CELLULAR STRESS RESPONSES TO CADMIUM CONTAMINATION AS MEASURE OF SENSITIVITY IN INTERTIDAL MOLLUSCAN SPECIES

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

IN ZOOLOGY

FACULTY OF SCIENCE

UNIVERSITY OF STELLENBOSCH

MARCH 2007

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DECLARATION

I, the undersigned hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

Signature:…….. **Date**:…….

Acknowledgements

Hereby I wish to thank the following people and institutions that made this study possible.

- My promotors, Prof. A.J. Reinecke & Prof. S.A. Reinecke for their guidance and support.
- Special thanks to Prof. A.J. Reinecke for his input on species selection criterion and the neutral red retention method used.
- Mr. Patrick Beneke for his assistance in field work.
- My fellow postgraduate students at the Ecotoxicology Laboratory of the Department of Botany & Zoology, University of Stellenbosch.
- The NRF for finacial assistance.
- My fiance for her support and encouragement.

Abstract

The ability of various molluscan species to accumulate toxicants such as cadmium from natural waters in quantities that are many orders of magnitude higher than background levels are well-known. This phenomenon of bioaccumulation might cause certain stress responses in these organisms at the cellular level, which can be measured using biomarkers. A biomarker response test known as the neutral red retention assay was employed in this study to measure responses in four intertidal species. Specimens of *Cymbula oculus* (Born), *Scutellastra longicosta* (Lamarck), *Cymbula granatina* (Linnaeus) and *Scutellastra granularis* (Linnaeus) were collected at two localities on the coast of False Bay, South Africa. Laboratory exposures in static flow tanks at three different concentrations i.e. 0.8, 1 and 1.2 mg/L of $CdCl_2$ were done respectively for each species over a three day exposure period i.e. each exposure concentration had an exposure period of 24, 48 and 72 hours. After every 24 hour exposure period the lysosomal membrane integrity was determined using the neutral red retention method to establish which species is the most sensitive to Cd. Both control and exposure groups for all species showed a decrease in retention times with an increase in Cd concentration over the exposure period. This decrease was particularly prominent at the highest exposure concentration after 72 hours. At 0.8 and 1.2 mg/L $CdCl_2$ exposures an indirectly proportional relationship between neutral red retention time and heavy metal concentration was prominent in *C. oculus*, indicating a dose related response. In all species there was a moderate increase in heavy metal concentration over the 72 hour exposure period. EC_{50} values indicated that *S. granularis* and *C. granatina* had a “high” sensitivity to Cd contamination, while *C. oculus* had “medium” sensitivity and *S. longicosta* “low” sensitivity to Cd contamination. The sensitivity data obtained from the analysis of the experimental species in this study may contribute to the eventual establishment of a species sensitivity distribution model (SSD).

Opsomming

Die vermoë van verskeie molluske spesies om toksikante soos kadmium te akkumuleer vanuit natuurlike waterbronne in kwantiteite wat verskeie ordegroottes hoër is as agtergrond vlakke is wel bekend. Die verskynsel van bioakkumulasie mag sekere stresresponse in hierdie organismes op ‘n sellulêre vlak veroorsaak wat deur middel van biomerkers gemeet kan word. ‘n Biomerker responstoets wat bekend staan as die neutraal rooi retensie toets is in hierdie studie gebruik om die response van vier tussengety spesies te bepaal. Monsters van *Cymbula oculus* (*Born*), *Scutellastra longicosta* (*Lamarck*), *Cymbula granatina (Linnaeus)* en *Scutellastra granularis (Linnaeus)* is versamel by twee lokaliteite aan die kus van Valsbaai, Suid Afrika. Laboratorium blootstellings in statiese vlooi tenke by drie verskillende konsentrasies i.e. 0.8, 1 en 1.2 mg/L van $CdCl_2$ was afsonderlik gedoen vir elke spesie oor ‘n drie dag blootstellings periode i.e. elke blootstellings konsentrasie het ‘n blootstellings tydperk van 24, 48 en 72 uur. Na elke 24 uur blootstellings periode was die lisosoom membraanintegriteit bepaal deur van die neutraal rooi retensie metode gebruik te maak om vas te stel watter spesie die mees sensitiefste is vir Cd. Beide die kontrole- en blootstellingsgroepe vir al die spesies het ‘n afname in retensie tyd getoon met ‘n toename in Cd konsentrasie oor die blootstellingsperiode. Hierdie afname was veral prominent by die hoogste blootstellingskonsentrasie na 72 uur. ‘n Omgekeerd eweredige verwantskap tussen neutraal rooi retensietyd en swaar-metaal konsentrasie in *C. oculus* by die 0.8 en 1.2 mg/L $CdCl_2$ blootstellings was prominent en dui op ‘n dosisverwante respons. Daar was ‘n matigde verhoging in swaarmetaal konsentrasie oor die 72 uur blootstellingstydperk in al die spesies. EC_{50} waardes het aangedui dat *S. granularis* en *C. granatina* ‘n “hoë” sensitiwiteit tot Cd kontaminasie het terwyl *C. oculus* ‘n “medium” sensitiwiteit en *S. longicosta* ‘n “lae” sensitiwiteit vir Cd kontaminasie het. Die sensitiwiteitsdata wat verkry is vanaf die analise van die eksperimentele spesies in hierdie studie beoog om ‘n bydrae te maak tot die uiteindelike konstruksie van ‘n spesie-sensitiwiteits verspreidingsmodel (SSV).

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1. Introduction

1.1 General introduction

Heavy metals have been increasing in areas of human activity largely due to industrial processes which indicate that levels are in the region of 100 – 1000 times higher than which is found in the earth's crust (Carral et al. 1995). By the same token living organisms, aquatic and terrestrial, can be exposed to much higher levels of these metals in the environment (Carral et al. 1995). Certain chemical agents are essential to support biological activities, but heavy metals in high concentrations can be detrimental to living organisms (Roesijadi and Robinson 1994). Coastal waters become polluted through anthropogenic activities (Fan 1996) and long term negative effects such as changes in sensitivity to heavy metal exposure along salinity and pollution gradients (De Wolf et al. 2004), reproductive fitness (Kammenga and Riksen 1996) and heavy metal accumulation (Regoli et al. 1991) may occur in certain marine vertebrate and invertebrate populations (Luoma 1996). Certain heavy metals are essential to human, plant and animal health but ever increasing non-essential substances such as Cd, Ni, Hg, As and many more pollute the ecosystem (Klavins et al. 1998). Due to increasing levels of heavy metals found in the environment today (Dietz et al. 2000) it becomes clear that precautionary measures to limit pollution of the environment need to be taken to ensure that species diversity is maintained.

Metals can be introduced into the marine environment by discharge of rivers which will lead to the contamination of estuaries and the coastal zone. Accumulation of these metals will inevitably occur in sediments and can in turn be assimilated by living organisms (Wright and Mason 1999). The level of heavy metals in the environment is partly dependand on the naturally occurring levels found in biota and the geological environment (Bryan et al. 1985). Pollutants have different impacts on the environment and species have different sensitivities

to these substances. These impacts might influence the environment differently in each case.

The study of these influences or impacts heavy metals and toxic substances have on the environment is called ecotoxicology. Ecotoxicology therefore ‘integrates the ecological and toxicological effects of chemical pollutants on populations, communities and ecosystems with the fate (transport, transformation and breakdown) of such pollutants in the environment‘(Forbes and Forbes 1994). The question inevitably is how widespread is contamination in the marine environment? Due to the improvement of scientific methods and analytical advances it is possible for scientists to analyze marine contamination with more accuracy and indicate where heavy metal pollution point sources occur, and whether they might be detrimental to animal and plant life (Luoma 1996). Estuaries, coastal zones and some off shore areas are affected by contamination of heavy metals and other chemicals by human activities (Sanudo-Wilhelmy and Flegal 1992 as cited by Luoma 1996).

Currently trace chemical contamination is restricted to the coastal and estuarine areas due to the nature of the population distribution (Sanudo-Wilhelmy and Flegal 1992 as cited by Luoma 1996). In some areas of the world near the continental shelves sludge dumping occur illegally and this will contribute to open ocean water contamination (Bothner et al. 1994 as cited by Luoma 1996). It is these levels of contamination and exposure to heavy metals that will lead to long term adverse effects on population scale and therefore contribute to changes in sensitivities of individuals and populations. We know that pollution and heavy metal contamination in general are highest in close proximity to humans due to our wasteful activities (Serricano et al. 1990; Hanson et al. 1993; Daskalakis and O’Connor 1995) but these levels will vary depending on the density of the population in specific areas. Levels are also dependant on natural processes such as distribution through mixing and absorption through biota while concentrations in severely contaminated ecosystems might also be extremely

variable (Luoma 1990). Taljaard et al. (2000) found pollution sources from storm water outlets, sewage works discharges and industrial discharges into False Bay to contribute a significant amount of heavy metals such as Pb, Cu, Cd, Zn and Ni to intertidal waters.

This heavy metal contribution may be deposited and assimilated in different ways in the environment, which will lead to an increase in dissolved metals that occur more freely in the ecosystems and are in most cases more homogenous than metal pollutants found in sediments (Flegal et al. 1991). These contaminants can affect biological activities such as phytoplankton blooms, while changes in contaminated episodic or seasonal inflows may affect background levels. Elevated levels of pollution in specific areas may well be reflective of specific pollution sources within resources such as sediments, water, plant matter and the tissue of some sessile biota (O'Connor and Huggett 1988; Luoma and Phillips 1988). Monitoring pollution levels can therefore play an important role in furthering the current body of ecotoxicological knowledge but effective implementation and monitoring of these programs are still a difficult challenge. It is therefore important to obtain adequate background information about heavy metals in specific study areas with respect to the impact they might have on the biota in those areas.

The levels of heavy metal pollution in the sea water around the South African coastline (Taljaard et al. 2000) may not have such a detrimental effect on the biota on the short term but may negatively affect them over a much longer period (Hyne and Maher 2003). Runoff from storm water and roads after a heavy spell of rain can contribute a significant amount of heavy metals to the coastal waters. Heavy metal pollution runoff might be influencing the coastal environment in such a way, that long term reactions to this type of pollution might have repercussions on the population scale (Freedman 1989 as cited by El-Sikaily et al. 2003). Heavy metals such as Cd, Cu, Hg and Zn also enter the environment in elevated concentrations through storm water runoff and waste-water discharged from

agricultural and industrial activities (El-Sikaily et al. 2003). Mdzeke (2004) found an increase in Cd levels from 0.04 – 10.4 µg/l in sea water at seven sites in False Bay, South Africa measured over five seasons from winter 2000 to 2001. This could be attributed to the runoff of agricultural chemicals after heavy rain spells (Schulz and Peall 2001) into nearby rivers, which add to this mixture of chemicals which may take many years to break down into their basic components.

Heavy metals such as Cd are increasingly found in coastal waters (Dietz et al. 1996). They subsequently cause adverse reactions and extreme negative effects depending on dosage in ecosystems. These effects are mainly due to high toxicity, the usage pattern of a specific heavy metal, emissions from fossil fuel combustion and industrial production of these heavy metals (Erk et al. 2005). Cd falls into this category of distribution and fits all of the above mentioned traits. Other anthropogenic sources are mining and metal smelting, automobile manufacturing and incineration of industrial wastes (Mdzeke 2004). Cd is also a by-product of zinc and lead mining and smelting (Klaassen 2001). All of these applications increase the possibility for Cd to enter the environment in unusual ways and in various quantities that can cause adverse effects to ecosystems and humans.

It is this negative impact at organismal level that will inevitably lead to cellular tissue damage within the organism due to the toxicity of the Cd ions (Erk et al. 2005). This is done by substitution of essential cations such as Zn^{2+} and Cu^{2+}. These cations serve as co-factors in a number of enzymes. Cd may also be taken up through the Ca channels since it has an atomic radius very similar to that of Ca (Sidoumou et al. 1997). Intracellular ligands such as metallothioneins (MTs) act as binding sites for Cd (Erk et al. 2005). Metallothioneins are found in many different marine organisms where they aid in the response process of Cd contamination (Roesijadi1994). According to Viarengo and Nott (1993) metallothioneins may be involved in the detoxification of Cd ions which enter the organism and may potentially regulate the intracellular availability of essential

metals such as Zn and Cu. Many studies have been carried out on the effects of Cd on many aquatic organisms, especially concerning the relationship with MT induction where Hidalgo et al. (1985) found increased levels of Cd in the liver of female dogfish, *Scyliorhinus canicula*, with an increase in exposure time and attributed to the high amount of Cd binding proteins. Stone et al. (1986) found that soluble Cd in the scallop, *Pecten maximus*, is mainly distributed between three weight classes where different proteins bind with this heavy metal. In the detritivore, *Chaetozone setosa,* Cd associates with medium molecular weight proteins (Eriksen et al. 1990). In the rainbow trout, *Salmo gairdneri,* Cd exposed individuals exhibited a rapid and significant loss of the heavy metal within 3 hours from the whole body of the fish (Norey et al. 1990a, b). This was attributed to the synthesis of MT's which sequestrated the Cd in the liver, kidney's and gills. Pederson et al. (1997) assesed the potential use of MT's and stress proteins (stress-70) as biomarkers of trace metal exposure and adverse effects.

Due to the availability and chemical nature of some heavy metals, sub-lethal toxicant levels may persist in the ocean waters and may cause physiological problems and toxicity in invertebrates and other marine organisms. Invertebrates such as molluscs, crustaceans and many other groups within this lineage are able to accumulate high levels of heavy metals in their tissues and still survive in the heaviest polluted areas (Rainbow 1997). This may largely be due to the ability of these organisms to actively regulate the levels of heavy metal concentrations within their body tissues and in some cases store these heavy metals in parts of the body where it can later be eliminated (Rainbow 2002). The most prominent changes will inevitably be noted at the cellular level and expressed as changes in sensitivity of biomarker responses such as cell membrane fragility (Lowe et al. 1995), biochemical mechanisms such as metallothioneins (MTs) (Bebianno et al. 2003) and acetylcholinesterase (AChE) activity (Brown et al. 2004).

Not only are there changes in sensitivity to heavy metals of individual organisms but species differ in sensitivity to xenobiotic as well as to essential substances (Dietz et al. 1996, 2000, Wu and Chen 2005). It is therefore difficult to say what a poison really is. Paracelsus already in the 1400's asked the question: "What is there that is not a poison? All substances are poisons and nothing is without poison. It is only the dose that determines whether a substance is a poison." This places us in an awkward situation. How do we determine what levels of toxicants in the environment is acceptable? The answer to this question seems easy. Due to each species having a different sensitivity to toxic substances (Van Straalen 2002a) we can deduce that certain species are more sensitive to certain substances than others. Therefore if we only protect the most sensitive species we will indirectly protect all other species that are not as sensitive for that specific substance. This sounds easy but how do we determine what an acceptable level of toxicant in the environment is? If the level of protection is too high then we might be spending too much money in order to uphold this level and if it is too low we might be neglecting certain species and loose species biodiversity (Reinecke and Reinecke 2003).

This is one of the ecotoxicologist's nightmares. The only way to maintain a close to accurate level is to apply constant biomonitoring studies to each specific field. One method for the marine environment is to monitor the levels of pollution in intertidal organisms such as limpets, mussels and clams. These invertebrates can potentially serve as sentinels in pollution monitoring due to their ability to accumulate heavy metals to a high degree (Shiber and Shatila 1978, Lobel et al. 1982, Ramelow 1985, Sericano et al. 1990, Mdzeke 2004). A possible reason for this could be due to the fact that some are sessile and some motile over short distances, therefore being exposed to heavy metals for extended periods. However the bioavailability of some heavy metals influence the absorption and assimilation of heavy metals in the shell and soft tissue which will lead to increased concentrations of heavy metals in the organism (Cravo et al. 2004).

Molluscan species can accumulate numerous pollutants from natural waters in quantities that are many orders of magnitude higher than background levels (Nickless et al. 1972, Brooks and Rumsby 1965 as cited by Howard and Nickless 1977, Hung et al. 2001) and could therefore serve as possible biomonitors of heavy metal pollution. It is therefore important to implement biomonitoring studies to ascertain how intertidal species are affected by heavy metal contamination over time. The concentration levels of heavy metals found in these species can be used to asses species sensitivities by using biomarker test such as the neutral red retention assay (NRR) (Lowe et al. 1995) or the comet assay (Singh et al. 1988).

Biomarker test are increasingly being used to asses individual responses on a cellular level due to exposure to xenobiotics (Hyne and Maher 2003). Biomarkers are defined as any biochemical, histological, or physiological alterations or manifestations of environmental stress (NRC 1987 as cited by Hyne and Maher 2003). According to Huggett et al. (1992) as cited by Luoma (1996) “biomarkers are measurable signals of the changes in cellular or biochemical processes, structures or functions that are induced by pollutant exposure”. Many authors have challenged this definition (Adams 1990, Depledge et al. 1995, McCarty and Munkittrick 1996, Engel and Vaughan, 1996) but the term biomarker is now used in a more restrictive sense. Biomarkers can indicate sublethal exposure of xenobiotics in individuals before effects are noticed on community or population scale (Hyne and Maher 2003). It is also not indicative of an overall effect of a pollutant but more of the fact that exposure to pollution has occurred (Neumann and Galvez 2002). It is the sublethal effects of heavy metals and other toxicants on a cellular level that are now more easily detected by biomarkers such as the NRR assay. Cellular stress here is apparent due to reduced lysosomal stability at low concentrations of pollutants. Using biomarkers in toxicity testing has now opened new doors in biomonitoring studies that use biomarkers to determine acceptable levels of pollutants in the environment. The information obtained from these types of monitoring studies would therefore be highly beneficial in the

efforts of scientists to determine specific environmental quality standards for natural areas.

Various techniques have been used to determine the sensitivities of invertebrates to heavy metal contamination (Lowe et al. 1995, Hauton et al. 1998, Spurgeon et al. 2000, Brown et al. 2004, Svendsen et al. 2004). One of the more simple techniques used by researchers is the NRR assay. This method focuses on a subcellular histochemical staining technique which employs the lysosomal probe, neutral red (Weeks and Svendsen 1996). The NRR assay provides the researcher with a fast and relatively easy method to determine effects on a cellular level (See Section 2.3). It has been used successfully in the marine (Lowe et al. 1995, Hauton et al. 1998, Brown et al. 2004) as well as terrestrial (Weeks and Svendsen 1996, Spurgeon et al. 2000, Svendsen et al. 2004) fields to determine sensitivity distributions. Brown et al. (2004) found invertebrate cellular and neurotoxic pathways to be more sensitive to disruption by heavy metal contamination than physiological processes. This indicates the potential value of the NRR assay in sensitivity tests and can therefore be employed in toxicity testing in the laboratory as well as the field (Brown et al. 2004). This will in turn provide sensitivity data that can be implemented in species sensitivity distributions (SSD) (Wheeler et al. 2002).

Organisms exhibit different effects at levels of taxonomy, life history, physiology and morphology which mean that these biological differences constitute a change in the way species respond to different compounds at a given concentration (i.e. different species have different sensitivities) (Posthuma et al. 2002). This leads us to the species sensitivity distribution model or SSD. The SSD concept is based on the assumption that the sensitivities of a set of species can be described by some distribution, usually parametric or nonparametric (Posthuma et al. 2002). Using the available ecotoxicological data obtained from sensitivity tests of individuals to a range of exposures to xenobiotics, the parameters of a SSD can be determined and the values gathered from the

variance in sensitivity among test species and the mean, used to calculate the concentration expected to be safe for the majority of species of interest (Posthuma et al. 2002). This model can potentially determine which species are more sensitive than others. The only problem is that it is nearly impossible to measure the sensitivity of all species to all known substances in the environment.

According to Van Straalen (2002) the idea is to use the mean and standard deviation of laboratory data obtained from inter species variation (considering species as a sample from a community distribution) to estimate the 5^{th} percentile in the left tail of the species distribution (Figure 1). This point is known as the HC5 (hazardous concentration for 5% of the species). This is a concentration that will exceed no more than 5% of species effects levels, usually based on “chronic no observed effect concentrations” (NOECs) (Wheeler et al. 2002). The 5^{th} percentile of a chronic toxicity distribution has been used in the early literature regarding SSDs (Kooijman 1987, Van Straalen and Denneman 1989) and is regarded as a safe concentration level and therefore protective of most of the species in a community.

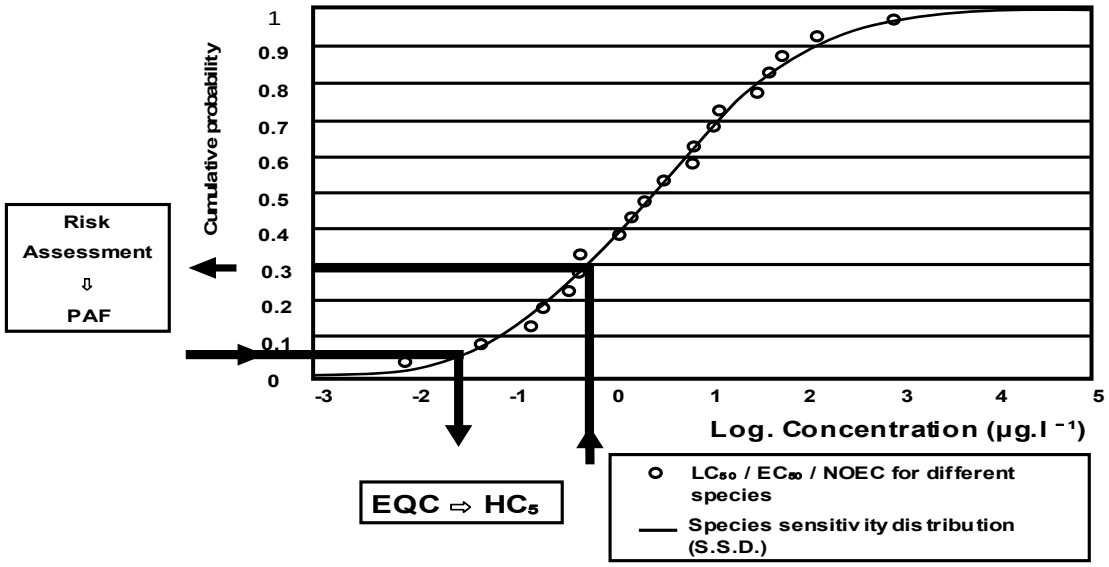

Figure 1: Species sensitivity distribution (SSD) model indicating the 5th percentile as HC_5 (hazardous concentration for 5% of population) and the cumulative probability of a species to experience a sensitivity reaction to any substance concentration (LC_{50} – lethal concentration for 50% of population, EC_{50} – effect concentration for 50% of population, NOEC – no observed effect concentration, PAF – p affected fraction, EQC – environmental quality criterion). Adapted from Posthuma et al. (2002).

The SSD concept can be used in a “forward” as well as “inverse” way (Van Straalen and Denneman 1989), where the inverse use of this model for example with the derivation of environmental quality criteria, selects a cutoff percentage known as *p* (Posthuma et al. 2002). Therefore to protect 1-*p* percent of species the desired “safe” concentration (HC_p) can then be calculated. Lately (Posthuma et al. 2002) this level has become known as the 95% protection criterion. Species falling within this percentile will therefore be protected whereas 5% of species will remain below critical concentrations and exposed to different substances at above acceptable concentrations. Only by obtaining more data from sensitivity studies can science contribute towards more accurate representation of

environmental quality concentrations for heavy metals and other substances in high concentrations. By using dose-response plots, sensitivity distributions of species to heavy metals can be obtained and these values can therefore be used to set environmental quality concentrations.

In order to determine which species to select for biomonitoring purposes certain criteria must be met. Due to economic and practical reasons a single species is normally chosen to perform toxicity tests on. From these tests sensitivity distributions will be obtained and used in a SSD model. The species selected must preferably meet most of the following selection criteria: 1. it must be a local or endemic species, 2. the organism must show a noticeable response to a wide range of pollutant concentrations, 3. it must have a wide geographic distribution and be easy accessible, 4. it must be ecologically important, 5. it must preferably hold a position in the food chain which might lead to higher trophic levels, 6. the species must be able to survive in captivity and 7. species must be easy to identify and information on the biology, physiology etc. must be readily obtainable (Reinecke, A. J., pers. comm.). Shore et al. (1975) used similar selection criteria and selected *Patella vulgata* as their study organism. Following these criteria, I chose representative species of the family *Patellidae* i.e. *Cymbula oculus* (Born) formerly *Patella oculus*, *Scutellastra longicosta* (Lamarck) formerly *Patella longicosta*, *Scutellastra granularis* (Linnaeus) formerly *Patella granularis* and *Cymbula granatina* (Linnaeus) formerly *Patella granatina*. All of the selected study species met the selection criteria with minor variations to the rules. All of the study species are endemic to South Africa, have a wide geographic distribution (Table 1) and are easily accessible at low tide. These species are ecologically important as they are prey to predators' occuring at higher trophic levels (Branch 1981). Limpets feed on microalgae in the intertidal zone and some of these species keep their own algal gardens (Branch 1981). As far as predators are concerned some fish species feed on limpets as well as certain crabs and birds (Branch 1981). All of the study species survived in captivity as proven by a

pilot study and the identification of these species were done using identification manuals of shells of Southern Africa (Richards 1981).

Table 1: Biology and ecology of patellid species selected for this study (adapted from Richards 1981).

Species	Distribution	Shell features	Zonation	Feeding Regime	Spawning period
Cymbula oculus	West Coast to Kei River (Endemic)	Somewhat flattened shell with slightly ridged buff exterior. Interior is dark brown with pale centre and muscle scar is brown or cream.	Live on exposed rocks at the mid-tide level.	Forms a home scar on rocks. Feeds on diatoms and various algae.	September
Cymbula granatina	West Coast, only rarely in False Bay (Endemic)	Exterior ridged, grey-brown with zigzag markings. Star-like projections around the edge. Not as pronounced as in *S. longicosta*. Interior blue and orange with a very dark centre.	Live on rocks at the mid-tide level often found in pools.	Forms a home scar which it leaves when the tide comes in, in order to feed. West Coast - feeds on surface deposits of diatoms. South Coast - feeds on various algae.	Autumn and Winter

Table 1 Cont: Ecology and biology of patellid species chosen for this study (adapted from Richards 1981).

Species	Distribution	Shell features	Zonation	Feeding Regime	Spawning period
Scutellastra granularis	West Coast to Natal (Endemic)	Exterior ridged and granular. Interior edge black becoming paler, with a brown or orange muscle scar at the centre.	Live on exposed rocks at the mid-tide level.	Home scars are sometimes formed, feeds at anytime provided the rocks are moist. Feeds mainly on black lichens and lower down the shore on any prostrate or creeping algae.	Winter
Scutellastra longicosta	Table Bay, False Bay to Natal	Flattened shell with pronounced projections, exterior black or brown. Interior white with dark band around margin. Can grow up to 100mm.	Lower mid-tde region	Each adult lives on a home scar surrounded by a garden of the encrusting brown seaweed *Ralfsia expansa* on which it feeds.	October - November

Many patellid species occur around the coastline of South Africa with False Bay having ten species (Richards 1981). Limpets inhabit the intertidal zone of the ocean and have a cosmopolitan distribution. They are primarily grazers and feed on different species of algae. Limpets have a hard, flat cone-shaped shell to protect against wave action and desiccation (Branch 1981). With its strong muscular foot the animal can maintain a tight seal against almost any surface which permits the animal to retain water under its mantle when the tide falls. Many species excavate a shallow home scar to which the shell margin conforms exactly. Some species may leave their home scar to feed but will return later (Branch 1981). Extensive reviews on the biology (Branch 1981) and ecology (Griffiths and Branch 1991) of endemic limpets (*Patella* sp) as well as the ecotoxicological effects of heavy metals (Boyden 1974, Shore et al. 1975, Howard and Nickless 1977, Shiber and Shatila 1978) on European limpet species have been done. Each of these species occupy a different niche in the intertidal zone and therefore possible differences in sensitivity between species with regard to feeding regime, desiccation tolerance (Branch 1975), oxygen consumption (Branch and Newell 1978) and reproduction may occur. When biomarker tests are subsequently used they are likely to exhibit differences due to these factors (Brown et al. 2004). Preliminary testing in a pilot study suggested that the patellid limpets would be an ideal group of species to work with because dose related responses were obtained. There is also adequate literature available on species endemic to South Africa as well as species which are found outside of South African borders. The fact that almost no literature were found on the ecotoxicological effects of heavy metals on endemic limpets supports the basis of this study which aimed to obtain sensitivity data with regard to heavy metal contamination for four patellid species.

1.2 Significance of the study

The study site was areas of rocky coastline of False Bay situated at 34° 15 ‘ S, 18° 40 ‘ E. Since varying levels of heavy metal pollution (Mdzeke 2004) were found in this area with certain “pollution hot-spots” occurring on the periphery of False Bay, the motivation for this study is concerned with the levels of heavy metal pollution inside this bay. These levels of heavy metal pollution may affect invertebrate species inhabiting the ocean. Invertebrates occurring here could be under environmental and toxicological stress resulting in physiological changes due to constant high levels of pollution.

These animals could aid in biomonitoring programmes to determine toxic stress even before a change in population numbers is noticed. Molluscan species such as mussels, limpets, barnacles, clams and snails have the potential to serve as test or monitoring species in which biomarker responses could be measured. These animals can accumulate heavy metals in their soft tissue and shells in very high concentrations (Howard and Nickless 1977, Shiber and Shatila 1978). By using assays such as the neutral red retention assay (Lowe et al. 1995) or the comet assay (Singh et al. 1988) we can determine what type of biomarker response the test organisms’ cells will have to a type of pollutant. Biomarkers are very effective tools in determining which species are more sensitive to xenobiotics than others (Neumann and Galvez 2002). Due to their ability to detect changes at a cellular level, biomarkers can therefore be used as very effective tests to obtain sensitivity distributions for individuals and species (Hyne and Maher 2003).

The NRR assay therefore serve as a tool in environmental pollution studies where biomarkers are used to determine the response or sensitivity of an organism to certain pollutants. When sensitivity distribution models (Posthuma et al. 2002) based on the information of responses of many species to pollutants are compiled we will be able to ascertain which species to protect so as to

ensure that we do not loose species diversity. It will be important to establish environmental quality standards for False Bay and the sensitivity of patellid species to Cd pollution could contribute towards establishing such standards. Due to the difficulty of measuring sensitivity changes of patellid species to heavy metals *in situ,* it was decided that a static flow tank system set-up in a laboratory would be most sufficient to achieve this goal. This study was therefore concerned with the relative sensitivity of patellid species when exposed to Cd in a laboratory and not the actual sensitivity of the species when found under prevailing conditions in the ocean.

1.3 Aims of the study

The aim of this study was to compare the sensitivities of different patellid species for cadmium by measuring a cellular response such as the neutral red retention in order to contribute towards establishing of species sensitivity distribution data for a model (SSD) (Van Straalen and Van Leeuwen 2001). Sensitivities were obtained by using the neutral red retention (NRR) method which measures the capacity of lysosomes to take up and retain, over time, the cationic probe neutral red that is used as an indicator of damage of the lysosome membrane due to heavy metal contamination (Lowe et al. 1995). This method could provide a dose-related response to Cd for each species which in turn can be used to compare the sensitivities of the different species.

2. Materials and Methods

2.1 Sample collection

Experimental animals were collected at two localities i.e. Gordon's Bay (34° 10 '0S, 18° 52 '0E) and Rooiels (34° 17 '60 S, 18° 49 '0 E) (Figure 2). Gordon's Bay, which is situated in the Sir Lowry's Pass catchment, was selected due to its location inside False Bay and the relatively high abundance of different *Patella*

species in this area. Here land uses range from formal housing, agriculture (vineyards and vegetable farms), holiday accommodation, a fishing-yacht harbour, a solid waste dumpsite and a wastewater treatment plant (Taljaard et al. 2000). These are all contributing factors for possible heavy metal pollution within this bay. However preliminary results indicated low concentrations of Cd in *Patella* species in False Bay. Mdzeke (2004) found water samples from the Gordon's Bay site to contain Cd levels, which varied between 0.04 and 2.47µg/ml. Therefore it was expected that Cd levels will be relatively low in *Patella* species and that they could serve as a potential species for biomonitoring studies as well as for experimental studies. Selection of the four patellid species was largely restricted by the site chosen and availability of species. At the Gordon's Bay site two species i.e. *C. oculus* and *S. longicosta* were chosen due to their relative abundance and the absence of any other major contributing patellid species. The Rooiels site had a better representation of other genera within the family where *S. granularis* and *C. granatina* were collected.

Figure 2: Map of False Bay (South Africa) and surrounding areas indicating the specimen collecting sites for this study by two star symbols (Adapted from Taljaard et al. 2000).

Collecting of specimens occurred during the sampling period of June 2004 – September 2005, at low tide when the rocky shore of the bay was exposed. A total of 62 individuals were collected for each exposure concentration and each species. These individuals were of similar shell length and were removed by means of a flat blade that was carefully pushed in under the shell of the animal to dislodge it from the rock. The animals were placed in 20-L plastic buckets containing site water and transported back to the lab for further laboratory exposures. All patellid species in this study were collected within the necessary specifications of sample collection and permit conditions as set out by the national government (Marine Living Resources Act – Act No. 18 of 1998). This act states that there is no minimum size restriction on the collection of limpets, the maximum number of limpets collected in one day may not exceed 15

individuals per person and the collection method is only by hand or with an implement with a blade or flat edge not exceeding 12mm.

2.2 Experimental design

Laboratory exposures at three different concentrations of $CdCl_2$ were done respectively for each species over a three day exposure period to determine which species is the most sensitive to Cd. At the laboratory two individuals were selected at random from the sampled group of limpets from each collecting trip and frozen for analysis of cadmium content. Each of the three exposure concentrations for each of the four species selected was done in conjunction with a negative control. Two 50-L glass aquaria containing 40 litres of constantly aerated sea water were used to maintain the collected limpets in. The two aquaria served as an exposure- and control group respectively. The aquaria were covered by a Perspex plate to minimize water evaporation. The exposure- and control aquaria contained 30 individuals respectively which were randomly selected from the collected samples and placed on Perspex plates used as the attachment surface. These plates were placed horizontally in the aquaria on top of four small glass beakers. Below the glass beakers, filters were placed to separate the animals from the bottom of the aquaria and any faeces that were excreted. The Perspex plates prevented the animals from moving up (out of the water) or down (onto the bottom). The aquaria were kept in a climate room which were maintained at 20 ± 1 °C. The animals were placed in the aquaria containing site water collected from one specific site in Gordon's Bay. The site water was regarded as ''clean'' with respect to the low concentration of Cd (as found in a preliminary control group study – see Appendix Figure 6) in the water. The animals remained in the aquaria for a period of two days prior to the start of the first exposure period, which allowed for the total depuration of gut contents since it could have influenced the results of the whole body analysis (Anon 1980) and contribute to variations in the heavy metal concentrations. The heavy metal dosages used for the exposures were 0.8, 1, and 1.2 mg / L $CdCl_2$ respectively.

These concentrations have previously been determined to be sub-lethal for the bivalve *Donacilla cornea* by Regoli et al. (1991) and fell within the range of heavy metal concentration (0.69 – 31.67 µg/g dry weight) found in *C. oculus* occurring in False Bay by Mdzeke (2004). After 48 hours of gut depuration the first exposure commenced. Exposure periods of 24, 48 and 72 hours were used. At the end of each exposure period ten individuals from the exposure as well as the control group were selected at random, their neutral red retention time (NRRT) determined and frozen at –20 °C for analysis of Cd content. The sistematic sampling of the negative control together with the exposure group at each exposure time and concentration ensured that minimal variation in the mean value for the negative control over the three day exposure period selected for the exposure organisms would be maintained.

2.3 Neutral red retention (NRR) assay

The neutral red retention (NRR) assay was used as a biomarker response to assess the relative sensitivity of *Patella* species in this study to Cd contamination. The NRR assay, which measures contaminant-induced lysosomal membrane damage (Svendsen et al. 2004, Spurgeon et al. 2000, Lowe et al. 1995), was conducted on cells present in limpet haemolymph. For this assay, haemocytes were collected using an invasive technique. This involved inserting a fine-needled syringe, containing 20 µl molluscan physiological ringer (Mdzeke 2004) into the foot muscle of the limpet. The syringe was filled with 20µl of haemolymph using a gentle drawing action. This technique has been found to be suitable for collecting live (viability 85%) intact limpet or snail haemocytes by Snyman (2001) and Mdzeke (2004). To determine the neutral-red retention time, a stock solution of 20 mg neutral red powdered dye dissolved in 1 ml of dimethyl sulphoxide (DMSO) was prepared. Ten microlitres of the stock solution were then diluted with 2.5 ml of molluscan ringer to give a neutral-red working solution. The working solution was renewed every hour because of the crystallization of nonpolar neutral red in the aqueous ringer with time. Collected limpet

haemocytes were placed on a microscope slide and allowed to adhere to the surface for at least 30 seconds. Twenty microlitres of neutral-red working solution were applied to the cells on the microscope slide and covered with a cover slip. The microscope slide with cells was scanned under a light microscope (Nikon) at 40x magnification for 2 min, during which time several fields of view were chosen at random and the number of unstained cells and cells with a stained cytosol (exhibiting dye loss from the haemocytes to the cytosol) counted. Cells were counted by using a manual sheep counter. Following each observation period, the slide was returned to a humidity chamber for a further 2 min prior to the next observation. This was done to ensure that the cell suspension on the microscope slide did not dry out due to the high light intensity of the microscope.

Observations were stopped when the number of stained cells exceeded 50% of the total. This time was taken as the neutral-red retention time. Neutral-red measurements were conducted for ten limpets from each exposure time at each of the exposure concentrations as well as the control. Two replicate measurements were done on each animal in an attempt to reduce the effect of individual differences. Because NRR is based on the observation of dye loss from the lysosome through the lysosomal membrane the observers judgment may in some way be biased due to human distinction differing when judging colour and time. Although it has been proved that a single observer will maintain a certain level of observations with the same errors right through his/her observations the technique can not be flawed on this reason alone (Reinecke and Reinecke 1999, Reinecke, A. J., pers. comm.). Therefore there might be differences in results between observers but these results will still be sufficiently consistent within each observer's observations.

2.4 Heavy metal analysis

The heavy metal analysis was carried out spectrophotometrically at the Department of Physics of the University of Stellenbosch. The method of sample

preparation and extraction by acid digestion is described by Katz and Jenniss (1983). For this purpose the frozen specimens were thawed, the soft tissue separated from the shells and weighed before being acid digested using the following method: samples were digested by adding 10ml of nitric acid and left to stand over night. The samples were then heated up to 40 – 60 °C for 2 hours and then to 110 – 120 °C for ± 1 hour or until brown fumes appeared. The samples were left to cool down for ± 1 hour, where after 1 ml of perchloric acid was added. The samples were mixed gently but well and heated up between 110 – 120 °C until brown fumes appeared. The samples were left to cool down again and then 5 ml of distilled water were added. It was mixed gently but well and heated up between 110 – 120 °C until white fumes appeared (after about 15 – 20 minutes). The samples were left overnight to cool down completely. Filtration of samples into 20 ml volumetric flasks using Whatman no 6 filterpaper and a small funnel were done the next day. The sample was filled up to 20 ml using distilled water and filtered again using 0.45 µl cellulose nitrate filter paper into film boxes. All the samples were stored in a dry place, wrapped in black plastic bags to prevent most of the fumes from escaping, until time for analysis. The Cd analysis were done by using an atomic absorption spectrophotometer (Varian AA – 1275), with acetylene-air flame. Standards of 1, 5, and 10 mg/L Cd were used for the extraction. Extraction efficiency was at least 80% of the initial sample concentration.

2.5 Water sampling

Site water from each of the 18 collecting trips was sampled at 0.5m below the water surface using a 1L plastic polyethylene container which was sealed with a lid and taken to the laboratory to be frozen for further analysis of heavy metal content. Water samples were also collected from the “clean site water” which was used for the static flow control and exposure experiments in the laboratory.

2.6 Statistical analysis

Significant differences in retention time and heavy metal concentration between species sampled, exposure time and heavy metal exposure concentration were determined by using analysis of variance (ANOVA). Where interactions were too complex to determine by one way ANOVA's and residuals were non-normal a Bootstrap analysis was done yielding 95% bootstrap confidence intervals. These manipulations were done by using the STATISTICA 7.0 statistical software package (StatSoft Inc, Tulsa, OK, USA). Probit analysis (Finney 1962) of dose response plots were done to determine the sensitivities of patellid species to Cd. From these analyses effect concentration (EC_{50}) values were determined for all species at the three exposure times.

3. Results

3.1 Cd concentration in sea water

Heavy metal analysis done on sea water samples from each collection trip indicated a varied distribution of Cd in both sample sites. Over the sampling period of June 2004 – September 2005 values ranged between 0.1 – 0.5 mg/L Cd (Figure 3 and 4). One way ANOVA (Appendix Table 100 and 101), Bootstrap analysis and a Mann-Whitney *U*-Test indicated no significant differences between study sites ($p > 0.05$) and due to only one sea water sample being analysed per sample trip for Cd content, no statistical analysis could be done to determine whether differences between sampling days were significant.

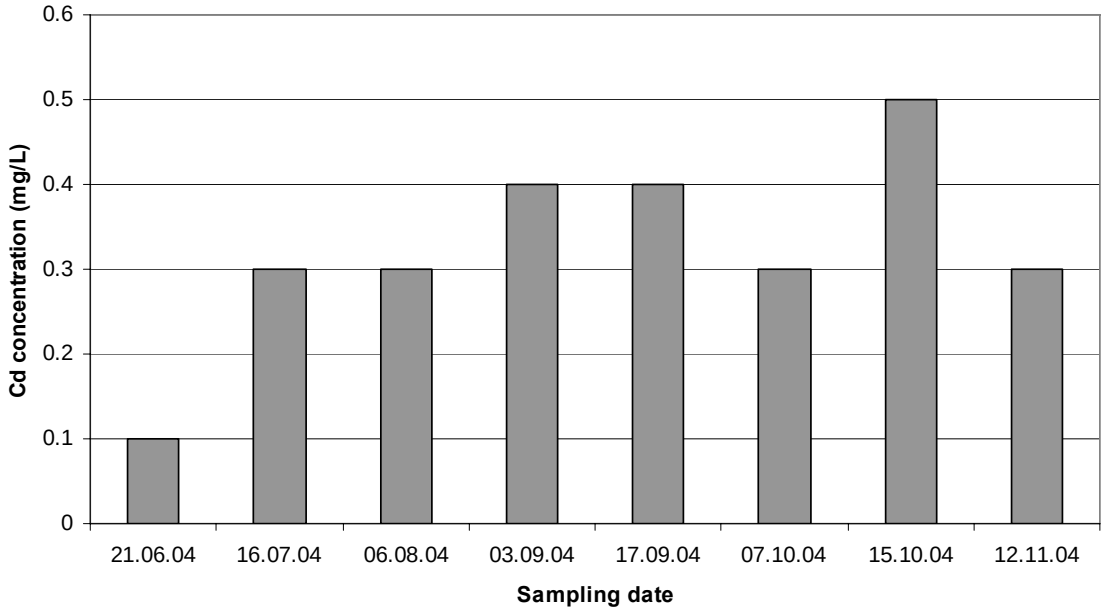

Figure 3: Cd concentration (mg/L) in sea water samples taken from each sample collection trip made to the Gordon's Bay study site, indicating the fluctuations over the sample period (n=8).

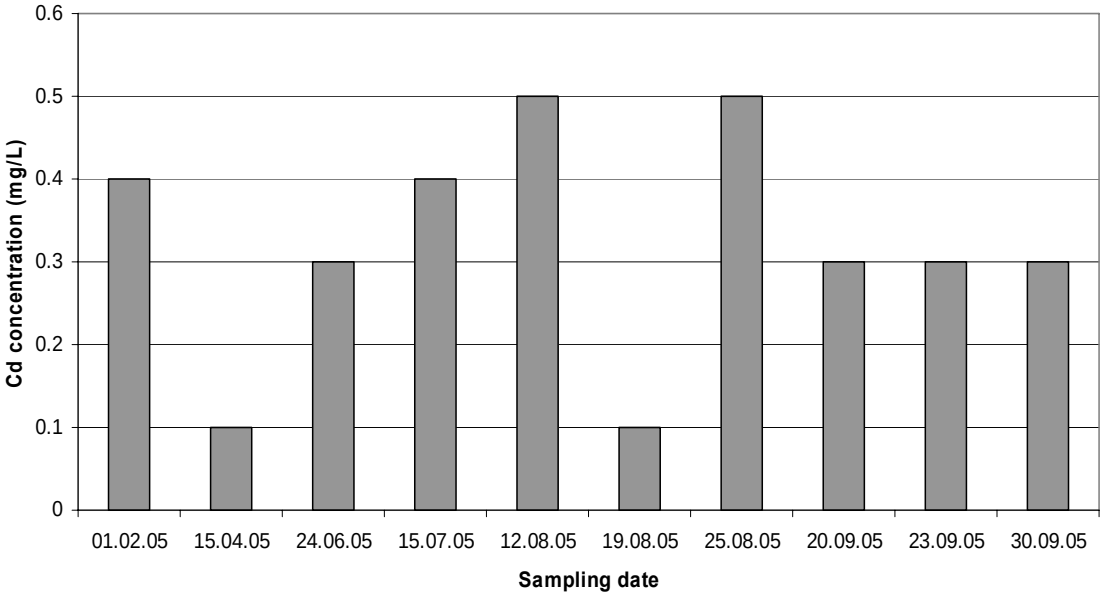

Figure 4: Cd concentration (mg/L) in sea water samples taken from each sample collection trip made to the Rooiels study site, indicating the fluctuations over the sample period (n=10).

3.2 Cadmium concentrations in experimental species

3.2.1 *Cymbula oculus*

Comparisons of the results at all three exposure concentrations and exposure times through two-way ANOVA have yielded no significant differences ($p > 0.05$, Appendix Table 102) in the metal concentrations between samples. Bootstrap analysis indicated significant differences between the control and exposure groups for each exposure time individually ($p < 0.05$, bootstrap corrected value) except for the control and 1mg/L CdCl2 exposure groups at 24 and 72 hour exposure times (Appendix Figure 96). Cd body concentrations varied between 4.56 – 21.41µg/g wet weight in exposure groups over the three day exposure period (Table 2, Appendix Table 64 – 68).

Table 2: Mean values for heavy metal body load (µg/g) of *C. oculus* at three exposure concentrations (mg/L $CdCl_2$) and exposure times (hours) (n=10 for each exposure concentration and exposure time). Control group done in conjunction with the exposure group (n=28).

Exposure conc. (mg/L $CdCl_2$)	Exposure Time (hours)	Mean Cd conc. (µg/g)	Std.Err.	-95.00%	+95.00%	N
Control	24	1.76	1.47	-1.14	4.67	28
Control	48	1.65	1.47	-1.26	4.55	28
Control	72	3.54	1.47	0.63	6.44	28
Cd 0.8	24	10.22	2.46	5.36	15.09	10
Cd 0.8	48	13.65	2.46	8.78	18.51	10
Cd 0.8	72	13.93	2.46	9.07	18.79	10
Cd 1	24	5.01	2.46	0.15	9.87	10
Cd 1	48	4.56	2.46	-0.30	9.42	10
Cd 1	72	5.82	2.46	0.96	10.69	10
Cd 1.2	24	8.40	2.46	3.53	13.26	10
Cd 1.2	48	10.26	2.46	5.39	15.12	10
Cd 1.2	72	21.41	2.46	16.55	26.27	10

3.2.2 *Scutellastra longicosta*

Mean Cd concentrations from soft tissue samples of *S. longicosta* were below the 20 µg/g Cd level (Table 3 and Appendix Table 72 - 77). Although ANOVAS indicated no significant differences between exposure times and exposure concentrations as variables (Appendix Table 103), significant individual differences ($p < 0.05$, bootstrap corrected) were found (Appendix Figure 97). A Bonferroni test confirmed these individual differences between Cd concentration means (Appendix Table 107). The control group differed significantly ($p < 0.05$, bootstrap corrected) from the 0.8 and 1 mg/L $CdCl_2$ exposures at 48 and 72 hours but not at the 24 hours exposure time (Appendix Figure 97). Mean Cd body concentrations from the 1.2 mg/L $CdCl_2$ exposure group at 24 and 48 hours

exposure, differed significantly ($p < 0.05$, bootstrap corrected) from the 0.8 and 1 mg/L $CdCl_2$ exposures at the same exposure time, but did however not differ at the 72 hours exposure time (Appendix Figure 97).

