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以系統層級動態研析吳郭魚暴露於擾動金屬濃度之生態生理反應

Systems-level dynamics to quantify ecophysiological responses for tilapia *Oreochromis mossambicus* exposed to fluctuating metals

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謝　誌

Where the mind is without fear
Where knowledge is free
Where words come out from the depth of truth
Where tireless striving stretches its arms towards perfection
Where the mind is led forward by thee into ever-widening though and action—
Into that heaven of freedom, my advisor let my idea awake.

Revised from GiTanJiali by Tagore

時光荏苒，在結束時總是會想起開始，憶起初次踏入老師辦公室時帶著雀躍又徬徨的心情，見到老師後就傻呼呼地猛問「老師願意收學生嗎？」，收到老師肯定的回應後，就此開啟這四年的旅程。

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ABSTRACT

Fluctuation exposure of contaminant is ubiquitous in aquatic environments. Traditional standard laboratory toxicity tests were performed at constant exposure scenarios typically did not elucidate the short-term pulsed exposure toxicity to aquatic organisms. Little is known about copper (Cu) and arsenic (As) toxic effects with pulsed and fluctuation exposures on aquatic organisms. The purpose of this dissertation was to develop a quantitative systems-level approach utilizing toxicokinetics, toxicodynamics, bioavailability, and bioenergetics mechanisms to elucidate the ecophysiological response of tilapia (*Oreochromis mossambicus*) to fluctuating or sequential pulse Cu and As stresses. This study investigated the relationship among bioavailable metal, accumulative concentration and critical damage level induced growth toxicity for tilapia based on biotic ligand model (BLM), threshold damage model (TDM), and ontogenetic growth-based dynamic energy budgets in toxicology (DEBtox) model.

This study conducted the sequential pulsed Cu exposure bioassays on tilapia population to provide Cu acute/chronic toxicokinetics information. The 10-day and 28-day sequential pulsed Cu exposure experiments were conducted to obtain the bioconcentration factor (BCF) for tilapia population. This study linked bioavailability and bioaccumulation mechanisms to estimate the time and water chemistry dependent BCF. This also study analyzed the As exposure experimental data and pulsed Cu exposure bioassays of tilapia with growth inhibition response by using the proposed systems-level mechanistic model with periodic pulses and fluctuating exposures to simulate and compare the outputs. The ontogenetic growth-based DEBtox model was used to estimate growth coefficient (A_0) based on chronic growth bioassay, for assessing Cu and As chronic growth toxicities to tilapia.

The experimental results indicated that larvae had the highest BCF of 1116.10 mL g^{-1} that was greater than those of juveniles 225.50 mL g^{-1} and adults 94.00 mL g^{-1} in acute pulsed Cu exposure, whereas juveniles had the highest BCF 154.54 mL g^{-1} than that of adults 23.10 mL g^{-1} in chronic pulsed Cu exposure. Besides, tilapia had a higher Cu accumulation capacity than that of As (BCF=2.89 mL g^{-1}). Results also showed that BCF value depended significantly on water chemistry conditions and ions concentration. Moreover, BCF value decreased with the increasing of exposure duration. This study also found that tilapia in response to low-frequency Cu/As pulsed exposure had longer 50% safe probability time (ST50) than that of high-frequency pulsed exposure, whereas the longer ST50 was found in high frequency for Cu/As fluctuating exposure. The results indicated that the regulations were triggered between the pulsed intervals. The accumulation of the second Cu pulsed exposure was positively influenced by first Cu pulsed exposure that was consistent with the results of model simulation. The growth coefficients were estimated to be 0.029± 0.0015 $g^{1/4}$ d^{-1} (Mean±SE) for control and 0.019±0.0017 $g^{1/4}$ d^{-1} for pulsed Cu exposures in tilapia. The results indicated that growth coefficient depends positively on the exposure concentrations, revealing that Cu concentration inhibited growth energy and affected the growth of tilapia. The estimated dimensionless mass ratio revealed that sequential and fluctuating Cu exposure could increase tilapia energy acquisition than that of sequential and fluctuating As exposure for overcoming externally fluctuation-driven environments.

This study showed that the dynamics of physiological responses were dependent on the pulsed and fluctuating concentrations, duration, frequency, and different chemical exposure characters in tilapia. Moreover, the time and ions-dependent BCF provided a tool to assess the relationship between accumulation and toxic effect in the

field situation. We anticipated that this study could provide a completed quantitative systems-level dynamic approach for understating the ecophysiological response of aquatic organisms in response to metal stresses in the field situations. We also hoped that the proposed dynamics of ecophysiological response mechanistic model could successfully assess the long-term metal exposure risk for tilapia population in the field situation of metal exposure impact.

***Keywords*:** Arsenic; Copper; Tilapia; Bioaccumulation; Bioavailability; Bioenergetics ; Pulsed/fluctuating exposure toxicity; Systems-level

中文摘要

水域環境普遍存在污染物的擾動現象。傳統標準實驗室毒性試驗皆於持續不變之暴露濃度情境下進行，但卻無法呈現短期脈衝暴露對水域生物之毒性效應。此外，銅與砷之脈衝與擾動暴露型態對水域生物所造成之效應甚少被研究。本論文之研究目的主要在於發展一量化系統層級法，利用毒理動力、毒理動態、生物可獲取率及生物能量機制解釋吳郭魚 (*Oreochromis mossambicus*)暴露於連續脈衝與擾動銅、砷濃度之生態生理反應。本研究以生物配體模式 (biotic ligand model, BLM)、閾值損害模式 (threshold damage model)及以毒理學之動態能量支出 (dynamic energy budgets in toxicology, DEBtox)為基礎之個體成長模式，探討可獲取的金屬、累積濃度及關鍵損害程度三者之相關性對成長毒性的影響。

