Ecotoxicology and Environmental Safety 74 (2011) 1011–1020

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Bioaccumulation and toxicity of copper in outdoor freshwater microcosms

Tham C. Hoang [a], Rachel L. Pryor [a], Gary M. Rand [a,*], Robert A. Frakes [b]

[a] Ecotoxicology & Risk Assessment Laboratory, Southeast Environmental Research Center, Department of Earth and Environment, Florida International University, 3000 NE 151st Street, North Miami, FL 33181, USA
[b] US Fish and Wildlife Service, Vero Beach, Florida, USA

ARTICLE INFO

Article history:
Received 21 October 2010
Received in revised form
20 January 2011
Accepted 21 January 2011
Available online 22 February 2011

Keywords:
Copper
Freshwater microcosms
Florida apple snails
Pomacea paludosa

ABSTRACT

This study characterizes the effects of copper (Cu) on Florida apple snails (*Pomacea paludosa*) and mosquito fish (*Gambusia affinis*) using a replicated outdoor microcosm design. Soils used in this study were collected from two Cu-enriched citrus agricultural sites in South Florida (Agler property (AGLR) in St. Lucie County and Sunrise Boys property (SRB) in Palm Beach County) and a reference site (Equus property) in St. Lucie County. The study included a 5-week aging phase, an 11 month exposure phase, and a 3 month post-treatment (exposure) phase. The aging phase was initiated by flooding agricultural soils with rainwater in 4 m^3 fiberglass microcosm tanks. Introducing juvenile apple snails ($\leq$7 d old) and mosquito fish (2–3 cm) into the microcosm tanks initiated the exposure phase. Survival, growth, and reproduction of apple snails and fish, and Cu uptake in apple snails, fish, and periphyton were determined in this study. Water chemistry (e.g., dissolved Cu concentration, dissolved organic carbon and dissolved oxygen concentrations, pH, hardness, alkalinity, etc.) was measured daily or weekly during the study. Initial soil Cu concentrations in Equus, SRB, and AGLR microcosms were 7, 55, and 99 mg/kg dw, respectively. Dissolved Cu concentrations in Equus, SRB and AGLR microcosms at the beginning of the study were 3, 82, and 43 μg/L, respectively and decreased to low saturation levels of about $\leq$9 μg/L Cu after the first 3 months of the study. The decrease of dissolved Cu concentrations was likely due to the dilution of rainwater. Snail and fish mortality appeared to be higher in SRB microcosms than in Equus and AGLR microcosms. There was no significant difference in growth of the snails between treatments. Snail growth data followed the von Bertalanffy Model. The maximum shell length, shell height, and shell width of the snails calculated by the von Bertalanffy Model (L_∞) were 2.76, 2.05, and 2.18 cm, respectively. The maximum wet weight was 9.38 g. Growth rate (k) of the snails increased in order of shell height (0.459), shell length (0.550), and shell weight (0.598). There was no reproduction in the snails in any treatments including the reference during the exposure phase. However, Cu did not affect reproduction of fish during this period. Copper concentrations in periphyton from Equus, SRB, and AGLR microcosms ranged from 2 to 62, 31 to 371, and 13 to 478 mg/kg, respectively. Copper concentrations in fish at the beginning, days 30 and 150 of the study ranged from 3.19 to 7.53 mg/kg and were not significantly different from the different treatments. Average Cu concentrations in the soft tissue of dead snails from SRB and AGLR microcosms were 4602 mg/kg dw (ranged from 2913 to 8370 mg/kg dw) and 2824 mg/kg dw (ranged from 2118 to 3600 mg/kg dw), respectively. The Cu concentrations in the soft tissue of dead snails found in this study were higher than the tissue Cu concentrations in live aquatic organisms reported in the literature. These high Cu concentrations in edible apple snail soft tissue might pose a risk to Florida apple snail predators, including the snail kite. The post-exposure phase, with snails exposed to only water (i.e., no soils) showed depuration of copper from apple snails and reproduction in all treatments.

1. Introduction

Copper (Cu) use in the form of copper sulfate and copper hydroxide in Florida as an algaecide, fungicide and soil amendment extends back to the early 1900s (Alva et al., 1995; USDA, 2005). The latter, along with high sorption capacity has resulted in Cu accumulation of soils overtime (SFWMD, 2001–2006). Implementation of the Comprehensive Everglades Restoration Plan (CERP) under the Water Resources Development Act of 2000 requires acquisition of thousands of acres of land for maintaining hydrologic buffer areas and for the creation of storm-water treatment areas, water storage reservoirs, and wetlands (Everglades National Park,

* Corresponding author. Fax: +1 305 919 5887.
E-mail address: randg@fiu.edu (G.M. Rand).

0147-6513/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2011.01.015

2001). A large portion of these lands is currently or was formerly agriculture-managed for row crops and citrus fruit orchards treated with fertilizers and pesticides, including Cu. Under the CERP, these Cu-enriched soils will be flooded, converting dry aerobic environments to inundated (perennially or intermittently) relatively undisturbed anaerobic sediments which will likely promote the release of Cu from these soils. Sediment is therefore a natural sink for Cu (Eisler, 1998).

A comparison of aqueous Cu concentrations in agricultural and non-agricultural watersheds shows higher concentrations in runoff where agriculture was practiced compared to runoff near non-agriculture land (Dietrich et al., 2001). Copper loads in surface runoff are related to total Cu in soils, soil properties, metal characteristics, and environmental factors, especially in sandy soils (He et al., 2006). Furthermore, total soil Cu increases proportionally to the age of citrus production (Reuther and Smith, 1952, 1953). Therefore, although annual contributions may be small, as a result of application, Cu concentrations in sandy soils in Florida with long-term citrus production show accumulation and are significantly greater than similar soils with native vegetation (Alva et al., 1995; Zhu and Alva, 1993).

Agricultural areas with Cu-enriched soils which become flooded and converted to storage basins or wetlands under the CERP will ultimately become habitat for sensitive aquatic receptors, like the Florida apple snail (and/or the exotic apple snail). The Florida apple snail (*Pomacea paludosa*) is a periphyton-grazing (i.e., microphyto-phagous, including plant detritus) and macrophyte-consuming freshwater prosobranch gastropod mollusc (Turner et al., 2001) and a key species in the Everglades ecosystem since it is the sole food source of the federally endangered Florida snail kite (*Rostrhamus sociabilis plumbeus*) and a prey species for other birds (e.g., limpkins), fish (e.g., redear sunfish), reptiles, and mammals (Sharfstein and Steinman, 2001). The literature indicates that the highest Cu accumulations are generally found in molluscan soft tissue and the lowest Cu accumulation is in vertebrates (e.g., fish). The marine gastropod (whelk), *Busycon canaliculatum*, can accumulate and store Cu and use it in the synthesis of hemocyanin (i.e., a Cu respiratory pigment) (Betzer and Yevich, 1975).

P. paludosa is particularly vulnerable to Cu accumulation and toxicity because it spends most of its life cycle submersed, except for the females during oviposition (Turner, 1994; Turner et al., 2001). Furthermore, their diet consists of periphyton and macrophytes which bioconcentrate Cu (Eisler, 1998). Cu-contaminated sediment may also be ingested by apple snails since sediment particles are used to facilitate digestion (Imlay, 1983). Estivation, a cyclical activity of the apple snail family (Ampullariidae), may be important, especially during periods of drought or other conditions (i.e., "dry downs") that reduce water levels (Darby et al., 2008) since it has been shown that sediment-bound Cu is readily available to deposit feeding clams, especially from anoxic sediments at low pH (Bryan and Langston, 1992). The Florida apple snail based on its behavioral ecology is therefore potentially in intimate contact with all three routes of Cu exposure: water, sediment (i.e., direct dermal and ingestion), and dietary uptake.

Classical, acute single-species laboratory toxicity studies with *P. paludosa* exposed to Cu in water-only indicate that the 96 h LC50 increases with age (2 d old: 34 μg/L and 120 d old: 182 μg/L) and that unlike other aquatic organisms hardness has no effect on Cu toxicity (Rogevich et al., 2008). During chronic 9 month laboratory exposures of *P. paludosa* to 8 and 16 μg/L Cu in water there was high Cu accumulation and significantly reduced clutch production (8 and 16 μg/L) and egg hatching (16 μg/L) (Rogevich et al., 2009). In addition, laboratory toxicity studies showed that *P. paludosa* exposed to flooded Cu-enriched Florida agriculture soils (i.e., a single flooding) for 28 d exhibited decreased survival and growth and a 12–23 fold increase in Cu tissue concentrations compared to controls (Hoang et al., 2008a). Furthermore, the mean Bioconcentration Factor (BCF) for juvenile apple snails exposed to Cu in water was 1493 with a depuration half-life of less than two weeks (Hoang et al., 2008b). In adult snails exposed to separate combinations of Cu-enriched soil or Cu-enriched food in water, dietary uptake produced the highest bioaccumulation factors compared to uptake from soil and most Cu was in the edible soft tissue (viscera plus foot) (Hoang et al., 2008b).