Table 3: Mean values for heavy metal body load (µg/g) of *S. longicosta* at three exposure concentrations (mg/L $CdCl_2$) and exposure times (hours) (n=10 for each exposure concentration and exposure time). Control group done in conjunction with the exposure group (n=30).

Exposure conc. (mg/L $CdCl_2$)	Exposure Time (hours)	Mean Cd conc. (µg/g)	Std.Err.	-95.00%	+95.00%	N
Control	24	4.66	1.28	2.12	7.19	30
Control	48	2.18	1.28	-0.35	4.72	30
Control	72	2.21	1.28	-0.33	4.74	30
Cd 0.8	24	19.58	2.22	15.19	23.97	10
Cd 0.8	48	9.42	2.22	5.03	13.81	10
Cd 0.8	72	11.70	2.22	7.31	16.09	10
Cd 1	24	7.51	2.22	3.12	11.90	10
Cd 1	48	6.93	2.22	2.54	11.32	10
Cd 1	72	7.68	2.22	3.29	12.07	10
Cd 1.2	24	1.18	2.22	-3.21	5.57	10
Cd 1.2	48	2.31	2.22	-2.08	6.70	10
Cd 1.2	72	3.01	2.22	-1.38	7.40	10

3.2.3 *Scutellastra granularis*

Two-way ANOVA indicated that significant differences ($p < 0.01$) between the exposure concentrations and exposure times as variables existed (Appendix Table 104). Mean Cd body concentrations were the highest of all sampled species reaching a level of 148 µg/g Cd at the highest exposure concentration (Table 4, Appendix Table 81 – 86). This level differed significantly ($p < 0.05$, Appendix Table 108) from all other sample means except for the 0.8 mg/L $CdCl_2$

exposure group at 72 hours exposure time and the 1 mg/L $CdCl_2$ exposure group at 24 hours exposure time (Appendix Figure 98). The control group exhibited very high background Cd body concentration levels (39 – 53 µg/g Cd, Table 4 and Appendix Table 81 - 86) compared to the other sample species (1 – 8.5 µg/g Cd, Table 2, 3 and 5).

Table 4: Mean values for heavy metal body load (µg/g) of *S. granularis* at three exposure concentrations (mg/L $CdCl_2$) and exposure times (hours) (n=10 for each exposure concentration and exposure time). Control group done in conjunction with the exposure group (n=30).

Exposure conc. (mg/L $CdCl_2$)	Exposure Time (hours)	Mean Cd conc. (µg/g)	Std.Err.	-95.00%	+95.00%	N
Control	24	38.56	9.24	20.32	56.80	30
Control	48	52.52	9.24	34.28	70.76	30
Control	72	44.21	9.24	25.97	62.45	30
Cd 0.8	24	51.52	16.00	19.93	83.11	10
Cd 0.8	48	77.11	16.00	45.52	108.70	10
Cd 0.8	72	145.90	16.00	114.30	177.49	10
Cd 1	24	75.49	16.00	43.90	107.08	10
Cd 1	48	42.82	16.00	11.23	74.42	10
Cd 1	72	8.83	16.00	-22.76	40.42	10
Cd 1.2	24	32.65	16.00	1.05	64.24	10
Cd 1.2	48	64.79	16.00	33.20	96.38	10
Cd 1.2	72	147.99	16.00	116.40	179.58	10

3.2.4 *Cymbula granatina*

Appendix Table 105 indicates that significant differences were determined for mean Cd body concentrations of *C. granatina* over three concentration exposures and three exposure times as variables ($p > 0.05$). A general increase in Cd body concentration was noted at all exposure concentrations from 24 – 48

hours exposure time (Table 5 and Appendix Figure 99) but according to the Bonferroni test (Appendix Table 109) this increase is not significant ($p > 0.05$). Mean Cd body concentrations decrease from 0.8 – 1.2 mg/L $CdCl_2$ over the three day exposure period (Table 5) except for a marginal increase at the highest exposure concentration on 72 hours exposure time.

Table 5: Mean values for heavy metal body load (µg/g) of *C. granatina* at three exposure concentrations (mg/L $CdCl_2$) and exposure times (hours) (n=10 for each exposure concentration and exposure time). Control group done in conjunction with the exposure group (n=30).

Exposure conc. (mg/L $CdCl_2$)	Exposure Time (hours)	Mean Cd conc. (µg/g)	Std.Err.	-95.00%	+95.00%	N
Control	24	6.86	0.62	5.63	8.08	30
Control	48	8.46	0.62	7.24	9.69	30
Control	72	7.65	0.62	6.43	8.87	30
Cd 0.8	24	10.04	1.07	7.92	12.15	10
Cd 0.8	48	12.72	1.07	10.60	14.83	10
Cd 0.8	72	14.13	1.07	12.01	16.24	10
Cd 1	24	9.31	1.07	7.19	11.43	10
Cd 1	48	12.56	1.07	10.44	14.67	10
Cd 1	72	10.91	1.07	8.79	13.03	10
Cd 1.2	24	7.27	1.07	5.15	9.39	10
Cd 1.2	48	8.62	1.07	6.50	10.73	10
Cd 1.2	72	13.06	1.07	10.95	15.18	10

3.3 Neutral red retention time (NRRT)

3.3.1 *Cymbula oculus*

Two-way ANOVA (Appendix Table 114) indicates no significant differences ($p > 0.05$) between exposure times and exposure concentrations for *C. oculus*

samples. The main effects however differ significantly from each other ($p < 0.01$) with the NRR time decreasing as the exposure concentration increases (Appendix Figure 1 – 5 and 133). There were no significant differences ($p > 0.05$) between the control and 0.8 mg/L $CdCl_2$ exposure concentrations but significant differences ($p < 0.05$) between these two groups and the 1 and 1.2 mg/L $CdCl_2$ groups did exist (Appendix Table 135). Retention times varied between 11.2 – 28.02 minutes in the 1.2 mg/L $CdCl_2$ exposure and control group respectively (Table 6).

Table 6: Mean NRR times (min) of *C. oculus* at three exposure concentrations (mg/L $CdCl_2$) and exposure times (hours) (n=10 for each exposure concentration and exposure time). Control group done in conjunction with the exposure group (n=28).

Exposure conc. (mg/L $CdCl_2$)	Exposure Time (hours)	Mean NRRT (min)	Std.Err.	-95.00%	+95.00%	N
Control	24	28.02	0.91	26.22	29.81	28
Control	48	23.38	0.91	21.58	25.17	28
Control	72	22.77	0.91	20.97	24.56	28
Cd 0.8	24	26.45	1.52	23.45	29.45	10
Cd 0.8	48	24.60	1.52	21.60	27.60	10
Cd 0.8	72	18.50	1.52	15.50	21.50	10
Cd 1	24	23.55	1.52	20.55	26.55	10
Cd 1	48	18.40	1.52	15.40	21.40	10
Cd 1	72	16.45	1.52	13.45	19.45	10
Cd 1.2	24	20.75	1.52	17.75	23.75	10
Cd 1.2	48	16.15	1.52	13.15	19.15	10
Cd 1.2	72	11.20	1.52	8.20	14.20	10

3.3.2 *Scutellastra longicosta*

Significant differences ($p < 0.01$) between exposure times and exposure concentrations were found by a two-way ANOVA done on NRR times of *S. longicosta* samples (Appendix Table 115). The 0.8 mg/L $CdCl_2$ exposure group showed significant ($p < 0.05$) higher retention times across all exposure concentrations compared to the control, 1 and 1.2 mg/L $CdCl_2$ exposures (Appendix Figure 111 and Appendix Table 118). Retention times were highest at the 0.8 mg/L $CdCl_2$ exposure, varying from 24.05 – 28.95 minutes during the exposure times and lowest at the 1.2 mg/L $CdCl_2$ exposure, varying from 9.55 – 18.65 minutes during the exposure times (Table 7). Appendix Figure 124 indicates a significant dose response ($p < 0.05$) between the exposure concentrations (and control) and the NRR time at 24 hours exposure. No correlation ($p > 0.05$ and $r^2 = 0.02$ and 0.03 respectively) between the exposure concentration and the response (NNRT) were found at the 48 and 72 hour exposure times (Appendix Figure 125 and 126).

Table 7: Mean NRR times (min) of *S. longicosta* at three exposure concentrations (mg/L $CdCl_2$) and exposure times (hours) (n=10 for each exposure concentration and exposure time). Control group done in conjunction with the exposure group (n=30).

Exposure conc. (mg/L $CdCl_2$)	Exposure Time (hours)	Mean Cd conc. (µg/g)	Std.Err.	-95.00%	+95.00%	N
Control	24	20.23	0.71	18.83	21.63	30
Control	48	17.28	0.71	15.88	18.68	30
Control	72	15.43	0.71	14.03	16.83	30
Cd 0.8	24	25.00	1.23	22.58	27.42	10
Cd 0.8	48	24.05	1.23	21.63	26.47	10
Cd 0.8	72	28.95	1.23	26.53	31.37	10
Cd 1	24	18.65	1.23	16.23	21.07	10
Cd 1	48	15.35	1.23	12.93	17.77	10
Cd 1	72	12.25	1.23	9.83	14.67	10
Cd 1.2	24	18.65	1.23	16.23	21.07	10
Cd 1.2	48	13.65	1.23	11.23	16.07	10
Cd 1.2	72	9.55	1.23	7.13	11.97	10

3.3.3 *Scutellastra granularis*

Univariate test of significance determined by two-way ANOVA (Appendix Table 116) indicated significant differences ($p < 0.01$) between exposure concentrations and exposure times for *S. granularis*. The control group showed significant ($p < 0.01$) higher NRR times over all exposure times compared to the 0.8 – 1.2 mg/L $CdCl_2$ exposure concentration groups (Appendix Figure 113 and Table 8). The only exception to the aforementioned result is the higher NRR time of the 1 mg/L $CdCl_2$ exposure concentration at the 24 hour exposure time where the NRR times was 26.05 minutes (Appendix Figure 113 and Table 8). The Bonferroni test (Appendix Table 119) indicated significant differences ($p < 0.05$) between group means of exposure concentration and exposure time in *S. granularis*. The NRRT

response was erratic as depicted by the values at the 24 hour exposure time (Appendix Figure 127) and a poor dose response correlation was found ($p > 0.05$ and $r^2 = 0.02$). Statistical analysis of NRR times vs exposure concentration yielded a dose response at the 48 and 72 hour exposure times (Appendix Figure 128 and 139).

Table 8: Mean NRR times (min) of *S. granularis* at three exposure concentrations (mg/L $CdCl_2$) and exposure times (hours) (n=10 for each exposure concentration and exposure time). Control group done in conjunction with the exposure group (n=30).

Exposure conc. (mg/L $CdCl_2$)	Exposure Time (hours)	Mean Cd conc. (µg/g)	Std.Err.	-95.00%	+95.00%	N
Control	24	18.48	0.49	17.52	19.45	30
Control	48	19.22	0.49	18.25	20.18	30
Control	72	20.45	0.49	19.49	21.41	30
Cd 0.8	24	11.40	0.85	9.73	13.07	10
Cd 0.8	48	12.30	0.85	10.63	13.97	10
Cd 0.8	72	9.45	0.85	7.78	11.12	10
Cd 1	24	26.05	0.85	24.38	27.72	10
Cd 1	48	12.40	0.85	10.73	14.07	10
Cd 1	72	10.80	0.85	9.13	12.47	10
Cd 1.2	24	11.55	0.85	9.88	13.22	10
Cd 1.2	48	8.85	0.85	7.18	10.52	10
Cd 1.2	72	7.85	0.85	6.18	9.52	10

3.3.4 *Cymbula granatina*

Mean *C. granatina* NRR times were low varying from 9.15 – 17.15 minutes over the exposure concentrations and exposure times (Table 9). Two-way ANOVA indicated that significant differences ($p < 0.01$) existed between group means of exposure concentration and exposure time (Appendix 120). A decrease in NRR

time was noted from 24 to 48 hours in the control, 0.8 and 1 mg/L $CdCl_2$ exposure groups ($p > 0.05$) while a increase in NRR time was evident for the 1.2 mg/L $CdCl_2$ exposure group over the same time ($p < 0.01$) (Appendix Figure 113 and Appendix Table 120). This decrease in NRR time was followed by an increase in NRR time from 48 to 72 hours exposure time for the control, 0.8 and 1 mg/L $CdCl_2$ exposure groups but was however not significant ($p > 0.05$) except for the control group ($p < 0.01$, Appendix Table 120). Poor correlation between the concentration exposures and the NRR response was evident in Appendix 133 – 135 over the three exposure times.

Table 9: Mean NRR times (min) of *C. granatina* at three exposure concentrations (mg/L $CdCl_2$) and exposure times (hours) (n=10 for each exposure concentration and exposure time). Control group done in conjunction with the exposure group (n=30).

Exposure conc. (mg/L $CdCl_2$)	Exposure Time (hours)	Mean Cd conc. (µg/g)	Std.Err.	-95.00%	+95.00%	N
Control	24	14.03	0.57	12.91	15.15	30
Control	48	13.07	0.57	11.95	14.19	30
Control	72	17.15	0.57	16.03	18.27	30
Cd 0.8	24	12.60	0.98	10.66	14.54	10
Cd 0.8	48	11.40	0.98	9.46	13.34	10
Cd 0.8	72	15.35	0.98	13.41	17.29	10
Cd 1	24	11.40	0.98	9.46	13.34	10
Cd 1	48	9.15	0.98	7.21	11.09	10
Cd 1	72	11.85	0.98	9.91	13.79	10
Cd 1.2	24	12.35	0.98	10.41	14.29	10
Cd 1.2	48	17.95	0.98	16.01	19.89	10
Cd 1.2	72	15.70	0.98	13.76	17.64	10

3.4 Probit analysis and effect concentrations (EC_{50}) of Cd.

EC_{50} values obtained from dose response plots in probit analysis (Appendix Figure 139 – 150) are given in Table 10. It is clear that there are differences in sensitivity between species with respect to exposure time. All of the EC_{50} values for the four species show a good correlation coefficient at the 48 hour exposure time. Only *C. oculus* and *S. longicosta* showed EC_{50} values that had a strong correlation at the 24, 48 and 72 hour exposures. Although EC_{50} values were obtained from all sampled species, only 66% of values yielded good correlations.

Table 10: Effect concentration values with correlation coefficients for four patellid species over three exposure times.

Species	Exposure Time	EC_{50} (mg/L Cd)	Correl.coeff
C. oculus	24	1.58	-0.997286017
S. longicosta	24	8.04	-0.9999961
S. granularis	24	0.07	0.063665987
C. granatina	24	31.9	-0.310397187
C. oculus	48	1.29	-0.943214904
S. longicosta	48	1.57	-0.977392884
S. granularis	48	1.2	-0.807512891
C. granatina	48	0.61	0.609623639
C. oculus	72	1.92	-0.970125931
S. longicosta	72	1.28	-0.957102076
S. granularis	72	0.78	-0.488180187
C. granatina	72	0.001	0.062646979

3.4.1 *Cymbula oculus*

Probit analysis for *C. oculus* indicated a dose response reaction at all exposure times (Appendix Figure 139 – 141). The lowest effect was observed at the

0.8 mg/L $CdCl_2$ concentration exposure at 48 hours exposure time (Appendix Figure 140). At 72 hours exposure time the dose response effect was the highest in all exposure concentrations (Appendix Figure 141). Correlations between the dose at all exposure concentrations and response were strong over all three the exposure times (Table 9). EC_{50} values were 1.58 mg/L Cd at 24 hours exposure time and 1.92 mg/L Cd at 72 hours exposure time (Table 10).

3.4.2 *Scutellastra longicosta*

A lower effect was evident over the exposure concentrations at the 24 hour exposure time for *S. longicosta* (Appendix Figure 142) while an increase in dose effect was observed at all exposure concentrations at 48 and 72 hours exposure concentration (Appendix Figure 143 – 144). At the 0.8 mg/L $CdCl_2$ exposure concentration over 72 hours exposure a very low effect was observed (Appendix Figure 144). The EC_{50} values for *S. longicosta* decreased from 8.04 to 1.28 mg/L Cd as exposure time increased (Table 10) and strong correlations between the exposure concentrations and the dose effect were observed.

3.4.3 *Scutellastra granularis*

S. granularis samples indicated high dose effects at all exposure concentrations and exposure times except for the low effect at the 1 mg/L $CdCl_2$ exposure at 24 hours exposure time (Appendix Figure 145 – 147). EC_{50} values were low ranging between 0.07 and 1.20 mg/L $CdCl_2$ over the three exposure times (Table 10). Correlations between dose concentrations were poor except for the 48 hour exposure time (Table 10).

3.4.4 *Cymbula granatina*

Probit analysis indicate minor effects at all exposure concentrations and exposure times (Appendix Figure 148 – 150) with the lowest effect being observed at the 1.2 mg/L $CdCl_2$ exposure concentration at 48 hours exposure time (Appendix Figure 149). EC_{50} was 31.9 mg/L Cd at the 24 hours exposure time and decreased to 0.001 mg/L Cd at 72 hours exposure time (Table 10). Correlations between dose concentrations were very poor indicating variation in dose response over the exposure period.

4. Discussion

4.1 Heavy metals in the sampling area

Heavy metal content (Cd) of sea water samples taken from the Gordon's Bay and Rooiels sample localities were lower than previous levels found by Mdzeke (2004). A increase in background concentration levels from 0.1 to 0.4 mg/L Cd at the Gordon's Bay site (Figure 3) between June and September '04, as well as an increase in concentration levels at the Rooiels site (Figure 4) from 0.1 to 0.5 mg/L Cd between April and August '05 could have been attributed to the winter rainfall during this period. Heavy rainfall increases the level of Cd (originating from car gas emissions) in surrounding intertidal waters in the form of runoff from major roads (Perdikaki and Mason 1999). Rainfall data from the South African Weather Service (2006) indicated that winter seasonal rainfall values were between 200 – 300mm during the July to August '04 rainfall period and between 100 – 300mm during the July to September '05 rainfall period. Subsequent to the rainfall season in the Western Cape, Cd levels for the Rooiels site indicated a sudden drop from 0.5 to 0.1 mg/L Cd, where after the background levels increased to 0.5 mg/L Cd again and then decreased to 0.3 mg/L Cd (Figure 4). This level was then maintained for the remainder of the study period. No significant differences between sample sites were found for this study (Appendix

Table 100 and 101). No statistical comparison could be made between individual sample trips due to only one water sample being collected each time.

A previous study done on the contamination levels of intertidal invertebrates in False Bay (Mdzeke 2004), where the current study was also undertaken, found relatively high background levels for Cd (0.04 – 10.4 µg/ml), Cu (0.60 – 4.65 µg/ml), Ni (0.15 – 10.74 µg/ml), Pb (0.14 – 11.40 µg/ml) and Zn (1.90 – 48.05 µg/ml) in sea water compared to levels found by Taljaard et al. (2000). An increase in Cd levels was evident in sea water samples from the Gordon's Bay site during the winter '00 / '01 season from 0.04 – 2.47 µg/g Cd as found by Mdzeke (2004). Background levels of Cd in sea water obtained from the Gordon's Bay and Rooiels study sites during the present study were lower than previous levels found by Mdzeke (2004) in the same localities. Although current background concentration values obtained are comparatively low, these levels could still serve as an indication of potentially high levels in invertebrate species occurring in the intertidal zone.

4.2 Heavy metals in patellids.

A continuous supply of heavy metals to the coastal zone (in high concentrations) may potentially result in bioaccumulation by organisms. Shore et al. (1975) found a tissue cadmium range for *Patella vulgata* along the Bristol Channel, ranging from 27 ± 6 mg/l – 537 ± 137mg/l Cd (dry weight). Mdzeke (2004) found Cd levels of between 0.69 and 31.67 µg/g Cd dry weight in *Cymbula oculus* (previously *Patella oculus*) from False Bay. Cravo et al. (2004) found levels of 6.0 ± 1.7 µg/g Cd in a clean marine site and a level of 1.6 µg/g Cd in an estuarine contaminated site on the south west coast of Portugal. Comparisons of Cd concentrations found by previous studies are given in Appendix Table 59. Due to the lack of data relating to heavy metal contamination in the literature for South Afican endemic patellid species it was difficult to obtain relevant information for comparison of endemics to global species.

Factors affecting the uptake and retention of heavy metals in marine organisms are numerous (Coombs 1977, George and Coombs 1977a, b). In marine organisms the soft tissue of the animal has the highest concentration of heavy metals, and this level is even higher than the background level in sea water (Lobel et al. 1982). According to Phillips (1980) the level of a heavy metal in the organism is often proportional to the level of the heavy metal in sea water. This means that the organism may be used as a biological indicator of heavy metal pollution (Phillips 1980). Due to the high level of heavy metals in the body of the organism, metallothioneins (MT's) are induced to eliminate the harmful heavy metals from the organism which is then excreted through the renal system or stored in the body for example the shell in limpets, where it can cause no harm (Bebianno et al. 2003). The MT's are used as a biomarker of heavy metal pollution which indicates the level of sensitivity of a species to a toxicant (Bebianno et al. 2003). Therefore the heavy metal body concentrations can in turn be used for determining species sensitivity.

4.2.1 Cd content in *Cymbula oculus*.

The heavy metal body concentration of *C. oculus* indicated elevated levels of Cd in the soft tissue after exposure for three days to $CdCl_2$ (Table 2, Appendix Figure 6, 8, 10, Appendix Table 64, 66 and 68). The decline of heavy metal body concentration in *C. oculus* from 0.8 to 1 mg/L $CdCl_2$ followed by the increase at 1.2 mg/L $CdCl_2$ (Table 2), may indicate a possible means of regulation of Cd by *C. oculus* at the 1mg/L $CdCl_2$ exposure concentration. This organism may potentially actively regulate certain heavy metals in its body. Some metals may be stored in parts of the body such as the shell where they are not harmful to the animal and show less variability in heavy metal concentration between the soft tissue and the shell (Cravo et al. 2004). Here they are preserved even after the animal has died and hold valuable information about past levels of exposure to investigating scientists (Cravo et al. 2004).

4.2.2 Cd content in *Scutellastra longicosta*.

S. longicosta exhibited some interesting behaviour. From Table 3 it is clear that there is a decrease in heavy metal body concentration from the 0.8 – 1.2 mg/L $CdCl_2$ exposure concentrations at all exposure times. The decrease in heavy metal body concentration from 0.8 – 1 mg/L $CdCl_2$ was however not significant ($p > 0.05$) but the decrease from 1 – 1.2 mg/L $CdCl_2$ was significant ($p < 0.05$, bootstrap corrected). From this experimental data it can be speculated that *S. longicosta* may be able to regulate Cd in some way, resulting in the decrease in body concentrations while the exposure concentrations were increased. The "potential regulation ability" of Cd by *S. longicosta* declines slightly from the 1 – 1.2 mg/L $CdCl_2$ exposure concentration as observed by the difference in body concentrations at 0.8 and 1.2 mg/L $CdCl_2$ ($p < 0.05$, bootstrap corrected) respectively (Table 3). A fluctuation at the 48 hour exposure time in heavy metal body concentration is visible at the 0.8 and 1 mg/L $CdCl_2$ exposure concentrations ($p > 0.05$). The period between 24 and 48 hours may therefore be the potential optimum point for Cd regulation by *S. longicosta* at the lower exposure concentrations. As soon as the organism is exposed for longer than 48 hours to the heavy metal the Cd body concentration starts to increase again towards the 72 hour exposure time (Appendix Figure 97, Table 3).

4.2.3 Cd content in *Scutellastra granularis*

S. granularis had the highest mean heavy metal body concentration after the laboratory exposures of all four patellid species sampled from the sample sites in False Bay. This phenomenon can possibly be explained by its smaller size compared to the other exposed species. It only grows to a maximum length of 69mm whereas the other three species chosen can attain sizes of between 90 – 110mm (Kilburn and Rippey 1982). Possibly the best known factor influencing heavy metal concentration is the size of the organism (Boyden 1974, 1977).

Mean Cd body concentrations increased ($p < 0.05$, bootstrap corrected) from 24 – 72 hours exposure time at the 0.8 mg/L $CdCl_2$ exposure concentration as would be expected when compared to the dose response relationship but was followed by a decrease ($p < 0.05$, bootstrap corrected) in the mean heavy metal body load at 1 mg/L $CdCl_2$ exposure from 24 – 72 hours. This occurrence may indicate "potential regulation ability" at the 1mg/L $CdCl_2$ exposure time but can however not be proved as there is no adequate literture available on this topic to substantiate these claims and may therefore only be speculated. This decrease was followed by a distinct increase at 1.2 mg/L $CdCl_2$ exposure concentration but was only significant at the 72 hour exposure concentration ($p < 0.05$, bootstrap corrected) (Appendix Figure 98). *S. granularis* may therefore potentially be able to regulate Cd in some way as seen by the fluctuations in heavy metal body concentration (Table 4 and Appendix Table 81 – 86).

4.2.4 Cd content in *Cymbula granatina.*

C. granatina exhibited a decrease in mean heavy metal body load with an increase in exposure concentration ($p < 0.05$, Appendix Figure 99 and Appendix Table 105) but showed an increase in heavy metal body load ($p < 0.05$, bootstrap corrected) over the three day exposure period for the 0.8 and 1.2 mg/L $CdCl_2$ exposure concentrations (Appendix Figure 99). The only significant differences between mean heavy metal body concentrations were between the 0.8 and 1.2 mg/L $CdCl_2$ exposure concentrations at the 24 and 48 hour exposures ($p < 0.05$, bootstrap corrected) (Appendix Figure 99). Appendix Table 109 indicates the individual significant differences between mean Cd body concentrations over the three exposure concentrations and exposure times where it is clear that *C. granatina* also appear to be regulating Cd actively although the decrease in heavy metal body concentration is only significant between 0.8 and 1.2 mg/L $CdCl_2$ as mentioned earlier. It appears that *C. granatina* loses the ability to regulate Cd at the 1.2 mg/L $CdCl_2$ exposure concentration from the 48 hour

exposure time, where the mean heavy metal body concentration increase from 8.62 – 13.06 µg/g Cd (Table 5 and Appendix Figure 99).

4.3 Cellular responses in patellids.

Most heavy metals will affect intertidal organisms on a cellular level when exposed to sub-lethal levels. Some of these intertidal organisms have developed specific biochemical mechanisms to cope with elevated environmental heavy metal levels (Bebianno et al. 2003). In the limpet *Patella vulgata* one of these mechanisms is employed i.e. metallothioneins (MT's). According to Bebianno et al. (2003) "MTs are a class of heat-stable, low molecular weight metal binding proteins of non-enzymatic nature, characterised by a unique amino acid composition with very high cysteine content (22–33 mol%), absence of aromatic amino acids or disulfide bonds that bind between 5 and 7 g atoms of group IIB heavy metals (such as Ag, Cd, Cu, Hg and Zn) per mole of protein." As a result of elevated levels of heavy metals in the environment, MTs are induced in the cells of the organism where they play an important role in the distribution and detoxification of non-essential metals and the regulation of the concentration of essential heavy metals (Bebianno et al. 2003). Using the neutral red retention test as a biomarker of response to heavy metal contamination, Brown et al. (2004) found retention times in *Patella vulgata* to vary between 21 (exposure to 6.1µg Cu l^{-1}) and 30 (control) min. Subsequently in their study *P. vulgata* was also the most sensitive to Cu contamination when a suit of biomarker tests were conducted on this species. Brown et al. (2004) also indicated that *P. vulgata* is not the best test organism to use in studies where high levels of contaminants are used to assess species sensitivity but can rather be used to measure the more subtle effects of heavy metal exposure at lower concentrations.

4.3.1 Cellular response of *Cymbula oculus* to Cd.

The mean NRR times for *C. oculus* over the three concentration exposures decreased ($p > 0.05$) over the three day exposure period (Appendix Figure 1 – 5 and 110). These differences were however not significant when the exposure time was compared to the exposure concentration (Appendix Table 114). The main effects however indicated a significant ($p < 0.05$) decrease in the mean NRR time from the control group to the 1.2 mg/L $CdCl_2$ exposure concentration (Appendix Figure 133 and Appendix Table 134 - 135). This indicates a strong dose response relationship between the exposure concentrations and the NRR time. Over the three day exposure period mean NRR times also indicated a strong dose response relationship between exposure time and NRR time (Appendix Figure 136 and Appendix Table 137 - 138). Judging from these results it is apparent that due to the increase in heavy metal concentration the mean NRR time of *C. oculus* decreased as the exposure time increased.

4.3.2 Cellular response of *Scutellastra longicosta* to Cd.

Mean NRRT values for *S. longicosta* indicated a decrease in NRR time from the 0.8 – 1.2 mg/L $CdCl_2$ exposure concentrations at all exposure times except for the 0.8 mg/L $CdCl_2$ exposure at 72 hours (Appendix Table 69 – 71 and Appendix Figure 111). In *S. longicosta* the dose response relationship is evident at the 1 and 1.2 mg/L $CdCl_2$ concentration exposures (Appendix Figure 111). Significant differences between mean NRR times and exposure times as well as mean NRR times and exposure concentrations exist (Appendix Table 115 and 118). The control group illustrated lower retention times than the 0.8 mg/L $CdCl_2$ exposure concentration. Possible reasons for this might be due to handling and stress during sampling activities. Although laboratory conditions were kept constant it is still almost impossible to duplicate the natural habitat of this organism and different stresses will have a negative impact on the NRR time during the exposure period. In the exposure groups the ability of *S. longicosta* to possibly

regulate or store Cd in the soft tissue or shell seems to diminish as the exposure concentration is increased. During the 0.8 mg/L $CdCl_2$ exposure, the NRR time marginally decreases from 24 to 48 hours exposure time but then increases again towards the 72 hour exposure time (Appendix Figure 111). This might indicate that the 0.8 mg/L $CdCl_2$ exposure concentration can well be an acceptable level of heavy metal exposure for *S. longicosta.*

4.3.3 Cellular response of *Scutellastra granularis* to Cd.

S. granularis samples indicated a decrease ($p < 0.01$) in NRR times over all concentration exposures from the 24 to 72 hour exposure times (Appendix Figure 112 and Appendix Table 116). The control group had significant ($p < 0.01$) higher NRR times compared to the exposure concentrations at all exposure times except for the 24 hours exposure at 1 mg/L $CdCl_2$ exposure concentration (Appendix Table 78 – 80). The majority of mean NRR times at all exposure concentrations and exposure times of *S. granularis* are the lowest compared to the other three sample species (Appendix Table 61 – 63, 69 – 71, 78 – 80 and 87 – 89). A Bonferroni test (Appendix Table 119) for mean NRR times of *S. granularis* that indicate significant individual differences in mean NRR times between exposure concentrations and exposure times illustrates the most significant differences between the control and exposure concentrations. These differences may be of importance when trying to explain the higher NRR times in the control group. When the mean NRR times of the control groups of all the tested species are compared against the exposure concentrations, the control group mean NRR times of *S. granularis* are much higher than those of the other species (Appendix Figure 153). This phenomenon can possibly be explained by the zonation of *S. granularis*. It lives on exposed rocks at the mid- tide level where it is only covered by water at spring and neap high tide. This may therefore indicate that *S. granularis* are used to extreme environmental conditions and that collecting and handling did not affect the control group animals in such a way that a very low NRR time was resultant.

4.3.4 Cellular response of *Cymbula granatina* to Cd.

C. granatina had comparatively the lowest NRR times of all species (Appendix Figure 151 – 153). EC_{50} values indicate that *C. granatina* is the most sensitive to Cd at the 48 and 72 hour exposures but least sensitive at the 24 hour exposure (Table 10). The fluctuation in mean NRR times between exposure concentrations from 24 to 48 hours and 48 to 72 hours exposure were not significant but in this case may indicate the potential regulation of Cd by *C. granatina*. At the highest exposure of 1.2 mg/L $CdCl_2$ the possible regulatory ability decreases from 48 to 72 hour exposure time. *C. granatina* are the second largest species tested in this study and occur predominantly in the mid-tide level in pools. It is therefore less exposed to the air for prolonged periods. NRR times were low compared to the other species and may possibly be due to its zonation, causing stress in the animals during collection and transport to the laboratory.

5. Conclusion

Mean NRR time values in this study indicated that a decrease in retention time with an increase in exposure concentration was evident (Appendix Figure 151 – 153). Control groups showed a slight decrease in retention times over the three day exposure experiment but this could be due to factors such as handling or stress caused by temperature, light, or varying oxygen levels which were not fully compensated for in this study. A general increase in heavy metal body concentration was noticed in all species as the exposure concentration and exposure time increased. From EC_{50} values determined for each species by probit analysis it is clear that *S. granularis* is the most sensitive to Cd contamination at 24 hours exposure time and *C. granatina* is the most sensitive at 48 and 72 hours exposure time. Sensitivity differences between species were evident and each species exhibited a unique sensitivity to Cd. *S. granularis* and *C. granatina* therefore have a “high” sensitivity to Cd contamination. *C. oculus*

exhibited a “medium” sensitivity over all exposure concentrations and exposure times, while *S. longicosta* exhibited a “low” sensitivity. The sensitivity data obtained in the analysis of the four sampled species in this study present a contribution for the eventual establishment of a SDD model.

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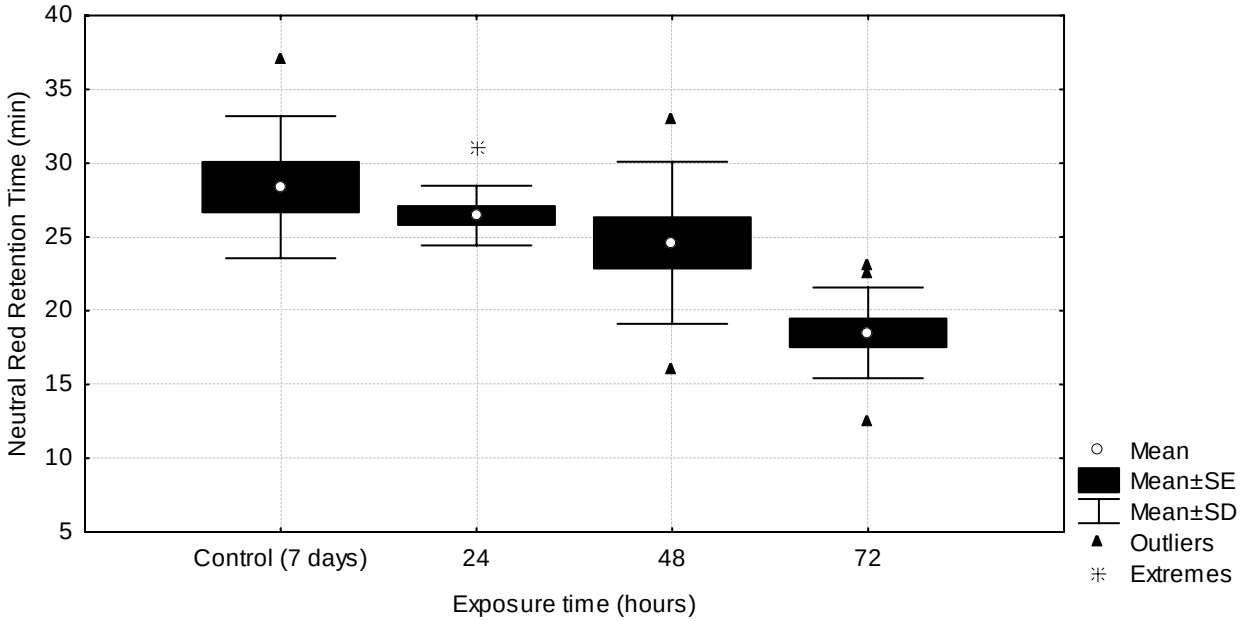
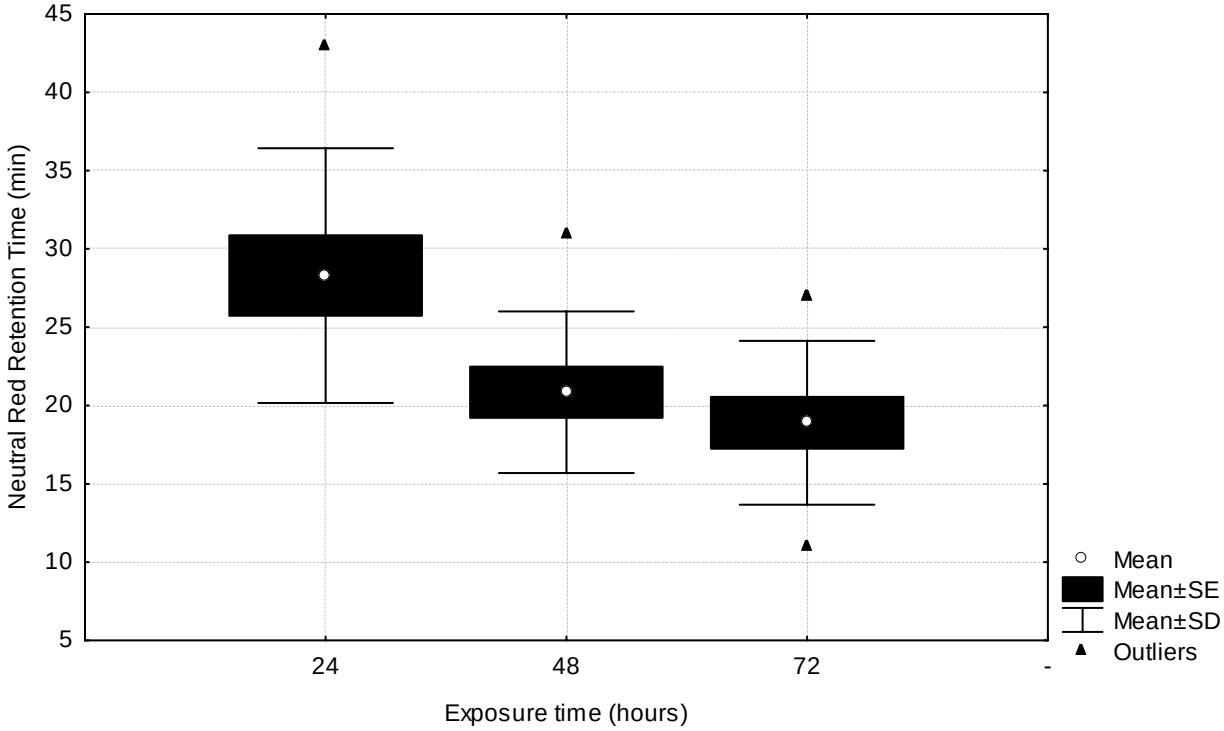
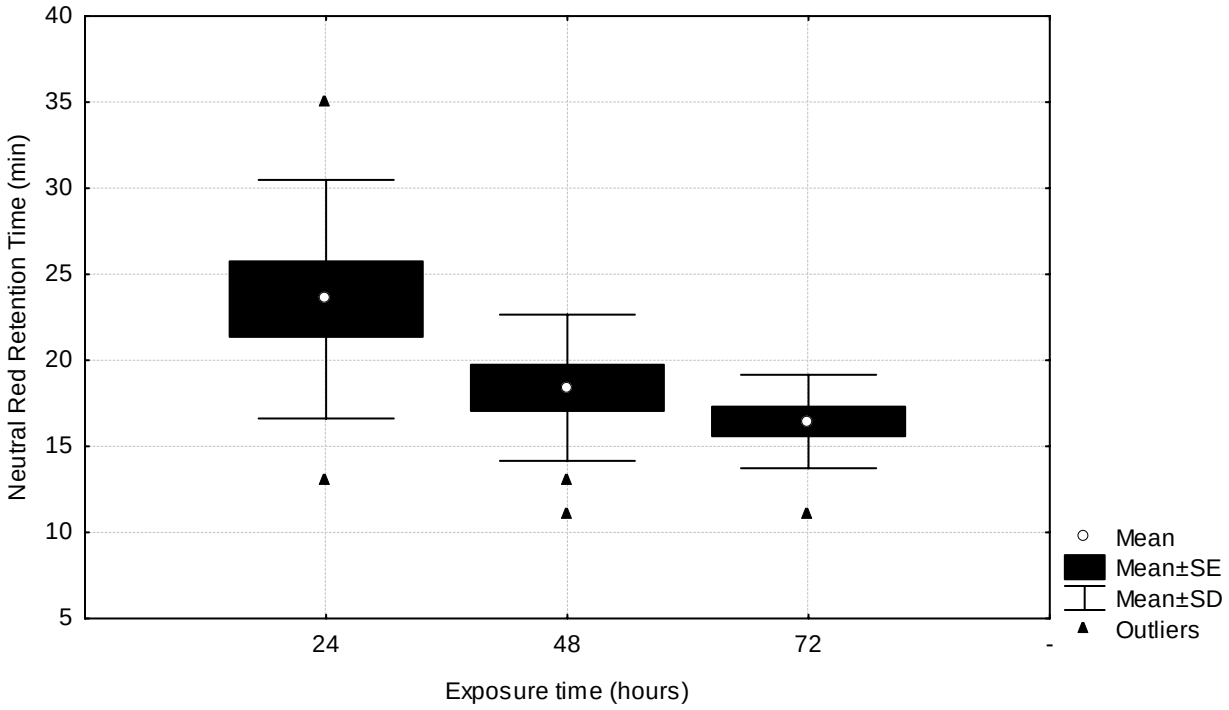
7. Appendices



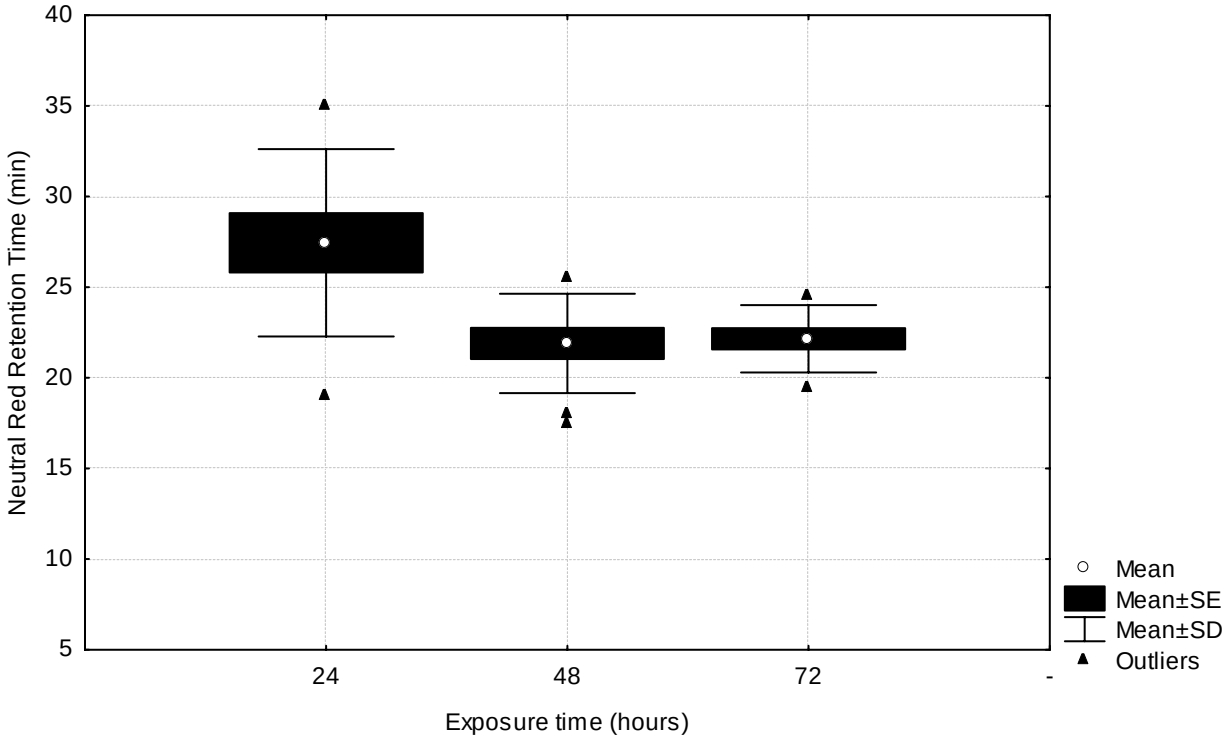
Appendix Figure 1: Neutral red retention times (min) for *C. oculus* over three exposure times (hours) at 0.8 mg/L $CdCl_2$ exposure concentration. Graph includes control group measured after 7 days of containment in tanks.



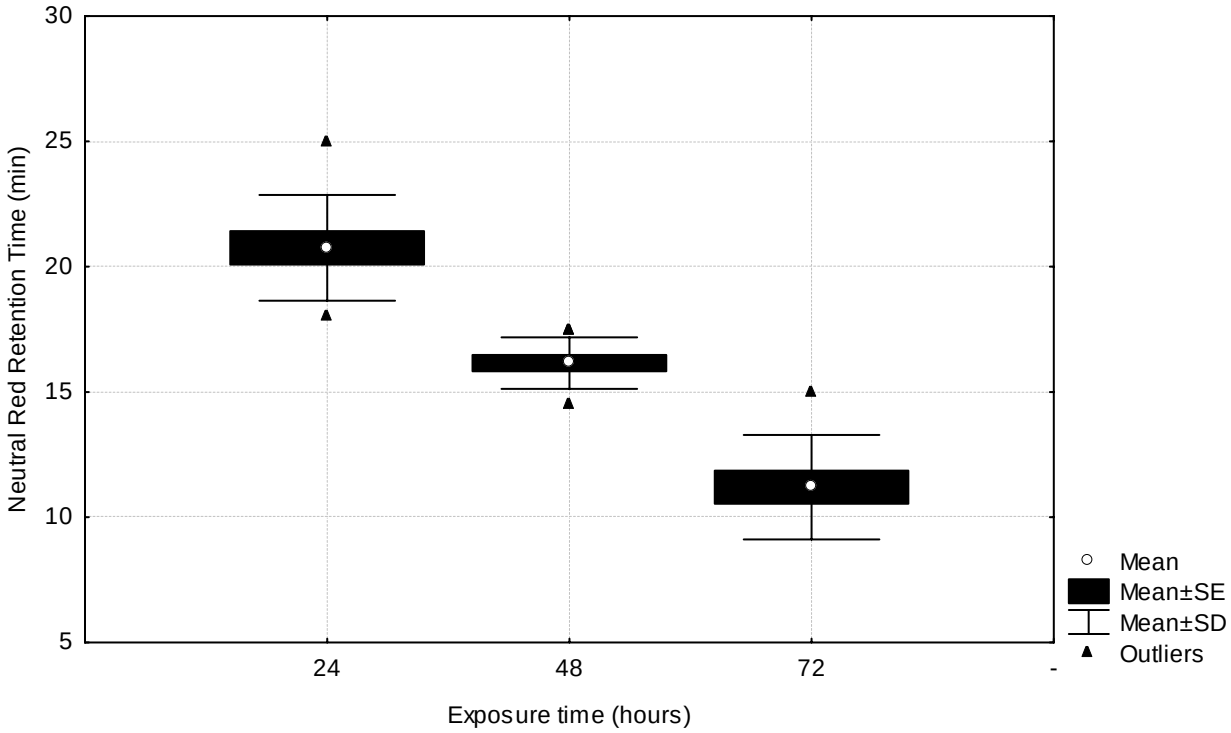
Appendix Figure 2: Neutral red retention times (min) for the control group of *C. oculus* done in conjunction with the exposure experiment at 1 mg/L $CdCl_2$ over three exposure times (hours).