本研究建構一吳郭魚暴露於脈衝銅濃度之生物試驗以提供急性與慢性暴露之毒理動力參數資訊。建構 10 天與 28 天之連續脈衝銅暴露實驗，以求得吳郭魚族群之生物濃縮因子 (bioconcentration factor, BCF)。亦結合生物可獲取率及生物累積機制，提供一方法推估隨時間及水化學而變化之生物濃縮因子。此外，本研究分析吳郭魚的成長抑制反應之砷暴露實驗數據及脈衝銅暴露生物試驗結果，運用本研究所提出之以系統層級機制模式，利用週期性脈衝及擾動暴露進行模擬與比較其模擬結果。再利用毒理學之動態能量支出為基礎之個體成長模式及慢性試驗數據推估成長係數 (A_0)，並評估吳郭魚對銅、砷之慢性成長毒性。

實驗結果顯示在急性脈衝銅暴露下，吳郭魚稚魚對銅之生物濃縮因子為 1116.10 mL g^{-1}，高於幼魚之 225.50 mL g^{-1} 及成魚之 94.00 mL g^{-1}。而在慢性脈衝銅暴露之 BCF 值則以幼魚的 154.54 mL g^{-1} 大於成魚之 23.10 mL g^{-1}。於結合生物可獲取率及生物累積機制之結果，顯示生物濃縮因子確實會受水化學條件、離子

濃度多寡而有所影響，且隨著暴露的時間愈長，其生物濃縮因子會愈小。此外，吳郭魚對銅的累積能力(BCF=94 mL g^{-1}) 高於對砷的累積能力(BCF=2.89 mL g^{-1})。本研究亦發現吳郭魚暴露於低頻率銅、砷脈衝暴露之 50%安全機率時間(ST50)大於高頻率暴露，而在擾動暴露則以 50%安全機率時間大於低頻率暴露。此結果顯示，吳郭魚會因不同的脈衝時間間距而啟動調節機制，且第二次脈衝銅暴露之累積程度確實受第一次脈衝銅暴露之影響，其結果均符合模式模擬之結果。而在吳郭魚控制組所推估之 A_0 為 0.029± 0.0015 $g^{1/4}$ d^{-1} (平均值±標準誤差)，而在脈衝銅暴露 A_0 為 0.019±0.0017 $g^{1/4}$ d^{-1}。此結果顯示成長係數確實會受暴露濃度所影響，表示銅濃度確實會抑制成長能量且影響吳郭魚之成長。由推估之無因次成長質量比例結果顯示在連續脈衝與擾動銅暴露下，吳郭魚會增加能量消耗大於連續脈衝與擾動砷暴露。

本研究顯示吳郭魚之生態生理反應動態會因脈衝及擾動濃度、持續時間、頻率及不同化學暴露特性而有所影響。再者，隨時間與離子濃度變化之生物濃縮因子可用以評估現地暴露狀況之毒性效應。因此，由本論文結果可知，運用量化系統層級動態法可了解水域生物在現地暴露狀況之生態生理反應。並期望所提出生態生理反應動態機制模式可成功地描述現地金屬暴露之影響狀態，而吳郭魚族群長期暴露於金屬之風險亦可精確地被評估。

關鍵字：砷；銅；吳郭魚；生物累積；生物可獲取率；生物能量；脈衝/擾動暴露毒性；系統層級

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NOMENCLATURE

[ ]	Dissolved ion concentration (g L^{-1})
{}	Free ion activity (M)
A	Constants depend on the water permittivity constant and temperature
A_0	Biological species-specific growth coefficient ($g^{1/4}$ d^{-1})
AUC	Area under the metal concentration in organism *versus* time curve (μg d g^{-1})
[a]	$K_{CuBL+}K_{CuOHBL}\{OH^-\}+K_{CuCO_3BL}K_{CuCO_3}\{CO_3^{2-}\}$ (M^{-1})
BCF	Bioconcentration factor (mL g^{-1})
BL	Biotic ligand (mol g^{-1})
BLM	Biotic ligand model
C	Chemical concentration (mg L^{-1})
C_0	Background or base line metal concentration (mg L^{-1})
C_1	Pulsed or amplitude metal concentration (mg L^{-1})
C_b	Metal concentration in the body (μg g^{-1})
$C_{e,0}$	External threshold chemical concentration (mg L^{-1})
C_i	Analytic ion concentration (M)
$C_{i,0}$	Internal threshold chemical concentration (μg g^{-1})
C_w	External waterborne metal concentration (mg L^{-1})
D	Damage (–)
DAM	Damage assessment model
$D_{E,50}$	Damage for 50% effect (–)
$D_{E,50}/k_a$	Compound equivalent toxic damage level for 50% effect (μg g^{-1} d)
DEBtox	Dynamic energy budgets in toxicology
d	Phase without metal exposure (–)
dH	Hazard rate (–)

E	Effect elicited by chemical (%)
E_0	Initial (background) effect (%)
$EC50$	Chemical concentration causing the half of maximum effect (%)
E_{max}	Maximum effect causing by chemical (%)
E_{c0}	Metabolic energy required to create a new cell (J)
E_{c0}	Metabolic energy required to create a new cell in control condition (J)
FIAM	Free ion activity model
$f_{CuBL}^{50\%}$	Fraction of total number of Cu binding site occupied by Cu at 50% effect
G	Growth rate (%)
G_0	Growth rate of fresh body biomass without metal exposure (%)
GI	Growth inhibition rate (%)
G_m	Growth rate of fresh body biomass with metal exposure (%)
GSIM	Gill surface interaction model
H	Cumulative hazard (–)
I	Total metal ionic strength (M)
k_3	Proportion between damage and hazard (–)
k_a	Damage accumulation rate (g μg^{-1} d^{-1})
k_e	Elimination rate constant (d^{-1})
$k_{g,0}$	Daily growth rate without chemical exposure (% d^{-1})
$k_{g,m}$	Daily growth rate with chemical exposure (% d^{-1})
k_k	Killing rat constant (g μg^{-1} d^{-1})
k_r	Damage recovery rate constant (d^{-1})
k_u	Uptake rate constant (mL g^{-1} d^{-1})
K_{MBL}	Stability constant for the binding of metal to the biotic ligand (M^{-1})
K_{ML}	Stability constant for the binding of metal to the ligand (M^{-1})