Laboratory aquatic toxicity tests are limited because they are conducted under laboratory controlled environmental conditions and therefore may not be completely reflective of what is actually occurring under natural environmental conditions. Model ecosystems like outdoor microcosms provide a more typical temporal environment, incorporate interactions between population members and between populations, and also allow for test substance transformations that regulate exposures.

To our knowledge there are no outdoor aquatic microcosm studies that have investigated uptake of copper, and its accumulation and effects in Florida apple snails (*P. paludosa*), mosquito fish (*G. affinis*), and periphyton resulting from flooding Cu-enriched agricultural soils. Therefore, an outdoor replicated aquatic microcosm study with flooded (freshwater) Cu-enriched agricultural soils was conducted to determine the total quantity (i.e., concentration) of Cu in periphyton and edible tissue of Florida apple snails (exposed in these systems) that could potentially be present (available) and transferred to predators. We also measured Cu concentrations in mosquito fish in these systems and evaluated the biological effects of Cu on survival/growth/reproduction in native Florida apple snails and mosquito fish. Data on Cu accumulation from flooded Cu-enriched agricultural soils from land acquired under the CERP will be especially useful for the Everglades restoration effort.

2. Methods

2.1. Soil collection

Soils were collected from three agriculture sites; two in St. Lucie County (Equus and Agler (AGLR) properties) and one in Palm Beach County (Sunrise Boys (SRB) property) Florida, USA. At each site, 0.4 m^3 of soils were sampled from the surface to a depth of 6 cm. Soils were transported to the Ecotoxicology and Risk Assessment Laboratory on the Biscayne Bay Campus of Florida International University (FIU) in North Miami, Florida. Soils were physically characterized (pH, % sand, silt, clay, OC, etc.) and chemically analyzed for background concentrations of metals and pesticides.

2.2. Test organisms

Organisms introduced into microcosm tanks included Florida apple snails (*P. paludosa*) and mosquito fish (*G. affinis*). Florida apple snails and mosquito fish are native to south Florida freshwater ecosystems. Juvenile apple snails ($\leq$7 d old) were obtained from the snail stock laboratory culture at the FIU Ecotoxicology and Risk Assessment Laboratory. Apple snails were cultured in laboratory freshwater under flow-through conditions, at a temperature between 22 and 27 °C and a photoperiod of 16 h light and 8 h dark. In the laboratory, snails were fed daily with romaine lettuce that had a Cu concentration of 2.4 mg/kg dw. Laboratory freshwater was North Miami city water that was treated with a carbon filter and UV-sterilized. Water hardness, alkalinity, pH, and conductivity of the laboratory freshwater were 60 mg/L as $CaCO_3$, 50 mg/L as $CaCO_3$, 7.8, and 350 μS/m, respectively.

Juvenile mosquito fish (2–3 cm) were purchased from a commercial grower and were acclimated to laboratory freshwater at a temperature between 22 and 27 °C and a photoperiod of 16 h light and 8 h dark for two weeks prior to study initiation. Fish were fed daily with brine shrimp flake food during the acclimation period.

2.3. Study preparation and design

The outdoor microcosm study was conducted from May 12, 2008 (day 0) to August 27, 2009 (day 430) at the outdoor microcosm facility of the Ecotoxicology

and Risk Assessment Laboratory at FIU. The Ecotoxicology & Risk Assessment Laboratory at FIU is a NELAP-accredited laboratory which operates under federal regulations (Public Health Service) through the FIU Institutional Animal Care and Use Committee (IACUC) to conduct our research with live vertebrate animals. We conform to the applicable federal guidelines, policies, and procedures that govern animal care and use. These procedures address the acquisition of animals, their transportation, use and care, efforts to minimize pain and distress, consideration of alternatives to the use of animals, and training of personnel.

The length of the microcosm study attempted to encompass the life cycle of *P. paludosa*, which is approximately 12–18 months (Darby et al., 2008). The microcosm methods were adapted from Rand et al. (2000a, b) and Rand (2004). The study consisted of nine 4 m^3 round fiberglass tanks (1.52 m depth × 1.83 m diameter). The tanks were spaced 1.2 m apart and buried in the ground at a depth of 0.7 m to buffer fluctuations of ambient temperature. There were two Cu-enriched soil treatments (SRB, AGLR) and a reference soil (Equus). Three replicates were used for each treatment. The study included three phases for a total of 430 days: aging (May 12, 2008–June 19, 2008), exposure (June 20, 2008–May 19, 2009 (day 331)), and post-treatment (post-exposure) (May 20, 2009–August 27, 2009 (day 430)). Neither food nor aeration was provided during the study.

The aging phase was started by randomly transferring 0.1 m^3 of soil into each replicate microcosm tank followed by 0.5 m^3 of rainwater. Soils were homogenized prior to introduction into microcosm tanks to ensure that the benthic community and physicochemical characteristics of the sediment would be as uniform as possible. Soils were physically and chemically characterized. Microcosm tanks were filled with rainwater which was also characterized for metals before the study was initiated. The soil and water flooding resulted in a depth of approximately 7 cm of soil and 18 cm of overlying water. Only rainwater events were used to replenish evaporated water from tanks. The exposure phase was initiated by introducing 30 juvenile mosquito fish and 50 juvenile Florida apple snails into each microcosm tank. The post-treatment phase exposed all apple snails remaining at the end of the exposure phase to water-only (i.e., surface water from the exposure phase) in the outdoor microcosms.

2.4. Sampling and monitoring

Samples were taken during the three distinct phases of the study. Each phase consisted of biological, chemical, and physical monitoring at random sites in each tank. This paper describes the physical–chemical monitoring, and survival, growth, reproduction in snails and fish and Cu tissue analyses of fish, snails, and periphyton.

Water samples were collected weekly during the aging phase and monthly (i.e., when fish and snails were collected) during the exposure phase for analysis of major anions (e.g., Cl, $SO_4^{=}$, and $PO_4^{=}$) and cations (e.g., Ca^{2+}, Mg^{2+}, Na^{+}, etc.), dissolved Cu and dissolved organic carbon (DOC) concentrations. Water samples were collected from a composite of five samples from each tank for dissolved Cu and DOC analyses. For dissolved Cu analysis, water samples were passed through a 0.45 μm filter, placed in 15 mL polypropylene tubes, and acidified with nitric acid to pH 2, prior to analysis. Water samples for DOC and ion analyses were filtered with 0.45 μm filter and placed in 30 mL brown glass bottles prior to analysis.

Results of water chemistry of months one and ten of the exposure phase were used to determine the chemical speciation of Cu for each soil using the Visual Minteq Model (http://www.lwr.kth.se/English/OurSoftware/vminteq).

Water temperature and dissolved oxygen concentration were measured daily during the aging phase and two times per day during the exposure phase (morning and afternoon) and post-treatment phases using a YSI meter (YS Inc, Yellow Springs, Ohio, USA). Conductivity, water hardness, alkalinity, and pH were measured weekly during the study from a composite of five samples from each tank. Conductivity was measured using a YSI meter (YS Inc, Yellow Springs, Ohio, USA). Hardness and alkalinity were measured by titrating with 0.01 M Ethylene Diamine Tetra Acetate (EDTA), 0.02 H_2SO_4 solutions, and an Accumet® meter (Fisher Scientific, Pittsburgh, PA, USA), respectively. Ammonia was measured weekly during the exposure phase with an Accumet® Ammonia Electrode (Fisher Scientific, Pittsburgh, PA, USA). Rainfall was also documented.

Live snails and fish ($n=3$) were collected from each replicate microcosm tank at test initiation, monthly during the exposure phase and at the end of exposure and post-treatment phases (i.e., snails only) for tissue Cu analysis. Snails were collected directly by hand and fish were collected using fish traps. When snails aestivated in soils and/or fish did not enter traps on sample collection days, sample sizes were smaller than three. Dead fish and snails were also collected for tissue Cu analysis. Snails and fish were rinsed three times with Deionized (DI) water and frozen at −20 °C, until analysis. Growth was measured by determining the shell length, shell width, shell height, and wet weight of the snails (adapted from methods by Teo, 2004; Hoang et al. 2008a) and the standard length and wet weight of the fish. Length measurements of the snails and fish were conducted using a Spi-2000 Caliper and a standard ruler (with increments of 1 mm), respectively. Wet weight of the snails and fish were determined with an AG135 Mettler Toledo Balance (Columbus, Ohio, USA). To assess reproduction of apple snails, Burma grass stems were added to each microcosm since egg-laying (oviposition) typically occurs when snails ascend from the water onto the stems of emergent freshwater plants to lay clutches.

