of free Cd^{2+} in the exposure medium having dropped from ~4.4 nM to ~1.5 nM. Overall, ~45% of the Cd associated with the algae was accumulated within *P. subcapitata* whereas the remaining ~55% was measured in the Na_2-EDTA rinse (Table 6.2). Overall, Cd concentrations in algae had reached the desired Cd concentration although the mean Cd concentration in the algae used for the second feeding experiment (test B) was significantly lower than the first (Table 6.3).

Table 6.2: Mean dissolved Cd (nmol ± SE) measured in the algal exposure medium before and after *Pseudokirchneriella subcapitata* was exposed to the medium for 24 h, and mean Cd (nmol ± SE) measured in the Na_2EDTA solution used to rinse the algae. Two 1-L batches of algae were contaminated for each experiment and three aliquots were sampled from each batch. / Moyennes de Cd dissous (nmol ± erreur type de la moyenne) mesurées dans le milieu d'exposition algale avant et après l'exposition de 24 h de *Pseudokirchneriella subcapitata*, ainsi que la moyenne de Cd dissous (nmol ± erreur type de la moyenne) mesurée dans la solution de Na_2EDTA utilisée pour rincer les algues. Deux cultures de 1-L ont été contaminées pour chaque expérience et trois échantillons ont été prélevés de chaque culture.

	Test A			Test B		
	Initial Cd (nmol)	Final Cd (nmol)	Na_2EDTA (nmol)	Initial Cd (nmol)	Final Cd (nmol)	Na_2EDTA (nmol)
Batch 1 (n = 3)	4.24 ± 0.03	1.83 ± 0.01	0.75 ± 0.01	4.31 ± 0.02	1.56 ± 0.09	0.62 ± 0.03
Batch 2 (n = 3)	4.65 ± 0.02	1.77 ± 0.02	0.73 ± 0.01	4.19 ± 0.03	1.43 ± 0.40	0.53 ± 0.06
Mean ± SE	4.45 ± 0.26	1.80 ± 0.02	0.74 ± 0.01	4.26 ± 0.19	1.47 ± 0.36	0.57 ± 0.05

Table 6.3: Mean Cd concentrations (nmol·m^{-2} and nmol·g^{-1} dry wt ± SE) accumulated in *Pseudokirchneriella subcapitata* after a 24-h exposure to dissolved Cd (~4.4 nM). Two 1-L batches of algae were contaminated for each experiment and three aliquots were sampled from each batch. / Concentrations moyennes de Cd (nmol·m^{-2} et nmol·g^{-1} poids sec ± erreur type de la moyenne) accumulées dans l'algue verte *Pseudokirchneriella subcapitata* après une exposition de 24-h au Cd dissous (~4,4 nM). Deux cultures de 1-L ont été contaminées pour chaque expérience et trois échantillons ont été prélevés de chaque culture.

	Test A		Test B	
	nmol·m^{-2}	nmol·g^{-1}	nmol·m^{-2}	nmol·g^{-1}
Batch 1 (n = 3)	2.78 ± 0.06	95.9 ± 3.3	1.88 ± 0.03	71.9 ± 3.6
Batch 2 (n = 3)	2.04 ± 0.12	70.2 ± 6.4	2.09 ± 0.10	64.8 ± 1.4
Mean ± SE	2.41 ± 0.11	83.0 ± 6.6	1.98 ± 0.10*	68.3 ± 2.3*

* The mean Cd concentration in Test B was lower than in Test A (Student's t-test, $p < 0.05$)

6.3.2 Cadmium uptake by *Pyganodon grandis*

The highest cadmium concentrations were observed in the digestive gland followed by the intestines and gills (Table 6.4). Although Cd was measured in the intestines, these results are difficult to interpret as it is unknown whether the Cd measured in this organ was due in part to the presence of Cd-contaminated algae remaining in the intestines. In terms of burden, ~40% of the total Cd burden for *P. grandis* was found in the digestive gland, followed by the gills (~30%), the miscellaneous organs (~20%), the mantle (~10%), the intestines (~2%) and the foot (<1%) (Table 6.5). An ANOVA performed on the arcsine-transformed percentages showed that the proportion of Cd in each organ was relatively insensitive to the difference in Cd concentrations accumulated by the different algal batches. For example, no significant difference was observed in the relative contributions of the gills, digestive gland, mantle and foot between the two tests. For the miscellaneous organs and intestines, the proportions were somewhat more variable (Table 6.5), but this variability was not related to the Cd concentration in the algae.

The relative Cd burden measured in the organs of *P. grandis* changed throughout the 8-d depuration period (Figure 6.2). Immediately following the 4-d feeding period (i.e., 0 d of depuration), ~90% of the total Cd burden was found in the miscellaneous organs and intestines. The relative Cd burden associated with these organs decreased steadily during the depuration period as the proportion of the Cd burden in the gills and digestive gland increased over time (Figure 6.2). These results illustrate why it was necessary to depurate the animals by feeding them uncontaminated food in order to purge any Cd remaining in the intestines and stomach.

Table 6.4: Mean cadmium concentrations (pmol·g^{-1} fresh wt ± SE) in *Pyganodon grandis* exposed to diet-borne cadmium. / Concentrations moyennes de cadmium (pmol·g^{-1} poids frais ± erreur type de la moyenne) observées chez *Pyganodon grandis* exposée au cadmium alimentaire.

Test	Gills	Digestive Gland	Mantle	Miscellaneous Organs	Intestines	Foot
A (n = 8)	1.38 ± 0.27	10.4 ± 2.2	0.67 ± 0.18	0.97 ± 0.16	1.32 ± 0.20	0.17 ± 0.04
B (n = 9)	2.46 ± 0.44	11.3 ± 1.3	0.89 ± 0.16	0.92 ± 0.13	5.04 ± 1.74	0.25 ± 0.04
Mean	**1.95 ± 0.29**	**10.9 ± 1.2**	**0.79 ± 0.12**	**0.94 ± 0.10**	**3.06 ± 0.88**	**0.21 ± 0.03**

Table 6.5: Relative Cd burdens (% ± SE) in *Pyganodon grandis* organs after exposure to diet-borne cadmium. / Pourcentage de la charge totale en cadmium (avec l'erreur type de la moyenne) observé dans les différents organes de *Pyganodon grandis* après exposition des bivalves au cadmium alimentaire.

Test	Gills	Digestive Gland	Mantle	Miscellaneous Organs	Intestines	Foot
A (n = 8)	24 ± 4^{a}	46 ± 6^{a}	12 ± 2^{a}	15 ± 2^{a}	3 ± 0.5^{a}	0.6 ± 0.1^{a}
B (n = 9)	29 ± 2^{a}	38 ± 2^{a}	11 ± 1^{a}	20 ± 1^{b}	0.6 ± 0.1^{b}	0.7 ± 0.1^{a}
Mean	**27 ± 2**	**42 ± 3**	**11 ± 1**	**18 ± 1**	**2 ± 0.4**	**0.6 ± 0.1**

Superscripts a and b denote significant differences obtained by means of a one-way ANOVA ($p<0.05$) and a posteriori comparisons using a Tukey test.

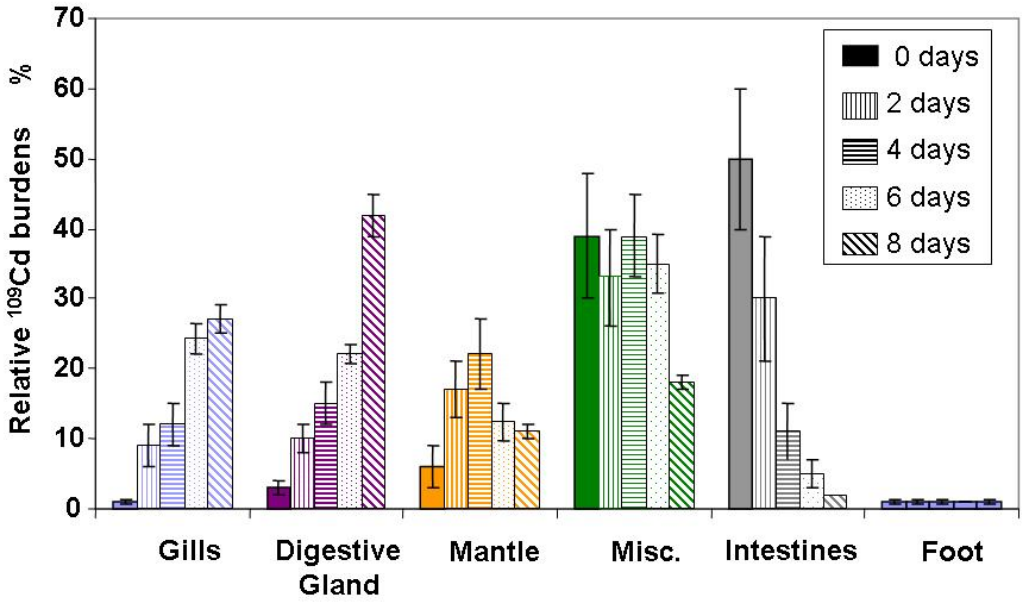


Figure 6.2: Relative Cd burdens (% ± SE) observed in *Pyganodon grandis* organs during the 8-day depuration period after exposure of the bivalves to diet-borne cadmium. / Pourcentage de la quantité totale du cadmium (avec l'erreur type de la moyenne) observé dans les différents organes de *Pyganodon grandis* durant la période de dépuration après l'exposition des bivalves au cadmium alimentaire.

6.3.3 Bivalve filtration rates, ingestion rates and cadmium assimilation efficiencies

To facilitate comparisons among bivalves, the results in this section will focus on those bivalves that fed during all 4 days of the study; bivalves that did not feed for the full 16 h were not considered. Bivalve filtration rates ranged from 12 to 48 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt) for an average of 25 ± 3 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt ± SE; n = 14) (Table 6.6). These values were similar to those reported in Section 5. Bivalve ingestion rates ranged from 9 to 54 µg dry wt of $algae \cdot h^{-1} \cdot g^{-1}$ bivalve (fresh wt) for an overall mean of 22 ± 3 µg dry wt of $algae \cdot h^{-1} \cdot g^{-1}$ bivalve (fresh wt ± SE; n = 14) (Table 6.6). Assimilation efficiency of diet-

borne Cd in whole bivalves ranged from 5 to >100% for an overall average of 57 ± 9% (± SE; n = 14) (Table 6.6). Assimilation of Cd in bivalves was inversely related to ingestion rates (Figure 6.3); AE values decreased as ingestion rates increased ($R = 0.88$, $p < 0.001$) suggesting that lower ingestion rates allow more complete digestion of the algal food and higher assimilation of the associated Cd.

Table 6.6: Mean bivalve filtration rates (FR: $mL \cdot h^{-1} \cdot g^{-1}$ fresh wt), ingestion rates (IR: µg dry wt of algae$\cdot h^{-1} \cdot g^{-1}$ bivalve fresh wt) and Cd assimilation efficiencies (AE: %) measured in individual specimens of *Pyganodon grandis*. Mean ± SE (n = 14) for each column are in bold. / Taux de filtration (FR: $mL \cdot h^{-1} \cdot g^{-1}$ poids frais), taux d'ingestion (IR: µg poids sec algue$\cdot h^{-1} \cdot g^{-1}$ bivalve poids frais) et efficacité d'assimilation du Cd (AE: %) moyennes observés chez des spécimens individuels de *Pyganodon grandis*. Les moyennes ± erreur type de la moyenne (n = 14) sont inscrites au bas de chaque colonne en caractères gras.

Test A			Test B		
FR	IR	AE	FR	IR	AE
27	17	87	38	25	23
24	26	24	13	12	94
25	29	35	20	14	85
17	21	69	24	9	155*
30	25	38	21	16	71
32	35	10	12	10	149*
48	54	5			
22	17	59			
28 ± 3	**28 ± 4**	**41 ± 10**	**22 ± 4**	**14 ± 2**	**78 ± 12**

* Two of the AE values in Test B were greater than 100%, suggesting that ingestion rates were underestimated for these bivalves. In calculating the average AE for test B, these two aberrant values have been set at 100%.

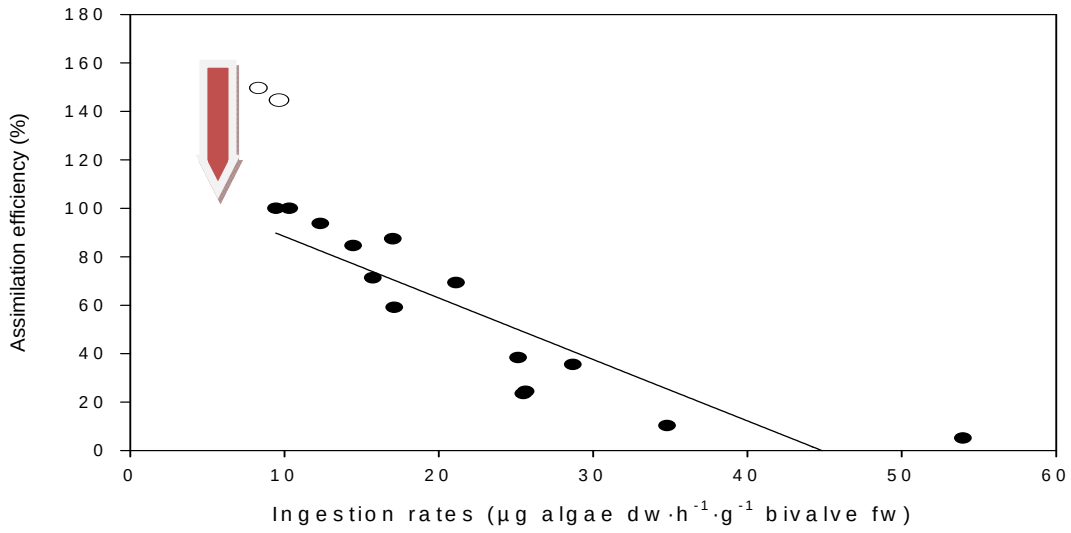


Figure 6.3: Relationship between Cd assimilation efficiency in whole organism (%) and bivalve ingestion rates (µg dry wt of algae·h^{-1}·g^{-1} bivalve fresh wt) for *Pyganodon grandis* exposed to dietary Cd. / Relation entre l'efficacité d'assimilation du Cd chez l'organisme entier et les taux d'ingestion des bivalves exposés au cadmium alimentaire (µg poids sec algue·h^{-1}·g^{-1} bivalve poids frais).

Note: The two AE values that exceeded 100% were set at 100% (arrow) for the regression calculation.

6.4 Discussion

6.4.1 Cadmium accumulation by *Pseudokirchneriella subcapitata*

There is much experimental evidence to suggest that the responses of algae to cadmium exposure (e.g., growth rate) are related to changes in the concentration of the free Cd ion and not directly to the total Cd concentrations. Reported results for Cd concentrations in *Pseudokirchneriella subcapitata* are summarized in Table 6.7. It is important to note that Cd speciation was not controlled during the present experiment, unlike the studies summarized in Table 6.7 that reported an influence of Cd speciation on Cd uptake by algal cells. Overall, our values are comparable to those reported in the studies cited below.

Table 6.7: Summary of accumulated Cd concentrations (nmol·m^{-2} and nmol·g^{-1} dry wt) measured in *Pseudokirchneriella subcapitata* exposed to various concentrations of dissolved Cd (nM) in the laboratory. / Résumé des concentrations de Cd accumulées (nmol·m^{-2} et nmol·g^{-1} poids sec) chez *Pseudokirchneriella subcapitata* exposée en laboratoire à des concentrations différentes de Cd dissous (nM).

[Cd] water (nM)	pH	Time	[Cd]algae (nmol·m^{-2})	[Cd]algae (nmol·g^{-1} dw)	Reference
14	5	45 min	0.55	18.9	(Vigneault 2000)
9	7	30 min	7.00	241	(Vigneault 2000)
69	7	1 h	-	2400	(Maloney 2007)
4.4	7	24 h	2.19	75.7	Present study

In comparison, relatively few studies have investigated metal uptake in algae in natural settings. Sigg (1987) studied the role of settling particles in metal cycles in a eutrophic lake. The field data indicated that biological particles, which included algae, played an important role in removing dissolved metals from the water column and regulating their concentrations. Sigg (1987) reported that, depending on pH, 10 to 50% of the total Cd concentration in the water column could be associated with suspended biological particles. Reported results for Cd concentrations measured in suspended material, which include algae, collected from various lakes are summarized in Table 6.8. The mean accumulated Cd concentrations in *P. subcapitata* reported for the present study are just below or exceed somewhat the upper limits reported in the studies cited below.

Table 6.8: Range and mean Cd concentrations measured in natural phyto-plankton collected from freshwater lakes. / Gamme de concentrations de Cd mesurées chez le phytoplancton prélevé dans des lacs d'eau douce.

Range (dry wt)	Mean (dry wt)	Reference
23 to 92 nmol·g^{-1} biomass	58 nmol·g^{-1} biomass	(Sigg 1987)
5 to 33 nmol·g^{-1} settling particles	14 nmol·g^{-1} settling particles	(Sigg 1987)
1 to 80 nmol·g^{-1} algae	55 nmol·g^{-1} algae	(Knauer *et al.* 1998)
75 to 120 nmol·g^{-1} algae	90 nmol·g^{-1} algae	Present study

6.4.2 Dietary cadmium uptake in *Pyganodon grandis*

The results from the present study suggest that the digestive gland is the main target organ when *P. grandis* is exposed to dietary Cd. This is in agreement with other studies that have noted that, in general, a dietary source of metal will be accumulated in the digestive gland. Harvey and Luoma (1985) reported that accumulated Cd concentrations were highest in the digestive gland of *Macoma balthica* after a 22-d exposure to radiolabelled bacteria. Graney *et al.* (1984) and Wang *et al.* (1996) also noted that concentrations of recently accumulated Cd were higher in the digestive gland than in the other organs of *Corbicula fluminea* and *Mytilus edulis* after a dietary Cd exposure.

The present results contrast with those previously discussed in Section 5 where the gills were the main site of Cd accumulation when *P. grandis* was exposed to aqueous Cd. This difference is likely due to differential uptake mechanisms that are dependent on the function of the cells at each of the uptake sites. Accumulation of dissolved Cd requires cellular uptake by either a specific carrier-mediated process or pinocytosis by tissue epithelial cells (Simkiss and Taylor 1989). In contrast, Cd bound within algal cells enters the digestive gland where the algal cells are engulfed through phagocytosis and subsequently digested (Widdows *et a*l. 1979). Assimilated food is presumably passed from the base of the digestive cell into the hemocoelic blood for transport to other body parts (Widdows *et a*l. 1979).

The transport of Cd from the gut via the circulatory system presumably accounts for the presence of radiolabelled Cd in the gills (30%) following diet-borne Cd exposure. Other studies have also observed the presence of Cd in the gills of bivalves exposed to a dietary source of Cd. Tran *et al*. (2002) noted that, after feeding *C. fluminea* a Cd-contaminated diet, 60 to 70% of the total Cd burden was located in the visceral mass (which contained the digestive gland), followed by the gills (15-20%), the muscles and foot (9-15%) and the mantle (6-8%). Wang *et al.* (1996) also noted that a dietary source of Cd could result in Cd being present in other than digestive organs. They reported that, after feeding *M. edulis* radiolabelled algae for 7 days, >60% of the total Cd burden was

located in the digestive gland, followed by the mantle (~30%), the foot and muscles (~10%), and the gills (<5%).

It is known that the digestive gland of bivalves is a dynamic organ, which may undergo a sequence of cytological changes during the course of a digestive cycle (Hemelraad et Herwig 1988). These cytological changes may influence the amount of Cd available for transport from the digestive gland to the other organs. Another factor that may affect the movement of Cd from one organ to another is the length of the exposure and depuration periods (Wang *et a*l. 1996). This redistribution of Cd among organs may be related to changes in the subcellular partitioning of Cd in the digestive gland during the depuration period. This possibility is explored in Section 7.

Ingestion rates reported for this study are lower than those reported for marine bivalve species but are higher than the ingestion rates reported for *Corbicula fluminea*, a freshwater mussel (Table 6.9). Although ingestion rates had little effect on the total amount of Cd accumulated in the digestive gland of *P. grandis*, unlike the relationship observed between dissolved Cd and $[Cd]_{gills}$ (Section 5), ingestion rates did significantly influence the assimilation efficiency of dietary Cd in *P. grandis*. In general, AE was higher when ingestion rates were low (Figure 6.3). Similar relationships between AE and ingestion rates have been reported for other mussel species (Chong et Wang 2000a; Wang and Fisher 1996a) as well as gastropods (Cheung et Wang 2005) and zooplankton (Munger et Hare 2000).

Table 6.9: Ingestion rates as reported for other bivalve species in the literature and transformed into µg of food dry weight·h^{-1}·(g bivalve)$^{-1}$ fresh weight. / Taux d'ingestion décrits dans la littérature pour d'autres espèces de bivalves et transformés en µg nourriture poids sec·h^{-1}·(g bivalve)$^{-1}$ poids frais.

Species	Food	Ingestion rates		Reference
		Literature	Data expressed on common basis (bivalve fresh wt)	
Marine species				
Mytilus edulis	NS	1,900 µg dw·h^{-1}·g^{-1} dw	8,400 µg dw·h^{-1}·g^{-1} fw	(Wang *et a*l. 1996)
Mytilus edulis	A	110 to 1,540 µg dw·h^{-1}·animal^{-1} dw	490 to 6,840 µg dw·h^{-1}·g^{-1} fw	(Wang *et al.* 1995)
Mytilus galloprovincialis	D	130 µg dw·h^{-1}·animal^{-1} dw	315 µg dw·h^{-1}·g^{-1} fw	(Fisher *et a*l. 1996)
Freshwater species				
Corbicula fluminea	A	0.5 to 2 µg dw·h^{-1}·animal^{-1} dw	3 to 12 µg dw·h^{-1}·g^{-1} fw	(Tran *et a*l. 2002)
Pyganodon grandis	A		22 µg dw of algae·h^{-1}·g^{-1} fw bivalve	Present study

A: algae assemblages; D: diatoms; NS: natural seston.

Similarly to bivalve ingestion rates, the assimilation efficiency of Cd varied greatly among individual specimens of *P. grandis*. Reported results for mean Cd assimilation efficiencies calculated for various marine and freshwater mussels are summarized in Table 6.10. Generally, there was a good agreement between the AEs of Cd determined in the present study for *P. grandis* and the studies cited in Table 6.10.

Table 6.10: Cadmium assimilation efficiencies (%) calculated for bivalves fed a variety of Cd-contaminated food particles. / Efficacités d'assimilation du cadmium (%) calculées pour des bivalves ayant été nourris avec une variété de particules contaminées en Cd.

Species	Food	AE (%)	Reference
Marine species			
Crassostrea virginica	A	69	(Reinfelder *et a*l. 1997)
Macoma balthica	S	4 to 42	(Decho and Luoma 1994)
	NS	13 to 21	(Lee and Luoma 1998)
	S + A	8 to 20	(Lee and Luoma 1998)
	A	33 to 51	(Lee and Luoma 1998)
	A	69 to 88	(Reinfelder *et a*l. 1997)
Mercenaria mercenaria	A	66 to 83	(Reinfelder *et a*l. 1997)
Mytilus edulis	A	19 to 30	(Borchardt 1983)
	D	19 to 26	(Fisher *et a*l. 1996)
	D	37 to 41	(Reinfelder *et a*l. 1997)
	A, D, S	11 to 34	(Wang and Fisher 1996a)
	D	11 to 40	(Wang *et a*l. 1996)
Mytilus trossulus	S + D	36 to 92	(Arifin and Bendell-Young 2000)
Potamocorbula amurensis	S	1 to 49	(Decho and Luoma 1994)
	NS	20 to 48	(Lee and Luoma 1998)
	S + A	8 to 16	(Lee and Luoma 1998)
	A	26 to 45	(Lee and Luoma 1998)
Perna viridis	S + D	11 to 48	(Ke and Wang 2002)
	D, S	5 to 23	(Wang et Wong 2003)
Freshwater species			
Dreissena polymorpha	A, NS	19 to 48	(Roditi and Fisher 1999)
	NS	23	(Roditi *et a*l. 2000a)
Pyganodon grandis	A	5 to >100	Present study

A: alga assemblages; D: diatoms; NS: natural seston; S: sediment; S+A: sediment-algae mixture; S+D: sediment-diatom mixture

The factors that influence AE are known (i.e., the nature of the food, the animals digestive strategy, the form of metal that is released from digestion, how the metal is taken up by the intestines or digestive gland), but their precise contributions in an individual bivalve at a given time are not as well known (Luoma et Rainbow 2008). It has been suggested that species-specific feeding behaviour may influence metal assimilation from food. For example, Decho and Luoma (1994) compared two bivalves, *Macoma balthica* and *Potamocorbula amurensis*, having different digestive strategies. In *M. balthica,* Cd was accumulated in the bivalve after an intestinal digestion whereas *P. amurensis* processed a large proportion of its food through glandular digestion. As a result, *P. amurensis* had higher assimilation efficiencies for Cd, and therefore higher overall Cd concentrations in its organs than did *M. balthica*, due to its more effective digestive strategy. Longer gut residence times have also been shown to increase the AE of metals by bivalves as they may enable food to be subjected to more rigorous digestion (Wang and Fisher 1996a). However, Decho and Luoma (1994) suggest that gut retention is not the critical factor in metal assimilation in bivalves as longer gut retention times did not increase metal assimilation in either *M. balthica* or *P. amurensis*.

Assimilation efficiency of Cd will also vary within the same species depending on the type of food the mussels consume (Table 6.10). The AE of metals associated with sediments is generally much lower than the AE from algae and diatoms (Chong and Wang 2000b; Gagnon et Fisher 1997; Griscom *et al.* 2000). Lee and Luoma (1998) demonstrated that the assimilation efficiencies of Cd were higher in *P. amurensis* and *M. balthica* when the mussels were fed contaminated algae than when they were fed sediments or a sediment-algae mixture. Contrary to *P. amurensis* and *M. balthica,* the assimilation efficiency of Cd varied little in *M. edulis* regardless of whether the mussel was fed diatoms, algae or natural seston (Table 6.10). One reason that Cd assimilation was higher for *P. amurensis* and *M. balthica* fed algae may be that Cd present in the algal cytoplasm is more readily available for assimilation by these organisms (Reinfelder et Fisher 1991; 1994). Moreover, if animals are fed a mixture of particles, different AEs may be observed among species as a result of a selective ingestion of particles as

different bivalve species will feed on different types of suspended particles (Lee and Luoma 1998).

Additional important biological processes that may influence metal assimilation from food include chemical reactions in the digestive tract (Mayer *et al.* 1996; Reinfelder and Fisher 1994). For example, lower gut pH (<5.0) was shown to increase metal assimilation from food in mussels. Griscom *et al.* (2002) observed that *M. balthica* had consistently higher metal AEs than *M. edulis* and suggested that this was partly the result of a lower gut pH in *M. balthica* (pH <5.0) compared to a gut pH of >5.5 in *M. edulis.* Yonge (Yonge 1949) suggested that low pH conditions in the guts of bivalves aid in digestion by creating an optimum pH for extracellular digestion which enhances the accumulation of metals. However, gut pH has been shown to vary spatially (in digestive tubules) and temporally. For example, the gut pH of *M. balthica* has been shown to vary temporally from <5.0 to 6.8 (Griscom *et al.* 2002; Purchon 1971). Such variability would, therefore, influence metal assimilation efficiencies from food.

The focus of the present study was to measure Cd accumulation in *P. grandis* after dietary exposure from one algal species. The goal was not to simulate a complex, realistic exposure to dietary Cd by feeding *P. grandis* a mixture of algae and inorganic particles. Several studies have reported that bivalve ingestion rates, metal assimilation efficiencies and metal uptake vary greatly depending on food mixtures, algal species, algal densities in the medium and the subcellular partitioning of metals within the algal cells. Such topics are discussed further in Chapter 8 and will be taken into consideration when establishing a Cd bioaccumulation model for *P. grandis*.

This study does, however, differ from many previous dietary metal exposure experiments conducted under laboratory conditions using mussels. One of the few limitations associated with the radiotracer technique is that some of the radioactive material adsorbed onto the food particles will desorb from the particle surface and into the ambient medium, thus creating a dissolved metal exposure. For this study, desorption

of Cd from the algal cells was reduced by rinsing the algae with Na_2EDTA prior to feeding the bivalves. Release of Cd into the medium by microbial degradation or by the leaching of soluble material from feces is also a potential source of dissolved Cd. The availability of any potential leaked Cd was minimized in the present study by the addition of Na_2EDTA (10^{-4} M). These precautions ensured that any Cd that had accumulated in *P. grandis* was truly from a dietary source and not from solution.

6.5 Conclusions

The present study is the first to measure Cd accumulation in the freshwater bivalve *P. grandis* solely from the diet. Cadmium concentrations and relative burdens were highest in the digestive gland, contrasting with the results obtained from a dissolved Cd exposure in which the gills were the main organ of accumulation. Unlike the gills, however, no significant relationship was established between $[Cd]_{digestive\ gland}$ and bivalve filtration or ingestion rates. Cadmium assimilation efficiencies were variable among individual bivalves but were similar in magnitude to those reported for other mussel species. The results obtained from the present study will be used in Chapter 8 to develop a Cd bioaccumulation model that will take into account Cd accumulation from both food and water.

7. SUBCELLULAR PARTITIONING OF CADMIUM

7.1 Introduction

Bivalves have been used worldwide as sentinel organisms to evaluate the health of aquatic ecosystems and to determine whether contaminants in a given geographical area have reached levels that may pose a threat to other organisms. They are commonly used as bioindicators for metal pollution because of their ability to bioaccumulate and integrate metals present at very low concentrations in water or sediment (Beckvar *et a*l. 2000; Roesijadi 1996). Bivalves are capable of accumulating high internal concentrations of metals in their gills and digestive gland; the gills typically accumulate metals from the dissolved phase whereas the digestive gland can accumulate metals from both aqueous and dietary sources. The ability of bivalves to tolerate high internal concentrations of metal has been attributed to the presence of metal-binding proteins and calcium concretions that sequester and bind metals, reducing their bioavailability within the organism (Roesijadi 1992; Langston *et al.* 1998; Roesijadi 1992; Viarengo 1989; Wallace *et al.* 2003). However, measuring only the total metal content in a specific organ does not give any information about the potentially toxic effects the metal may induce at the subcellular level.

Once absorbed into the circulatory system, metals are transported to specific organs, where they may be utilized in normal metabolism, eliminated or, if they cannot be eliminated, sequestered. When an essential metal, such as copper, is incorporated into a molecule (e.g., a metalloenzyme) it enters the routine metabolic pathways. When a toxic non-essential metal, like cadmium, enters the cell, it is usually complexed and removed, either by sequestration or excretion (Rainbow 2002). Metal-binding ligands, such as metallothioneins (MT), are now known to be ubiquitous among mollusc species (Langston *et a*l. 1998). Metallothioneins participate in the homeostasis of essential metals such as Cu and Zn, and in the detoxification of non-essential and toxic metals such as Ag, Cd, and Hg. Laboratory experiments suggest that the biosynthesis of these metalloproteins may be induced by exposure to metals such as Ag and Cd (Langston *et*

*a*l. 1998). Although not necessarily induced by metal exposure, granules can also play a role in the detoxification of metals (Simkiss 1981) and are found in the hepatopancreas (Simkiss and Taylor 1989; Simkiss 1981) and gills of bivalves (Silverman *et a*l. 1983; Silverman *et al.* 1987), where they also serve as a source of calcium for embryonic shell development. Although marine bivalves have similar granules, they are not produced in such high concentrations as is characteristic of unionids and other freshwater bivalves. Several field and laboratory studies have shown that the relative contributions of MT and granules to total metal burdens are highly dependent on the target organ and the biochemical processes occurring within cells (Bonneris *et a*l. 2005b; Bonneris *et a*l. 2005a; Langston *et a*l. 1998; Bebianno et Langston 1992).

It has been suggested that if metal accumulation exceeds the binding capacity of available metallothionein (MT) or granules, then the metals may bind to other intracellular ligands, a phenomenon termed spillover. It is when metals are bound to these other ligands that signs of cellular toxicity may appear (Brown et Parsons 1978; Mason and Jenkins 1995; Sanders et Jenkins 1984). The binding of an "inappropriate" metal to a metal-sensitive site, like organelles or enzymes, is often associated with a failure of detoxification mechanisms and could be an indicator of metal-induced stress (Wallace *et a*l. 2003; Campbell *et al.* 2005).

Few studies have addressed cadmium accumulation in different organelles, in particular in mitochondria, which are the primary site of ATP production and a key target for cadmium toxicity (Kesseler et Brand 1994). Cadmium was shown to inhibit mitochondrial bioenergetics in oysters, *Crassostrea virginica*, exposed to a range of dissolved Cd concentrations (10^{-7} to 10^{-6} M), by impairing the organisms' ability to produce ATP (Sokolova 2004). Consequently, cadmium accumulation in mitochondria may result in serious disturbances of tissue energy balance and eventually cell death (Sokolova *et al.* 2005b). Another key intracellular compartment involved in Cd accumulation is the lysosome. Lysosomes are an important type of organelle in which molluscs sequester metals, especially in their hepatopancreas (Marigomez *et al.* 2002).

Increased metal concentrations in the lysosomal subcellular fraction in response to an increase in ambient metal concentrations have been reported in bivalves (Giguère *et a*l. 2003; Bonneris *et a*l. 2005b), which may reflect compensatory changes to increased cellular metal concentrations. Viarengo *et al.* (1989) suggested that MTs may also be involved in the transfer of metals to lysosomes, thus serving as a link between the cytosolic and lysosomal systems of metal sequestration.

Recent studies have shown that the freshwater bivalve, *Pyganodon grandis*, has potential as a sentinel organism for metal pollution. It is tolerant of a wide range of metal contamination and is capable of concentrating many metals, suggesting that it must be able to mount an effective detoxification response (Couillard *et a*l. 1993; Couillard *et a*l. 1995b; Couillard *et a*l. 1995a; Wang *et a*l. 1999). Spatial studies have consistently shown a relationship between Cd concentrations in *P. grandis* and Cd concentrations in its environment (Tessier *et a*l. 1993; Couillard *et a*l. 1993; Perceval *et a*l. 2002), and recently these studies have been extended to consider not just total metal accumulation but also the subcellular partitioning of metal (Cd, Cu, Zn) in the gills and the digestive gland – see Giguère *et al*. (2003) and Bonneris *et al.* (2005b; 2005a). These studies demonstrated that in both organs, metal concentrations in the tissues responded in a concentration-dependent manner to changes in ambient metal levels, although the strength of the relations was weaker for the digestive gland. At the subcellular level, the differences between the gills and the digestive gland were even more pronounced. For instance, in the gills all three metals were located largely in calcium concretions, whereas in the digestive gland these granules played a minor role in metal accumulation and other sub-cellular compartments, such as the "heat-stable proteins" fraction and, to a lesser extent, the "lysosomes + microsomes" and "mitochondria" fractions, assumed greater importance. In both organs, metal concentrations in the "heat-denaturable protein" fraction remained low and constant, suggesting reasonably effective metal detoxification. These studies concluded that the sub-cellular distribution of Cd, Cu and Zn was linked to the metabolic orientation of the tissue and to the relative affinity of the various cellular ligands for each metal.

However, no short-term laboratory studies focusing on the subcellular partitioning of metals have been conducted with *P. grandis*. Results derived from the laboratory would complement those already obtained from the field and should allow us to answer some questions, such as: Does the subcellular distribution of a metal differ in a short-term exposure compared to a long-term exposure? Does subcellular partitioning vary as a function of the exposure pathway (aqueous vs. dietary exposure)? Does the subcellular distribution of metals change during the depuration phase? And lastly, can the metals be redistributed among organs or subcellular fractions after metal exposure? The purpose of this study was to measure the subcellular distribution of Cd in *P. grandis* after a short-term laboratory-exposure to a low, environmentally relevant, Cd concentration (5 nM) and a high, unrealistic, Cd concentration (275 nM). The results are compared to those previously obtained from field-collected animals. We also observed the subcellular partitioning of Cd in *P. grandis* throughout a depuration period after the animals were fed Cd-contaminated algae to see if (a) the subcellular distribution of Cd differed with metal uptake pathway, and (b) to observe whether there was any redistribution of Cd among organs and subcellular fractions during the depuration phase.