L	Ligand (mol g^{-1})
$LC50$	Median lethal concentration (mg L^{-1})
M^+	Metal ion concentration (mole L^{-1}, M)
MBL	Metal ion concentration in biotic ligand (mole L^{-1}, M)
ML	Metal complex concentration (mole L^{-1}, M)
MOA	Mode of action
m	Average resting metabolic rate (J d^{-1})
m_0	Taxon-specific constant (–)
m_c	Metabolic rate of a single cell (J d^{-1})
N	Total number of cell in the organims
n	Hill coefficient (–)
n_i	Pulsed frequency of this exposure duration (–)
P	Permittivity constant (78.3)
r	Dimensionless biomass ratio (–)
S	Safe probability (–)
$S_{control}$	Safe probability resulting from the control effect (–)
$ST50$	50% safe probability time (d)
T	Exposure timing or periods (d)
TDM	Threshold damage model
T_s	Solution temperature (K)
W	Body biomass (g)
W_0	Body biomass at birth (g)
W_i	Fresh body biomass at beginning (g)
W_c	Mass of cell (g)
W_{max}	Maximum body biomass (g)

W_{max0}	Maximum body biomass in control group (g)
W_t	Fresh body biomass at time t (g)
WHAM	Windermere humic aqueous model
Z	Valence charge number of ion in the solution
γ	Activity coefficient (–)
δ	Dirac delta function

CHAPTER 1. INTRODUCTION

For the water quality management in aquatic ecosystems, it is important to be able to predict the impact of metal and toxic effects on aquatic organisms. Traditional standard laboratory toxicity tests are performed at constant exposure scenarios to develop water quality criteria. Yet, fluctuation exposure is ubiquitous in nature. Aquatic organisms are always exposed to temporal fluctuations of contaminants. In reality, fluctuating/pulsed exposure may be the prevalent form in the field situation (Handy, 1994; Reinert et al., 2002; Nimick et al., 2007). Many factors could affect chemical characteristics, such as wastewater flow, sunshine, rainfall, temperature, and the various effluent ions changed contamination exposure. Hence, incorporating exposure timing and frequency into the realistic exposures to predict the metal toxic effects can improve the robustness of environmental risk assessment.

Fish are commonly used bioindicators for metal contamination due to their sensitivity, wide distribution, abundance, and tolerant to various environmental conditions. Moreover, by including their changes in behaviors, e.g., swimming and avoidance response patterns, it can be measured immediately as responses to the occurrence of contaminants (van der Schalie et al., 2001; Gerhardt et al., 2005, 2006; Zhou et al, 2008). Behaviors could be used as ecophysiological activity parameters for providing ecological relevance to standard toxicity testing. Consequently, the predictions of ecophysiological mechanisms of aquatic organisms subjected to time-varying exposure patterns may provide a practical implication for species growing, cultivation strategies, and risk assessment in realistic situations.

CHAPTER 2. BACKGROUND AND RESEARCH OBJECTIVES

2.1. Background

Arsenic (As) and copper (Cu) are widespread in the environment from anthropogenic and natural processes. As is a hazardous trace element that existing in both organic and inorganic states in the environment. Previous investigations indicated that As could be accumulated in tissues of freshwater organisms. Consumption of these As contaminated tissues may pose adverse health risk (Williams et al., 2006). It is known that As inhibits more than 200 enzymes and causes adverse effects, leading to serious disorders to organisms (Abernathy et al., 1999).

There had also been serious Cu pollution caused by electroplating industrial discharges in Erhjen River and by computer-related high-tech industrial wastewater in Sien San area of Taiwan, known as green oyster incidents (Lee et al., 1996; Lin and Hsieh, 1999). In addition, copper sulfate ($CuSO_4$) has been widely used to exterminate phytoplankton and control skin lesion of fish in cultured ponds (Carbonell and Tarazona, 1993; Chen and Lin, 2001; Chen et al., 2006). The contamination of various rivers and coastal areas by Cu has been received increasing attention in Taiwan.

Based on previous studies, effluent metal concentration measured in aquatic environment fluctuated with time (Gammons et al., 2005, 2007; Diamond et al., 2005). Aquatic organisms living in aquatic systems would positively experience pulsed/fluctuating exposures. Water quality standard toxicity tests rely on the exposure magnitude and duration. Typically, standard tests did not investigate the sequential pulsed and fluctuating exposure toxicities to aquatic organisms (USEPA,

1995, 2002, 2003). Thus, it is important to be able to predict As and Cu toxic effects with pulsed and fluctuation exposures on aquatic organisms.

Based on the toxicological principles in aquatic ecosystems, the chemical toxicity depends on the external (water chemical) and the internal (biological) factors. The chemical toxicity was affected by external factors such as temperature, pH, hardness, specific ion levels, and chemical reaction by influencing the mechanisms of the bioavailability to aquatic organisms (De Schamphelaere and Janssen, 2002; Niyogi and Wood, 2004). Knowledge of the major ion competition and complex effects on chemistry species are quite important. The water chemical mechanistic approach can greatly improve the site-specific ambient water quality criteria for metals. In view of the biological factors, the metal toxicity to aquatic organisms were performed by two phases: (i) metal accumulative capacity through absorption, distribution, metabolism, and excretion (toxicokinetics), and (ii) metal caused adverse effect that acts at the site of action or target site (toxicodynamics).

Farming tilapia (*Oreochromis mossambicus*) is the most promising aquatic products in Taiwan because of its high market value and is also one of major food sources of human. Therefore, the tolerances of aquatic organism to sequential pulsed As and Cu toxicities are needed to be estimated.