Water samples were collected from each replicate microcosm tank for dissolved Cu and DOC analyses when fish and snails were collected. Floating artificial periphyton samplers (Eaton et al., 1995), supporting 25 × 75 mm glass slides were anchored at one location in each microcosm and sampled every two weeks for the first 5 months of the exposure phase and weekly after the fifth month of the exposure phase for Cu analysis. Periphyton was not analyzed during post-treatment.

Snails were dissected into three sections (shell, foot, and viscera) following an adaptation of a method published by Gomot-de Vaufleury and Pihan (2002). Each section of the snails, fish (as whole body), periphyton, and soils were digested separately with HNO_3 based on the U.S. EPA Method 3050B for tissue Cu analysis (U.S.EPA, 1996). Regarding snail tissue and consumption by predators, only soft tissue that includes viscera and foot is consumed by predators. Therefore, Cu concentrations in the snail soft tissue (viscera plus foot) were also calculated based on the Cu concentrations in snail viscera plus foot. The digestion was conducted in the FIU Ecotoxicology and Risk Assessment Laboratory. Water, soil, periphyton, and snail tissue Cu analysis were conducted in the Agricultural Service Laboratory at Clemson University, Clemson, South Carolina. The soil pesticide analyses and concentration of DOC in water were analyzed in the Environmental Analysis Laboratory at the University of Georgia, Athens, Georgia. Soil physical characteristic analyses were conducted at Brookside Laboratories Inc., New Knoxville, Ohio.

There was no reproduction in Florida apple snails for all three soil–water systems including the reference soil treatment (Equus) during the exposure phase of the study. To examine whether the snails would reproduce following cessation of soil Cu exposures, the study was extended for 3 months (post-treatment) by collecting water-only and the remaining live snails from each microcosm tank and transferring them into microcosm tanks without soils. During the post-treatment phase, snails were fed romaine lettuce daily because there would be little to no source of natural food (i.e., periphyton) in these newly developed microcosm tanks. Measurement of water quality was conducted as described above. Snail and water samples were collected for Cu analysis at the end of months 1, 2, and 3 post-treatment.

2.5. Data analysis

Treatment comparisons with the reference soil for Cu concentrations in periphyton and fish were performed using *T*-tests; for snails, comparisons of tissue Cu concentrations between treatments and reference were performed using analysis of covariance (time as covariance). All treatment comparisons were conducted with SAS 9.2 (SAS Institute Inc., Cary, NC, USA). An effect with a *p*-value ≤ 0.05 was considered significant.

Growth data of the snails (shell length, shell width, shell height, and wet weight) were analyzed using von Bertalanffy growth models (Bertalanffy 1938, 1960), as described

$$L_t = L_\infty(1-\exp(-k(t-t_0))) \quad (1)$$

where L_t is the length at time t, L_∞ is the theoretical maximum length that the snails would reach at age ∞, the parameter k is the growth coefficient which represents a growth rate at which maximum size is reached, t_0 is the theoretical age at length equals zero. The value of t_0 is usually negative or equals zero. In this study, t_0 was set to zero.

The Ford–Walford plot method (Walford, 1946) was used to estimate the values of L_∞ and k and is described as follows:

$$L_\infty = \varepsilon/(1-\beta) \quad (2)$$

$$k = -\ln(\beta) \quad (3)$$

where β and ε are the slope and intercept of the linear relationship between length at time t (L_t (independent variable)) and length at time $t+1$ (L_{t+1} (dependent variable)). The linear relationship was analyzed using SAS (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Chemistry

Results of physical and chemical characteristics of soils are presented in Table 1. The soils contained a high percent of sand (>90%). Based on the USDA system of nomenclature for soil texture, all three soils in this study are classified as sandy soils. AGLR soil had the highest total cation exchange capacity (18.7 ME/100 g). The pH of soils (6–7) was in the neutral pH range. Except Cu, other toxic metals (e.g., As, Cd, and Se) were not

Table 1
Soil physical and chemical characteristics.

Soils	Equus	SRB	AGLR
Clay (%)	5.63	2.47	4.76
Silt (%)	2.18	0.47	4.88
Sand (%)	92.19	97.06	90.36
Organic matter (%)	1.97	1.52	2.38
Total exchange capacity (ME/100 g)	8.32	7.39	18.75
pH (H_2O 1:1)	6.0	6.0	7.3
Estimated nitrogen release (lb/A)	59	50	68
Soluble sulfur (mg/kg)	18	35	74
Phosphorus (mg/kg as P)	63	113	55
Calcium (mg/kg)	951	2354	4646
Magnesium (mg/kg)	162	141	289
Potassium (mg/kg)	141	113	120
Manganese (mg/kg)	11	108	35
Iron (mg/kg)	1366	1279	643
Boron (mg/kg)	ND[a]	ND	ND
Aluminum (mg/kg)	871	844	1260
Arsenic (mg/kg)	ND	ND	ND
Cadmium (mg/kg)	ND	ND	ND
Selenium (mg/kg)	ND	ND	ND
Zinc on day 0 (mg/kg)	6	44	37
Copper on day 0 (mg/kg)	7	55	99
Copper on day 331 (mg/kg)	8	47	87

The concentration is in dry weight base.

[a] ND=not detected.

detected. Concentrations of Zn were $\leq$44 mg/kg dw and were less than the sediment quality guideline (Threshold Effect Concentration (TEC)=121 mg/kg dw) from the Florida Department of Environmental Protection (FDEP, 2003).

Initial Cu concentrations in Equus, SRB, and AGLR soils were 7, 55, and 99 mg/kg dw, respectively, (Table 1). For SRB and AGLR soils, Cu concentrations appeared to be slightly lower at the end of the study than at the beginning. This may be due to desorption of Cu from soils to water. The copper concentrations in SRB and AGLR soils exceeded the sediment TEC for Cu (31.6 mg/kg dw) in Florida (FDEP, 2003). The copper concentration in the reference soil (Equus=7 mg/kg dw) was approximately 4-times lower than the Cu sediment TEC of FDEP. Pesticides (organochlorine and organophosphate) were not detected in any of the three soils.

Concentrations of DOC in all treatments ranged from 16 to 51 mg/L. Due to the similarity of the DOC concentrations in the first 10 months of each treatment, DOC concentrations were not measured for months 11 and 12. The concentrations of DOC measured in this study are within a range of the DOC concentrations in surface freshwaters of south Florida (South Florida Water Management District's DBHYDRO environmental monitoring database (www.sfwmd.gov)).

Total water hardness, alkalinity, and pH ranged from 25 to 135 mg/L as $CaCO_3$, 19 to 140 mg/L as $CaCO_3$, and 6.4 to 9.7 for all treatments, respectively. In general, total hardness, alkalinity, and pH appeared to increase in order of Equus < SRB < AGLR. Water chemistry of the microcosm water fluctuated from month to month. The combination of dilution from rainwater and ion concentrating affect due to water evaporation might explain the fluctuation. Based on the recommended composition of reconstituted freshwaters for aquatic toxicity testing, the microcosm water in this study is characterized as soft to moderately hard water (Eaton et al., 1995). The average concentration of total ammonia in each microcosm was low ($\leq$0.3 mg/L). The average conductivity of the microcosm waters ranged from 75 to 153, 80 to 186, and 150 to 434 μS/cm for Equus, SRB, and AGLR, respectively.

The daily average DO concentrations in the microcosm water ranged from 3 to 17 mg/L. The DO concentrations in surface freshwater of south Florida ranged from 0.4 to 16 mg/L (South Florida Water Management District's DBHYDRO environmental monitoring database (1985–2000) (www.sfwmd.gov)). The DO concentrations in the microcosm water were usually low in the early morning, especially in the Equus microcosm (as low as 0.24 mg/L) and high in the afternoon. This is consistent with studies by Cioffi and Gallerano (2001) and Marsili-Libelli (2004) who observed low DO concentrations in lagoons in the morning (down to 2 mg/L) and high DO concentrations in the afternoon (up to 17 mg/L). The fluctuation of DO concentrations was likely due to respiration in the systems, sediment oxygen demand and intense phytoplankton activity. Daily average temperature ranged from 13 °C in February to over 35 °C in June but also fluctuated during the day. Temperature was usually lower in the morning and higher in the afternoon. The temperature in this study is within a range of the temperatures in surface waters of south Florida from 1985 to 2000 (10–39 °C) (South Florida Water Management District's DBHYDRO environmental monitoring database (www.sfwmd.gov).

Results of dissolved Cu concentrations in the microcosm water are illustrated in Fig. 1. Dissolved Cu concentrations (average) in Equus, SRB, and AGLR microcosms at the beginning of the study were 3, 82, and 43 μg/L, respectively. For the first few months overlying surface water from flooded Cu-enriched agricultural soils (n=2; SRB and AGLR sites) contained dissolved Cu concentrations that were many fold higher than the U.S.EPA numerical freshwater criterion (13 μg/L) (U.S.EPA, 2003) and the 10th centile for acute effects to plants and algae (5.5 μg/L) and the 10th centile for chronic effects to freshwater organisms (3.9 μg/L) (Schuler et al., 2008). Overlying surface water from the flooded reference soil microcosm (Equus site) contained dissolved Cu concentrations that were below the U.S.EPA numerical freshwater criterion based on Biotic Ligand Model simulation.