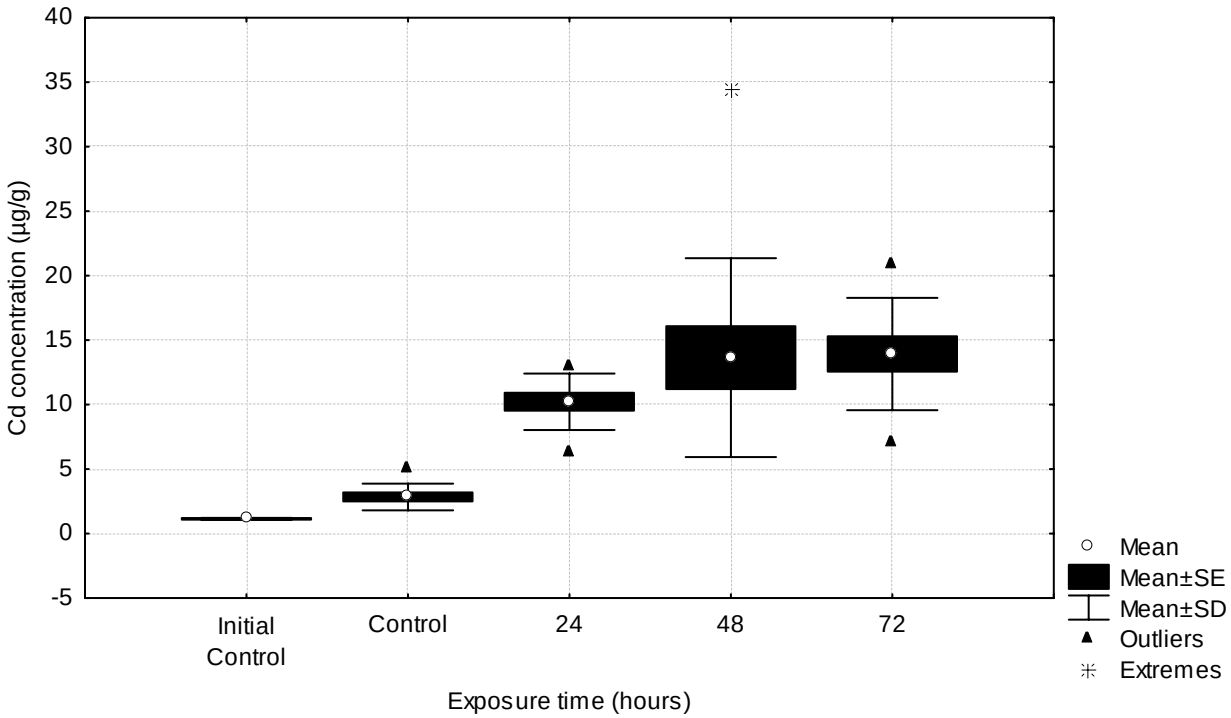
Appendix Figure 3: Neutral red retention times (min) for *C. oculus* over three exposure times (hours) at 1 mg/L $CdCl_2$ exposure concentration.



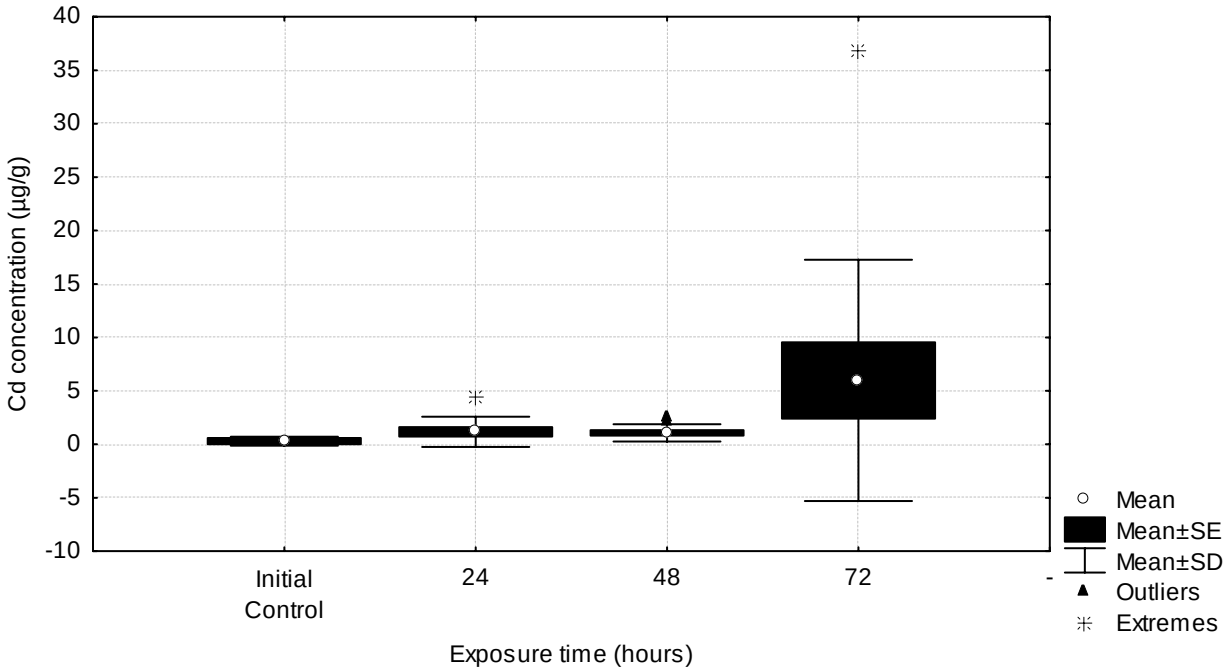
Appendix Figure 4: Neutral red retention times (min) for the control group of *C. oculus* done in conjunction with the exposure experiment at 1.2 mg/L $CdCl_2$ over three exposure times (hours).



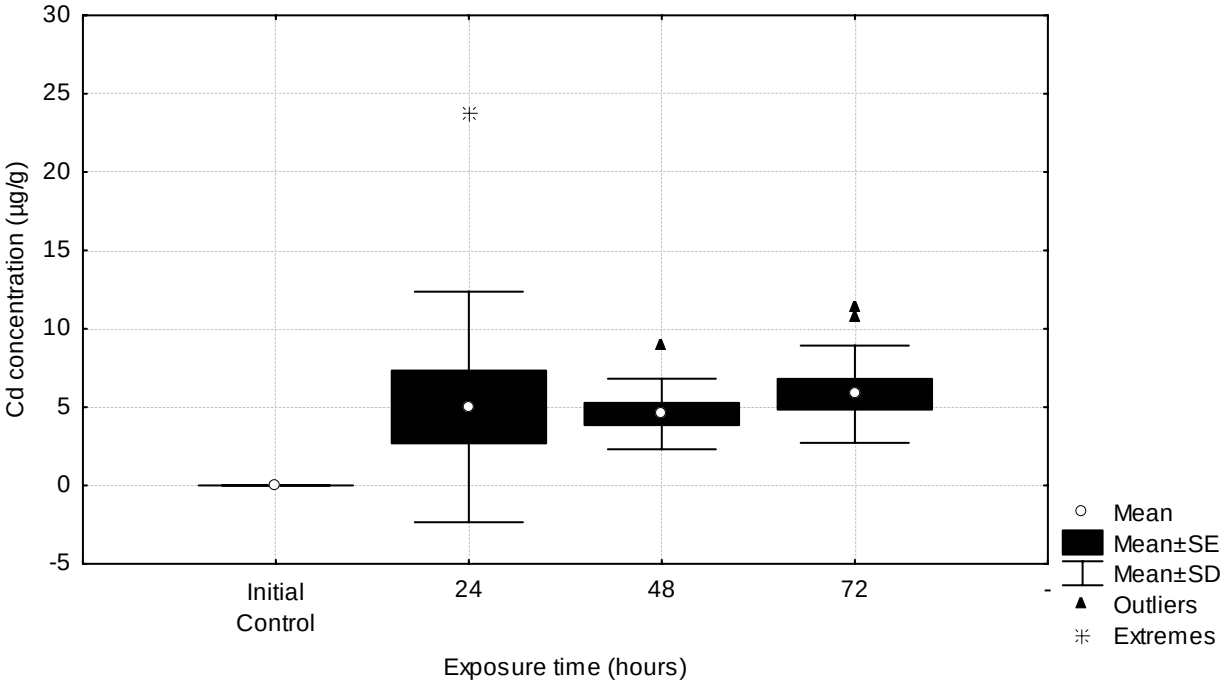
Appendix Figure 5: Neutral red retention times (min) for *C. oculus* over three exposure times (hours) at 1.2 mg/L $CdCl_2$ exposure concentration.



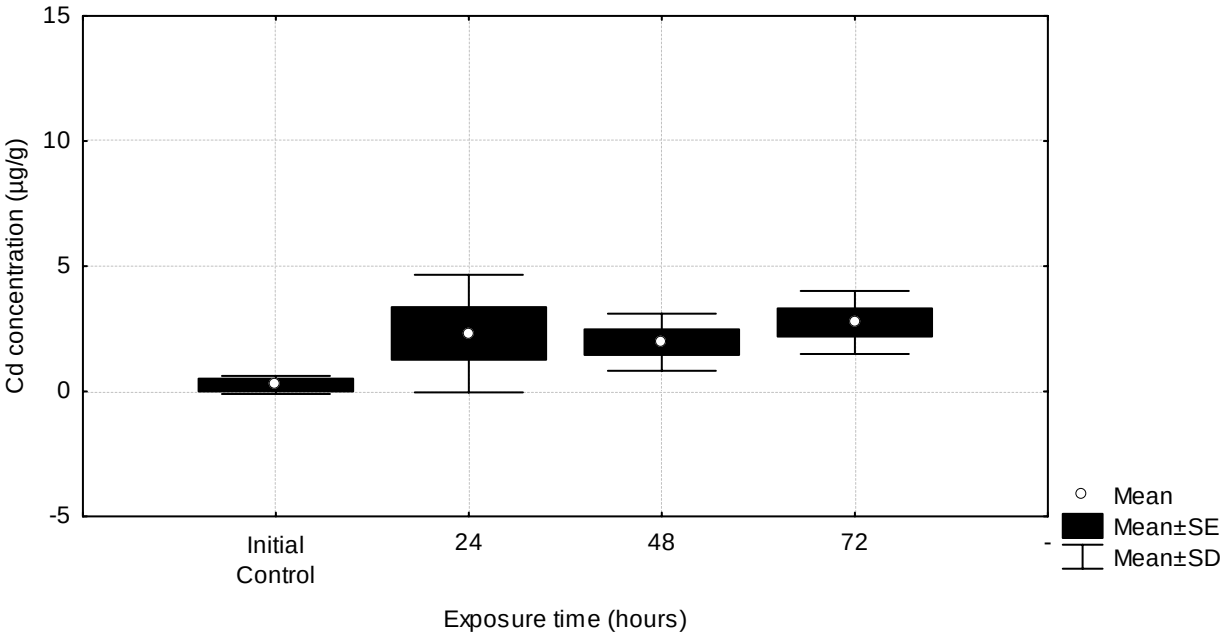
Appendix Figure 6: Heavy metal body concentrations (µg/g) of *C. oculus* over three exposure times (hours) at 0.8 mg/L $CdCl_2$ exposure concentration. Graph includes an initial control (done at 0 hours) and control group (done at 7 days). Data from preliminary pilot study.



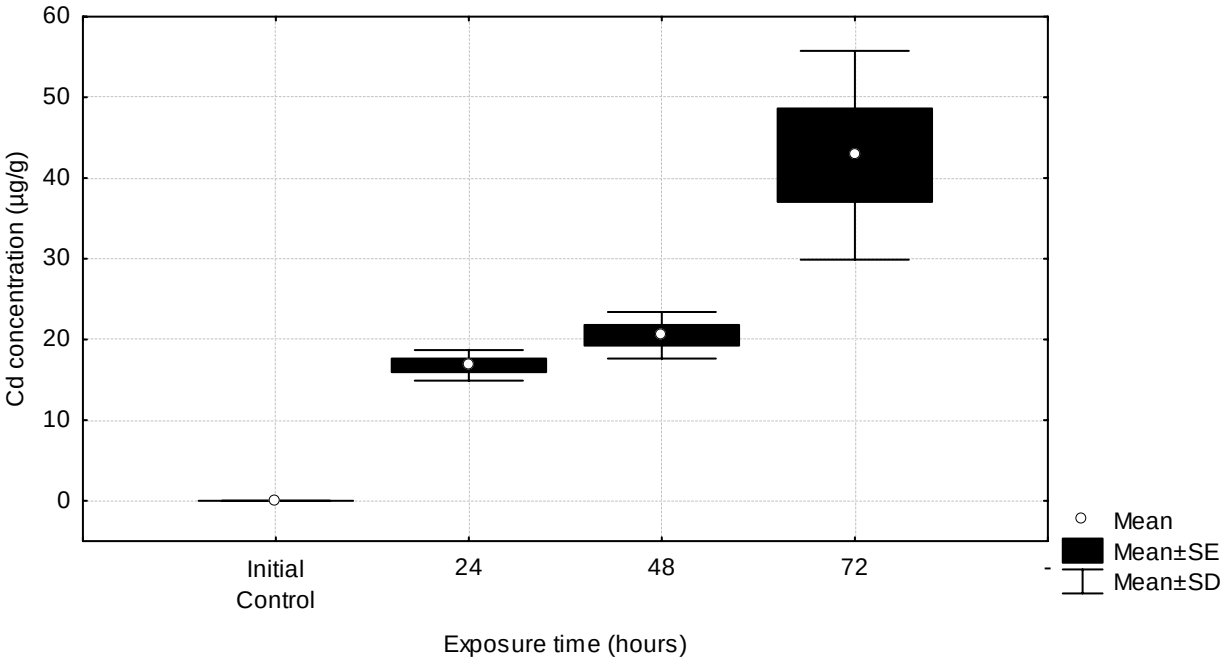
Appendix Figure 7: Heavy metal body concentrations (µg/g) of *C. oculus* for the control group done in conjunction with the 1 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



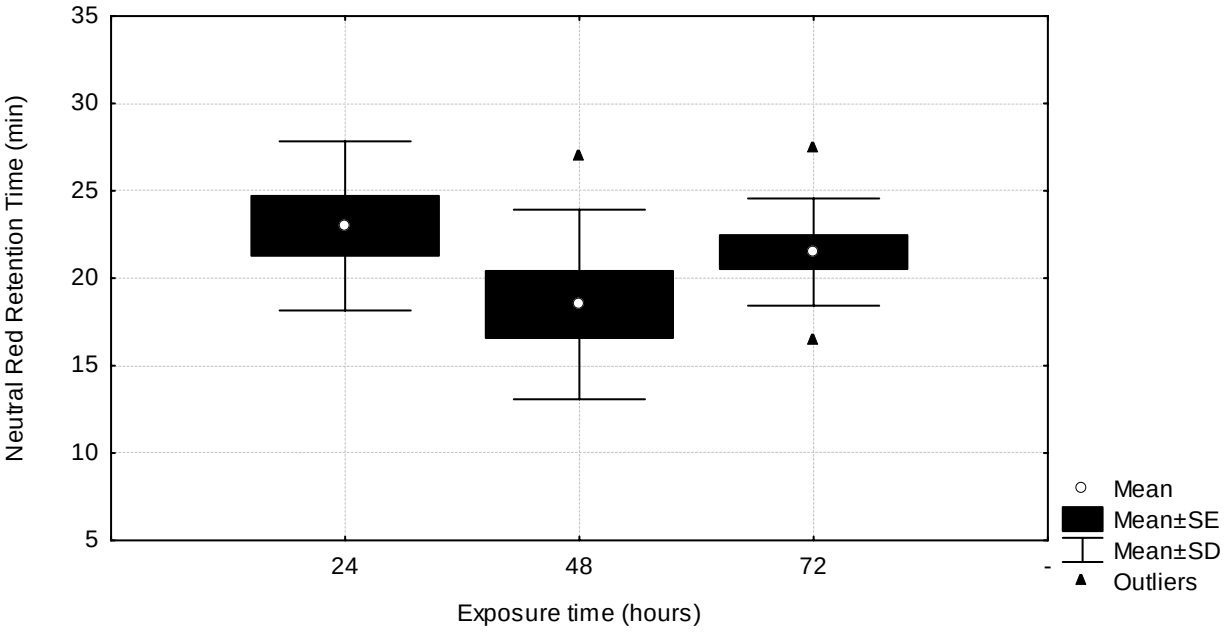
Appendix Figure 8: Heavy metal body concentrations (µg/g) of *C. oculus* over three exposure times (hours) at 1 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



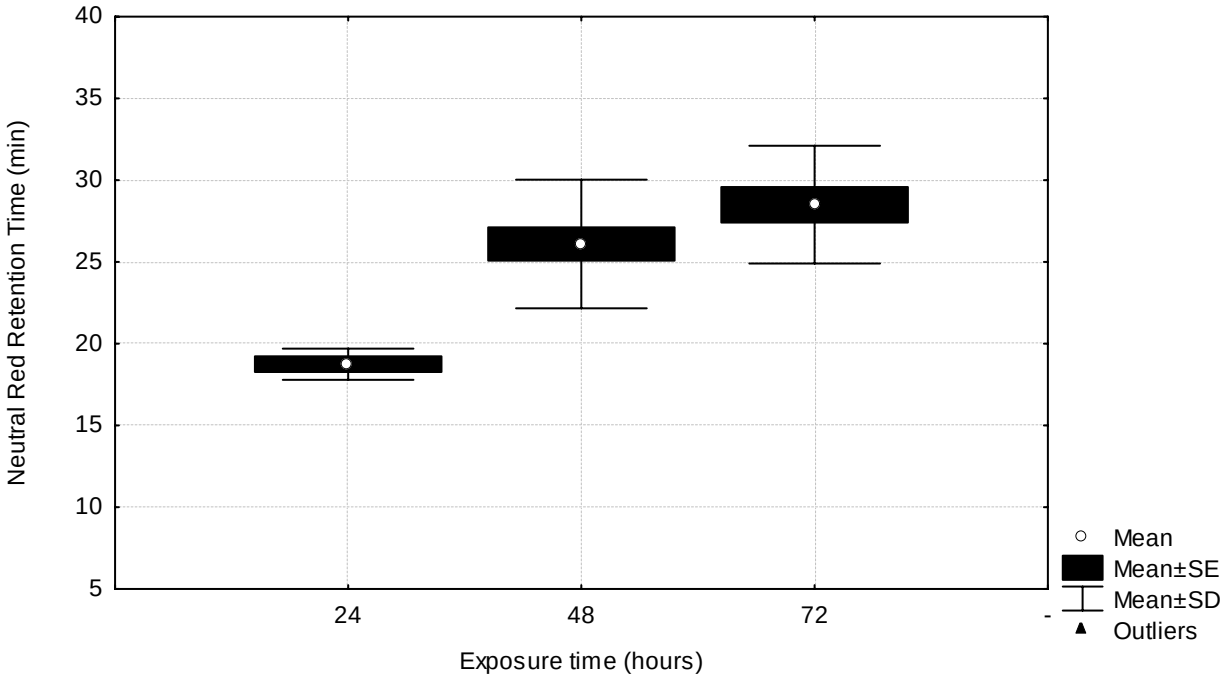
Appendix Figure 9: Heavy metal body concentrations (µg/g) of *C. oculus* for the control group done in conjunction with the 1.2 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



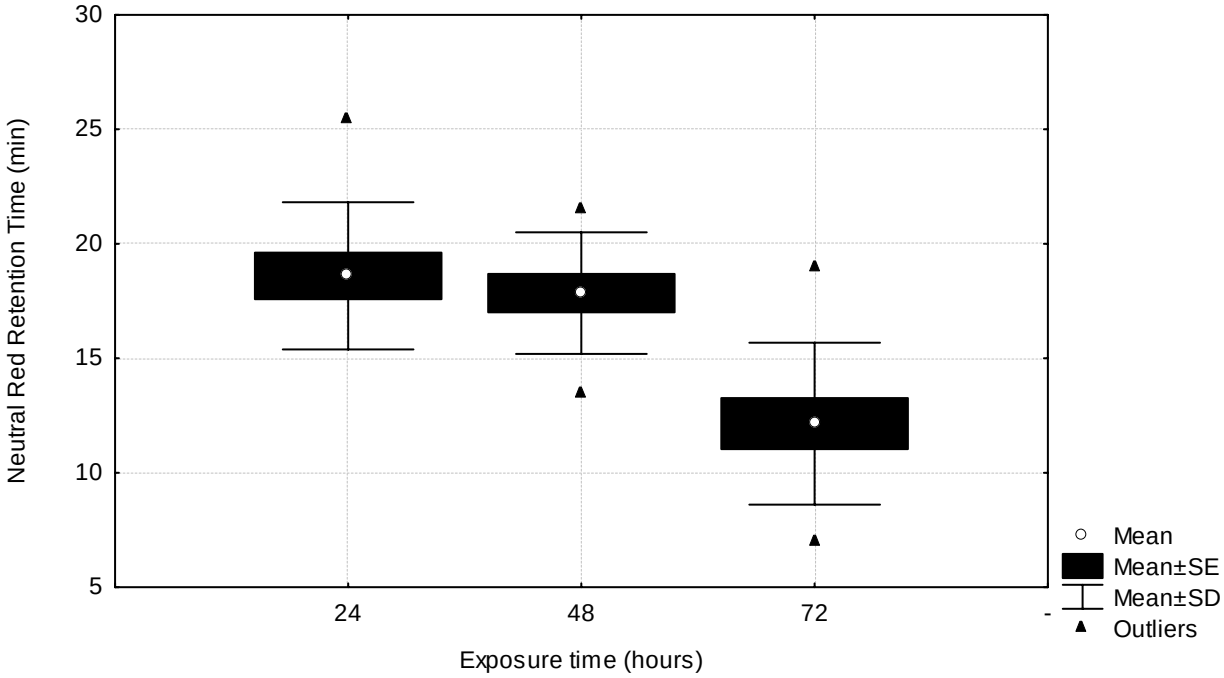
Appendix Figure 10: Heavy metal body concentrations (µg/g) of *C. oculus* over three exposure times (hours) at 1.2 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



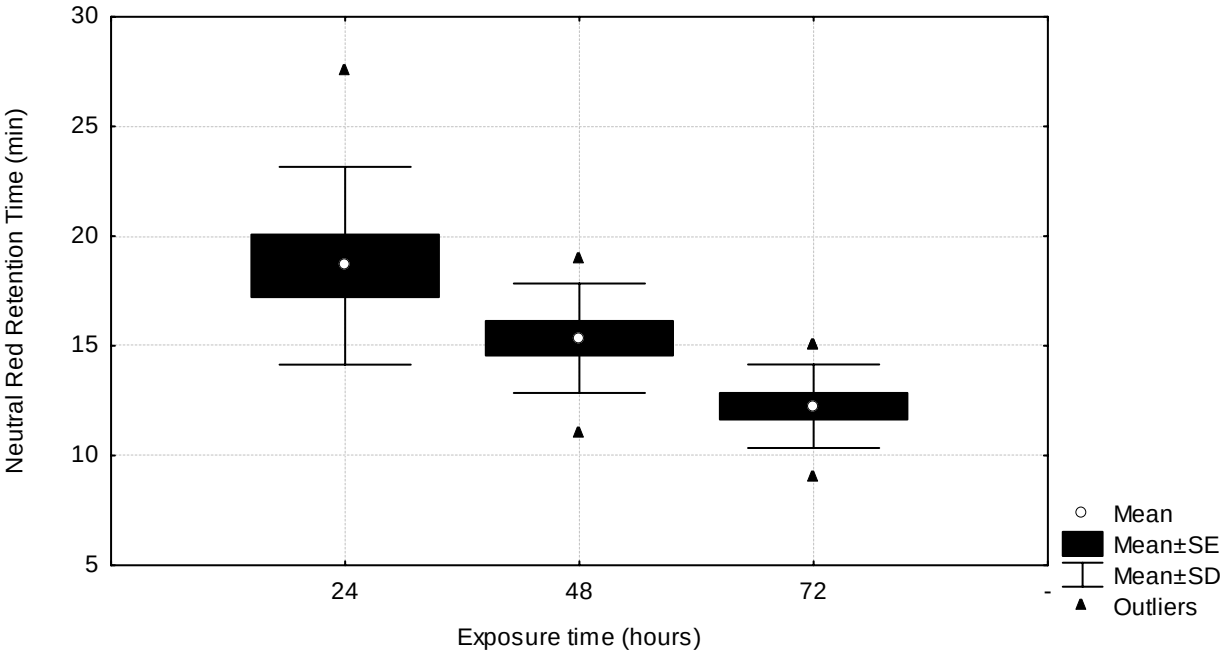
Appendix Figure 11: Neutral red retention times (min) for the control group of *S. longicosta* done in conjunction with the exposure experiment at 0.8 mg/L $CdCl_2$ over three exposure times (hours).



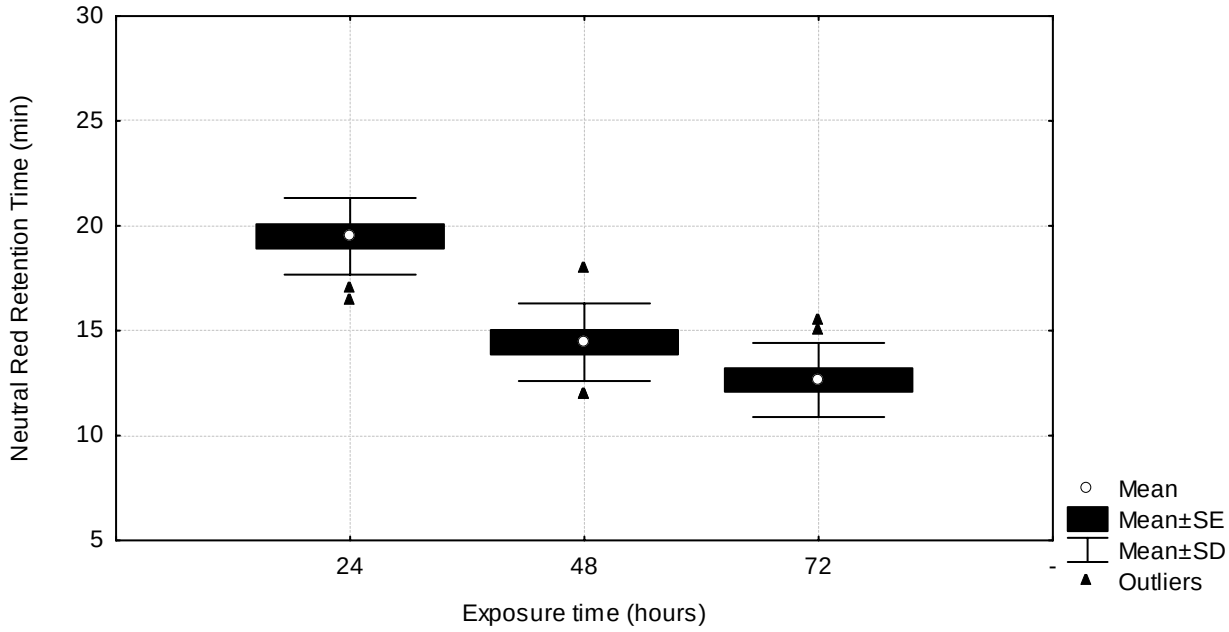
Appendix Figure 12: Neutral red retention times (min) for *S. longicosta* over three exposure times (hours) at 0.8 mg/L $CdCl_2$ exposure concentration.



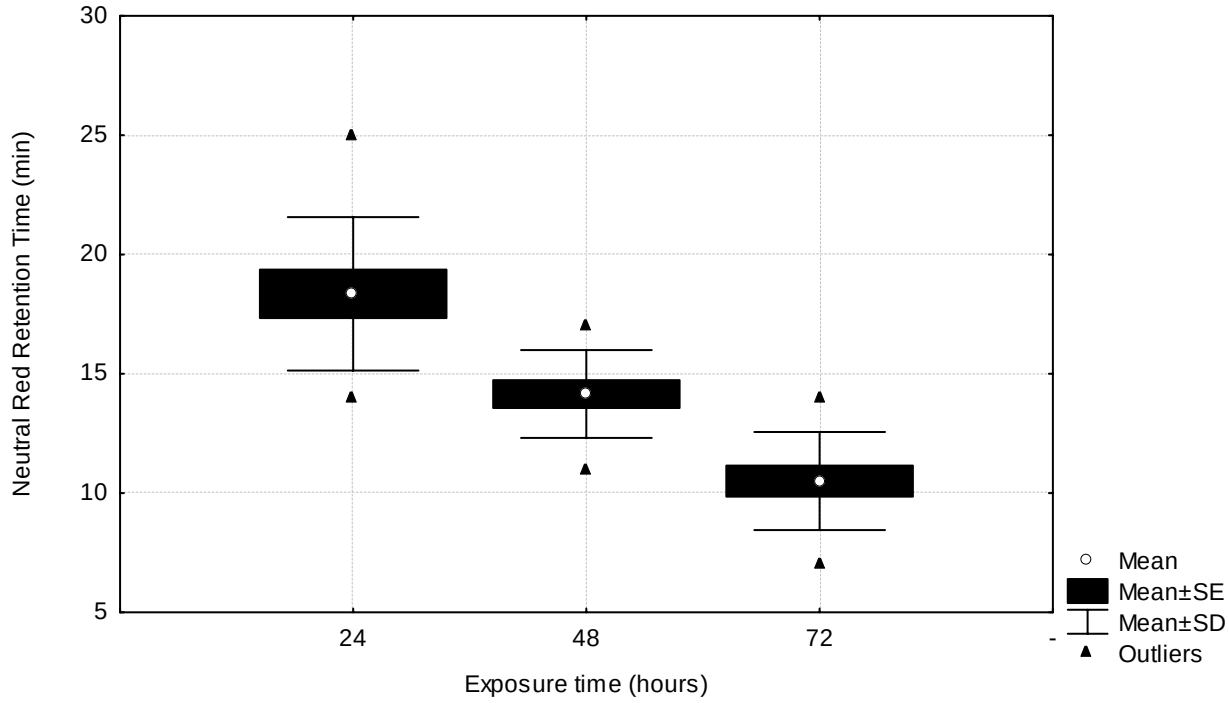
Appendix Figure 13: Neutral red retention times (min) for the control group of *S. longicosta* done in conjunction with the exposure experiment at 1 mg/L $CdCl_2$ over three exposure times (hours).



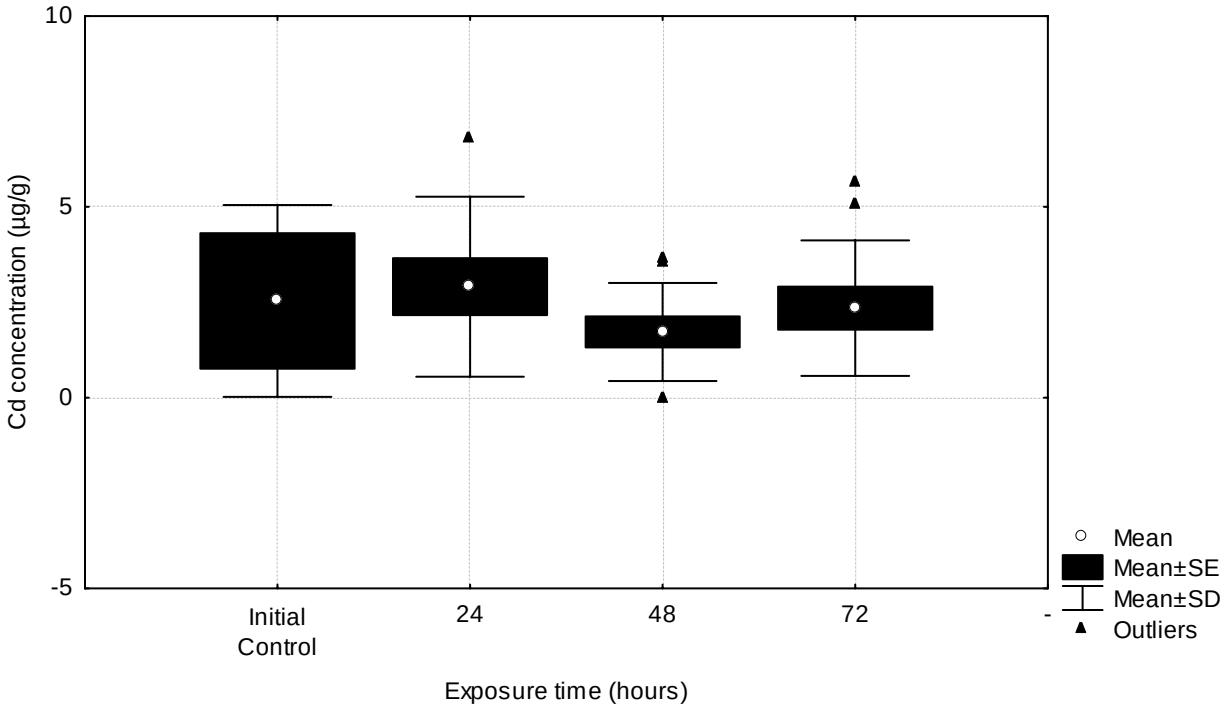
Appendix Figure 14: Neutral red retention times (min) for *S. longicosta* over three exposure times (hours) at 1 mg/L $CdCl_2$ exposure concentration.



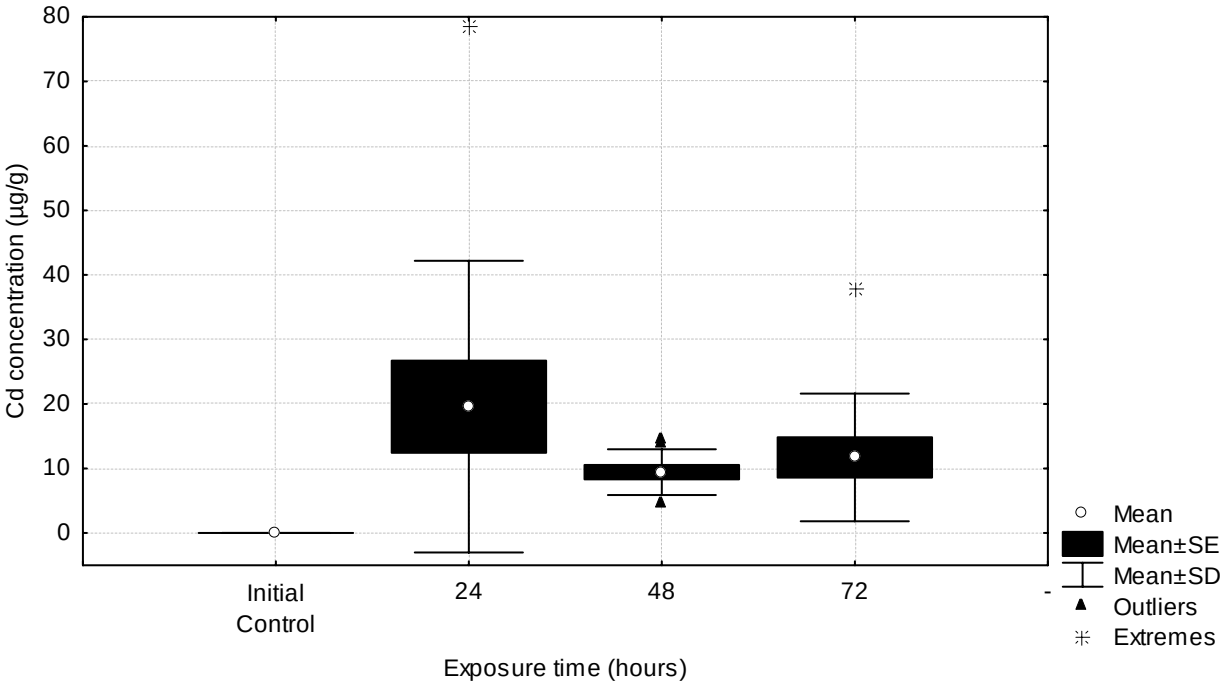
Appendix Figure 15: Neutral red retention times (min) for the control group of *S. longicosta* done in conjunction with the exposure experiment at 1.2 mg/L $CdCl_2$ over three exposure times (hours).



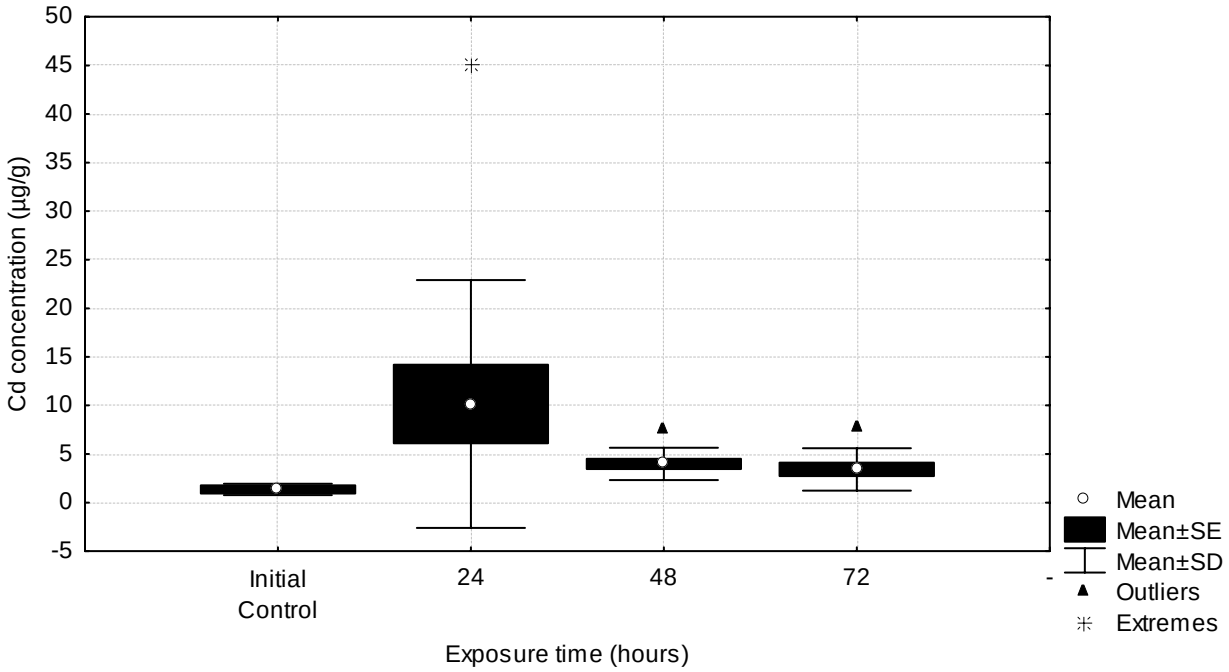
Appendix Figure 16: Neutral red retention times (min) for *S. longicosta* over three exposure times (hours) at 1.2 mg/L $CdCl_2$ exposure concentration.



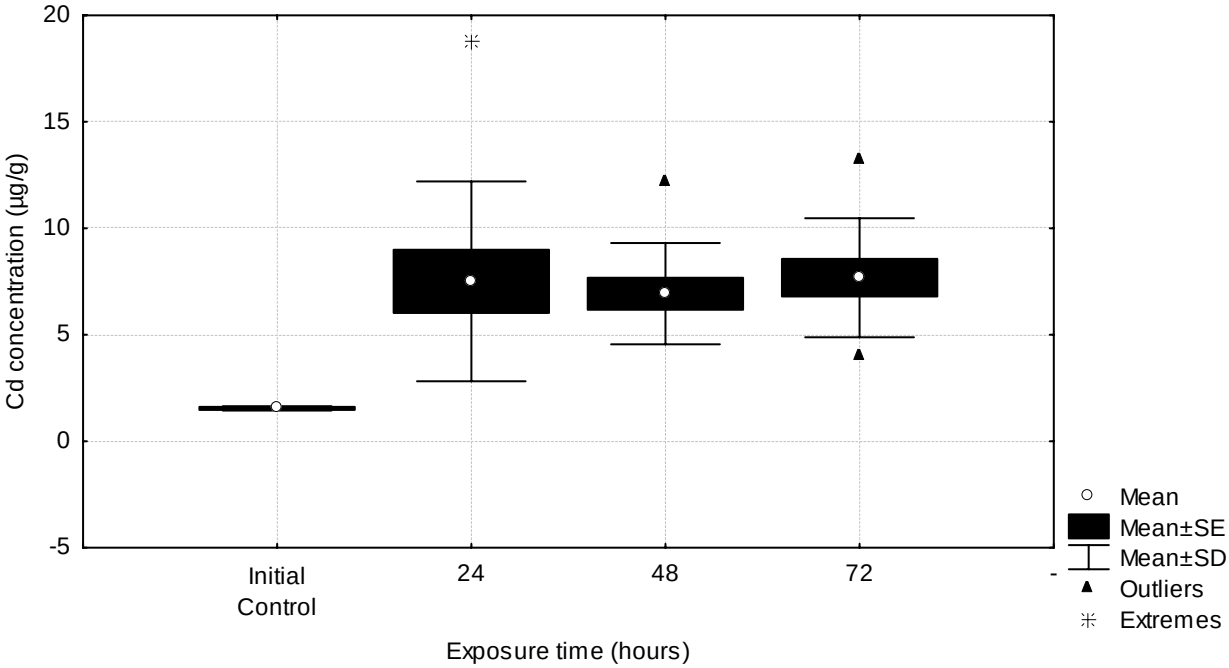
Appendix Figure 17: Heavy metal body concentrations (µg/g) of *S. longicosta* for the control group done in conjunction with the 0.8 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



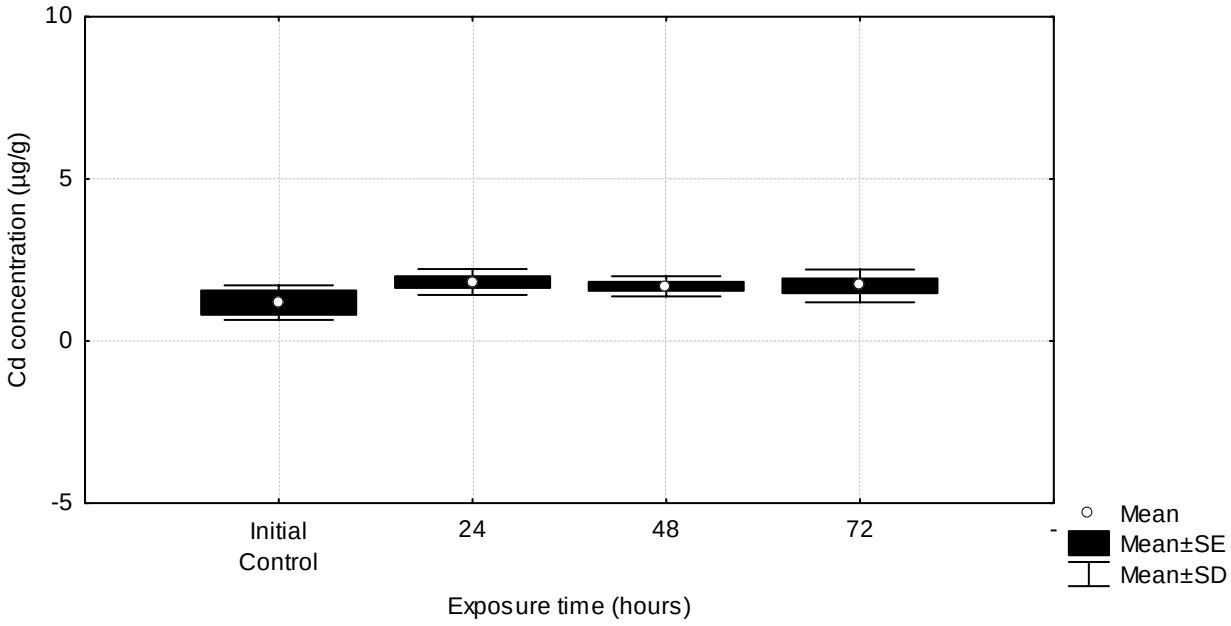
Appendix Figure 18: Heavy metal body concentrations (µg/g) of *S. longicosta* over three exposure times (hours) at 0.8 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



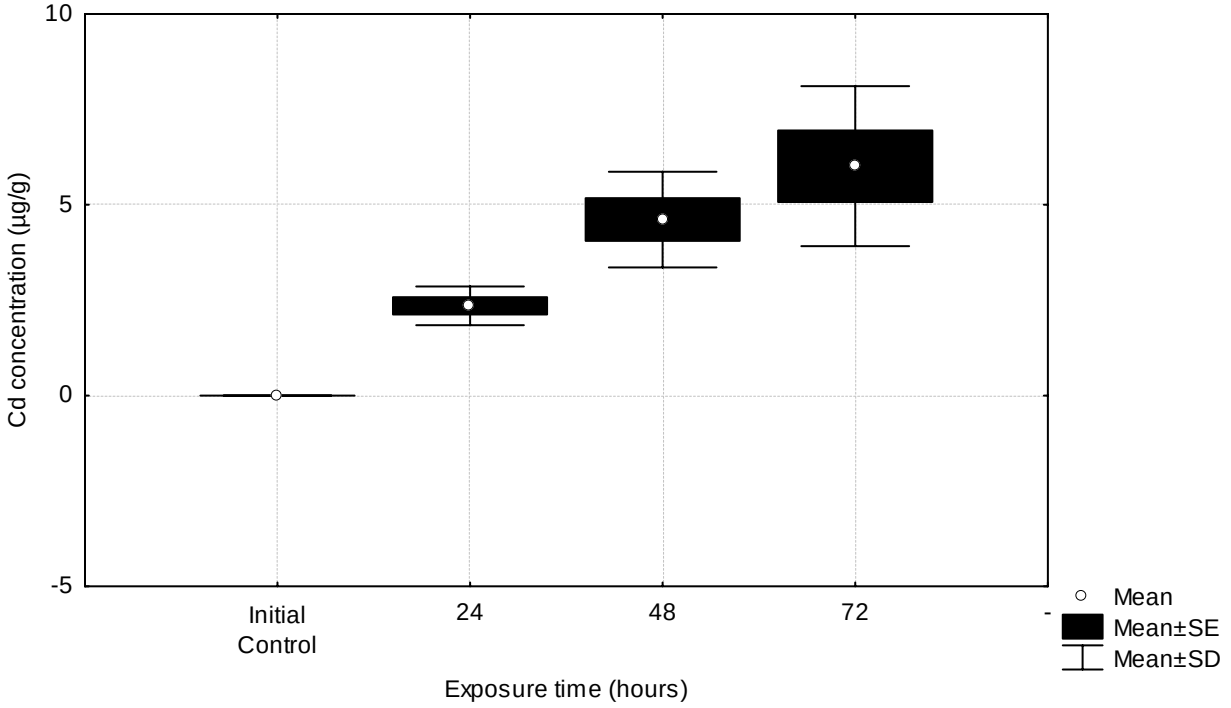
Appendix Figure 19: Heavy metal body concentrations (µg/g) of *S. longicosta* for the control group done in conjunction with the 1 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