This study presents an integrated methodology and framework to develop a quantitative systems-level modeling approach based on bioavailability and toxicokinetic/toxicodynamic mechanisms to predict ecophysiological responses (i.e. bioengeretics) of tilapia to sequential pulsed As and Cu concentrations. The overall

conceptual framework of this study is illustrated in Fig. 2.1.

Available published experimental database of As-tilapia system together with the proposed Cu-tilapia bioassays could provide an extensive range of physiological characterization including bioaccumulation and ontogenetic growth inhibition response with waterborne metal exposure. This study constructed an integrated model based on toxicologically mechanistic models to develop ecophysiological response model to elucidate ecobehaviors of fish under the ecologically relevant metal exposure patterns.

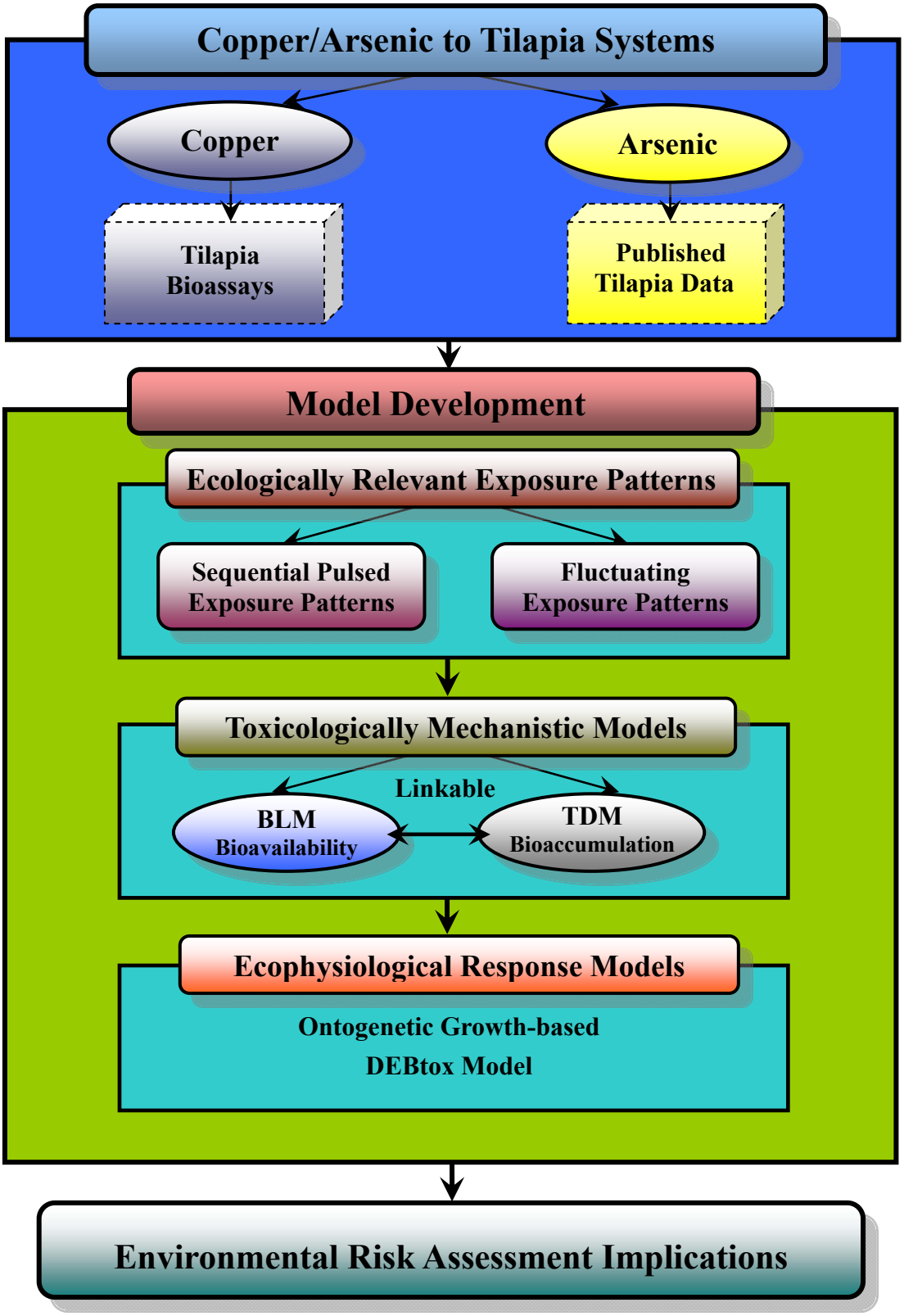


Figure 2.1. A conceptual framework revealing the interaction among experimental data, ecologically relevant exposure pattern, toxicologically mechanistic models, ecophysiologcal response models, and evaluating the environmental risk assessment.

2.2. Research Objectives

Specifically, the major objectives of this study are fivefold:

1. To develop a quantitative systems-level modeling approach to elucidate the ecophysiological response of tilapia to sequential pulsed/fluctuating Cu and As concentrations.

2. To conduct a pulsed Cu acute and chronic exposure experiments to provide information on the toxicokinetics of Cu in tilapia after sequential pulsed exposure patterns.

3. To link the proposed quantitative systems–level modeling approach to bioavailability and bioaccumulation mechanisms for simulating internal effect of tilapia in response to sequential pulsed and fluctuating Cu and As concentrations.

4. To link bioavailability and bioaccumulation mechanisms for simulating bioenergetics of tilapia to sequential pulsed or fluctuating Cu and As concentrations in response to sequential pulsed/fluctuating patterns.

5. To comprise the bioavailability, toxicokinetics, toxicodynamics, and bioenergetics knowledge to illustrate a more reliable prediction for long-term exposure risk assessment in site-specific settings.