The dissolved Cu concentrations decreased to low saturation levels of $\leq$9 μg/L Cu, after the first 3 months of the study.

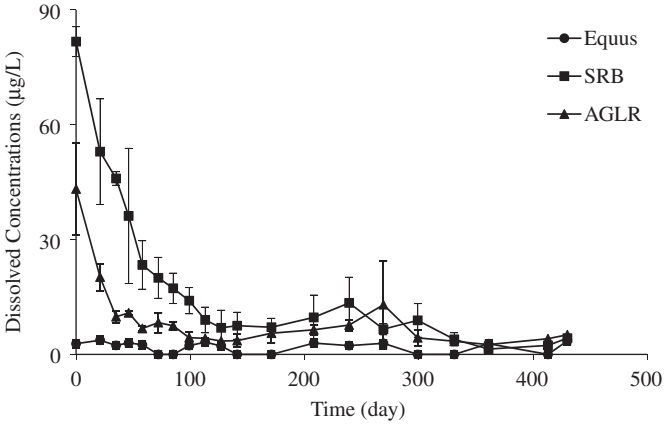

Fig. 1. Dissolved copper concentrations in the microcosm waters (points represent mean values and error bars represent standard deviations).

Table 2
Copper concentrations in mosquito fish (μg/g dw).

Study day[a]	Equus	SRB	AGLR
0 (background)	5.58 ± 0.16	5.58 ± 0.16	5.58 ± 0.16
30	3.19 ± 0.77	6.26 ± 2.69	4.86 ± 0.51
150	5.18 ± 2.02	5.69 ± 1.55	7.53 ± 3.74

Data are mean ± standard deviation of three replicates. There were no significant differences among treatments.

[a] From the start of exposure phase.

The decrease of dissolved Cu concentrations was likely due to dilution by rainwater. Although soil Cu concentrations were higher in AGLR soil than in SRB soil, water Cu concentrations were generally higher in SRB microcosms than in AGLR microcosms. In general, desorption of metals from soils is dependent on soil characteristics (Hoang et al., 2008b). Soils with a higher percent of total ion exchange capacity, silt, clay, organic matter, and pH retain more metals (Pedersen et al., 1997; Impellitteri et al., 2003; He et al., 2006). The values of these soil characteristics were higher for AGLR soil than SRB soil. Therefore, Cu desorption from AGLR soil will be less than Cu desorption from SRB soil.

Results of the Minteq model prediction indicated that the copper–organic matter complex (Cu–DOC) was the dominant species and accounted for 99% of total dissolved Cu in the microcosm water. Other Cu species were less than 1% of total dissolved Cu. This result is in agreement with the results of our earlier study (Hoang et al., 2009) for different soils in Florida. He et al. (2006) also reported that up to 70% of total dissolved Cu released from Florida soils was in organic complexes.

3.2. Copper accumulation

Results of Cu concentrations in mosquito fish, periphyton, dead snails, and live snails throughout the study are shown in Tables 2–5, respectively. Copper concentrations in fish at the beginning, day 30 and 150 of the study ranged from 3.19 to 7.53 mg/kg and were not significantly different. This indicates that there was no uptake of Cu. Copper was not bioavailable for uptake because of complexation of Cu and DOC, resulting in low Cu bioavailability. Since there was no significant Cu uptake in fish the first 5 months of the study, fish were not collected at later time periods for Cu analyses.

Copper concentrations in periphyton are shown in Table 3. No sample for periphyton indicates that there was insufficient biomass to make measurements. Copper concentrations in periphyton from Equus, SRB, and AGLR microcosms ranged from 2 to 62, 31 to 371, and 13 to 478 mg/kg, respectively. This is similar to Cu concentrations in field-collected periphyton samples from similar agricultural sites in South Florida (13–136 mg/kg) (Frakes et al., 2008).

Table 3
Copper concentrations (mean ± std.dev.) in periphyton (μg/g, dw).[a]

Treatment/day	Equus	SRB	AGLR
30	17 ± 3	371 ± 61	382 ± 458
58	ND	NS	478 ± 300
72	62 ± 20	301 ± 20	168 ± 8
85	48 ± 4	281 ± 89	242 ± 107
99	11	233 ± 126	222
113	13	105 ± 44	NS
127	26	83 ± 66	79
141	NS	31	NS
171	NS	162	NS
208	NS	67 ± 20	13
239	NS	38 ± 23	NS
269	1	42	NS
299	ND	35 ± 27	NS
331	2	39 ± 14	NS

ND = not detected.
NS = no sample.

[a] No standard deviation indicate single replicate measurement and no sample for other two replicates.

Table 4
Copper concentrations in dead Florida apple snails on dry weight basis.

Treatment	Collected day	Replicate	Number dead snails	Shell (μg/g)	Foot (μg/g)	Viscera (μg/g)	Foot+viscera (μg/g)
SRB	64	2	2	139	2340	4062	3412
	65	3	1	ND	1744	5777	3514
	72	1	1	8	2420	7072	4822
	72	2	2	98	1859	4280	3130
	72	3	1	ND	2830	4173	3652
	78	3	2	ND	2618	4672	3815
	80	3	1	4	2170	3423	2913
	85	2	2	13	NS	NS	NS
	89	3	2	4	2352	3462	2935
	92	3	1	ND	2131	3426	2863
	95	2	1	ND	2366	2755	2595
	99	3	1	ND	5013	5524	5326
	101	3	1	103	NS	NS	NS
	108	3	1	ND	NS	NS	NS
	117	3	1	65	NS	NS	NS
	171	3	3	ND	4244	10340	8370
	208	2	1	ND	NS	NS	NS
	239	1	1	5	NS	7790	NS
	318	1	1	ND	NS	NS	NS
AGLR	92	3	1	128	NS	7977	NS
	103	1	1	ND	NS	2401	NS
	178	3	1	ND	970	3158	2118
	201	3	1	ND	2085	3300	2717
	208	1	1	138	NS	NS	NS
	218	3	1	ND	2429	2566	2511
	235	3	1	ND	1839	2823	2418
	239	1	2	ND	4611	1909	3600
	239	2	1	ND	3098	3941	3579
	246	1	1	9	NS	NS	NS
	246	3	1	167	NS	NS	NS
	318	2	1	ND	7347	5485	6066

ND = not detected.
NS = no sample (tissue was discomposed).

Table 5
Tissue copper concentrations in live Florida apple snails on dry weight basis.

Treatment	Exposure time (day)	Shell		Foot		Viscera		Soft tissue (Foot +Viscera)	
		Mean (μg/g)	Std. dev. (μg/g)	Mean (μg/g)	Std. dev. (μg/g)	Mean (μg/g)	Std. dev. (μg/g)	Mean (μg/g)	Std. dev. (μg/g)
Initial background	0							35[a]	1
Equus	27	19	15	51	20	62	70	56	38
	58	ND		65	33	121	44	92	37
	85	ND		99	11	139	27	119	10
	113	ND		82	54	197	138	147	104
	141	ND		69	26	140	40	106	39
	171	ND		86	49	152	63	123	60
	208	ND		129	3	166	9	149	4
	239	ND		105	7	131	5	120	8
	269	ND		97	10	125	42	108	23
	299	ND		75	6	140	20	118	12
	331	ND		86	6	108	15	97	10
	372	ND		48	10	108	16	82	8
	401	ND		49	17	96	6	74	6
	430	ND		33	12	44	21	38	17
SRB	27	1	3	712	337	829	327	782	332
	58	4	0	1121	295	1337	275	1220	302
	85	1	0	1409	434	2256	639	1943	563
	113	ND		1749	474	2831	1479	2414	1120
	141	7	11	2694	925	3166	887	2978	907
	171	ND		1898	982	2763	582	2431	676
	208	2	0	1756	491	2747	369	2344	455
	239	40		2187	1061	2517	1135	2373	1121
	269	7	6	1345	39	2268	440	1850	281
	299	1	0	929	270	1296	307	1136	287
	331	ND		918	139	1381	232	1205	198
	372	NS		NS		NS		NS	
	401	ND		426		299		341	
	430	ND		287	92	361	234	451	245
AGLR	27	ND		651	109	444	280	505	232
	58	ND		660	39	975	126	850	90
	85	ND		803	160	1152	214	1013	193
	113	ND		942	248	1183	209	1145	144
	141	ND		890	189	1564	170	1312	85
	171	ND		1103	299	1723	295	1524	296
	208	ND		1222	467	2258	627	1814	584
	239	ND		1127	521	2255	719	1923	*517*
	269	ND		1736	435	2200	380	1969	331
	299	ND		1634	1257	2261	1572	2036	1410
	331	ND		1271	458	2142	813	1816	675
	372	ND		447	70	606	251	624	101
	401	ND		234		251		328	
	430	ND		242	139	211	140	270	151

ND=not detected.
NS=no sample.