7.2 Methods

7.2.1 Aqueous and dietary cadmium

Prior to the exposure experiments, six bivalves were collected from the "stock culture" (Section 3.2) and were sacrificed to be used as controls during this study. The gills and digestive glands were removed and stored at -80ºC until analysis. The remaining organs (mantle, foot and miscellaneous organs) were discarded.

For the dissolved Cd exposures, bivalves were exposed to either 5 nM (n = 36) or 275 nM (n = 16) of dissolved Cd (radioactive ^{109}Cd + cold ^{112}Cd; specific activity of radioactive ^{109}Cd: 0.5 μCi·μg Cd^{-1}). Before an experiment, bivalves were removed from their "stock culture" and placed separately in 2-L beakers filled with uncontaminated synthetic medium (described in Section 3.3). Bivalves were allowed to acclimate in the

experimental set-up for 4-5 days and were fed daily with the green alga *Pseudokirchneriella subcapitata.* To reduce the presence of organic matter in the exposure aquaria, bivalves were starved during the last 24 h of acclimation to minimize the production of pseudofeces. For every Cd concentration, four 6-L aquaria (3 experimental aquaria containing 1 group of bivalves each + 1 control aquarium without bivalves) were filled with synthetic medium that had been contaminated with dissolved Cd. For the 5 nM exposure, bivalves were exposed to the dissolved Cd for 6 h (n = 12), 12 h (n = 12) or 24 h (n = 12). For the 275 nM exposure, bivalves were exposed to the dissolved Cd for either 24 h (n = 6) or 96 h (n = 6). Bivalves were fed during the 96-h exposure experiment as described in Section 5.2.1 (Figure 5.1) but not in the shorter experiments. Bivalves from both dissolved exposures were allowed to depurate overnight in a separate beaker filled with uncontaminated synthetic medium prior to dissection.

For the dietary exposure, 24 bivalves were fed Cd-contaminated *Pseudokirchneriella subcapitata* as described in Section 6.2 (Figure 6.1), with 4 x 4 h feedings. Once the exposure period was completed, the bivalves were transferred into feeding beakers that contained fresh medium spiked with EDTA (10^{-4} M) and allowed to depurate. Three bivalves were collected after 0, 2, 4 and 6 d of depuration and sacrificed while the remaining 12 bivalves were allowed to depurate for 8 d. During the depuration period, bivalves were fed continuously with uncontaminated *P. subcapitata* so that they would purge any contaminated algae from their gut.

During dissection, organs (gills, digestive gland, mantle, foot and miscellaneous organs) were separated and rinsed with EDTA (10^{-3} M) for 20 min to remove any Cd adsorbed on their surface. The mantle, foot and miscellaneous organs were weighed separately, placed in a scintillation vial and analyzed for ^{109}Cd using a Gamma counter. Total Cd was later calculated as described in Section 3.4. The gills and digestive gland were placed in Eppendorf® tubes and stored at -80ºC until they could be homogenized and fractionated as described below. After fractionation, the pellets were lyophilized (72 h)

and all six cellular fractions were analyzed for ^{109}Cd using a Gamma counter. Cadmium concentrations in the organs and the cellular fractions were calculated according to the description provided in Section 3.4. As a quality control measure, we compared the sum of Cd recovered in the six fractions with the initial total Cd burden as determined in a subsample removed from the original homogenate, before the fractionation procedure. Agreement between the two values was good – gill control (Cd: 94 ± 7%); digestive gland control (Cd: 96 ± 9%).

7.2.2 Subcellular partitioning procedure

The gills and digestive gland of control and experimental bivalves were partially thawed, homogenized on ice and separated into subcellular fractions. The subsequent subcellular fractionation procedure was adapted from that described by Giguère *et al.* (2006) and Wallace *et al*. (1998) and is illustrated in Figure 7.1. To minimize disruption of cellular organelles, tissues were manually homogenised on ice (20 turns of the pestle for the gills and 12 turns of the pestle for the digestive gland) in a glass grinder (Glas-Col product). Homogenisation was performed in 25 mM Tris buffer (OmniPur) adjusted to pH 7.2 at 4°C; the tissue-to-buffer ratio was adjusted to 1:2 (wet weight tissue: volume of buffer). A sub-sample of this homogenate was removed for determination of total tissue metal concentrations. Another subsample (3 mL) of tissue homogenate was centrifuged at 800*g* for 15 min to obtain cells, membranes, nuclei and granules. To isolate granules (Gr), this first pellet was resuspended in water (500 µL) and heated to 100°C for 2 min. An equal volume of 1 N NaOH (99.998%, Aldrich) was added and the mixture was heated between 60 and 70°C for 60 min before a final centrifugation (10,000*g* for 10 min). The supernatant from the initial 800*g* separation was re-centrifuged at 10,000*g* for 30 min to obtain the mitochondrial fraction (Mit) and a supernatant. The mitochondrial supernatant was centrifuged at 100,000*g* for 60 min. This step yielded the lysosomal and microsomal fraction (Lyso) and a supernatant or cytosol, which was heated at 80°C for 10 min and then cooled for 60 min and centrifuged at 50,000*g* for 10 min in order to obtain a pool of heat-denaturable proteins (HDP) and a final soluble supernatant of heat-stable proteins (HSP). Subcellular fractions were stored at -20°C until lyophilisation

and, in the case of the non-radioactive control bivalves, the two supernatants were acidified with 65% nitric acid (Fisher Scientific, trace metal grade; 1:1 (v/v) nitric acid added). Acidification was not necessary in the case of the supernatants collected from the bivalves that had been exposed to ^{109}Cd as samples do not need to be acidified when analyzing ^{109}Cd with a Gamma counter.

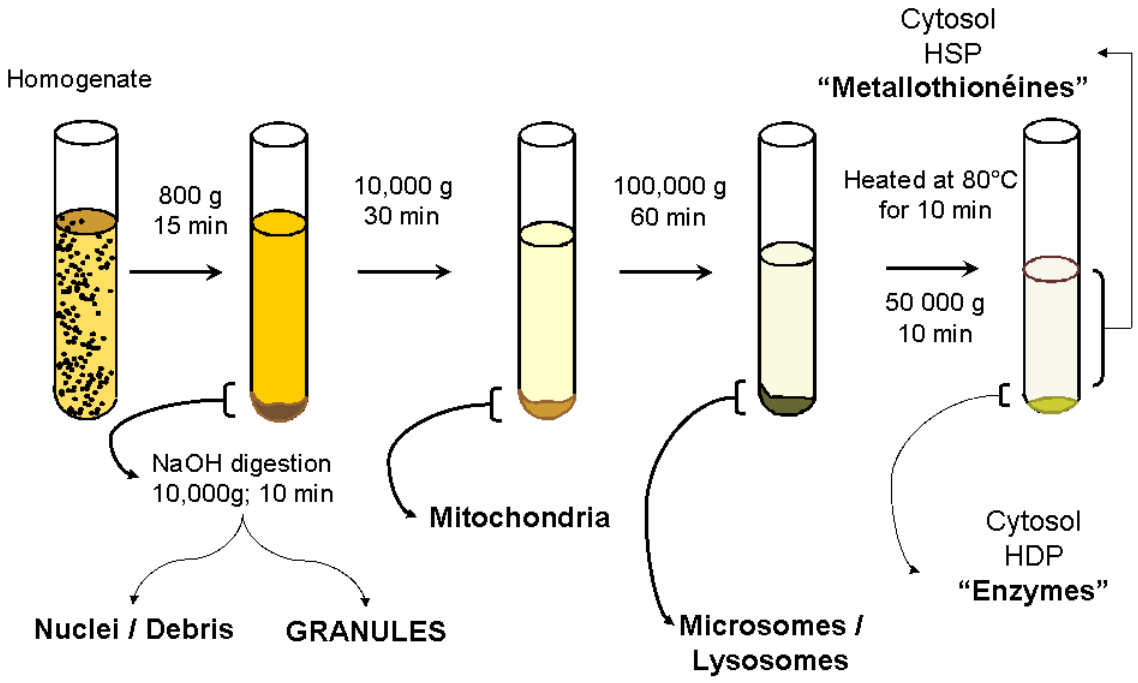


Figure 7.1: Protocol for subcellular partitioning by differential centrifugation. / Protocole de la répartition subcellulaire par centrifugation différentielle.

7.2.3 Metal analyses and analytical quality control for control bivalves

The fractionated gills and digestive gland of control bivalves were analysed for Cd, Cu, Zn and Ca by atomic emission spectrometry (Varian Vista ICP-AES). Solid samples were lyophilized (72 h) and then subjected to acid digestion prior to analysis. The dry tissues and certified reference material (lobster hepatopancreas reference material for metals: TORT 2, National Research Council Canada, Halifax, NS, Canada) were digested in 65% nitric acid for 24 h, transferred to Teflon (PTFE) bombs for digestion under pressure (124 kPa, 240°C) for 3 h and diluted before analysis. Analytical procedural blanks and standard reference materials (NIST 1640, National Institute of Standards and Technology, Gaithersburg, MD, USA; multi-element, Plasma CAL; multi-element SCP Science) were analysed during each run. An internal standard (yttrium) was added to the matrix modifier (certified caesium chloride 99.99%, Fisher Scientific) to control for variations in the atomization procedure. The procedural blanks indicated no appreciable contamination (< detection limit) and the aqueous certified reference samples were quantitatively recovered in samples of gills (Cd (214.4 nm): 100 ± 2%, Cu (327.4 nm): 99 ± 2%, Zn (213.8 nm): 102 ± 8%) and for the digestive gland (Cd (214.4 nm): 99 ± 3%, Cu (327.4 nm): 101 ± 6%, Zn (213.8 nm): 100 ± 5%). The percent recovery of TORT 2 reference samples (n = 6) was within the certified range (Cd: 26.4 ± 0.9 $\mu g \cdot g^{-1}$ dry wt; Cu: 105 ± 11 $\mu g \cdot g^{-1}$ dry wt; Zn: 178 ± 8 $\mu g \cdot g^{-1}$ dry wt). Low to negligible contamination was detected in digestion blanks.

As a final quality control measure, for these control bivalves, we compared the sum of the metal quantities recovered in the six fractions with the initial total metal burden as determined in a subsample removed from the original homogenate, before the fractionation procedure. Agreement between the two values for the control bivalves (i.e., not exposed to ^{109}Cd) was consistently good – gill control (Cd: 99 ± 5%; Cu 100 ± 7%; Zn 93 ± 3%); digestive gland control (Cd: 102 ± 7%; Cu 99 ± 6%; Zn 103 ± 10%).

7.2.4 Data analysis

Unlike previous sections, results are presented as mean Cd concentrations ($nmol \cdot g^{-1}$ or $pmol \cdot g^{-1}$ dry wt) ± standard deviations (SD). This facilitated comparisons between the results presented in this study and those from previous studies that were presented as means ± SD (Bonneris *et al.* 2004, 2005a, 2005b). Differences in metal concentrations in the organs and subcellular fractions of control and experimental bivalves were compared using a one-way analysis of variance (ANOVA; $p < 0.05$) or Wilcoxon's rank test for non-parametric data (i.e., when $n = 3$). The total Cd burden, expressed as percentages, in bivalve organs obtained for each Cd treatment was transformed (arcsine) and compared using an ANOVA ($p < 0.05$) or Wilcoxon's rank test. When the general variance model was significant for parametric data, multiple comparisons were conducted using the Tukey test. Statistical analyses were performed using SYSTAT and Sigma Plot software.

7.3 Results

7.3.1 Control animals

The total metal concentrations and subcellular distribution of Cd, Cu and Zn in the gills and digestive gland of control bivalves were compared with those previously measured for bivalves collected from Lake Opasatica in October 2002 and October 2004 (Michaud 2005; Bonneris 2004). The designation "control bivalves" in this context means bivalves collected from Lake Opasatica in 2006 that were never exposed to (radio-labelled) Cd.

For the gills, no significant differences in the total concentrations of Cd, Cu and Zn of control bivalves were observed among the three bivalve groups (2002, 2004, and 2006: Table 7.1). Similarly, the percentages of Cd, Cu and Zn in the different subcellular fractions of the gills also did not significantly differ among the three groups (Table 7.2). Comparison of the absolute concentrations of Cd, Cu and Zn concentrations in each subcellular fraction did show, however, that metal concentrations measured in the HDP fraction of the control bivalves for our experiments (2006) were somewhat lower than

those observed for the Lake Opasatica bivalves sampled on previous occasions (Figures 7.2 a,b,c). Overall, Cd, Cu and Zn were mainly found in the granule fraction of the gills, although all three metals were also found in the cytosolic compartment.

In the digestive gland, total concentrations of Cd, Cu and Zn in the control bivalves were significantly lower than those observed for the bivalves collected in 2002 and 2004 (Table 7.1). The percentages of Cd, Cu and Zn in the subcellular fractions of the digestive gland were roughly similar among the three bivalve groups, except for the HSP fraction; the percentages of Cd, Cu and Zn in this fraction isolated from the digestive gland of 2006 control bivalves were significantly higher than those observed for the bivalves collected in 2002 and 2004 (Table 7.3). In addition, the percentages of Cu and Zn in the granule fraction were significantly lower in the 2006 control bivalves. The total Cd, Cu and Zn concentrations measured in many of the subcellular fractions isolated from the digestive gland of the control bivalves (notably the granule, mitochondria and HDP fractions) were significantly lower than those observed for the Lake Opasatica bivalves collected in earlier years. In contrast, Cu concentrations were significantly higher in the HSP fraction of control bivalves (Figures 7.4 a,b,c).

In general, Cd, Cu and Zn were mainly stored in the cytosolic fraction and, to a lesser extent, in the mitochondria and lysosomes fractions of the digestive gland. Although metals do accumulate in the nuclei and debris cell fraction of both organs, we cannot interpret those results toxicologically (due to the ambiguous nature of this operationally-defined fraction; Bonneris *et al*. (2005b); Giguère *et al*. (2006)), and they will therefore be ignored.

Having established the baseline partitioning of non radioactive Cd (and other metals) in our control bivalves, we will now consider how the bivalves handled radiolabelled ^{109}Cd from waterborne and diet-borne sources.

Table 7.1: Comparison between the total Cd, Cu and Zn concentrations (nmol·g^{-1} dry wt ± SD) measured in the gills and digestive gland of control bivalves and those measured for bivalves collected from Lake Opasatica in October 2002 and October 2004. / Comparaison entre les concentrations totales en Cd, Cu et Zn (nmol·g^{-1} poids sec ± écart-type) mesurées dans les branchies et glandes digestives des bivalves témoins et les bivalves échantillonnés du Lac Opasatica en octobre 2002 et octobre 2004.

	Year	n	[Cd]	[Cu]	[Zn]	Reference
Gills	2002	3	98 ± 14	378 ± 90	5240 ± 875	Bonneris (2004)
	2004	4	104 ± 5	342 ± 75	5720 ± 577	Michaud (2005, unpublished)
	2006	6	94 ± 21	320 ± 85	6450 ± 628	Present study
Digestive gland	2002	3	160 ± 3	366 ± 28	2110 ± 83	Bonneris (2004)
	2004	4	189 ± 64	458 ± 20	2100 ± 83	Michaud (2005, unpublished)
	2006	6	108 ± 35*	231 ± 77*	1600 ± 96*	Present study

An asterisk (*) denotes a significant difference among bivalve groups for a given metal and organ as determined by Wilcoxon's rank test.

Table 7.2: Percentages of Cd, Cu and Zn (± SD) in the subcellular fractions isolated from the gills of control bivalves (2006; n = 6) and bivalves collected from Lake Opasatica in October 2002 (n = 3) and October 2004 (n = 4). / Pourcentages de Cd, Cu et Zn (± écart-type) dans les fractions subcellulaires des branchies des bivalves témoins (2006; n = 6) et des bivalves récoltés dans le Lac Opasatica en octobre 2002 (n = 3) et octobre 2004 (n = 4).

	Year	Cd	Cu	Zn	Reference
Particulate fractions					
Granules	2002	50 ± 15	52 ± 6*	76 ± 6	Bonneris (2004)
	2004	56 ± 9	73 ± 5	75 ± 7	Michaud (2005, unpublished)
	2006	50 ± 12	67 ± 6	75 ± 7	Present study
Mitochondria	2002	2.0 ± 0.3	2.6 ± 0.2	1.1 ± 0.1	Bonneris (2004)
	2004	2.4 ± 0.8	3.3 ± 1.4	1.9 ± 0.9	Michaud (2005, unpublished)
	2006	2.5 ± 0.7	3.1 ± 1.2	1.4 ± 0.4	Present study
Lysosomes	2002	2.4 ± 0.4	2.3 ± 0.4	1.2 ± 0.4	Bonneris (2004)
	2004	1.2 ± 0.3	1.7 ± 1.1	1.1 ± 0.4	Michaud (2005, unpublished)
	2006	1.9 ± 0.7	1.9 ± 0.7	1.3 ± 0.6	Present study
Nuclei + debris	2002	24 ± 4	33 ± 5	18 ± 4	Bonneris (2004)
	2004	20 ± 3	14 ± 4*	18 ± 5	Michaud (2005, unpublished)
	2006	25 ± 4	20 ± 6	19 ± 5	Present study
Cytosolic fractions					
Heat-denaturable proteins	2002	12 ± 9	5 ± 2	4 ± 1	Bonneris (2004)
	2004	7 ± 4	2 ± 1	4 ± 1	Michaud (2005, unpublished)
	2006	7 ± 3	3 ± 1	3 ± 1	Present study
Heat-stable proteins	2002	10 ± 3	4 ± 1	0.4 ± 0.1	Bonneris (2004)
	2004	14 ± 2	6 ± 2	0.5 ± 0.1	Michaud (2005, unpublished)
	2006	14 ± 5	5 ± 2	0.6 ± 0.2	Present study

An asterisk (*) denotes significant difference among bivalve groups for a given metal and fraction as determined by Wilcoxon rank test. Note that data were transformed (arcsine) before performing the non-parametric test.

Table 7.3: Percentages of Cd, Cu and Zn (± SD) in the subcellular fractions isolated from the digestive gland of control bivalves (2006; n = 6) and bivalves collected from Lake Opasatica in October 2002 (n = 3) and October 2004 (n = 4). / Pourcentages de Cd, Cu et Zn (± écart-type) dans les fractions subcellulaires des glandes digestives des bivalves témoins (2006; n = 6) et des bivalves récoltés dans le Lac Opasatica en octobre 2002 (n = 3) et octobre 2004 (n = 4).

	Year	Cd	Cu	Zn	Reference
Particulate fractions					
Granules	2002	4 ± 3	7 ± 2	10 ± 1	Bonneris (2004)
	2004	15 ± 12	9 ± 3	7 ± 2	Michaud (2005, unpublished)
	2006	2 ± 1*	3 ± 1*	3 ± 1*	Present study
Mitochondria	2002	7 ± 2	11 ± 2	13 ± 2*	Bonneris (2004)
	2004	10 ± 1	17 ± 3*	17 ± 2	Michaud (2005, unpublished)
	2006	7 ± 3	11 ± 1	20 ± 5	Present study
Lysosomes	2002	6 ± 2	6 ± 1	11 ± 3	Bonneris (2004)
	2004	6 ± 3	9 ± 4	15 ± 6	Michaud (2005, unpublished)
	2006	7 ± 3	7 ± 1	14 ± 2	Present study
Nuclei + debris	2002	30 ± 12*	29 ± 3*	34 ± 4	Bonneris (2004)
	2004	18 ± 4	18 ± 4	24 ± 6	Michaud (2005, unpublished)
	2006	15 ± 2	18 ± 2	31 ± 5	Present study
Cytosolic fractions					
Heat-denaturable proteins	2002	28 ± 7	18 ± 3	28 ± 5	Bonneris (2004)
	2004	24 ± 9	12 ± 2	31 ± 6	Michaud (2005, unpublished)
	2006	28 ± 7	12 ± 4	24 ± 3	Present study
Heat-stable proteins	2002	25 ± 5	28 ± 5	5 ± 1	Bonneris (2004)
	2004	26 ± 5	34 ± 4	5 ± 1	Michaud (2005, unpublished)
	2006	41 ± 12*	48 ± 7*	10 ± 3*	Present study

An asterisk (*) denotes a significant difference among bivalve groups for a given metal and fraction as determined by Wilcoxon rank test. Note that data were transformed (arcsine) before performing the non-parametric test.

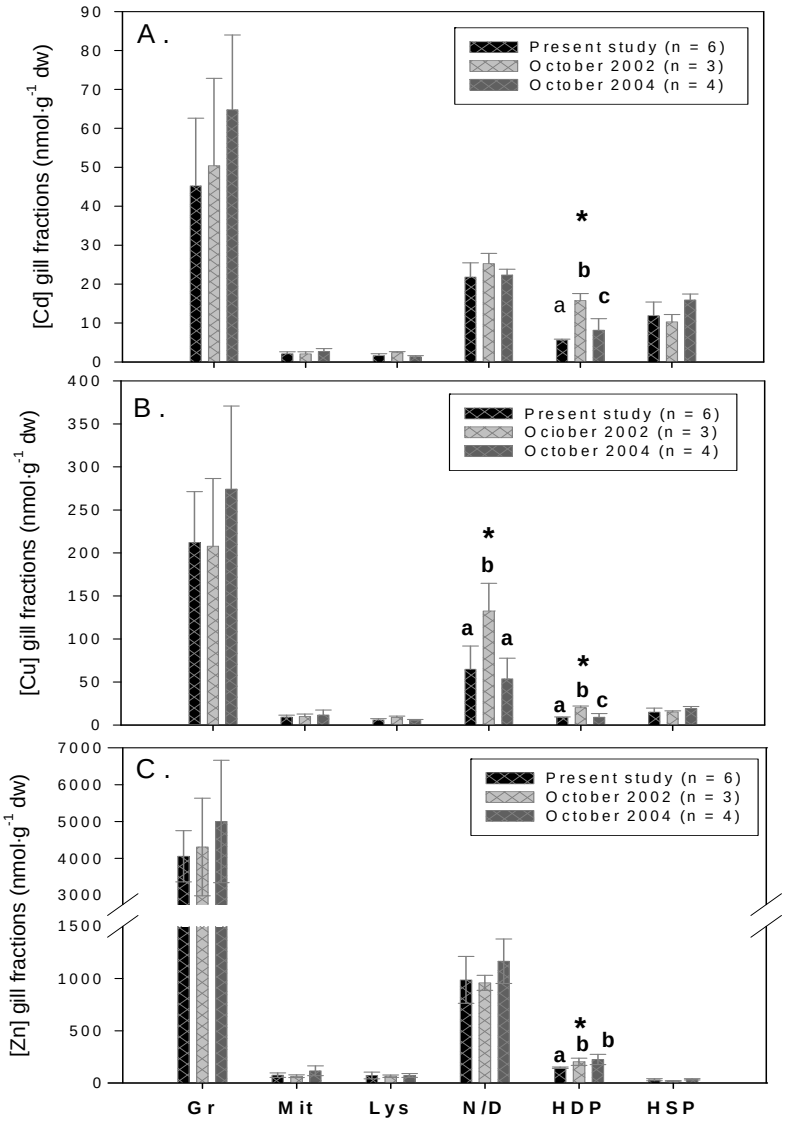


Figure 7.2: Cadmium (A), copper (B) and zinc (C) concentrations (nmol·g^{-1} dry wt ± SD) measured in the cell fractions isolated from the gills of control bivalves and bivalves collected from Lake Opasatica in October 2002 and October 2004. An asterisk denotes a significant difference among groups as determined by a Wilcoxon rank test ($p < 0.05$). / Concentrations (nmol·g^{-1} poids sec ± écart-type) de cadmium (A), cuivre (B) et zinc (C) mesurées dans les fractions subcellulaires des branchies des bivalves témoins et des bivalves récoltés dans le Lac Opasatica en octobre 2002 et octobre 2004. Un astérisque indique une différence significative entre les groupes (Wilcoxon; $p < 0,05$).

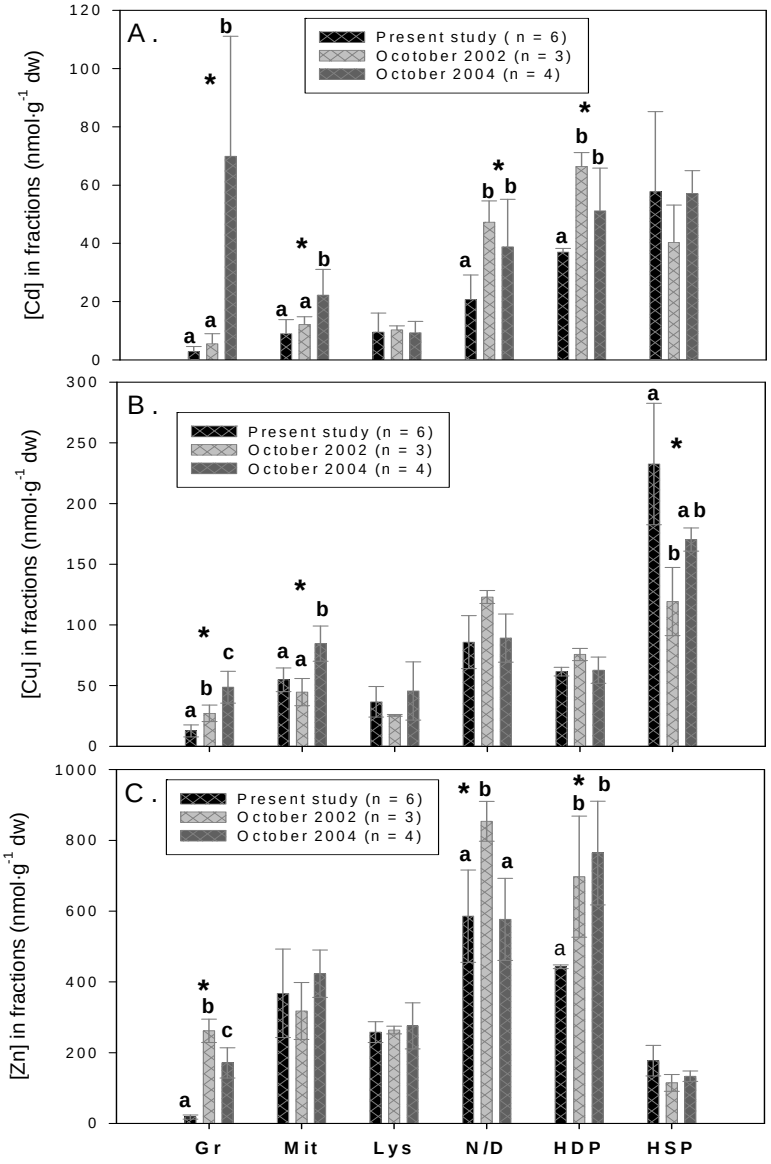


Figure 7.3: **Cadmium (A), copper (B) and zinc (C) concentrations (nmol·g^{-1} dry wt ± SD) measured in the cell fractions isolated from the digestive gland of control bivalves and bivalves collected from Lake Opasatica in October 2002 and October 2004. An asterisk denotes a significant difference among groups as determined by a Wilcoxon rank test ($p < 0.05$). / Concentrations (nmol·g^{-1} poids sec ± écart-type) de cadmium (A), cuivre (B) et zinc (C) mesurées dans les fractions subcellulaires des glandes digestives des bivalves témoins et des bivalves récoltés dans le Lac Opasatica en octobre 2002 et octobre 2004. Un astérisque indique une différence significative entre les groupes (Wilcoxon; $p < 0,05$).**

7.3.2 Aqueous exposures

Accumulated Cd in the gills and digestive gland of bivalves increased as both exposure duration and dissolved Cd concentrations increased, and (surprisingly) the absolute concentrations (nmol $Cd \cdot g^{-1}$ dry weight) were similar in both organs (Figures 7.4 a,b). The percentages of Cd in the subcellular fractions of the gills did not change with increasing exposure length or between dissolved Cd exposures, but the percentages of Cd in the mitochondria, lysosomes and HDP fractions were significantly higher than those observed for the non radioactive Cd in the control bivalves (Table 7.4). Cadmium concentrations significantly increased in all the gill subcellular fractions with increased length of exposure, although there was no significant difference between Cd concentrations measured in the bivalves exposed to 5 nM dissolved Cd for 12 h or 24 h (Figures 7.5 a). The most notable increases in Cd concentrations occurred in the granule and the cytosolic fractions.

As in the case of the gills, the percentages of Cd in the subcellular fractions of the digestive gland did not change with increasing exposure length or between dissolved Cd exposures. However, the percentages of Cd in the granule and mitochondria fractions were significantly higher than those observed for non radioactive Cd in the control bivalves. The percentage of Cd in the lysosome fraction was also higher in the experimental bivalves than in the control bivalves, but not significantly. In the cytosolic fraction, the percentage of Cd in the HSP fraction was significantly lower in the experimental bivalves (Table 7.5). Similarly to the gills, Cd concentrations significantly increased in all of the cell fractions isolated from the digestive gland, with the bivalves exposed to 5 nM dissolved Cd for up to 24 h and to 275 nM dissolved Cd for up to 96 h having the highest Cd concentrations (Figures 7.6 a,b). The most notable increases in Cd concentrations occurred in the HDP, mitochondria and granule fractions.

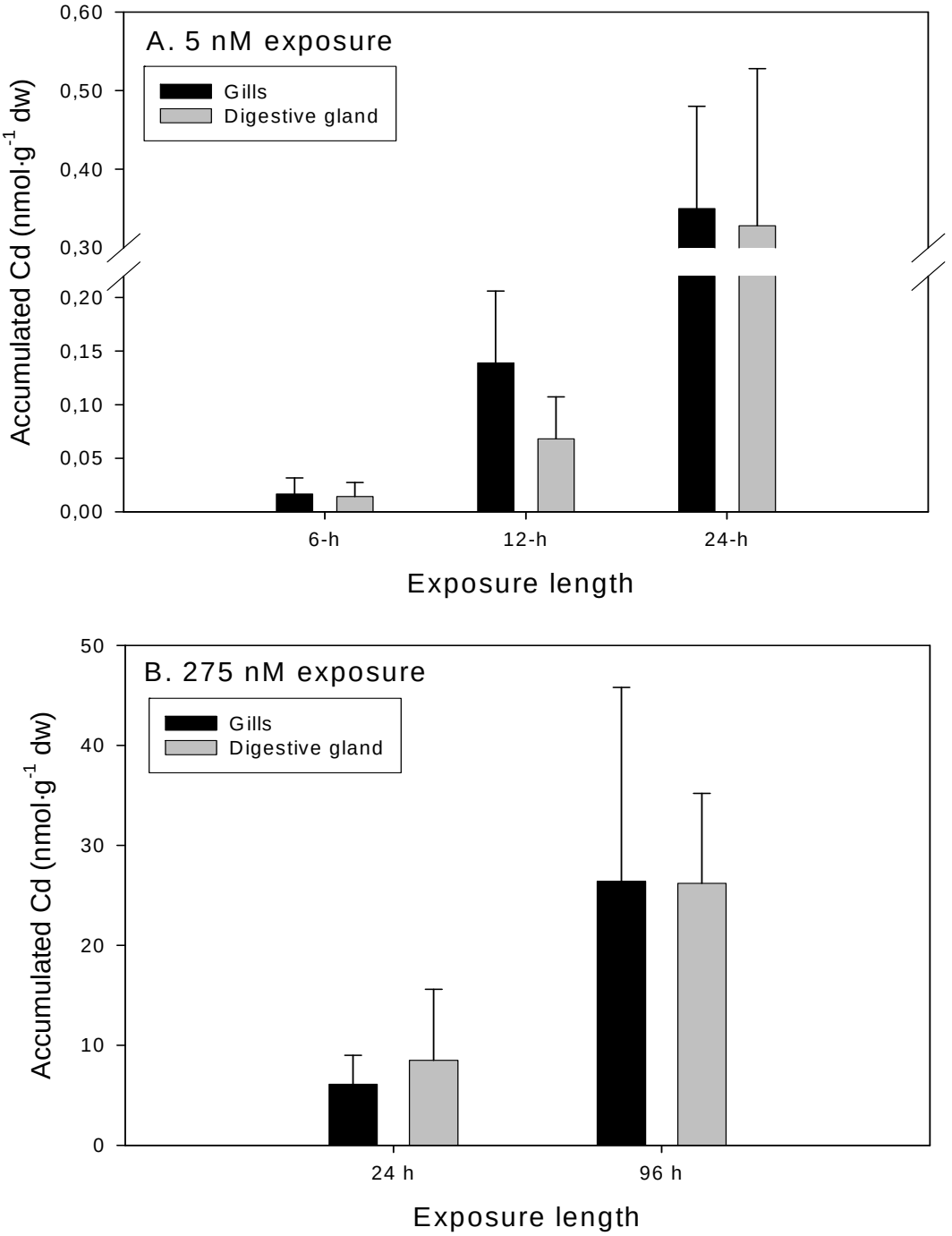


Figure 7.4: Accumulated Cd (nmol·g^{-1} dry wt ± SD) in the gills and digestive gland of bivalves exposed to radio-labelled aqueous Cd: 5 nM (A; n = 12) or 275 nM (B; n = 6). / Accumulation totale de Cd (nmol·g^{-1} poids sec ± écart-type) dans les branchies et la glande digestive des bivalves exposés au Cd aqueux radioactif : 5 nM (A; n =12) ou 275 nM (B; n = 6).

Table 7.4: Percentages of Cd accumulated in the subcellular fractions isolated from the gills of bivalves exposed to radio-labelled aqueous Cd (5 nM or 275 nM) or from the gills of control bivalves. / Pourcentages de Cd accumulé dans les fractions subcellulaires des branchies de bivalves exposés au Cd aqueux radioactif (5 nM ou 275 nM) ou des branchies de bivalves témoins.

	5 nM			275 nM		Control
	6-h	12-h	24-h	24-h	96-h	
Particulate fractions						
Granules	39 ± 10	41 ± 12	55 ± 8	39 ± 11	53 ± 15	50 ± 12
Mitochondria	6 ± 4	6 ± 2	4 ± 1	5 ± 2	6 ± 1	2.5 ± 0.7*
Lysosomes	6 ± 3	4 ± 1	3 ± 1	4 ± 1	5 ± 2	1.9 ± 0.7*
Nuclei + Debris	27 ± 5	25 ± 8	18 ± 4	31 ± 8	19 ± 5	25 ± 4
Cytosolic fractions						
Heat-denaturable proteins	14 ± 4	15 ± 3	11 ± 3	9 ± 3	10 ± 4	7 ± 3*
Heat-stable proteins	9 ± 4	8 ± 2	9 ± 3	12 ± 3	8 ± 4	14 ± 5

An asterisk (*) denotes significant difference among groups (comparison between hour and dissolved Cd treatment), as determined by ANOVA ($p<0.05$) and compared using a Tukey test. Note that data were transformed (arcsine) before performing the ANOVA. N = 12 for all three bivalve groups exposed to 5 nM of dissolved Cd and n = 6 for both bivalve groups exposed to 275 nM of dissolved Cd. Means ± SD.

Table 7.5: Percentages of Cd accumulated in the subcellular fractions isolated from the digestive gland of bivalves exposed to radio-labelled aqueous Cd (5 nM or 275 nM) or from the digestive gland of control bivalves. / Pourcentages de Cd accumulé dans les fractions subcellulaires des glandes digestives de bivalves exposés au Cd aqueux radioactif (5 nM ou 275 nM) ou des glandes digestives de bivalves témoins.