CHAPTER 3. LITERATURE REVIEW

3.1. Ecologically Relevant Metal Exposure Pattern

In the aquatic ecosystems, the variation in toxicant concentrations can generate either pulsed or fluctuating exposures. The rainfall, accidental spillage of wastes, the periodic emission of anthropogenic contaminants into the waterborne, and precipitations of airborne contaminant can generate pulsed patterns. The metal cycles, pulsed or fluctuating exposure scenarios were dependent on the geology and hydrology (Astruc, 1989; Campbell and Tessier, 1989; Pereira et al., 2009). The diel metal cycles of biogeochemical process are greatly dynamics and short-term (daily and bihourly) variations in metal concentration and speciation (Nagorski et al., 2003; Authman and Abbas, 2007; Tercier-waeber et al., 2009).

Tercier-waeber et al. (2009) indicated that water chemistry conditions were positively affected by the metal cycles rather than hydrological conditions, the solubility, and availability of metal. The occurrence of diel variations in the As, Cu, and other heavy metal concentrations were found in the surface water (Gammons et al., 2005; 2007; Diamond et al., 2005; Parker et al., 2007; Pereira et al., 2009; 2010). It is positively confirmed that many factors could affect diel variations in the water chemistry characteristics in As- and Cu-rich aquatic ecosystems. Previous studies indicated the positive relationship between As and pH or temperature (Fig. 3.1A) (Fuller and Davis, 1989; Gammons et al., 2007), whereas the Cd and Zn had negative relationship with pH and temperature (Fig. 3.1B) (Nimick et al., 2007). The Cu concentrations were positively correlated with water flow and dissolved organic carbon (Heier et al., 2010). However, these studies indicated that temperature and pH were the dominant factors for controlling diel fluctuations in water chemistry in the

metal-rich stream (Nimick et al., 2003, 2007; Gammons et al., 2005, 2007).

Standard laboratory bioassays carried out the continuous exposure to investigate the chemical induced adverse effect for aquatic organisms that would inaccurately estimate the chemical toxicity and risk (Hoang et al., 2007). Beside, some studies indicated that pulsed/fluctuating exposure toxicity would be induced the latent effect to aquatic organisms (Diamond et al., 2005; Hoang et al., 2007; Nimick et al., 2007). Nimick et al. (2007) have been conducted the field bioassays to monitor the diel waterborne metal cycle and measure the survival probability for cutthroat trout (*Oncorhynchus Clarki Lewisi*). Nimick et al. (2007) revealed that the latent mortality and the mortality related prior fluctuating events occurred in the cutthroat trout bioassays (Fig. 3.2). Nimick et al. (2007) suggested that exposure to diel-fluctuating concentration was less toxic than exposure to the constant concentration for cutthroat trout when the average concentration for the fluctuating and a constant was the same. Moreover, the low concentration periods during the diel cycle would give a sufficient regulating time to repair the gill or tissue damages (McDonald and Wood, 1993; Reinert et al., 2002; Diamond et al., 2005, 2006; Bearr et al., 2006; Hoang et al., 2007).