[a] Whole body concentration.

Copper concentrations in periphyton samples from SRB and AGLR microcosms appeared to be higher in the first 3 months than in later months of the study. This may be due to the decrease in water Cu concentrations as result of rainwater dilution. Most of the time, Cu concentrations in periphyton samples from SRB and AGLR microcosms were significantly higher than Cu concentrations in periphyton samples from Equus. This may be due to the higher water Cu concentrations in SRB and AGLR microcosms than in Equus microcosms (Fig.1).

Average Cu concentrations in the soft tissue (foot plus viscera) of dead snails from SRB and AGLR microcosms during the exposure phase were 3946 mg/kg dw (ranged from 2595 to 8370 mg/kg dw) and 3287 mg/kg dw (ranged from 2118 to 6066 mg/kg dw), respectively, (Table 4). Although tissue Cu concentrations reported in the literature are usually for live organisms, the Cu concentrations in soft tissue of dead snails found in this study were significantly higher than the tissue Cu concentrations in live aquatic organisms reported in the literature (Ingersoll et al., 1994); Adewunmi et al., 1996; Bu-Olayan and Subrahmanyam, 1997; Gomot and Pihan, 1997; Scheifler et al., 2003; Heng et al., 2004; Northwood et al., 2007; Hoang et al. 2008a,b). Note that during exposure and post-treatment phases, decomposition of tissue occurred in some snails found dead in microcosms without sufficient tissue for any Cu analyses measurements.

Results of Cu concentrations in the foot, viscera, and shell of live Florida apple snails are shown in Table 5 and Fig. 2. An $n \geq 3$ was used for each point. Copper concentrations in snail shell were below the detection limit or ≤ 40 mg/kg dw. In general, Cu concentrations were highest in snail viscera and lowest in snail shell. This is in agreement with Hoang et al. (2008a) and Laskowski and Hopkin (1996) who reported that 60% of accumulated Cu was in snail viscera and Cu accumulation in the snail shell did not exceed 5% of total body copper. Desouky (2006) also reported that up to 80% of accumulated metals (Al, Cd, Zn)

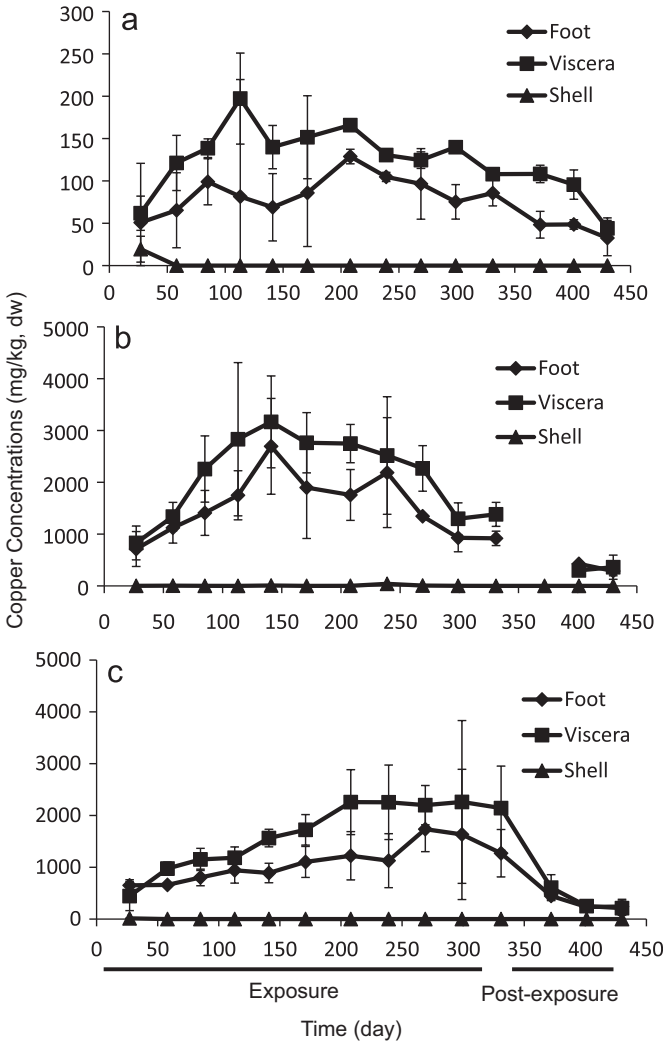

Fig. 2. Copper uptake in various parts of live Florida apple snails (points represent mean values and error bars represent standard deviations, (a) Equus soil, (b) SRB soil, and (c) AGLR soil).

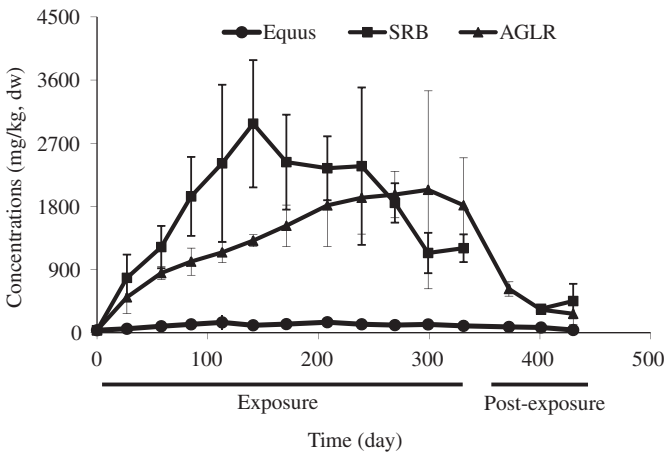

Fig. 3. Copper uptake in soft tissue (foot+viscera) of Florida apple snails (points represent mean values and error bars represent standard deviations).

were found in snail viscera. With concern for Cu transfer via the food chain, viscera and foot (soft tissue) are the two edible parts that comprise what is consumed by snail predators.

Copper concentrations in the soft tissue of live snails ranged from 56 to 149, 782 to 2978, and 505 to 2036 mg/kg dw for snails from Equus, SRB, and AGLR microcosms, respectively, during the exposure phase (Table 5, Fig. 3). These soft tissue Cu concentrations (e.g., 2978 mg/kg dw) are up to three times higher than the tissue Cu concentrations reported by Frakes et al. (2008) for field-collected Florida apple snails from similar agricultural sites in South Florida. Also, these soft tissue Cu concentrations are higher than the tissue Cu concentrations in aquatic organisms reported in the literature, regardless of route of uptake (Ingersoll et al. 1994; Adewunmi et al. 1996; Bu-Olayan AH and Subrahmanyam, 1997; Gomot and Pihan, 1997; Scheifler et al. 2003; Heng et al. 2004; Northwood et al. 2007; Hoang et al. 2008a,b). The snail tissue Cu concentrations in this study are some of the highest reported for aquatic organisms (see Table 3 in Eisler, 1998). These high Cu concentrations in apple snail soft tissue might pose a risk to apple snail predators, such as snail kites.

Copper concentrations in snail soft tissue from SRB microcosms increased with time in the first half and decreased in the second half of the exposure phase. However, in AGLR microcosms, Cu concentrations in snail soft tissue increased with time and reached a plateau after 7 months. In general, Cu concentrations in the snail soft tissue were higher for snails from SRB and AGLR microcosms than snails from Equus microcosms. Copper concentrations in soils, periphyton, and water in SRB and AGLR microcosms were generally higher than those in Equus microcosms (Tables 1 and 3, Fig. 1). This explains the higher snail tissue Cu concentrations from SRB and AGLR microcosms than from Equus microcosms because metal uptake via diet (periphyton), soil ingestion, and soil dermal contact are the important factors for Cu uptake in snails (Gomot-de Vaufleury and Pihan, 2002; Frakes et al., 2008; Hoang et al., 2008b).

Periphyton was the main food source for snails in the outdoor microcosm study. Snails were not artificially fed during the study, as organisms are in laboratory toxicity studies. In addition, aquatic plants, bioconcentrate Cu (see Table 5; Eisler, 1998). As pointed out by many investigators, diet is a very significant route for Cu exposure in aquatic organisms (Meyer et al., 2005). Furthermore, in this study, the high DOC concentrations (16–51 mg/L) and low dissolved Cu concentrations in microcosm waters indicate that the bioavailable Cu fraction will be minimal and thus provide support that diet, dermal contact, and direct ingestion of soil particles are the main routes of Cu exposure for apple snails.

There was significant depuration of copper in snails during the post-treatment phase when snails were placed in water-only (i.e., surface water from original soil–water system) microcosms (Table 5). During the 100 days of the post-treatment phase, Cu concentrations in the soft tissue of apple snails from Equus microcosms decreased to the background level (35 mg/kg). For SRB and AGLR microcosms, approximately between 83% and 67%, respectively, of Cu in the snail soft tissue were depurated. However, Cu concentrations in the snail soft tissues from SRB and AGLR microcosms were still 10 times higher than the background level.