	5 nM			275 nM		Control
	6-h	12-h	24-h	24-h	96-h	
Particulate fractions						
Granules	10 ± 5	10 ± 5	12 ± 4	8 ± 3	4 ± 2*	2 ± 1*
Mitochondria	15 ± 5	16 ± 3	15 ± 3	8 ± 1	14 ± 5	7 ± 3*
Lysosomes	11 ± 3	15 ± 5	9 ± 2	7 ± 1	10 ± 2	7 ± 3
Nuclei + Debris	20 ± 6	19 ± 5	22 ± 5	25 ± 6	20 ± 6	15 ± 2*
Cytosolic fractions						
Heat-denaturable proteins	28 ± 9	30 ± 6	30 ± 12	34 ± 6	41 ± 10	28 ± 7
Heat-stable proteins	16 ± 6	9 ± 4	11 ± 4	18 ± 9	12 ± 4	41 ± 12*

An asterisk (*) denotes significant difference among groups (comparison between hour and dissolved Cd treatment), as determined by ANOVA ($p<0.05$) and compared using a Tukey test. Note that data were transformed (arcsine) before performing the ANOVA. N = 12 for all three bivalve groups exposed to 5 nM of dissolved Cd and n = 6 for both bivalve groups exposed to 275 nM of dissolved Cd. Means ± SD.

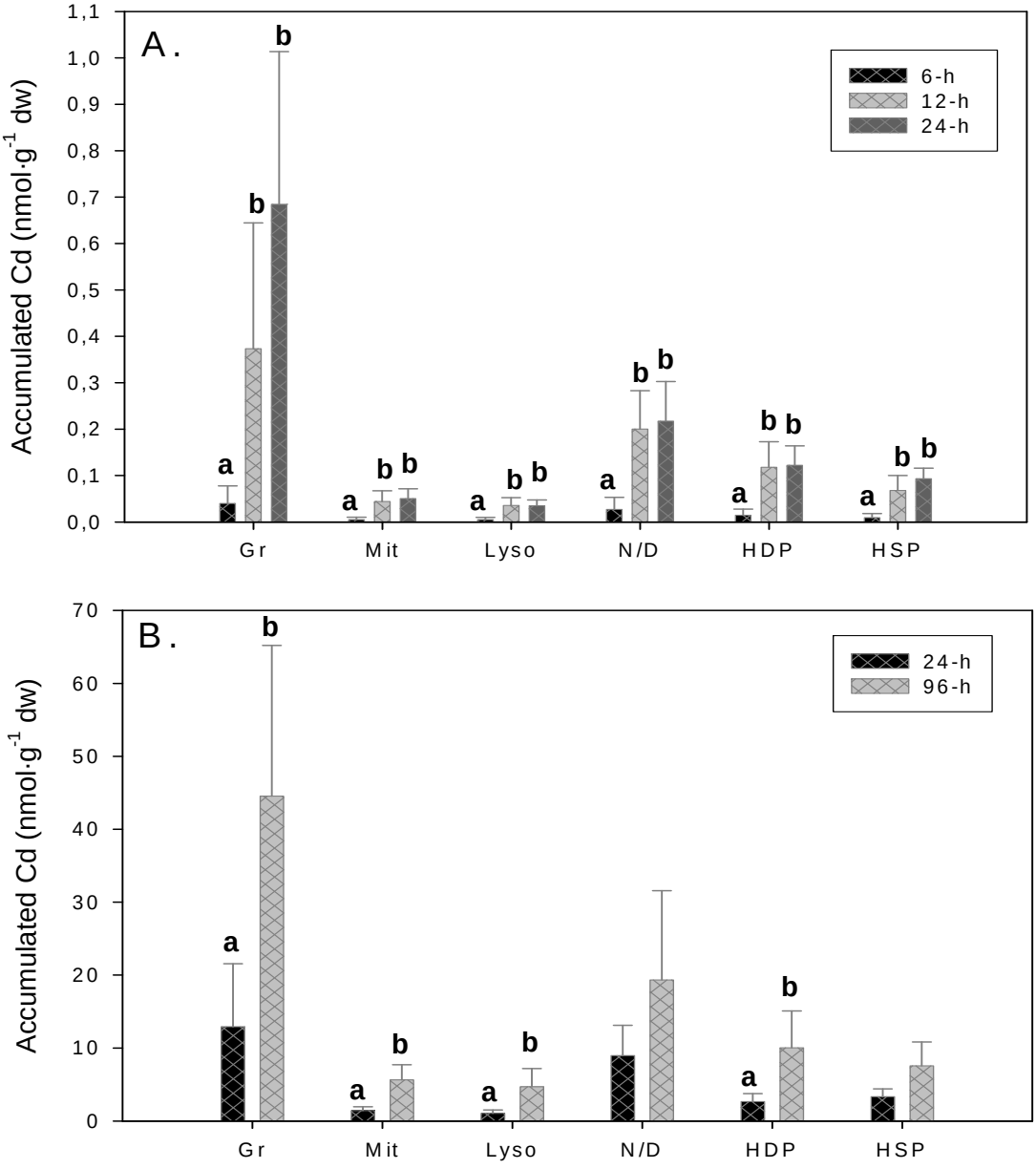


Figure 7.5: **Accumulated Cd (nmol·g^{-1} dry wt ± SD) in the subcellular fractions isolated from the gills of bivalves exposed to radio-labelled aqueous Cd: 5 nM (A; n = 12) or 275 nM (B, n = 6), for different time periods. / Accumulation totale de Cd (nmol·g^{-1} poids sec ± écart-type) dans les fractions subcellulaires des branchies de bivalves exposés au Cd aqueux radioactif: 5 nM (A; n = 12) ou 275 nM (B; n = 6), pendant des périodes de temps différentes.**

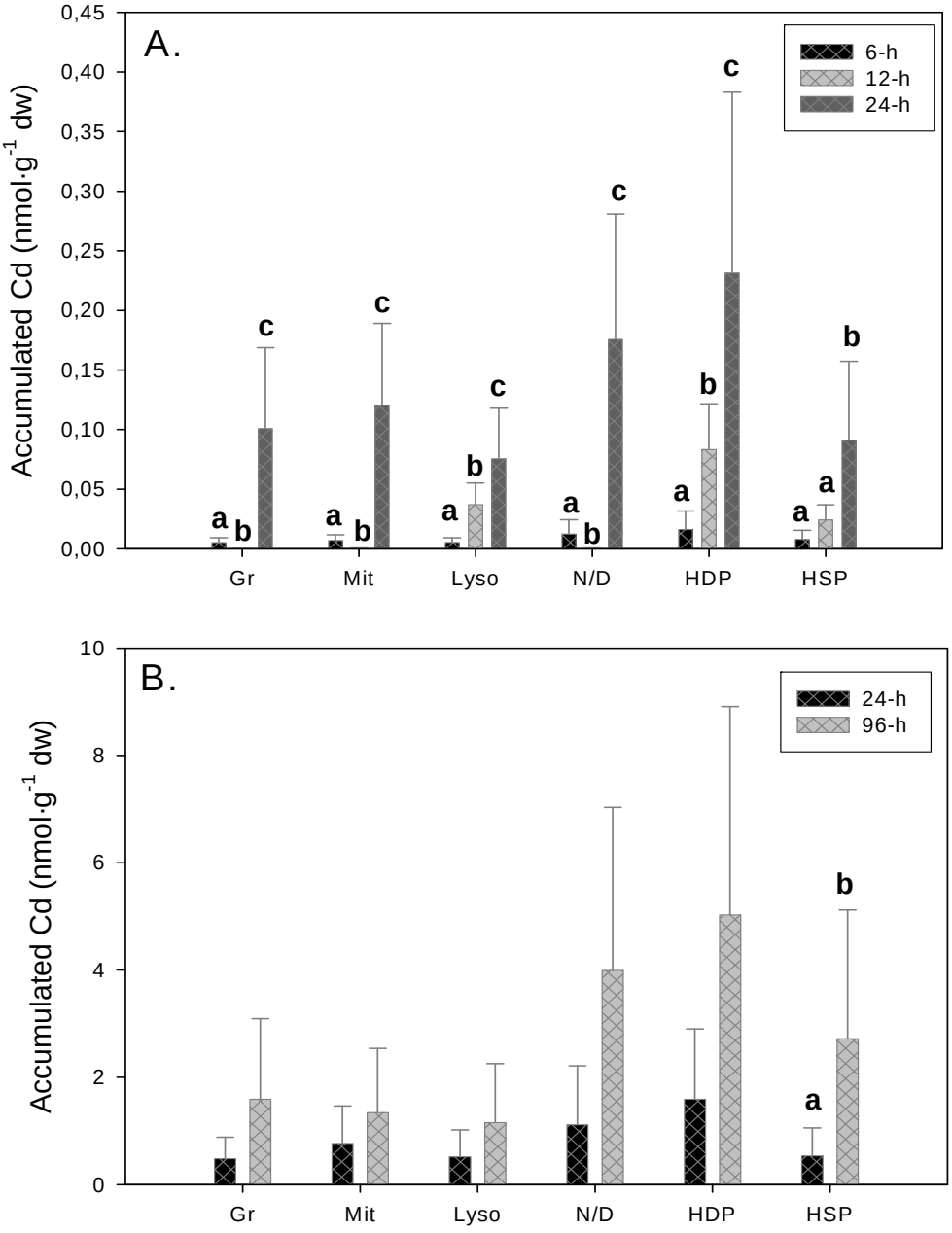


Figure 7.6: Accumulated Cd (nmol·g^{-1} dry wt ± SD) in the subcellular fractions isolated from the digestive gland of bivalves exposed to radio-labelled aqueous Cd: 5 nM (A ; n = 12) or 275 nM (B ; n = 6) , for different time periods. / Accumulation totale de Cd (nmol·g^{-1} poids sec ± écart-type) dans les fractions subcellulaires des glandes digestives de bivalves exposés au Cd aqueux radioactif : 5 nM (A; n =12) ou 275 nM (B; n = 6), pendant des périodes de temps différentes.

7.3.3 Dietary exposure

Recall that bivalves were fed radiolabelled algae and then allowed to depurate. Thus, unlike the case for waterborne exposures, the time course applies to the depuration phase, not the uptake phase of the experiment; t = 0 d corresponds to bivalves that have been exposed to diet-borne ^{109}Cd for 4 d. For these bivalves, total accumulated Cd was higher in the digestive gland than in the gills (Figure 7.7, t = 0 d: 23 ± 5 pmol·g^{-1} (dry wt) for the digestive gland *vs.* 12 ± 6 pmol·g^{-1} (dry wt) for the gills). Accumulated Cd concentrations in the gills increased during the first 3-4 days of depuration and remained stable at ~30 pmol·g^{-1} (dry wt) after the fourth day of depuration (Figure 7.7). Total accumulated Cd in the digestive gland remained reasonably constant for the first 6 days of the depuration phase but increased markedly between day 6 and day 8 (Figure 7.7).

The percentages of Cd in the subcellular fractions of the gills did not change during the depuration period and the distribution of Cd among the fractions in the experimental bivalves did not differ significantly from that in the control bivalves (Table 7.6). With respect to the absolute Cd concentrations, the most notable increase occurred in the granule fraction, which increased 4-fold over the first four days of the depuration and then remained stable at ~20 pmol·g^{-1} (dry wt) (Figure 7.8 a). Unlike the gills, significant changes did occur in the percentages of Cd associated with the subcellular fractions of the digestive gland (Table 7.7). When compared to the control bivalves, the percentages of Cd in the granule fraction were significantly higher in the experimental bivalves depurated for 0 to 6 d but then dropped on Day 8. In contrast, the percentage of Cd in the HSP fraction was initially significantly lower in the same bivalves, but increased markedly between Day 6 and Day 8.

The absolute accumulated Cd concentrations in the digestive gland also significantly changed throughout the depuration period. Accumulated Cd concentrations in the HDP fractions were significantly higher than in the other fractions on Day 0, but then suddenly dropped on Day 2 (Figure 7.8 b). No significant changes in Cd concentrations occurred in the other cell fractions until the last two days of the depuration: with the

exception of the granule fraction, Cd concentrations increased significantly in all the cell fractions between Day 6 and Day 8. The most notable increases were observed in the HDP and HSP fractions (Figure 7.8 b).

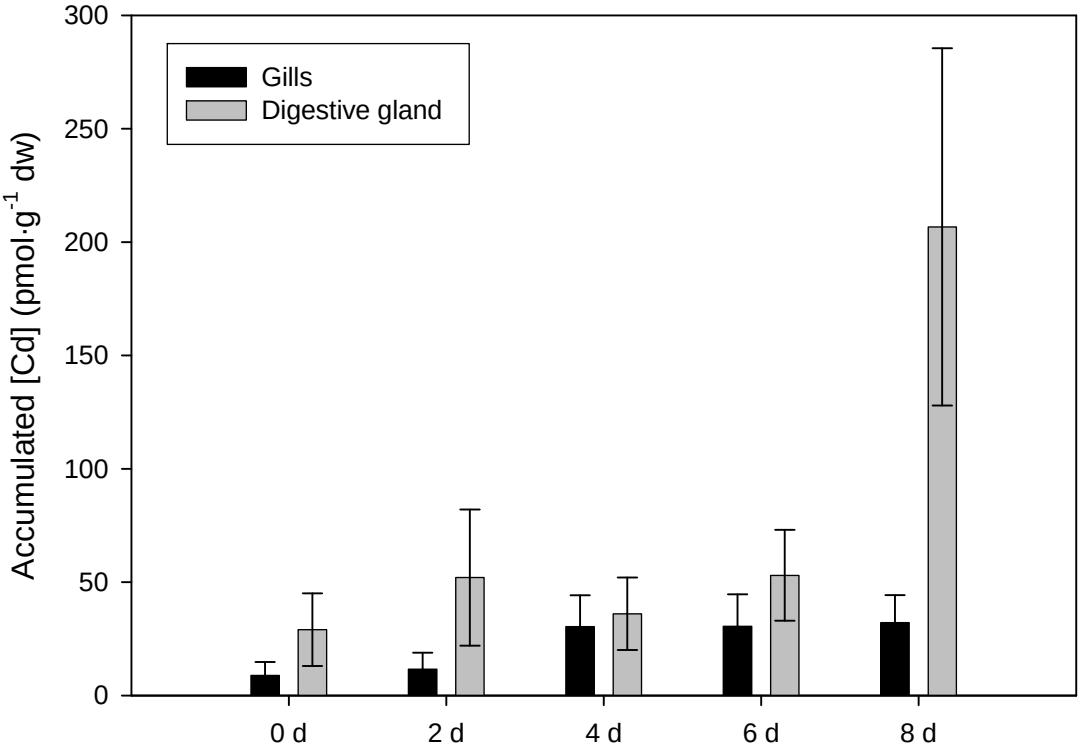


Figure 7.7: Total accumulated Cd (pmol·g^{-1} dry wt ± SD) in the gills and digestive gland of the bivalves exposed to dietary Cd and allowed to depurate for 0, 2, 4, 6 (n = 3 per group) and 8 d (n = 12). / Accumulation totale de Cd (pmol·g^{-1} poids sec ± écart-type) dans les branchies et la glande digestive de bivalves exposés au Cd alimentaire et dépurés pendant 0, 2, 4, 6 (n = 3 par groupe) et 8 j (n = 12).

Table 7.6: Percentages of Cd found in the subcellular fractions isolated from the gills of bivalves exposed to dietary Cd and allowed to depurate for 0, 2, 4, 6 and 8 d. Also presented are the percentages of Cd found in the subcellular fractions of control bivalves. / Pourcentages de Cd retrouvés dans les fractions subcellulaires isolées des branchies de bivalves exposés au Cd alimentaire et dépurés pendant 0, 2, 4, 6 et 8 j. Les pourcentages de Cd retrouvés dans les fractions subcellulaires de bivalves témoins sont aussi présentés.

	Depuration					
	0 d	2 d	4 d	6 d	8 d	Control
Particulate fractions						
Granules	53 ± 4	47 ± 10	64 ± 12	62 ± 12	50 ± 10	50 ± 12
Mitochondria	5 ± 3	3 ± 1	3 ± 1	3 ± 1	3 ± 1	2.5 ± 0.7
Lysosomes	4 ± 2	3 ± 1	2 ± 1	3 ± 1	2 ± 1	1.9 ± 0.7
Nuclei + Debris	25 ± 2	24 ± 8	22 ± 10	25 ± 10	35 ± 9	25 ± 4
Cytosolic fractions						
Heat-denaturable proteins	7 ± 2	7 ± 2	6 ± 1	5 ± 1	4 ± 1	7 ± 3
Heat-stable proteins	7 ± 1	7 ± 4	3 ± 1	3 ± 1	6 ± 3	14 ± 5

N = 3 for days 0, 2, 4, and 6 and n = 12 for day 8. Means ± SD.

Table 7.7: Percentages of Cd found in the subcellular fractions isolated from the digestive glands of bivalves exposed to dietary Cd and allowed to depurate for 0, 2, 4, 6 and 8 d. Also presented is the percentages of Cd found in the subcellular fractions of control bivalves. / Pourcentages de Cd retrouvés dans les fractions subcellulaires isolées des glandes digestives de bivalves exposés au Cd alimentaire et dépurés pendant 0, 2, 4, 6 et 8 j. Les pourcentages de Cd retrouvés dans les fractions subcellulaires de bivalves témoins sont aussi présentés.

	Depuration					
	0 d	2 d	4 d	6 d	8 d	Control
Particulate fractions						
Granules	12 ± 5	10 ± 5	9 ± 3	12 ± 6	4 ± 1*	2 ± 1*
Mitochondria	10 ± 2	15 ± 3	13 ± 1	11 ± 6	10 ± 2	7 ± 3*
Lysosomes	7 ± 2	10 ± 1	10 ± 1	8 ± 5	8 ± 2	7 ± 3
Nuclei + Debris	22 ± 10	20 ± 11	28 ± 12	20 ± 5	19 ± 8	15 ± 2
Cytosolic fractions						
Heat-denaturable proteins	35 ± 14	32 ± 6	20 ± 9	25 ± 3	36 ± 9	28 ± 7
Heat-stable proteins	13 ± 4	14 ± 3	20 ± 5	15 ± 3	33 ± 10*	41 ± 12*

An asterisk (*) denotes significant difference among groups as determined by Wilcoxon rank test ($p<0.05$). Note that data were transformed (arcsine) before performing the non-parametric test. N = 3 for days 0, 2, 4, and 6 and n = 12 for day 8. Means ± SD.

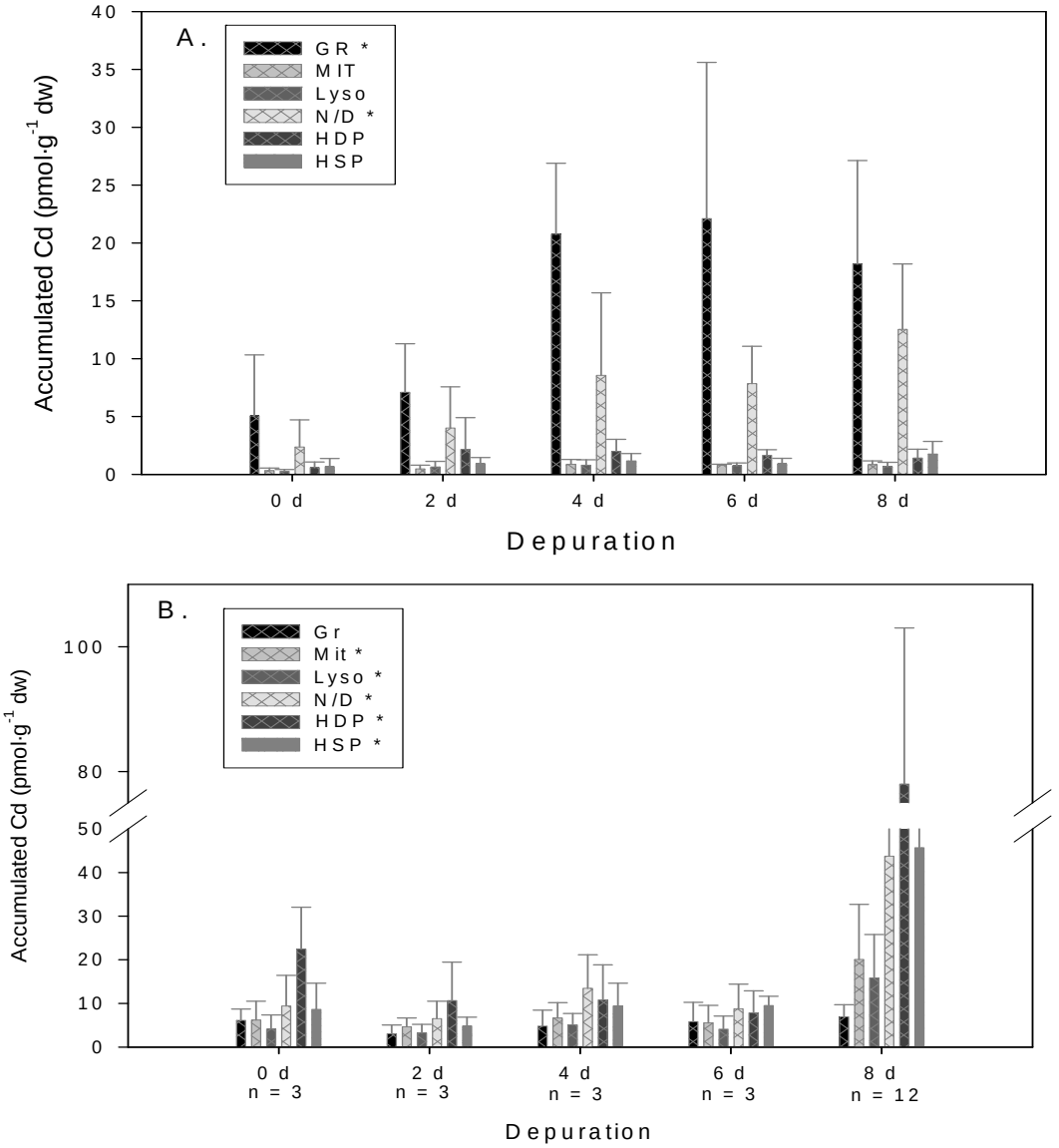


Figure 7.8: Accumulated Cd in the subcellular fractions isolated from the gills (A) and digestive gland (B) of bivalves exposed to dietary Cd and allowed to depurate for 0, 2, 4, 6 and 8 d. An asterisk denotes that significant changes in [Cd] were observed in the cell fraction as determined by a Wilcoxon rank test. Means ± SD./ Cadmium accumulé dans les fractions subcellulaires des branchies (A) et glandes digestives (B) de bivalves exposés au Cd alimentaire et dépurés pendant 0, 2, 4, 6 et 8 j. Un atérisque indique un changement significative dans [Cd] observé pour une fraction subcellulaire (Wilcoxon). Moyenne ± écart-type.

7.4 Discussion

The subcellular distribution of freshly accumulated Cd was investigated in the freshwater bivalve *Pyganodon grandis* after the animals were exposed to either a dissolved or dietary source of Cd. Although metals were found in the "nuclei and debris" fraction of the gills and digestive gland, we have not attempted to interpret the metal concentrations in this cell fraction, due to its ambiguous nature. Some researchers group the nuclei and debris fraction in the detoxified compartment, along with the granules, as they consider the metals that accumulate in the nuclei and debris fraction to be biologically inactive and detoxified (Cain *et al.* 2004; Ng et Wang 2005; Shi et Wang 2004b; Shi et Wang 2004a), but this seems unjustified since the relative importance of this fraction varies as a function of the intensity (efficacy) of the homogenization step. It should be noted that centrifugation does not perfectly separate each fraction (De Duve 1975), and various potential artifacts, such as breakage or clumping of particles and leakage of soluble constituents from organelles into other fractions, can complicate the interpretation of the results (Wallace *et a*l. 2003; Giguère *et a*l. 2006). Therefore, the separation and designation of each fraction as either "granules", "mitochondria", "lysosomes + microsomes", etc. should be considered with caution. Similarly, the grouping of the fractions into "potentially metal-sensitive" (HDP, mitochondria, microsomes) and "detoxified" (granules and metallothionein-like or HSP) categories (Wallace *et a*l. 2003) is likely an oversimplification. Nevertheless, relative comparisons among treatments do yield useful results.

7.4.1 Control bivalves

As a quality control measure, the total concentration and subcellular distribution of Cd, Cu, and Zn in the gills and digestive glands of non-radioactive control bivalves, collected and sacrificed from the stock culture, were compared with those previously measured in Lake Opasatica bivalves collected in October 2002 and October 2004 by Bonneris (2004) and Michaud (2005). The purpose of this comparison was to ensure that (a) the subcellular partitioning of metals in control bivalves collected from the stock culture did not differ from what was previously observed in wild bivalves; (b) the

subcellular partitioning procedure, done by differential centrifugation, could be performed in a reproducible manner by different individuals without creating any significant difference in the distribution of metals.

For the gills, no significant difference in total metal concentrations or subcellular distribution of metals was observed between the 2006 control bivalves and the Lake Opasatica bivalves collected in 2002 and 2004. The results suggest that, for the gills at least, the differential centrifugation protocol can be performed by different individuals without introducing any significant difference in the data due to human error. Total metal concentrations in the digestive gland of 2006 control bivalves were significantly lower than the values measured in the Lake Opasatica bivalves collected in October 2002 and October 2004 (Table 7.1). However, in their spatial study Bonneris *et al.* (2005b) reported total Cd, Cu, and Zn concentration in the digestive glands of Lake Opasatica bivalves (Cd: 123 ± 15; Cu: 402 ± 30; Zn: 1692 ± 39 nmol·g^{-1} dry wt ± SD) that were similar to those measured in the present study. It is thus likely that the differences in total metal concentrations among the October 2002, 2004 and 2006 bivalve groups were due to temporal variability in the bivalve population and are not the result of an analytical error.

The distribution of metals among the subcellular compartments in the digestive gland also differed between the 2006 control bivalves and the Lake Opasatica bivalves collected in 2002 and 2004, notably in the granule and cytosolic fractions (Figure 7.3 a,b,c). Metal concentrations in the granule and HDP fractions were generally higher in the 2002 and 2004 Lake Opasatica bivalves, whereas metal concentrations, notably Cu, were higher in the 2006 control bivalves. It is widely known that proteins, especially metallothioneins, can be induced by physico-chemical and biological factors other than an increase in ambient metal concentrations (Jenny *et a*l. 2004; George and Langston 1994). Baudrimont *et al*. (1997a) reported seasonal changes in MT concentrations in the freshwater bivalve *Corbicula fluminea* and attributed this variability to changes in hormone concentrations associated with the bivalve's reproductive cycle. Cooper (2008)

noted that the MT concentrations in the digestive gland of *P. grandis* decreased by a factor of ~1.5x between the months of June and October. The minimal values in MT concentrations corresponded to the incubation and maturation of glochidia (October) and the maximum values occurred during the maturation of the gonads (May). However, in the present case the control bivalves were collected and transported to the laboratory during the month of October, and therefore MT concentrations should have been similar among all three bivalve groups. It is possible that captivity may have affected bivalve protein metabolism as the sudden change in diet and habitat may have stressed the animals into modifying their metabolism, thus altering the distribution of metals in the digestive gland.

7.4.2 Aqueous exposure

A number of laboratory studies have investigated the subcellular distribution of metals in bivalve species, but unfortunately the animals were exposed to very high dissolved metal concentrations during several of these studies, as summarized in Table 7.8. Such concentrations are unrealistic compared to levels seen in the environment and it is difficult to interpret the results obtained under such conditions since the influx of metals might well overwhelm the bivalve's metal detoxification capabilities, triggering a range of cellular responses that normally would not be initiated. Recent studies have confirmed that even low metal exposure concentrations are capable of inducing changes in subcellular metal partitioning (Shi and Wang 2004a; Erk *et a*l. 2005).

Our study is the first to measure the subcellular partitioning of Cd in *P. grandis* over a short-term exposure to low (5 nM) or high (275 nM) dissolved Cd concentrations. Cadmium accumulated mostly in the granule fraction of the gills whereas Cd concentrations were highest in the HDP fraction of the digestive gland. Interestingly, Cd concentrations in the other subcellular fractions (excluding the HDP fraction) in the digestive gland increased significantly between 12 and 24 h for bivalves exposed to 5 nM dissolved Cd, whereas the Cd concentration was stabilizing in the same cell fractions in the gills. Although the distribution of Cd among the subcellular fractions

did not change significantly in either organ during the exposure period, regardless of exposure concentration, the percentages of Cd in the mitochondria, lysosomes and HDP fractions in the gills were significantly higher in the Cd-exposed bivalves compared to the control bivalves. For the digestive gland, the percentage of Cd was higher in the granule and mitochondria fractions of the Cd-exposed bivalves, whereas the percentage of Cd in the HSP fraction was significantly lower in the Cd-exposed bivalves than in the control animals. The lower Cd concentrations associated with the HSP/metallothionein-like protein fraction is not surprising since the induction of MT is not instantaneous and, therefore, Cd would have to accumulate elsewhere until MT can be synthesized.

Table 7.8: Summary of studies in which the subcellular distribution of Cd in marine mussels was investigated after the animals had been exposed to very high concentrations of dissolved metals. / Résumé des études qui ont observé la répartition subcellulaire du Cd chez les mollusques marins après avoir exposé les animaux à des concentrations élevées de métaux dissous.

Species	Dissolved [Cd] (nM)	Duration	Reference
Crassostrea virginica	225	2, 7, 21 d	(Sokolova *et al.* 2005a)
Mactra veneriformis	20, 90, 445	1 h	(Shi and Wang 2004b)
Mytilus edulis	0.5, 18, 445	21 d	(Erk *et a*l. 2005)
Mytilus galloprovincialis	900	40 d	(Bebianno et Serafim 1998)
Perna viridis	18	24 h	(Ng and Wang 2005)
	9, 35, 135, 270	7 to 35 d	(Shi and Wang 2004a)
Ruditapes decussatus	900	40 d	(Bebianno and Serafim 1998)
	35, 360	7 to 40 d	(Serafim et Bebianno 2007)
Ruditapes philippinarum	20, 90, 445	1 h	(Shi and Wang 2004b)

The results of this study indicate that several pools of ligands are responsible for metal binding during the early stages of dissolved metal uptake. Although Cd was rapidly trapped and sequestered in the granules, the Cd concentration increased in the mitochondria, lysosomes and HDP fractions of the gills. Generally, the accumulation of toxic metals, like Cd, in the metal-sensitive organelles such as mitochondria and lysosomes is regarded as a precursor of toxicity. The "spillover" of Cd into metal-

sensitive fractions occurs when detoxifying ligands, such as MT, become overwhelmed and saturated. Over the short exposure period to which the bivalves were subjected, it would appear that MT induction was insufficient to prevent the Cd from binding to the putative metal-sensitive fractions immediately after dissolved exposure. This interpretation is consistent with the observation that the Cd concentrations in the mitochondria, lysosomes and HDP fraction stabilized after between 12 and 24 h in the gills of bivalves exposed to 5 nM of dissolved Cd but continued to increase significantly in the granules.

The presence of Cd in the HDP fraction, notably in the digestive gland, differs from what was previously observed in the field with *P. grandis* collected along a metal concentration gradient. Bonneris *et al.* (2005b; 2005b; 2005a) reported that, although Cd concentrations in the mitochondria and the lysosome fractions increased along the Cd exposure gradient, Cd did not accumulate in the HDP fraction, except in bivalves collected from the most contaminated lake. Typically, ~40% of the Cd accumulated in the digestive gland of *P. grandis* was associated with the HSP fraction (Bonneris *et a*l. 2005b; Bonneris *et a*l. 2005a), whereas in the present study <15% of the Cd was found in this HSP fraction. Metallothioneins, which are a major constituent of the HSP fraction, are induced in an organism after the animal has been subjected to a sudden stress, such as increase in ambient metal concentrations. However, it may take several days before MT is produced in sufficient concentration by the organism (Langston *et a*l. 1998; Jenny *et a*l. 2004). Therefore, it is likely that the accumulation of Cd in the HDP fraction in the digestive gland of Cd-exposed bivalves is a temporary phenomenon until the animals have produced the necessary MT for Cd detoxification. This "rescue function" for metallothionein has been reviewed by Roesijadi and Robinson (1994).

Similarly to the present study, Sokolova *et al.* (2005a) observed Cd accumulation in the mitochondria and lysosomes in both the gills and digestive glands of *Crassostrea virginica* exposed to dissolved Cd within 48 h of exposure. In terms of relative distribution, Cd in the mitochondria and lysosomes represented only ~10% of the total

Cd accumulated in *C. virginica*, whereas 75-83% of the total tissue Cd load was associated with the cytosolic fraction. Very little Cd (~5%) was associated with the granule fraction in the gills (Sokolova *et a*l. 2005a), unlike our study. Erk *et al*. (2005) observed an important Cd accumulation in the HDP fraction isolated from whole specimens of *C. virginica* within the first 7 days of dissolved metal exposure. Cadmium continued to increase in the HDP fraction of *C. virginica* throughout the 21 d exposure. Contrary to other studies, the subcellular distribution of Cd in *P. grandis* did not undergo any time-dependent changes during the first 24 h of dissolved Cd exposure. Ng and Wang (2005) reported changes in the subcellular partitioning of Cd in the cytosolic fraction in *Perna viridis* within 24 h of exposure. The relative contribution of Cd in the granule fraction remained stable at ~50% whereas the percentage of Cd in the HSP fraction increased over time as the Cd in the HDP fraction decreased. In the clam *Ruditapes decussates*, Cd was accumulated mainly in the cytosol and associated with soluble proteins after 48 h of exposure. After 7 d of exposure, the percentage of Cd in the cytosol had decreased, suggesting a redistribution of the soluble Cd to the particulate fraction (Romeo et Gnassia-Barelli 1995).

7.4.3 Dietary exposure

Under natural conditions, bivalves are simultaneously exposed to metals from different sources (e.g., aqueous and particulate metals) throughout their lifetime. Although several studies have investigated dietary metal uptake in marine mussels (see Section 6), few have studied the subcellular partitioning of metals after a dietary exposure (Ng and Wang 2005; Shi and Wang 2004b; Shi and Wang 2004a), and even fewer have investigated the subcellular partitioning of metals in freshwater bivalves, let alone after a dietary exposure.

In the present study, *P. grandis* was fed Cd-contaminated algae for 4 d and allowed to depurate, after the last feeding, for 8 d during which the bivalves were fed continuously with uncontaminated algae. Bivalves were sacrificed and analyzed after 0, 2, 4, 6, and 8 d of depuration. Similarly to the results obtained after dissolved exposure, Cd was

predominantly associated with the granule fraction in the gills. Cadmium concentrations in the digestive gland remained unchanged in all of the subcellular fractions during the first 6 d of depuration and then suddenly increased between Day 6 and Day 8. The most notable increase in Cd concentration occurred in the cytosolic fraction. The relative distribution of Cd did not change in the gills throughout the depuration period and the percentage of Cd in each subcellular fraction was similar to what was observed for the control (unfed) bivalves. The relative distribution of Cd in the digestive gland, however, did change over time – the percentage of Cd in the granule fraction decreased from 12 to 4%, whereas the contribution of the HSP fraction increased from 13 to 33%.

The granules seem to play an important role in the detoxification of Cd in the digestive gland during the early stages of dietary metal uptake. As the depuration period progressed, Cd was detoxified by the cytosolic fraction, notably the HSP fraction. Although the HSP fraction did sequester a large portion of the Cd by the 8th day of depuration, a portion of the Cd did escape detoxification in the digestive gland as Cd concentrations in the mitochondria, lysosomes and HDP fractions also increased significantly between Day 6 and Day 8 of the depuration. This partial detoxification or delay in the "rescue" function of the HSP fraction is also evident in the gills as Cd concentrations in the granule fraction of the gills increased during the first 4 d of depuration.

Ng and Wang (2005) also observed the subcellular distribution of Cd in whole specimens of *Perna viridis* over a 28 d depuration period that was preceded by a 6-d feeding period during which the oysters were fed radiolabelled diatoms. Similarly to our results, the percentage of Cd in the HDP fraction remained stable throughout the depuration period, whereas the percentage of Cd decreased in the granules and increased in the HSP fraction during 28 d of depuration. Redistribution of Cd among the subcellular fractions in *P. grandis* was observed during the 8 days of depuration and may have continued beyond that time frame. Shi and Wang (2004b) studied the effects of pre-exposure of Cd on the subcellular partitioning on whole specimens of *Mactra*

veneriformis and *Ruditapes philippinarum* exposed to dietary Cd. They reported that mussels collected from the uncontaminated sites accumulated more Cd in the granule fraction contrary to mussels collected from contaminated sites which accumulated more Cd in the cytosolic fraction. They concluded that the MT "rescue function" had been activated in the contaminated mussels as a result of their pre-exposure to metals.