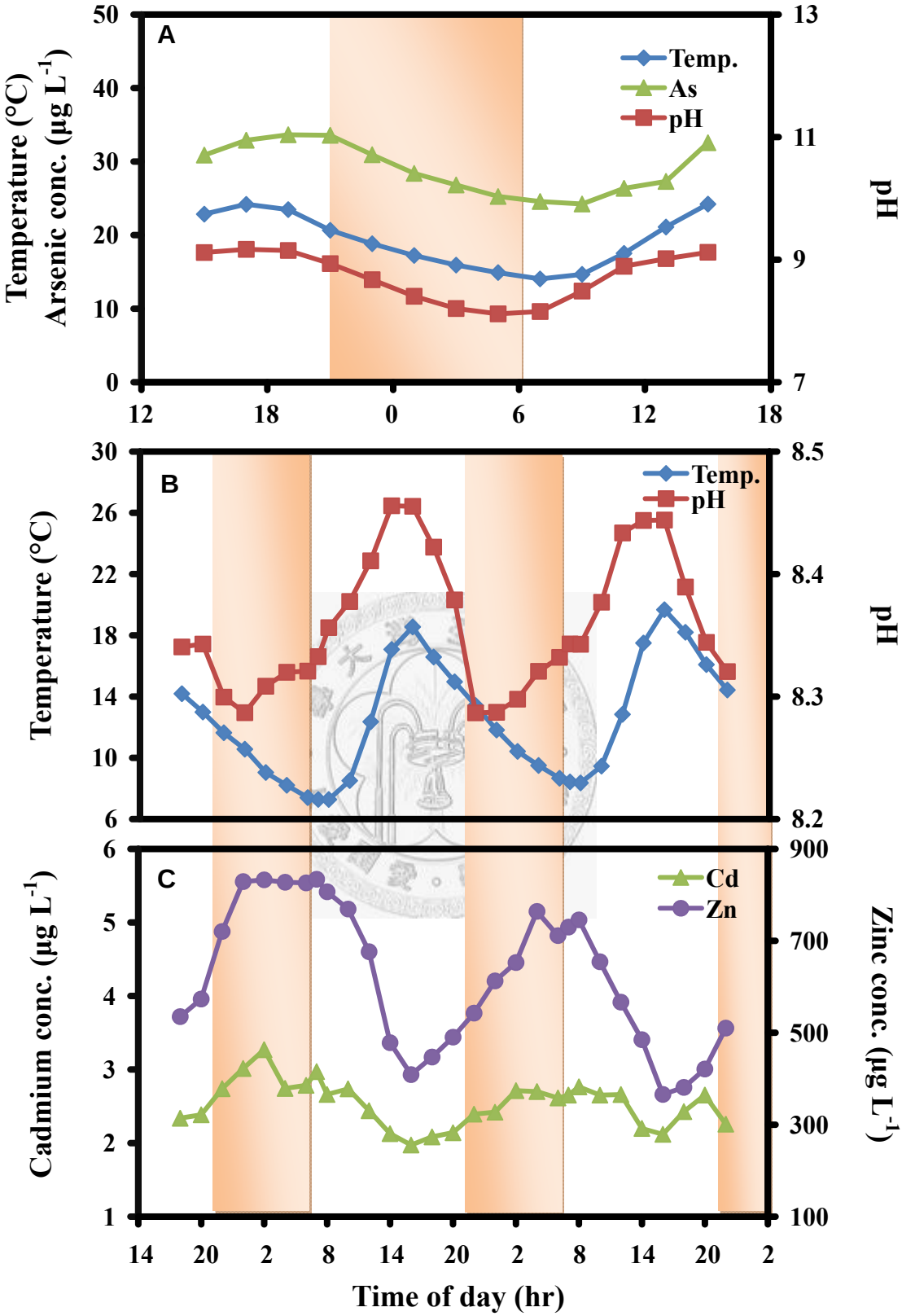


Figure 3.1. (A) Relationship among temperature, pH, and As in Clark Fork River (Gammons et al., 2007). (B) Relationship among temperature, pH, Cd, and Zn High Ore Creek, Montana, USA. Shaded area denote night time hours (Nimick et al., 2007).

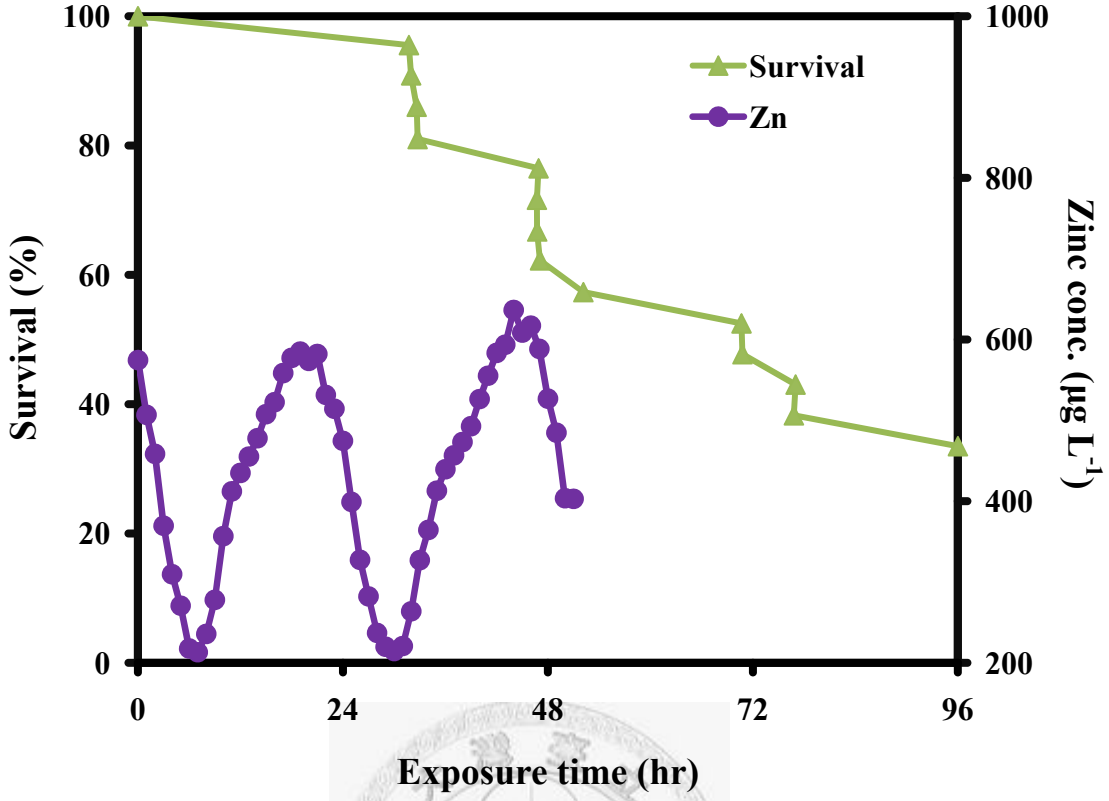


Figure 3.2. Relationship between the time-dependent Zn exposure concentration and survival percentage of cutthroat trout (Nimick et al., 2007).

3.2. Metal Toxic Effects in Aquatic Ecosystems

3.2.1. Cu toxicity

Numerous researches reported that Cu in fish (*Megalops cyprinoids, Chanos chanos*, *Liza macrolepis*, *Mugil cephalus*, *Oreochromis sp*) and oyster (*Crassostrea gigas*) ranged from 0.39 – 1 μg g^{-1} dry wt and 1.3 – 988 μg g^{-1} dry wt, respectively (Han et al., 2000; Huang et al., 2001; Chen et al., 2004), whereas the Cu in tilapia ranged from 1.524 – 18 μg g^{-1} dry wt (Lin et al., 2005a). Copper plays an essential role in cellular metabolism of aquatic organisms (Prasad, 1984; Cousins, 1985). Several previous studies demonstrated that Cu accumulates in the chloride cell and positively inhibit brachial Na^+/K^+-ATPase activities decreasing Na^+ transport in gill of fish (Li et al., 1998; Grosell and Wood, 2002; Paquin et al., 2002; Wu et al., 2003; De Boeck et al., 2007). That could cause cardiovascular and mortality in fish because the high Cu levels could induce the disruption of branchial ion regulation.

Previous studies have been carried out the acute toxicity and the Cu exposure bioassays to determine the effective Cu concentration of induced mortality and accumulation for tilapia, indicating that the water chemistry and other environmental conditions such as water hardness, humic substance, and pH were positively affected the toxic effect and accumulative capacity (Pelgrom et al., 1995; Nussey et al., 1996; de Vera and Pocsidio, 1998; James et al., 1998; Straus, 2003; Wu et al., 2003; Abdel-Tawwab and Mousa, 2005; Naigaga and Kaiser, 2006; van Aardt and Hough, 2006; Kosai et al., 2009). The 96-h median lethal concentration (LC50) of tilapia species from numerous studies were listed in Table 3.1. The rank of waterborne Cu accumulation in organ-specific of tilapia was liver > intestine > kidney > gill (Pelgrom et al., 1995). Generally, the excess Cu was most stored in the liver since it is

an important storage organ for aquatic organisms (Buck, 1978; Shearer, 1984).