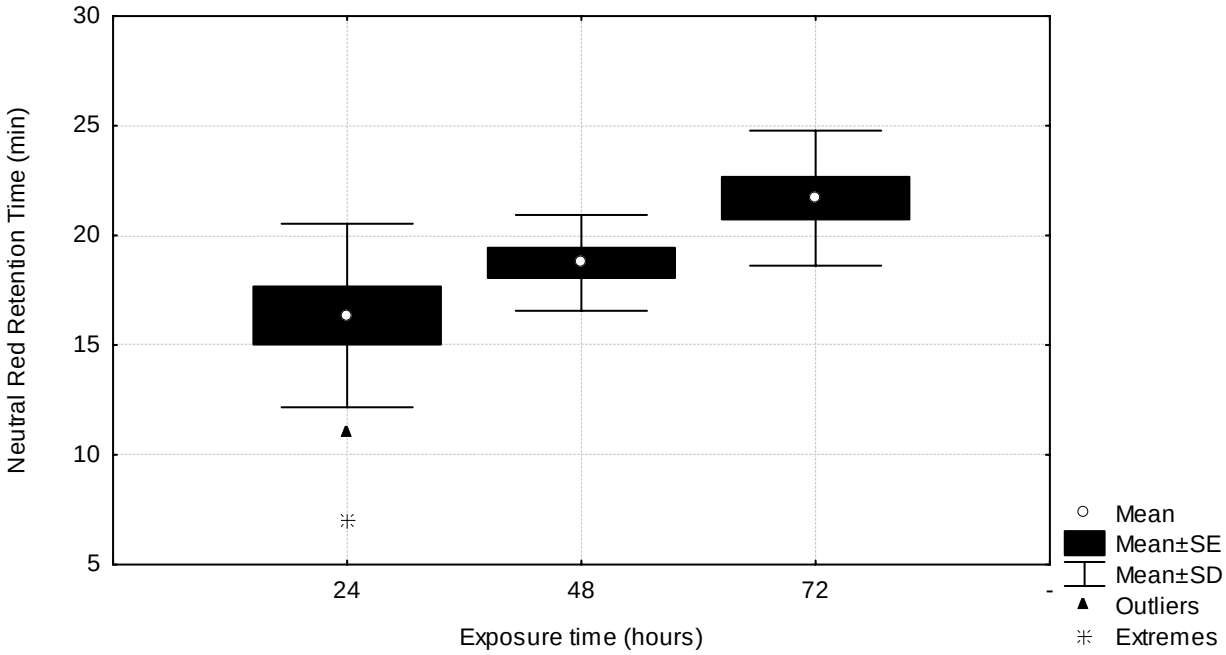
Appendix Figure 20: Heavy metal body concentrations (µg/g) of *S. longicosta* over three exposure times (hours) at 1 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



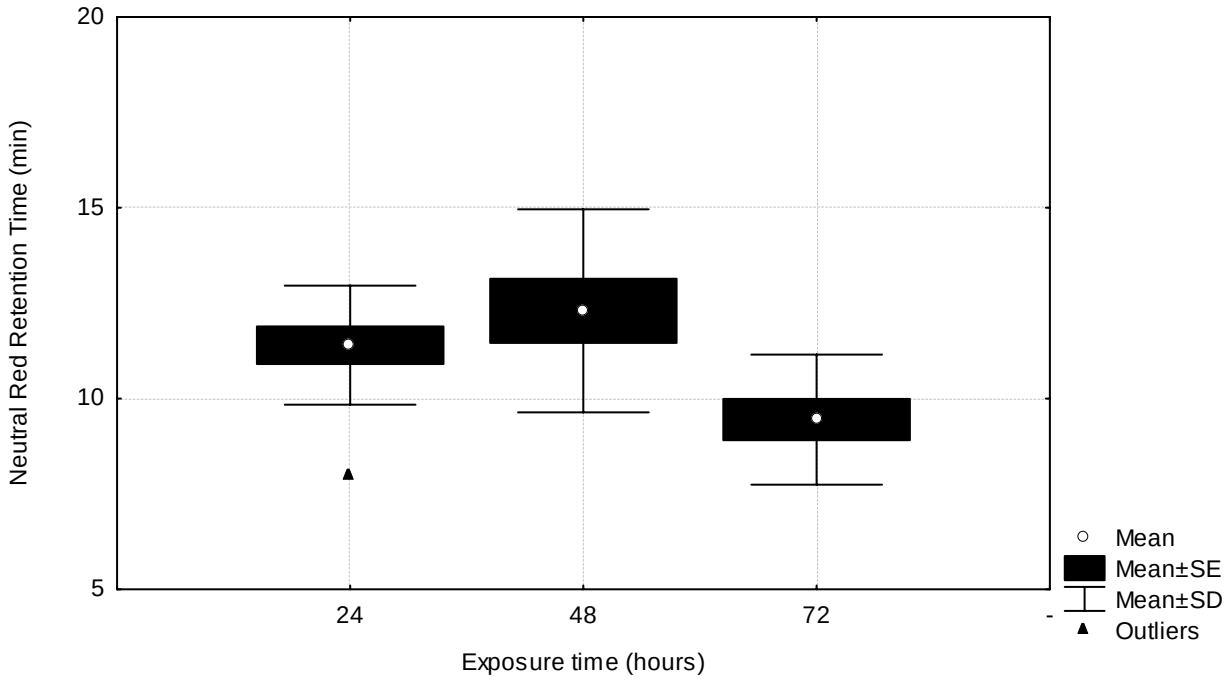
Appendix Figure 21: Heavy metal body concentrations (µg/g) of *S. longicosta* for the control group done in conjunction with the 1.2 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



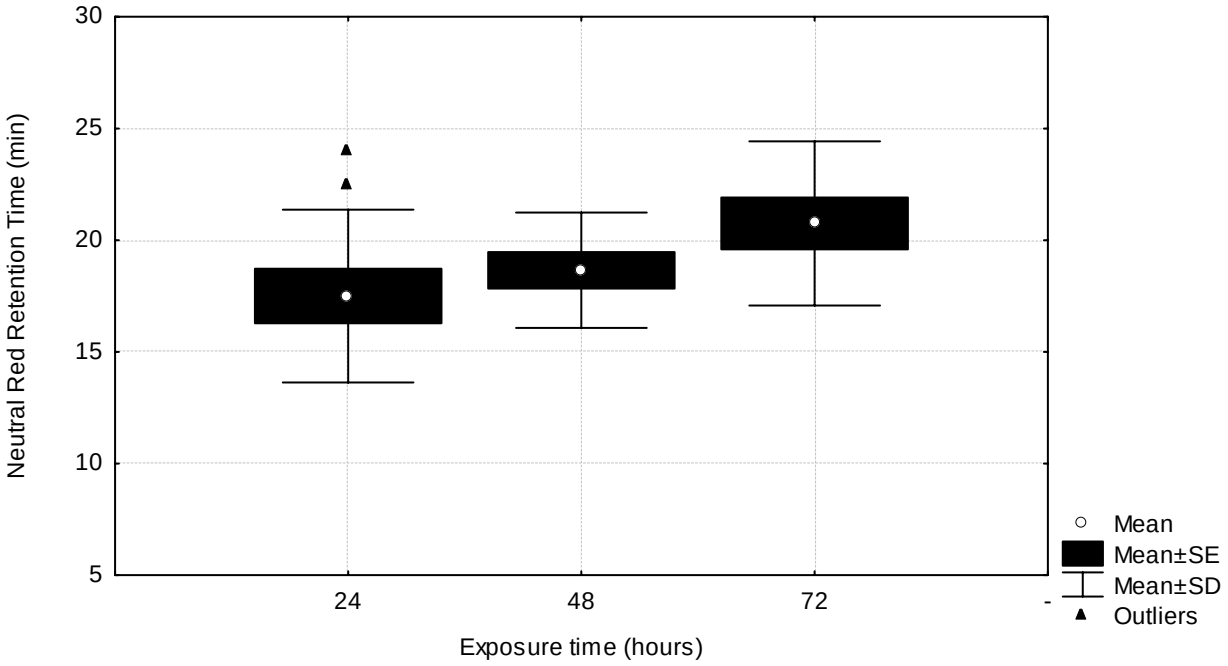
Appendix Figure 22: Heavy metal body concentrations (µg/g) of *S. longicosta* over three exposure times (hours) at 1.2 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



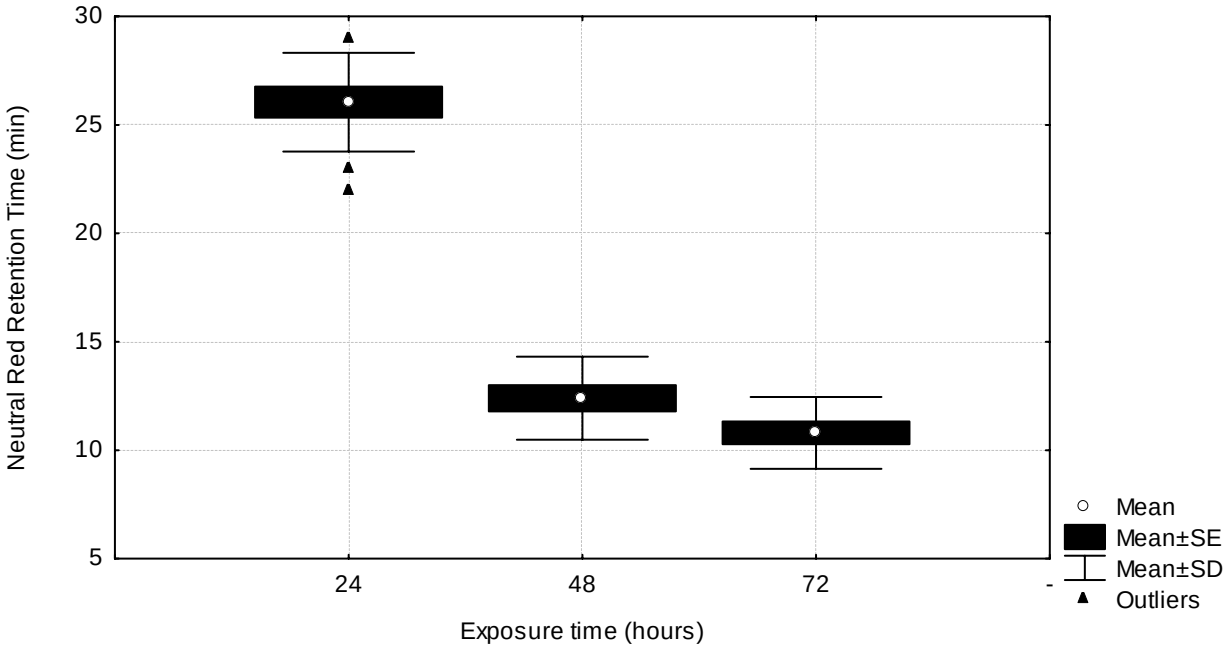
Appendix Figure 23: Neutral red retention times (min) for the control group of *S. granularis* done in conjunction with the exposure experiment at 0.8 mg/L $CdCl_2$ over three exposure times (hours).



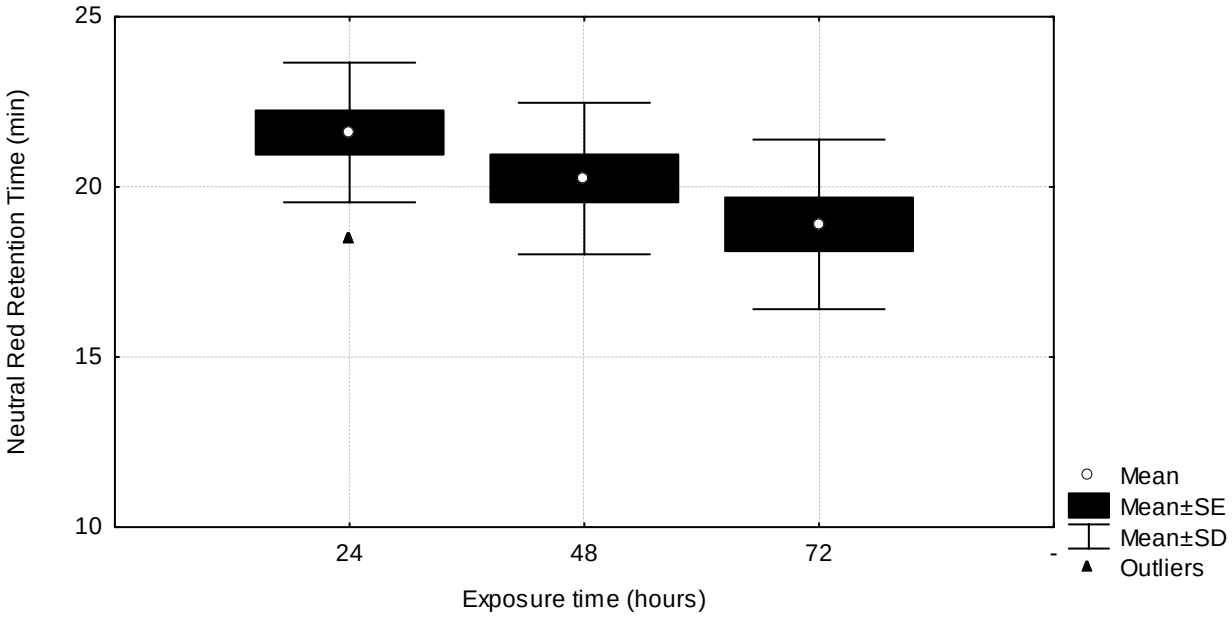
Appendix Figure 24: Neutral red retention times (min) for *S. granularis* over three exposure times (hours) at 0.8 mg/L $CdCl_2$ exposure concentration.



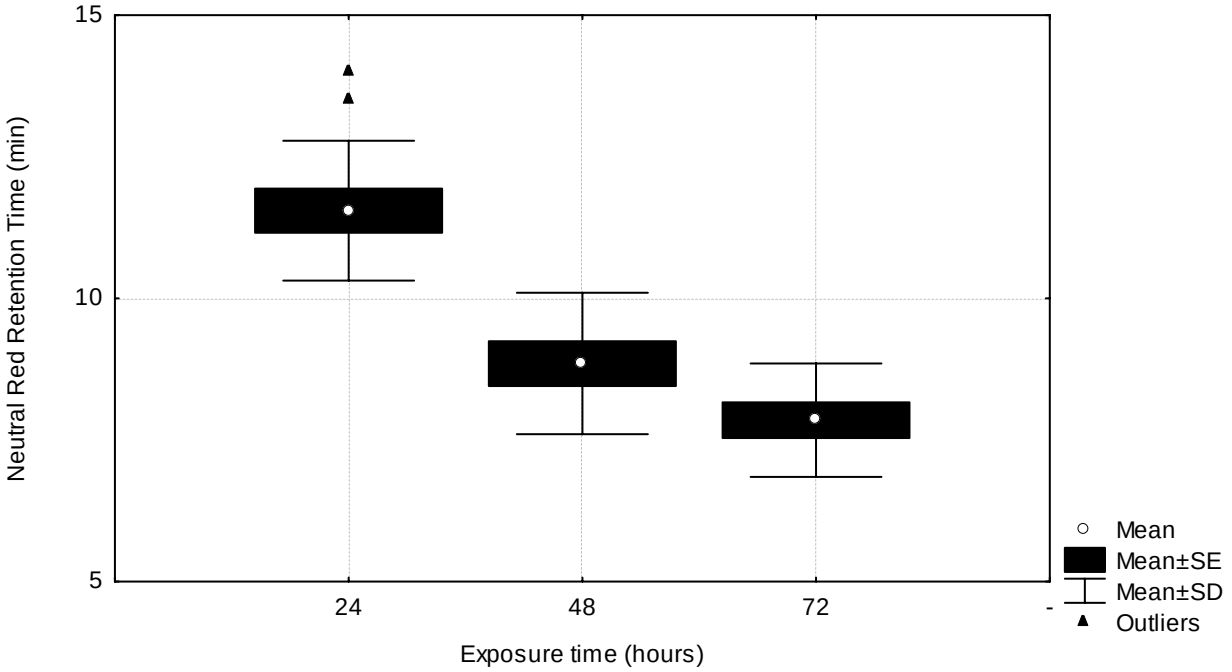
Appendix Figure 25: Neutral red retention times (min) for the control group of *S. granularis* done in conjunction with the exposure experiment at 1 mg/L $CdCl_2$ over three exposure times (hours).



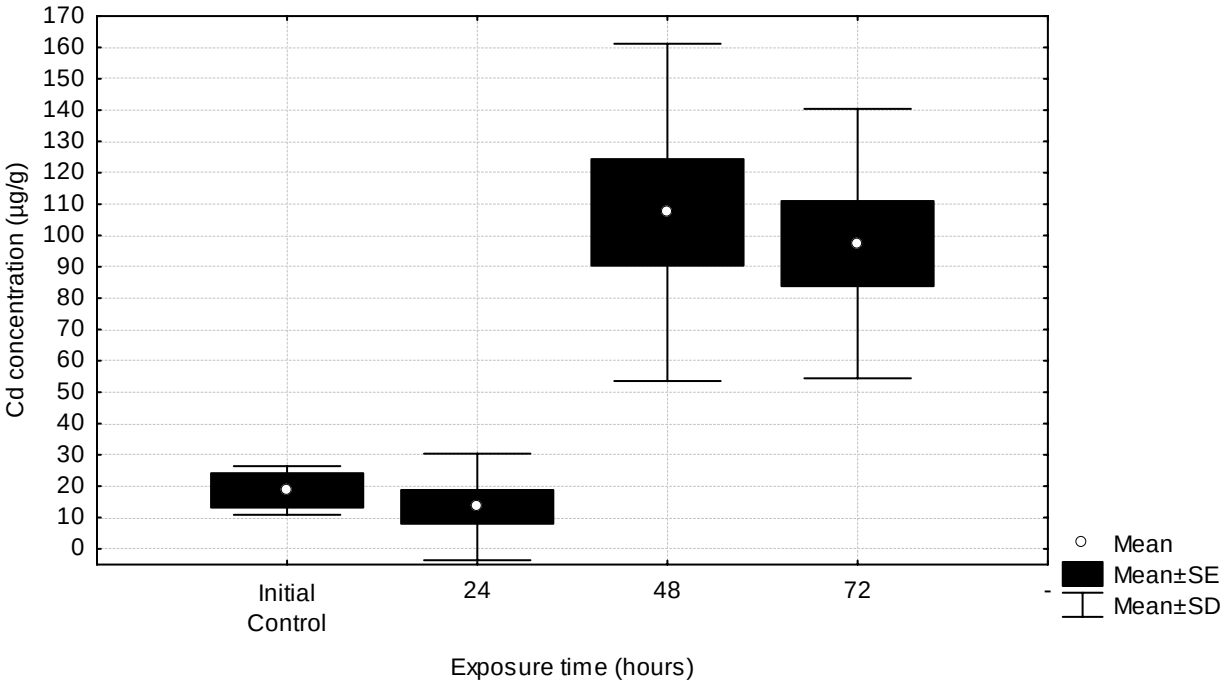
Appendix Figure 26: Neutral red retention times (min) for *S. granularis* over three exposure times (hours) at 1 mg/L $CdCl_2$ exposure concentration.



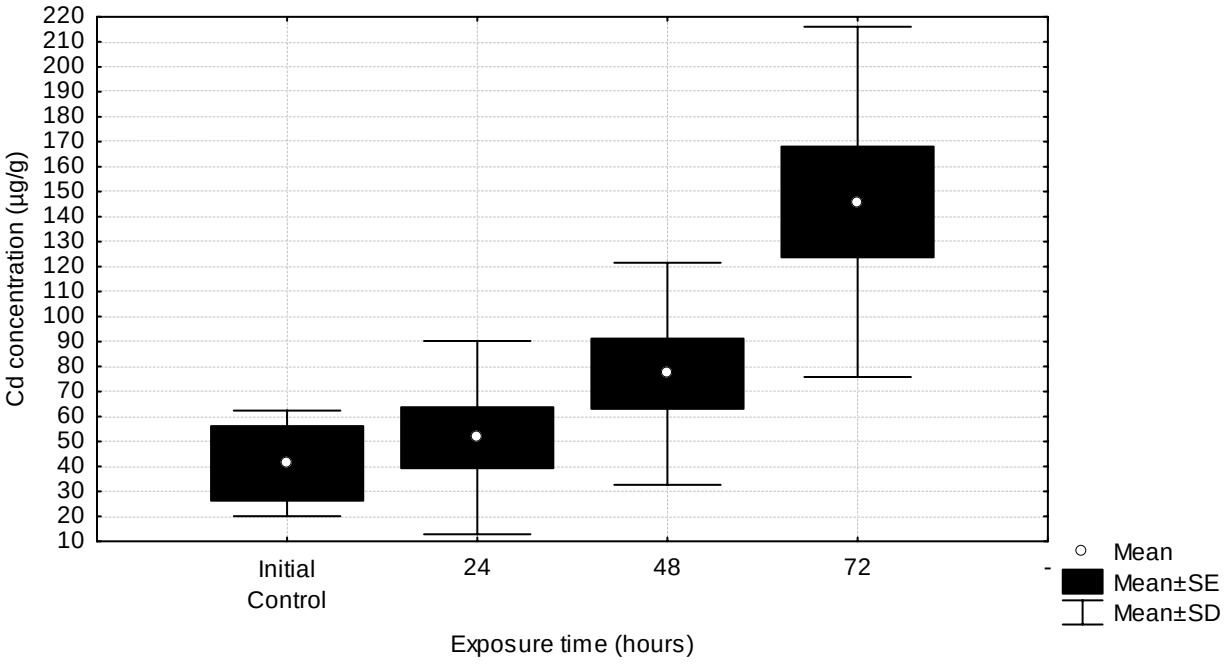
Appendix Figure 27: Neutral red retention times (min) for the control group of *S. granularis* done in conjunction with the exposure experiment at 1.2 mg/L $CdCl_2$ over three exposure times (hours).



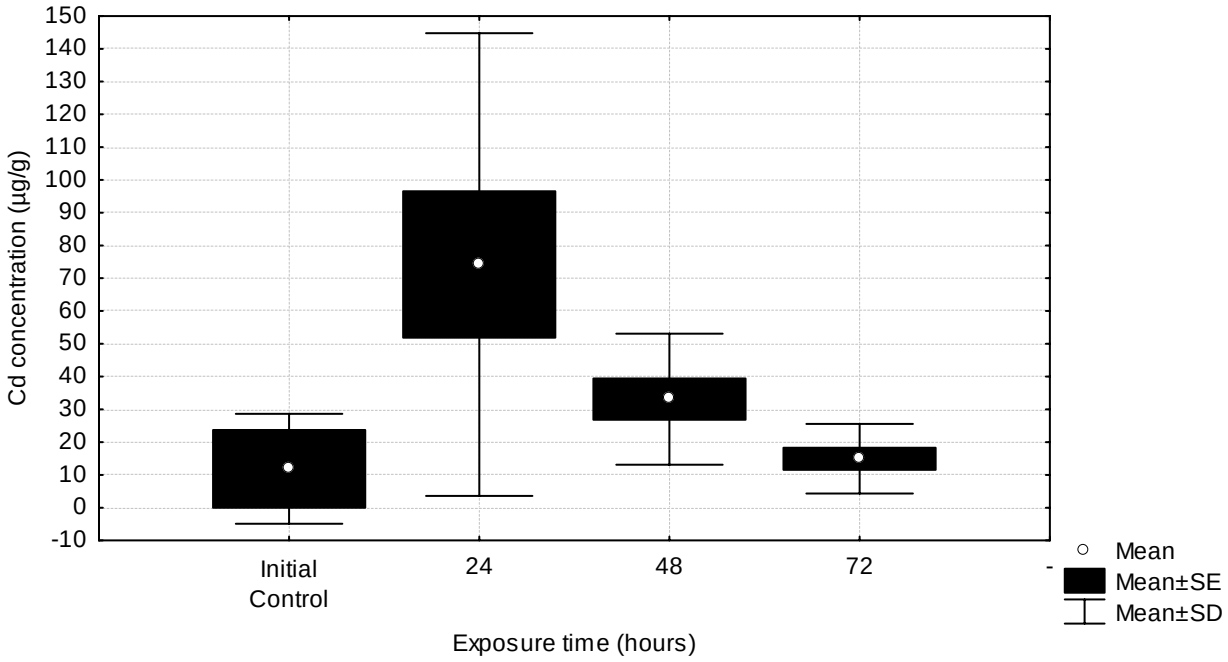
Appendix Figure 28: Neutral red retention times (min) for *S. granularis* over three exposure times (hours) at 1.2 mg/L $CdCl_2$ exposure concentration.



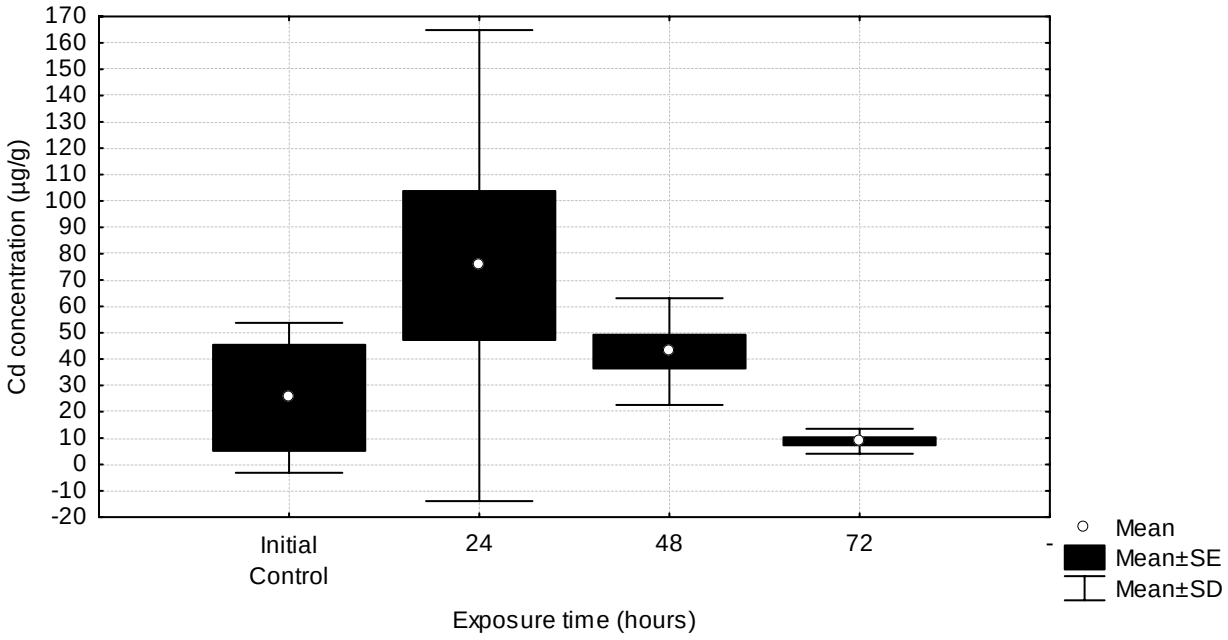
Appendix Figure 29: Heavy metal body concentrations (µg/g) of *S. granularis* for the control group done in conjunction with the 0.8 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



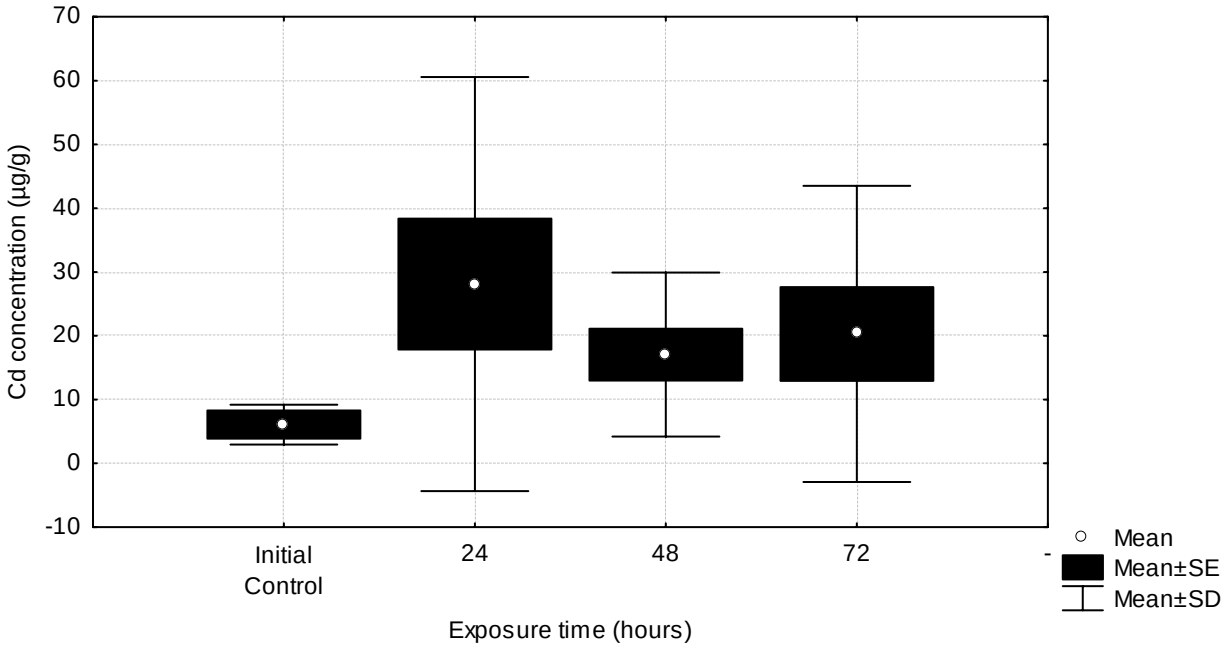
Appendix Figure 30: Heavy metal body concentrations (µg/g) of *S. granularis* over three exposure times (hours) at 0.8 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



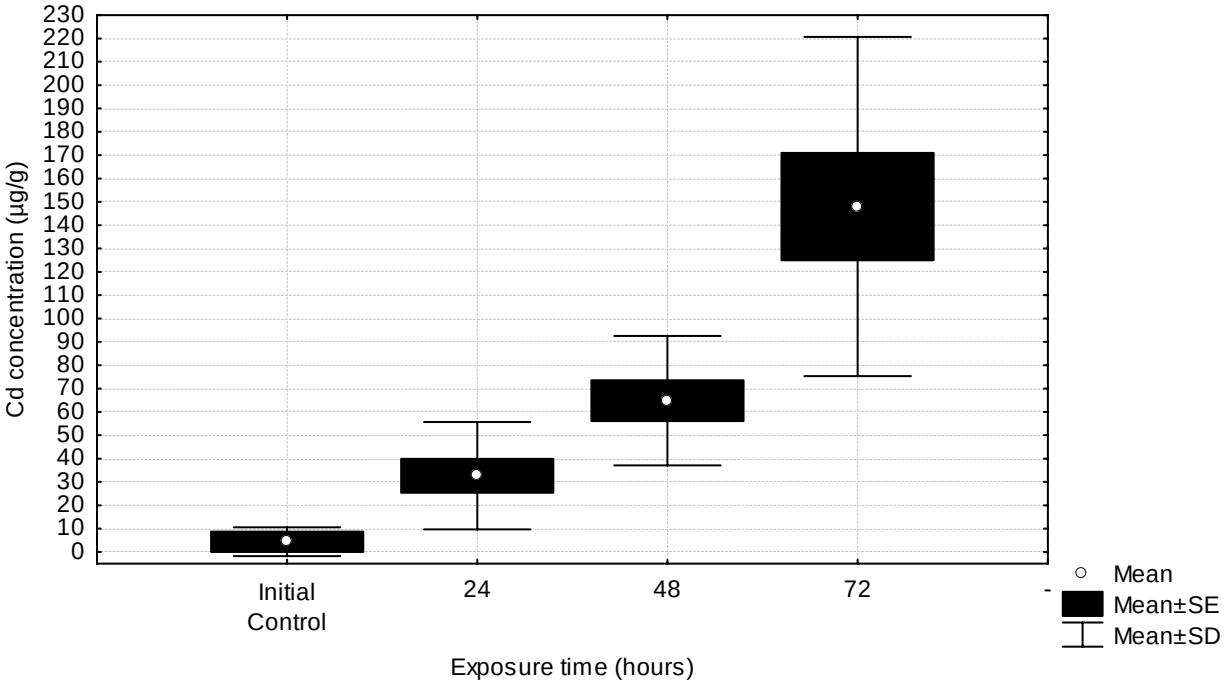
Appendix Figure 31: Heavy metal body concentrations (µg/g) of *S. granularis* for the control group done in conjunction with the 1 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



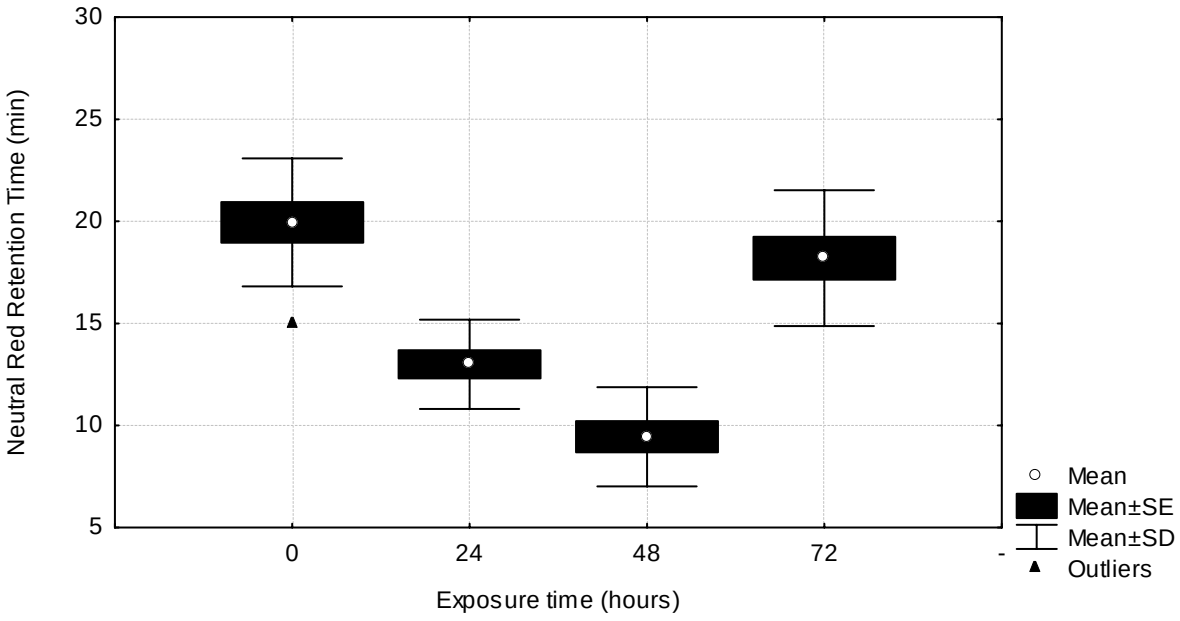
Appendix Figure 32: Heavy metal body concentrations (µg/g) of *S. granularis* over three exposure times (hours) at 1 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



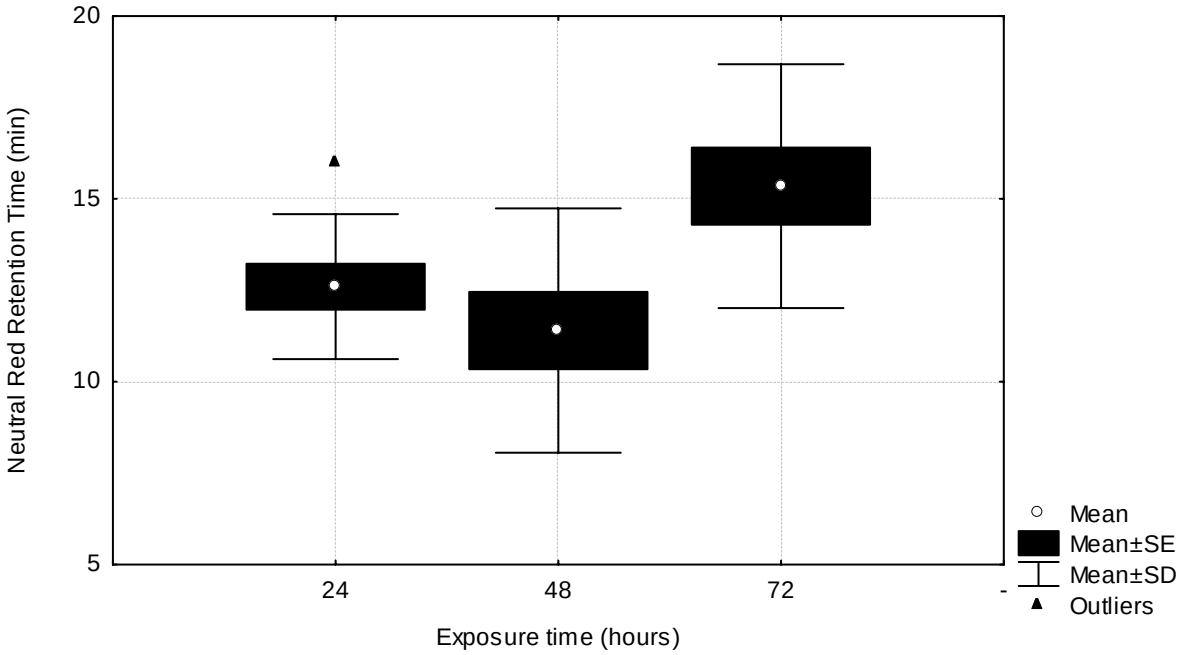
Appendix Figure 33: Heavy metal body concentrations (µg/g) of *S. granularis* for the control group done in conjunction with the 1.2 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



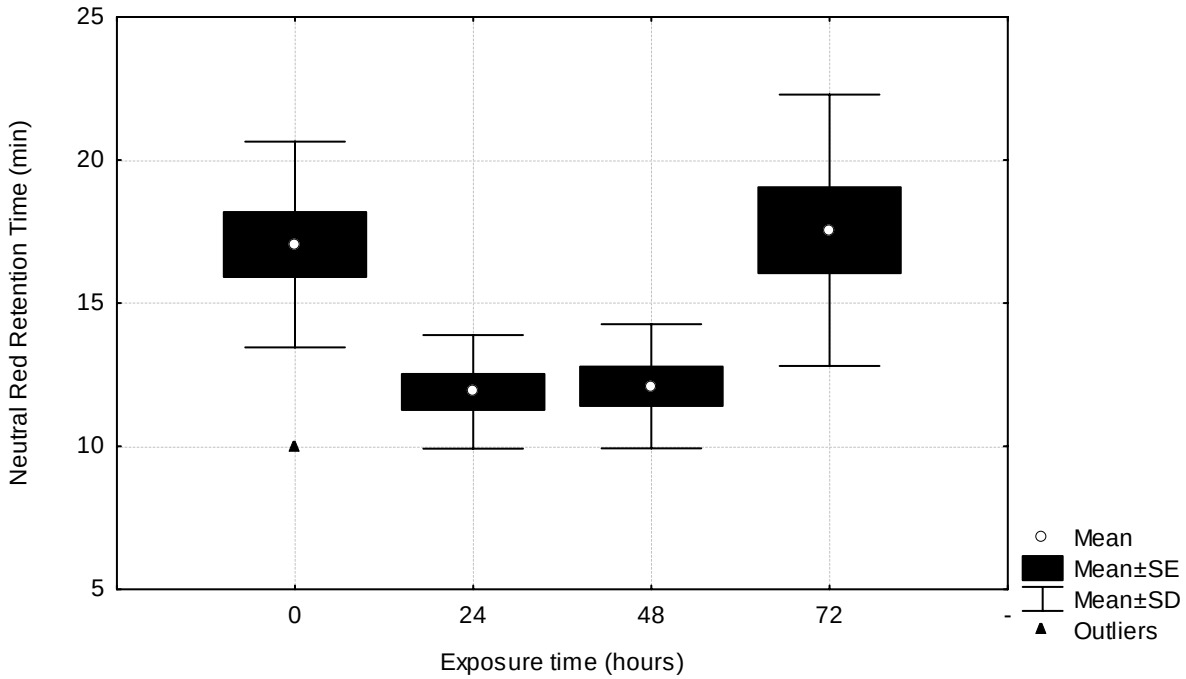
Appendix Figure 34: Heavy metal body concentrations (µg/g) of *S. granularis* over three exposure times (hours) at 1.2 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



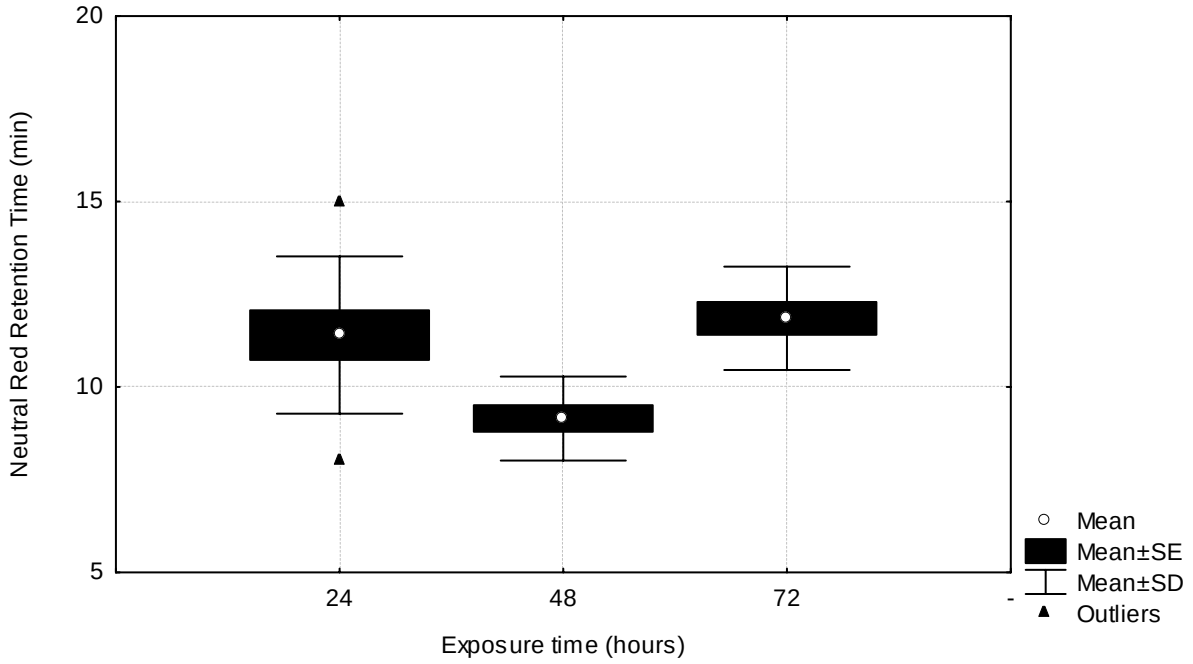
Appendix Figure 35: Neutral red retention times (min) for the control group of *C. granatina* done in conjunction with the exposure experiment at 0.8 mg/L $CdCl_2$ over three exposure times (hours). Graph includes field control at 0 hours.



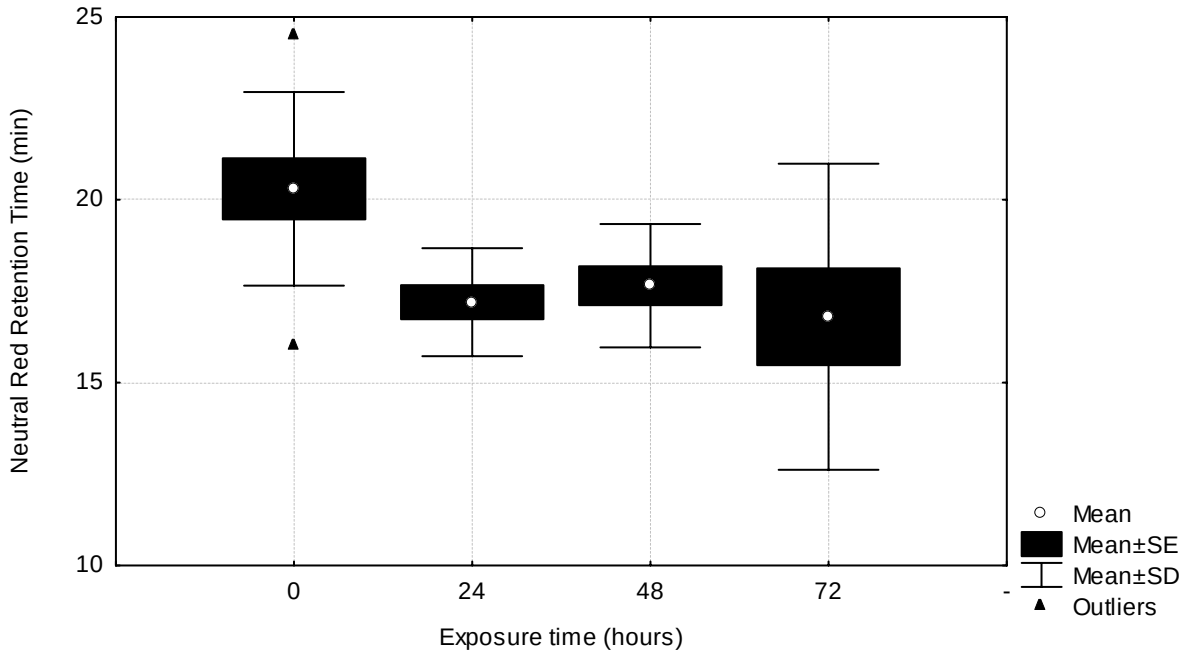
Appendix Figure 36: Neutral red retention times (min) for *C. granatina* over three exposure times (hours) at 0.8 mg/L $CdCl_2$ exposure concentration.



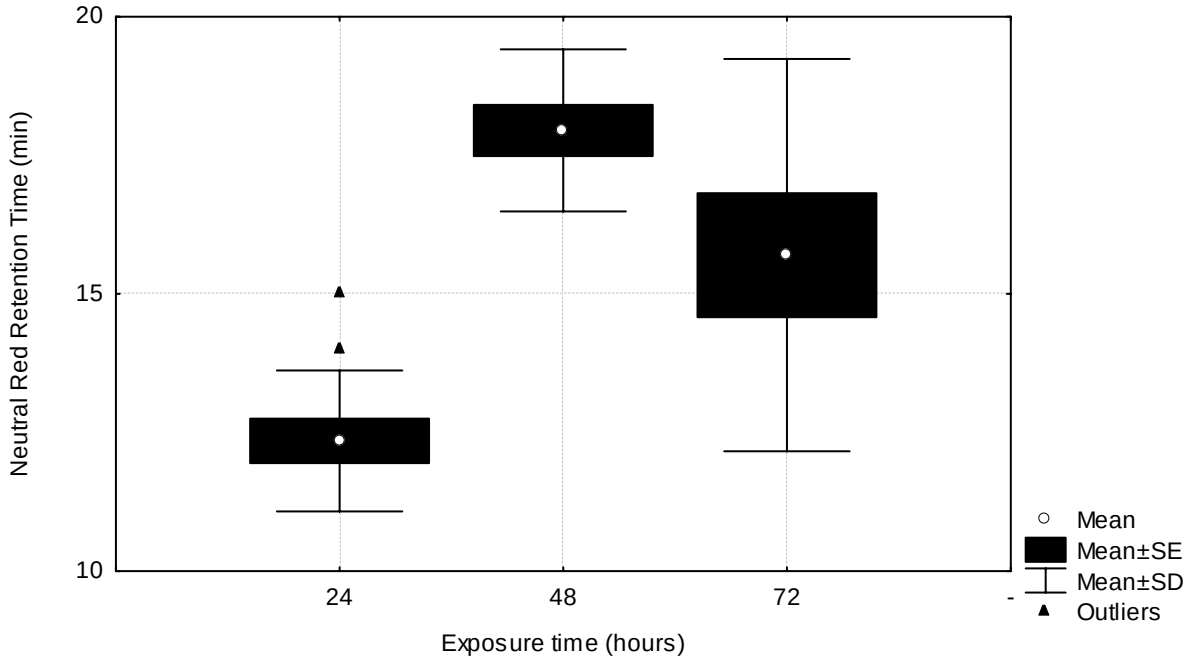
Appendix Figure 37: Neutral red retention times (min) for the control group of *C. granatina* done in conjunction with the exposure experiment at 1 mg/L $CdCl_2$ over three exposure times (hours). Graph includes field control at 0 hours.