The increase in Cd concentrations observed in the gills and digestive gland is related to the circulation of blood and other fluids within the organism. Blood flows from the heart (located near the miscellaneous organs and intestines), to the mantle, to the digestive gland and finally to the gills from where it is pumped back towards the heart (Gosling 2003). This is consistent with the results presented in Figure 6.2, which illustrates the changes in Cd concentrations among the organs as dietary Cd is first located in the intestines and miscellaneous organs and then transported to the other organs as the algae are digested during the depuration period. Cadmium concentrations in the miscellaneous organs and intestines decrease as less Cd-contaminated algae enter the animal and Cd is transported to the digestive gland and gills. The mass balance calculated in Table 7.9 also supports this interpretation in that the total amount of Cd within the bivalve remains more or less constant, whereas the amount of Cd associated with an individual organ varies considerably during the 8 d depuration period.

Table 7.9: Calculated Cd mass balance (pmol) for bivalves during depuration. The bivalves were pre-exposed to dietary Cd for 4 d prior to depuration. N = 3 for days 0, 2, 4 and 6; n = 12 for day 8. / Bilan massique (pmol) du cadmium calculé pour les bivalves durant la période de dépuration. Les bivalves étaient exposés préalablement Cd alimentaire pendant 4 j. N = 3 pour les jours 0, 2, 4, et 6; n = 12 pour jour 8.

Length of depuration (d)	Gills	Digestive gland	Mantle	Foot	Misc. organs	Intestines	Total (pmol)
0	0.5	1.1	1.7	0.14	7.9	11.2	**22.6**
2	1.0	2.2	4.0	0.18	6.1	3.4	**16.8**
4	2.4	4.1	3.5	0.16	5.6	1.7	**17.4**
6	4.3	5.7	2.0	0.11	5.9	0.9	**19.0**
8	4.2	7.9	2.6	0.15	3.9	0.3	**19.0**

7.4.4 Aqueous exposure vs. dietary exposure

P. grandis displayed a dynamic pattern in metal detoxification during both the dissolved and dietary exposures as the gills and digestive gland demonstrated different detoxification strategies for each exposure pathway. Although Cd accumulated in the mitochondria, lysosomes and HDP fractions of the gills, a large proportion of the Cd was associated with the granule fraction. It is evident that the granules are an important site of metal sequestration for *P. grandis*, even in the short term, in contrast to the results obtained for marine mussel species. In general, MT or metallothionein-like proteins seem to play a more important role in the detoxification of metals for marine species. Although marine species do contain granules within their organs, they are found in much lower concentrations than in freshwater species (Simkiss 1989). For the digestive gland, Shi and Wang (2004b) showed that while MTs were not induced within a 24-h or 96-h period, Cd accumulated initially in the HDP and mitochondria fractions.

The subcellular distribution of Cd after dietary exposure was different from that after dissolved exposure. Our study demonstrated that Cd was distributed evenly among the different subcellular fractions in the gills which play a lesser role in metal accumulation in *P. grandis* after a dietary exposure than for the waterborne exposures. The most

dynamic changes occurred in the digestive gland where Cd concentrations suddenly increased in all subcellular fractions and the percentage of Cd increased in the HSP (or MTLP) fraction between Day 6 and Day 8 of the depuration.

Redistribution of Cd occurred among organs after *P. grandis* was exposed to Cd via both exposure pathways. During the dissolved exposure, Cd concentrations in the mitochondria, lysosomes and cytosolic fractions were stable after 12 h of exposure whereas Cd concentrations increased significantly between 12 and 24 h in the granule, mitochondria, HDP and HSP fractions of the digestive gland, suggesting that Cd was transferred from the gills into the digestive gland. Smith (1975) also observed a redistribution of metals in *P. grandis* after a dissolved Hg exposure. Mercury concentrations increased in the muscles of bivalves during an 8 week depuration period whereas the concentration of Hg significantly decreased in the digestive gland. They suggested that as Hg is slowly eliminated from the digestive gland, it accumulates in the muscles to be excreted later.

Likewise in our study, after dietary exposure, Cd was transferred from the digestive gland into the gills, as illustrated notably by an increase in Cd concentrations in the granule fraction of the gills. However, the redistribution of Cd was only temporary as Cd concentrations in the gills granule fraction stabilized after 4 d of depuration. This stabilization of Cd concentrations in the granule fraction of the gills suggests that steady-state had been reached in the gills whereas the Cd concentration in the subcellular fractions of the digestive gland increased after 4 d.

7.5 Conclusion

The distribution of Cd in *P. grandis* differed in the short-term dissolved exposure compared to the distribution of Cd in bivalves that are chronically exposed to Cd. Cadmium accumulated in the metal-sensitive fractions (mitochondria, lysosomes and HDP) in both the gills and digestive gland of bivalves during the short-term exposures. Although Cd has been shown to accumulate in the mitochondria and lysosomes fractions

of bivalves collected from the field, notably in bivalve collected from highly contaminated lakes, Cd typically does not accumulate in the HDP fraction of these bivalves. It would seem that the chronically exposed bivalves may have adapted to their surroundings and are able to cope with the influx of internal Cd.

For bivalve gills, the distribution of Cd among the subcellular fractions was insensitive to the exposure route, i.e.. both waterborne and diet-borne Cd ended up bound largely to the granules fraction. Although Cd did accumulate in all subcellular fractions of the digestive gland during the aqueous exposure, a significant change in the relative distribution of Cd, notably in the granules and HSP fractions, was observed in bivalves during the depuration period after a dietary Cd exposure. These results further illustrate that the gills are the main target organ during dissolved metal exposure whereas the digestive gland is the main site of Cd accumulation during a dietary exposure. However, longer feeding studies (months or years) would be required to determine if, at steady-state, the subcellular partitioning in the digestive gland is sensitive to the route of exposure.

8. RELATIVE IMPORTANCE OF FOOD AND WATER AS SOURCES OF CADMIUM TO *PYGANODON GRANDIS*

8.1 Introduction

Effects of metal contamination on aquatic ecosystems have received considerable attention over the past 30 years. Monitoring programs using aquatic invertebrates, such as bivalves, have been developed in several countries to investigate the risk such contamination may pose for aquatic organisms and ecosystems (Jernelov 1996). Early risk assessment or biomonitoring programs were based on the assumption that dissolved metals were the main source of contamination in mussels (Schlekat *et a*l. 2001). However, mussels and bivalves are exposed to metals from both the particulate and dissolved phases and studies have revealed that metal uptake from food can be an important source of metal for mussels (Wang *et a*l. 1996; Luoma *et a*l. 1992; Reinfelder *et al.* 1998; Reinfelder *et a*l. 1997). These recent findings suggest that dietary exposures can be at least as important as aqueous exposures and have important implications for risk assessment and biomonitoring. The most important implication is that the dissolved-only assumption could lead to underestimates of metal exposure under natural conditions if animals take up metals from both dietary and aqueous sources, particularly if the diet-borne metal is disproportionately bioavailable (Hook et Fisher 2001). The recognition of the potential importance of dietary metal exposures emphasizes the need to quantify the relationship between ambient metal concentrations and mussel metal concentrations and in particular to determine the sources of metals (food *versus* water) for these animals.

Previous efforts to explain metal accumulation in marine and freshwater bivalves, performed using regression analyses, determined that tissue metal concentrations can be a function of the aqueous concentration of the free metal ion (as computed for Cd by Tessier *et al*. (1993) for *Pyganodon grandis*) or correlated with metal concentrations in food (particularly for Zn, Cd, Cu and Hg; Thomann *et al*. (1995) for *Mytilus edulis*). However, these studies did not directly quantify the uptake pathways or uptake kinetics of metals from food and water into the bivalves. Other mathematical models can be used to evaluate the relationship between metal accumulation in bivalves and environmental metal concentrations. Landrum *et al.* (1992) described three forms of mechanistically-based, dynamic bioaccumulation models that can characterize dietary and

dissolved exposures to metals: 1) compartmental models, 2) physiological-based pharmacokinetic models and 3) bioenergetic models. Of these, bioenergetic models are the most widely used as they allow for multiple uptake pathways. A crucial assumption in this approach is that metal accumulation in an organism is an additive function of aqueous and dietary uptake pathways. If this assumption is accepted, then the kinetics of each pathway can be calculated independently (Landrum *et al.* 1992). Bioenergetic-based kinetic models have been developed to evaluate the bioaccumulation of metals and other contaminants in aquatic animals, including mussels. These models require information on uptake efficiencies from food and water pathways, efflux rates of contaminants from the animals, and filtration, ingestion, and growth rates, and they can be solved for steady-state tissue concentrations under any set of environmental conditions. These models are therefore very flexible and applicable to diverse environments with varying conditions (Schlekat *et a*l. 2001; Landrum *et a*l. 1992).

Researchers have worked extensively on developing metal bioaccumulation models for marine and freshwater bivalves. Kinetic bioaccumulation models have been used to determine the relative importance of food in the uptake of trace elements such as Cd (Reinfelder *et a*l. 1997), Cr (Wang *et a*l. 1997; Decho and Luoma 1991), and Se (Reinfelder and Fisher 1994; Luoma *et a*l. 1992) for various marine mussel species. In many of these studies, the kinetic model was used not only to determine the relative importance of metal bioaccumulation from food and water, but also to provide site-specific predictions of metal concentrations in mussels. Luoma *et al.* (1992) found that model-predicted Se concentrations (1.1–8.6 $\mu g \cdot g^{-1}$) in *Macoma balthica* compared well with values (2.9-6.7 $\mu g \cdot g^{-1}$) measured in mussels collected from San Francisco Bay. This approach has also been applied to a number of metals in the mussel *Mytilus edulis*. Wang *et al.* (1996) determined metal uptake and depuration kinetics for *M. edulis* as well as the assimilation efficiencies of Ag, Cd, and Zn in mussels fed natural seston under laboratory conditions. The relative importance of food as a source of Ag, Cd and Zn was variable depending on how efficiently mussels assimilated metals from food (43-69% for Ag; 24-49% for Cd and 48-67% for Zn). Predicted metal concentrations in *M. edulis* were in good agreement with those measured independently from mussels collected from San Francisco Bay and Long Island Sound (Wang *et a*l. 1996). Fisher *et al*. (1996) examined the bioaccumulation of Ag, Am, Cd, Co, Pb

and Zn in *Mytilus galloprovincialis* exposed to dissolved and dietary metals separately under laboratory conditions. The uptake and efflux rates, along with assimilation efficiencies obtained during the experiments, were used in a kinetic model to estimate the relative importance of food and water as sources of metals to *M. galloprovincialis* and to predict metal concentrations in mussels under different field conditions. The authors concluded that laboratory-derived estimates of AEs for *M. galloprovincialis* could be applied to predict metal concentrations in field collected mussels.

In one of the rare studies on freshwater species, Roditi and Fisher (1999) exposed the zebra mussel, *Dreissena polymorpha*, to both aqueous and dietary sources of Ag, Cd, Cr, Hg and Se under laboratory conditions to measure their assimilation efficiencies, absorption efficiencies, and efflux rates. These parameters were later used in a kinetic model to predict the concentration of these metals in zebra mussels collected from various sites along the Laurentian Great Lakes. The model-predicted mean body concentrations of Ag, Cr, Hg, and Se were similar to those measured in mussels collected from all the sites, whereas predictions for Cd only matched values measured at two sites (Roditi *et a*l. 2000a). Furthermore, the model predicted that, under natural conditions, Ag, Cd, and Hg were predominantly accumulated in the zebra mussel from ingested particles, that Cr was accumulated mostly from the dissolved phase, and that the dominant uptake pathway for Se was dependent on environmental conditions (Roditi *et a*l. 2000a).

Previous research has recognized *Pyganodon grandis* as a potential sentinel organism to assess spatial and temporal trends in bioavailable Cd concentration (Giguère *et a*l. 2003; 1995a; Couillard *et a*l. 1995b; Bonneris *et a*l. 2005b; 2005a). This species is widely distributed in freshwater lakes and rivers across North America and could be utilized as a sentinel species for metal contamination, especially in freshwater systems that have been perturbed by industrial and mining activities. Although consideration has been given to using *P. grandis* as a biosentinel, no previous study has quantified the relative importance of different uptake pathways of metals into *P. grandis*, and kinetic parameters (e.g., filtration rates, ingestion rates and assimilation efficiencies) that may assist in the development of a bioaccumulation model for metals in this bivalve have not been evaluated. We therefore conducted a series of laboratory experiments to determine the absorption efficiency for dissolved Cd, the assimilation efficiency for Cd in *P.*

grandis fed Cd-labelled algae (specifically *Pseudokirchneriella subcapitata*), as well as the bivalve filtration and ingestion rates. Results from these experiments can also be used to evaluate the potential of using *P. grandis* as a sentinel organism for Cd contamination in freshwater systems. Based on the parameters obtained in the laboratory, we parameterized a kinetic model of Cd bioaccumulation for *P. grandis* (Section 8.2) and compared the results predicted by the model with those obtained from field studies. This study was motivated by our interest in enhancing the value of programs that use freshwater bivalves as a monitoring organism which to date only utilize zebra mussels in the Great Lakes.

8.2 Parameters of bioaccumulation model

Kinetic models of metal bioaccumulation are based on a simple conceptual model in which the concentration of a metal in an animal is controlled by the balance between uptake, elimination, and growth. Metal (M) uptake includes contributions from food ($AE \cdot IR \cdot [M]_f$) and water ($\alpha_w \cdot FR \cdot [M]_w$). Overall, metal loss from an organism occurs through growth (by dilution) and elimination. The time-dependent concentration of Cd in a bivalve is described by the following equation:

$$[Cd]_{bivalve} = \left(\frac{\alpha_w \cdot FR \cdot [Cd]_w}{k_{ew} + k_g}\right) \cdot \left(1 - e^{-(k_{ew} + k_g)t}\right) + \left(\frac{AE \cdot IR \cdot [Cd]_f}{k_{ef} + k_g}\right) \cdot \left(1 - e^{-(k_{ef} + k_g)t}\right) \qquad (8.1)$$

At steady-state, uptake is balanced by Cd elimination and dilution due to bivalve growth to give a constant concentration in the bivalve:

$$[Cd]_{bivalve}^{ss} = \left(\frac{\alpha_w \cdot FR \cdot [Cd]_w}{k_{ew} + k_g}\right) + \left(\frac{AE \cdot IR \cdot [Cd]_f}{k_{ef} + k_g}\right) \qquad (8.2)$$

where $[Cd]_{bivalve}^{ss}$ = Cd concentration in a bivalve at steady state (nmol·g^{-1} fresh wt), α_w = Cd absorption efficiency (0.01→1.0) from the dissolved phase (unitless), FR = bivalve filtration rate (L·d^{-1}·g^{-1} fresh wt bivalve), $[Cd]_w$ = dissolved Cd concentration (nmol·L^{-1}), AE = metal assimilation efficiency (0.01→1.0) from ingested food (unitless), IR = ingestion rate (g dry wt algae·d^{-1}·g^{-1} fresh wt bivalve), $[Cd]_f$ = Cd concentration in ingested particles (nmol·g^{-1} dry wt

algae), k_{ew} = efflux rate constant of Cd taken up from water (d^{-1}), k_{ef} = efflux rate constant for Cd following food uptake (d^{-1}), k_g = growth rate constant (d^{-1}).

During an exposure experiment (either from food or water), factors such as the animal's filtration rate, metal assimilation efficiency, ingestion rate, and efflux rates need to be measured/estimated as they will have a significant influence on the overall metal accumulation in an aquatic organism. Reasonably good estimates of these four parameters are needed in order to make even first-order predictions of metal accumulation in aquatic animals from both food and water using the kinetic modeling approach.

In our study, filtration rates were measured by determining the volume of water cleared of algae by the bivalve per unit of time in a 2-L experimental beaker. Coughlan's equation (Coughlan 1969; Tran *et a*l. 2000) was used to calculate FR, the bivalve's filtration rate:

$$FR = \frac{V \cdot \left\{ \left(\ln(d_o) - \ln(d_f) \right) - \left(\ln(d_o') - \ln(d_f') \right) \right\}}{(t \times M)} \qquad (8.3)$$

where FR is the filtration rate ($L \cdot h^{-1} \cdot g^{-1}$ fresh wt); *V* is the volume of water in each beaker (L); $\ln(d_o)$ and $\ln(d_f)$ are the natural logarithms of algal densities ($algae \cdot mL^{-1}$) at the beginning and end of the measurement period, respectively; $\ln(d_o')$ and $\ln(d_f')$ are the natural logarithms of algal densities ($algae \cdot mL^{-1}$) in the control chamber without a bivalve, at the beginning and end of the measurement period, respectively; *t* is the duration of each exposure period (h); and *M* is body mass (fresh weight, g). It is important to note that four assumptions are associated with the use of eq. 8.3, that is: the concentration of algae in the water was homogeneous; decreases in algal density were due only to animal filtration; all the algae passing over the gills were retained; and the filtration rate was constant throughout the measurement period. Filtration rates measured during the dissolved Cd exposure experiments (Section 5) varied from < 1 to 140 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt) for an average ventilation rate of 24 ± 17 $mL \cdot h^{-1} \cdot g^{-1}$ (± SE, fresh wt). It is important to note that to eliminate exposure to dietary Cd, filtration rates were measured while the bivalves were feeding and not when they were exposed to dissolved Cd. Both the average filtration rate and the range in filtration rates will be used in the bioaccumulation model.

Cadmium absorption efficiency from the dissolved phase (α_w) represents the proportion of dissolved cadmium that the bivalve accumulated with respect to the total that it pumped over a given time period:

$$\alpha_w = \left[\Delta[Cd]_{bivalve} / \left(FR \cdot t \cdot [Cd]_w \right) \right] \quad (8.4)$$

where α_w = metal absorption efficiency from the dissolved phase (unitless), $\Delta[Cd]_{bivalve}$ = increase in Cd concentration in the bivalve after Cd-exposure (nmol·g^{-1} fresh wt), FR = filtration rate (L·h^{-1}·g^{-1} fresh wt), t = exposure duration (60 h), and $[Cd]_w$ = dissolved Cd concentration (nmol·L^{-1}). For example, Cd concentrations in the bivalves after a 60 h exposure to 5 nM of dissolved Cd were, on average, 0.207 nmol·g^{-1}; if the average FR was 0.026 L·h^{-1}·g^{-1}, then α_w equals 0.027 (value entered in eq. 8.2) or 2.7% (when expressed as a percentage; the value presented in figures and tables). This value may change, however, based on bivalve filtration rates and dissolved Cd concentrations.

Hourly bivalve ingestion rates were calculated for each individual bivalve using the amount of algae consumed by the bivalve during the 4-h feeding period, and taking into account algal growth in the control beakers without bivalves:

$$IR = \left[V \times \frac{\left((d_0 - d_f) + \left(d_f' - d_0' \right) \right)}{t} \times 18.3 \times 10^{-13} \right] \times 1/M \quad (8.5)$$

where IR = the ingestion rate (g dry wt algae·h^{-1}·g^{-1} fresh wt bivalve); V = the volume of water in each beaker (mL); d_o = the initial algal density at the beginning of the feeding period (cells·mL^{-1}); d_f = the final algal density at the end of the feeding period (cells·mL^{-1}); d_o' = algal densities (cells·mL^{-1}) in the control beaker without a bivalve, at the beginning of the feeding period; d_f' = algal densities (cells·mL^{-1}) in the control beaker without a bivalve, at the end of the feeding period; t = the duration of each exposure period; M = body mass (fresh wt, g) and 18.3 x 10^{-13} is the weight of one algal cell (g dry wt ·cell^{-1}). Ingestion rates measured during the dietary

Cd exposure experiments (Section 6) varied from 9 to 54 μg dry wt of algae·h^{-1}·g^{-1} bivalve (fresh wt) for an average ingestion rate of 22 ± 3 μg dry wt of algae·h^{1}·g^{-1} bivalve (± SE, fresh wt, n = 14) (Table 8.1). Both the average ingestion rate and the range in ingestion rates will be used in the bioaccumulation model.

Assimilation efficiencies from food were calculated by dividing the total amount of Cd accumulated in the gills, in the digestive gland or in the whole bivalve by the amount of Cd consumed by each bivalve in a 16-h period:

$$AE = \left[\left(Cd_{bivalve} \right) / \left(IR \cdot \left[Cd \right]_f \cdot t \right) \right] \tag{8.6}$$

where AE = the assimilation efficiency of Cd per bivalve (unitless); IR = mean ingestion rate measured for each bivalve during four 4-h feeding periods (g dry wt algae·h^{-1}·g^{-1} bivalve fresh wt); $[Cd]_f$ = the mean Cd concentration in the algae (nmol·g^{-1} dry weight); t = the length of the feeding experiment (4 x 4 = 16 h); and $Cd_{bivalve}$ = the final amount of Cd in the bivalve (gills, digestive gland or whole organism; nmol Cd) accumulated after the feeding experiment, which includes the four separate 4-h feeding periods and an 8-d depuration period. As with α_w, the calculated value is added to equation 8.2, then the value is multiplied by 100 to be presented as a percentage (%) in figures and tables. Assimilation efficiencies measured during the dietary Cd exposure experiments (Section 6) varied from 5 to >100% for an average AE of 57 ± 9% (± SE; n = 14)[10] (Table 8.1). Both the average assimilation efficiency and the range in AE will be used in the bioaccumulation model and will be explored further in the Results section.

Efflux rates are a measurement of the physiological turnover rate of assimilated metals. For the purpose of the laboratory experiments, as exposure periods were relatively short, efflux and growth rates were considered negligible. Later, when comparing the calculated values obtained using the results measured in the laboratory experiments with values measured in bivalves collected from nature, we will use Cd-efflux rate and growth rate contants found in the literature.

[10] For the calculation of the average IR and AE, bivalves that did not feed for the full 16 h of the experiment were not considered. Moreover, individual AE values greater than 100% were set to 100%.

Table 8.1: Mean (± SE) and range in bivalve filtration rates (FR), absorption efficiencies of dissolved Cd (α_w), ingestion rates (IR), and assimilation efficiencies of ingested Cd (AE) measured during separate waterborne and dietary Cd exposures (N = number of bivalves; [Cd] = 0.1 to 5 nM). / Moyenne (± erreur type de la moyenne) et gamme observées pour le taux de filtration (FR), l'efficacité d'absorption du Cd dissous (α_w), le taux d'ingestion (IR), et l'efficacité d'assimilation du Cd ingéré chez les bivalves exposés séparément au Cd aqueux et alimentaire (N = nombre de bivalves; [Cd] = 0,1 à 5 nM).

	Mean (± SE)	Range
Dissolved exposure (N = 45)		
FR ($mL \cdot h^{-1} \cdot g^{-1}$ fw bivalve)	24 ± 17	< 1 to 140
α_w (%) (for gills)	7 ± 3	1 to 11
α_w (%) (for digestive gland)	3 ± 1	1 to 6
α_w (%) (whole body)	18 ± 4	3 to 47
Dietary exposure (N = 14)		
FR ($mL \cdot h^{-1} \cdot g^{-1}$ fw bivalve)	24 ± 2	8 to 48
IR (µg dry wt of algae$\cdot h^{-1} \cdot g^{-1}$ fw bivalve)	22 ± 3	9 to 54
AE (%) (for gills)	4 ± 3	1 to 18
AE (%) (for digestive gland)	71 ± 6	21 to >100
AE (%) (whole body)	57 ± 9	5 to >100

8.3 Results

8.3.1 Laboratory experiments

The mean filtration rate and absorption efficiency measured during the aqueous Cd exposures and the mean ingestion rate and assimilation efficiency measured during the dietary Cd exposures were used in the bioaccumulation model (eq. 8.2) to determine the relative importance of water and food as sources of Cd for *P. grandis*. Note that the denominators in eq. 8.2, i.e., (k_{ew} + k_g) and (k_{ef} + k_g), were considered to be identical for the waterborne and diet-borne exposure experiments, meaning that the relative contributions of food and water could be estimated by comparing the numerators, ($\alpha_w \cdot FR \cdot [Cd]_w$) and ($AE \cdot IR \cdot [Cd]_f$). The mean filtration rate (24 $mL \cdot h^{-1} \cdot g^{-1}$ fresh wt) was obtained from all dissolved Cd exposures combined (see Section 5). An ANOVA ($p < 0.05$) was performed on the filtration rates measured for each Cd treatment and showed that there was no significant difference among filtration rates for bivalves exposed to

0.1, 0.5, 5 or 20 nM dissolved Cd (i.e., Cd treatment level had no effect on bivalve filtration rates). The results presented in Table 8.2 show that for our laboratory conditions water was the main source of Cd for *P. grandis* gills, regardless of dissolved Cd treatment. Water was also the main source for the whole organism, except when bivalves were exposed to the lowest dissolved Cd concentration (0.1 nM).

Table 8.3 summarizes the effects of varying filtration rates on the relative importance of water and food as sources of Cd for the gills of *P. grandis* (range 5 to 140 $mL \cdot h^{-1} \cdot g^{-1}$ fresh wt). For ingestion rates, the highest and lowest values observed during the feeding experiments were used in the simulations, since IR is necessarily a function of the filtration rate (i.e., IR should increase with increasing FR), although the actual IR for a FR of 140 $mL \cdot h^{-1} \cdot g^{-1}$ (fresh wt) is unknown. In general, water remains the main source of Cd for *P. grandis*, except for the low filtration rate (5 $mL \cdot h^{-1} \cdot g^{-1}$ fresh wt) and the lowest value of $[Cd]_w$ (0.1 nM Cd). Note, however, that the value for $[Cd]_f$ was not varied in the simulations shown in Table 8.3 (whereas in the field one would expect decreases in $[Cd]_w$ from 5 to 0.1 nM to be accompanied by decreases in $[Cd]_f$).

Increasing the assimilation efficiency of Cd from ingested food to 100% increased the relative importance of food as source of Cd for *P. grandis* gills, notably when bivalves were exposed to 0.1 and 0.5 nM dissolved Cd. Water remained the primary source of Cd when bivalves were exposed to 5 nM dissolved Cd (AE = 100%) or when AE was 1% (Table 8.4a). Food becomes the main source of Cd only when the dissolved Cd concentration is below 0.5 nM. However, as mentioned above, the importance of food in these cases is presumably overestimated as the Cd concentration in the food would be lower if the algae had been exposed to the same dissolved Cd concentration as the bivalves (recall that when the algal cells were labelled with ^{109}Cd, the final concentration of dissolved Cd in the exposure medium was ~1.5 nM). To take this factor into account, the value of $[Cd]_f$ was varied over the range from 10 to 300 $nmol \cdot g^{-1}$ dry wt to reflect those observed in nature (Knauer *et al*. (1998); see Table 6.8). As before, food becomes the main source of Cd only when dissolved Cd concentrations are below 0.5 nM and when $[Cd]_f$ is relatively high. At the lowest $[Cd]_f$ value, water becomes the only meaningful source of Cd to *P. grandis* (Table 8.4b). The final factor to be considered was the algal density, which was varied between 400,000 $cells \cdot mL^{-1}$ (approximately the concentration of algal cells to which the bivalves

were exposed in the feeding experiments) and 2,000,000 cells·mL^{-1} (an upper range for algal densities in Rouyn-Noranda lakes; Campbell *et al*., unpublished data, chlorophyll-a and pheopigment concentrations in 23 lakes, summer 1997). A higher algal density leads to a higher ingestion rate and, hence, increases the relative importance of food as a source of Cd, notably at the lowest dissolved Cd concentrations (0.1 nM; Table 8.4c).

8.3.2 Field comparison

The values obtained for the various parameters of the biodynamic model from the laboratory experiments were used to predict steady-state Cd concentrations in the gills of bivalves collected from the field. The calculated results were then compared with those measured by Bonneris *et al.* (2005a) in *P. grandis* collected from various lakes in the mining region of Rouyn-Noranda (Table 8.5). Because aqueous Cd seems to be the main source of Cd for *P. grandis*, the bioaccumulation model was initially run with dietary uptake set to zero, i.e., with Cd uptake from the dissolved phase alone:

$$[Cd]_{gills}^{ss} = \left(\frac{\alpha_w \cdot FR \cdot [Cd]_w}{k_{ew} + g} \right) \tag{8.7}$$

For this calculation, an efflux rate constant (k_{ew}) of 0.0022 d^{-1} was used, based on the biological half-life of Cd (315 d) determined by Tessier *et al*. (1987) ($k_e = 0.693/t_{½}$)[11]. A growth rate constant (k_g) of 0.00044 d^{-1} was used, as measured by Perceval *et al.* (2004) for bivalves in the Rouyn-Noranda area. The total dissolved Cd concentrations in lake water were taken from Bonneris *et al.* (2005a). These calculations were first performed on the gills, as they were the main site of Cd accumulation when *P. grandis* was exposed to dissolved Cd.

The calculated Cd concentrations for the gills presented in Table 8.5 are much lower (Student paired t-test, $p < 0.05$) than the Cd concentrations measured in the gills of *P. grandis* and reported by Bonneris *et al*. (2005a), suggesting that the contribution of food in the field may be more important than what was observed during the laboratory experiments. Accordingly, the

[11] Note that this value of k_e, obtained from a field transplantation experiment, includes both k_{ew} and k_{ef}; it is used here as the best available estimate of k_{ew}.

calculations were repeated for the gills but with both water and food as vectors for Cd accumulation (Table 8.6). For this simulation, a high ingestion rate was used (80 µg dry wt algae·h^{-1}·g^{-1} fresh wt bivalve). Values for $[Cd]_f$ were calculated for each lake based on Cd uptake by the alga, *P. subcapitata*, during the labelling experiments that proceded the feeding trials:

$$[Cd]_f = \left([Cd]_{lake} / 1.5\right) \cdot 76 \qquad (8.8)$$

where $[Cd]_f$ = Cd concentration in the food calculated for each lake (nmol·g^{-1} dw); $[Cd]_{lake}$ = total the dissolved Cd concentration measured in the water of each lake; 1.5 corresponds to the final dissolved [Cd] in the experiments where *P. subcapitata* was labelled with ^{109}Cd (nmol·L^{-1}); and 76 corresponds to the mean [Cd] accumulated by the algae after a 24-h exposure to dissolved Cd (nmol·g^{-1} dry wt algae). For this calculation, we assumed a linear relationship between $[Cd]_f$ and total dissolved [Cd] in lake water.

Finally, similar calculations were performed for the digestive gland (Table 8.7).

Table 8.2: Relative importance (%) of water and food as sources of Cd (gills and whole organism) for the freshwater bivalve, *Pyganodon grandis*. All variables were measured under laboratory conditions. / Importance relative (%) de l'eau et de la nourriture comme sources de Cd (branchies et l'organisme entier) chez le bivalve d'eau douce, *Pyganodon grandis*. Tous les paramètres ont été obtenus en laboratoire.

Organ	Measured [Cd]	Uptake from water			Uptake from food			$[Cd]_{bivalve}$ from:		Relative importance	
		$[Cd]_w$	FR	α_w	$[Cd]_f$	IR	AE	Water	Food	Water	Food
Gills	0.51	5.0	24	7	76	22	4	8.4E-03	6.7E-05	99	1
Gills	0.06	0.5	24	7	76	22	4	8.4E-04	6.7E-05	93	7
Gills	0.01	0.1	24	7	76	22	4	1.7E-04	6.7E-05	72	28
Whole	1.30	5.0	24	18	76	22	57	2.2E-02	9.5E-04	96	4
Whole	0.125	0.5	24	18	76	22	57	2.2E-03	9.5E-04	70	30
Whole	0.025	0.1	24	18	76	22	57	4.3E-04	9.5E-04	30	70

Measured [Cd] in gills or whole organism: $nmol \cdot g^{-1}$ fresh wt (mean); $[Cd]_w$: dissolved Cd concentration ($nmol \cdot L^{-1}$); FR: mean filtration rate ($mL \cdot h^{-1} \cdot g^{-1}$ fresh wt); α_w: mean absorption efficiency of dissolved Cd (%); $[Cd]_f$: mean Cd concentration in algae ($nmol \cdot g^{-1}$ dry wt); IR: mean ingestion rate (µg dw algae$\cdot h^{-1} \cdot g^{-1}$ fresh wt); AE: mean assimilation efficiency of Cd from food (%); $[Cd]_{bivalve}$ taken up from water and food: $nmol \cdot g^{-1}$ fresh wt (water = $\alpha_w \cdot FR \cdot [Cd]_w$, and food = $AE \cdot IR \cdot [Cd]_f$).

Table 8.3: Effects of filtration rate (FR) on the relative importance (%) of water and food as sources of Cd for *Pyganodon grandis* gills. All variables were measured under laboratory conditions. / Influence du taux de filtration sur l'importance relative (%) de l'eau et de la nourriture comme sources de Cd chez les branchies de *Pyganodon grandis*. Tous les paramètres ont été obtenus en laboratoire.

Measured [Cd]	Water			Food			$[Cd]_{bivalve}$ from:		Relative importance	
	$[Cd]_w$	FR	α_w	$[Cd]_f$	IR	AE	Water	Food	Water	Food
0.51	5.0	140	7	76	54	4	4.9E-02	1.6E-04	99	1
0.06	0.5	140	7	76	54	4	4.9E-03	1.6E-04	97	3
0.01	0.1	140	7	76	54	4	1.0E-03	1.6E-04	86	24
0.51	5.0	5	7	76	9	4	1.8E-03	2.7E-05	99	1
0.06	0.5	5	7	76	9	4	2.0E-04	2.7E-05	87	13
0.01	0.1	5	7	76	9	4	4.0E-05	2.7E-05	56	44

Measured [Cd] in gills: $nmol \cdot g^{-1}$ fresh wt (mean); $[Cd]_w$: dissolved Cd concentration ($nmol \cdot L^{-1}$); FR: mean filtration rate ($mL \cdot h^{-1} \cdot g^{-1}$ fresh wt); α_w: mean absorption efficiency of dissolved Cd (%); $[Cd]_f$: mean Cd concentration in algae ($nmol \cdot g^{-1}$ dry wt); IR: mean ingestion rate (µg dw algae$\cdot h^{-1} \cdot g^{-1}$ fresh wt); AE: mean assimilation efficiency of Cd from food (%); $[Cd]_{bivalve}$ taken up from water and food: $nmol \cdot g^{-1}$ fresh wt (water = $\alpha_w \cdot FR \cdot [Cd]_w$, and food = $AE \cdot IR \cdot [Cd]_f$).

Table 8.4: Effects of assimilation efficiency (AE), Cd concentration in food ($[Cd]_f$) and ingestion rate (IR; 5x algal density) on the relative importance (%) of water and food as sources of Cd for *Pyganodon grandis* gills. All parameters were obtained under laboratory conditions. / Influence du AE, de la concentration du Cd dans la nourriture et de la densité algale sur l'importance relative (%) de l'eau et de la nourriture comme sources de Cd chez les branchies de *Pyganodon grandis*. Tous les paramètres ont été obtenus en laboratoire.