Authman and Abbas (2007) have measured the seasonal waterborne Cu concentration and organ-specific (gill, muscle, liver) concentration and bioaccumulation factor for tilapia (*Tilapia zillii*) in Lake Qarun, Egypt. The relationships among waterborne Cu concentration, accumulation concentration, and bioaccumulation factor were found positive (Fig. 3.3). The influential factors included variant temperatures (22.8, 31.2, 30.2, and 19.8 °C), pH (7.8, 8.1, 7.8, and 7.5), and total dissolved solids (19.2, 27.4, 18.7, and 9.3 g L^{-1}) varied with different seasons. The hydrological factors with the seasonal variation could affect metal bioavailability and the accumulated metal concentration (Luoma and Rainbow, 2008).

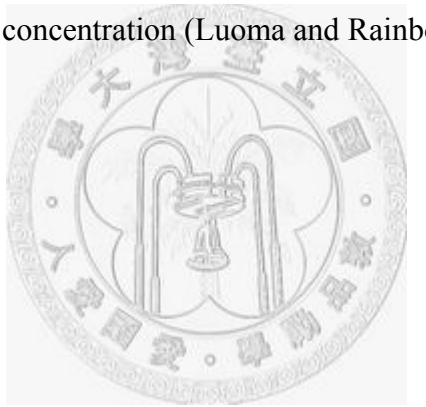


Table 3.1. 96-h median lethal concentration (LC50) of Cu compound and exposure condition on tilapia species

Species	Weight (g wet wt)	Life stage	96-h LC50 (mg L^{-1})	Cu compound	Temperature (°C)	pH	Alkalinity (mg L^{-1})	Hardness (mg L^{-1})	References
O. mossambicus	NA	Larva	0.25	$CuSO_4$	26 – 28	8.2 – 8.7	ND [a]	146.6±5.6	Wu et al. (2003)
	5 – 23.6	Juvenile	2.78	$CuCl_2 \cdot 2H_2O$	19±1	7.70 – 8.08	76	79	Nussey et al. (1996)
	5 – 23.6	Juvenile	2.61	$CuCl_2 \cdot 2H_2O$	29±1	7.36 – 8.12	77	80	Nussey et al. (1996)
	ND	Juvenile	1.52	ND	ND	ND	ND	ND	Lam et al. (1998)
	30 – 50	Adult	6.5	$CuSO_4 \cdot 5H_2O$	27 – 29	7.2 – 7.5	ND	93	de Vera and Pocsidio (1998)
	11.3±0.7	Adult	4.27	$CuSO_4 \cdot 7H_2O$	29.1±0.6	7.7±0.06	ND	90±3.8	James et al. (1998)
	6 – 20	ND	5	$CuSO_4$	26 – 28	7.5	ND	165	Jafri and Shaikh (1989)
	18	ND	1.5	$CuSO_4$	25	8.5	98	115	Qureshi and Saksena (1980)
	6	ND	133.5	$CuSO_4$	27	6.5	115	268	Mukhopadhyay and Konar (1984)
	6	ND	3F5	$CuSO_4$	27	7.0	115	268	Mukhopadhyay and Konar (1984)
	6	ND	18	$CuSO_4$	27	8.5	115	268	Mukhopadhyay and Konar (1984)
	3.7±0.7	ND	0.2	$CuSO_4 \cdot 5H_2O$	20.1±0.4	7.4	15.5	7.0	Straus (2003)
	3.7±0.7	ND	0.7	$CuSO_4 \cdot 5H_2O$	20.1±0.4	8.1	57.1	28.2	Straus (2003)
	3.7±0.7	ND	6.6	$CuSO_4 \cdot 5H_2O$	20.1±0.4	8.4	111.8	57.5	Straus (2003)
	3.7±0.7	ND	43.1	$CuSO_4 \cdot 5H_2O$	20.1±0.4	8.7	224.9	114.2	Straus (2003)
O. niloticus	1.8 – 2.5	Juvenile	5.03	ND	26 – 28	8 – 8.5	160 – 200	120 – 150	Abdel-Tawwab and Mousa (2005)
	15 – 20	ND	185.75	$CuSO_4 \cdot 5H_2O$	26.0±1.0	6.9 – 7.2	62 – 65	50 – 60	Kosai et al. (2009)
Tilapia sparrmanii	30 ± 8	ND	4.36	$CuCl_2 \cdot 2H_2O$	20	7.9	ND	ND	van Aardt and Hough (2006)

[a] ND indicates no data reported for the characteristics.