3.3. Survival, growth, and reproduction

Table 6 is a summary of the total number of snails and fish collected for tissue Cu analyses, total number of dead snails and fish, the total number of live snails and fish, up until day 331 (end of exposure phase) and snail mortality during the post-treatment phase. Live snails remaining at the end of the exposure phase and overlying water were transferred to microcosm tanks for the post-treatment phase. Fish tissue showed no significant Cu concentrations and, therefore, there was no post-treatment phase for this group.

At the end of the exposure phase (day 331), the total number of dead snails was highest in SRB microcosms (total

Table 6
Survival and mortality of snails and fish.

Treatment	Organisms	Survival (day 0)	Collection for tissue Cu analysis (alive, day 0–331)	Survival (day 331)	Mortality (during exposure)	Mortality (post-exposure)
Equus	Snail	150	77	30	43	3
SRB		150	77	13	60	8
AGLR		150	86	20	44	5
Equus	Fish	90	75	224	2[a]	
SRB		90	68	484	43[a]	
AGLR		90	93	197	0[a]	

Survival and mortality data are total number of organisms in each microcosm.

[a] Total number of dead fish collected during the study.

mortality=60) compared to AGLR (total mortality=44), and Equus (total mortality=43), the reference. In one out of the three replicates (rep 2) of Equus, all fish and snails were dead by the beginning of the third month (snails and fish were not observed in this replicate after the second month and at the end of the exposure phase and snail shells were not found, most likely a result of snail mortality in their early life stage and shell decomposition). The dead snails and fish in Equus were likely due to low dissolved oxygen (as low as 0.24 mg/L in mornings). The total number of mortalities in snails at the end of the post-treatment phase were 3, 5, and 8 for Equus, AGLR, and SRB, respectively.

Results of snail growth are shown in Fig. 4. Initial (background-day 0) shell length, shell height, and shell width of the snails ranged from 0.41, 0.33, and 0.37 cm at the beginning of the study to 3.37, 2.61, and 2.62 cm at the end of the exposure phase (day 331), respectively. This is similar with the shell length of apple snails after one year on a diet of lettuce (Turner et al., 2001). The initial background average wet weight of snails (i.e., from a subsample of 20 apple snails) was 0.02 g at the beginning of the study and 9.51 g at the end of the exposure phase (i.e., of all apple snails from all microcosms). The size and weight of Florida apple snails found in this study are similar to the size and weight of Florida apple snails collected from the Everglades (Eisemann et al., 1997) and from our laboratory (Rogevich et al., 2009). The shell length, shell height, shell width, and wet weight of the snails were significantly higher after the first month of the exposure phase than at the beginning of the study. There was no significant difference between shell length, shell height, shell width, and wet weight of the snails from SRB, AGLR and Equus microcosms at the end of the exposure phase. This indicates Cu did not affect snail growth.

The growth data of the snails followed the von Bertalanffy growth model (Table 7). This is similar to the growth data reported in the literature for various organisms (Li et al., 1997; Fieber et al., 2005; Katsanevakis, 2006; Ohnishi and Akamine, 2006). The predicted and measured data overlapped, indicating a good fit of the model. Snail size increased with time and reached a plateau after 3 months. The maximum shell length, shell height, and shell width of the snails calculated by von Bertalanffy model (L_∞) were 2.76, 2.05, and 2.18 cm, respectively. The maximum model predicted wet weight was 9.38 g compared to actual wet weight of 9.51 g. Growth rate (k) of the snails increased in order of shell height (0.459), shell length (0.550), and shell weight (0.598).

Results of fish growth are shown in Table 7. Standard length and wet weight ranged from 2.1 cm and 0.18 g at the beginning of the study (day 0) to 4.4 cm and 0.9 g at the end of the exposure phase, respectively. The final growth measurements are based on several age classes which could not be separated. Adult mature females reach a maximum overall length of 7 cm (2.5 in), while males reach only 4 cm (1.5 in) (Simon and Wallus, 1990). At the optimal temperature range and with the presence of mosquito larvae in the microcosm water of this study, mosquito fish could go through their life cycle in less than 30 days (Sholdt et al., 1972). Therefore, monthly measurements of length and weight may not represent cumulative growth of fish. In general, standard length and wet weight of fish at the beginning were lower than those of fish during the exposure phase, except for Equus and month 5, wet weight was not significantly different from background. There was no effect of Cu on fish growth.

There was no reproduction in snails during the exposure phase in any replicate which was 11 months in duration and therefore covered the reproductively active phase of the Florida apple snail. The life span of the Florida apple snail is estimated to be between 12 and 18 months (Hanning, 1978; Darby, 1998: Darby et al., 2008) and their life ends in a post-reproductive die-off (Darby et al., 2003). Egg clusters are usually produced and eggs hatch from February–November. Reproduction was first observed on days 52, 76, and 64 in the post-treatment phases of Equus, SRB, and AGLR microcosms, respectively. A total of 655, 338, and 529 eggs, were observed from Equus, SRB, and AGLR microcosms, respectively. Reproduction during the post-exposure phase is coincident with a decrease in Cu concentrations in the snails due to depuration and the daily supplement of uncontaminated food (lettuce). Note that we did not know the male–female sex ratio per microcosm tank at the beginning of the study because young snails could not be sexed and the total number of snails per tank was different during post-treatment because it was based on the remaining number that survived the exposure phase. Therefore, we could not quantify and statistically compare egg production during post-treatment. Furthermore, although it was assumed that high water depths in microcosms, as a result of rainfall events, might have been the cause for a lack of reproduction during the exposure phase, this is clearly not the cause since high water depths also occurred during post-treatment.

Reproduction occurred in fish during the exposure phase. The total number of fish recovered at the end of the exposure phase was higher than the total number of fish introduced at the beginning of the study for all microcosms. SRB microcosms contained the highest total number of fish but note that the one replicate microcosm (rep 2) of Equus contained no fish. Using the endpoint of total number of fish from beginning to the end of the study indicates that there was no effect of Cu on fish reproduction. Low dissolved copper and low Cu bioavailability (free Cu^{+2}) in the microcosm water (less than 1% of the total dissolved Cu) would explain no effect of Cu on fish reproduction.

4. Conclusion

In the outdoor freshwater microcosm study (430 days), for the first few months overlying water from flooded Cu-enriched agricultural soils (n=2; SRB and AGLR sites) contained dissolved

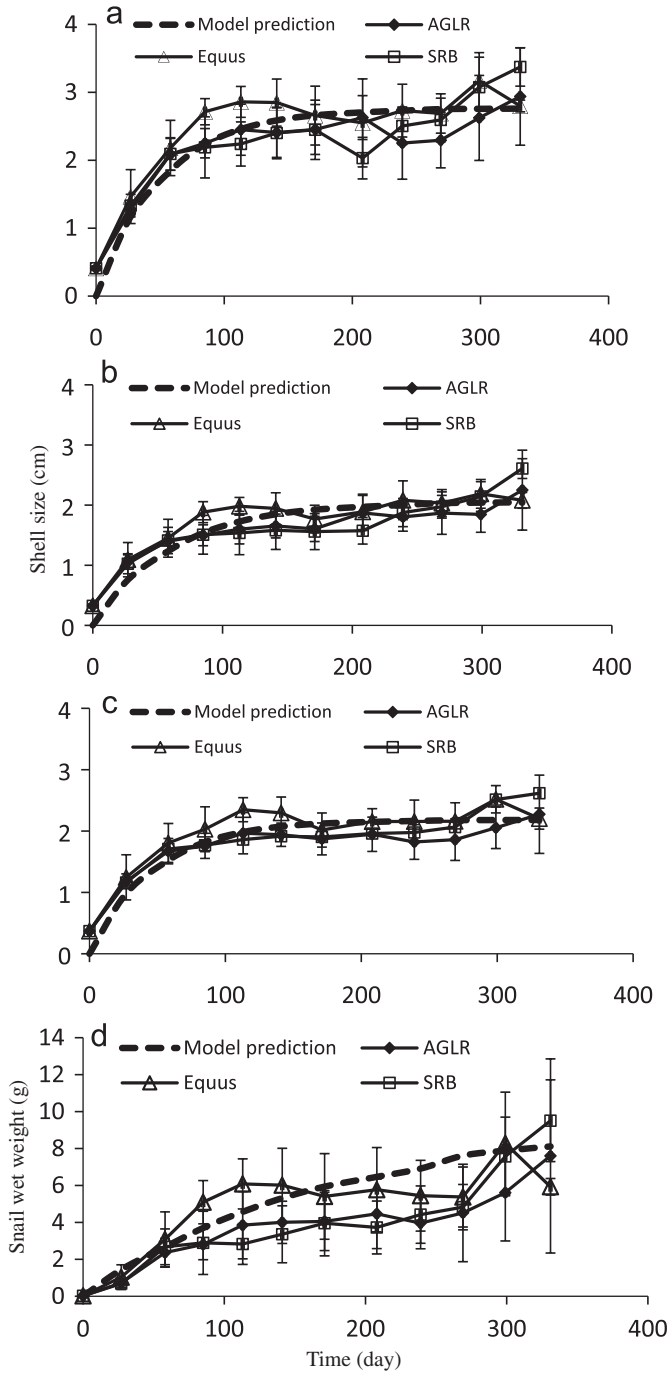

Fig. 4. Snail growth and von Bertalanffy model prediction ((a) shell length, (b) shell height, (c) shell width, and (d) whole body wet weight. There was no significant difference in shell length, shell height, shell width, or wet weight of the snails between treatments (SRB, AGLR) and reference (Equus)).