Changes	Water			Food			$[Cd]_{bivalve}$ from:		Relative importance	
	$[Cd]_w$	FR	α_w	$[Cd]_f$	IR	AE	Water	Food	Water	Food
(a)										
Higher AE	5.0	24	7	76	22	100	8.4E-03	1.7E-03	83	17
Higher AE	0.5	24	7	76	22	100	8.4E-04	1.7E-03	33	67
Higher AE	0.1	24	7	76	22	100	1.7E-04	1.7E-03	9	91
Lower AE	5.0	24	7	76	22	1	8.4E-03	1.7E-05	99	1
Lower AE	0.5	24	7	76	22	1	8.4E-04	1.7E-05	98	2
Lower AE	0.1	24	7	76	22	1	1.7E-04	1.7E-05	91	9
(b)										
Higher $[Cd]_f$	5.0	24	7	300	22	4	8.4E-03	2.6E-04	97	3
Higher $[Cd]_f$	0.5	24	7	300	22	4	8.4E-04	2.6E-04	76	24
Higher $[Cd]_f$	0.1	24	7	300	22	4	1.7E-04	2.6E-04	40	60
Lower $[Cd]_f$	5.0	24	7	10	22	4	8.4E-03	8.8E-07	100	0
Lower $[Cd]_f$	0.5	24	7	10	22	4	8.4E-04	8.8E-07	99	1
Lower $[Cd]_f$	0.1	24	7	10	22	4	1.7E-04	8.8E-07	95	5
(c)										
Higher IR (5x algal density)	5.0	24	7	76	110	4	8.4E-03	3.3E-04	96	4
Higher IR (5x algal density)	0.5	24	7	76	110	4	8.4E-04	3.3E-04	76	28
Higher IR (5x algal density)	0.1	24	7	76	110	4	1.7E-04	3.3E-04	33	67

$[Cd]_w$: dissolved Cd concentration ($nmol \cdot L^{-1}$); FR: mean filtration rate ($mL \cdot h^{-1} \cdot g^{-1}$ fresh wt); α_w: mean absorption efficiency of dissolved Cd (%); $[Cd]_f$: Cd concentration in algae ($nmol \cdot g^{-1}$ dry wt); IR: mean ingestion rate (µg dw algae$\cdot h^{-1} \cdot g^{-1}$ fresh wt); note that in section (c) the IR was increased 10-fold, reflecting an increase not in ventilation rate but in algal density; AE: assimilation efficiency of Cd from food (%); $[Cd]_{bivalve}$ taken up from water and food: $nmol \cdot g^{-1}$ fresh wt (water = $\alpha_w \cdot FR \cdot [Cd]_w$, and food = $AE \cdot IR \cdot [Cd]_f$).

Table 8.5: Comparison between calculated steady-state gill Cd concentrations in *Pyganodon grandis* (waterborne exposure only, eq. 8.7) and those measured in the gills of bivalves collected from lakes in the Rouyn-Noranda region (Bonneris *et al.* 2005a). / Comparaison entre les concentrations de Cd chez les branchies de *Pyganodon grandis*, telles que calculées pour un état stationnaire et en tenant compte uniquement de la prise en charge à partir de l'eau (éq. 8.7), et les concentrations de Cd mesurées dans les branchies de bivalves recueillis dans les lacs de la région de Rouyn-Noranda (Bonneris *et al.* 2005a).

Lake	Water			Calculated $[Cd]_{gills}^{ss}$		Measured $[Cd]_{gills}^{ss}$
	$[Cd]_w$	FR	α_w	$nmol \cdot g^{-1}$ fw	$nmol \cdot g^{-1}$ dw	$nmol \cdot g^{-1}$ dw
Évain	0.06	24	7	92	18	160
Opasatica	0.07	24	7	107	21	95
Ollier	0.07	24	7	107	21	53
Dufay	0.19	24	7	290	58	426
Joannès	0.30	24	7	458	92	1000
Héva	0.34	24	7	519	104	641
Caron	0.52	24	7	794	160	243
Vaudray	0.57	24	7	870	174	1470
Bousquet	0.55	24	7	840	168	979

$[Cd]_w$: total dissolved Cd concentrations measured in each lake ($nmol \cdot L^{-1}$); FR: mean filtration rate observed in the laboratory ($mL \cdot h^{-1} \cdot g^{-1}$ fresh wt); α_w: mean absorption efficiency of dissolved Cd in gills observed in the laboratory (%); Calculated [Cd] at steady-state accumulated in the gills from water: $nmol \cdot g^{-1}$ fresh wt and dry wt (Dry wt/Wet wt ratio of gills: 0.05); Measured [Cd] in gills of bivalves collected from field: $nmol \cdot g^{-1}$ dry wt (mean).

Table 8.6: Comparison between calculated steady-state gill Cd concentrations in *P. grandis* (water + diet-borne exposures adjusted for maximum ingestion rate) and those measured in the gills of bivalves collected from lakes in the Rouyn-Noranda region (Bonneris *et al.* 2005a). / Comparaison entre les concentrations de Cd chez les branchies de *P. grandis*, telles que calculées pour un état stationnaire et en tenant compte de la prise en charge à partir de l'eau et de la nourriture et ajustée pour un taux d'ingestion maximum, et les concentrations de Cd mesurées dans les branchies de bivalves recueillis dans les lacs de la région de Rouyn-Noranda (Bonneris *et al.* 2005a).

Lake	Water			Food			$[Cd]_{bivalve}$ from:		Calculated $[Cd]_{gills}^{SS}$	Measured $[Cd]_{gills}^{SS}$	Dissolved [Ca]
	$[Cd]_w$	FR	α_w	$[Cd]_f$	IR	AE	Water	Food	nmol·g^{-1} (dw)	nmol·g^{-1} (dw)	(µM)
Évain	0.06	24	7	3.0	80	4	2.4E-03	2.3E-04	20	160	166
Opasatica	0.07	24	7	3.5	80	4	2.8E-03	2.7E-04	23	95	203
Ollier	0.07	24	7	3.5	80	4	2.8E-03	2.7E-04	23	53	314
Dufay	0.19	24	7	9.6	80	4	7.7E-03	7.4E-04	64	426	71
Joannès	0.30	24	7	15.2	80	4	1.2E-02	1.2E-03	100	1000	174
Héva	0.34	24	7	17.2	80	4	1.4E-02	1.3E-03	114	641	50
Caron	0.52	24	7	26.3	80	4	2.1E-02	2.0E-03	174	243	275
Vaudray	0.57	24	7	27.9	80	4	2.3E-02	2.2E-03	191	1470	104
Bousquet	0.55	24	7	28.9	80	4	2.2E-02	2.1E-03	184	979	76

$[Cd]_w$: total dissolved Cd concentrations measured in each lake (nmol·L^{-1}); FR: mean filtration rate observed in the laboratory (mL·h^{-1}·g^{-1} fresh wt); α_w: mean absorption efficiency of dissolved Cd in gills observed in the laboratory (%); $[Cd]_f$: Cd concentration in algae (nmol·g^{-1} dry wt) N.B $[Cd]_f$ was calculated using equation 8-8; IR: adjusted ingestion rate (µg dw algae·h^{-1}·g^{-1} fresh wt) N.B. IR increases as algal density increases; AE: assimilation efficiency of Cd from food (%); $[Cd]_{bivalve}$ taken up from water and food: nmol·g^{-1} fresh wt; Calculated [Cd] at steady-state accumulated in the gills from water: nmol·g^{-1} dry wt; Measured [Cd] in gills of bivalves collected from field: nmol·g^{-1} dry wt (mean); total dissolved Ca concentrations measured in each lake (µM).

Table 8.7: Comparison between calculated steady-state digestive gland Cd concentrations in *P. grandis* (water + diet-borne exposures adjusted for maximum ingestion rate) and those measured in the digestive glands of bivalves collected from lakes in the Rouyn-Noranda region (Bonneris *et al.* 2005a). / Comparaison entre les concentrations de Cd chez les glandes digestives de *P. grandis*, telles que calculées pour un état stationnaire et en tenant compte de la prise en charge à partir de l'eau et de la nourriture et ajustée pour un taux d'ingestion maximum, et les concentrations de Cd mesurées dans les glandes digestives de bivalves recueillis dans les lacs de la région de Rouyn-Noranda (Bonneris *et al.* 2005a).

Lake	Water			Food			[Cd]bivalve from:		Calculated $[Cd]_{DG}^{ss}$	Measured $[Cd]_{GD}^{ss}$	Dissolved [Ca]
	$[Cd]_w$	FR	α_w	$[Cd]_f$	IR	AE	Water	Food	nmol·g^{-1} (dw)	nmol·g^{-1} (dw)	(μM)
Évain	0.06	24	3	3.0	80	71	1.0E-03	4.1E-03	49	150	166
Opasatica	0.07	24	3	3.5	80	71	1.2E-03	4.8E-03	57	123	203
Ollier	0.07	24	3	3.5	80	71	1.2E-03	4.8E-03	57	55	314
Dufay	0.19	24	3	9.6	80	71	3.3E-03	1.3E-02	155	300	71
Joannès	0.30	24	3	15.2	80	71	5.2E-03	2.1E-02	245	325	174
Héva	0.34	24	3	17.2	80	71	5.8E-03	2.3E-02	278	388	50
Caron	0.52	24	3	26.3	80	71	9.0E-03	3.6E-02	425	245	275
Vaudray	0.57	24	3	27.9	80	71	9.9E-03	3.9E-02	465	452	104
Bousquet	0.55	24	3	28.9	80	71	9.5E-03	3.8E-02	449	969	76

$[Cd]_w$: total dissolved Cd concentrations measured in each lake (nmol·L^{-1}); FR: mean filtration rate observed in the laboratory (mL·h^{-1}·g^{-1} fresh wt); α_w: mean absorption efficiency of dissolved Cd in digestive gland observed in the laboratory (%); $[Cd]_f$: Cd concentration in algae (nmol·g^{-1} dry wt) N.B $[Cd]_f$ was calculated using equation 8-8; IR: adjusted ingestion rate (μg dry wt algae·h^{-1}·g^{-1} fresh wt) **N.B.** IR increases as algal density increases; AE: assimilation efficiency of Cd from food (%); $[Cd]_{bivalve}$ taken up from water and food: nmol·g^{-1} fresh wt; Calculated [Cd] at steady-state accumulated in the digestive gland from water: nmol·g^{-1} dry wt; Measured [Cd] in digestive gland of bivalves collected from field: nmol·g^{-1} dry wt (mean); total dissolved Ca concentrations measured in each lake (μM).

8.4 Discussion

8.4.1 Laboratory experiments

This study confirms that under our laboratory conditions dissolved Cd was the main source of Cd for the freshwater bivalve *Pyganodon grandis*. This observation is consistent with the results reported by Tessier *et al*. (1993) who observed a positive relationship between $[Cd]_{bivalve}$ and $[Cd^{2+}]$ in their study of lakes. However, Tessier *et al.* (1993) could not rule out food as a possible source of Cd to *P. grandis*, since Cd concentrations in the food (e.g., phytoplankton) would presumably reflect those in the water column. Our results also agree with those of Wang *et al*. (1996) who reported that Cd was predominantly accumulated from the dissolved phase by the blue mussel, *Mytilus edulis*. Our results do contrast, however, with those reported by Roditi *et al*. (2000a) who suggested that the zebra mussel, *Dreissena polymorpha*, accumulated Cd mainly from ingested particles.

Uptake of Cd by *P. grandis* from solution is dependent on the dissolved Cd concentration, the metal's absorption efficiency and the bivalve's filtration rate. Filtration rates for *P. grandis* were measured using only one algal species and at a constant algal concentration. Many factors, namely food quality and quantity, have been shown to influence bivalve filtration rates. For example, lower algal densities have been shown to lead to increased bivalve filtration rates, as demonstrated by Tran *et al.* (2002) and Fournier *et al*. (2005) for *Corbicula fluminea*. The calculated results in Table 8.3 suggest that filtration rates have little effect on the relative importance of water and food as sources of Cd for *P. grandis* gills as water remained the main source of Cd for all the simulations. Indeed, an increase in filtration rate will increase both the amount of dissolved Cd and the number of algal cells that enter the mantle cavity. Note, however, that the calculation assumes that α_w is constant (7%), regardless of the filtration rate, and thus may overestimate the importance of waterborne Cd at high filtration rates; under real-world conditions, α_w would likely decrease at high filtration rates, a trend that has been reported for *Corbicula fluminea* (Tran *et a*l. 2001).

The absorption efficiency of Cd by *P. grandis* calculated for the dissolved metal exposure is higher than the values for α_w reported for Cd for marine species, which range from 0.03 to 0.7% depending on bivalve species (Chong and Wang 2001; Wang 2001; Wang *et a*l. 1996). Our results do, however, fall in the range of those observed by Tran *et al*. (2002) for the freshwater bivalve *C. fluminea*. These latter authors measured the extraction coefficient of dissolved Cd (E_{Cd}) for bivalves exposed to 18 nM dissolved Cd for 15 d. The extraction coefficient is analogous to the absorption efficiency, although the technical approach used to calculate it is different. Tran *et al.* (2002) reported extraction coefficients that varied from 2 to 12% depending on filtration rates, water temperature and food density. It is not surprising that the absorption efficiency of dissolved Cd is higher in freshwater species since Cd is strongly complexed by chloride in seawater, reducing its bioavailability and hence its uptake in marine animals (Langston and Bebianno 1990).

Although the absorption efficiency of dissolved Cd is lower than the assimilation efficiency of Cd from ingested food, uptake from the dissolved phase can contribute significantly to body burdens, particularly if the metal is predominantly in the dissolved phase, as is illustrated in the present study. In fact, after converting the Cd concentrations in the food (76 nmol Cd·g^{-1} dry wt algae) and the concentrations of food (~1 mg dry wt algae·L^{-1}) into concentrations of particulate Cd in the dietary exposure flasks (70 pmol Cd·L^{-1}), it is obvious that the dissolved Cd concentration in the inhaled water was much greater than the particulate Cd concentration. For a given filtration rate, the bivalves were exposed from 100 to 1000 times more Cd in the dissolved phase than in the particulate phase.

To determine if this large difference between the Cd concentrations of aqueous and particulate Cd was environmentally realistic, we considered the average Cd concentrations in the algae used during the feeding experiments. Using the K_D for Cd as calculated by Sigg (1987) for suspended particles collected in two Swiss lakes (50-500 L·g^{-1} dry wt), and considering dissolved Cd concentrations ranging from 0.1 to 0.5 nM,

one can calculate that the concentration of Cd in the particulate phase would range from 5 to 250 nmol Cd·g^{-1} particles (dry wt) (e.g., (0.1 nmol·L^{-1})·(50 L·g^{-1}) = 5 nmol·g^{-1}). The Cd concentration in the algae for the present study was ~76 nmol·g^{-1} dry wt. This value falls within the calculated range and suggests that the algae were not unreasonably contaminated during our experiments and that the diet-borne Cd concentrations approach those that might be observed in a natural setting.

One potential limitation of our study is that bivalves were fed only one species of algae during the food exposures. Studies have demonstrated that the ingestion rates and the assimilation efficiency of metals may be influenced by food type, algal assemblages and whether sediments were present in the suspended particulate matter. For example, the AE of metals associated with sediments is generally much lower than the AE for uptake from algae and diatoms (Gagnon and Fisher 1997; Griscom *et a*l. 2000; Chong and Wang 2000b). Lee and Luoma (1998) demonstrated that the assimilation efficiencies of Cd were higher in *P. amurensis* and *M. balthica* when the mussels were fed contaminated algae than when they were fed sediments or a sediment-algae mixture. One reason that Cd bioavailability is enhanced in algae may be that Cd accumulated in the algal cytoplasm is readily extracted and assimilated by the mussels (Reinfelder and Fisher 1991; 1994). *Pyganodon grandis* has been observed ingesting inorganic material in the laboratory (e.g. latex beads) and sediments have been found in the gut of bivalves collected from the field suggesting that these animals do ingest inorganic matter. It is, therefore, possible that the AE of metals by bivalves will be lower in the field due to the presence of sediments in the suspended particulate matter.

A bivalve's gut residence time may also influence metal assimilation efficiency. A shorter gut residence time may reduce the amount of metals that would accumulate in the animal as (a) less food would be digested and (b) only a superficial digestion will be performed on the ingested material. With a longer gut residence time, more non-cytosolic metal might be absorbed by the animal. Different AE from the natural particle assemblages could also result if bivalves selectively ingested different particles from an

assemblage, a consideration that cannot be addressed in studies with pure cultures. Such biological considerations should be more fully incorporated into conceptual models of factors affecting metal bioavailability (Lee and Luoma 1998).

The assimilation efficiency of a metal may vary seasonally due to changes in the concentration of phytoplankton found in the suspended material. Given that Lee and Luoma (1998) noted that the AE of Cd and Zn in the clams *P. amurensis* and *M. balthica* increased as the proportion of microalgae increased in the suspended material, microalgal blooms may be a period of increased exposure of bivalves to metals like Cd and Zn and a time when the risk to adverse effects increases. The results reported by Lee and Luoma (1998) also suggest experiments using only sediment exposures that do not include a realistic living food component in the particle assemblages could be underestimating bioavailability from particle ingestion.

Another factor that may have influenced the AE of Cd in *P. grandis* during this study is the algal species used for our feeding experiments. Although *P. subcapitata* was ingested by *P. grandis* and much of the Cd found within the algae was assimilated by the bivalve, it is possible that AE would have been lower had *P. grandis* been fed another algal species, one that is not as digestible for *P. grandis*. Moreover, the subcellular distribution of Cd within *P. subcapitata* may also differ from other algal species. Metal assimilation efficiencies in aquatic herbivores generally correlate with the cytoplasmic distribution of metals in algal food (Wang and Fisher 1996a; Reinfelder and Fisher 1994). Reinfelder *et al.* (1997) demonstrated that metals that accumulated in the cytoplasmic fraction of ingested phytoplankton were assimilated more efficiently by bivalves than metals that are not concentrated in this fraction. However, some bivalve species are capable of assimilating a significant portion of metals associated with the non-cytoplasmic fraction of algae. Enhanced assimilation as a result of prolonged gut retention may explain greater assimilation. Recent results on the subcellular partitioning of Cd in *P. subcapitata* show that the contributions of the subcellular fractions decrease

in the order: heat-stable phytochelatin-like peptide fraction > organelles > heat-denaturable proteins (HDP) (Lavoie *et al.* 2009).

8.4.2 Laboratory to field extrapolations

If we assume that our laboratory estimates for the variables in equation 8.7 are accurate, then the field results summarized in Table 8.5 suggest that food may be an important source of Cd for *P. grandis* in the field. Thus steady-state Cd concentrations, as calculated for bivalve gills on the basis of uptake from water alone, were significantly lower than the Cd concentrations measured in the gills of adult bivalves collected from various lakes located in the Rouyn-Noranda region.

In the light of these initial calculations, a second simulation was performed to take into account both water and food as sources of Cd for the gills (Table 8.6). Despite adding food as a source of Cd, calculated steady-state Cd concentrations in bivalve gills remained significantly lower than the Cd concentrations measured in the gills of adult bivalves collected from various lakes located in the Rouyn-Noranda region (Figure 8.1). In addition, the predicted progressive increase from Lake Évain (the lowest [Cd] in lake water) to Lake Bousquet (the highest [Cd] in lake water) did not correspond to the measured lake-to-lake trend (Figure 8.1).

A possible reason for this discrepancy might have been that the algal densities used to calculate the ingestion rate were lower than would normally be the case in a natural setting. As illustrated in Table 8.4(c), algal densities will have a significant effect on bivalve ingestion rate, which in turn will affect the relative importance of food as a source of Cd. Based on the chlorophyll-a concentrations measured in the Rouyn-Noranda lakes that were studied by Bonneris *et al*. (2005a), live phytoplankton levels may be between 60 and 190 $\mu g \cdot L^{-1}$ (dry wt) in the water columns of these lakes (Campbell, unpublished data). Furthermore, concentrations of phaeopigments are relatively high in these lakes, ranging from 2 to 5 times the chlorophyll concentrations (phaeopigments = dead algae, still presumably available to filter-feeding bivalves). It

follows that the total concentration of algal cells (living and dead) in the water columns of the Rouyn-Noranda lakes could be as high as 1100 µg dry wt·L^{-1} (i.e., (5+1)·190). During the feeding experiments, the bivalves were exposed to ~400,000 algal cells·mL^{-1} or ~730 µg·L^{-1} (dry wt). In other words, algal densities in the feeding experiments were already at the high end of what might be found in the Rouyn-Noranda lakes; increasing the algal densities to 1100 µg·L^{-1} would only increase the ingestion rate by a factor of about 1.5 (1100/730).

Because food was the main Cd source for the digestive gland of *P. grandis*, a final simulation was conducted with equation 8.2 to predict steady-state Cd concentrations in the digestive gland of bivalves collected from the Rouyn-Noranda region. A maximum ingestion rate of 80 µg algae dry wt·d^{-1}·g^{-1} bivalve fresh wt was used (based on the maximum ingestion rate observed experimentally (Table 6.6: 54 µg algae dry wt·h^{-1}·g^{-1} bivalve fresh wt) and the factor of 1.5 calculated in the preceding paragraph). Contrary to the gills, the model successfully predicted the Cd concentrations in bivalve digestive glands, notably in the moderately highly contaminated lakes (Table 8.7). Although calculated Cd concentrations for bivalve digestive glands were sometimes significantly lower (for non-contaminated lakes and Lake Bousquet) (Student paired t-test, $p < 0.05$) than the measured Cd concentrations in the digestive gland of *P. grandis* reported by Bonneris *et al.* (2005a), the differences between the predicted and measured Cd concentrations were less than that for the gills (Figure 8.2). Moreover, the relative importance of water and food as calculated in Table 8.7 was 20/80, unlike that for the gills where the relative importance of each pathway was generally 90/10 (water/food). These results support the idea that the relative importance of food may be higher than previously calculated (see Tables 8.2, 8.3 and 8.4), especially for the digestive gland.

The waterborne and diet-borne exposures carried out in the laboratory were not designed to mimic conditions observed in the field; physico-chemical factors that were not taken into account during our laboratory experiments may have contributed to the divergence between calculated and measured Cd concentrations. For example, higher dissolved Ca

concentrations were correlated with lower Cd accumulation in *P. grandis* in the field (Perceval *et a*l. 2002). The dissolved Ca concentration in the synthetic media used for the waterborne and diet-borne exposure experiments (625 μM) was chosen to match the Ca concentrations in the tap water used to maintain the bivalves in their stock aquariums; this concentration was considerably higher than the concentrations measured in the Rouyn-Noranda lakes (Table 8.6). The highest mean dissolved Ca concentration was reported for Lake Caron (275 μM), and interestingly the gill Cd concentration calculated for bivalves from this lake is similar to the measured value. High dissolved Ca concentrations in the ambient medium may have also reduced Cd accumulation in the digestive gland, although to a lesser degree than the gills since the digestive gland is not in direct contact with the medium, unlike the gills.

Metal speciation, the concentration of DOM and pH also differ among lakes and would also be expected to influence Cd accumulation in *P. grandis* gills. For the digestive gland, differences in the composition of the algal community may play an important role in Cd uptake in this organ. Dissolved Cd uptake by algae will vary among species, as will their susceptibility to digestive processes; the assimilation efficiency of diet-borne Cd would be expected to vary for various ingested algal species. However, algal species composition can vary greatly among lakes in response to water quality differences, making it difficult to define the actual relative importance of food as a source of Cd to *P. grandis*. The quality of the suspended matter may significantly affect AE as a higher proportion of inorganic material in the suspended matter may reduce AE. It is therefore possible, on a lake to lake basis, that the AE observed under laboratory conditions may be either overestimated or underestimated. For example, when AE is increased (~90%), the model's capability to predict $[Cd]_{digestive\ gland}$ is improved for bivalves collected from the least contaminated lakes.

8.5 Conclusions

The results from the present study suggest that both water and food contribute to Cd accumulation in the freshwater bivalve *P. grandis*, with water predicted to be the dominant source under field conditions. Attempts to predict steady-state Cd concentrations in bivalves collected along a metal contamination gradient in the field were unsuccessful; predicted Cd concentrations in the gills were always lower than observed concentrations, with the ratio «observed [Cd]/calculated [Cd]» ranging from 1.3 to 9.2. Although the model adequately predicted Cd concentrations in the digestive gland for bivalves collected from a majority of the lakes, the model underestimates $[Cd]_{digestive\ gland}$ in the least contaminated lakes and Lake Bousquet, suggesting that AE may vary from lake to lake and differ from the average AE measured under laboratory conditions.

Predictions using the biodynamic model were hampered by a number of uncertainties, notably with respect to:

- algal densities and species in the field (only chlorophyll-a data were available);
- Cd concentrations in phytoplankton in the Rouyn-Noranda lakes (no data were available);
- assimilation efficiency of Cd from natural phytoplankton (AE values for *P. subcapitata* were used);
- water chemistry in the Rouyn-Noranda lakes (which would influence Cd speciation and bioavailability, e.g., through complexation of Cd and through competition between Cd^{2+} and other cations such as Ca^{2+} or Mn^{2+}).

Future work should focus on these uncertainties.

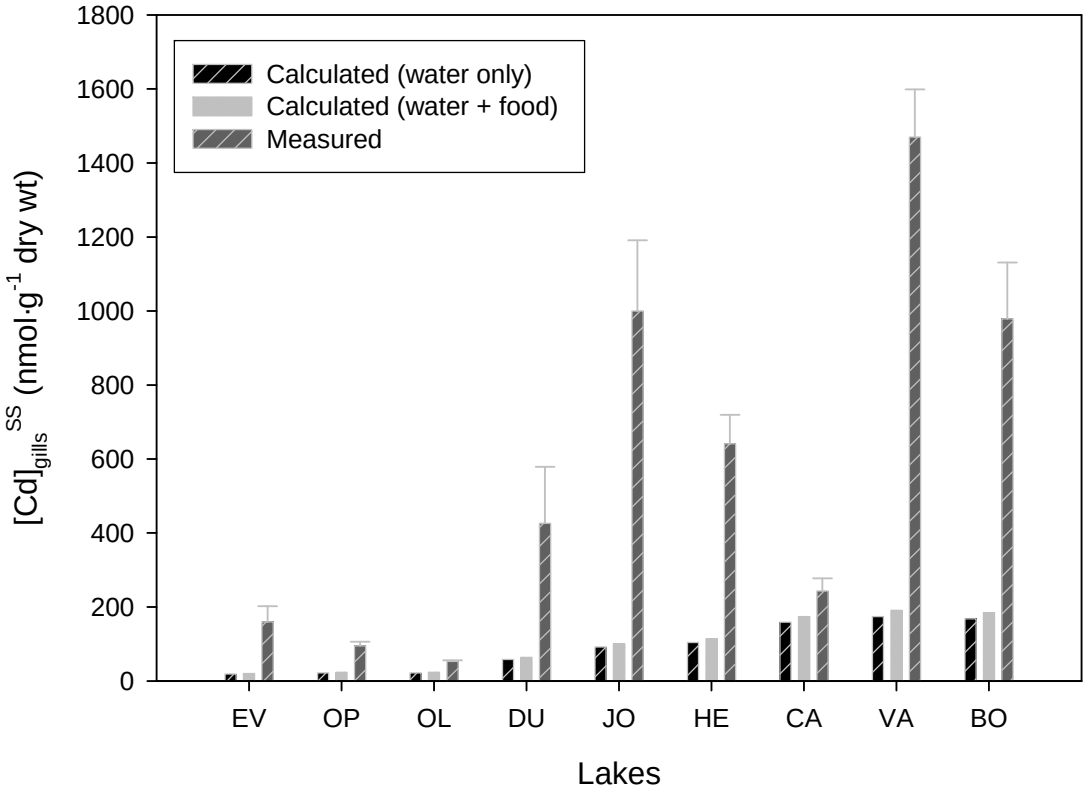


Figure 8.1: Comparison between calculated steady-state gill Cd concentrations (± SD) in *P. grandis* exposed to waterborne Cd only or to waterborne + diet-borne Cd (adjusted for a higher algal density) and those measured in the gills of bivalves collected from lakes in the Rouyn-Noranda region (Bonneris *et al.* 2005a). / Comparaison entre les concentrations de Cd chez les branchies de *P. grandis*, telles que calculées pour un état stationnaire et en tenant compte de la prise en charge à partir de l'eau seulement et à partir de l'eau et de la nourriture et ajustée pour une densité algale plus élevée, et les concentrations de Cd mesurées (± écart-type) dans les branchies de bivalves recueillis dans les lacs de la région de Rouyn-Noranda (Bonneris *et al.* 2005a).

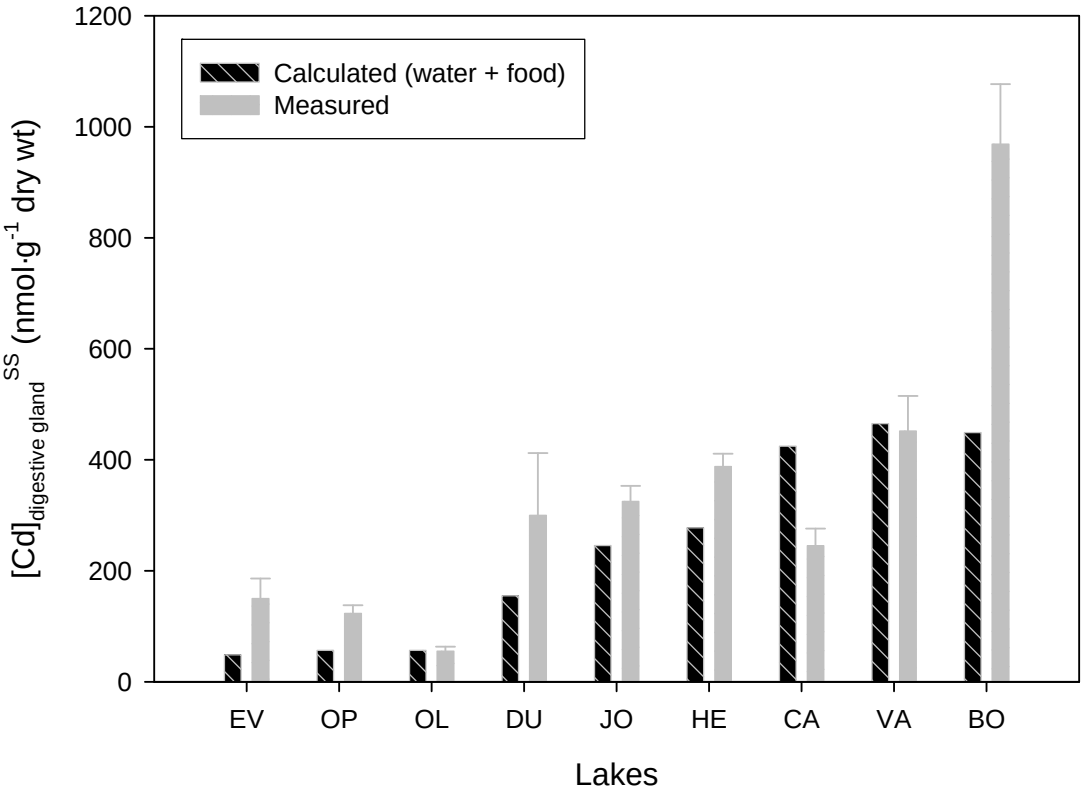


Figure 8.2: Comparison between calculated steady-state digestive gland Cd concentrations (± SD) in *P. grandis* exposed to waterborne and diet-borne Cd (adjusted for a higher algal density) and those measured in the digestive glands of bivalves collected from lakes in the Rouyn-Noranda region (Bonneris *et al.* 2005a). / Comparaison entre les concentrations de Cd chez les glandes digestives de *P. grandis*, telles que calculées pour un état stationnaire et en tenant compte de la prise en charge à partir de l'eau et de la nourriture et ajustée pour une densité algale plus élevée, et les concentrations de Cd mesurées (± écart-type) dans les glandes digestives de bivalves recueillis dans les lacs de la région de Rouyn-Noranda (Bonneris *et al.* 2005a).

9. CONCLUSIONS

9.1 Water and food as Cd sources for Pyganodon grandis

The main objective of the present study was to determine the relative importance of water and food as sources of cadmium for the freshwater bivalve *Pyganodon grandis*. Bivalves were exposed to waterborne and diet-borne Cd separately, and bivalve filtration rates (water- and diet-borne exposures), ingestion rates (diet-borne exposures) and assimilation efficiencies of aqueous and dietary Cd were measured during the experiments. These parameters were then used in a bioaccumulation model to predict the relative importance of each pathway as a source of Cd for *P. grandis*.

The results from the present study suggest that, under our laboratory conditions, water is the major source of Cd for *P. grandis*. Various simulations in which the parameters of the bioaccumulation model were altered according to results obtained in the laboratory (e.g., higher and lower filtration rates, higher and lower AE) showed that water remains the main source of Cd for *P. grandis*, regardless of these changes. However, under certain conditions (e.g., higher ingestion rates, higher AE, higher algal abundance) the importance of food as a source of Cd increases, suggesting that both water and food could contribute to Cd accumulation in *P. grandis* under field conditions.

The study also illustrated that the short-term fate of cadmium within *P. grandis* is dependent on its uptake source. When the bivalve is exposed to dissolved Cd, a large proportion of the accumulated Cd is associated with the gills, notably in the calcium-rich granules. Under these conditions, less Cd accumulates in the digestive gland than in the gills, and most of it accumulates in the cytosolic fraction of the digestive gland cells. In contrast, after exposure to diet-borne Cd, the metal is predominantly associated with the digestive gland, although some of the Cd accumulated by the digestive gland is transferred into the gills (where it again is largely bound to the granule fraction). This transfer is linked to marked changes in the subcellular partitioning of Cd within the

digestive gland. Contrary to the results obtained in field studies, spillover of Cd into the potentially metal-sensitive HDP fraction was observed during these short-term exposures, suggesting that bivalves were unable to cope with the sudden increase in ambient and internal Cd concentrations.

Bivalve filtration rates were also shown to have a significant influence on Cd accumulation from the dissolved phase as $[Cd]_{gills}$ generally increased as bivalve filtration rates increased, a trend that was also observed for *Corbicula fluminea* by Tran *et al*. (2002). However, it is unclear whether this increase in $[Cd]_{gills}$ was related to an increase in water flow over the gill surface area or an increase in gill absorption efficiency brought about by an increase in blood flow. Nonetheless, the present study further illustrates the need to take into account ventilatory activity in dissolved metal accumulation studies.

9.2 Pyganodon grandis *as a sentinel species for Cd*

Pyganodon grandis possess many of the characteristics needed of a good biomonitor (Phillips and Rainbow 1993). They can be found in large numbers in lakes and rivers across North America, they are sedentary and easily identifiable, and they offer sufficient tissue for contaminant analysis. They have also shown to be adaptable and are capable of surviving a new environment when transplanted from their original setting (Couillard *et a*l. 1995a; Perceval *et a*l. 2006; Cooper 2008; Tessier *et al*. 1987). More importantly, *P. grandis* is tolerant of a wide range of ambient metal concentrations and concentrates many metals (Couillard *et a*l. 1993; 1995a; Couillard *et a*l. 1995b; Wang *et a*l. 1999).

Given the results of the present study, indicating that water is the major source of Cd for *P. grandis*, the interpretation of spatial and temporal variations in Cd concentrations accumulated in the whole body or in individual target organs is less ambiguous: it can now be presumed that such changes in accumulated Cd reflect changes in bioavailable waterborne Cd. Moreover, our findings also simplify the interpretation of results

stemming from the use of *P. grandis* as a biomonitor as now we will only have to focus on aqueous sources of metals and not on what is found in suspension. If food had been the main source of Cd, we would have had to know the algal species found in every lake. This is difficult as the algal community found in a lake is greatly dependent on pH, temperature, dissolved Ca concentrations and other physico-chemical parameters.

Changes in ambient Cd concentrations can occur quite rapidly, particularly in lotic environments, but it must be remembered that Cd accumulation in *P. grandis* is a very slow process (Cooper 2008; 1995a; Couillard *et a*l. 1995b; Perceval *et a*l. 2006). Therefore, *P. grandis* can only be used to monitor long-term changes (i.e., years) in environmental and aqueous Cd concentrations. The monitoring of episodic, short-term changes (i.e., days or weeks) in aqueous [Cd] would require an organism whose internal metal concentrations can rapidly attain steady-state, such as *Chaoborus* (Hare et Tessier 1998).

9.3 Bioaccumulation model for Pyganodon grandis

Biodynamic models have been used to evaluate the relative importance of dietary versus aqueous uptake pathways for contaminants in molluscs, as well as to make site-specific predictions of contaminant concentrations in these animals. We used the following model to describe steady state Cd concentrations in *P. grandis*:

$$[Cd]_{bivalve}^{ss} = \left(\frac{\alpha_w \cdot FR \cdot [Cd]_w}{k_{ew} + k_g} \right) + \left(\frac{AE \cdot IR \cdot [Cd]_f}{k_{ef} + k_g} \right)$$

where $[Cd]_{bivalve}$ = metal concentration in bivalve at steady state (nmol·g^{-1} fresh wt), α_w = metal assimilation efficiency from the dissolved phase (unitless), FR = filtration rate (L·d^{-1}·g^{-1} fresh wt bivalve), $[Cd]_w$ = dissolved metal concentration (nmol·L^{-1}), AE = metal assimilation efficiency from ingested food (unitless), IR = ingestion rate (g algae·h^{-1}·g^{-1} fresh wt bivalve), $[Cd]_f$ = metal concentration in ingested particles (nmol·g^{-1} fresh wt algae), k_{ew} = efflux rate constant following dissolved uptake (d^{-1}), k_{ef} = efflux rate constant following food uptake (d^{-1}), k_g = growth rate constant (d^{-1}).