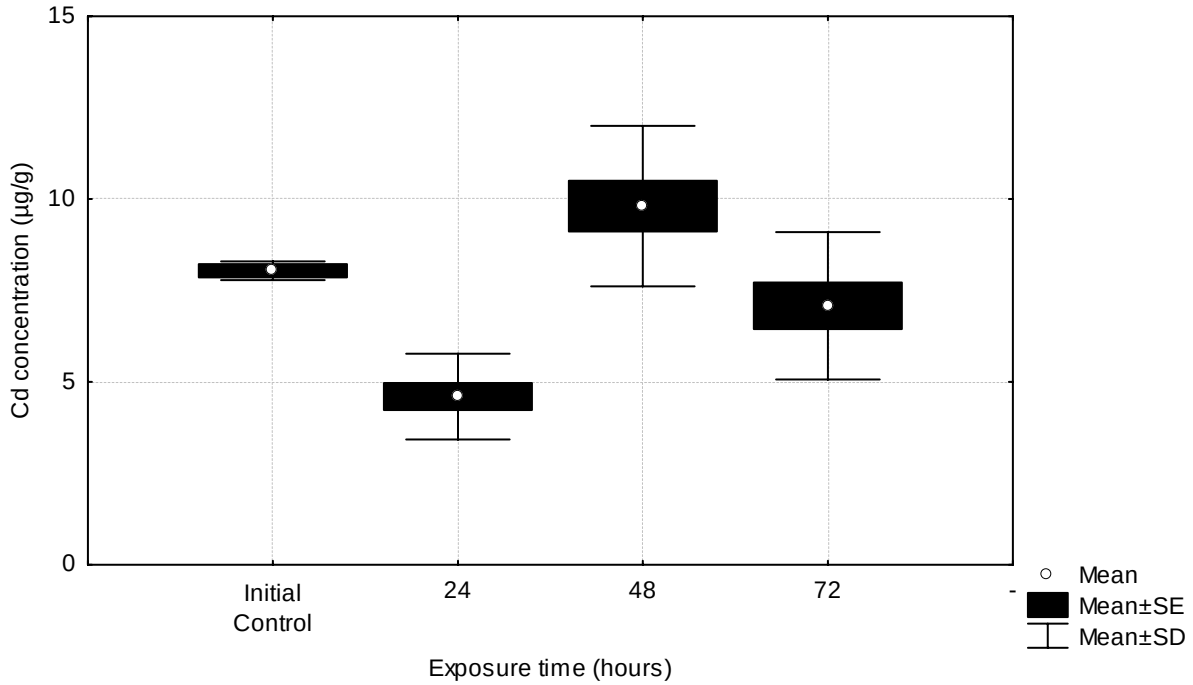
Appendix Figure 38: Neutral red retention times (min) for *C. granatina* over three exposure times (hours) at 1 mg/L $CdCl_2$ exposure concentration.



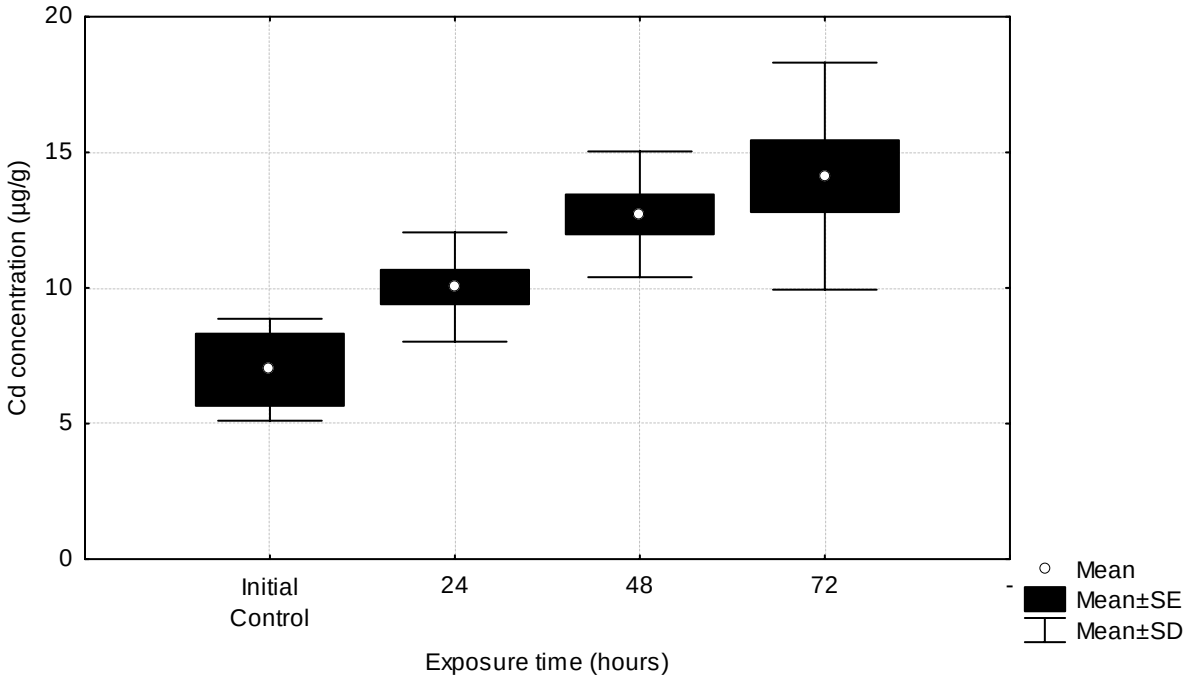
Appendix Figure 39: Neutral red retention times (min) for the control group of *C. granatina* done in conjunction with the exposure experiment at 1.2 mg/L $CdCl_2$ over three exposure times (hours). Graph includes field control at 0 hours.



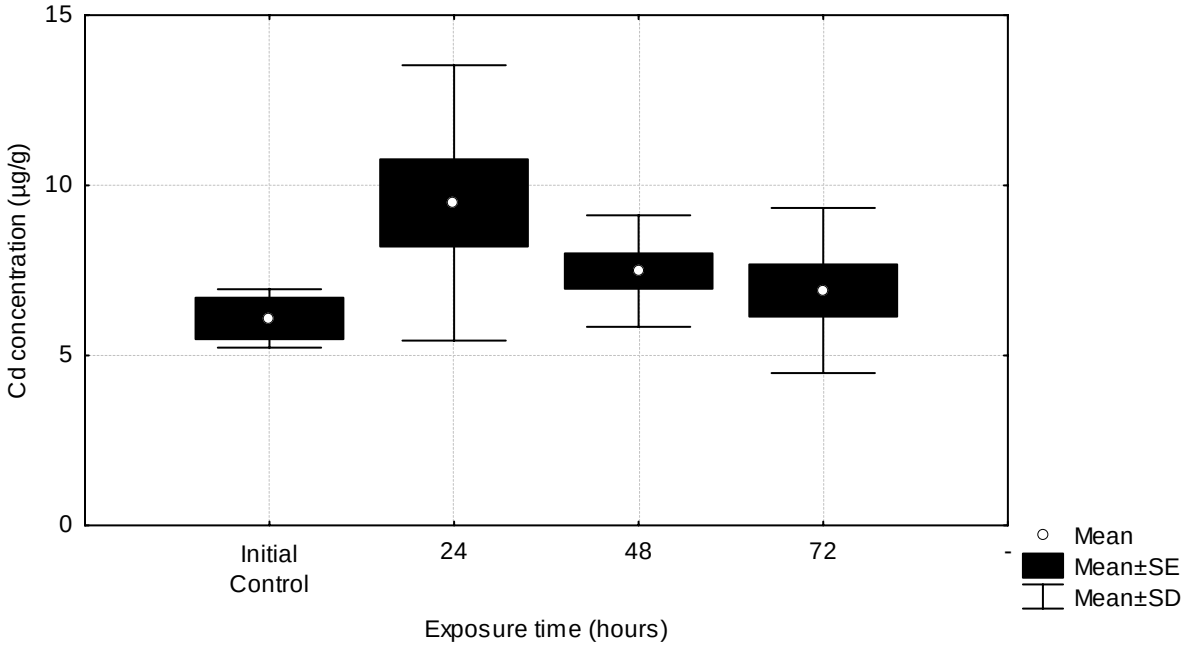
Appendix Figure 40: Neutral red retention times (min) for *C. granatina* over three exposure times (hours) at 1.2 mg/L $CdCl_2$ exposure concentration.



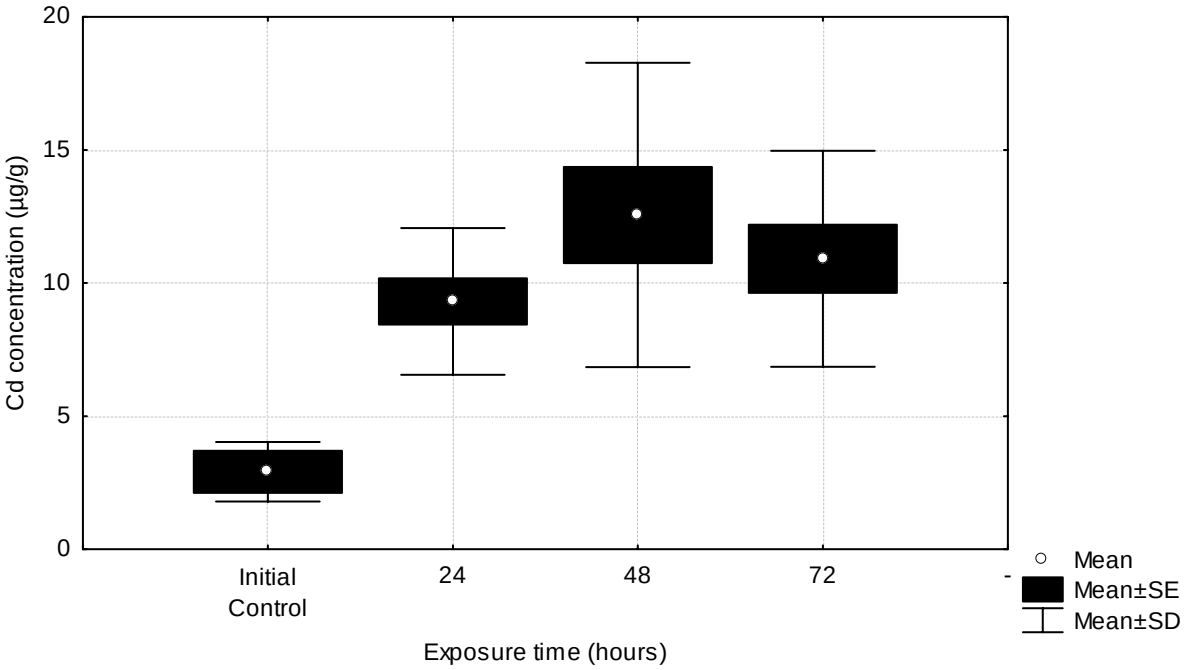
Appendix Figure 41: Heavy metal body concentrations (µg/g) of *C. granatina* for the control group done in conjunction with the 0.8 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



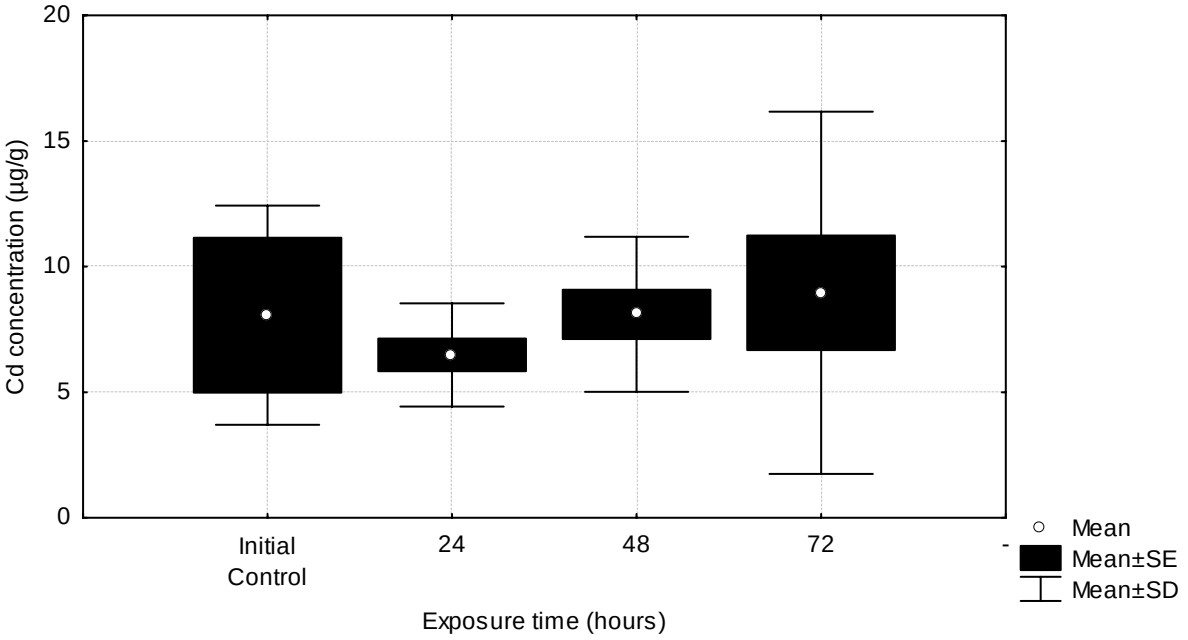
Appendix Figure 42: Heavy metal body concentrations (µg/g) of *C. granatina* over three exposure times (hours) at 0.8 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



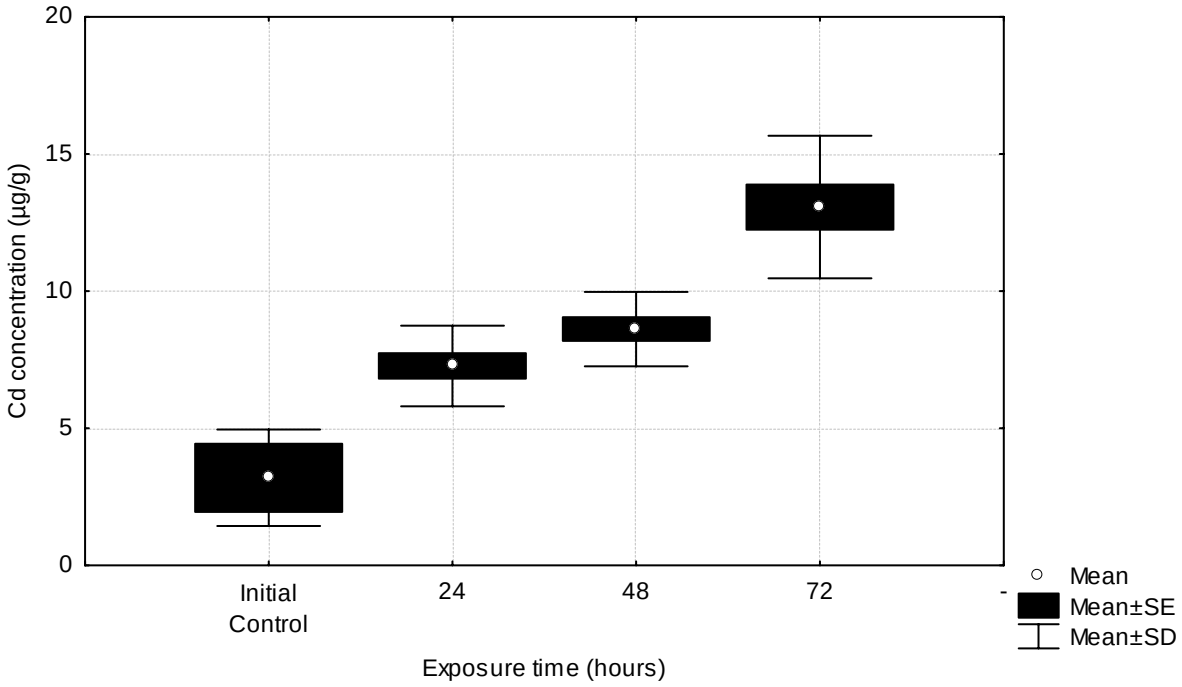
Appendix Figure 43: Heavy metal body concentrations (µg/g) of *C. granatina* for the control group done in conjunction with the 1 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



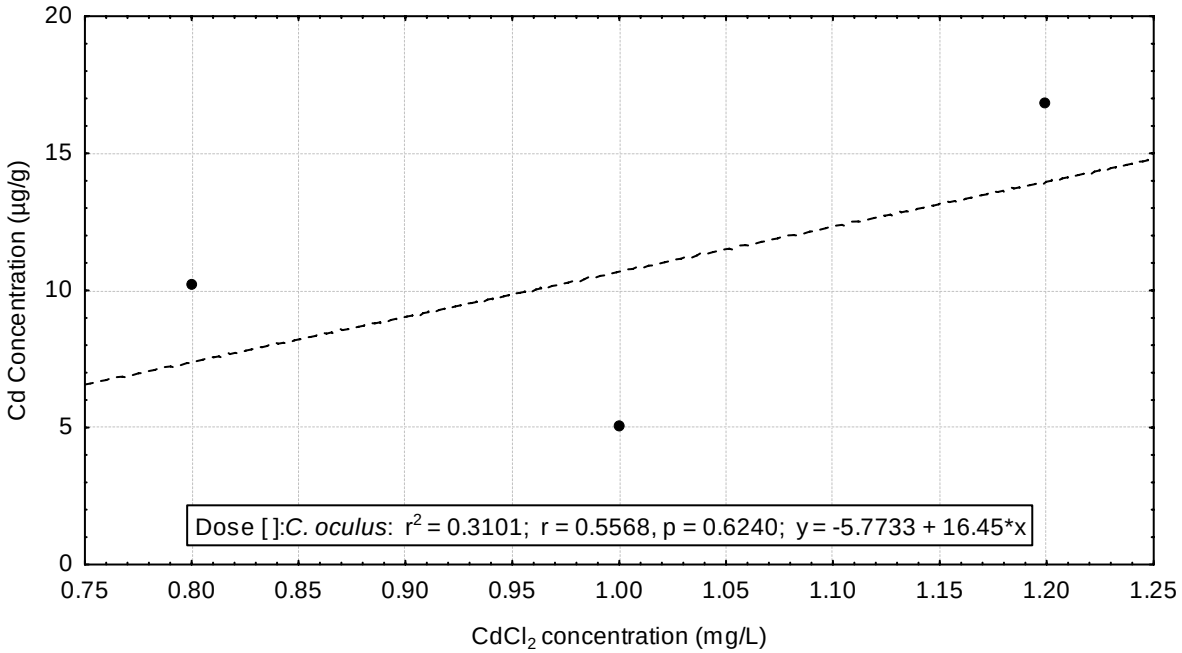
Appendix Figure 44: Heavy metal body concentrations (µg/g) of *C. granatina* over three exposure times (hours) at 1 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



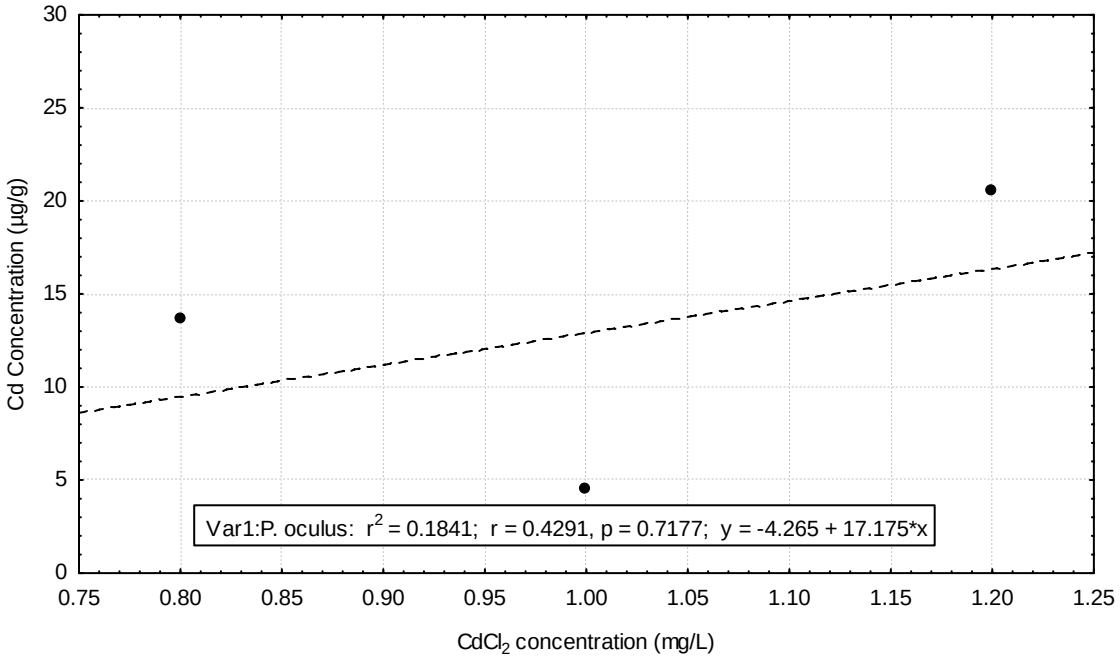
Appendix Figure 45: Heavy metal body concentrations (µg/g) of *C. granatina* for the control group done in conjunction with the 1.2 mg/L $CdCl_2$ exposure experiment. Graph includes initial control done at 0 hours.



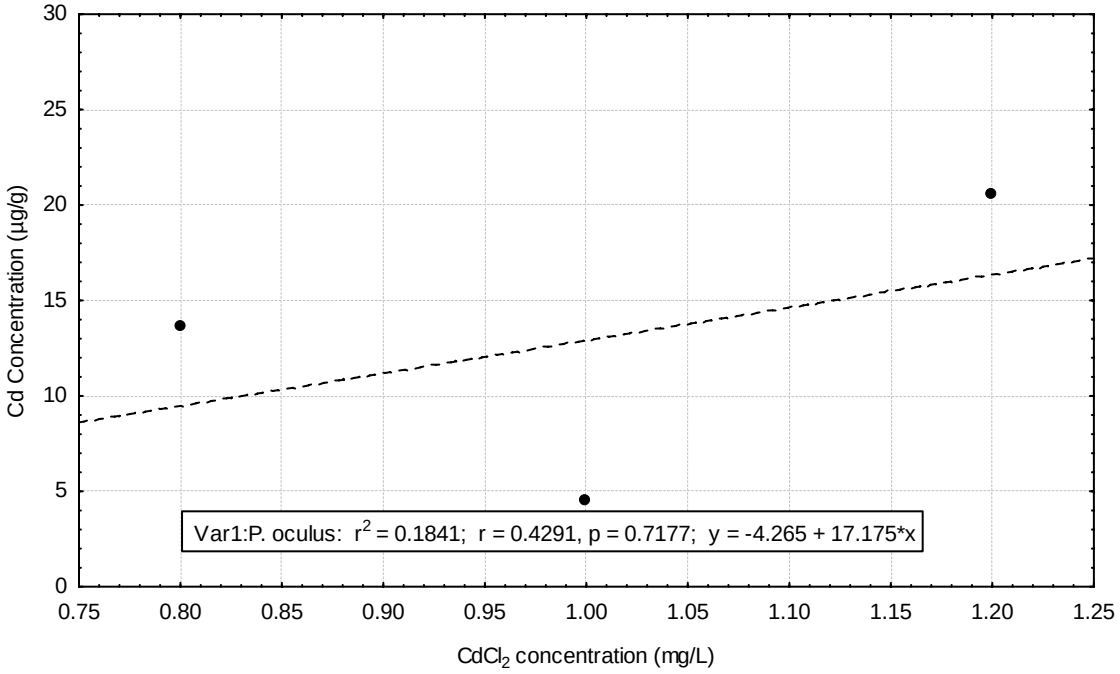
Appendix Figure 46: Heavy metal body concentrations (µg/g) of *C. granatina* over three exposure times (hours) at 1.2 mg/L $CdCl_2$ exposure concentration. Graph includes initial control at 0 hours.



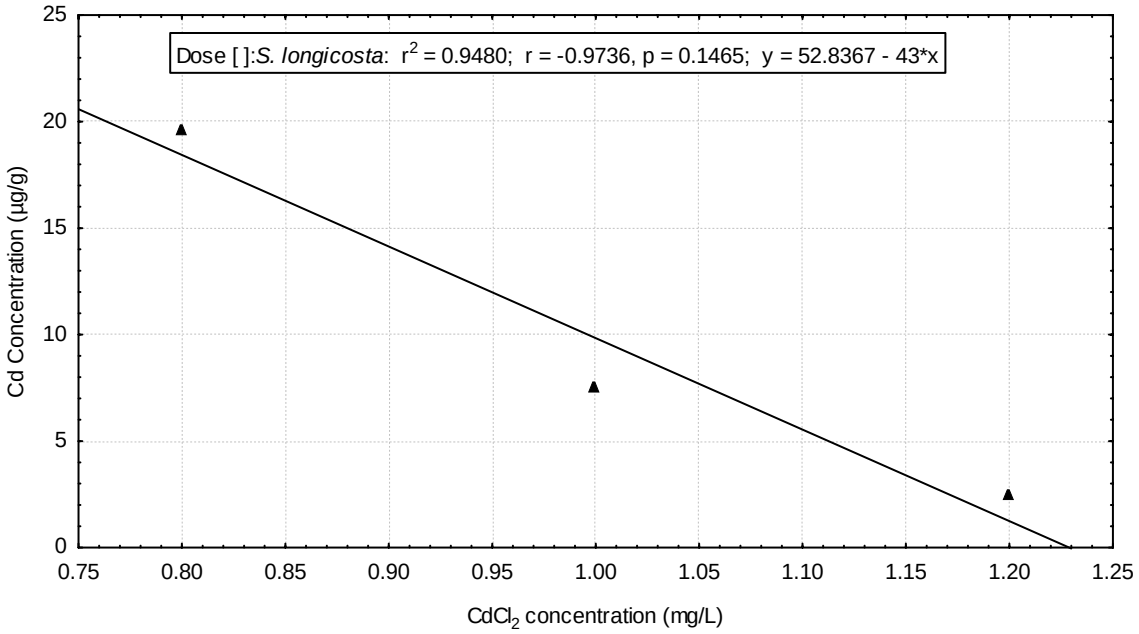
Appendix Figure 47: Mean heavy metal body load concentrations (µg/g) for *C. oculus* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 24 hours exposure time.



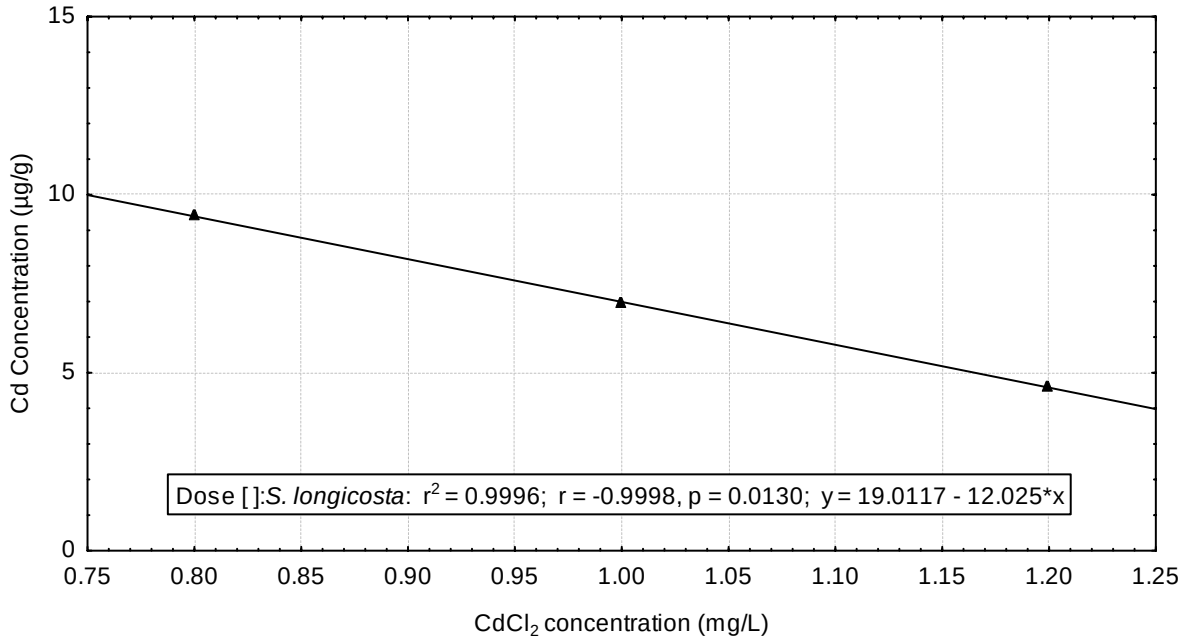
Appendix Figure 48: Mean heavy metal body load concentrations (µg/g) for *C. oculus* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 48 hours exposure time.



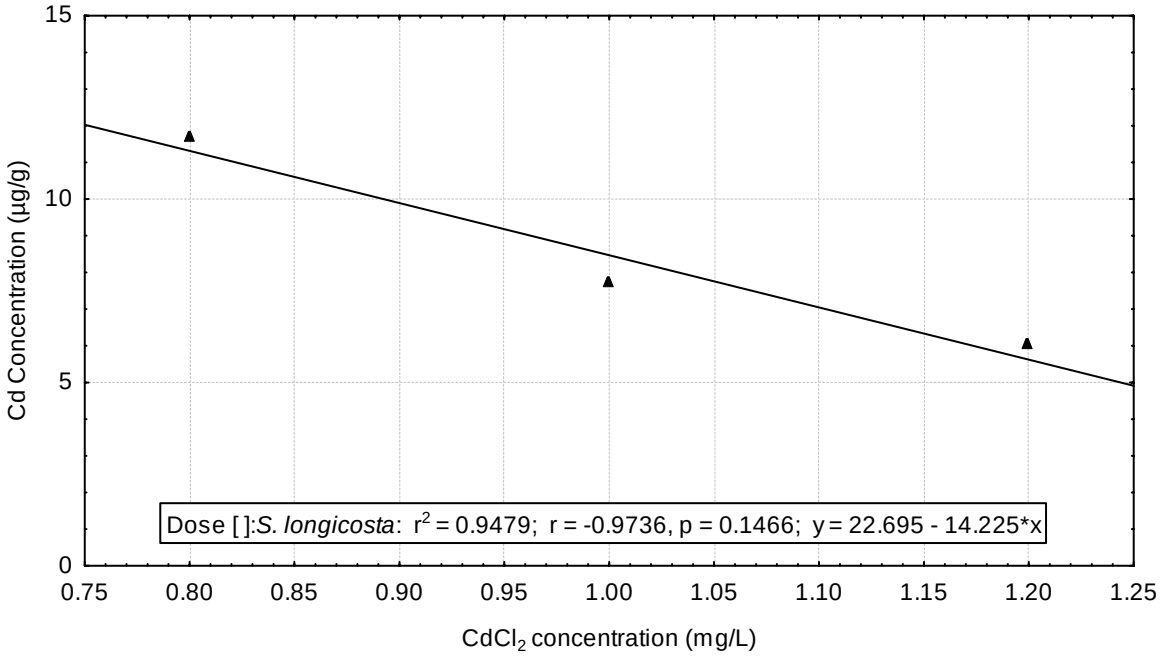
Appendix Figure 49: Mean heavy metal body load concentrations (µg/g) for *C. oculus* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 72 hours exposure time.



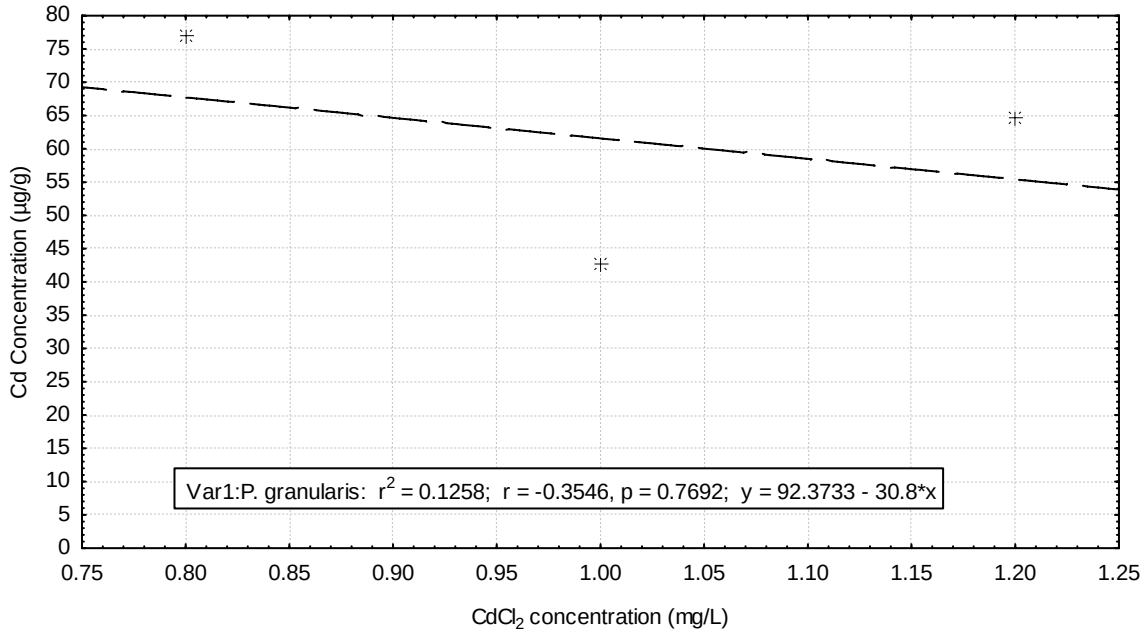
Appendix Figure 50: Mean heavy metal body load concentrations (µg/g) for *S. longicosta* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 24 hours exposure time.



Appendix Figure 51: Mean heavy metal body load concentrations (µg/g) for *S. longicosta* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 48 hours exposure time.

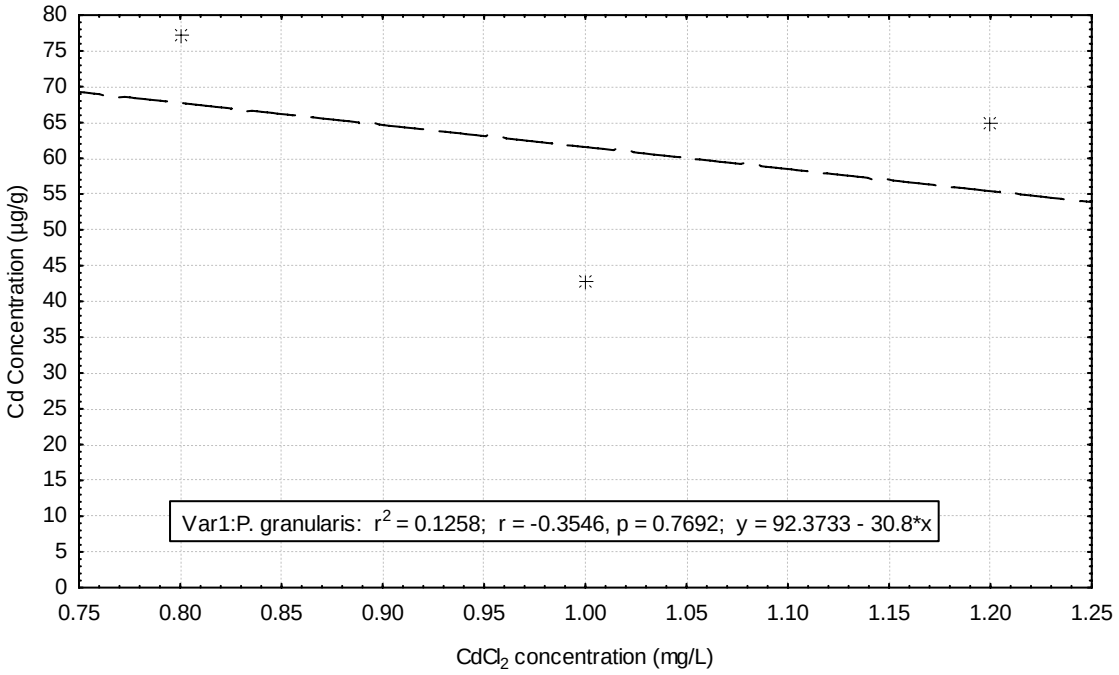


Appendix Figure 52: Mean heavy metal body load concentrations (µg/g) for *S. longicosta* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 72 hours exposure time.

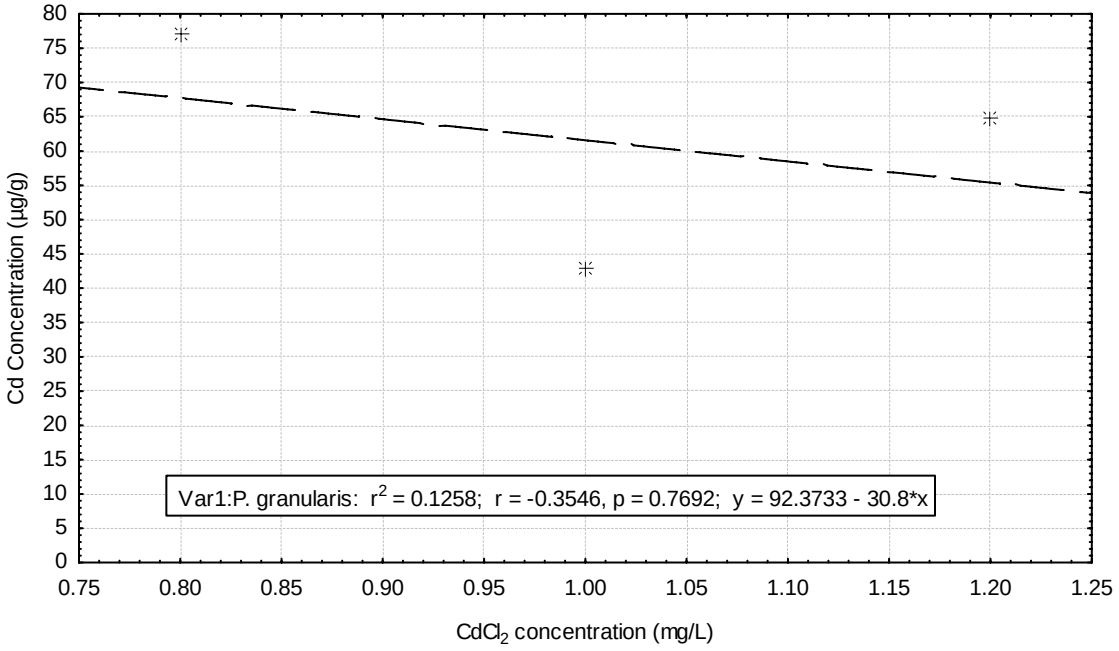


Appendix Figure 53: Mean heavy metal body load concentrations (µg/g) for

S. granularis vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 24 hours exposure time.

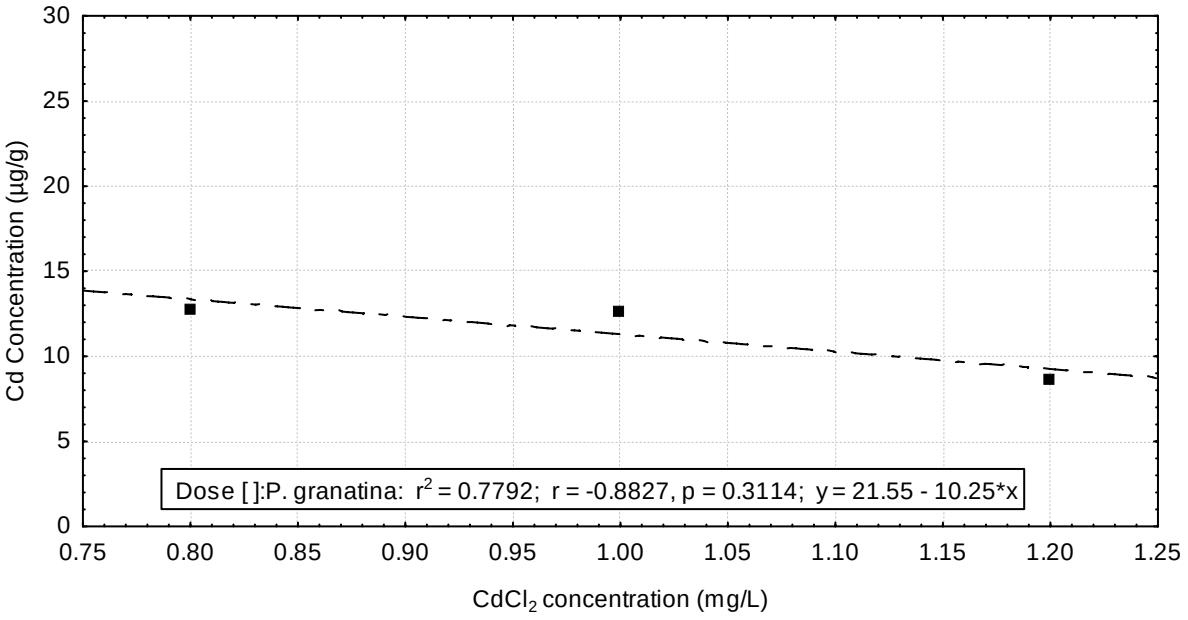


Appendix Figure 54: Mean heavy metal body load concentrations (µg/g) for *S. granularis* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 48 hours exposure time.

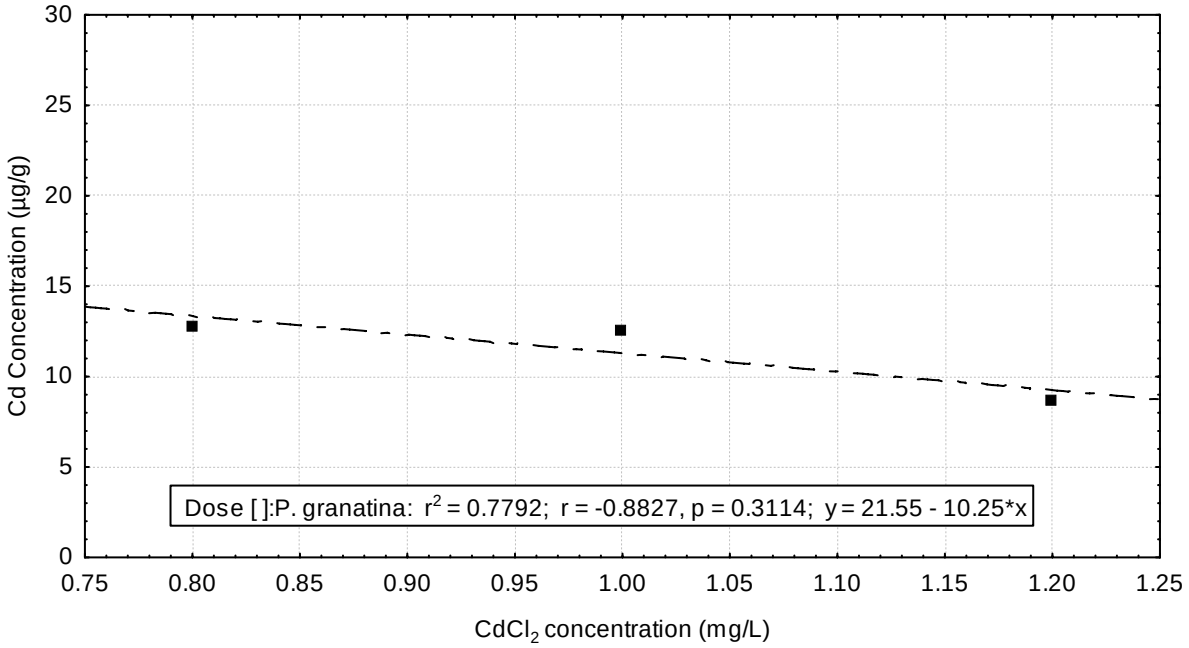


Appendix Figure 55: Mean heavy metal body load concentrations (µg/g) for

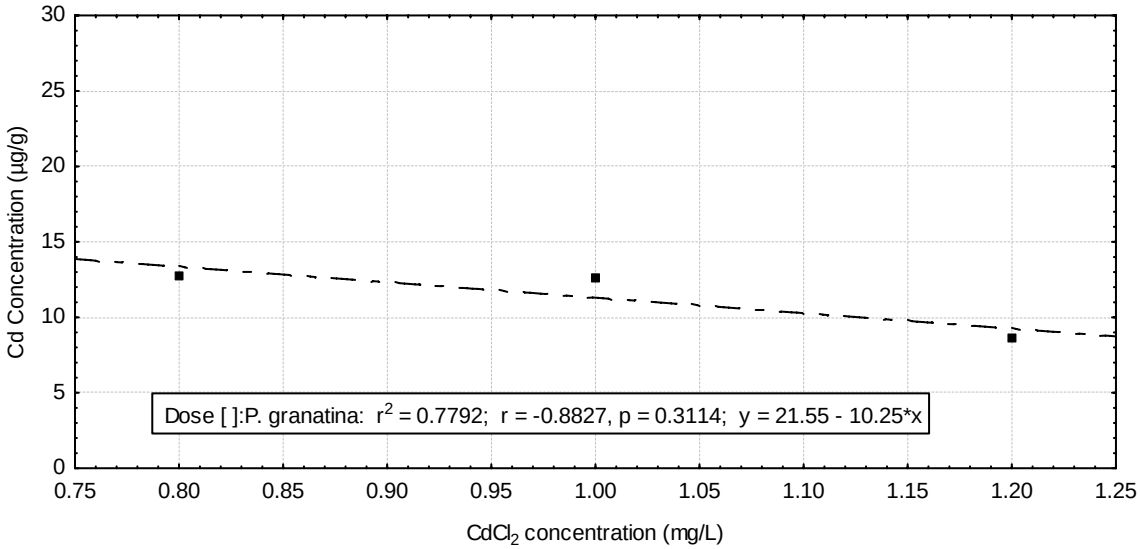
S. granularis vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 72 hours exposure time.



Appendix Figure 56: Mean heavy metal body load concentrations (µg/g) for *C. granatina* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 24 hours exposure time.



Appendix Figure 57: Mean heavy metal body load concentrations (µg/g) for *C. granatina* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 48 hours exposure time.



Appendix Figure 58: Mean heavy metal body load concentrations (µg/g) for *C. granatina* vs heavy metal laboratory exposure concentrations (mg/L $CdCl_2$) in sea water for three exposure concentrations (mg/L $CdCl_2$) at 72 hours exposure time.

Appendix Table 59: Cd concentration in body tissues of limpets from previous studies.

AUTHOR	YEAR	LIMPET SPECIES	Cd [ ] µg/g	AREA ANALYSED	SITES ANALYSED
Howard & Nickless	1977	P. vulgata	3.14 (wet weight) 62.7 (wet weight) <1 (wet weight) <1 (wet weight)	Soft tissue	4
Shore *et al.*	1975	P. vulgata	537 ± 137 (dry weight) 419 ± 25 (dry weight) 257 ± 44 (dry weight) 116 ± 40 (dry weight) 27 ± 6 (dry weight)	Digestive gland	5
Ramelow	1985	Patella sp.	2.1 - 30.3 (dry weight)	Soft tissue	3
Preston *et al.*	1972	Patella sp.	2.8 - 35 (dry weight)	Soft tissue	2
Bryan *et al.*	1977	Patella sp.	3.9 (dry weight)	Soft tissue	3
Klump & Peterson	1979	Patella sp.	13.2 - 17.2 (dry weight)	Soft tissue	2
Leatherland & Burton	1974	Patella sp.	2.7 (dry weight)	Soft tissue	4
Lande	1976	Patella sp.	2 - 22 (dry weight)	Soft tissue	2
Stenner & Nickless	1975	Patella sp.	1.1 - 7.1 (dry weight)	Soft tissue	1
Shiber & Shatila	1978	P. caerulea	0.4 - 4.7 (dry weight) 0.1 - 1.1 (wet weight)	Soft tissue	2
Cravo *et al.*	2004	P. aspera	3.5 - 9.1 (dry weight) 1.0 - 2.6 (dry weight)	Soft tissue	2
Catsiki *et al.*	1991	P. aspera	3.7 - 11.4 5	Soft tissue	2
Bebianno et al.	1991	P. aspera	4.7 5.3 1.7 1.9	Soft tissue	2
Bryan *et al.*	1985	P. vulgata	3.3 - 7.4 2.7 - 289	Soft tissue	3
Miramand & Bentley	1992	P. vulgata	3.6	Soft tissue	2
Conti & Cecchetti	2003	P. caerulea	2.89–4.06 (dry weight)	Soft tissue	2

Appendix Table 60: Summary of major biomarkers of exposure presently used to assess impaired biological function

Biomarker	Tissue	Use
Mixed-function oxidases	Liver	Indicator of exposure to organic chemicals such as PAHs and PCBs
Glutathione S-transferases	Liver	Indicator of exposure to pesticides and metalloids
Cellulase/carbohydrase	Stomach	Indicator of exposure to pesticides
Acetylcholinesterase	Brain	Indicator of exposure to organophosphorus or carbamate pesticides
Carboxylesterase	Various	Indicator of exposure to pyrethroid and carbamate pesticides
DNA strand breakage, adduct formation, chromatid exchange	Various	Indicator of exposure to alkylating or arylating agents
Aminolevulinic acid dehydratase	Blood	Indicator of exposure to lead
Metallothionein	Various	Indicator of exposure to metals
Retinoids	Liver	Indicator of exposure to dioxin and furans
Porphyrins	Liver	Indicator of exposure to chlorinated aromatic hydrocarbons
Adenylate energy change and ATP/ADP ratio	Various	Indicator of exposure to stress
Stress proteins	Various	Indicator of cells experiencing stress
Glutathione	Liver	Indicator of oxidative stress

Note: From Hyne and Maher (2003) as adapted from Peakall (1992), McCarthy and Shugart (1990), Benson and DiGiulio (1992).