Cu concentrations that were many fold higher than the U.S.EPA numerical freshwater criterion (13 μg/L). Overlying surface water from the flooded reference soil microcosm (Equus site) contained dissolved Cu concentrations that were below the U.S.EPA numerical freshwater criterion. Cu (total) concentrations in the two Cu-enriched soils were similar from day 0 until day 331 (last day of flooding) and were both higher than the Florida DEP SQAG TEC of 31.6 mg/kg. Apple snail and mosquito fish mortality were higher in the two flooded Cu-enriched agricultural soil microcosms (SRB, AGLR) compared to the reference agricultural soil during the exposure phase. Mosquito fish did reproduce during the exposure phase of the microcosm study but apple snails only

Table 7
Standard length and wet weight of fish and the von Bertalanffy model parameters for snail growth.

Snails			Fish			
Growth endpoint[a]	L_∞	k	Treatment	Time (month)	Standard length[b] (cm)	Wet weight[b] (g)
Shell length (cm)	2.76	0.550	Background	0	2.1 ± 0.2	0.18 ± 0.06
Shell height (cm)	2.05	0.459	Equus	1	2.9 ± 0.4	0.60 ± 0.35
Shell width (cm)	2.18	0.598	SRB	1	3.0 ± 0.5	0.69 ± 0.36
Wet weight (g)	9.38	0.167	AGLR	1	3.1 ± 0.6	0.87 ± 0.54
			Equus	5	2.7 ± 0.3	0.30 ± 0.21[a]
			SRB	5	3.1 ± 0.5	0.59 ± 0.31
			AGLR	5	3.0 ± 0.8	0.66 ± 0.56
			Equus	11	3.3 ± 0.9	0.55 ± 0.24
			SRB	11	4.4 ± 0.8	1.83 ± 0.77
			AGLR	11	3.8 ± 0.7	1.14 ± 0.62

[a] L_∞ is the theoretical maximum length that the snails would reach at age ∞, the parameter k is the growth coefficient and represents a growth rate at which maximum size is reached.

[b] There was no significant difference in either standard length or wet weight of fish between microcosms (SRB, AGLR) and the reference (Equus). Standard length and wet weight of fish were significantly higher in months 1, 5, and 11 than background, except wet weight for Equus and month 5 (not significantly different from background).

reproduced during the post-treatment phase in all microcosms. The latter effect is related to Cu exposures.

Copper concentrations were higher in periphyton from the two Cu-enriched soil microcosms at the beginning of the exposure then at the end of the exposure phase. Fish tissue contained very low Cu concentrations at the beginning and end of the exposure phase (3–8 mg/kg). However, soft tissue (i.e., edible) from dead apple snails during the exposure phase from the two Cu-enriched soils contained average Cu concentrations of 3946 mg/kg dw (range: 2595–8370 mg/kg) and 3728 mg/kg dw (range: 1835–6066 mg/kg) for SRB and AGLR soils, respectively. The range of Cu concentrations in soft tissue (i.e., edible) from live apple snails during the exposure phase in the two Cu-enriched soils and the reference soil microcosms were 782–2978, 505–2036, and 38–149 mg/kg, respectively. High accumulated tissue concentrations of Cu in Florida apple snails in the microcosm study support previous high Cu tissue concentrations in apple snails collected in a field study at similar agricultural sites by the U.S. Fish & Wildlife Service. During the 3 months of the post-treatment phase, apple snails from Equus, SRB, and AGLR depurated approximately 100%, 67%, and 83% of the accumulated Cu.

Note that there are CERP-acquired agricultural sites with soil Cu concentrations higher than the two Cu-enriched soils (SRB, AGLR) used in the microcosm study indicating high Cu accumulation potential in Florida apple snails. In the outdoor freshwater microcosm study, Cu concentrations in apple snail tissue were highest in viscera and foot. The shell contained a low percentage of copper. This supports our laboratory data and the published literature.

Acknowledgment

This study was funded by the U.S, Fish and Wildlife Service under Cooperative Agreement no. 401816J034. This is SERC contribution number 510.

References

Adewunmi, C.O., Becker, W., Kuehnast, O., Oluwole, F., Dorfler, G., 1996. Accumulation of copper, lead and cadmium in freshwater snails in southwestern Nigeria. Sci. Total Environ 193, 69–73.

Alva, A.K., Graham, J.H., Anderson, C.A., 1995. Soil-pH and copper effects on young Hamlin orange trees. Soil Sci. Soc. Am. J. 59, 481–487.

Bertalanffy, L. von, 1938. A quantitative theory of organic growth (Inquiries on growth laws. II). Human Biol. 10, 181–213.

Bertalanffy, L. von, 1960. Principles and theory of growth. In: Nowinski, W.W. (Ed.), Fundamental aspects of normal and malignant growth. Elsevier, Amsterdam, pp. 137–259.

Betzer, S.B., Yevich, P.P., 1975. Copper toxicity in *Busycon canaliculatum* L. Biol. Bull. 148, 16–25.

Bryan, G.W., Langston, W.J., 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: A Review. Environ. Pollut. 76, 89–131.

Bu-Olayan, A.H., Subrahmanyam, M.N.V., 1997. Accumulation of copper, nickel, lead and zinc by snail, *Lunella coronatus* and pearl oyster, *Pinctada radiata* from the Kuwait coast before and after the gulf war oil spill. Sci. Total Environ. 197, 161–165.

Cioffi, F., Gallerano, F., 2001. Management strategies for the control of eutrophication processes in Fogliano lagoon (Italy): A long-term analysis using a mathematical model. App. Mathemat. Model 25, 385–426.

Darby, P.C. 1998. Florida apple snail (*Pomacea paludosa*) life history in the context of a hydrologically fluctuating environment. Ph.D. thesis, University of Florida, Gainesville.

Darby, P.C., Valintine-Darby, P.L., Percival, H.F., 2003. Dry season survival in a Florida apple snail (*Pomacea paludosa Say*) population. Malacologia 45, 179–184.

Darby, P.C., Bennetts, R.E., Percival, H.F., 2008. Dry down impacts on apple snail (*Pomacea paludosa*) demography: implications for wetland water management. Wetlands 28, 204–214.

Desouky, M.M.A., 2006. Tissue distribution and subcellular localization of trace metals in the pond snail *Lymnaea stagnalis* with special reference to the role of lysosomal granules in metal sequestration. Aquat. Toxicol. 77, 143–152.

Dietrich, A.M., Gallagher, D.L., Klawiter, K.A., 2001. Inputs of copper-based crop protectants to coastal creeks from pasticulture runoff. J. Am.. Water Resour. Assoc. 37, 281–293.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E., 1995. Standard Methods for the Examination of Water and Wastewater, 19th Ed. American Public Health Association, Washington, DC.

Eisemann, J.D., Beyer, W.N., Bennetts, R.E., Morton, A., 1997. Mercury residues in south Florida apple snails (*Pomacea paludosa*). Bull. Environ. Contam. Toxicol. 58, 739–743.

Eisler, R. 1998. Copper Hazards to fish, wildlife, and invertebrates: a synoptic review. US Geological Survey, Biological Resources Division, Biological Science Report USGS/BDR/BSR 1997-0002, Containment Hazard Reviews Report 33.

Everglades National Park, 2001. Everglades National Park Strategic Plan 2001–2005. National Park Service, U.S. Department of the Interior, Homestead, FL.

Fieber, L.A., Schmale, M.C., Jordi, N., Orbesen, E., Diaz, G.A., Capa, T.R., 2005. Von Bertalanffy growth models for Hatchery-reared Aplysia California. Bull. Mar. Sci. 76, 95–104.

Florida Department of Environmental Protection. 2003. Development and Evaluation of Numerical Sediment Quality Assessment Guidelines for Florida Inland Waters. Technical Report. Twin Towers Office Building, 2600 Blair Stone Road, Tallahassee, Florida 2399–2400.

Frakes, R.A., Bargar, T.A., Bauer, E.A., 2008. Sediment copper bioavailability to freshwater snails in south Florida: risk implications for the Everglade snail kite (*Rostrhamus sociabilis plumbeus*). Ecotoxicology 17, 598–604.