Agreement between the gill Cd concentrations predicted with the biodynamic model and those observed in native bivalves collected from 9 Rouyn-Noranda lakes was poor; the model consistently underestimated Cd accumulation in *P. grandis* gills, even after food was added as a Cd source. On the other hand, the model successfully predicted $[Cd]_{digestive\ gland}$ in many of the lakes, notably for bivalves collected from the moderately and highly contaminated lakes; digestive gland Cd concentrations were somewhat underestimated in bivalves from the non-contaminated lakes and Lake Bousquet. However, the differences between predicted and observed $[Cd]_{digestive\ gland}$ were significantly smaller than what was observed for the gills. These results suggest that certain parameters (e.g., efflux rates and AE) may have been overestimated or that certain physico-chemical factors, such as dissolved [Ca], need to be taken into account in the model. Moreover, the results obtained for the digestive gland suggest that food may be more important as a source of Cd for *P. grandis* than previously thought, although for the whole organism water remains the main source of cadmium.

These modelling results contrast with those reported by Kraemer *et al*. (2008) for the yellow perch, *Perca flavescens*. As in the present case for *P. grandis*, Kraemer *et al*. (2006) showed that dissolved Cd was the predominant source of Cd in the gills of *P. flavescens*. They therefore used a bioaccumulation model that took into account Cd accumulation only from the dissolved phase. The model established by Kraemer *et al*. (2008) greatly over-predicted steady-state gill Cd concentrations in wild yellow perch. Based on their results, Kraemer *et al*. (2008) suggested that *P. flavescens* are able to control Cd accumulation in the gills by invoking a feedback mechanism whereby the perch reduce Cd uptake from the dissolved phase when internal Cd concentrations reach a certain threshold. Our results suggest that *P. grandis* does not have this capacity to control Cd uptake and will continue to accumulate Cd, albeit slowly.

9.4 Further work

Given the relative importance of dissolved Cd as a source of Cd for *P. grandis*, further studies are needed to investigate the influence of filtration rates on the accumulation of Cd in this bivalve. Bivalves were not fed during the aqueous Cd exposures and the relationship observed between $[Cd]_{gills}$ and bivalve filtration rates was established using filtration rates measured during the feeding period. Due to the variability observed in bivalve filtration rates, it is unlikely that filtration rates were constant between the feeding and exposure periods. Additional studies are needed to develop a protocol that would allow the researcher to measure filtration rates during a dissolved Cd exposure without exposing the bivalves to a dietary source of Cd. Additional studies are also needed to determine if factors such as pH, temperature and food quality may have an influence on bivalve filtration rates. As several of the suggested experiments involve the study of *P. grandis* in captivity, additional information regarding ammonia and CO_2 production, oxygen consumption, and the amount of pseudofeces and feces produced by the animals would be useful. Determining the scope for growth of bivalves maintained in captivity may help improve bivalve health and survival.

Further work is also needed to investigate the influence of dissolved Ca on Cd accumulation in *P. grandis*. The tendency of the biodynamic model to under-predict gill Cd concentrations in wild specimens of *P. grandis* may be the result of the relatively high dissolved Ca concentrations that were used during the laboratory experiments. Additional studies conducted under laboratory conditions that expose bivalves to a variety of dissolved Ca and Cd concentration are needed to determine whether dissolved Ca does in fact influence the accumulation of waterborne Cd accumulation by *P. grandis*. Numerous uncertainties also remain regarding dietary Cd uptake in *P. grandis* and how algal density and algal species can influence assimilation efficiencies and Cd uptake from food. Moreover, studies that measure Cd concentrations in natural populations of phytoplankton are lacking and any additional information regarding metal concentrations in phytoplankton would enhance our knowledge of the importance of food as a source of metals in *P. grandis* .

10.REFERENCE LIST

Adhiya, J., Cai, X., Sayre, R.T., et Traina, S.J. 2002. Binding of aqueous cadmium by the lyophilized biomass of *Chlamydomonas reinhardtii*. Coll. Surf. **210**: 1-11.

Ahsanullah, M. et Florence, T.M. 1984. Toxicity of copper to the marine amphipod *Allorchestes compressa* in the presence of water- and lipid-soluble ligands. Mar. Biol. **84**: 41-45.

Almer, B., Dickson, W., Ekstrom, C., et Hornstrom, E. 1978. Sulfur pollution and the aquatic ecosystem. *In* Sulfur in the Environment Part II: Ecological Impacts. *Edited by* J.O. Nriagu. John Wiley & Sons, New York, NY, USA, pp. 271-311.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., et Rainbow, P.S. 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxifixation and their use as biomarkers. Aquat. Toxicol. **76**: 160-202.

Amiard, J.-C., Geffard, A., Amiard-Triquet, C., et Crouzet, C. 2007. Relationship between the lability of sediment-bound metals (Cd, Cu, Zn) and their bioaccumulation in benthic invertebrates. Est. Coast. Shelf Sci. **72**: 511-521.

Anandraj, A., Marshall, D.J., Gregory, M.A., et McClurg, T.P. 2002. Metal accumulation, filtration and O_2 uptake rates in the mussel *Perna perna* (Mollusca: Bivalvis) exposed to Hg^{2+}, Cu^{2+} and Zn^{2+}. Comp. Biochem. Physiol. **132C**: 355-363.

Ardisson, P.-L. 1985. Évaluation de facteurs qui contrôlent l'échange de métaux traces entre des mollusques bivalves (*Anodonta grandis*) et leur environnement. M.Sc. thesis, Université du Québec, INRS, INRS-Eau, 108p.

Arifin, Z. et Bendell-Young, L. 2000. Influence of a selective feeding behaviour by the blue mussel (*Mytilus trossulus*) on the assimilation of ^{109}Cd from environmentally relevant seston matrices. J. Exp. Mar. Biol. Ecol. **192**: 181-193.

Baudrimont, M., Lemaire-Gony, S., Ribeyre, F., Metivaud, J., et Boudou, A. 1997a. Seasonal variations of metallothionein concentrations in the Asiatic clam (*Corbicula fluminea*). Comp. Biochem. Physiol. **118C**: 361-367.

Baudrimont, M., Metivaud, J., Maury-Brachet, R., Ribeyre, F., et Boudou, A. 1997b. Bioaccumulation and metallothionein response in the Asiatic clam (*Corbicula fluminea*) after experimental exposure to cadmium and inorganic mercury. Environ. Toxicol. Chem. **16**: 2096-2102.

Bayne, B.L. 1998. The physiology of suspension-feeding by bivalve mollusks - an introduction to the Plymouth "TROPHEE" Workshop. J. Exp. Mar. Biol. Ecol. **219**: 1-19.

Bayne, B.L., Hawkins, A.J.S., et Navaarro, E. 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in a mixture of silt and algae cells at low concentrations. J. Exp. Mar. Biol. Ecol. **111**: 1-12.

Bayne, B.L., Iglesias, J.I.P., Hawkins, A.J.S., Navarro, E., Heral, M., et Deslous-Paoli, J.M. 1993. Feeding behavior of the mussel, *Mytilus edulis*: responses to variations in quantity and organic content of the seston. J. Mar. Biol. Assoc. **73**: 813-829.

Bayne, B.L. et Newell, R.C. 1983. Physiological energetics of marine molluscs. *In* The Molluscs. *Edited by* A.S.M. Saleuddin and K.M.Wilbur. Academic Press, New York, NY, USA, pp. 407-515.

Bebianno, M.J. et Langston, W.J. 1991. Metallothionein induction in *Mytilus edulis* exposed to cadmium. Mar. Biol. **108**: 91-96.

Bebianno, M.J. et Langston, W.J. 1992. Metallothionein induction in *Littorina littorea* (Mollusca: Prosobranchia) on exposure to cadmium. J. Mar. Biol. Assoc. U. K. **72**: 329-342.

Bebianno, M.J. et Serafim, M.A. 1998. Comparison of metallothionein induction in response to cadmium in the gills of the bivalve molluscs *Mytilus galloprovincialis* and *Ruditapes decassatus*. Sci. Tot. Environ. **214**: 123-131.

Beckvar, N., Salazar, S., Salazar, M., et Finkelstein, K. 2000. An in situ assessment of mercury contamination in the Sudbury River, Massachusetts, using transplanted freshwater mussels (*Elliptio complanata*). Can. J. Fish. Aquat. Sci. **57**: 1103-1112.

Bewers, J.M., Barry, P.J., et MacGregor, D.J. 1987. Distribution and cycling of cadmium in the environment. *In* Cadmium in the aquatic environment. *Edited by* J.O. Nriagu and J.B.Sprague. J. Wiley & Sons, Ltd., New York, NY, USA, pp. 1-18.

Bias, R. et Karbe, L. 1985. Bioaccumulation and partitioning of cadmium within the freshwater mussel *Dreissena polymorpha* Pallas. Internat. Rev. Ges. Hydrobiol. **70**: 113-125.

Bjerregaard, P. et Depledge, M.H. 1994. Cadmium accumulation in *Littorina littorea, Mytlius edulis* and *Carcinus maenas*: the influence of salinity and calcium ion concentrations. Mar. Biol. **119**: 385-395.

Block, M. et Wicklund Glynn, A. 1992. Influence of xanthates on the uptake of ^{109}Cd by eurasian dace (*Phoxinus phoxinus*) and rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. **11**: 873-879.

Bonneris, E. 2004. Étude de l'implication de différents ligands cellulaires dans la séquestration du cadmium, du cuivre, et du zinc chez des Mollusques bivalves d'eau douce exposés à une contamination environnementale. Ph.D. thesis, École Doctorale Ingénièrie pour la Santé, la Cognition et l'Environnement, Université Joseph Fourier, Grenoble, France, 198p.

Bonneris, E., Giguère, A., Perceval, O., Buronfosse, T., Masson, S., Hare, L., et Campbell, P.G.C. 2005a. Sub-cellular partitioning of metals (Cd, Cu, Zn) in the gills of a freshwater bivalve, *Pyganodon grandis*: role of calcium concretions in metal sequestration. Aquat. Toxicol. **71**: 319-334.

Bonneris, E., Perceval, O., Masson, S., Hare, L., et Campbell, P.G.C. 2005b. Sub-cellular partitioning of Cd, Cu and Zn in tissues of indigenous unionid bivalves living along a metal exposure gradient and links to metal-induced effects. Environ. Pollut. **135**: 195-208.

Borchardt, T. 1983. Influence of food quantity on the kinetics of cadmium uptake and loss via food and sea water in *Mytilus edulis*. Mar. Biol. **76**: 67-78.

Borchardt, T. 1985. Relationships between carbon and cadmium uptake in *Mytilus edulis*. Mar. Biol. **85**: 233-244.

Braddock, J.F. et Brown, E.J. 1994. Phosphate-uptake by the yeast, *Rhodotorula rubra*, and the green alga, *Selenastrum caricornutum* Printz, after phosphate additions to steady-state continuous cultures. FEMS Microbiol. Ecol. **14**: 111-119.

Brown, D.A. et Parsons, T.R. 1978. Relationship between cytoplasmic distribution of mercury and toxic effects to zooplankton and chum salmon (*Oncorhynchus keta*) exposed to mercury in a controlled ecosystem. J. Fish. Res. Board Can. **35**: 880-884.

Bryan, G.W. et Hummerstone, L.G. 1978. Heavy metals in the burrowing bivalve *Scrobicularia plana* from contaminated and uncontaminated estuaries. J. Mar. Biol. Assoc. U. K. **58**: 401-419.

Byrne, R.H., Kemp, L.R., et Cantrell, K.J. 1988. The influence of temperature and pH on trace metal speciation in seawater. Mar. Chem. **25**: 163-181.

Cain, D.J., Luoma, S.N., et Wallace, W.G. 2004. Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. Environ. Toxicol. Chem. **23**: 1463-1473.

Campbell, P.G.C. 1995. Interactions between trace metals and organisms: a critique of the free-ion activity model. *In* Metal Speciation and Bioavailability in Aquatic Systems. *Edited by* A. Tessier and D.Turner. J. Wiley & Sons, Chichester, UK, pp. 45-102.

Campbell, P.G.C., Chapman, P.M., et Hale, B.A. 2006. Risk assessment of metals in the environment. Issues Environ. Sci. Technol. **21**: 102-131.

Campbell, P.G.C. et Couillard, Y. 2004. Prise en charge et détoxication des métaux chez les organismes aquatiques. *In* Écotoxicologie moléculaire - Principes fondamentaux et perspectives de développement. *Edited by* E. Pelletier, P.G.C.Campbell, and F.Denizeau. Les Presses de l'Université du Québec, Ste-Foy, Quebec, Canada, pp. 9-61.

Campbell, P.G.C., Errécalde, O., Fortin, C., Hiriart-Baer, V.P., et Vigneault, B. 2002. Metal bioavailability to phytoplankton - applicability of the Biotic Ligand Model. Comp. Biochem. Physiol. **C133**: 185-202.

Campbell, P.G.C., Giguère, A., Bonneris, E., et Hare, L. 2005. Cadmium-handling strategies in two chronically exposed indigenous freshwater organisms - the yellow perch (*Perca flavescens*) and the floater mollusc (*Pyganodon grandis*). Aquat. Toxicol. **72**: 83-97.

Campbell, P.G.C. et Tessier, A. 1987. Metal speciation in natural waters: influence of environmental acidification. *In* Sources and Fates of Aquatic Pollutants. ACS Advances in Chemistry Series, Vol. No. 216. *Edited by* R.A. Hites and S.J.Eisenreich. Amer.Chem.Soc., Washington, DC, USA, pp. 185-207.

Cassini, A., Tallandini, L., Favero, N., et Albergoni, V. 1986. Cadmium bioaccumulation studies in the freshwater molluscs *Anodonta cygnea* and *Unio elongatulus*. Comp. Biochem. Physiol. **84C**: 35-41.

Chapman, P.M. et Wang, F. 2000. Issues in ecological risk assessment of inorganic metals and metalloids. Human Ecol. Risk Assess. **6**: 965-988.

Cheung, M.S. et Wang, W.X. 2005. Influence of subcellular metal compartmentalization in different prey on the transfer of metals to a predatory gastropod. Mar. Ecol. Progr. Ser. **286**: 155-166.

Chong, K. et Wang, W.X. 2000a. Assimilation of cadmium, chromium, and zinc by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. Environ. Toxicol. Chem. **19**: 1660-1667.

Chong, K. et Wang, W.X. 2000b. Bioavailability of sediment-bound Cd, Cr and Zn to the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*. J. Exp. Mar. Biol. Ecol. **255**: 75-92.

Chong, K. et Wang, W.-X. 2001. Comparative studies on the biokinetics of Cd, Cr, and Zn in the green mussel *Perna viridis* and Manila clam *Ruditapes philippinarum*. Environ. Pollut. **115**: 107-121.

Clarke, A.H. 1981. The freshwater molluscs of Canada. National Museum of Canada, D.W. Freisen & Sons Ltd., Ottawa, ON, Canada.

Cooper, S.A. 2008. Subcellular partitioning of metals (Cd, Cu, Zn) in the gills and digestive glands of a freshwater bivalve, *Pyganodon grandis*, transplanted along a metal exposure gradient. Université du Québec, INRS, INRS-ETE, Rapport interne, Québec, QC, Canada.

Cossa, D. 1989. A review of the use of *Mytilus* spp as quantitative indicators of cadmium and mercury contamination in coastal waters. Oceanol. Acta **12**: 417-432.

Cossa, D., Bourget, E., Pouliot, D., Piuze, J., et Chanut, J.P. 1980. Geographical and seasonal variations in the relationship between trace metal content and body weight in *Mytilus edulis.* Mar. Biol. **58**: 7-14.

Coughlan, J. 1969. The estimation of filtering rate from clearance of suspensions. Mar. Biol. **2**: 356-358.

Couillard, Y., Campbell, P.G.C., Pellerin-Massicotte, J., et Auclair, J.-C. 1995a. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. II. Metallothionein response to metal exposure, evidence for cytotoxicity, and links to effects at higher levels of biological organization. Can. J. Fish. Aquat. Sci. **52**: 703-715.

Couillard, Y., Campbell, P.G.C., et Tessier, A. 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. Limnol. Oceanogr. **38**: 299-313.

Couillard, Y., Campbell, P.G.C., Tessier, A., Pellerin-Massicotte, J., et Auclair, J.-C. 1995b. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. I. Temporal changes of metallothionein and metal (Cd, Cu, Zn) concentrations in soft tissues. Can. J. Fish. Aquat. Sci. **52**: 690-702.

Croteau, M.-N., Hare, L., et Tessier, A. 1998. Refining and testing a trace metal biomonitor (*Chaoborus*) in highly acidic lakes. Environ. Sci. Technol. **32**: 1348-1353.

Croteau, M.-N., L.Hare, et A.Tessier 2002. Difficulties in relating Cd concentrations in the predatory insect *Chaoborus* to those of its prey in nature. Can. J. Fish. Aquat. Sci. **60**: 800-808.

Croteau, M.-N., Luoma, S.N., et Stewart, A.R. 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnol. Oceanogr. **50**: 1511-1519.

De Duve, C. 1975. Exploring cells with a centrifuge. Science **189**: 186-194.

De Zwann, A., Cortesi, P., van der Thillart, G., Roos, J., et Storey, K.B. 1991. Different sensitivities to hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. Mar. Biol. **111**: 343-351.

Decho, A.W. et Luoma, S.N. 1991. Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: significance to the absorption of carbon and chromium. Mar. Ecol. Prog. Ser. **78**: 303-314.

Decho, A.W. et Luoma, S.N. 1994. Humic and fulvic acids: sink or source in the availability of metals to the marine bivalves *Macoma balthica* and *Potamocorbula amurensis*? Mar. Ecol. Progr. Ser. **108**: 133-145.

Depledge, M.H., Weeks, J.M., et Bjerregaard, P. 1994. Heavy metals. *In* Handbook of ecotoxicology. *Edited by* P. Calow. Blackwell Scientific, London, UK, pp. 79-105.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., et Santore, R.C. 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. **20**: 2383-2396.

Elliott, N.G., Swain, R., et Ritz, D.A. 1986. Metal interaction during accumulation by the mussel *Mytilus edulis planulatus*. Mar. Biol. **93**: 395-399.

Environment Canada 1994. Cadmium and its compounds. *In* Canadian Environmental Protection Act: First Priority Substances List (PSL1). Assessment Report. Cat. No. 40-215/40E. National Press (Ottawa).

Erk, M., Ruus, A., Ingebrigtsen, K., et Hylland, K. 2005. Cadmium accumulation and Cd-binding proteins in marine invertebrates - A radiotracer study. Chemosphere **61**: 1651-1664.

Errécalde, O. et Campbell, P.G.C. 2000. Cadmium and zinc bioavailability to *Selenastrum capricornutum* (Chlorophyceae): accidental metal uptake and toxicity in the presence of citrate. J. Phycol. **36**: 473-483.

Errécalde, O., Seidl, M., et Campbell, P.G.C. 1998. Influence of a low molecular weight metabolite (citrate) on the toxicity of cadmium and zinc to the unicellular green alga *Selenastrum capricornutum*: an exception to the Free-Ion Model. Wat. Res. **32**: 419-429.

Ferrarello, C.N., De la Campa, M.D.R.F., Muniz, C.S., et Sanz-Medel, A. 2000. Metal distribution patterns in the mussel *Mytilus edulis* cytosols using size-exclusion chromatography and double focusing ICP-MS detection. Analyst **125**: 2223-2229.

Fisher, N.S., Teyssié, J.-L., Fowler, S.W., et Wang, W.-X. 1996. Accumulation and retention of metals in mussels from food and water: a comparison under field and laboratory conditions. Environ. Sci. Technol. **30**: 3232-3242.

Foe, C. et Knight, A. 1986. Growth of *Corbicula fluminea* (Bivalvia) fed artificial and algal diets. Hydrobiologia **133**: 155-164.

Fournier, E., Adam, C., Massabuau, J.C., et Garnier-Laplace, J. 2005. Bioaccumulation of waterborne selenium in the Asiatic clam *Corbicula fluminae*: influence of feeding-induced ventilatory activity and selenium species. Aquat. Toxicol. **72**: 251-260.

Frazier, J.M. 1986. Cadmium-binding proteins in the mussel, *Mytilus edulis*. Environ. Health Perspect. **65**: 39-43.

Friberg, L., Piscator, M., Nordberg, G.G., et Kjellstrom, T. 1974. Cadmium in the environment. CRC Press, Cleveland, OH, USA.

Gagnon, C. et Fisher, N.S. 1997. The bioavailability of sediment-bound Cd, Co, and Ag to the mussel *Mytilus edulis*. Can. J. Fish. Aquat. Sci. **54**: 147-156.

George, S.G. et Langston, W.J. 1994. Metallothioneins as an indicator of water quality - Assessment of the bioavailability of cadmium, copper, mercury and zinc in aquatic animals at the cellular level. *In* Water Quality and Stress Indicators in Marine and Freshwater Ecosystems: Linking Levels of Organisation (Individuals, Populations, Communities). *Edited by* D.W. Sutcliffe. Freshwater Biological Association, Cumbria, UK, pp. 138-153.

Giguère, A., Campbell, P.G.C., Hare, L., et Couture, P. 2006. Sub-cellular partitioning of cadmium, copper, nickel and zinc in indigenous yellow perch (*Perca flavescens*) sampled along a polymetallic gradient. Aquat. Toxicol. **77**: 178-189.

Giguère, A., Couillard, Y., Campbell, P.G.C., Perceval, O., Hare, L., Pinel-Alloul, B., et Pellerin, J. 2003. Steady-state distribution of metals among metallothionein and other cytosolic ligands and links to cytotoxicity in bivalves living along a polymetallic gradient. Aquat. Toxicol. **64**: 185-200.

Gorsuch, J.W., Janssen, C.R., Lee, C.M., et Reiley, M.C. 2002. Special Issue: The Biotic Ligand Model for Metals - Current Research, Future Directions, Regulatory Implications. Comp. Biochem. Physiol. **133C**: 1-343.

Gosling, E. 2003. Circulation, respiration, excretion and osmoregulation. Wiley-Blackwell, Oxford, UK.

Graney, R.L., Cherry, D.S., et Cairns, J. 1984. The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. Wat. Res. 833-842.

Green, R.H. 1980. Role of a unionid clam population in the calcium budget of a small arctic lake. Can. J. Fish. Aquat. Sci. **37**: 219-224.

Griffin, J.J., Koide, M., Hodge, V., et Goldberg, E.D. 1980. Estimating the ages of mussels by chemical and physical methods. *In* Isotope Marine Chemistry. *Edited by* E.D. Goldberg, Y.Horide, and K.Saruhashi. Uchida Roka-Kuho Publ. Co., Tokyo, Japan, pp. 193-209.

Griscom, S.B., Fisher, N.S., Aller, R.C., et Lee, B.G. 2002. Effects of gut chemistry in marine bivalves on the assimilation of metals from ingested sediment particles. J. Mar. Res. **60**: 101-120.

Griscom, S.B., Fisher, N.S., et Luoma, S.N. 2000. Geochemical influences on assimilation of sediment-bound metals in clams and mussels. Environ. Sci. Technol. **34**: 91-99.

Hare, L. et Tessier, A. 1998. The aquatic insect *Chaoborus* as a biomonitor of trace metals in lakes. Limnol. Oceanogr. **43**: 1850-1859.

Harvey, R.W. et Luoma, S.N. 1985. Separation of solute and particulate vectors of heavy metal uptake in controlled suspension-feeding experiments with *Macoma balthica*. Hydrobiol. **121**: 97-102.

Hassler, C.S., Slaveykova, V.I., et Wilkinson, K.J. 2004. Some fundamental (and often overlooked) considerations underlying the free ion activity model and the biotic ligand models. Environ. Toxicol. Chem. **23**: 283-291.

Hayton, W.L. et Barron, M.G. 1990. Rate-limiting barriers to xenobiotic uptake by the gill. Environ. Toxicol. Chem. **9**: 151-157.

Hemelraad, J. et Herwig, H.J. 1988. Cadmium kinetics in freshwater clams. IV. Histochemical localization of cadmium in *Anodonta cygnea* and *Anodonta anatina*, exposed to cadmium chloride. Arch. Environ. Contam. Toxicol. **17**: 333-343.

Hemelraad, J., Holwerda, D.A., et Zandee, D.I. 1986. Cadmium kinetics in freshwater clams I: Pattern of cadmium accumulation in *Anodonta cygnea*. Arch. Environ. Contam. Toxicol. 1-7.

Hemelraad, J., Kleinveld, H.A., De Roos, A.M., Holwerda, D.A., et Zandee, D.I. 1987. Cadmium kinetics in freshwater clams. III. Effects of zinc on uptake and distribution of cadmium in *Anodonta cygnea*. Arch. Environ. Contam. Toxicol. **16**: 95-101.

Herreid, C.F. 1980. Hypoxia in invertebrates. Comp. Biochem. Physiol. **67A**: 311-320.

Hochachka, P.W. 1986. Defence strategies against hypoxia and hypothermia. Science. **231**: 234-241.

Hoffman, D.J. 1995. Handbook of ecotoxicology. Lewis Publishers, Boca Raton, FL, USA.

Holwerda, D.A., De Knecht, J.A., Hemelraad, J., et Veenhof, P.R. 1989. Cadmium kinetics in freshwater clams, uptake of cadmium by the excised gill of *Anodonta anatina*. Bull. Environ. Contam. Toxicol. **42**: 382-388.

Holwerda, D.A., Hemelraad, J., Veenhof, P.R., et Zandee, D.I. 1988. Cadmium accumulation and depuration in *Anodonta anatina* exposed to cadmium chloride or cadmium-EDTA complex. Bull. Environ. Contam. Toxicol. **40**: 373-380.

Hook, S.E. et Fisher, N.S. 2001. Sublethal effects of silver in zooplankton: Importance of exposure pathways and implications for toxicity testing. Environ. Toxicol. Chem. **20**: 568-574.

Hornbach, D.L., Way, C.M., Wissing, T.E., et Burky, A.J. 1984. Effects of particle concentration and season on the filtration rates of the freshwater clam, *Sphaerium striatinum* Lamark (Bivalvia: Pisidiidae). Hydrobiologia. **108**: 83-96.

Horne, A.J. et Goldman, C.R. 1994. Limnology. McGraw-Hill, Inc., New York, NY, USA.

Hoskin, W.M.A. 1991. Cadmium. *In* Canadian Minerals Year Book 1990. Energy Mines and Resources Canada, Ottawa, ON, Canada.

Hudson, R.J.M. 1998. Which aqueous species control the rates of trace metal uptake by aquatic biota? Observations and predictions of non-equilibrium effects. Sci. Total Environ. **219**: 95-115.

Huebner, J.D., Malley, D.F., et Donkersloot, K. 1990. Population ecology of the freshwater mussel *Anodonta grandis grandis* in a Precambrian Shield lake. Can. J. Zool. **68**: 1931-1941.

Hughes, G.M. et Flos, R. 1978. Zinc content of the gills of rainbow trout (*Salmo gairdneri*) after treatment with zinc solutions under normoxic and hypoxic conditions. J. Fish Biol. **13**: 717-728.

Hung, Y.-W. 1982. Effects of temperature and chelating agents on cadmium uptake in the American oyster. Bull. Environ. Contam. Toxicol. **28**: 546-551.

Inza, B., Ribeyre, F., MauryBrachet, R., et Boudou, A. 1997. Tissue distribution of inorganic mercury, methylmercury and cadmium in the Asiatic clam (*Corbicula fluminea*) in relation to the contamination levels of the water column and sediment. Chemosphere **35**: 2817-2836.

Jackim, E., Morrison, G., et Steele, R. 1977. Effects of environmental factors on radiocadmium uptake by four species of marine bivalves. Mar. Biol. **40**: 303-308.

Jansen, S., Blust, R., et van Leeuwen, H.P. 2002. Metal speciation dynamics and bioavailability: Zn(II) and Cd(II) uptake by mussel *(Mytilus edulis)* and carp *(Cyprinus carpio)*. Environ. Sci. Technol. **36**: 2164-2170.

Jansen, W.A. et Hanson, J.M. 1991. Estimates of the number of glochidia produced by clams (*Anodonta grandis simpsoniana*), attaching to yellow perch (*Perca flavescens*), and surviving to various ages in Narrow Lake, Alberta. Can. J. Zool. **69**: 973-977.

Jansen, W.A. 1991. Seasonal prevalence, intensity of infestation, and distribution of glochidia of *Anodonta grandis simpsoniana* Lea on yellow perch, *Perca flavescens*. Can. J. Zool. **69**: 964-972.

Jenny, M.J., Ringwood, A.H., Schey, K., Warr, G.W., et Chapman, R.W. 2004. Diversity of metallothioneins in the American oyster, *Crassostrea virginica*, revealed by transcriptomic and proteomic approaches. Environ. J. Biochem. **271**: 1702-1712.

Jernelov, A. 1996. The International Mussel Watch: a global assessment of environmental levels of chemical contaminants. Sci. Tot. Environ. **188**: S37-S44.

Jorgensen, C.B. 1975a. Comparative physiology of suspension feeding. Annu. Rev. Physiol. **37**: 57-79.

Jorgensen, C.B. 1975b. On gill function in the mussel *Mytilus edulis* L. Ophelia. **13**: 187-232.

Jorgensen, C.B. 1990. Bivalve filter feeding: hydrodynamics, bioenergetics, physiology and ecology. Olsen & Olsen, Denmark.

Kay, S.H. 1985. Cadmium in aquatic food webs. Residue Rev. **96**: 13-43.

Ke, C.H. et Wang, W.X. 2002. Trace metal ingestion and assimilation by the green mussel *Perna viridis* in a phytoplankton and sediment mixture. Mar. Biol. **140**: 327-335.

Kesseler, A. et Brand, M.D. 1994. Effects of cadmium on the control and internal regulation of oxidative phosphorylation in potato tuber mitochondria. Eur. J. Biochem. **225**: 907-922.

Kliza, D. et Telmer, K. 2001. Phase I: Lake sediment studies in the vicinity of the Horne smelter in Rouyn-Noranda, Québec. GSC Open File D2952. Geological Survey of Canada, Natural Resources Canada, Ottawa, ON, Canada.

Klumpp, D.W. et Burdon-Jones, C. 1982. Investigations of the potential of bivalve molluscs as indicators of heavy metal levels in tropical marine waters. Aust. J. Mar. Freshwater Res. **33**: 285-300.

Knauer, K., Ahner, B.A., Xue, H.-B., et Sigg, L. 1998. Metal and phytochelatin content in phytoplankton from freshwater lakes with different metal concentrations. Environ. Toxicol. Chem. **17**: 2444-2452.

Kraak, M.H.S., Lavy, D., Peeters, W.H.M., et Davids, C. 1992. Chronic ecotoxicity of copper and cadmium to the zebra mussel *Dreissena polymorpha*. Arch. Environ. Contam. Toxicol. **23**: 363-369.

Kraak, M.H.S., Schoon, H., Peeters, W.H.M., et Van Straalen, N.M. 1993. Chronic ecotoxicity of mixtures of Cu, Zn, and Cd to the zebra mussel *Dreissena polymorpha*. Ecotoxicol. Environ. Safety **25**: 315-327.

Kraemer, L.D., Campbell, P.G.C., et Hare, L. 2008. Modeling Cd accumulation in indigenous yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. **65**: 1623-1634.

Kraemer, L.D., Campbell, P.G.C., Hare, L., et Auclair, J.C. 2006. A field study examining the relative importance of food and water as sources of cadmium for juvenile yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. **63**: 549-557.

Landrum, P.F., Lee, H., et Lydy, M.J. 1992. Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ. Toxicol. Chem. **11**: 1709-1725.

Langston, W.J. et Bebianno, M.J. 1990. Environmental change - Biological response; A comparative study of metallothionein induction and cadmium-binding characteristics in molluscs. J. Mar. Biol. Assoc. U. K. **70**: 657-677.

Langston, W.J., Bebianno, M.J., et Burt, G.R. 1998. Metal handling strategies in molluscs. *In* Metal metabolism in aquatic environments. *Edited by* W.J. Langston and M.J.Bebianno. Chapman & Hall, London, UK, pp. 219-283.

Langston, W.J. et Zhou, M. 1987. Cadmium accumulation, distribution and elimination in the bivalve *Macoma balthica*: neither metallothionein nor metallothionein-like proteins are involved. Mar. Environ. Res. **21**: 225-237.

Lares, M.L. et Orians, K.L. 2001. Differences in Cd elimination from *Mytilus californianus* and *Mytilus trossulus* soft tissues. Environ. Pollut. **112**: 201-207.

Lavergne, C. 1985. Gîtes minéraux à tonnage évalué et production minérale du Québec. Gouvernement du Québec, Ministère de l'Énergie et des Ressources No. Rapport no. DV 85-08, Québec, QC, Canada.

Lavoie, M., Le Faucheur, S., Fortin, C., et Campbell, P.G.C. 2009. Cadmium detoxification strategies in two phytoplankton species: Metal binding by newly synthesized thiolated peptides and metal sequestration in granules. Aquat. Toxicol. **92**: 65-75.

Lee, B.-G. et Luoma, S.N. 1998. Influence of microalgal biomass on absorption efficiency of Cd, Cr, and Zn by two bivalves from San Francisco Bay. Limnol. Oceanogr. **43**: 1455-1466.

Lee, J.G., Roberts, S.B., et Morel, F.M.M. 1995. Cadmium: a nutrient for the marine diatom *Thalassiosira weissflogii*. Limnol. Oceanogr. **40**: 1056-1063.

Lei, J., Payne, B.S., et Wang, S.Y. 1996. Filtration dynamics of the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. **53**: 29-37.

Lewandowski, K. et Stanczykowska, A. 1975. The occurrence and role of bivalves of the family Unionidae in Mikolajskie Lake. Ekol. Pol. **23**: 317-334.

Lewis, J.B. 1985. Breeding cycle of the freshwater mussel *Anodonta grandis* Say. Can. J. Zool. **63**: 2477-2478.

Lloyd, R. et Herbert, D.W.M. 1962. The effect of the environment on the toxicity of poisons to fish. Inst. Publ. Health Eng. J. **61**: 132-145.

Lobel, P.B., Bajdik, C.D., Belkhode, S.P., Jackson, S.E., et Longerich, H.P. 1991. Improved protocol for collecting mussel watch specimens taking into account sex, size, condition, shell shape and chronological age. Arch. Environ. Contam. Toxicol. **21**: 409-414.

Lobel, P.B., Belkhode, S.P., Jackson, S.E., et Longerich, H.P. 1989. A universal method for quantifying and comparing the residual variability of element concentrations in biological tissues using 25 elements in the mussel *Mytilus edulis* as a model. Mar. Biol. **102**: 513-518.

Lum, K.R. 1987. Cadmium in freshwaters: the Great Lakes and St-Lawrence river. *In* Cadmium in the aquatic environment. *Edited by* J.O. Nriagu and J.B.Sprague. John Wiley & Sons, Inc., New York, NY, USA, pp. 35-50.

Luoma, S.N. 1989. Can we determine the biological availability of sediment-bound trace elements? Hydrobiol. **176**: 379-396.

Luoma, S.N., Johns, C., Fisher, N.S., Steinberg, N.A., et Oremland, R.S. 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. Environ. Sci. Technol. **26**: 485-491.

Luoma, S.N. et Rainbow, P.S. 2008. Trace metal bioaccumulation. *In* Metal contamination in aquatic environments: science and lateral management. Cambridge University Press, Cambridge, UK, pp. 126-168.

MacGinitie, G.E. 1941. On the method of feeding of four pelecypods. Bull. Mar. Biol. **80**: 18-25.