Appendix Table 61: Mean neutral red retention times (min) (±SD) for samples of *C. oculus* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Control group analysed at 7 days also included (n=10 for each exposure time group and each sample were done in duplicate hence the mean was determined).

Samples	Control (7 days)	24 hr Exposure	48 hr Exposure	72 hr Exposure
1	37 ± 1.41	24 ± 0	27 ± 4.24	18.5 ± 0.71
2	30.5 ± 0.71	31 ± 4.24	33 ± 1.41	12.5 ± 0.71
3	26 ± 2.83	27.5 ± 0.71	16 ± 8.49	18 ± 5.66
4	23 ± 1.41	27 ± 1.41	19.5 ± 0.71	16 ± 2.83
5	25 ± 1.41	27 ± 4.24	24 ± 4.24	18.5 ± 2.12
6	31 ± 7.07	24.5 ± 2.12	18 ± 1.41	22.5 ± 0.71
7	23.5 ± 0.71	24.5 ± 0.71	29 ± 1.41	17 ± 1.41
8	31 ± 1.41	27 ± 1.41	29.5 ± 0.71	23 ± 4.24
9	NS	26 ± 0	23 ± 1.41	18.5 ± 0.71
10	NS	26 ± 1.41	27 ± 1.41	20.5 ± 0.71
MEAN case 1-10	28.375	26.45	24.6	18.5
MEDIAN case 1-10	28.25	26.5	25.5	18.5
SD case 1-10	4.82	2.02	5.49	3.07

*NS: not sampled

Appendix Table 62: Mean neutral red retention times (min) (±SD) for samples of *C. oculus* over three exposure times (hours) at 1mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24 hr Exposure	Control 24hr	48 hr Exposure	Control 48 hr	72 hr Exposure	Control 72 hr
1	30 ± 4.24	43 ± 7.07	13 ± 1.41	15 ± 1.41	19 ± 1.41	21 ± 1.41
2	35 ± 1.41	37 ± 1.41	22 ± 0	21 ± 1.41	19 ± 1.41	27 ± 1.41
3	21 ± 1.41	21 ± 1.41	23 ± 4.24	31 ± 1.41	16.5 ± 0.71	17 ± 4.24
4	28.5 ± 0.71	21 ± 1.41	21 ± 1.41	25 ± 1.41	19 ± 1.41	27 ± 1.41
5	21 ± 1.41	35 ± 1.41	16 ± 2.83	23 ± 4.24	19 ± 4.24	19 ± 1.41
6	23 ± 4.24	31 ± 1.41	21 ± 1.41	19.5 ± 6.36	14 ± 1.41	14.5 ± 9.19
7	29 ± 1.41	30.5 ± 2.12	17 ± 1.41	15 ± 1.41	15 ± 1.41	21 ± 1.41
8	15.5 ± 0.71	21 ± 1.41	23 ± 1.41	14.5 ± 2.12	15 ± 1.41	16.5 ± 2.12
9	19.5 ± 3.54	22 ± 8.49	11 ± 1.41	22 ± 2.83	17 ± 1.41	11 ± 1.41
10	13 ± 1.41	21.5 ± 6.36	17 ± 1.41	22.5 ± 3.54	11 ± 1.41	15 ± 7.07
MEAN case 1-10	23.55	28.3	18.4	20.85	16.45	18.9
MEDIAN case 1-10	22	26.25	19	21.5	16.75	18
SD case 1-10	6.93	8.13	4.25	5.15	2.71	5.23

Appendix Table 63: Mean neutral red retention times (min) (±SD) for samples of *C. oculus* over three exposure times (hours) at 1.2mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24 hr Exposure	Control 24hr	48 hr Exposure	Control 48 hr	72 hr Exposure	Control 72 hr
1	22 ± 2.83	19 ± 1.41	15.5 ± 0.71	18 ± 2.83	9 ± 1.41	22 ± 2.83
2	18 ± 2.83	25 ± 1.41	14.5 ± 0.71	24.5 ± 2.12	9 ± 1.41	19.5 ± 0.71
3	23 ± 1.41	21 ± 0	16 ± 2.83	22 ± 5.66	13 ± 4.24	24.5 ± 4.95
4	19 ± 1.41	27.5 ± 9.19	17.5 ± 3.54	24 ± 2.83	9 ± 1.41	23.5 ± 0.71
5	25 ± 1.41	35 ± 1.41	17 ± 4.24	19.5 ± 3.54	11 ± 1.41	20 ± 2.83
6	19.5 ± 4.95	32.5 ± 0.71	15.5 ± 2.12	22 ± 4.24	10.5 ± 2.12	20 ± 1.41
7	19 ± 4.24	33.5 ± 4.95	17.5 ± 3.54	23.5 ± 3.54	10 ± 1.41	21.5 ± 2.12
8	20.5 ± 4.95	26.5 ± 6.36	15.5 ± 2.12	25.5 ± 4.95	13 ± 0	22.5 ± 4.95
9	20.5 ± 0.71	28 ± 2.83	17 ± 2.83	22.5 ± 0.71	12.5 ± 0.71	24 ± 2.83
10	21 ± 4.24	26.5 ± 10.61	15.5 ± 0.71	17.5 ± 0.71	15 ± 0	24 ± 2.83
MEAN case 1-10	20.75	27.45	16.15	21.9	11.2	22.15
MEDIAN case 1-10	20.5	27	15.75	22.25	10.75	22.25
SD case 1-10	2.11	5.17	1.03	2.74	2.08	1.86

Appendix Table 64: Mean Cd concentrations (µg/g) for soft tissue samples of *C. oculus* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Control group analysed at 7 days also included (n=10 for each exposure and control time group, n=2 for initial control).

Samples	Initial Control	Control (7 days)	24 hr Exposure	48 hr Exposure	72 hr Exposure
1	1.08	5.14	7.87	9.36	20.98
2	1.21	2.90	12.45	15.80	19.40
3	NS	3.04	9.56	7.58	7.06
4	NS	1.68	11.11	9.89	17.32
5	NS	2.17	10.22	14.27	10.25
6	NS	2.03	9.71	12.69	14.08
7	NS	2.46	12.80	10.34	12.90
8	NS	2.56	6.27	10.70	12.90
9	NS	3.60	13.02	34.48	9.86
10	NS	NS	9.24	11.36	14.54
MEAN case 1-10	1.15	2.84	10.22	13.65	13.93
MEDIAN case 1-10	1.15	2.56	9.97	11.03	13.49
SD case 1-10	0.09	1.04	2.19	7.70	4.36

*NS: not sampled

Appendix Table 65: Mean Cd concentrations (µg/g) for soft tissue samples of *C. oculus* from the control group study done in conjunction with the exposure experiment at 1mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	0.59	ND	0.36	1.04
2	ND	2.59	1.39	36.88
3	NS	4.42	1.36	2.69
4	NS	ND	2.62	2.65
5	NS	1.77	0.58	10.27
6	NS	0.70	0.36	4.67
7	NS	0.41	0.49	0.34
8	NS	0.27	2.11	0.45
9	NS	0.31	1.18	0.44
10	NS	1.24	0.29	0.30
MEAN case 1-10	0.29	1.17	1.07	5.97
MEDIAN case 1-10	0.29	0.55	0.88	1.85
SD case 1-10	0.41	1.41	0.81	11.29

*NS: not sampled, ND: not detected

Appendix Table 66: Mean Cd concentrations (µg/g) for soft tissue samples of *C. oculus* over three exposure times (hours) at 1mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	ND	11.73	8.95	3.06
2	ND	2.51	5.60	10.68
3	NS	23.73	6.15	3.74
4	NS	4.56	5.17	11.36
5	NS	1.51	6.39	8.03
6	NS	1.06	2.93	4.42
7	NS	1.18	2.52	2.97
8	NS	1.77	2.89	3.96
9	NS	0.99	3.23	4.44
10	NS	1.06	1.78	5.54
MEAN case 1-10	ND	5.01	4.56	5.82
MEDIAN case 1-10	ND	1.64	4.20	4.43
SD case 1-10	ND	7.35	2.25	3.10

*NS: not sampled, ND: not detected

Appendix Table 67: Mean Cd concentrations (µg/g) for soft tissue samples of *C. oculus* from the control group study done in conjunction with the exposure experiment at 1.2mg/L $CdCl_2$ exposure (n=5 for each control time and n=2 for initial control group). *NS: not sampled, ND: not detected

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	ND	6.33	2.06	1.67
2	0.51	0.38	3.83	2.90
3	NS	1.31	1.74	4.76
4	NS	2.29	0.83	2.74
5	NS	1.24	1.35	1.69
MEAN case 1-5	0.25	2.31	1.96	2.75
MEDIAN case 1-5	0.25	1.31	1.74	2.74
SD case 1-5	0.36	2.35	1.14	1.26

Appendix Table 68: Mean Cd concentrations (µg/g) for soft tissue samples of *C. oculus* over three exposure times (hours) at 1.2mg/L $CdCl_2$ exposure. Initial control group included (n=5 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	ND	14.61	24.81	33.46
2	ND	17.29	21.03	49.11
3	NS	16.38	17.81	34.78
4	NS	15.97	17.89	33.95
5	NS	19.70	21.05	62.81
MEAN case 1-5	ND	16.79	20.52	42.82
MEDIAN case 1-5	ND	16.38	21.03	34.78
SD case 1-5	ND	1.89	2.88	12.94

*NS: not sampled, ND: not detected

Appendix Table 69: Mean neutral red retention times (min) for samples of *S. longicosta* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (each sample were done in duplicate hence the mean was determined).

Samples	24 hr Exposure	Control 24hr	48 hr Exposure	Control 48 hr	72 hr Exposure	Control 72 hr
1	49	19.5	31	17	24	24
2	49	26.5	31	17	31	22.5
3	61	24	25	15	25	22
4	83	17	32	25	31	20
5	NS	28.5	31	21	33	20.5
6	NS	29	25	15	35	22
7	NS	22.5	22.5	11	27.5	22
8	NS	17	28.5	27	29	18
9	NS	NS	23	NS	25	16.5
10	NS	NS	20	NS	26	27.5
11	NS	NS	23	NS	NS	NS
12	NS	NS	25	NS	NS	NS
13	NS	NS	22.5	NS	NS	NS
14	NS	NS	29	NS	NS	NS
15	NS	NS	23	NS	NS	NS
MEAN case 1-15	60.5	23	26.1	18.5	28.5	21.5
MEDIAN case 1-15	55	23.25	25	17	27.5	22
SD case 1-15	16.03	4.84	3.93	5.42	3.60	3.06

*NS: not sampled

Appendix Table 70: Mean neutral red retention times (min) for samples of *S. longicosta* over three exposure times (hours) at 1mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24 hr Exposure	Control 24hr	48 hr Exposure	Control 48 hr	72 hr Exposure	Control 72 hr
1	23	25.5	18	19	11	11
2	27.5	18	19	17.5	15	11
3	19	17	15	16	13	7
4	13	20.5	15.5	15.5	13	10
5	19	16	17	20.5	11	16.5
6	17	15	17	15.5	11	19
7	15	21	13	21.5	9	9
8	13	15	11	19	13	13.5
9	19	19	15	20.5	15	13
10	21	19	13	13.5	11.5	11.5
MEAN case 1-10	18.65	18.6	15.35	17.85	12.25	12.15
MEDIAN case 1-10	19	18.5	15.25	18.25	12.25	11.25
SD case 1-10	4.51	3.21	2.49	2.66	1.90	3.54

Appendix Table 71: Mean neutral red retention times (min) for samples of *S. longicosta* over three exposure times (hours) at 1.2mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24 hr Exposure	Control 24hr	48 hr Exposure	Control 48 hr	72 hr Exposure	Control 72 hr
1	21	20.5	17	13	9	11
2	21	20.5	13	14	11	12
3	19	21	13	15	7	12
4	20	21	13	14.5	9	15.5
5	18.5	17	11	12	7	11.5
6	15.5	18.5	14	16	9.5	11.5
7	17.5	21.5	12	15.5	11	10.5
8	17.5	16.5	13.5	14.5	10.5	13
9	18.5	20.5	15.5	18	11.5	14.5
10	18	18	14.5	12	10	15
MEAN case 1-10	18.65	19.5	13.65	14.45	9.55	12.65
MEDIAN case 1-10	18.5	20.5	13.25	14.5	9.75	12
SD case 1-10	1.70	1.83	1.72	1.85	1.59	1.76

Appendix Table 72: Mean Cd concentrations (µg/g) for soft tissue samples of *S. longicosta* from the control group study done in conjunction with the exposure experiment at 0.8mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	0.76	6.80	1.60	1.51
2	4.31	0.67	0.68	2.22
3	NS	3.74	0.59	0.74
4	NS	5.68	ND	5.06
5	NS	1.23	3.66	1.44
6	NS	4.93	3.57	3.42
7	NS	1.46	0.98	0.65
8	NS	0.52	2.63	1.57
9	NS	0.40	2.46	1.21
10	NS	3.66	1.06	5.65
MEAN case 1-10	2.53	2.91	1.72	2.35
MEDIAN case 1-10	2.53	2.56	1.33	1.54
SD case 1-10	2.51	2.36	1.29	1.78

*NS: not sampled, ND: not detected

Appendix Table 73: Mean Cd concentrations (µg/g) for soft tissue samples of *S. longicosta* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	ND	78.39	4.50	4.22
2	ND	16.35	7.26	1.97
3	NS	5.34	6.71	9.09
4	NS	27.27	10.95	9.98
5	NS	7.22	6.37	11.89
6	NS	29.06	6.99	14.21
7	NS	8.44	14.51	10.19
8	NS	4.52	12.78	7.06
9	NS	3.54	10.10	10.46
10	NS	15.65	14.06	37.96
MEAN case 1-10	ND	19.58	9.42	11.70
MEDIAN case 1-10	ND	12.04	8.68	10.09
SD case 1-10	ND	22.60	3.54	9.90

*NS: not sampled, ND: not detected

Appendix Table 74: Mean Cd concentrations (µg/g) for soft tissue samples of *S. longicosta* from the control group study done in conjunction with the exposure experiment at 1mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	1.77	10.84	7.65	5.36
2	0.94	2.83	4.55	5.27
3	NS	1.80	3.06	4.51
4	NS	10.50	5.40	1.99
5	NS	4.02	3.72	7.85
6	NS	9.01	4.72	1.73
7	NS	8.14	3.09	2.29
8	NS	45.03	2.04	1.41
9	NS	7.95	2.92	1.51
10	NS	1.34	2.62	2.26
MEAN case 1-10	1.36	10.15	3.98	3.42
MEDIAN case 1-10	1.36	8.05	3.40	2.28
SD case 1-10	0.59	12.76	1.66	2.19

*NS: not sampled

Appendix Table 75: Mean Cd concentrations (µg/g) for soft tissue samples of *S. longicosta* over three exposure times (hours) at 1mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	1.47	7.04	8.48	7.48
2	1.61	6.16	6.71	13.24
3	NS	4.37	12.20	8.80
4	NS	10.66	6.00	9.51
5	NS	2.60	7.75	9.97
6	NS	9.97	8.46	4.03
7	NS	6.57	5.25	5.97
8	NS	18.78	4.17	6.26
9	NS	4.44	4.98	7.21
10	NS	4.51	5.31	4.34
MEAN case 1-10	1.54	7.51	6.93	7.68
MEDIAN case 1-10	1.54	6.37	6.35	7.35
SD case 1-10	0.10	4.69	2.38	2.80

*NS: not sampled

Appendix Table 76: Mean Cd concentrations (µg/g) for soft tissue samples of *S. longicosta* from the control group study done in conjunction with the exposure experiment at 1.2mg/L $CdCl_2$ exposure (n=5 for each control time and n=2 for initial control group). *NS: not sampled

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	0.81	2.13	1.19	1.50
2	1.56	1.63	1.81	1.63
3	NS	2.20	1.59	1.35
4	NS	1.22	1.91	1.46
5	NS	1.90	1.94	2.59
MEAN case 1-5	1.18	1.82	1.69	1.70
MEDIAN case 1-5	1.18	1.90	1.81	1.50
SD case 1-5	0.53	0.40	0.31	0.51

Appendix Table 77: Mean Cd concentrations (µg/g) for soft tissue samples of *S. longicosta* over three exposure times (hours) at 1.2mg/L $CdCl_2$ exposure. Initial control group included (n=5 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	ND	1.52	3.74	9.66
2	ND	2.41	4.53	5.68
3	NS	2.82	3.30	5.44
4	NS	2.68	4.99	4.70
5	NS	2.34	6.52	4.58
MEAN case 1-5	ND	2.35	4.61	6.01
MEDIAN case 1-5	ND	2.41	4.53	5.44
SD case 1-5	ND	0.51	1.25	2.09

*NS: not sampled, ND: not detected

Appendix Table 78: Mean neutral red retention times (min) for samples of *S. granularis* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24 hr Exposure	Control 24hr	48 hr Exposure	Control 48 hr	72 hr Exposure	Control 72 hr
1	11	11	10.5	14	11	23.5
2	13	7	16	21	7.5	16
3	12.5	19	13.5	17	9.5	24.5
4	11	18	12.5	19	11.5	22
5	13	17.5	8.5	18	7	20
6	8	18.5	12	20.5	9	20.5
7	11	16	15	21	8	19.5
8	10	21	15.5	18	11	20
9	12.5	18	9.5	18.5	11.5	25
10	12	17.5	10	20.5	8.5	26
MEAN case 1-10	11.4	16.35	12.3	18.75	9.45	21.7
MEDIAN case 1-10	11.5	17.75	12.25	18.75	9.25	21.25
SD case 1-10	1.56	4.18	2.66	2.19	1.71	3.07

Appendix Table 79: Mean neutral red retention times (min) for samples of *S. granularis* over three exposure times (hours) at 1mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24 hr Exposure	Control 24hr	48 hr Exposure	Control 48 hr	72 hr Exposure	Control 72 hr
1	27	24	10	17	8.5	14.5
2	29	22.5	11.5	23	9	17.5
3	28	13	15	18	9	25
4	26.5	17	11	21.5	11	25.5
5	25	15	10	20.5	11.5	24.5
6	23	20.5	11	19.5	13.5	17.5
7	26	19	14	15	11	20
8	22	14	13	19.5	13	22
9	25.5	13.5	14	16	11	19
10	28.5	16.5	14.5	16.5	10.5	22
MEAN case 1-10	26.05	17.5	12.4	18.65	10.8	20.75
MEDIAN case 1-10	26.25	16.75	12.25	18.75	11	21
SD case 1-10	2.28	3.87	1.91	2.58	1.65	3.68

Appendix Table 80: Mean neutral red retention times (min) for samples of *S. granularis* over three exposure times (hours) at 1.2mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24 hr Exposure	Control 24hr	48 hr Exposure	Control 48 hr	72 hr Exposure	Control 72 hr
1	14	23.5	10	19.5	8.5	21
2	11.5	23.5	10.5	22.5	8.5	18.5
3	10.5	23.5	7.5	22	7	21.5
4	10.5	23	8	22.5	8	23
5	13.5	20	9	20	8	19.5
6	11	22	9.5	23	7.5	17.5
7	10.5	18.5	8	18	10	15.5
8	11.5	18.5	8.5	20.5	7	17.5
9	11	20.5	10.5	17.5	7.5	19.5
10	11.5	23	7	17	6.5	15.5
MEAN case 1-10	11.55	21.6	8.85	20.25	7.85	18.9
MEDIAN case 1-10	11.25	22.5	8.75	20.25	7.75	19
SD case 1-10	1.23	2.05	1.25	2.23	1.00	2.49

Appendix Table 81: Mean Cd concentrations (µg/g) for soft tissue samples of *S. granularis* from the control group study done in conjunction with the exposure experiment at 0.8mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	13.16	ND	177.94	18.09
2	24.14	2.66	77.28	156.33
3	NS	48.45	46.91	127.12
4	NS	7.13	132.19	79.14
5	NS	33.65	185.34	73.55
6	NS	ND	70.60	147.16
7	NS	ND	43.53	63.96
8	NS	13.79	84.16	74.37
9	NS	25.62	89.24	108.16
10	NS	2.58	166.62	126.30
MEAN case 1-10	18.65	13.39	107.38	97.42
MEDIAN case 1-10	18.65	4.90	86.70	93.65
SD case 1-10	7.76	16.99	53.79	42.98

*NS: not sampled, ND: not detected

Appendix Table 82: Mean Cd concentrations (µg/g) for soft tissue samples of *S. granularis* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	56.14	34.62	177.64	306.44
2	26.26	144.26	94.40	208.77
3	NS	35.87	40.26	68.74
4	NS	22.37	103.51	77.12
5	NS	51.73	44.84	130.34
6	NS	14.48	52.72	107.07
7	NS	70.21	38.72	177.93
8	NS	72.80	51.12	127.41
9	NS	53.51	56.92	124.47
10	NS	15.32	110.98	130.67
MEAN case 1-10	41.20	51.52	77.11	145.90
MEDIAN case 1-10	41.20	43.80	54.82	128.87
SD case 1-10	21.13	38.69	44.47	70.09

*NS: not sampled

Appendix Table 83: Mean Cd concentrations (µg/g) for soft tissue samples of *S. granularis* from the control group study done in conjunction with the exposure experiment at 1mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	ND	44.35	38.47	22.97
2	23.72	178.75	17.13	2.39
3	NS	4.83	38.11	4.88
4	NS	206.16	19.43	4.72
5	NS	23.51	16.11	5.27
6	NS	109.43	67.55	14.81
7	NS	91.99	29.61	12.31
8	NS	35.98	67.40	26.71
9	NS	27.89	15.80	23.69
10	NS	19.03	21.54	31.47
MEAN case 1-10	11.86	74.19	33.11	14.92
MEDIAN case 1-10	11.86	40.17	25.58	13.56
SD case 1-10	16.78	70.57	19.98	10.63

*NS: not sampled, ND: not detected

Appendix Table 84: Mean Cd concentrations (µg/g) for soft tissue samples of *S. granularis* over three exposure times (hours) at 1mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	5.18	15.29	90.36	4.37
2	45.40	88.75	27.38	18.72
3	NS	33.89	34.13	10.64
4	NS	55.65	37.05	7.81
5	NS	55.84	25.48	2.25
6	NS	31.21	25.93	6.98
7	NS	65.51	55.59	6.95
8	NS	323.18	43.55	6.18
9	NS	40.30	57.18	11.40
10	NS	45.30	31.60	12.96
MEAN case 1-10	25.29	75.49	42.82	8.83
MEDIAN case 1-10	25.29	50.47	35.59	7.40
SD case 1-10	28.44	89.36	20.25	4.75

*NS: not sampled

Appendix Table 85: Mean Cd concentrations (µg/g) for soft tissue samples of *S. granularis* from the control group study done in conjunction with the exposure experiment at 1.2mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	3.88	11.27	24.63	11.75
2	8.29	18.93	46.75	22.10
3	NS	20.74	25.96	82.73
4	NS	38.02	3.91	27.06
5	NS	115.05	6.46	8.15
6	NS	7.29	17.50	6.83
7	NS	36.59	5.23	4.32
8	NS	7.88	12.87	10.87
9	NS	14.78	14.96	21.37
10	NS	10.45	12.28	7.68
MEAN case 1-10	6.08	28.10	17.06	20.29
MEDIAN case 1-10	6.08	16.86	13.91	11.31
SD case 1-10	3.11	32.46	12.86	23.22

*NS: not sampled

Appendix Table 86: Mean Cd concentrations (µg/g) for soft tissue samples of *S. granularis* over three exposure times (hours) at 1.2mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	ND	12.36	78.36	93.97
2	8.73	59.48	118.12	95.77
3	NS	10.10	101.13	200.56
4	NS	21.32	56.38	175.82
5	NS	20.78	60.57	316.53
6	NS	52.20	45.41	186.88
7	NS	18.70	62.28	98.25
8	NS	11.26	59.35	106.47
9	NS	72.44	38.38	112.91
10	NS	47.80	27.91	92.77
MEAN case 1-10	4.37	32.65	64.79	147.99
MEDIAN case 1-10	4.37	21.05	59.96	109.69
SD case 1-10	6.17	22.99	27.70	72.71

*NS: not sampled, ND: not detected

Appendix Table 87: Mean neutral red retention times (min) for samples of *C. granatina* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24hr Exposure	Control 24hr	48hr Exposure	Control 48hr	72hr Exposure	Control 72hr
1	16	14.5	15.5	13	12	21
2	11.5	15.5	16	8.5	14	18.5
3	12	15	13	6	11	17
4	15	9.5	14.5	9	13	20.5
5	11.5	13	9.5	10.5	20	22
6	11	15	11.5	8.5	19.5	13.5
7	10.5	11	11	7	15	20.5
8	11	12.5	8	7.5	20	21
9	12.5	14	6	12	14	14
10	15	10	9	12.5	15	14
MEAN case 1-10	12.6	13	11.4	9.45	15.35	18.2
MEDIAN case 1-10	11.75	13.5	11.25	8.75	14.5	19.5
SD case 1-10	1.98	2.19	3.34	2.43	3.33	3.33

Appendix Table 88: Mean neutral red retention times (min) for samples of *C. granatina* over three exposure times (hours) at 1mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24hr Exposure	Control 24hr	48hr Exposure	Control 48hr	72hr Exposure	Control 72hr
1	8	9	7	11.5	10	11.5
2	11	11	9	11	14	23
3	11	11	8.5	10	11.5	23
4	14	13.5	10.5	11.5	11	15
5	12	15	9	9	11	16
6	9	10.5	8	15	13	14
7	12	11	10	13	10.5	11
8	10	12	10.5	13	14	18
9	12	11	10	16	11.5	21
10	15	15	9	11	12	23
MEAN case 1-10	11.4	11.9	9.15	12.1	11.85	17.55
MEDIAN case 1-10	11.5	11	9	11.5	11.5	17
SD case 1-10	2.12	1.98	1.13	2.17	1.40	4.74

Appendix Table 89: Mean neutral red retention times (min) for samples of *C. granatina* over three exposure times (hours) at 1.2mg/L $CdCl_2$ exposure. Control group done in conjunction with exposure group (n=10 for each exposure and control time group and each sample were done in duplicate hence the mean was determined).

Samples	24hr Exposure	Control 24hr	48hr Exposure	Control 48hr	72hr Exposure	Control 72hr
1	11	16	16	20	16	19
2	11.5	19	19	17	9	9
3	12	17	18.5	20	11	15
4	11	19.5	20.5	15	18	13
5	12	18	18	17.5	19	17
6	15	16.5	18	19.5	16	25
7	12.5	15	17	17	16.5	17
8	14	18	16.5	17	21	19
9	12	17.5	16.5	17.5	15.5	16
10	12.5	15.5	19.5	16	15	18
MEAN case 1-10	12.35	17.2	17.95	17.65	15.7	16.8
MEDIAN case 1-10	12	17.25	18	17.25	16	17
SD case 1-10	1.27	1.48	1.46	1.68	3.54	4.18

Appendix Table 90: Mean Cd concentrations (µg/g) for soft tissue samples of *C. granatina* from the control group study done in conjunction with the exposure experiment at 0.8mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	8.22	3.49	11.66	4.96
2	7.86	4.41	7.28	6.48
3	NS	7.49	10.66	7.76
4	NS	3.86	6.69	4.54
5	NS	3.83	10.32	10.12
6	NS	4.40	13.08	7.45
7	NS	4.35	11.40	7.48
8	NS	3.94	9.53	10.06
9	NS	4.58	10.60	7.38
10	NS	5.63	6.85	4.58
MEAN case 1-10	8.04	4.60	9.81	7.08
MEDIAN case 1-10	8.04	4.38	10.46	7.42
SD case 1-10	0.26	1.17	2.19	2.01

*NS: not sampled

Appendix Table 91: Mean Cd concentrations (µg/g) for soft tissue samples of *C. granatina* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	5.66	9.76	16.30	16.34
2	8.31	13.36	12.16	18.61
3	NS	9.41	12.62	12.55
4	NS	7.19	12.56	8.47
5	NS	10.36	9.25	7.27
6	NS	11.20	10.22	11.45
7	NS	7.76	11.18	16.20
8	NS	8.35	12.18	18.10
9	NS	10.23	15.82	18.94
10	NS	12.74	14.87	13.32
MEAN case 1-10	6.98	10.04	12.72	14.13
MEDIAN case 1-10	6.98	10.00	12.37	14.76
SD case 1-10	1.88	2.01	2.32	4.18

*NS: not sampled

Appendix Table 92: Mean Cd concentrations (µg/g) for soft tissue samples of *C. granatina* from the control group study done in conjunction with the exposure experiment at 1mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	5.48	12.37	7.21	6.90
2	6.70	7.57	5.32	4.93
3	NS	2.43	8.26	6.78
4	NS	16.66	9.60	12.05
5	NS	6.44	5.74	4.19
6	NS	7.75	5.71	8.43
7	NS	7.47	9.63	4.66
8	NS	11.76	8.57	5.80
9	NS	13.07	8.47	6.01
10	NS	9.31	6.31	9.32
MEAN case 1-10	6.09	9.48	7.48	6.91
MEDIAN case 1-10	6.09	8.53	7.73	6.40
SD case 1-10	0.86	4.05	1.64	2.43

*NS: not sampled

Appendix Table 93: Mean Cd concentrations (µg/g) for soft tissue samples of *C. granatina* over three exposure times (hours) at 0.8mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	2.12	6.58	10.27	8.22
2	3.70	11.50	17.19	12.67
3	NS	11.18	6.57	15.49
4	NS	5.31	22.34	13.86
5	NS	8.08	5.53	2.18
6	NS	6.56	15.68	7.18
7	NS	8.17	11.09	9.85
8	NS	10.76	19.31	13.57
9	NS	13.92	9.33	12.53
10	NS	11.01	8.24	13.59
MEAN case 1-10	2.91	9.31	12.56	10.91
MEDIAN case 1-10	2.91	9.47	10.68	12.60
SD case 1-10	1.12	2.76	5.72	4.06

*NS: not sampled

Appendix Table 94: Mean Cd concentrations (µg/g) for soft tissue samples of *C. granatina* from the control group study done in conjunction with the exposure experiment at 1.2mg/L $CdCl_2$ exposure (n=10 for each control time and n=2 for initial control group).

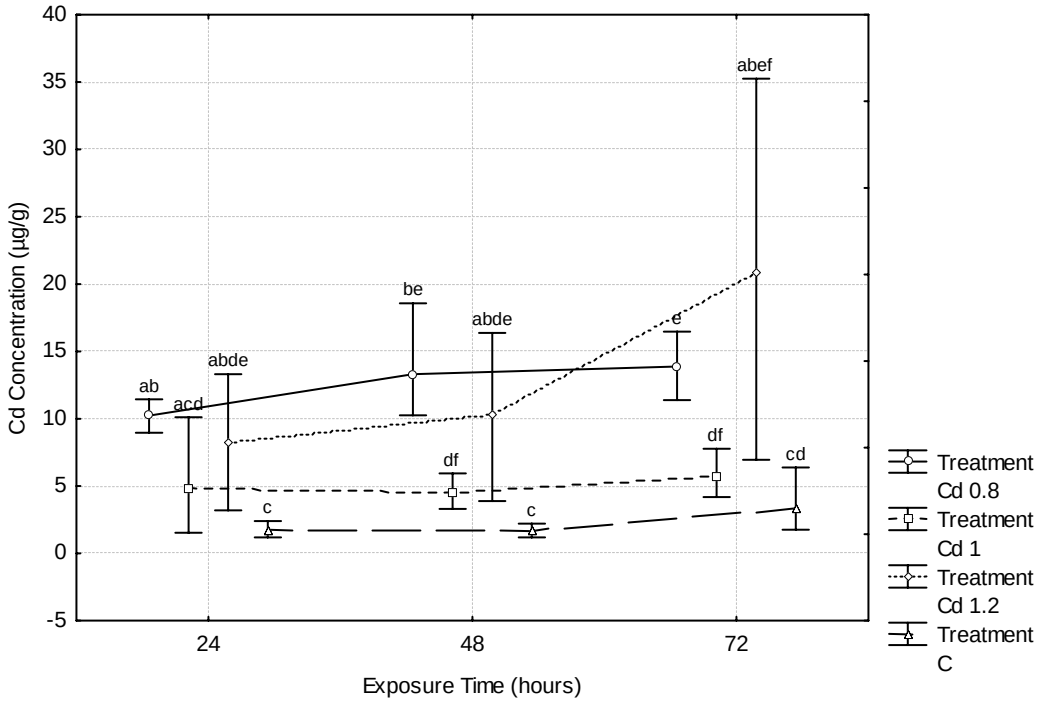
Samples	Initial Control	Control 24hr	Control 48hr	Control 72hr
1	4.98	11.73	8.52	4.38
2	11.15	4.14	9.01	29.11
3	NS	5.73	8.25	4.90
4	NS	6.97	15.19	7.45
5	NS	6.73	2.68	7.55
6	NS	4.88	7.06	6.27
7	NS	6.10	6.18	7.33
8	NS	7.04	7.60	8.86
9	NS	5.55	8.27	6.11
10	NS	6.02	8.25	7.61
MEAN case 1-10	8.07	6.49	8.10	8.96
MEDIAN case 1-10	8.07	6.06	8.25	7.39
SD case 1-10	4.36	2.05	3.09	7.21

*NS: not sampled

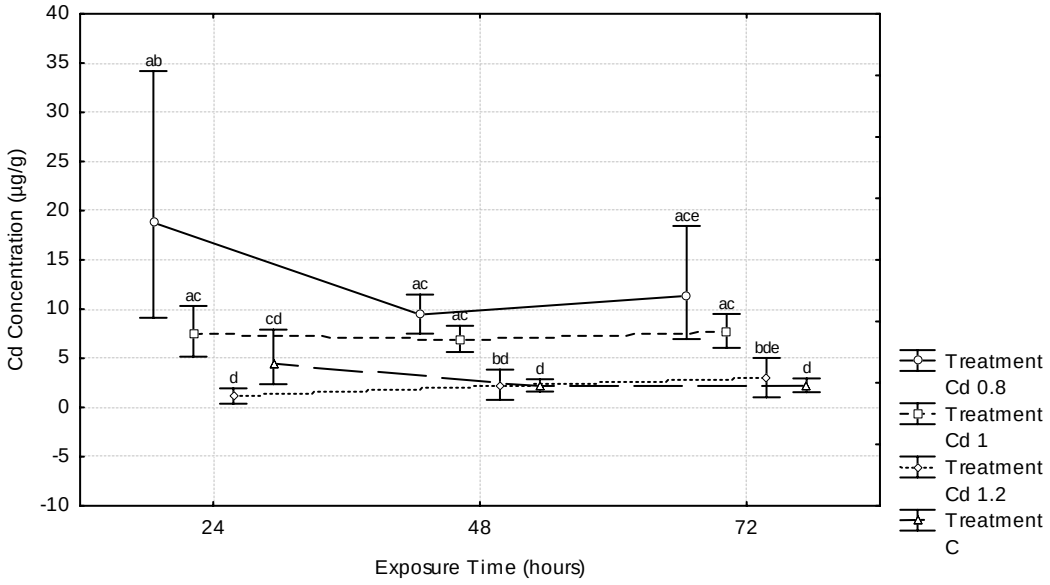
Appendix Table 95: Mean Cd concentrations (µg/g) for soft tissue samples of *C. granatina* over three exposure times (hours) at 1.2mg/L $CdCl_2$ exposure. Initial control group included (n=10 for each exposure time and n=2 for initial control group).

Samples	Initial Control	Exposure 24hr	Exposure 48hr	Exposure 72hr
1	1.95	6.70	9.22	17.49
2	4.44	7.10	11.09	13.34
3	NS	11.06	7.39	14.38
4	NS	6.25	7.08	9.76
5	NS	6.16	9.67	10.78
6	NS	7.89	8.18	11.78
7	NS	6.58	9.97	14.55
8	NS	6.25	8.76	9.33
9	NS	7.81	7.12	14.07
10	NS	6.89	7.70	15.17
MEAN case 1-10	3.19	7.27	8.62	13.06
MEDIAN case 1-10	3.19	6.80	8.47	13.70
SD case 1-10	1.76	1.47	1.36	2.60

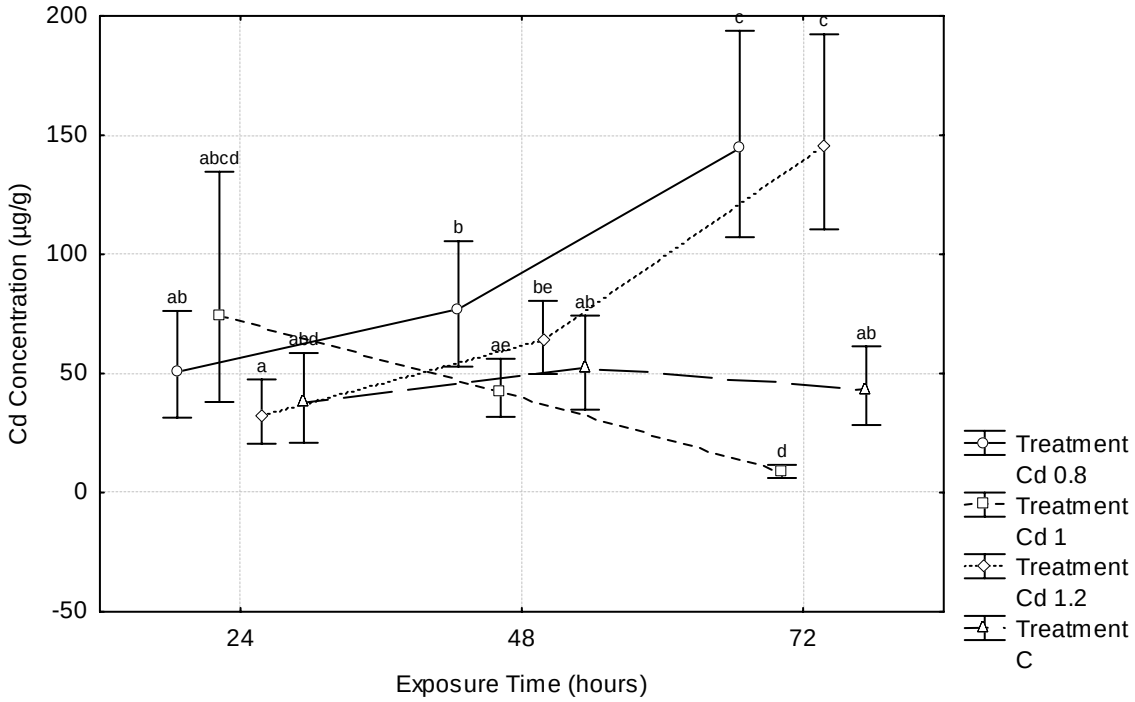
*NS: not sampled



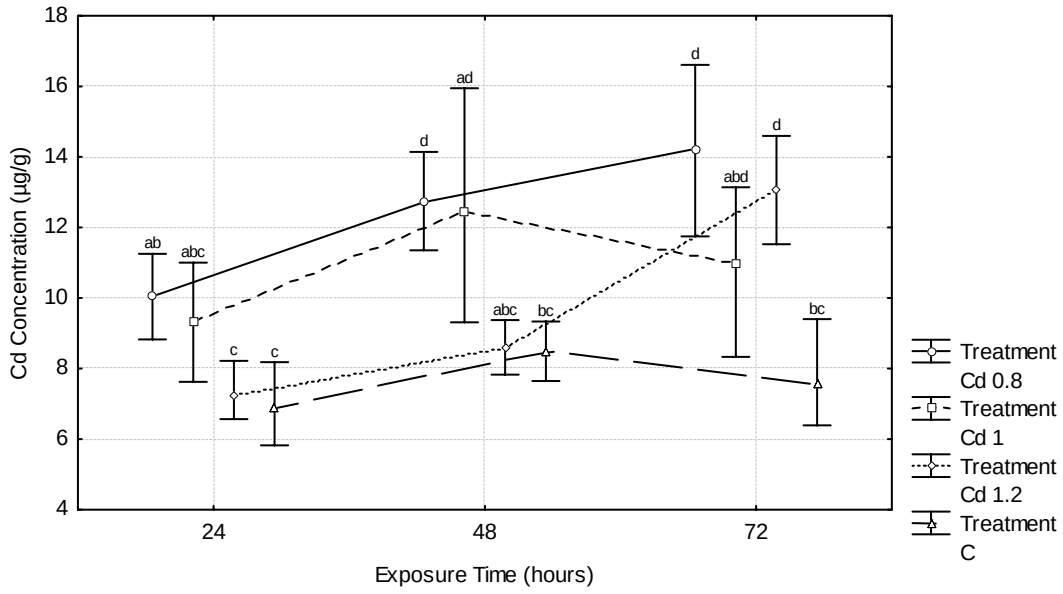
Appendix Figure 96: Mean Cd body concentrations (µg/g) (bootstrap corrected) for soft tissue samples of *C. oculus* at three concentration exposures (mg/L $CdCl_2$) and three exposure times (hours).Vertical bars denote 95% bootstrap confidence intervals. Control indicated by long dashed line.



Appendix Figure 97: Mean Cd body concentrations (µg/g) (bootstrap corrected) for soft tissue samples of *S. longicosta* at three concentration exposures (mg/L $CdCl_2$) and three exposure times (hours).Vertical bars denote 95% bootstrap confidence intervals. Control indicated by long dashed line.



Appendix Figure 98: Mean Cd body concentrations (µg/g) (bootstrap corrected) for soft tissue samples of *S. granularis* at three concentration exposures (mg/L $CdCl_2$) and three exposure times (hours). Vertical bars denote 95% bootstrap confidence intervals. Control indicated by long dashed line.



Appendix Figure 99: Mean Cd body concentrations (µg/g) (bootstrap corrected) for soft tissue samples of *C. granatina* at three concentration exposures (mg/L $CdCl_2$) and three exposure times (hours). Vertical bars denote 95% bootstrap confidence intervals. Control indicated by long dashed line.

Appendix Table 100: One-way ANOVA indicating the univariate tests of significance for Cd concentrations (µg/g) between the Gordon's Bay and Rooiels study sites.

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**1.84900**	**1**	**1.84900**	**109.166**	**0.00**
Location	0.00011	1	0.00011	0.0066	0.93645
Error	0.27100	16	0.01693		

Appendix Table 101: Mean Cd concentration (µg/g) of sea water samples collected from Gordon's Bay and Rooiels sample sites during the study period.

Location	Cd Conc. Mean	Cd Conc. Std. Err.	Cd Conc. -95.00 %	Cd Conc. +95.00 %	N
Gordon's Bay	0.32500	0.04601	0.22745	0.42254	8
Rooiels	0.32500	0.04115	0.23275	0.40724	10

Appendix Table 102: Two-way ANOVA indicating the univariate tests of significance for mean Cd concentrations (µg/g) of soft tissue samples (all exposure concentrations and exposure times) of *C. oculus*. Values in bold text indicate significant interaction with $p < 0.01$.

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**9969.81**	**1**	**9969.81**	**164.37**	**0.00**
Treatment	**4052.72**	**3**	**1350.91**	**22.27**	**0.00**
Exposure Time	**603.65**	**2**	**301.82**	**4.98**	**0.01**
Treatment*Exposure Time	658.13	6	109.69	1.81	0.10
Error	9826.03	162	60.65		

Appendix Table 103: Two-way ANOVA indicating the univariate tests of significance for mean Cd concentrations (µg/g) of soft tissue samples (all exposure concentrations and exposure times) of *S. longicosta*. Values in bold text indicate significant interaction with $p < 0.01$.

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**6139.82**	**1**	**6139.82**	**124.22**	**0.00**
Treatment	**2944.82**	**3**	**981.61**	**19.86**	**0.00**
Exposure Time	229.32	2	114.66	2.32	0.10
Treatment*Exposure Time	442.92	6	73.82	1.49	0.18
Error	8303.60	168	49.43		

Appendix Table 104: Two-way ANOVA indicating the univariate tests of significance for mean Cd concentrations (µg/g) of soft tissue samples (all exposure concentrations and exposure times) of *S. granularis*. Values in bold text indicate significant interaction with $p < 0.01$.

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**612120.18**	**1**	**612120.18**	**239.05**	**0.00**
Treatment	**72951.00**	**3**	**24317.00**	**9.50**	**0.00**
Exposure Time	**35668.58**	**2**	**17834.29**	**6.96**	**0.00**
Treatment*Exposure Time	**122173.46**	**6**	**20362.24**	**7.95**	**0.00**
Error	430192.60	168	2560.67		

Appendix Table 105: Two-way ANOVA indicating the univariate tests of significance for mean Cd concentrations (µg/g) of soft tissue samples (all exposure concentrations and exposure times) of *S. granatina*. Values in bold text indicate significant interaction with $p < 0.01$.

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**14780.11**	**1**	**14780.11**	**1283.69**	**0.00**
Treatment	**594.59**	**3**	**198.20**	**17.21**	**0.00**
Exposure Time	**241.33**	**2**	**120.66**	**10.48**	**0.00**
Treatment*Exposure Time	**171.88**	**6**	**28.65**	**2.49**	**0.02**
Error	1934.31	168	11.51		

Appendix Table 106: Bonferroni test for the mean Cd concentrations of *C. oculus* soft tissue samples over three exposure concentrations (mg/L $CdCl_2$) and three exposure times (hours) indicating significant interactions between samples. Values in bold text indicate significant interaction ($p < 0.05$).

	Treatment	Exposure Time	1	2	3	4	5	6	7	8	9	10	11	12
1	C	24		1.00	1.00	0.24	**0.00**	**0.00**	1.00	1.00	1.00	1.00	0.23	**0.00**
2	C	48	1.00		1.00	0.21	**0.00**	**0.00**	1.00	1.00	1.00	1.00	0.21	**0.00**
3	C	72	1.00	1.00		1.00	**0.04**	**0.03**	1.00	1.00	1.00	1.00	1.00	**0.00**
4	Cd 0.8	24	0.24	0.21	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.11
5	Cd 0.8	48	**0.00**	**0.00**	**0.04**	1.00		1.00	0.94	0.66	1.00	1.00	1.00	1.00
6	Cd 0.8	72	**0.00**	**0.00**	**0.03**	1.00	1.00		0.75	0.52	1.00	1.00	1.00	1.00
7	Cd 1	24	1.00	1.00	1.00	1.00	0.94	0.75		1.00	1.00	1.00	1.00	**0.00**
8	Cd 1	48	1.00	1.00	1.00	1.00	0.66	0.52	1.00		1.00	1.00	1.00	**0.00**
9	Cd 1	72	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	**0.00**
10	Cd 1.2	24	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	**0.02**
11	Cd 1.2	48	0.23	0.21	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		0.11
12	Cd 1.2	72	**0.00**	**0.00**	**0.00**	0.11	1.00	1.00	**0.00**	**0.00**	**0.00**	**0.02**	0.11	

Appendix Table 107: Bonferroni test for the mean Cd concentrations of *S. longicosta* soft tissue samples over three exposure concentrations (mg/L $CdCl_2$) and three exposure times (hours) indicating significant interactions between samples. Values in bold text indicate significant interaction ($p < 0.05$).