Gomot, A., Pihan, F., 1997. Comparison of the bioaccumulation capacities of copper and zinc in two snail subspecies (*Helix*). Ecotoxicol. Environ. Saf. 38, 85–94.

Gomot-de Vaufleury, A., Pihan, F., 2002. Methods for toxicity assessment of contaminated soil by oral or dermal uptake in land snails: Metal bioavailability and bioaccumulation. Environ. Toxicol. Chem. 21, 820–827.

Hanning, G.W., 1978. Aspects of reproduction in *Pomacea paludosa* (Mesogastropoda: Pilidae). Unpublished M.S. Thesis, Florida State University, Tallahassee, FL.

He, Z.L., Zhang, M.K., Yang, X.E., Stoffella, P.J., 2006. Release behavior of copper and zinc from sandy soils. Soil Sci. Soc. Am.. J. 70, 1699–1707.

Heng, L.Y., Mokhtar, M.B., Rusin, S., 2004. The bioaccumulation of trace essential metals by the freshwater snail, *Turritella sp.* found in the rivers of Borneo East Malaysia. J. Biol. Sci. 4, 441–444.

Hoang, T.C., Rogevich, E.C., Rand, G.M., Gardinali, P.R., Frakes, R.A., Bargar, T.A., 2008a. Copper desorption in flooded agricultural soils and toxicity to the Florida apple snail (*Pomacea Paludosa*): implications in Everglades restoration. Environ. Pollut. 154, 338–347.

Hoang, T.C., Rogevich, E.C., Rand, G.M., 2008b. Copper uptake and depuration by juvenile and adult Florida apple snails (*Pomacea paludosa*). Ecotoxicology 17, 605–615.

Hoang, T.C., Schuler, L.J., Rogevich, E.C., Bachman, P.M., Rand, G.M., Frakes, R.A., 2009. Copper Release, Speciation, and Toxicity Following Multiple Floodings of Copper Enriched Agriculture Soils: Implications in Everglades Restoration. Water Air Soil Pollut. 199, 79–93.

Imlay, M.J., 1983. Relation of gizzard stones to toxicity of copper. Abstract. Am. Malacol. Bull. 1, 97.

Impellitteri, C.A., Saxe, J.K., Cochran, M., Janssen, G., Allen, H.E., 2003. Predicting the bioavailability of copper and zinc in soils: Modeling the partitioning of potentially bioavailable copper and zinc from soil solid to soil solution. Environ. Toxicol. Chem. 22, 1380–1386.

Ingersoll, C.G., Brumbaugh, W.G., Dwyer., F.J., Kemble, N.E., 1994. Bioaccumulation of metals by *Hyalella azteca* exposed to contaminated sediments from the upper Clark Fork River, Montana. Environ. Toxicol. Chem. 13, 2013–2020.

Katsanevakis, S., 2006. Modelling fish growth: model selection, multi-model inference and model selection uncertainty. Fish.. Res. 81, 229–235.

Laskowski, R., Hopkin, S.P., 1996. Accumulation of Zn, Cu, Pb and Cd in the garden snail (*Helix aspersa*): implications for predators. Environ. Pollut. 91, 289–297.

Li, H., Liu, Q., Shen, Z., 1997. Investigation of an iterative method for fitting von Bertalanffy growth equation using data on the halfbeak fish *Hemirhamphus sajori*. Fish.. Res. 29, 289–294.

Marsili-Libelli, S., 2004. Fuzzy prediction of the algal blooms in the Orbetello lagoon. Environ. Modelling Software 19, 799–808.

Meyer ,J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. 2005. Toxicity of dietborne metals to aquatic organisms. In: Proceedings from the Pellston Workshop on Toxicity of Dietborne Metals to Aquatic Organisms. July 22–August 1 2002. Published by Society of Environmental Toxicology and Chemistry (SETAC). Pensacola, FL.

Northwood, W.P., Borgmann, U., Dixon, D.G., 2007. Interative effects of metals in mixtures on bioaccumulation in the amphipod Hyalella azteca. Aquat. Toxicol. 84, 255–267.

Ohnishi, S., Akamine, T., 2006. Extension of von Bertalanffy growth model incorporating growth patterns of soft and hard tissues in bivalve molluscs. Fish.. Sci. 72, 787–795.

Pedersen, M.B., Temminghoff, E.J.M., Marinussen, M., Elmegaard, N., van Gestel, C.A.M., 1997. Copper accumulation and fitness of *Folsomia candida* Willem in a copper contaminated sandy soil as affected by pH and soil moisture. App. Soil Ecol. 6, 135–146.

Rand, G.M., Clark, J.R., Holmes, C.M., 2000a. Use of outdoor freshwater pond microcosms: I. microcosm design and fate of pyridaben. Environ. Toxicol. Chem. 19, 387–395.

Rand, G.M., Clark, J.R., Holmes, C.M., 2000b. Use of outdoor freshwater pond microcosms: II. Responses of biota to pyridaben. Environ. Toxicol. Chem. 19, 396–404.

Rand, G.M., 2004. Fate and effects of the insecticide-miticide chlorfenapyr in outdoor aquatic microcosms. Ecotoxicol. Environ. Saf. 58, 50–60.

Reuther, W., Smith, P.F., 1952. Iron chlorosis in Florida citrus groves in relation to certain soil constituents. Proceedings of the Florida State Horticultural Society 65, 62–69.

Reuther, W., Smith, P.F., 1953. Effects of high copper content of sandy soil on growth of citrus seedlings. Soil Sci. 75, 219–224.

Rogevich, E.C., Hoang, T.C., Rand, G.M., 2008. The effects of water quality and age on the acute toxicity of copper to the Florida apple snail, *Pomacea paludosa*. Archives of Environ. Toxicol. Chem. 54, 690–696.

Rogevich, E.C., Hoang, T.C., Rand, G.M., 2009. Effects of sublethal chronic copper exposure on the growth and reproductive success of the Florida apple snail (*Pomacea paludosa*). Arch. Environ. Contam. Toxicol. 56, 450–458.

Scheifler, R., Brahim, M.B., Gomot-de Vaufleury, A., Carnus, J.M., Badot, P.M., 2003. A field method using microcosms to evaluate transfer of Cd, Cu, Ni, Pb, and Zn from sewage sludge amended forest soils to *Helix aspersa* snails. Environ. Pollut. 122, 343–350.

Schuler, L.J., Hoang, T.C., Rand, G.M., 2008. Aquatic risk assessment of copper in freshwater and saltwater ecosystems of South Florida. Ecotoxicology 17, 642–659.

Sharfstein, B., Steinman, A.D., 2001. Growth and survival of the Florida apple snail (Pomacea paludosa) fed 3 naturally occurring macrophyte assemblages. J. North Amer. Benthol. Soc. 20, 84–95.

Sholdt, L., Ehrhardt, D.A., Michael, A.G. 1972. A guide to the use of the mosquito fish, *Gambusia affinis*, for mosquito control, 1972. Navy Environmental and Preventive Medicine Unit No.2, Norfolk, Virginia, 23511.

Simon, T.P., Wallus, R., 1990. Reproductive Biology and Early Life History of Fishes in the Ohio River Drainage. Taylor & Francis, pp. 175. isbn:0849319218.

South Florida Water Management District, 2001–2006. Reports submitted to South Florida Water Management District for Phase I/II environmental site assessments. South Florida Water Management District, West Palm Beach, FL.

Teo, S.S., 2004. Biology of the golden apple snail, *Pomacea canaliculata* (Lamarck, 1982) with emphasis on responses to certain environmental conditions in Sabah, Malaysia. Mollus. Res. 24, 139–148.

Turner, R.L. 1994. The effects of hydrology on the population dynamics of the Florida apple snail (*Pomacea paludosa*): Final report for the St. Johns Water Management District: Special publication SJ94-SP3; Palatka, FL.

Turner, R.L., Hartman, M.C., Mikkelsen, P.M. 2001. Biology and management of the Florida apple snail. Final Report for Florida Fish and Wildlife Conservation Commission. Project NG88-105.

U.S. Department of Agriculture, 2005. Agricultural chemical usage summary. Fruit summary. National Agricultural Statistical Service. USDA, Washington, DC.

U.S. Environmental Protection Agency. 1996. Acid digestion of sediment, sludges and soils: EPA method 3050BSW-846 manual; Washington, DC.

U.S. Environmental Protection Agency. 2003. Draft update of ambient water quality criteria for copper: EPA 822-R-03-026; Office of Water, Office of Science and Technology, Washington, DC.

Walford, L.A., 1946. A new graphic method of describing the growth of animals. Biol. Bull. Mar. Biol. Lab. Woods Hole 90 (2), 141–147.

Zhu, B., Alva, A.K., 1993. Distribution of trace metals in some sandy soils under citrus production. Soi. Sci. Soc. Am.. J. 57, 350–355.