Mackey, D.J. 1983. Metal-organic complexes in seawater - an investigation of naturally occurring complexes of Cu, Zn, Fe, Mg, Ni, Cr, Mn and Cd using high performance liquid chromatography with atomic fluorescence detection. Mar. Chem. **16**: 169-180.

Mahler, B.J., Van Metre, P.C., et Callender, E. 2006. Trends in metals in urban and reference lake sediments across the United States, 1970 to 2001. Environ. Toxicol. Chem. **25**: 1698-1709.

Malley, D.F., Klaverkamp, J.F., Brown, S.B., et Chang, P.S.S. 1993. Increase in metallothionein in freshwater mussels *Anodonta grandis grandis* exposed to cadmium in the laboratory and the field. Wat. Pollut. Res. J. Can. **28**: 253-273.

Maloney, F. 2007. Influence de paramètres physico-chemiques (dureté, pH et présence d'un agent chelateur, l'EDTA) sur la réponse toxique au cadmium de l'algue verte d'eau douce, *Pseudokirchneriella subcapitata*. M.Sc. thesis, Université du Québec, INRS, INRS-ETE, 118p.

Marigomez, I., Soto, M., Cajaraville, M.P., Angula, E., et Giamberini, L. 2002. Cellular and subcellular distribution of metals in molluscs. Microsc. Res. Tech. **56**: 358-392.

Marina, G.L. et Enzo, O. 1983. Variability of zinc and manganese concentrations in relation to sex and season in the bivalve *Donax trunculus*. Mar. Pollut. Bull. **14**: 342-346.

Markich, S.J. et Jeffree, R.A. 1994. Absorption of divalent trace metals as analogues of calcium by Australian freshwater bivalves: an explanation of how water hardness reduces metal toxicity. Aquat. Toxicol. **29**: 257-290.

Martell, A.E. et Smith, R.M. NIST Critically Selected Stability Constants of Metal Complexes v. 8.0. NIST Standard Reference Data Base 46. 2004. Gaithersburg, MD, USA, National Institute of Standards and Technology.
Ref Type: Computer Program

Mason, A.Z. et Jenkins, K.D. 1995. Metal detoxification in aquatic organisms. *In* Metal Speciation and Bioavailability in Aquatic Systems. *Edited by* A. Tessier and D.Turner. J. Wiley & Sons, Chichester, UK, pp. 479-608.

Mayer, L.M., Chen, Z., Findlay, R.H., Fang, J., Sampson, S., Self, R.F.L., Jumars, P.A., Quetel, C., et Donard, O.F.X. 1996. Bioavailability of sedimentary contaminants subject to deposit- feeder digestion. Environ. Sci. Technol. **30**: 2641-2645.

Mcleese, D.W. et Ray, S. 1984. Uptake and excretion of cadmium, CdEDTA and zinc by *Macoma balthica*. Bull. Environ. Contam. Toxicol. **32**: 85-92.

McLusky, D.S., Bryant, V., et Campbell, R. 1986. The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. Oceanogr. Biol. Rev. **24**: 481-520.

Metcalfe-Smith, J.L. 1994. Influence of species and sex on metal residues in freshwater mussels (family Unionidae) from the St. Lawrence river, with implications for biomonitoring programs. Environ. Toxicol. Chem. **13**: 1433-1443.

Metcalfe-Smith, J.L., Green, R.H., et Grapentine, L.C. 1996. Influence of biological factors on concentrations of metals in the tissues of freshwater mussels (*Elliptio complanata* and *Lampsilis radiata radiata*) from the St. Lawrence River. Can. J. Fish. Aquat. Sci. **53**: 205-219.

Michaud, A. 2005. Répartition subcellulaire de métaux (Cd, Cu, Zn) chez des bivalves *Pyganodon grandis* transplantés (récolte 0ctobre 2004). Université du Québec, INRS, INRS-ETE No. Rapport interne, Québec, QC, Canada.

Millero, F.J. et Hawke, D.J. 1992. Ionic interactions of divalent metals in natural waters. Mar. Chem. **40**: 19-48.

Mills, S.C. et Reynolds, J.D. 2002. Mussel ventilation rates as a proximate cue for host selection by bitterling, *Rhodeus sericeus*. Oecologia **131**: 473-478.

Morel, F.M.M. 1983. Principles and Applications of Aquatic Chemistry. J. Wiley & Sons Ltd., New York, NY, USA.

Morel, F.M.M. et Hering, J.G. 1993. Principles and Applications of Aquatic Chemistry. J. Wiley & Sons Ltd., New York, NY, USA.

Munger, C. et Hare, L. 2000. Influence of ingestion rate and food types on cadmium accumulation by the aquatic insect *Chaoborus*. Can. J. Fish. Aquat. Sci. **57**: 327-332.

Munger, C., Hare, L., et Tessier, A. 1999. Cadmium sources and exchange rates for *Chaoborus* larvae in nature. Limnol. Oceanogr. **44**: 1763-1771.

Nelson, W.O. et Campbell, P.G.C. 1991. The effects of acidification on the geochemistry of Al, Cd, Pb and Hg in freshwater environments: a literature review. Environ. Pollut. **71**: 91-130.

Ng, T.Y.T. et Wang, W.-X. 2005. Dynamics of metal subcellular distribution and its relationship with metal uptake in marine mussels. Environ. Toxicol. Chem. **24**: 2365-2372.

NRCC 1979. Effects of cadmium in the environment. National Research Council of Canada, Associate Committee on Scientific Criteria for Environmental Quality No. No. 16743, Ottawa, ON, Canada.

Nriagu, J.O. 1989. A global assessment of natural sources of atmospheric trace metals. Nature **338**: 47-49.

Nriagu, J.O. et Pacyna, J.M. 1988. Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature **333**: 134-139.

O'Connor, T.P. 2002. National distribution of chemical concentrations in mussels and oysters in the USA. Mar. Environ. Res. **53**: 117-143.

Olendrzynski, K., Anderberg, S., Bartnicki, J., Pacyna, J.M., et Stigliani, W. 1996. Atmospheric emissions and depositions of cadmium, lead, and zinc in Europe during the period 1955-1987. Environ. Rev. **4**: 300-320.

Olsen, S., Masson, S., Campbell, P.G.C., Martel, L., et Parent, L. 2004. Développement d'outils d'évaluation de sites récepteurs servant à la définition d'objectifs environnementaux de rejets dans le processus d'intégration de l'industrie minière au Programme de Réduction de Rejets Industriels (PRRI). COREM, Ste-Foy, Qc No. Rapport final - Synthèse, Projet R000013.

Pacyna, J.M., Scholtz, M.T., et Li, Y.-F.A. 1995. Global budget of trace metal sources. Environ. Rev. **3**: 145-159.

Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., Delos, C.G., Di Toro, D.M., Dwyer, R.L., Galvez, F., Gensemer, R.W., Goss, G.G., Hogstrand, C., Janssen, C.R., McGeer, J.C., Naddy, R.B., Playle, R.C., Santore, R.C., Schneider, U., Stubblefield, W.A., Wood, C.M., et Wu, K.B. 2002a. The biotic ligand model: a historical overview. Comp. Biochem. Physiol. **133C**: 3-35.

Paquin, P.R., Voltay, V., Winfield, R.P., Wu, K.B., Mathew, R., Santore, R.C., et Di Toro, D.M. 2002b. Extension of the biotic ligand model of acute toxicity to a physiologically-based model of survival time of rainbow trout (*Oncorhynchus mykiss*) exposed to silver. Comp. Biochem. Physiol. **133C**: 305-343.

Perceval, O., Couillard, Y., Pinel-Alloul, B., et Campbell, P.G.C. 2006. Linking changes in subcellular cadmium distribution to growth and mortality rates in transplanted freshwater bivalves (*Pyganodon grandis*). Aquat. Toxicol. **79**: 87-98.

Perceval, O., Couillard, Y., Pinel-Alloul, B., Giguère, A., et Campbell, P.G.C. 2004. Metal-induced stress in bivalves living along a gradient of Cd contamination: relating sub-cellular metal distribution to population-level responses. Aquat. Toxicol. **69**: 327-345.

Perceval, O., Pinel-Alloul, B., Méthot, G., Couillard, Y., Giguère, A., Campbell, P.G.C., et Hare, L. 2002. Cadmium accumulation and metallothionein synthesis in freshwater bivalves (*Pyganodon grandis*): relative influence of the metal exposure gradient versus limnological variability. Environ. Pollut. **118**: 5-17.

Pessatti, M.L., Resgalla J.R., Reis Fo., R.W., Kuehn, J., Salomao, L.C., et Fontana, J.D. 2002. Variability of filtration and food assimilation rates, respiratory activity and multixenobiotic resistance (MXR) mechanism in the mussel *Perna perna* under lead influence. Braz. J. Biol. **62**: 651-656.

Phillips, D.J.H. et Rainbow, P.S. 1989. Strategies of trace metal sequestration in aquatic organisms. Mar. Environ. Res. **28**: 207-210.

Phillips, D.J.H. et Rainbow, P.S. 1993. Biomonitoring of trace aquatic contaminants. Elsevier Applied Science Publishers, London, UK.

Phinney, J.T. et Bruland, K.W. 1994. Uptake of lipophilic organic Cu, Cd and Pb complexes in the coastal diatom, *Thalassiosira weissflogii*. Environ. Sci. Technol. **28**: 1781-1790.

Pilgaard, L., Malte, H., et Jensen, F.B. 1994. Physiological-effects and tissue accumulation of copper in freshwater rainbow trout (*Oncorhynchus mykiss*) under normoxic and hypoxic conditions. Aquat. Toxicol. **29**: 197-212.

Poldoski, J.E. 1979. Cadmium bio-accumulation assays - their relationship to various ionic equilibria in Lake Superior water. Environ. Sci. Technol. **13**: 701-706.

Price, E.E., Parkerton, T.F., et Dickson, K.L. 1990. Use of *Selenastrum capricornutum* and Microfeast as food for *Daphnia pulex*. Bull. Environ. Contam. Toxicol. **44**: 59-66.

Prokopovich, N.P. 1969. Deposition of clastic sediments by clams. J. Sediment Petrol. **39**: 891-901.

Purchon, R.D. 1971. Digestion in filter feeding bivalves - a new concept. Proc. Malac. Soc. Lond. **39**: 253-262.

Rainbow, P.S. 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. **120**: 497-507.

Ray, S., Mcleese, D.W., et Pezzack, D. 1980. Accumulation of cadmium by *Nereis virens*. Arch. Environ. Contam. Toxicol. 1-8.

Reimann, C. et Garrett, R.G. 2005. Geochemical background - concept and reality. Sci. Tot. Environ. **350**: 12-27.

Reinfelder, J.R. et Fisher, N.S. 1991. The assimilation of elements ingested by marine copepods. Science **251**: 794-796.

Reinfelder, J.R. et Fisher, N.S. 1994. The assimilation of elements ingested by marine planktonic bivalve larvae. Limnol. Oceanogr. **39**: 12-20.

Reinfelder, J.R., Fisher, N.S., Luoma, S.N., Nichols, J.W., et Wang, W.-X. 1998. Trace elements trophic transfer in aquatic organisms: a critique of the kinetic model approach. Sci. Total Environ. **219**: 117-135.

Reinfelder, J.R., Wang, W.-X., Luoma, S.N., et Fisher, N.S. 1997. Assimilation efficiencies and turnover rates of trace-elements in marine bivalves - a comparison of oysters, clams and mussels. Mar. Biol. **129**: 443-452.

Ritz, D.S., Swain, R., et Elliott, N.G. 1982. Use of the mussel *Mytilus edulis planulatus* (Lamark) in monitoring heavy metals in seawater. Aust. J. Mar. Freshwat. Res. **33**: 491-506.

Robinson, W.E. et Ryan, D.K. 1986. Metal interactions within kidney gill and digestive gland of hard clam. Arch. Environ. Contam. Toxicol. 23-30.

Roditi, H.A. et Fisher, N.S. 1999. Rates and route of trace element uptake in zebra mussels. Limnol. Oceanogr. **44**: 1730-1749.

Roditi, H.A., Fisher, N.S., et Sanudo-Wilhelmy, S.A. 2000a. Field testing a metal bioaccumulation model for zebra mussels. Environ. Sci. Technol. **34**: 2817-2825.

Roditi, H.A., Fisher, N.S., et Sanudo-Wilhelmy, S.A. 2000b. Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature **407**: 78-80.

Roesijadi, G. 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat. Toxicol. **22**: 81-114.

Roesijadi, G. 1996. Metallothionein and its role in toxic metal regulation. Comp. Biochem. Physiol. **113C**: 117-123.

Roesijadi, G. et Fellingham, G.W. 1987. Influence of Cu, Cd and Zn preexposure on Hg toxicity in the mussel *Mytilis edulis*. Can. J. Fish. Aquat. Sci. **44**: 680-684.

Roesijadi, G., Kielland, S., et Klerks, P.L. 1989. Purification and properties of novel molluscan metallothioneins. Arch. Biochem. Biophys. **273**: 403-413.

Roesijadi, G. et Klerks, P.L. 1989. Kinetic analysis of cadmium binding to metallothionein and other intracellular ligands in oyster gills. J. Exp. Zool. **251**: 1-12.

Roesijadi, G. et Robinson, W.E. 1994. Metal regulation in aquatic animals: mechanisms of uptake, accumulation, and release. *In* Aquatic toxicology. Molecular, biochemical, and cellular perspectives. *Edited by* D.C. Malins and G.K.Ostrander. Lewis Publishers, Boca Raton, FL, USA, pp. 387-420.

Roesijadi, G. et Unger, M.E. 1993. Cadmium intake in gills of the mollusc *Crassostrea virginica* and inhibition by calcium channel blockers. Aquat. Toxicol. **24**: 195-206.

Romeo, M. et Gnassia-Barelli, M. 1995. Metal distribution in different tissues and in subcellular fractions of the Mediterranean clam *Ruditapes decussatus* treated with cadmium, copper, or zinc. Comp. Biochem. Physiol. **111C**: 457-463.

Sanders, B.M. et Jenkins, K.D. 1984. Relationship between free cupric ion concentrations in sea water and copper metabolism and growth in crab larvae. Biol. Bull. **167**: 704-711.

Schlekat, C.E., Lee, B.-G., et Luoma, S.N. 2001. Dietary metals exposure and toxicity to aquatic organisms: implications for ecological risk assessment. *In* Coastal and estuarine risk assessment. *Edited by* M.C. Newman, M.H.Roberts, and R.C.Hale. Lewis Publishers, Boca Raton, FL, USA, pp. 151-188.

Serafim, A. et Bebianno, M.J. 2007. Kinetic model of cadmium accumulation and elimination and metallthionein response in *Ruditapes decussatus*. Environ. Toxicol. Chem. **26**: 960-969.

Shi, D. et Wang, W.-X. 2004a. Modification of trace metal accumulation in the green mussel *Perna viridis* by exposure to Ag, Cu, and Zn. Environ. Pollut. **132**: 265-277.

Shi, D. et Wang, W.-X. 2004b. Understanding the differences in Cd and Zn bioaccumulation and subcellular storage among different populations of marine clams. Environ. Sci. Technol. **38**: 449-456.

Shick, J.M., Gnaiger, E., Widdow, J., Bayne, B.L., et De Zwann, A. 1986. Activity and metabolism in the mussel *Mytilus edulis* L. during intertidal hypoxia and aerobic recovery. Physiol. Zool. **59**: 627-642.

Sigg, L. 1987. Surface chemical aspects of the distribution and fate of metal ions in lakes. *In* Aquatic Surface Chemistry. *Edited by* W. Stumm. J. Wiley & Sons, New York, NY, USA, pp. 319-349.

Sijm, D.T.H.M., Verberne, M.E., Part, P., et Opperhuizen, A. 1994. Experimentally determined blood and water-flow limitations for uptake of hydrophobic compounds using perfused gills of rainbow trout (*Oncorhynchus mykiss*) - allometric applications. Aquat. Toxicol. **30**: 325-341.

Silverman, H., McNeil, J.W., et Dietz, T.H. 1987. Interaction of trace metals Zn, Cd, and Mn, with Ca concretions in the gills of freshwater unionid mussels. Can. J. Zool. **65**: 828-832.

Silverman, H., Steffens, W.L., et Dietz, T.H. 1983. Calcium concretions in the gills of a freshwater mussel serve as a calcium reservoir during periods of hypoxia. J. Exp. Zool. **227**: 177-189.

Silverman, H., Steffens, W.L., et Dietz, T.H. 1985. Calcium from extracellular concretions in the gills of freshwater unionid mussels is mobilized during reproduction. J. Exp. Zool. **236**: 137-147.

Simkiss, K. 1981. Cellular discrimination processes in metal accumulating cells. J. Exp. Biol. **94**: 317-327.

Simkiss, K. 1989. Structural and analytical studies on metal ion-containing granules. *In* Biomineralization, Chemical and Biochemical Perspectives. *Edited by* S. Mann, R.J.Webb, and R.J.P.Williams. VCH Verlags-gesellschaft, Weinhein, Germany., pp. 427-460.

Simkiss, K. et Taylor, M.G. 1989. Metal fluxes across membranes of aquatic organisms. Rev. Aquat. Sci. **1**: 174-188.

Slaveykova, V.I. et Wilkinson, K.J. 2005. Predicting the bioavailability of metals and metal complexes: Critical review of the Biotic Ligand Model. Environ. Chem. **2**: 9-24.

Smith, A.L., Green, R.H., et Lutz, A. 1975. Uptake of mercury by freshwater clams (family unionidae). J. Fish. Res. Board Can. **32**: 1297-1303.

Sobral, P. et Widdows, J. 1997. Effects of copper exposure on the scope for growth of the clam *Ruditapes decussatus* from southern Portugal. Mar. Pollut. Bull. **34**: 992-1000.

Sokolova, I.M. 2004. Cadmium effects on mitochondrial function are enhanced by elevated temperatures in a marine poikilotherm, *Crassostrea virginica* Gmelin (Bivalvis: Ostreidae). J. Exp. Biol **207**: 2639-2648.

Sokolova, I.M., Ringwood, A.H., et Johnson, C. 2005a. Tissue-specific accumulation of cadmium in subcellular compartments of eastern oyters *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). Aquat. Toxicol. **74**: 218-228.

Sokolova, I.M., Sokolov, E.P., et Ponnappa, K. 2005b. Cadmium exposure affects mitochondrial bioenergetics and gene expression of key mitochondrial proteins in the eastern oyster *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). Aquat. Toxicol. **73**: 242-255.

Southgate, T., Slinn, D.J., et Eastman, J.F. 1983. Mine-derived metal pollution in the Isle of Man. Mar. Pollut. Bull. **14**: 137-140.

Stephenson, M. et Mackie, G.L. 1988. Total cadmium concentrations in the water and littoral sediments of central Ontario Lakes. Wat. Air Soil Pollut. **38**: 121-136.

Stewart, A.R. 1999. Accumulation of Cd by a freshwater mussel (*Pyganodon grandis*) is reduced in the presence of Cu, Zn, Pb, and Ni. Can. J. Fish. Aquat. Sci. **56**: 467-478.

Stuijfzand, S.C., Jonker, M.J., Van Ammelrooy, E., et Admiraal, W. 1999. Species-specific responses to metals in organically enriched river water, with emphasis on effects of humic acids. Environ. Pollut. **106**: 115-121.

Stumm, W. et Morgan, J.J. 1996. Aquatic Chemistry. Chemical Equilibria and Rates in Natural Waters. John Wiley and Sons Inc., New York, NY, USA.

Sunda, W.G., Engel, D.W., et Thuotte, R.M. 1978. Effect of chemical speciation on toxicity of cadmium to grass shrimp, *Palaemonetes pugio:* importance of free cadmium ion. Environ. Sci. Technol. **12**: 409-413.

Tessier, A., Campbell, P.G.C., Auclair, J.-C., et Bisson, M. 1984. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata*. Can. J. Fish. Aquat. Sci. **41**: 1463-1472.

Tessier, A., Campbell, P.G.C., Auclair, J.-C., Schenck, R.C., Huizenga, D.L., et Dubreuil, B. 1987. Contribution à l'amélioration des critères scientifiques pour évaluer la signification biologique de métaux traces dans les sédiments. INRS-Eau, Rapport Scientifique, Environment Canada Report No. SB-43031745.

Tessier, A., Couillard, Y., Campbell, P.G.C., et Auclair, J.-C. 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. Limnol. Oceanogr. **38**: 1-17.

Thomann, R.V., Mahony, J.D., et Mueller, R. 1995. Steady-state model of biota sediment accumulation factor for metals in two marine bivalves. Environ. Toxicol. Chem. **14**: 1989-1998.

Tran, D., Boudou, A., et Massabuau, J.-C. 2003. Estimation of potential and limits of bivalve closure response to detect contaminants: Application to cadmium. Environ. Toxicol. Chem. **22**: 914-920.

Tran, D., Boudou, A., et Massabuau, J.-C. 2001. How water oxygenation level influences cadmium accumulation pattern in the Asiatic clam *Corbicula fluminea*: a laboratory and field study. Environ. Toxicol. Chem. **20**: 2073-2080.

Tran, D., Boudou, A., et Massabuau, J.-C. 2000. Mechanism for maintaining oxygen consumption under varying oxygenation levels in the freshwater clam *Corbicula fluminea*. Can. J. Zool. **78**: 2027-2036.

Tran, D., Boudou, A., et Massabuau, J.-C. 2002. Relationship between feeding-induced ventilatory activity and bioaccumulation of dissolved and algal-bound cadmium in the Asiatic clam *Corbicula fluminea*. Environ. Toxicol. Chem. **21**: 327-333.

Tran, D., Fournier, E., Durrieu, G., et Massabuau, J.C. 2007. Inorganic mercury detection by valve closure response in the freshwater clam *Corbicula fluminea:*

Integration of time and water metal concentration changes. Environ. Toxicol. Chem. **26**: 1545-1551.

Tremblay, Y. 2008. Détermination de la présence de thiosulfate en milieu naturel et évolution de son potentiel de stimulation du transport du Hg chez une algue verte. M.Sc. thesis, Université du Québec, INRS, INRS-ETE, 118p.

van Leeuwen, H.P. 1999. Metal speciation dynamics and bioavailability: inert and labile complexes. Environ. Sci. Technol. **33**: 3743-3748.

Vercauteren, K. et Blust, R. 1996. Bioavailability of dissolved zinc to the common mussel *Mytilus edulis* in complexing environments. Mar. Ecol. Progr. Ser. **137**: 123-132.

Vercauteren, K. et Blust, R. 1999. Uptake of cadmium and zinc by the mussel *Mytilus edulis* and inhibition by calcium channel and metabolic blockers. Mar. Biol. **135**: 615-626.

Viarengo, A. 1989. Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. CRC Crit. Rev. Aquat. Sci. **1#**: 295-317.

Viarengo, A., Pertica, M., Canesi, L., Mazzucotelli, A., Orunesu, M., et Bouquegneau, J.M. 1989. Purification and biochemical characterization of a lysosomal copper-rich thionein-like protein involved in metal detoxification in the digestive gland of mussels. Comp. Biochem. Physiol. **93C**: 389-395.

Vigneault, B. 2000. Interactions des substances humiques dissoutes avec les algues unicellulaires - mécanismes et implications. Ph.D. thesis, Université du Québec, INRS, INRS-ETE, 166p.

Voets, J., Bervoets, L., et Blust, R. 2004. Cadmium bioavailability and accumulation in the presence of humic acid to the zebra mussel, *Dreissena polymorpha*. Environ. Sci. Technol. **38**: 1003-1008.

Waitling, H.R. et Waitling, R.J. 1982. Comparative effects of metals on the filtering rate of brown mussel (*Perna perna*). Bull. Environ. Contam. Toxicol. **29**: 651-657.

Wallace, W.G., Lee, B.-G., et Luoma, S.N. 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar. Ecol. Progr. Ser. **249**: 183-197.

Wallace, W.G., Lopez, G.R., et Levinton, J.S. 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar. Ecol. Progr. Ser. **172**: 225-237.

Wang, D., Couillard, Y., Campbell, P.G.C., et Jolicoeur, P. 1999. Changes in subcellular metal partitioning in the gills of freshwater bivalves (*Pyganodon grandis*) living along an environmental cadmium gradient. Can. J. Fish. Aquat. Sci. **56**: 774-784.

Wang, W.-X. 2001. Comparison of metal uptake rate and absorption efficiency in marine bivalves. Environ. Toxicol. Chem. **20**: 1367-1373.

Wang, W.-X. et Fisher, N.S. 1996a. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Oceanogr. **41**: 197-207.

Wang, W.-X. et Fisher, N.S. 1996b. Assimilation of trace elements by the mussel *Mytilus edulis*: effects of diatom chemical composition. Mar. Biol. **125**: 715-724.

Wang, W.-X. et Fisher, N.S. 1997. Modeling metal bioavailability for marine mussels. Rev. Environ. Contam. Toxicol. **151**: 39-65.

Wang, W.-X., Fisher, N.S., et Luoma, S.N. 1995. Assimilation of trace elements ingested by the mussel *Mytilus edulis*: effects of algal food abundance. Mar. Ecol. Progr. Ser. **129**: 165-176.

Wang, W.-X., Fisher, N.S., et Luoma, S.N. 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Mar. Ecol. Progr. Ser. **140**: 91-113.

Wang, W.-X., Griscom, S.B., et Fisher, N.S. 1997. Bioavailability of Cr(III) and Cr(IV) to marine mussels from solute and particulate pathways. Environ. Sci. Technol. **31**: 603-611.

Wang, W.-X. et Widdows, J. 1993. Metabolic responses of the common mussel *Mytilus edulis* to hypoxia and anoxia. Mar. Ecol. Prog. Ser. **95**: 205-214.

Wang, W.-X. et Wong, R.C.K. 2003. Combined effects of food quantity and quality on Cd, Cr, and Zn assimilation to the green mussel, *Perna viridis*. J. Exp. Mar. Biol. Ecol. **290**: 49-69.

Way, C.M., Hornbach, D.J., Millller-Way, C.A., Payne, B.S., et Miller, A.C. 1990. Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia: Corbiculidae). Can. J. Zool. **68**: 115-120.

Widdows, J. 1985. Physiological measurements. *In* The effects of stress and pollution on marine animals. *Edited by* B.L. Bayne. Praeger, New York, NY, USA, pp. 161-179.

Widdows, J., Fieth, P., et Worrall, C.M. 1979. Relationship between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar. Biol. **50**: 195-207.

Widdows, J., Newell, R.I.E., et Mann, R. 1989. Effects of hypoxia and anoxia on survival, energy metabilism and feeding of oyster larvae (*Crassostera virginica*, Gmelin). Biol. Bull. **177**: 154-166.

Widmeyer, J.R. et Bendell-Young, L.I. 2007. Influence of food quality and salinity on dietary cadmium availability in *Mytilus trossulus*. Aquat. Toxicol. **81**: 144-151.

Widmeyer, J.R., Crozier, E.D., Moore, M.M., Jurgensen, A., et Bendell-Young, L.I. 2004. Role of *Leptothrix discophora* in mediating metal uptake in the filter-feeding bivalve *Mytilus trossulus*. Environ. Sci. Technol. **38**: 769-774.

Wilcock, R.J., Nagels, J.W., Mcbride, G.B., et Collier, K.J. 1998. Characteristics of lowland streams using a single-station diurnal curve analysis model with continuous monitoring data for dissolved oxygen and temperature. New Zealand J. Mar. Freshwat. Res. **32**: 67-79.

Wilson, D.J. et Chung, E. 2000. Bioturbation and oxidation of sulphide in sediments. J. Tenn. Acad. Sci. **75**: 76-85.

Wilson, D.N. Cadmium - market trends and influences. (Proceedings of the 6th International Cadmium Conference.London,Cadmium Association), 9-16. 1988.
Ref Type: Conference Proceeding

Wren, C.D., Harris, S., et Harttrup, N. 1995. Ecotoxicology of mercury and cadmium. *In* Handbook of ecotoxicology. Lewis Publishers, Boca Raton, FL, USA, pp. 392-423.

Wren, C.D., MacCrimmon, H.R., et Loescher, B.R. 1983. Examination of bioaccumulation and biomagnification of metals in a Precambrian Shield lake. Wat. Air Soil Pollut. **19**: 277-291.

Xue, H.B. et Sigg, L. 1998. Cadmium speciation and complexation by natural organic ligands in fresh water. Anal. Chim. Acta **363**: 249-259.

Yonge, C.M. 1949. On the structure and the adaptations of the Tellinacea, deposit-feeding Eulamellibranchia. Phil. Trans. Roy. Soc. Lond. **234**: 29-76.

Zamuda, C.D. et Sunda, W.G. 1982. Bioavailability of dissolved Cu to the American oyster *Crassostrea virginica*. I. Importance of chemical speciation. Mar. Biol. **66**: 77-82.

Zamuda, C.D., Wright, D.A., et Smucker, R.A. 1985. The importance of dissolved organic compounds in the accumulation of Cu by the American oyster. Mar. Environ. Res. **16**: 1-12.

APPENDIX A: BIVALVE MORTALITY AND CONDITION INDICES

Introduction

Keeping animals in captivity is sometimes necessary due to the difficulty of conducting ecotoxicological studies at the ecosystem level. However, to keep any wild creature in captivity for an extended period of time without any noticeable morbidity is a challenge. Proving that the task is not impossible, some researchers have successfully maintained wild fish and benthic organisms under laboratory conditions.

This appendix describes the methodology used to maintain *Pyganodon grandis*, a freshwater bivalve found in most Canadian Shield lakes and rivers, in a laboratory setting. Observations regarding bivalve behaviour in the laboratory and trends in mortality and condition will also be discussed.

Laboratory set-up and maintenance

Once in the laboratory, a stock culture was established in a cold room maintained at 15^{o}C (temperature of lake water at 4 m in Lake Opasatica depth during the summer months). Bivalves were placed in eight 55-L Rubbermaid plastic containers filled with 50-L of dechlorinated tap water and 5 cm of lake sediments. Bivalve density ranged from 30 to 45 bivalves per container. Each container was aerated with an air stone hooked up to an air pump. Fluorescent lamps and timers were placed in the cold room for a 12-h day/12-h night photoperiod.

Tap water, originating from the St-Lawrence River at Quebec City, was used for the stock culture. Water was dechlorinated by filling a 300-L reservoir and aerating the water for 6 days using a large air stone and air pump. Studies have shown that *P. grandis* prefers fine-grained sediment as opposed to a medium or coarse-grained sediment (Downing *et al*., 2000). Although a fine-grained sand or till would have sufficed for our

stock culture, we opted to use lake sediments from Lake Opasatica, believing that the bivalves would be more "comfortable" and less stressed in their sediments of origin.

At the beginning, bivalves were fed every two days with a 50/50 mixture of *Pseudokirchneriella subcapitata* (formerly named *Selenastrum capricornutum*) grown under laboratory conditions and a commercial food composed of marine algae (Phytoplex, Kent Marine). Later, bivalves were fed every day with double the food ration for reasons that will be discussed in the follow section. The Rubbermaid aquaria were cleaned once every week. This consisted of emptying the aquaria of half of their water, cleaning the sides to remove algae and feces, and slowly refilling the aquaria with fresh, clean, dechlorinated tap water. Pouring the water onto a piece of floating Styrofoam® avoided disturbing the sediments. It is important to regularly change the water to reduce the amount of ammonia produced by biological waste and uneaten food. Removing the water also facilitates the removal of any dead bivalves found in the aquarium.

It is important to remove any dead bivalves as soon as they are discovered, as their presence can lead to the mortality of other bivalves. A bivalve is dead when it is seen to be gaping (shell is wide open and it is obvious the bivalve is not filtering because the siphons are not visible). When removing a dead bivalve, **it is very important to tightly close the bivalve before lifting it from the aquarium**. If the bivalve is not closed, rotting tissues will fall into the aquarium. The aquarium will then have to be emptied and the mud sifted to remove decomposing bivalve parts.

Upon being added to their new environment, the bivalves will become very active. It is surprising how quickly they can move from one end of the aquarium to the other (50 cm in ~10 min). The bivalves will move around in the aquarium for at least a month and the water will remain very murky during this time. For this reason, the bivalves need an acclimation period of a month to allow them to settle into their new environment.

Trends in mortality

Between 250 and 350 bivalves were collected in May and October 2002 (Group 1); June 2003, May and October 2004, and May 2005 (Group 2); May and October 2006 (Group 3). No morbidity was noted in the first 2 months of captivity for bivalves collected in 2002 (Group 1). Mortality began after three months of captivity as 15% of the remaining bivalve population died during this period (Figure 1). The mortality steadily increased in the following months to reach ~50% after the 8th month of captivity (Figure 1).

Due to the high mortality rate observed for Group 1, food production was doubled in order to increase the amount of algae available to the bivalve. Bivalves were also fed every day instead of every second day. After these changes were implemented, bivalve mortality was significantly reduced, at least in the early months of captive, for Group 2 (bivalves collected between June 2003 and May 2005). Mortality occurred after 5 months of captivity and remained low (between 10% and 20%) for the following 4 months. The mortality eventually reached >60% after 10 months of captivity (Figure A1). However, some bivalves from this group lived in captivity beyond 16 months.

There are several other factors that may likely have contributed to this reduction in morbidity in Group 2. One reason is that Group 1 was the researcher's first attempt at keeping bivalves in captivity. Questions about proper maintenance of the stock culture were being worked out as the study progressed. An effective maintenance protocol had only been established a few weeks before the arrival of Group 2. As a result, bivalves in Group 2 were better fed and daily care of the stock culture was correctly maintained. Bivalve density in Group 3 was lower compared to the bivalve densities for Group 1, dropping from 45 to 30 bivalves per aquarium. This reduction in density, coupled with continuous removal of bivalves from the stock culture for experiments, may have reduced competition for food allowing for better survival.

Mortality for Group 3 (bivalves collected in 2006) was higher the previous 2 groups. Bivalve morbidity was observed within the first month of captivity and increased steadily with the following months (Figure A1). Bivalves did not survive beyond 7 months from this group. The consequence of these high mortality rates is that we were unable to conduct certain important laboratory tests and studies.

Reasons for this mortality were never clearly identified; however, it is believed that it may have been the results of the changes in the chemical properties of the tap water. An analysis of the tap water after the 7 days dechlorination period noted that there were still trace amounts of chlorine in the water. Bivalves are very sensitive to chlorine and its presence has been shown to be deleterious for bivalve health. Another possibility that may have led to an increase in bivalve mortality was food quality. Several batches of the commercial food, Phytoplex, were found to have rotted during this period and it is possible that the bivalves may have been poisoned due to the rotten food. This reduction in food quality coupled with the possible presence of chlorine in the water may have caused the increased mortality observed in Group 3.