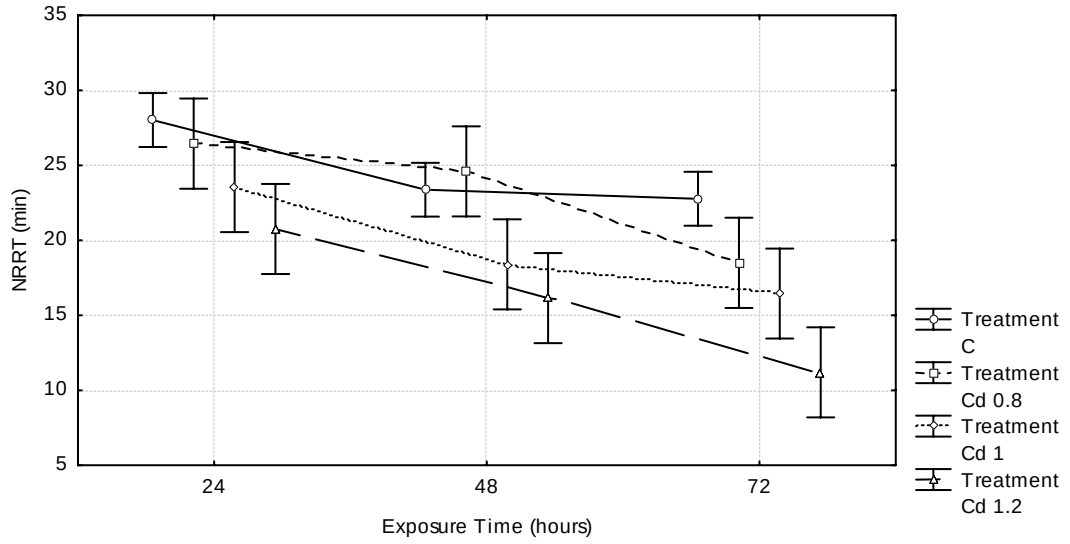
	Treatment	Exposure Time	1	2	3	4	5	6	7	8	9	10	11	12
1	C	24		1.00	1.00	**0.00**	1.00	0.44	1.00	1.00	1.00	1.00	1.00	1.00
2	C	48	1.00		1.00	**0.00**	0.35	**0.02**	1.00	1.00	1.00	1.00	1.00	1.00
3	C	72	1.00	1.00		**0.00**	0.36	**0.02**	1.00	1.00	1.00	1.00	1.00	1.00
4	Cd 0.8	24	**0.00**	**0.00**	**0.00**		0.10	0.87	**0.01**	**0.01**	**0.01**	**0.00**	**0.00**	**0.00**
5	Cd 0.8	48	1.00	0.35	0.36	0.10		1.00	1.00	1.00	1.00	0.63	1.00	1.00
6	Cd 0.8	72	0.44	**0.02**	**0.02**	0.87	1.00		1.00	1.00	1.00	0.07	0.21	0.42
7	Cd 1	24	1.00	1.00	1.00	**0.01**	1.00	1.00		1.00	1.00	1.00	1.00	1.00
8	Cd 1	48	1.00	1.00	1.00	**0.01**	1.00	1.00	1.00		1.00	1.00	1.00	1.00
9	Cd 1	72	1.00	1.00	1.00	**0.01**	1.00	1.00	1.00	1.00		1.00	1.00	1.00
10	Cd 1.2	24	1.00	1.00	1.00	**0.00**	0.63	0.07	1.00	1.00	1.00		1.00	1.00
11	Cd 1.2	48	1.00	1.00	1.00	**0.00**	1.00	0.21	1.00	1.00	1.00	1.00		1.00
12	Cd 1.2	72	1.00	1.00	1.00	**0.00**	1.00	0.42	1.00	1.00	1.00	1.00	1.00	

Appendix Table 108: Bonferroni test for the mean Cd concentrations of *S. granularis* soft tissue samples over three exposure concentrations (mg/L $CdCl_2$) and three exposure times (hours) indicating significant interactions between samples. Values in bold text indicate significant interaction ($p < 0.05$).

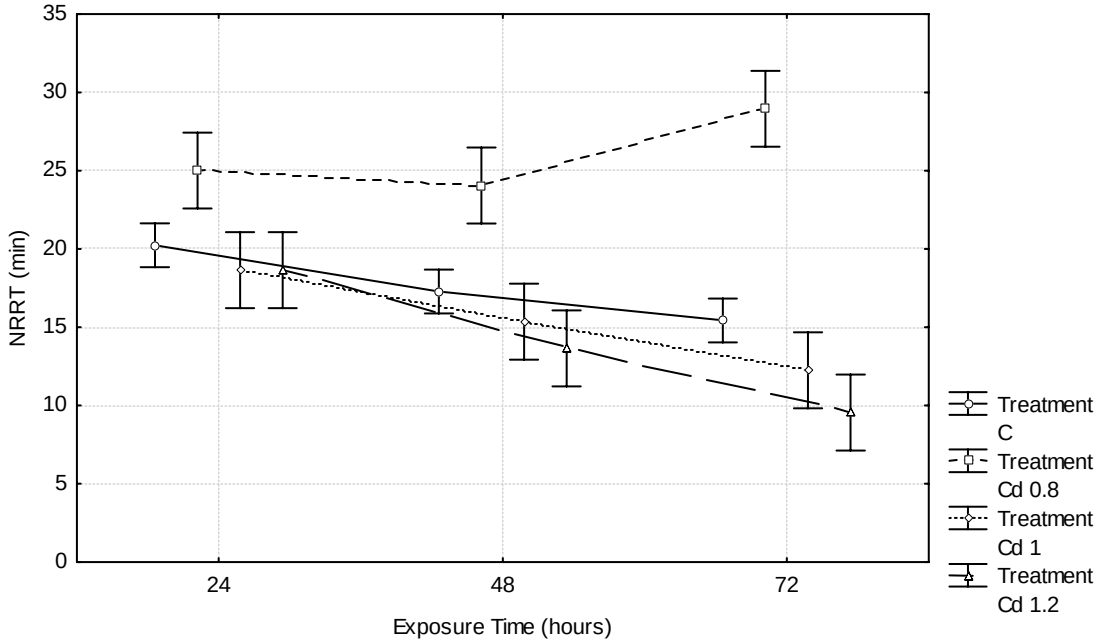
	Treatment	Exposure Time	1	2	3	4	5	6	7	8	9	10	11	12
1	C	24		1.00	1.00	1.00	1.00	**0.00**	1.00	1.00	1.00	1.00	1.00	**0.00**
2	C	48	1.00		1.00	1.00	1.00	**0.00**	1.00	1.00	1.00	1.00	1.00	**0.00**
3	C	72	1.00	1.00		1.00	1.00	**0.00**	1.00	1.00	1.00	1.00	1.00	**0.00**
4	Cd 0.8	24	1.00	1.00	1.00		1.00	**0.00**	1.00	1.00	1.00	1.00	1.00	**0.00**
5	Cd 0.8	48	1.00	1.00	1.00	1.00		0.18	1.00	1.00	0.19	1.00	1.00	0.14
6	Cd 0.8	72	**0.00**	**0.00**	**0.00**	**0.00**	0.18		0.14	**0.00**	**0.00**	**0.00**	**0.03**	1.00
7	Cd 1	24	1.00	1.00	1.00	1.00	1.00	0.14		1.00	0.24	1.00	1.00	0.11
8	Cd 1	48	1.00	1.00	1.00	1.00	1.00	**0.00**	1.00		1.00	1.00	1.00	**0.00**
9	Cd 1	72	1.00	1.00	1.00	1.00	0.19	**0.00**	0.24	1.00		1.00	0.95	**0.00**
10	Cd 1.2	24	1.00	1.00	1.00	1.00	1.00	**0.00**	1.00	1.00	1.00		1.00	**0.00**
11	Cd 1.2	48	1.00	1.00	1.00	1.00	1.00	**0.03**	1.00	1.00	0.95	1.00		**0.02**
12	Cd 1.2	72	**0.00**	**0.00**	**0.00**	**0.00**	0.14	1.00	0.11	**0.00**	**0.00**	**0.00**	**0.02**	

Appendix Table 109: Bonferroni test for the mean Cd concentrations of *C. granatina* soft tissue samples over three exposure concentrations (mg/L $CdCl_2$) and three exposure times (hours) indicating significant interactions between samples. Values in bold text indicate significant interaction ($p < 0.05$).

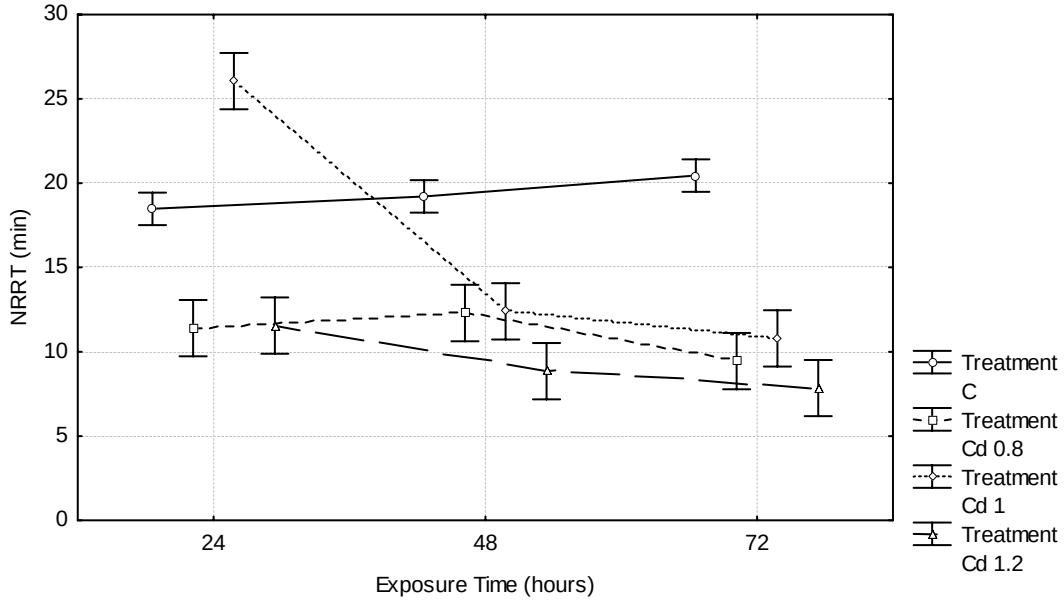
	Treatment	Exposure Time	1	2	3	4	5	6	7	8	9	10	11	12
1	C	24		1.00	1.00	0.74	**0.00**	**0.00**	1.00	**0.00**	0.09	1.00	1.00	**0.00**
2	C	48	1.00		1.00	1.00	**0.05**	**0.00**	1.00	0.08	1.00	1.00	1.00	**0.02**
3	C	72	1.00	1.00		1.00	**0.00**	**0.00**	1.00	**0.01**	0.61	1.00	1.00	**0.00**
4	Cd 0.8	24	0.74	1.00	1.00		1.00	0.51	1.00	1.00	1.00	1.00	1.00	1.00
5	Cd 0.8	48	**0.00**	**0.05**	**0.00**	1.00		1.00	1.00	1.00	1.00	**0.03**	0.50	1.00
6	Cd 0.8	72	**0.00**	**0.00**	**0.00**	0.51	1.00		0.12	1.00	1.00	**0.00**	**0.02**	1.00
7	Cd 1	24	1.00	1.00	1.00	1.00	1.00	0.12		1.00	1.00	1.00	1.00	0.94
8	Cd 1	48	**0.00**	0.08	**0.01**	1.00	1.00	1.00	1.00		1.00	**0.04**	0.68	1.00
9	Cd 1	72	0.09	1.00	0.61	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00
10	Cd 1.2	24	1.00	1.00	1.00	1.00	**0.03**	**0.00**	1.00	**0.04**	1.00		1.00	**0.01**
11	Cd 1.2	48	1.00	1.00	1.00	1.00	0.50	**0.02**	1.00	0.68	1.00	1.00		0.25
12	Cd 1.2	72	**0.00**	**0.02**	**0.00**	1.00	1.00	1.00	0.94	1.00	1.00	**0.01**	0.25	



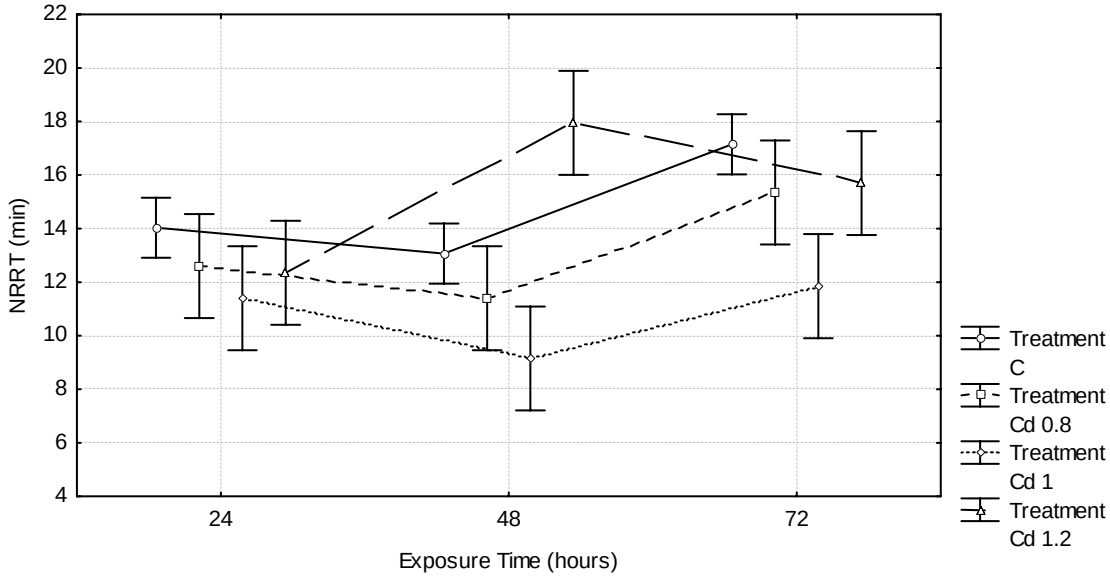
Appendix Table 110: Mean NRR times (min) for samples of *C. oculus* at three concentration exposures (mg/L $CdCl_2$) and three exposure times (hours) (n=10 for each concentration exposure, exposure time and control group). Vertical bars denote 95% confidence intervals ($p > 0.05$).



Appendix Figure 111: Mean NRR times (min) for samples of *S. longicosta* at three concentration exposures (mg/L $CdCl_2$) and three exposure times (hours) (n=10 for each concentration exposure, exposure time and control group). Vertical bars denote 95% confidence intervals ($p < 0.01$).



Appendix Figure 112: Mean NRR times (min) for samples of *S. granularis* at three concentration exposures (mg/L $CdCl_2$) and three exposure times (hours) (n=10 for each concentration exposure, exposure time and control group). Vertical bars denote 95% confidence intervals ($p < 0.01$).



Appendix Figure 113: Mean NRR times (min) for samples of *C. granatina* at three concentration exposures (mg/L $CdCl_2$) and three exposure times (hours) (n=10 for each concentration exposure, exposure time and control group). Vertical bars denote 95% confidence intervals ($p < 0.01$).

Appendix Figure 114: Two-way ANOVA indicating the univariate tests of significance for mean NRR times (min) for samples (all exposure concentrations and exposure times) of *C. oculus*. Values in bold text indicate significant interaction
($p < 0.01$).

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**62161.39**	**1**	**62161.39**	**2688.584**	**0.00**
Treatment	**1923.68**	**3**	**641.23**	**27.734**	**0.00**
Exposure Time	**1330.50**	**2**	**665.25**	**28.773**	**0.00**
Treatment*Exposure Time	173.71	6	28.95	1.252	0.28
Error	3745.52	162	23.12		

Appendix Table 115: Two-way ANOVA indicating the univariate tests of significance for mean NRR times (min) for samples (all exposure concentrations and exposure times) of *S. longicosta*. Values in bold text indicate significant interaction ($p < 0.01$).

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**47982.90**	**1**	**47982.90**	**3183.73**	**0.00**
Treatment	**2622.81**	**3**	**874.27**	**58.01**	**0.00**
Exposure Time	**433.38**	**2**	**216.69**	**14.38**	**0.00**
Treatment*Exposure Time	**516.61**	**6**	**86.10**	**5.71**	**0.00**
Error	2531.97	168	15.07		

Appendix Table 116: Two-way ANOVA indicating the univariate tests of significance for mean NRR times (min) for samples (all exposure concentrations and exposure times) of *S. granularis*. Values in bold text indicate significant interaction ($p < 0.01$).

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**28493.44**	**1**	**28493.44**	**3982.50**	**0.00**
Treatment	**3066.04**	**3**	**1022.01**	**142.85**	**0.00**
Exposure Time	**592.83**	**2**	**296.42**	**41.43**	**0.00**
Treatment*Exposure Time	**1355.56**	**6**	**225.93**	**31.58**	**0.00**
Error	1201.98	168	7.15		

Appendix Table 117: Two-way ANOVA indicating the univariate tests of significance for mean NRR times (min) for samples (all exposure concentrations and exposure times) of *S. granatina*. Values in bold text indicate significant interaction ($p < 0.01$).

Effect	SS	Degr. Of Freedom	MS	F	p
Intercept	**26244.00**	**1**	**26244.00**	**2712.34**	**0.00**
Treatment	**433.32**	**3**	**144.44**	**14.93**	**0.00**
Exposure Time	**166.81**	**2**	**83.41**	**8.62**	**0.00**
Treatment*Exposure Time	**261.13**	**6**	**43.52**	**4.50**	**0.00**
Error	1625.53	168	9.68		

Appendix Table 118: Bonferroni test for the mean NRR times (min) of *S. longicosta* samples over three exposure concentrations (mg/L $CdCl_2$) and three exposure times (hours) indicating significant interactions between samples. Values in bold text indicate significant interaction ($p < 0.05$).

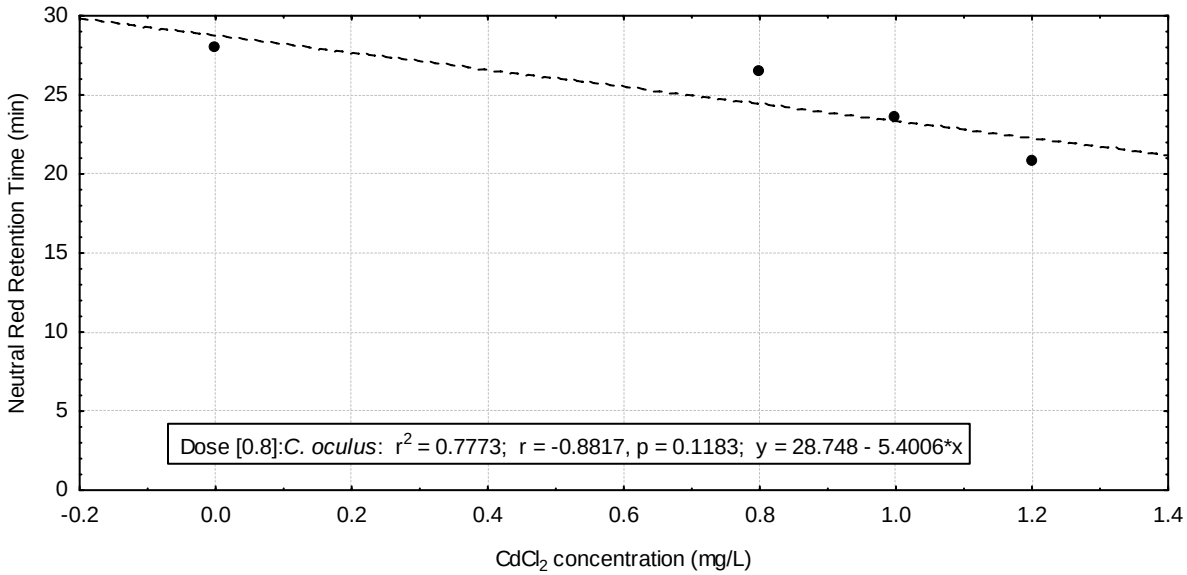
	Treatment	Exposure Time	1	2	3	4	5	6	7	8	9	10	11	12
1	C	24		0.24	**0.00**	0.06	0.52	**0.00**	1.00	**0.05**	**0.00**	1.00	**0.00**	**0.00**
2	C	48	0.24		1.00	**0.00**	**0.00**	**0.00**	1.00	1.00	**0.03**	1.00	0.74	**0.00**
3	C	72	**0.00**	1.00		**0.00**	**0.00**	**0.00**	1.00	1.00	1.00	1.00	1.00	**0.00**
4	Cd 0.8	24	0.06	**0.00**	**0.00**		1.00	1.00	**0.02**	**0.00**	**0.00**	**0.02**	**0.00**	**0.00**
5	Cd 0.8	48	0.52	**0.00**	**0.00**	1.00		0.35	0.14	**0.00**	**0.00**	0.14	**0.00**	**0.00**
6	Cd 0.8	72	**0.00**	**0.00**	**0.00**	1.00	0.35		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
7	Cd 1	24	1.00	1.00	1.00	**0.02**	0.14	**0.00**		1.00	**0.02**	1.00	0.30	**0.00**
8	Cd 1	48	**0.05**	1.00	1.00	**0.00**	**0.00**	**0.00**	1.00		1.00	1.00	1.00	0.07
9	Cd 1	72	**0.00**	**0.03**	1.00	**0.00**	**0.00**	**0.00**	**0.02**	1.00		**0.02**	1.00	1.00
10	Cd 1.2	24	1.00	1.00	1.00	**0.02**	0.14	**0.00**	1.00	1.00	**0.02**		0.30	**0.00**
11	Cd 1.2	48	**0.00**	0.74	1.00	**0.00**	**0.00**	**0.00**	0.30	1.00	1.00	0.30		1.00
12	Cd 1.2	72	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	0.07	1.00	**0.00**	1.00	

Appendix Table 119: Bonferroni test for the mean NRR times (min) of *S. granularis* samples over three exposure concentrations (mg/L $CdCl_2$) and three exposure times (hours) indicating significant interactions between samples. Values in bold text indicate significant interaction ($p < 0.05$).

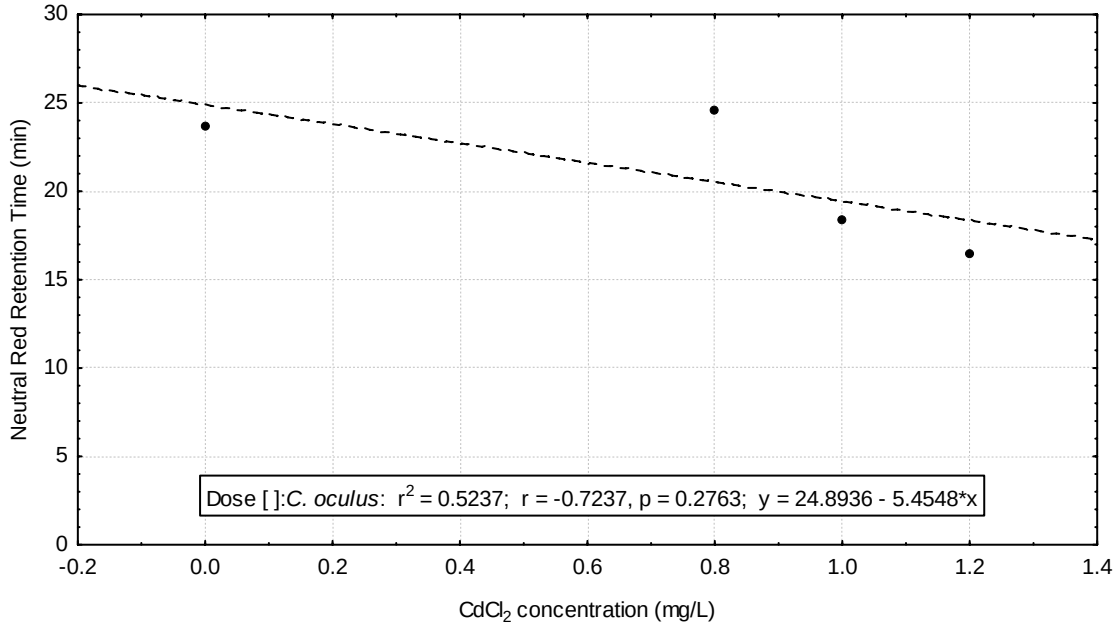
	Treatment	Exposure Time	1	2	3	4	5	6	7	8	9	10	11	12
1	C	24		1.00	0.33	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
2	C	48	1.00		1.00	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
3	C	72	0.33	1.00		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
4	Cd 0.8	24	**0.00**	**0.00**	**0.00**		1.00	1.00	**0.00**	1.00	1.00	1.00	1.00	0.23
5	Cd 0.8	48	**0.00**	**0.00**	**0.00**	1.00		1.00	**0.00**	1.00	1.00	1.00	0.29	**0.02**
6	Cd 0.8	72	**0.00**	**0.00**	**0.00**	1.00	1.00		**0.00**	0.97	1.00	1.00	1.00	1.00
7	Cd 1	24	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**		**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
8	Cd 1	48	**0.00**	**0.00**	**0.00**	1.00	1.00	0.97	**0.00**		1.00	1.00	0.23	**0.01**
9	Cd 1	72	**0.00**	**0.00**	**0.00**	1.00	1.00	1.00	**0.00**	1.00		1.00	1.00	0.97
10	Cd 1.2	24	**0.00**	**0.00**	**0.00**	1.00	1.00	1.00	**0.00**	1.00	1.00		1.00	0.15
11	Cd 1.2	48	**0.00**	**0.00**	**0.00**	1.00	0.29	1.00	**0.00**	0.23	1.00	1.00		1.00
12	Cd 1.2	72	**0.00**	**0.00**	**0.00**	0.23	**0.02**	1.00	**0.00**	**0.01**	0.97	0.15	1.00	

Appendix Table 120: Bonferroni test for the mean NRR times (min) of *S. granatina* samples over three exposure concentrations (mg/L $CdCl_2$) and three exposure times (hours) indicating significant interactions between samples. Values in bold text indicate significant interaction ($p < 0.05$).

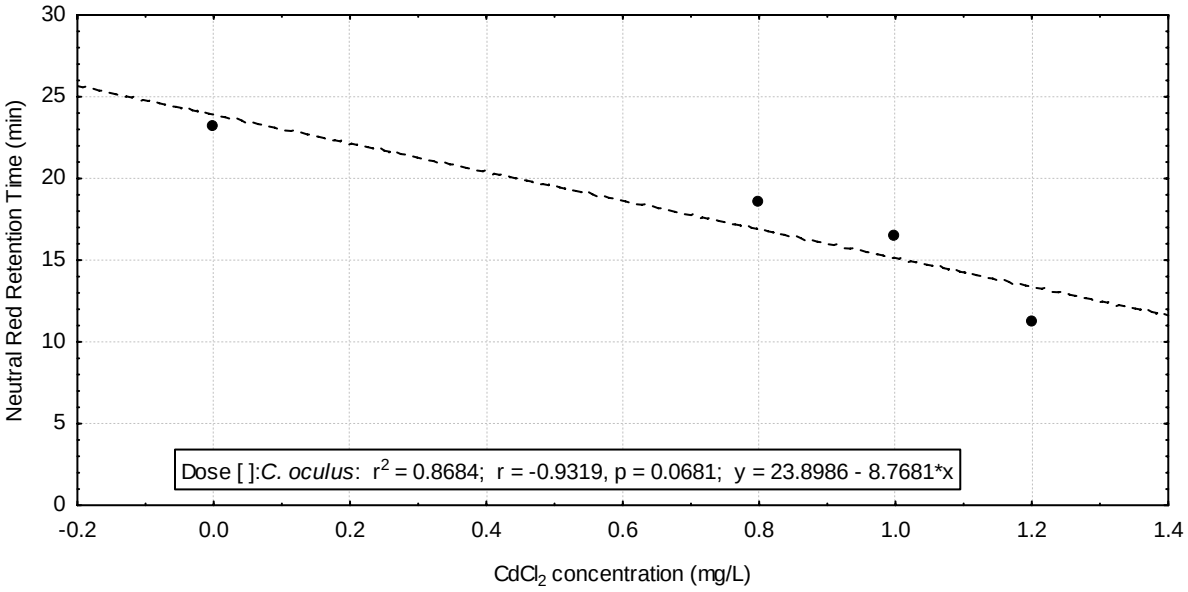
	Treatment	Exposure Time	1	2	3	4	5	6	7	8	9	10	11	12
1	C	24		1.00	**0.01**	1.00	1.00	1.00	1.00	**0.00**	1.00	1.00	**0.05**	1.00
2	C	48	1.00		**0.00**	1.00	1.00	1.00	1.00	**0.05**	1.00	1.00	**0.00**	1.00
3	C	72	**0.01**	**0.00**		**0.01**	**0.00**	1.00	**0.00**	**0.00**	**0.00**	**0.00**	1.00	1.00
4	Cd 0.8	24	1.00	1.00	**0.01**		1.00	1.00	1.00	0.93	1.00	1.00	**0.01**	1.00
5	Cd 0.8	48	1.00	1.00	**0.00**	1.00		0.34	1.00	1.00	1.00	1.00	**0.00**	0.15
6	Cd 0.8	72	1.00	1.00	1.00	1.00	0.34		0.34	**0.00**	0.85	1.00	1.00	1.00
7	Cd 1	24	1.00	1.00	**0.00**	1.00	1.00	0.34		1.00	1.00	1.00	**0.00**	0.15
8	Cd 1	48	**0.00**	**0.05**	**0.00**	0.93	1.00	**0.00**	1.00		1.00	1.00	**0.00**	**0.00**
9	Cd 1	72	1.00	1.00	**0.00**	1.00	1.00	0.85	1.00	1.00		1.00	**0.00**	0.41
10	Cd 1.2	24	1.00	1.00	**0.00**	1.00	1.00	1.00	1.00	1.00	1.00		**0.01**	1.00
11	Cd 1.2	48	**0.05**	**0.00**	1.00	**0.01**	**0.00**	1.00	**0.00**	**0.00**	**0.00**	**0.01**		1.00
12	Cd 1.2	72	1.00	1.00	1.00	1.00	0.15	1.00	0.15	**0.00**	0.41	1.00	1.00	



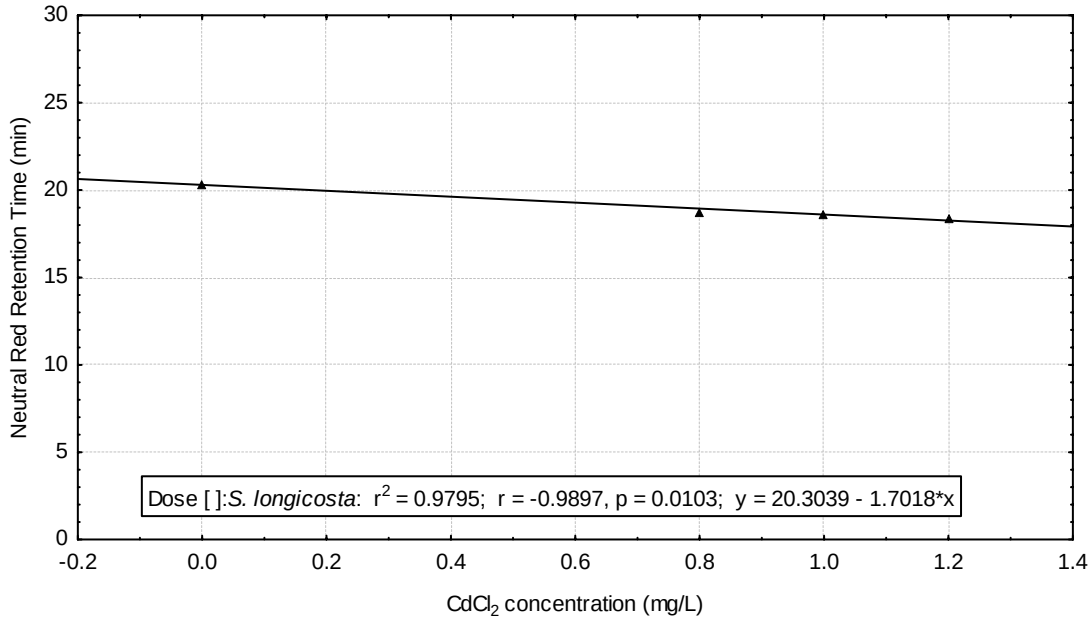
Appendix Figure 121: Mean neutral red retention time (min) for *C. oculus* after 24 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



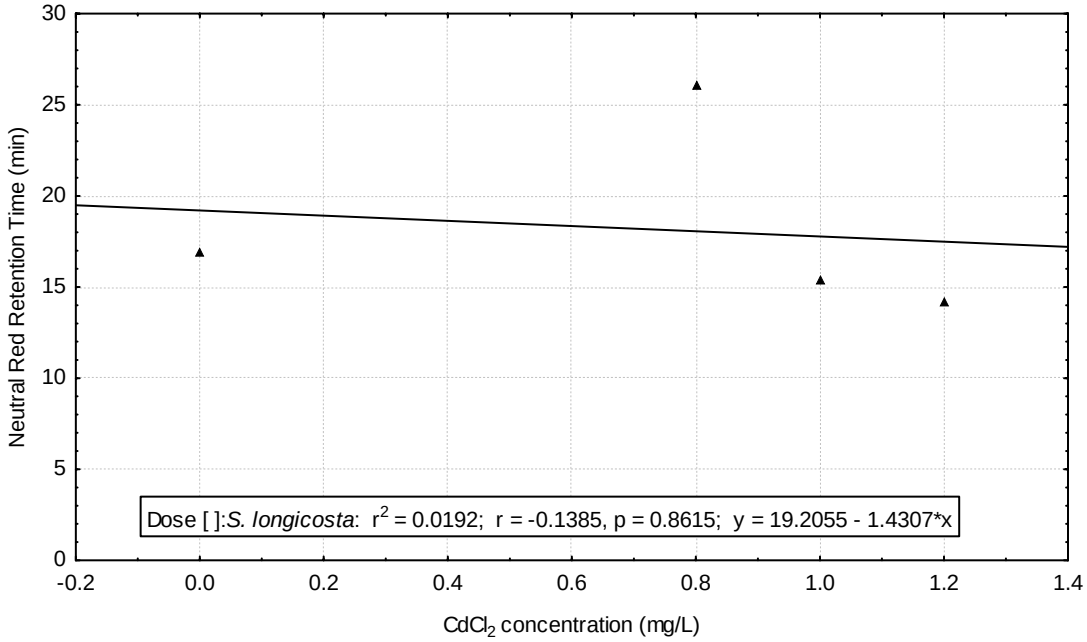
Appendix Figure 122: Mean neutral red retention time (min) for *C. oculus* after 48 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



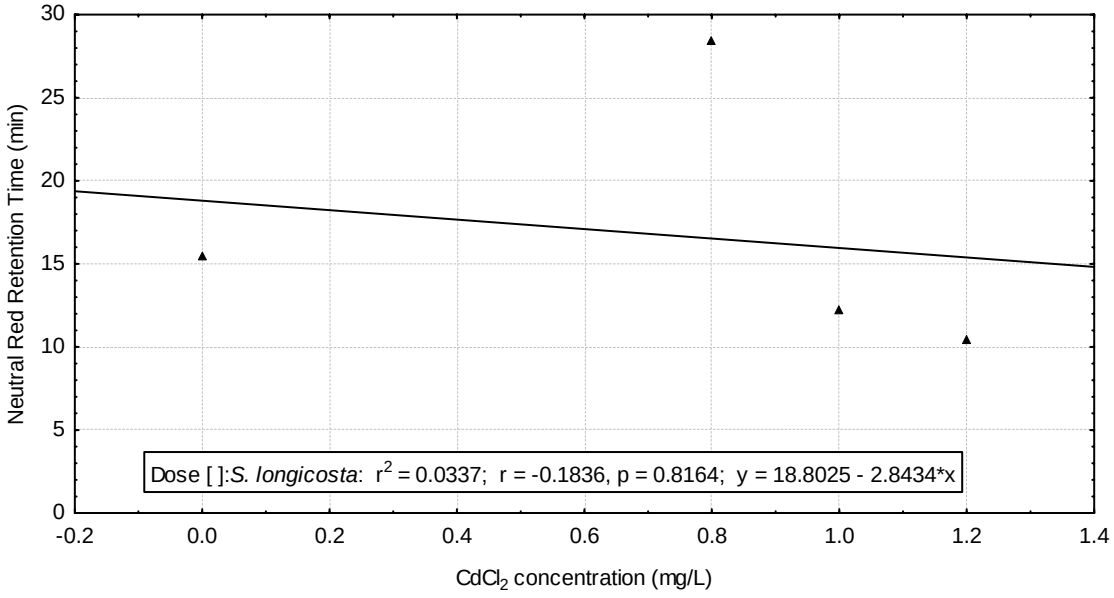
Appendix Figure 123: Mean neutral red retention time (min) for *C. oculus* after 72 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



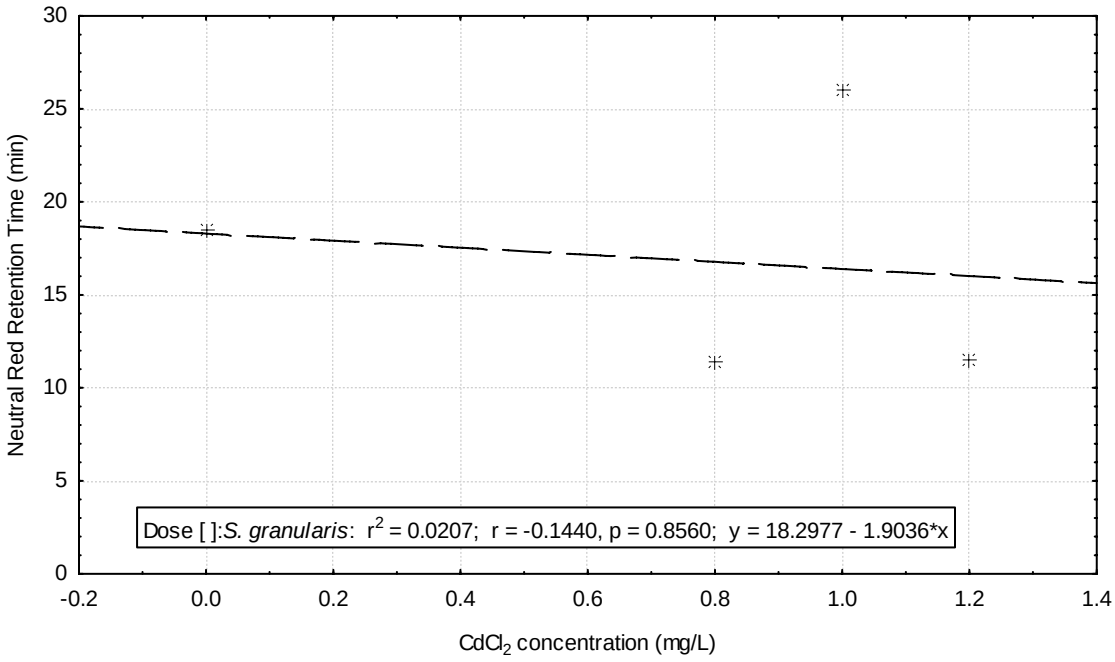
Appendix Figure 124: Mean neutral red retention time (min) for *S. longicosta* after 24 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



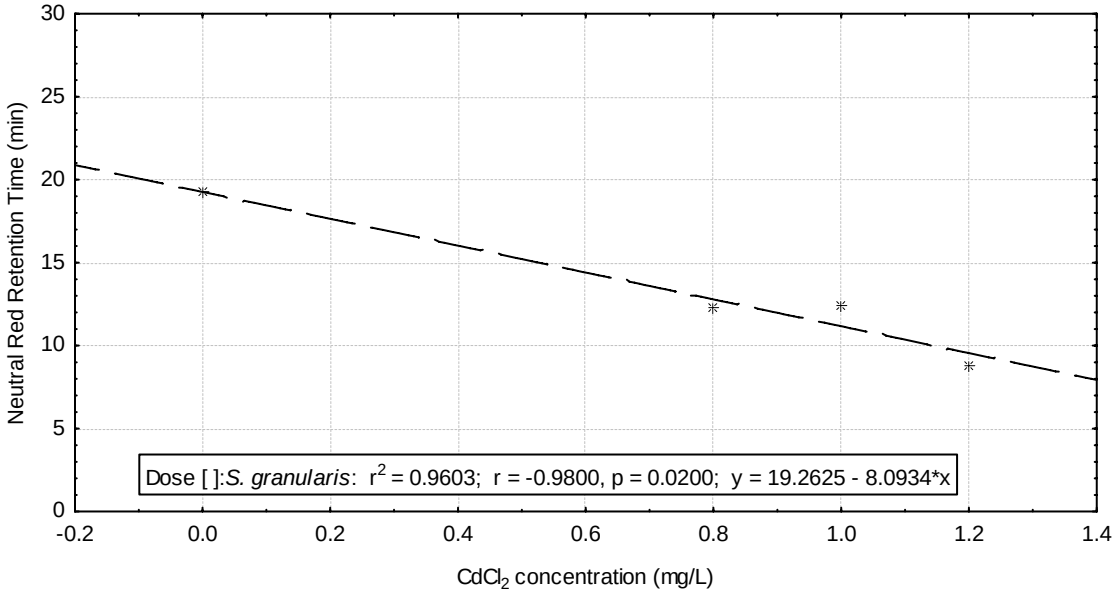
Appendix Figure 125: Mean neutral red retention time (min) for *S. longicosta* after 48 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



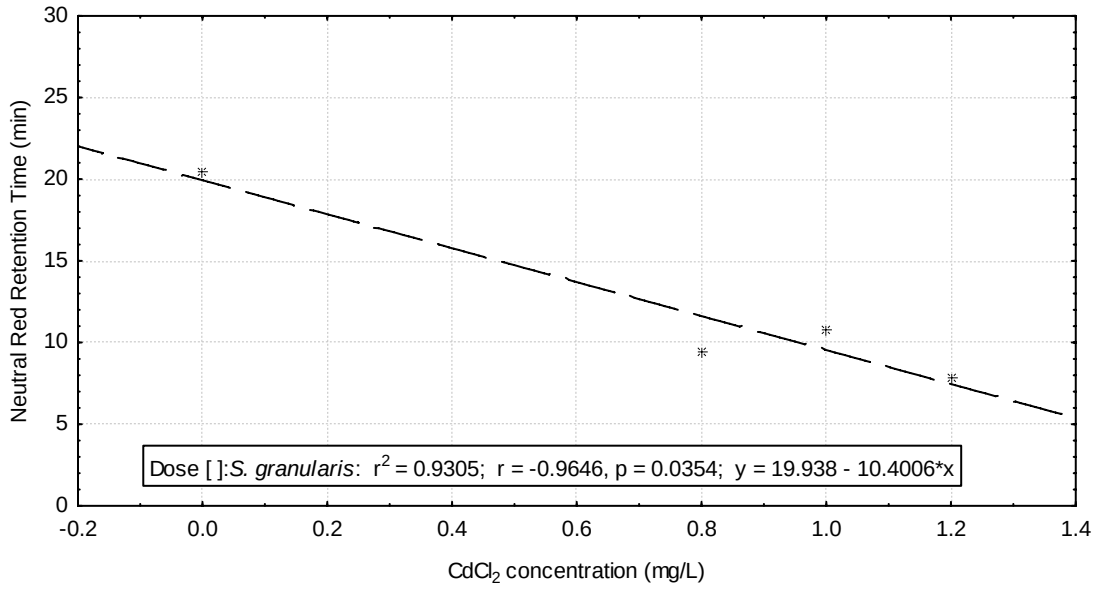
Appendix Figure 126: Mean neutral red retention time (min) for *S. longicosta* after 72 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



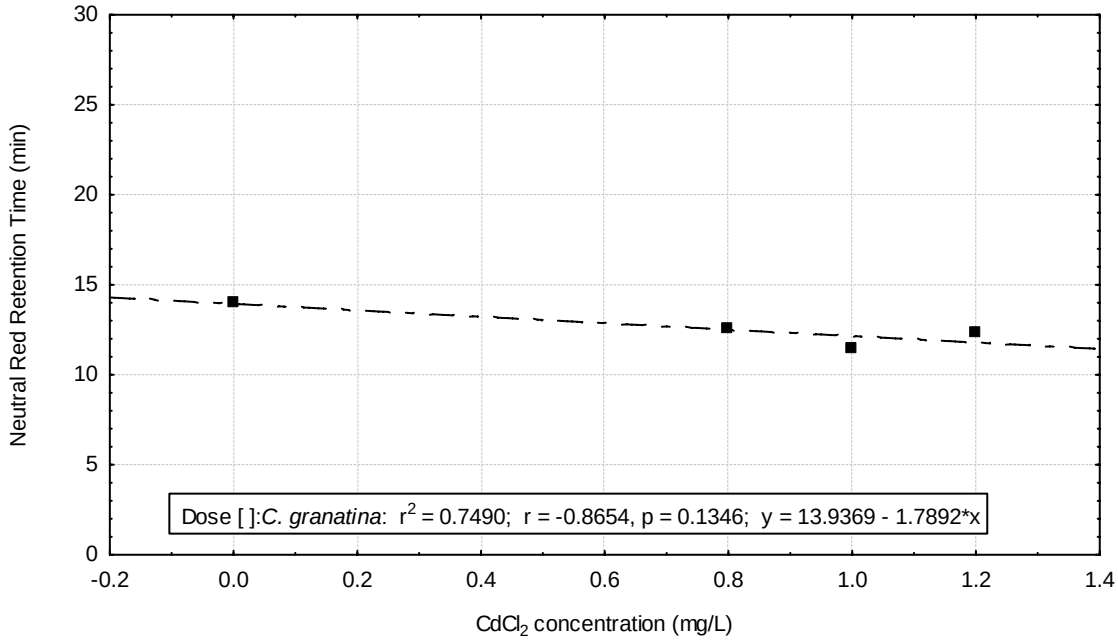
Appendix Figure 127: Mean neutral red retention time (min) for *S. granularis* after 24 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



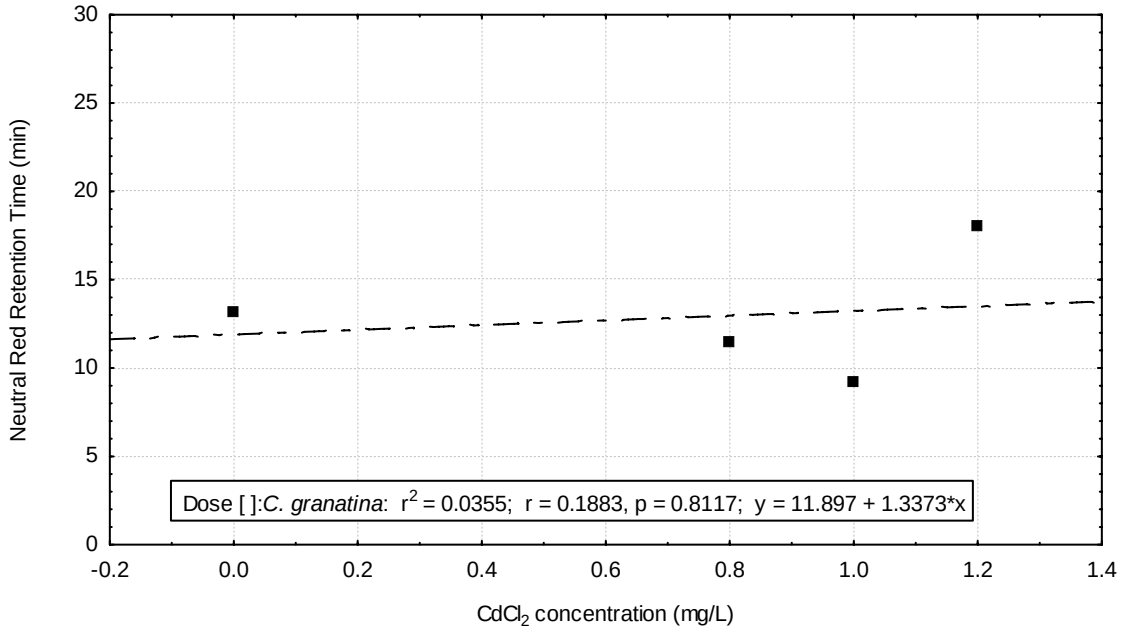
Appendix Figure 128: Mean neutral red retention time (min) for *S. granularis* after 48 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



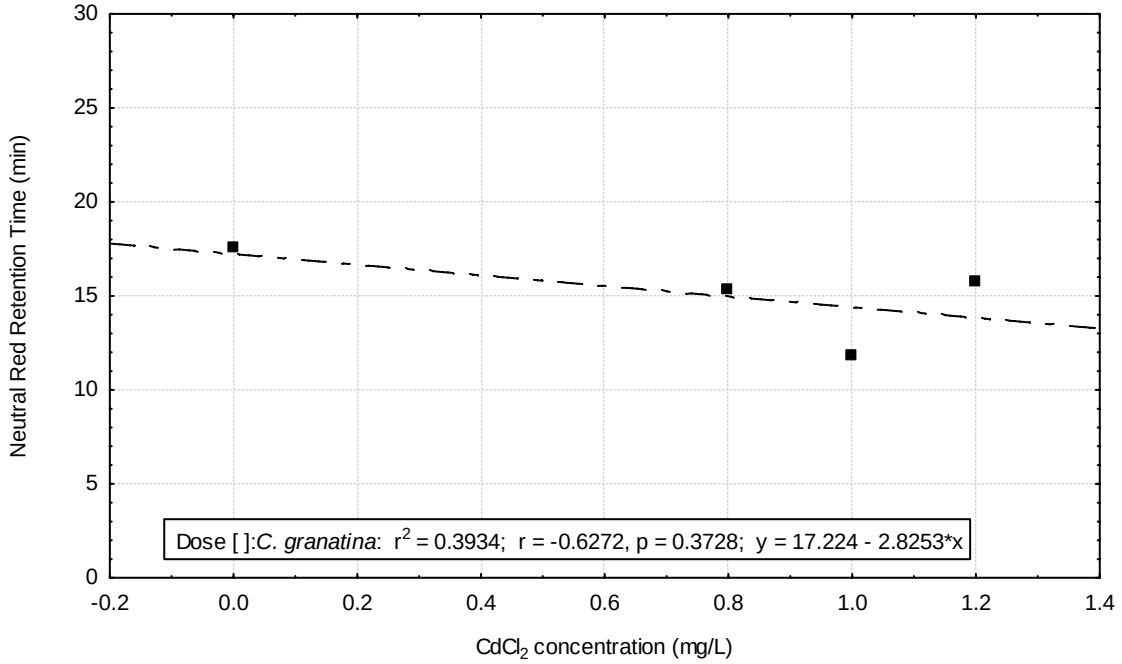
Appendix Figure 129: Mean neutral red retention time (min) for *S. granularis* after 72 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



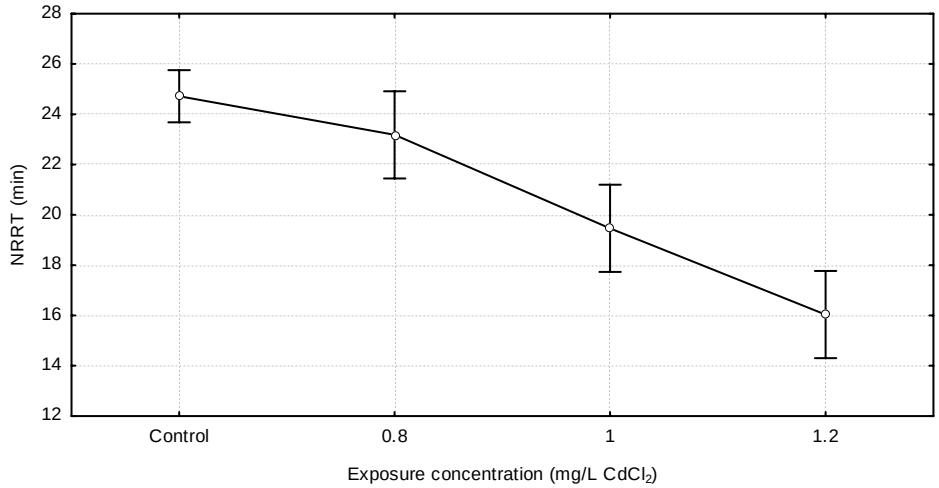
Appendix Figure 130: Mean neutral red retention time (min) for *S. granatina* after 24 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



Appendix Figure 131: Mean neutral red retention time (min) for *S. granatina* after 48 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



Appendix Figure 132: Mean neutral red retention time (min) for *S. granatina* after 72 hours exposure to 0.8, 1 and 1.2 mg/L $CdCl_2$ in sea water.



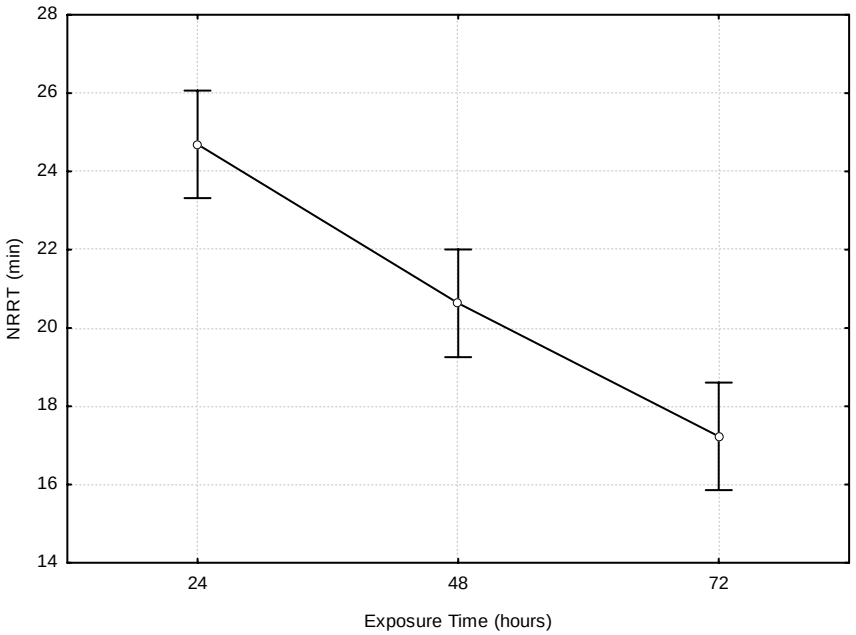
Appendix Figure 133: Mean NRR times (min) of *C. oculus* at three exposure concentrations (mg/L $CdCl_2$) (n=30 for each exposure concentration). Control group done in conjunction with the exposure group (n=84). Vertical bars indicate 95% confidence intervals.

Appendix Table 134: Mean NRR times (min) of *C. oculus* at three exposure concentrations (mg/L $CdCl_2$) (n=30 for each exposure concentration). Control group done in conjunction with the exposure group (n=84).

Exposure conc. (mg/L)	Mean NRRT (min)	Std.Err.	-95.00%	+95.00%	N
Control	24.72	0.52	23.68	25.76	84
Cd 0.8	23.18	0.88	21.45	24.92	30
Cd 1	19.47	0.88	17.73	21.20	30
Cd 1.2	16.03	0.88	14.30	17.77	30

Appendix Table 135: Bonferroni test for the mean NRR times (min) of *C. oculus* samples over three exposure concentrations (mg/L $CdCl_2$) indicating significant interactions between samples (See Appendix Figure 136). Values in bold text indicate significant interaction ($p < 0.05$).

	Exposure concentration (mg/L)	1	2	3	4
1	**Control**		0.81	**0.00**	**0.00**
2	**Cd 0.8**	0.81		**0.02**	**0.00**
3	**Cd 1**	**0.00**	**0.02**		**0.04**
4	**Cd 1.2**	**0.00**	**0.00**	**0.04**	



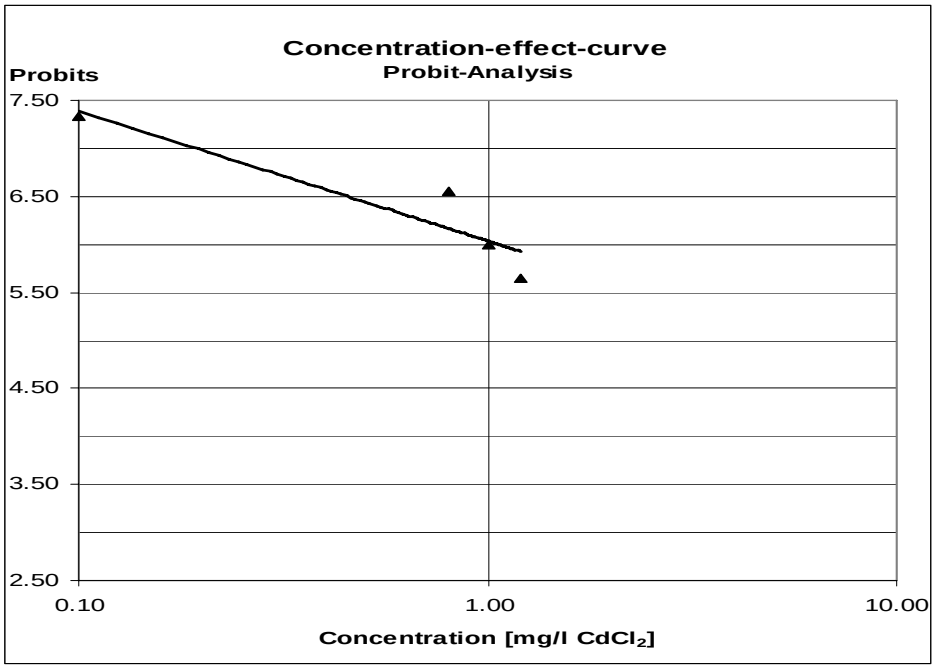
Appendix Figure 136: Mean NRR times (min) for *C. oculus* samples at three exposure times (hours) (n=58, control included). Mean values are the combined samples for each exposure concentration (mg/L $CdCl_2$) at a specific exposure time (hours). Vertical bars indicate 95% confidence intervals.

Appendix Table 137: Mean NRR times (min) for *C. oculus* samples at three exposure times (hours) (n=58, control included).

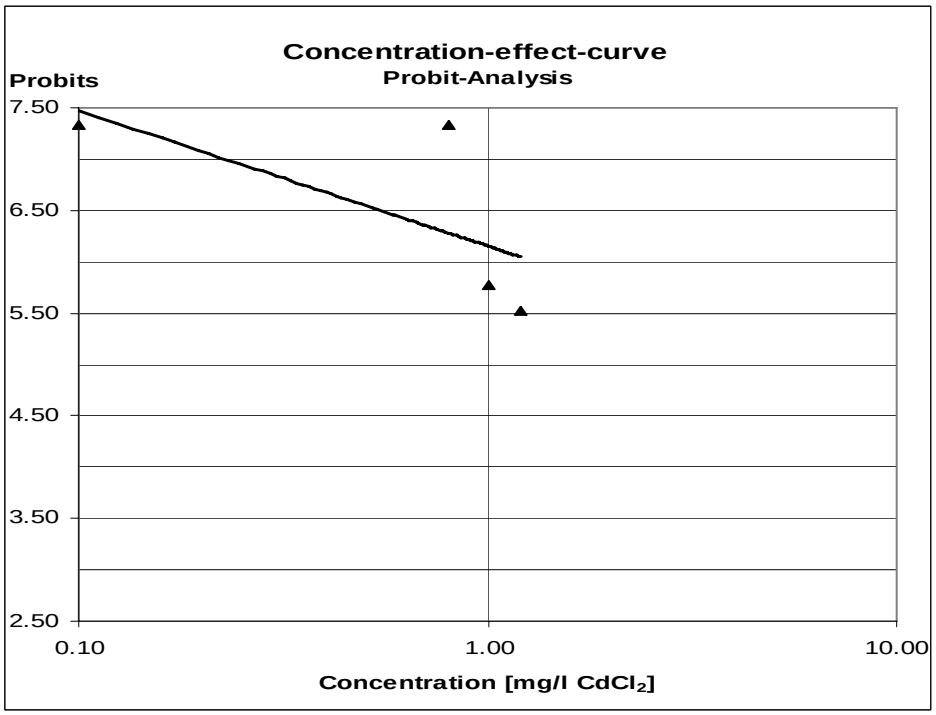
Exposure time (hours)	Mean NRRT (min)	Std.Err.	-95.00%	+95.00%	N
24	24.69	0.70	23.32	26.07	58
48	20.63	0.70	19.26	22.01	58
72	17.23	0.70	15.85	18.60	58

Appendix Table 138: Bonferroni test for the mean NRR times (min) of *C. oculus* samples over three exposure times (hours) indicating significant interactions between samples (See Appendix Figure 139). Values in bold text indicate significant interaction ($p < 0.05$).

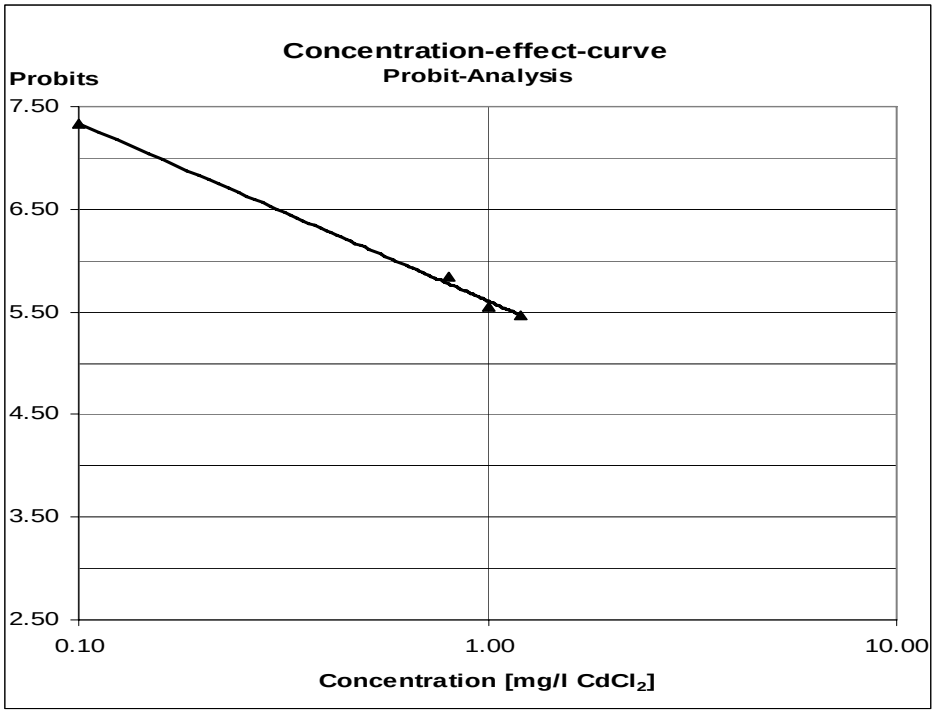
	Treatment	1	2	3
1	C		**0.00**	**0.00**
2	Cd 0.8	**0.00**		**0.02**
3	Cd 1	**0.00**	**0.02**	



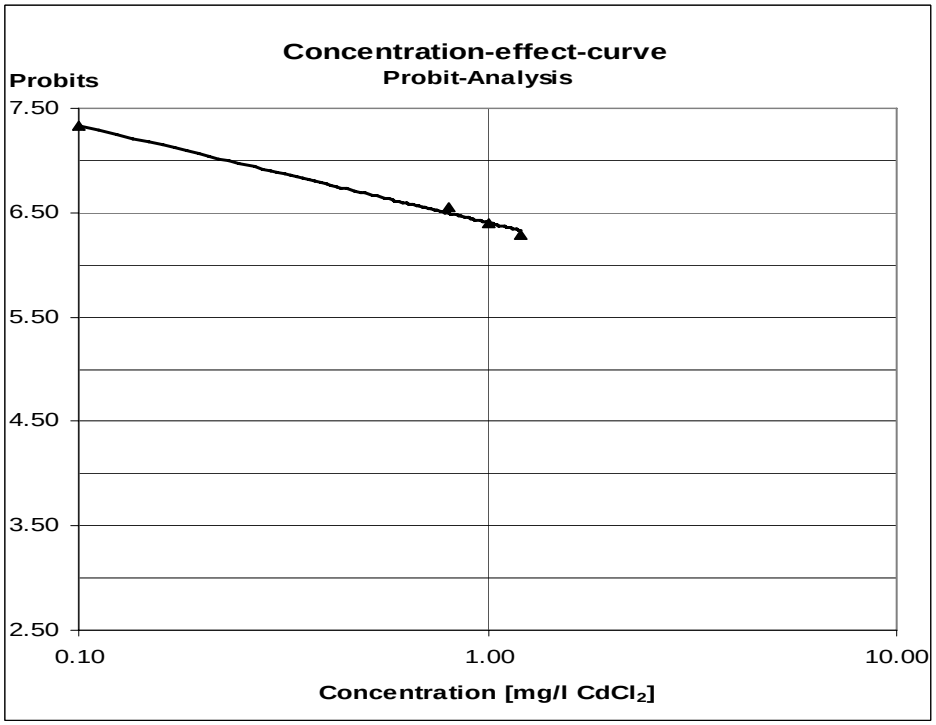
Appendix Figure 139: Probit analysis of the dose response plots for the 24 hours exposure period for *C. oculus*. The y-axis indicates the % response in probits.



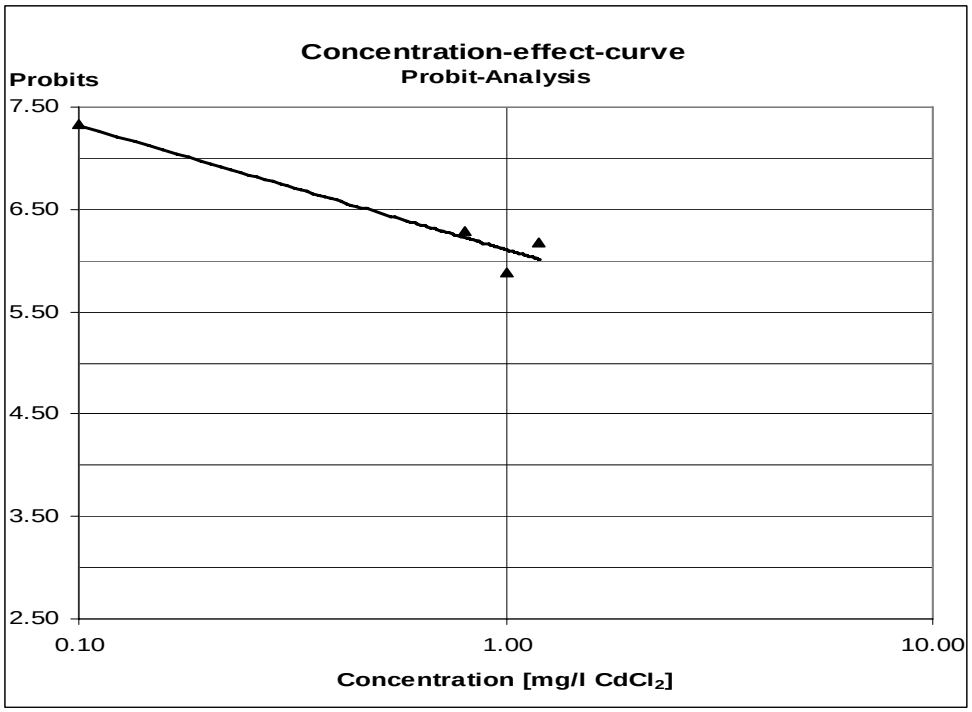
Appendix Figure 140: Probit analysis of the dose response plots for the 48 hours exposure period for *C. oculus*. The y-axis indicates the % response in probits.



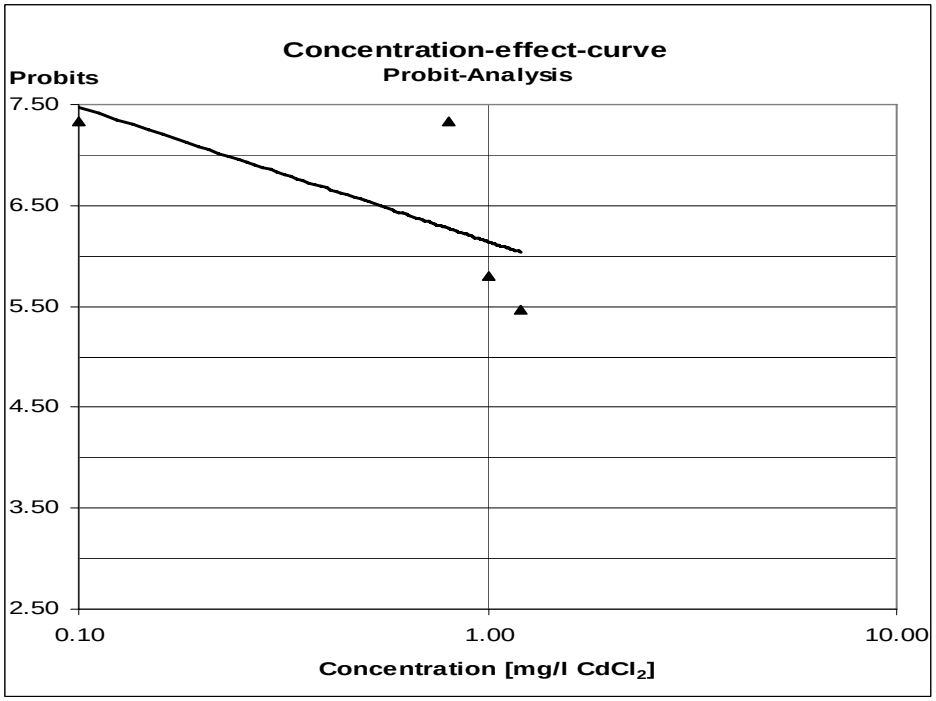
Appendix Figure 141: Probit analysis of the dose response plots for the 72 hours exposure period for *C. oculus*. The y-axis indicates the % response in probits.



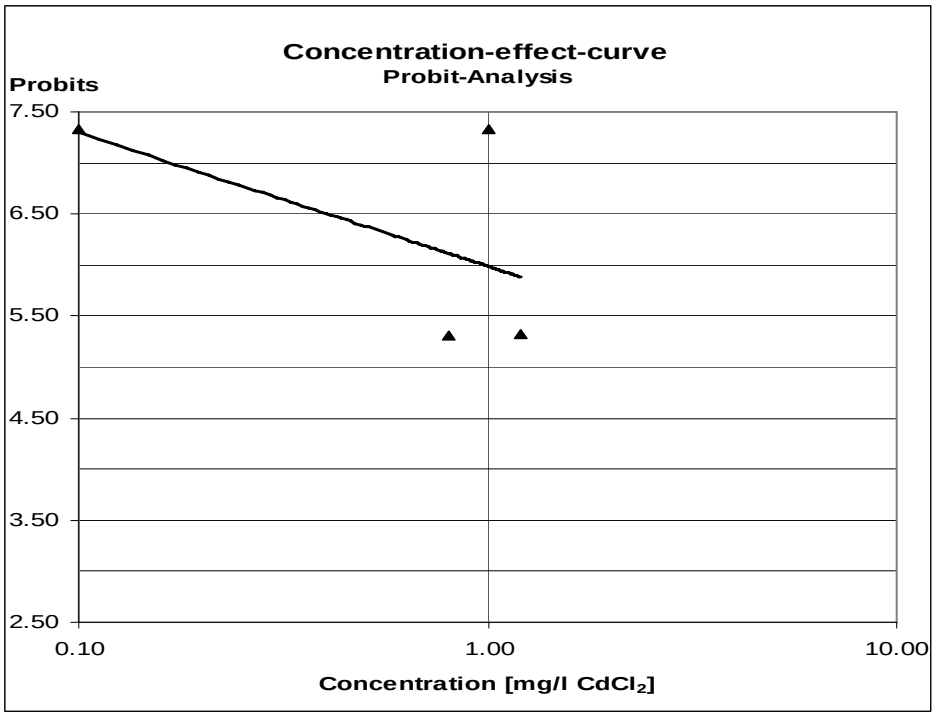
Appendix Figure 142: Probit analysis of the dose response plots for the 24 hours exposure period for *S. longicosta*. The y-axis indicates the % response in probits.



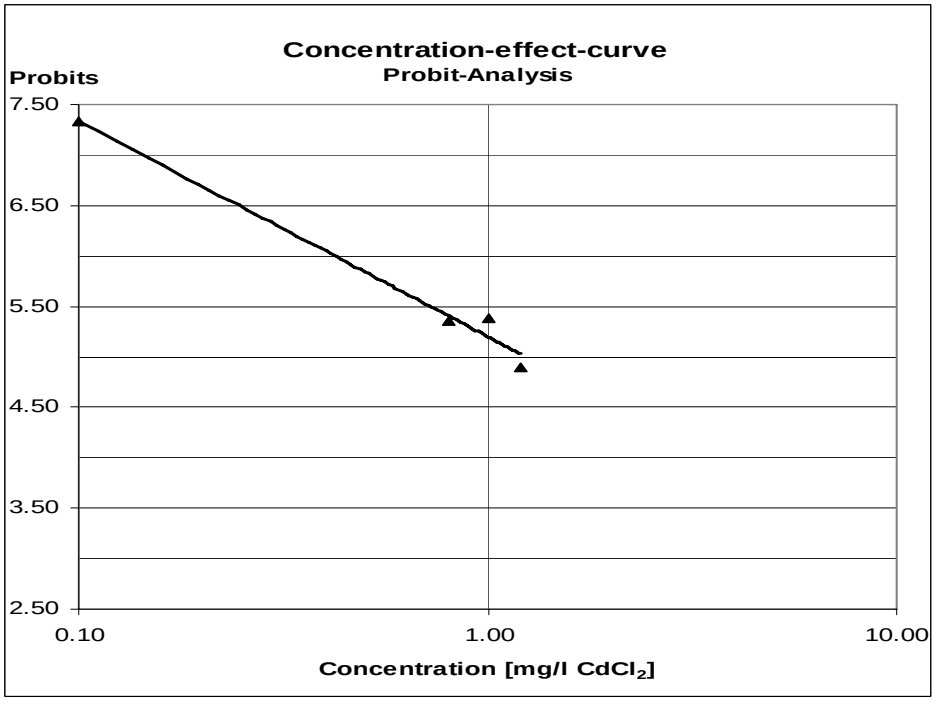
Appendix Figure 143: Probit analysis of the dose response plots for the 48 hours exposure period for *S. longicosta*. The y-axis indicates the % response in probits.



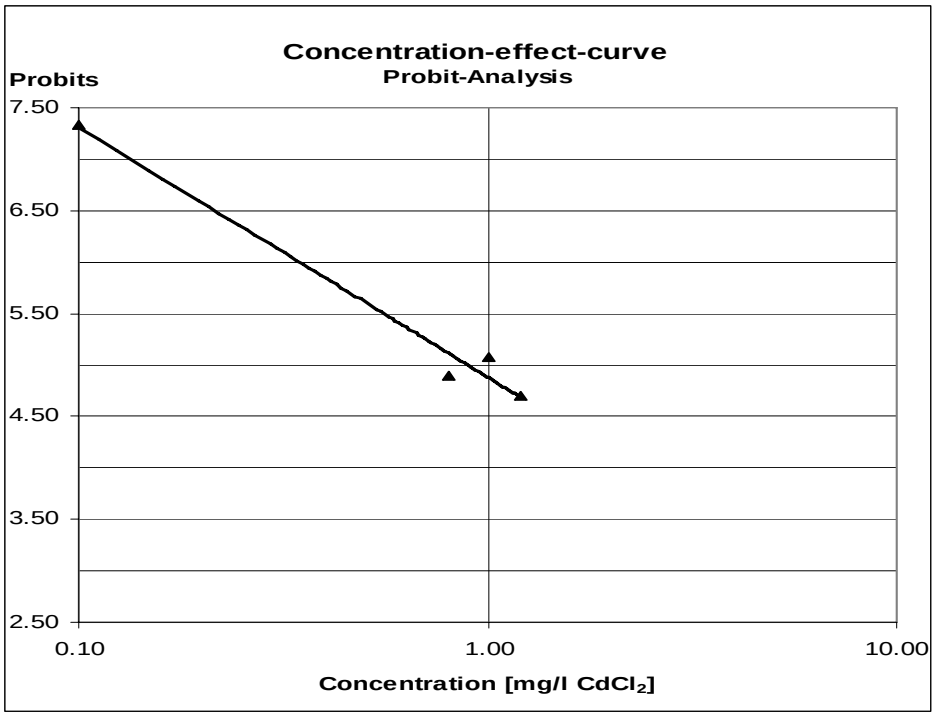
Appendix Figure 144: Probit analysis of the dose response plots for the 72 hours exposure period for *S. longicosta*. The y-axis indicates the % response in probits.



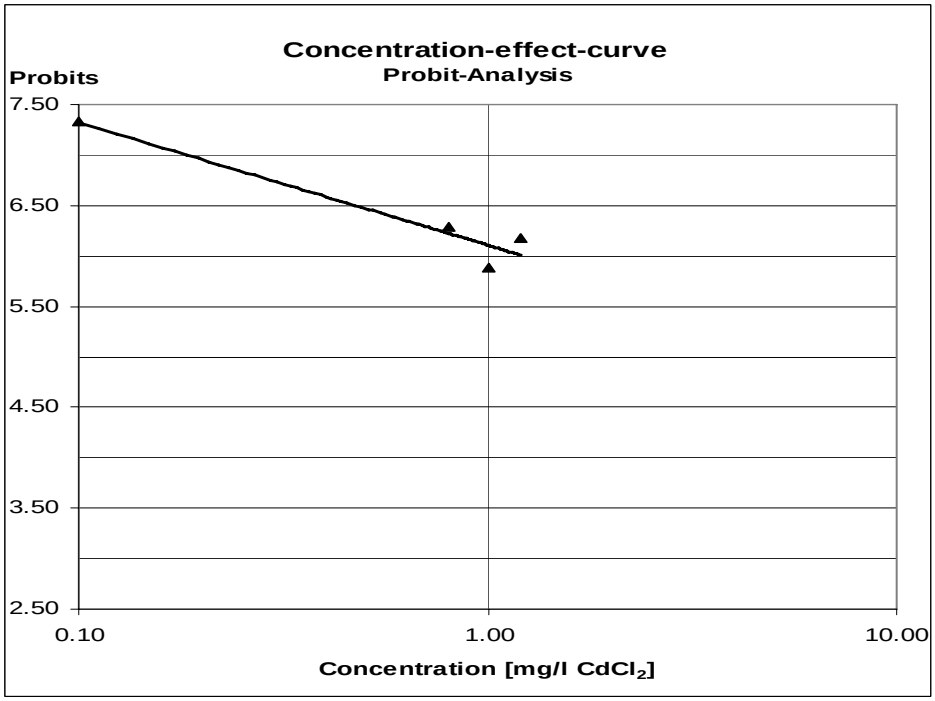
Appendix Figure 145: Probit analysis of the dose response plots for the 24 hours exposure period for *S. granularis*. The y-axis indicates the % response in probits.



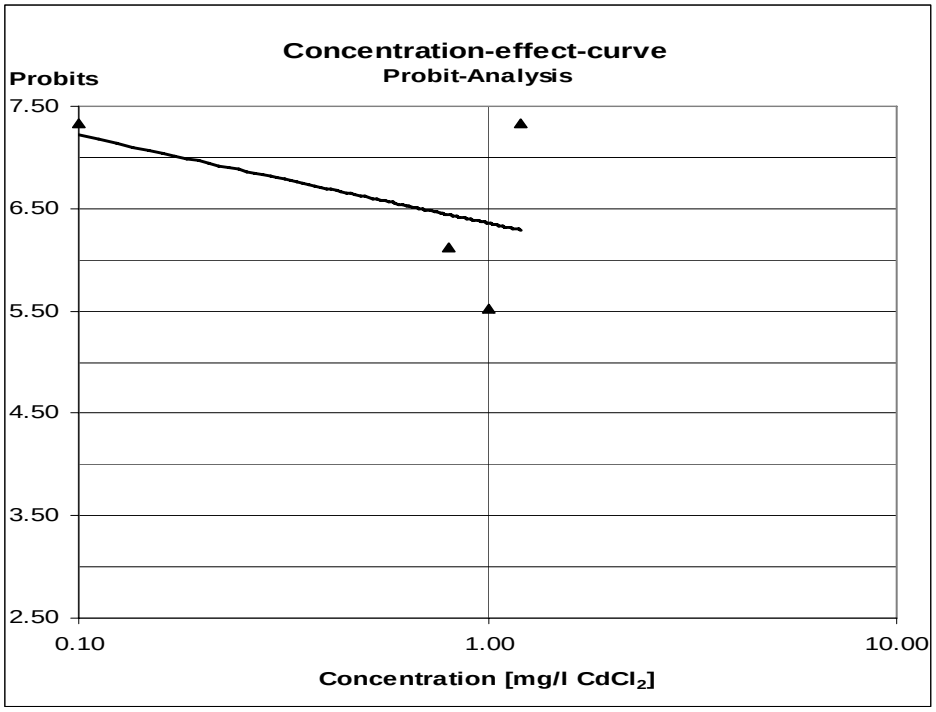
Appendix Figure 146: Probit analysis of the dose response plots for the 48 hours exposure period for *S. granularis*. The y-axis indicates the % response in probits.



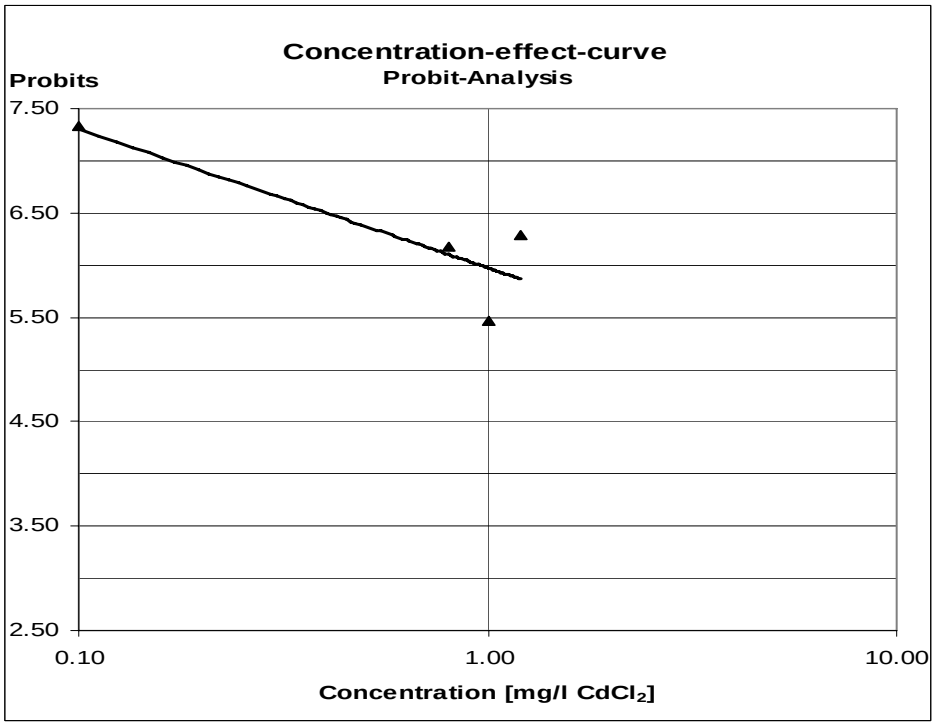
Appendix Figure 147: Probit analysis of the dose response plots for the 72 hours exposure period for *S. granularis*. The y-axis indicates the % response in probits.



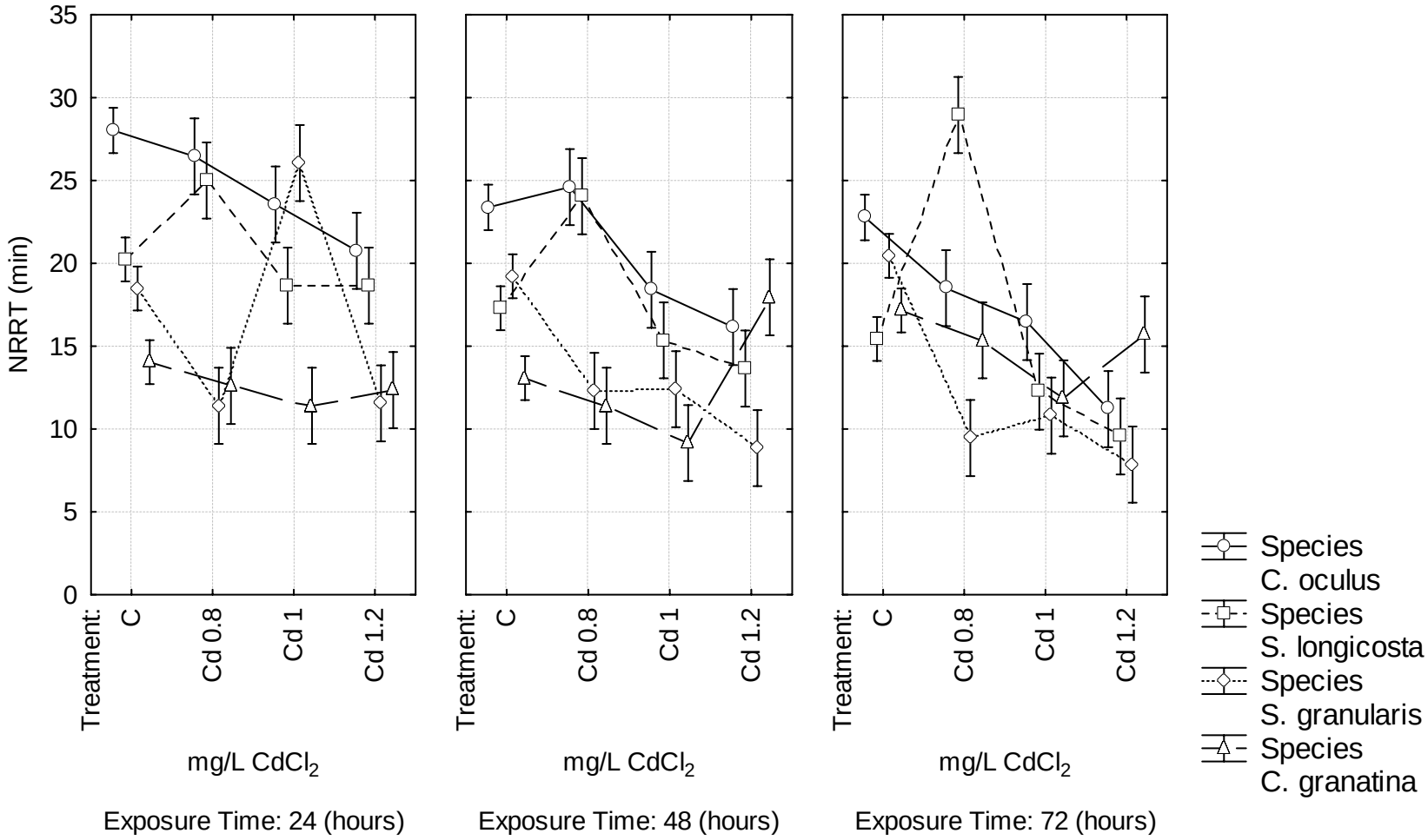
Appendix Figure 148: Probit analysis of the dose response plots for the 24 hours exposure period for *C. granatina*. The y-axis indicates the % response in probits.



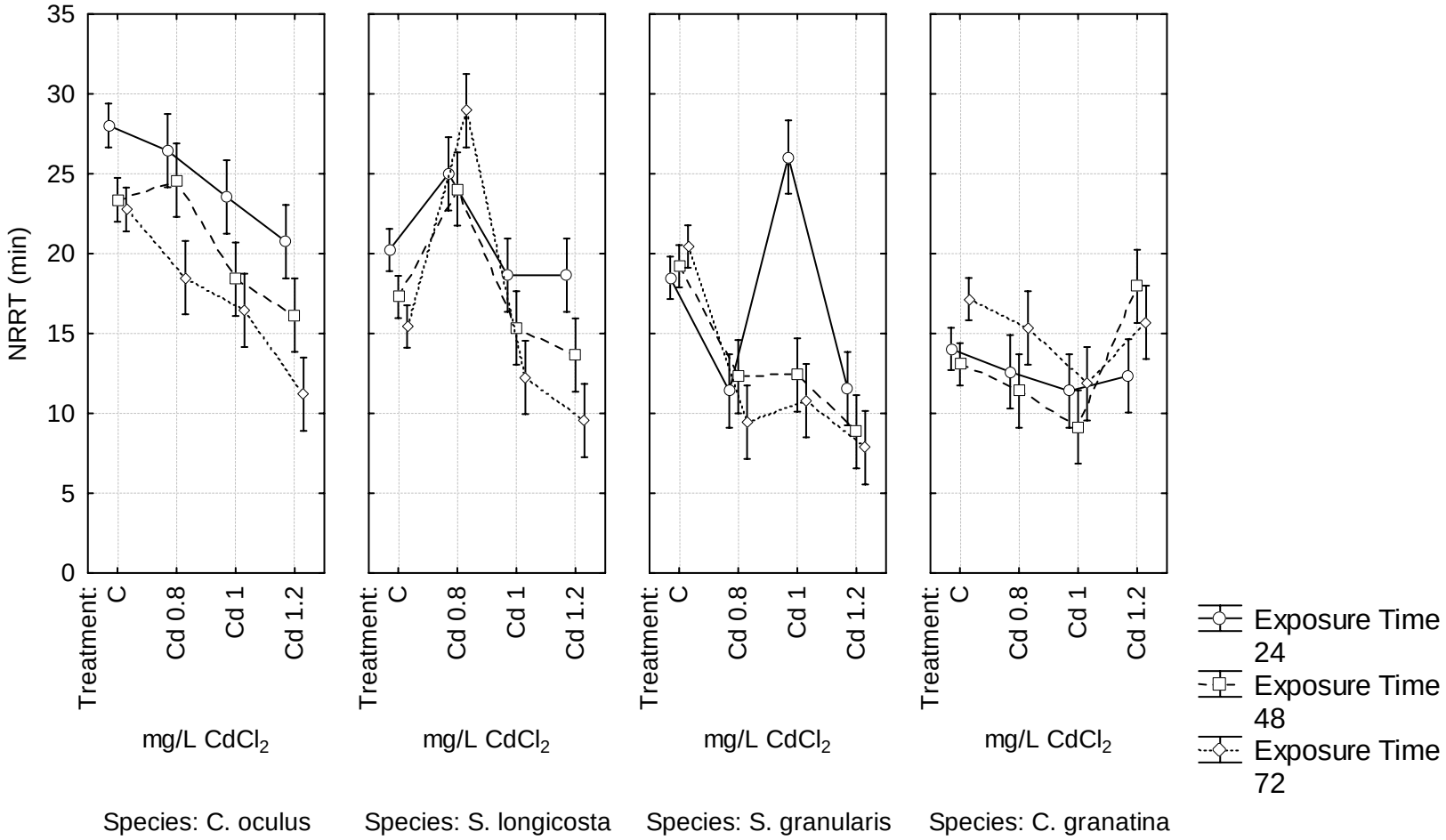
Appendix Figure 149: Probit analysis of the dose response plots for the 48 hours exposure period for *C. granatina*. The y-axis indicates the % response in probits.



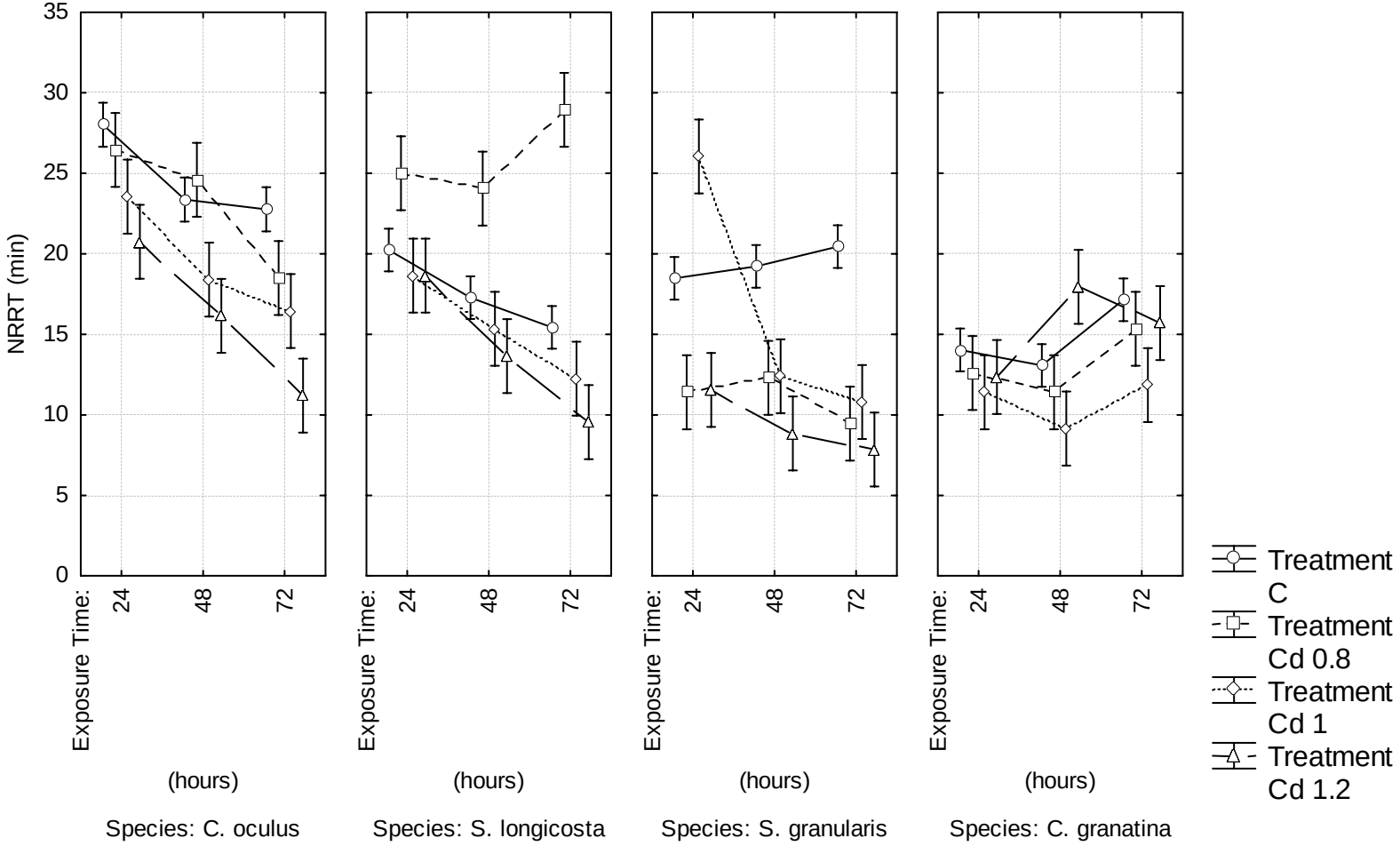
Appendix Figure 150: Probit analysis of the dose response plots for the 72 hours exposure period for *C. granatina*. The y-axis indicates the % response in probits.



Appendix Figure 151: Mean NRR times for *C. oculus, S. longicosta, S. granularis* and *C. grantina* over three exposure concentrations (x-axis, control included) and three exposure times (each block figure illustrates a different exposure time).



Appendix Figure 152: Mean NRR times for *C. oculus, S. longicosta, S. granularis* and *C. grantina* over three exposure concentrations (x-axis, control included) and three exposure times (each block figure illustrates a different species).



Appendix Figure 153: Mean NRR times for *C. oculus, S. longicosta, S. granularis* and *C. grantina* over three exposure times (x-axis) and three exposure concentrations and control (each block figure illustrates a different species, C = control).