Trends in bivalve condition

Bivalve condition was followed throughout the duration of captivity for all groups in order to observe if there was a decline in the overall fitness of the animals. Once a month, 10 bivalves were recovered from the stock culture, sacrificed, dried by lyophilisation and weighed to calculate the condition index (Couillard *et a*l. 1995a):

$$\text{C.I.} = \frac{\text{total dry wt of soft parts } (\text{g})}{\text{total dry wt of shell } (\text{g})}$$

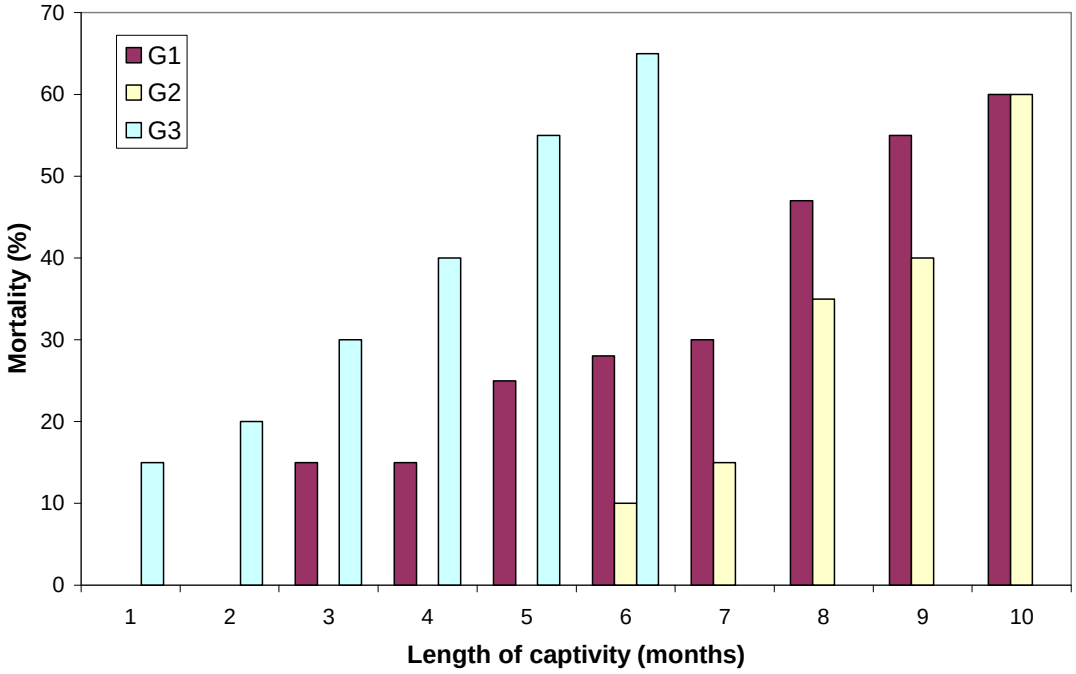


Figure A 1 : Mortality (%) observed in *Pyganodon grandis* kept in laboratory. / Mortalité (%) observée chez *Pyganodon grandis* durant leur captivité.

The average initial condition index for all Groups of bivalves was 0.120 ± 0.013 (S.E.). For Group 1, the index value dropped to 0.098 ± 0.005 (a 20% decline) after the first month of captivity. After seven months of captivity, the index value dropped an additional 35% to 0.067 (± 0.003) for an overall decrease of 55% in condition. The condition index remained fairly stable beyond seven months of captivity (Figure 2). For Group 2; the index value did not drop as dramatically as with Group 1 after the first month of captivity (0.110 ± 0.003 (S.E.)). The condition index remained stable at 0.090 (± 0.004) between months 2 and 5 of captivity. The index then dropped to 0.070 (± 0.004) after 8 months of captivity (Figure 2). Due to the high mortality rate, no bivalves from Group 3 were sacrificed in order to follow the trend in bivalve condition beyond the first month of captivity.

It is likely that nutrition and captivity played a role in the decline of bivalve fitness over time. The mixture of green algae and Phytoplex might be nutritionally inadequate and the bivalves' diet could be lacking important vitamins and minerals. Lastly, a change in habitat could stress the animals and thereby contibute to a decline in their fitness. It is possible that the animals also simply do not like being "caged". Couillard *et al*. (1995a) and Perceval *et al*. (2006) observed a decrease in bivalve condition in animals that were caged in their lake of origin.

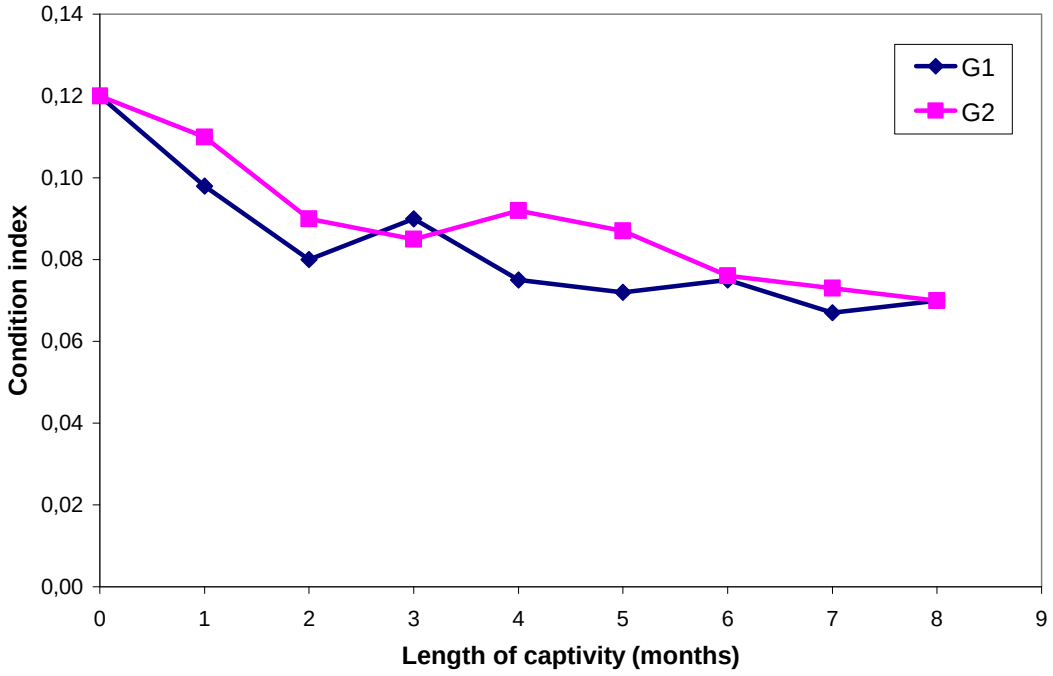


Figure A 2: Condition indices observed in captive bivalves. N = 10 for each month (CI = dry wt of soft tissue/ dry wt of shell). / Indices de condition observés chez les bivalves en captivité. N = 10 pour chaque mois (IC = poids sec des organes/poids sec de la coquille).

Because animal condition could affect filtration rates and, in turn, influence metal uptake by the bivalves, an ANOVA (Tukey, $p < 0.05$) was performed on the filtration rates measured in all of the bivalve groups that were used in our experiments (Figure A3). Group 1 corresponds to bivalves collected in May 2002, Group 2 to bivalves collected in October 2002, and Group 3 to bivalves collected in June 2003. The results show that a reduction in overall bivalve health, as shown by the decrease in condition, does not affect bivalve filtration rates, even after 10 months of captivity. However, the bivalves from Group 1 did have significantly higher filtration rates compared to the other groups. The researcher can not think of any reason (biological, seasonal or otherwise) why bivalves collected in May 2002 should be different from the other groups.

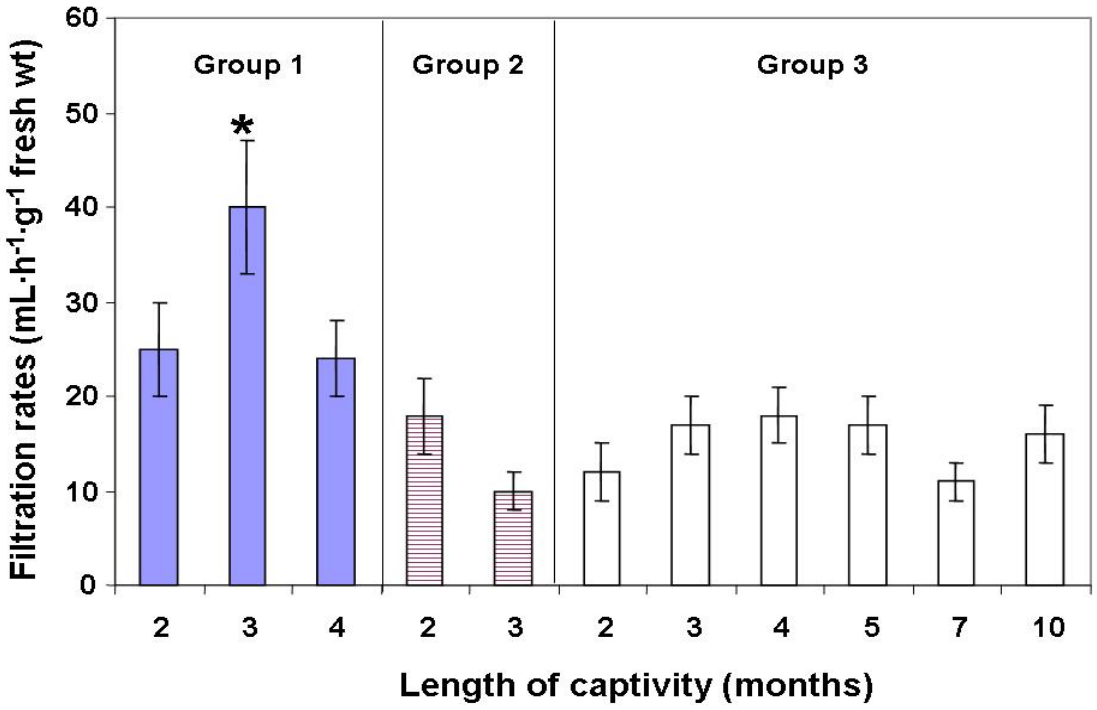


Figure A 3 : Filtration rates ($mL \cdot h^{-1} \cdot g^{-1}$ fresh wt ± SE) observed for bivalve groups used in our experiments. Group 1: May 2002, Group 2: October 2002, Group 3: June 2003. / Taux de filtration ($mL \cdot h^{-1} \cdot g^{-1}$ poids frais ± erreur type de la moyenne) observé chez les bivalves utilisés pour des expériences. Groupe 1 : mai 2002, Groupe 2 : octobre 2002, Groupe 3 : juin 2003.

References cited :

Couillard, Y., Campbell, P.G.C., Pellerin-Massicotte, J., et Auclair, J.-C. 1995. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. II. Metallothionein response to metal exposure, evidence for cytotoxicity, and links to effects at higher levels of biological organization. Can. J. Fish. Aquat. Sci. **52**: 703-715.

Perceval, O., Couillard, Y., Pinel-Alloul, B., et Campbell, P.G.C. 2006. Linking changes in subcellular cadmium distribution to growth and mortality rates in transplanted freshwater bivalves (*Pyganodon grandis*). Aquat. Toxicol. **79**: 87-98.

APPENDIX B: MEASURING BIVALVE FILTRATION RATES USING LATEX BEADS

Introduction

The freshwater bivalve *Pyganodon grandis* has been recognized as a potential sentinel organism to assess spatial and temporal trends in bioavailable Cd concentrations (Bonneris *et a*l. 2005b; Bonneris *et a*l. 2005a; Couillard *et a*l. 1995b; Couillard *et a*l. 1995a; Giguère *et a*l. 2003). However, no study has quantified the filtration or ingestion rates of this animal or the assimilation efficiency of Cd by *P. grandis*. Reasonably good estimates of these parameters are needed in order to predict Cd accumulation in *P. grandis* from both water and food using a kinetic model. More importantly, the filtration rate needs to be properly defined as this parameter will be used to estimate the ingestion rate and assimilation efficiencies.

Several studies have reported that metal accumulation in bivalves is influenced by the animal's filtration rate (Fournier *et a*l. 2005; Waitling and Waitling 1982; Tran *et a*l. 2002; Tran *et a*l. 2001), further illustrating the need to properly define this parameter. The results of the present study support these previous findings as filtration rates seemed to play an important role in dissolved Cd accumulation for *P. grandis* (see Section 5.4.1 of the thesis). In general, bivalves with the highest filtration rates accumulated higher concentrations of dissolved Cd in their gills. However, the filtration rates used to illustrate this relationship between bivalve filtration and $[Cd]_{gills}$ were measured during the 4 h feeding period and not during the 20 h exposure to aqueous Cd. Because bivalve filtration rates can vary greatly during a 24 h period (Section 4), it is possible that the bivalves were filtering at a different rate during the aqueous Cd exposure. The reason for this lack of data during the aqueous Cd exposure is that bivalve filtration rates were measured by determining the volume of water cleared of algae, in this case *Pseudokirchneriella subcapitata*, per unit of time. Using algae to measure filtration rates during the aqueous Cd exposure would have created a dietary source of Cd as *P.*

subcapitata would have accumulated Cd during the 20 h period. Therefore we needed to find a suspended material that would allow us to measure bivalve filtration rates without exposing the bivalves to any dietary Cd.

This short paper summarizes the results obtained from a series of tests that were conducted to observe whether bivalve filtration rates could be measured using labile latex beads (3 μm, polystyrene, Sigma-Aldrich). Our hope was that the latex beads would allow us to measure bivalve filtration rates during the aqueous Cd exposure without creating a dietary source of cadmium.

Preliminary test on latex beads

Test 1

Although the latex beads were positively charged, a test was conducted to ensure that Cd^{2+} would not stick onto their surface. Beads were suspended, using a magnetic stirrer, in a synthetic medium contaminated with radioactive $^{109}Cd^{2+}$ (5 nM) for 24 h at a concentration of 500,000 beads per mL. After 24 h, 3 x 10 mL samples of the medium were collected and filtered through two polycarbonate membranes (0.2 μm, Nuclepore). The first membrane served to collect the beads, whereas the second membrane was exposed only to dissolved ^{109}Cd and thus served as a filtration blank (accounting for any passive sorption of ^{109}Cd to the filter). The second filter was placed in a scintillation vial and the filtered exposure medium was also collected, both were analyzed in a Gamma Counter. The first filter was carefully removed and placed in a beaker containing 10 mL of synthetic medium. The beads were resuspended in the medium in order to properly rinse them. The rinse medium was filtered as before and each filter was placed in a separate scintillation vial, as well as the rinse medium, and analyzed in a Gamma counter.

Table B1 summarizes the results of this test and shows that rinsing the beads was unnecessary as most of the Cd^{2+} (95%) was associated with the filtered exposure medium. Any Cd found in the rinse medium may have been due to the difficulty in

filtering the exposure medium through a 0.2 µm filter. These results suggest that it is safe to use the latex beads to measure bivalve filtration rates during an aqueous Cd exposure without creating a source of dietary Cd.

Table B 1 : CPM (counts per minute) for radioactive Cd measured for filters, exposure and rinse medium, and latex beads. / CPM (comptes par minute) du Cd radioactive mesurés pour les filtres, les milieux d'exposition et de rinçage, et les billes en latex.

Sample (10 mL)	Medium			Filter		
	Initial	Exposure	Rinse	Blank 1	Blank 2	Beads
1	3138	2988	55	45	20	10
2	2893	2743	45	34	14	25
3	3009	2868	64	49	37	17
Mean	3013	2866	55	43	24	18

Test 2

One important assumption associated with measuring filtration rates is that the concentration of the suspended material in the water is homogeneous. During the experiments with *P. subcapitata*, the algae were suspended using air stones, which were successful at maintaining a homogeneous suspension of algae throughout the duration of an experiment (5 to 7 h). The following test was conducted to verify whether the air stones were capable of maintaining a homogeneous suspension of beads.

Beads were added to 4 beakers filled with 2 L of synthetic medium. The medium was stirred manually to create a homogeneous suspension after which the air stones were inserted into the beakers. A 1 mL sample of the medium was taken every hour during a 7 h period and bead density was measured using a Coulter counter. The beads were left suspended in the beakers overnight. A final sample was taken the next day to see whether bead density had decreased during the night. The results, shown in Figure B1, suggest that the air stones do not provide sufficient current to maintain a homogeneous

suspension. Bead density either dropped dramatically (30% from initial density) or was erratic during the 7 h period.

A second test, using magnetic stirrers, was conducted to see whether the stirrers would be better at maintaining a homogeneous suspension of beads then the air stones. As before, beads were added to 4 beakers filled with 2 L of synthetic medium. Knowing that future experiments would be conducted with bivalves sitting in a plastic cup (see Figure 3.1), each beaker contained a plastic cup that was raised on a platform, above the magnetic stirrer, allowing the stirrer to mix the water. A fake bivalve (empty shells glued together) was also added to see whether the presence of a bivalve would disrupt the current produced by the magnetic stirrer and reduce bead suspension. As with the first test, a 1 mL sample of the medium was taken every hour during a 7 h period to measure bead density, and a final sample taken after 24 h.

The results suggest that the magnetic stirrers were capable of maintaining a homogeneous suspension. Although there is an initial drop in bead density within the first hour, due to some of the beads sticking to the surface of the bivalve and plastic cup, bead density remains stable throughout the 7 h period (Figure B2). Bead density may drop after 24 h, although not as dramatically as was observed when the beads were suspended using the air stones.

Based on these results, it is advisable to use the magnetic stirrers to suspend and maintained a homogeneous bead suspension. The only drawback to this method is that only 3 or 4 bivalves can be studied at a time due to a lack of equipment (i.e., magnetic platforms) and space.

Measuring bivalve filtration rates using latex beads

Four bivalves were transferred from their acclimation beakers to a beaker placed on top of a magnetic platform. The experimental beaker was filled with synthetic medium and contained a magnetic stirrer. Once the stirrer was activated, latex beads were added to the medium and the system was allowed to stabilize for an hour. A 1 mL sample of the medium was taken every hour for 5 h and bead density was measured using a Coulter counter. Bivalve behaviour (e.g., open or closed valves) was also noted during the experiment. The experiment was repeated with four more bivalves given a total of eight bivalves (2 x 4 bivalves). After 3 h it was obvious that the bivalves did not filter the beads as bead density did not decrease. Moreover, the bivalves remained closed throughout the experiment.

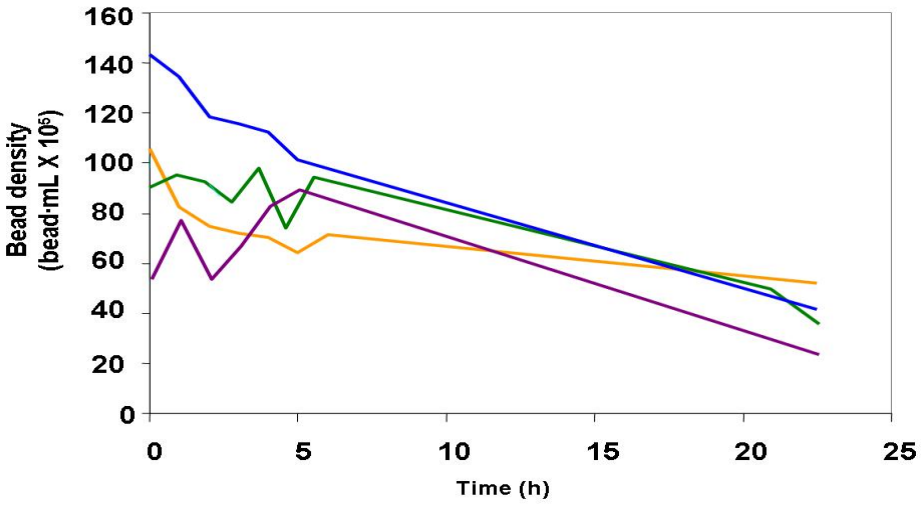


Figure B 1 : Patterns in bead concentrations observed when latex beads were suspended using air stones. / Changements de concentrations des billes observés lorsque les billes en latex étaient suspendues avec une pierre à air.

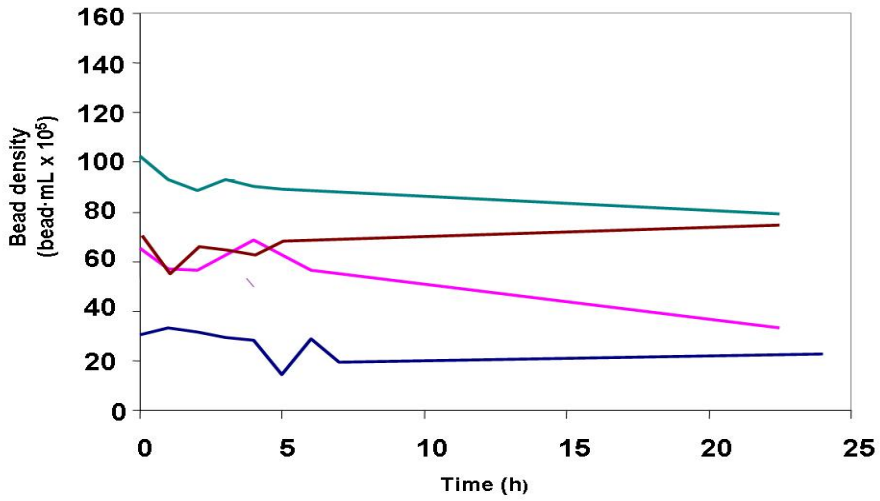


Figure B 2 : Patterns in bead concentrations observed when latex beads were suspended using a magnetic stirrer. / Changements de concentrations de billes observés lorsque les billes en latex étaient suspendues avec un agitateur magnétique.

A second experiment was conducted by exposing the bivalves to a suspended mixture of algae and beads. Four mixtures were prepared with the percentage of algae in the mixture ranging from 5 to 20% of the total suspended medium. The goal was to see which algae density was necessary to entice the bivalve to feed and filter. Six bivalves were exposed to each mixture regime (4 x 6 = 24 bivalves) for 4 h during 3 consecutive days following a similar experimental method described in Section 4.

All six bivalves exposed to the 5% algae – 95% bead mixture barely filtered the suspended medium as filtration rates were very low (4 ± 3 $mL \cdot h^{-1} \cdot g^{-1}$ fresh wt; mean ± SE) (Figure B3). Bivalve filtration rates increased to 12 ± 6 $mL \cdot h^{-1} \cdot g^{-1}$ (mean ± SE; fresh wt) when bivalves were exposed to the 10% algae – 90% bead mixture. However, this value is significantly lower than the 26 ± 4 $mL \cdot h^{-1} \cdot g^{-1}$ (mean ± SE; fresh wt; n = 9) observed when the bivalves were fed only algae. Bivalve filtration continued to increase as the concentration of algae increased in the suspended medium. Filtration rates were

18 ± 5 $mL \cdot h^{-1} \cdot g^{-1}$ (mean ± SE; fresh wt) for bivalves exposed to the 15% algae – 85% bead mixture and 23 ± 4 $mL \cdot h^{-1} \cdot g^{-1}$ (mean ± SE; fresh wt) for bivalves exposed to the 20% algae – 80% bead mixture (Figure B3).

The results from this study suggest that 20% of the suspended medium must be composed of algae for the bivalves to filter at a similar rate observed when the animals are fed only algae. This would allow us to measure bivalve filtration rates during an aqueous Cd exposure. However, the drawback to this method is that the bivalves will be exposed to a source of dietary Cd, albeit a small source. Nevertheless, the amount of ingested Cd could be estimated based on the amount of Cd accumulated by the algae during the 4-5 h experiment and the amount of algae consumed by the bivalve.

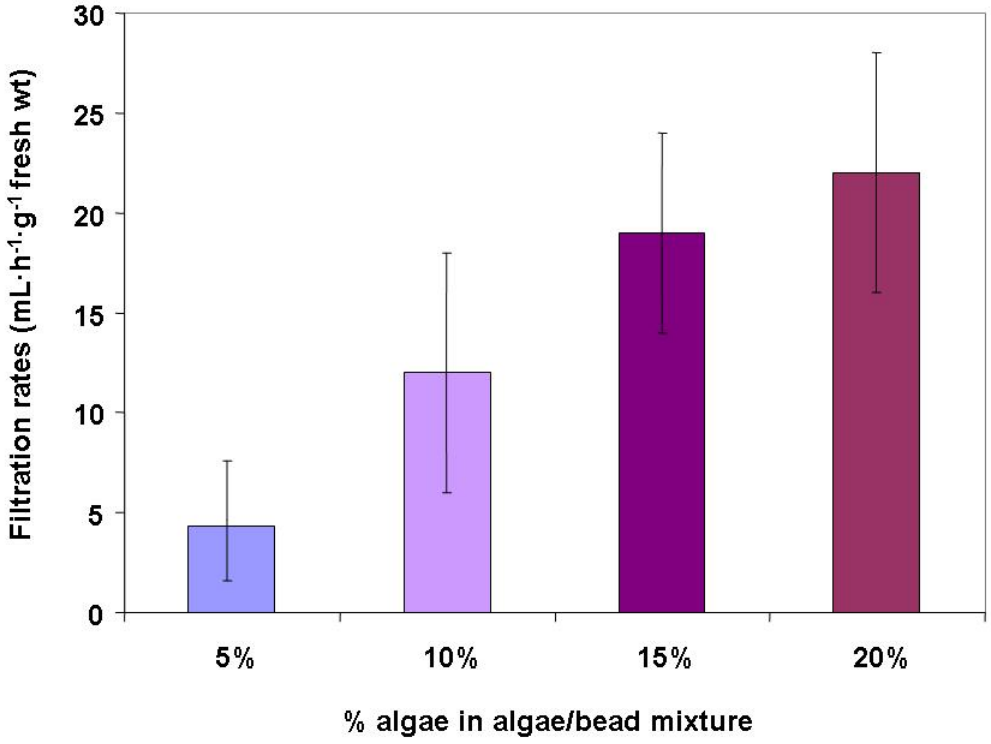


Figure B 3 : Mean filtration rates (mL·h^{-1}·g^{-1} fresh wt) measured for bivalves exposed to a suspended algae/bead mixture composed of 5, 10, 15 or 20% algae. N = 6 bivalves for each exposure group. / Taux de filtration moyens (mL·h^{-1}·g^{-1} poids frais) observés chez des bivalves exposés à un mélange de matières en suspension composé billes de latex et de 5, 10, 15 ou 20% d'algues vertes. N = 6 pour chaque groupe expérimental.

References cited :

Bonneris, E., Giguère, A., Perceval, O., Buronfosse, T., Masson, S., Hare, L., et Campbell, P.G.C. 2005a. Sub-cellular partitioning of metals (Cd, Cu, Zn) in the gills of a freshwater bivalve, *Pyganodon grandis*: role of calcium concretions in metal sequestration. Aquat. Toxicol. **71**: 319-334.

Bonneris, E., Perceval, O., Masson, S., Hare, L., et Campbell, P.G.C. 2005b. Sub-cellular partitioning of Cd, Cu and Zn in tissues of indigenous unionid bivalves living along a metal exposure gradient and links to metal-induced effects. Environ. Pollut. **135**: 195-208.

Couillard, Y., Campbell, P.G.C., Pellerin-Massicotte, J., et Auclair, J.-C. 1995a. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. II. Metallothionein response to metal exposure, evidence for cytotoxicity, and links to effects at higher levels of biological organization. Can. J. Fish. Aquat. Sci. **52**: 703-715.

Couillard, Y., Campbell, P.G.C., Tessier, A., Pellerin-Massicotte, J., et Auclair, J.-C. 1995b. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. I. Temporal changes of metallothionein and metal (Cd, Cu, Zn) concentrations in soft tissues. Can. J. Fish. Aquat. Sci. **52**: 690-702.

Fournier, E., Adam, C., Massabuau, J.C., et Garnier-Laplace, J. 2005. Bioaccumulation of waterborne selenium in the Asiatic clam *Corbicula fluminae*: influence of feeding-induced ventilatory activity and selenium species. Aquat. Toxicol. **72**: 251-260.

Giguère, A., Couillard, Y., Campbell, P.G.C., Perceval, O., Hare, L., Pinel-Alloul, B., et Pellerin, J. 2003. Steady-state distribution of metals among metallothionein and other cytosolic ligands and links to cytotoxicity in bivalves living along a polymetallic gradient. Aquat. Toxicol. **64**: 185-200.

Tran, D., Boudou, A., et Massabuau, J.-C. 2001. How water oxygenation level influences cadmium accumulation pattern in the Asiatic clam *Corbicula fluminea*: a laboratory and field study. Environ. Toxicol. Chem. **20**: 2073-2080.

Tran, D., Boudou, A., et Massabuau, J.-C. 2002. Relationship between feeding-induced ventilatory activity and bioaccumulation of dissolved and algal-bound cadmium in the Asiatic clam *Corbicula fluminea*. Environ. Toxicol. Chem. **21**: 327-333.

Waitling, H.R. et Waitling, R.J. 1982. Comparative effects of metals on the filtering rate of brown mussel (*Perna perna*). Bull. Environ. Contam. Toxicol. **29**: 651-657.

APPENDIX C: SIMULTANEOUS AQUEOUS CADMIUM AND MANGANESE EXPOSURES

The present study was conducted after results obtained by Olsen *et al.* (2004) showed an inverse relationship between the total Cd concentrations accumulated by *Pyganodon grandis* in the field (collected at sites in the Rivière Allard, near Matagami, QC) and the concentration of aqueous Mn in the ambient water. The goal of the present study was to verify whether dissolved Mn could interfere or reduce dissolved Cd uptake in *P. grandis*.

Bivalves were exposed simultaneously to a range of dissolved Mn concentrations (10, 30, 90, 150 and 300 $\mu g \cdot L^{-1}$) and a stable concentration of dissolved radioactive ^{109}Cd (0.05 $\mu g \cdot L^{-1}$ or 0.5 nM). Dissolved Mn concentrations were selected based on values observed for the Rivière Allard (from 2 to 80 $\mu g \cdot L^{-1}$). Seven 6 L aquaria (5 experimental + 2 controls) were filled with synthetic medium and contaminated with ^{109}Cd. The 5 experimental aquaria were contaminated with dissolved Mn whereas the control aquaria were only contaminated with ^{109}Cd. Three bivalves were placed inside each aquarium. The experimental protocol was similar to the one described in Section 5 of the thesis (bivalves remained in their exposure aquaria for 20 h, transferred into feeding beakers for 4 h, then returned to their aquaria for another 20 h period). In total, during the 96 h experiment, the bivalves were exposed to the contaminated medium for 80 h. Bivalves were sacrificed, dissected, rinsed with EDTA and their organs (gills, digestive gland, mantle, foot and miscellaneous tissues) were placed in separate scintillation vials. The organs were analyzed for ^{109}Cd using a Gamma counter.

Accumulated Cd concentrations measured in the gills of bivalves from this study were compared with those obtained for a reference group comprised of 9 bivalves that were exposed to 0.5 nM of dissolved Cd during an earlier experiment. No significant difference (Wilcoxon test, $p < 0.05$) was observed between the $[Cd]_{gills}$ for bivalves

exposed to Mn and Cd simultaneously and the $[Cd]_{gills}$ for reference and control bivalves (Figure C1).

Results obtained from the Rivière Allard suggest a decrease in the total Cd concentration in bivalves as the concentration of dissolved Mn increases in the water (Figure C2) (Olsen *et al*. 2004). This study conducted under laboratory condition does not support these findings as dissolved Mn was not shown to reduce $[Cd]_{gills}$ in bivalves (ANOVA, $p < 0.05$). However, animals in the field are chronically exposed to the conditions established in the laboratory and the duration of the experiment may not have been long enough to observe a relationship between dissolved Mn and $[Cd]_{gills}$. Moreover, in a natural habitat, bivalves are exposed to both aqueous and dietary source of metals. It is possible that a simultaneous exposure to both aqueous and dietary Cd and Mn might have shown a competition between the two metals.

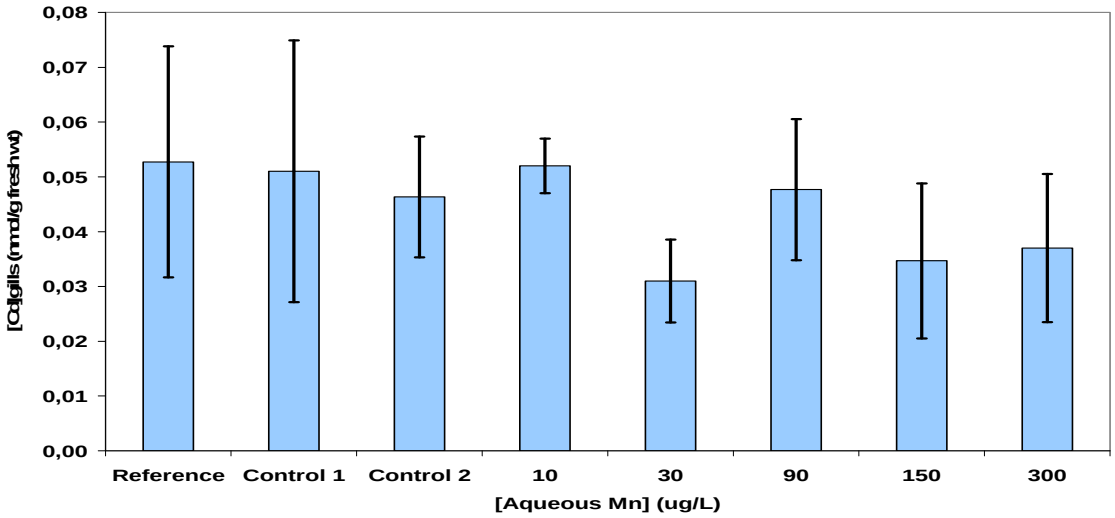


Figure C 1 : Cadmium concentrations observed in the gills of bivalves exposed simultaneously to dissolved Cd (0.5 nM) and a range in dissolved Mn concentrations. Bivalves in the reference and control groups were not exposed to dissolved Mn. N = 9 for the reference group and n =3 for the other groups. / Concentrations de Cd branchiale observées chez les bivalves exposés simultanément à 0,5 nM de Cd dissous et une gamme de concentrations de Mn dissous. Les bivalves dans les groupes référence et témoins n'ont pas été exposés au Mn dissous. N = 9 pour le groupe référence et n = 3 pour les autres groupes.

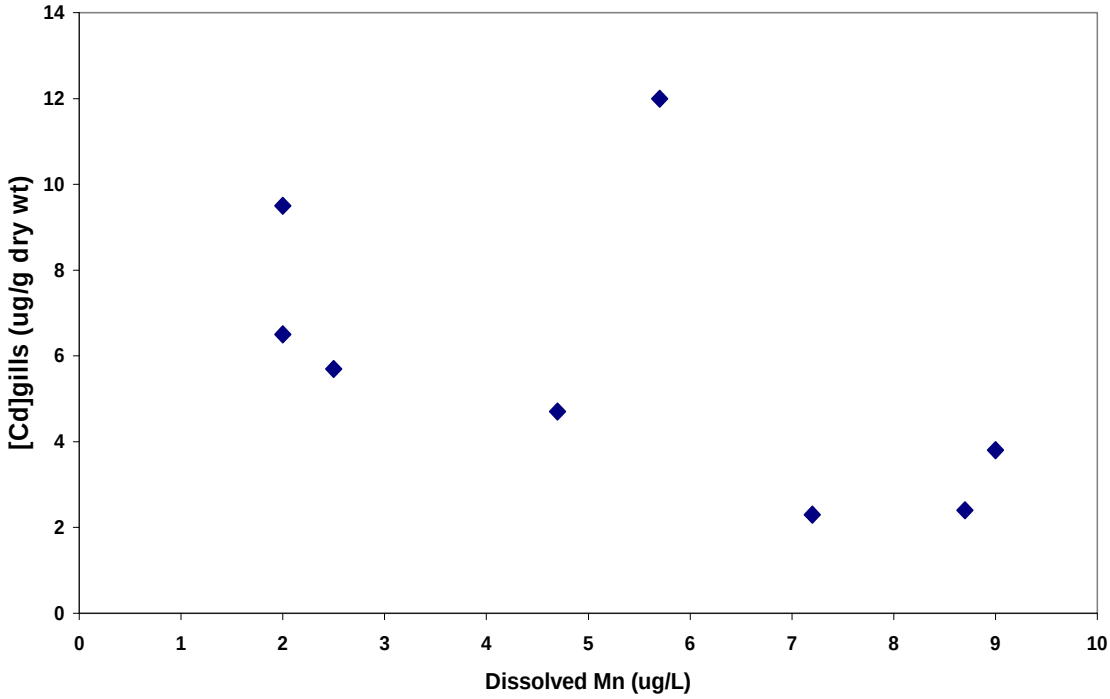


Figure C 2 : Relationship between [Cd]gills (µg·g^{-1} dry wt) and dissolved Mn observed in *P. grandis* collected from the Rivière Allard (S. Masson, unpublished data). / Relation entre [Cd]branchies (µg·g^{-1} dry wt) et le manganèse dissous chez *P. grandis* recueilli dans la rivière Allard (S. Masson, données non publiées).

Reference cited :

Olsen, S., Masson, S., Campbell, P.G.C., Martel, L., et Parent, L. 2004. Développement d'outils d'évaluation de sites récepteurs servant à la définition d'objectifs environnementaux de rejets dans le processus d'intégration de l'industrie minière au Programme de Réduction de Rejets Industriels (PRRI). COREM, Ste-Foy, QC No. Rapport final - Synthèse, Projet R000013.