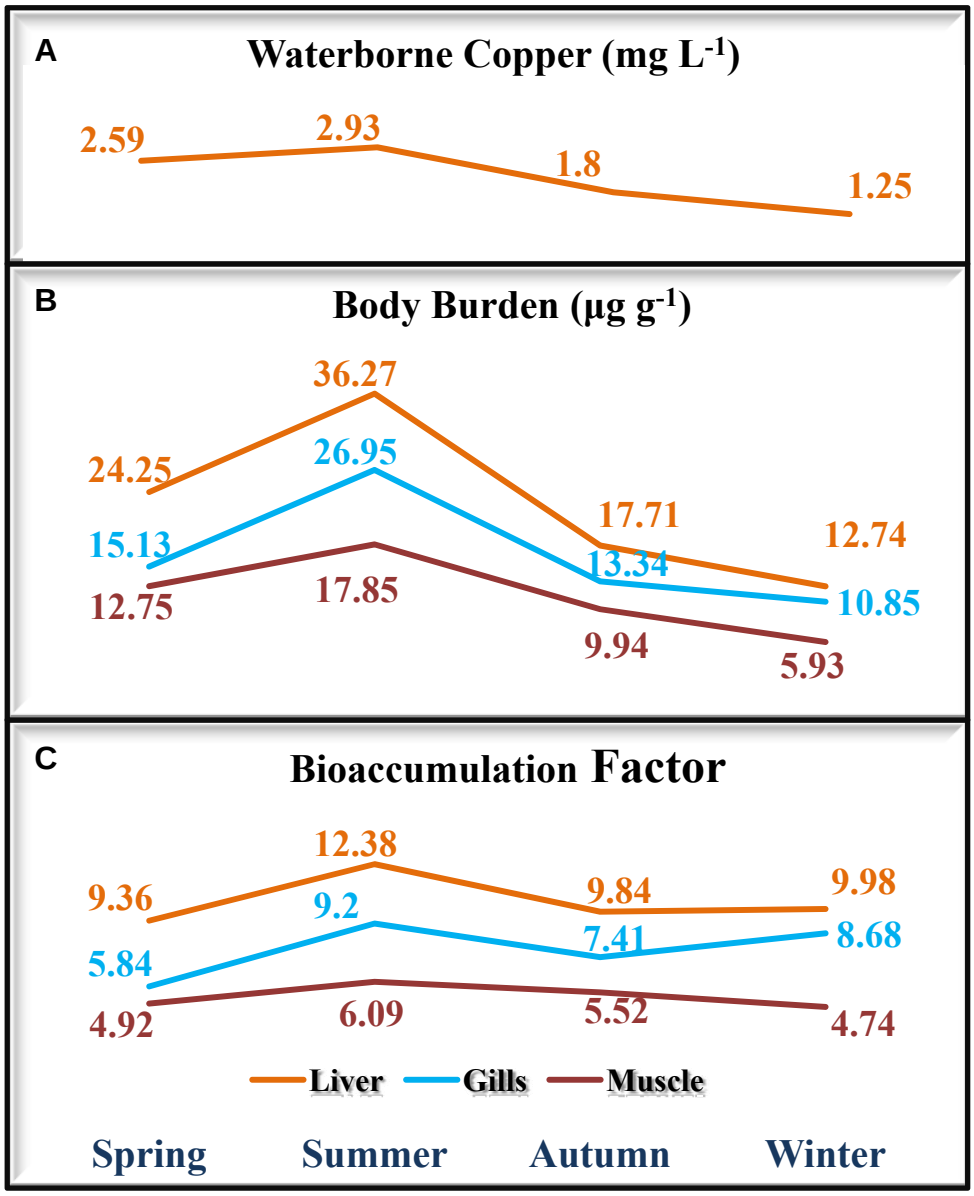


Figure 3.3. Relationship among (A) waterborne Cu concentration, (B) organ-specific Cu concentration and (C) bioaccumulation factor of tilapia with seasonal variation in Lake Qarun, Egypt (Authman and Abbas, 2007).

3.2.2. As toxicity

Arsenic is a metalloid element naturally occurring in the environment (Duker et al., 2005). As exists in both inorganic and organic forms and four oxidation states, As(III) (arsenite), As(V) (arsenate), As(0) (arsenic), and As(-III) (arsine) in the water, air, and soil, etc. The As toxicity depends on As speciation in that inorganic As species are more toxic than organic ones to the living organisms and As(III) is usually more toxic than As(V) (Goessler and Kuehnett, 2002; Ng, 2005). Neff (1997) indicated that the aquatic ecosystem plays a significant role in the global cycle of As. As concentration is usually less than 2 μg L^{-1} in the seawater (Ng and Kinniburgh, 2002; Ng, 2005). In the unpolluted surface water and groundwater, the average levels of As generally ranged from 1 to 10 μg L^{-1}, and freshwater varied typically from 0.15–0.45 μg L^{-1} (Smedley and Kinniburgh, 2002; Bissen and Frimmel, 2003a, b). The global As contamination in the aquatic system is summarized in the Table 3.2.

Previous investigations indicated that As could accumulate in tissues of aquatic organisms, and humans who consume these As contaminated tissues may pose adverse health risk (Williams et al., 2006). It is known that As leading to serious disorders such as blackfoot disease, skin lesions, and several cancers of bladder, kidney, liver, and vasculature to human (Chen et al., 2005).

Lin et al. (2001, 2005a, b), Huang et al. (2003), Liao et al. (2003), Chen et al. (2004), Liu et al. (2005, 2007, 2008), and Wang et al. (2007) have conducted a long-term study during 1998–2008 in southwestern Taiwan. The investigations indicated that As concentrations ranged from 40 – 900 μg L^{-1} in aquaculture water ponds including farm fish and shellfish, and the dominant As species was inorganic

As that % in of total As ranged from 83.6 – 97.9%. Moreover, the As(V) fraction in inorganic As were 84.2 – 100%. Furthermore, As concentrations in fish (tilapia *O. mossambicus* and *O.* sp., milkfish *Chanos chanos*, Indo-Pacific tarpon *Megalopsl cyprinoids*, striped mullet *Mugil cephalus*, and large-scale mullet *Liza macrolepis*) and shellfish (hard clam *Meretrix lusoria* and oyster *Crassostrea gigas*) ranged from 1 – 350 and 4 – 23 μg g^{-1} dry wt, respectively. Williams et al. (2006) summarized the As concentrations of freshwater fish ranging from 0.13 – 27.45 μg g^{-1} dry wt in USA. These indicated that As accumulations in fish in Taiwan were much more than those in USA.

Tsai and Liao (2006) indicated that aquatic organisms continue to accumulate and eliminate As with growth mechanism for the entire life. Liao et al. (2003) also revealed the negative correlations between body weight and As concentration of gill, liver, viscera, stomach, intestine, and muscle for tilapia.

Table 3.2 Global As contamination in aquatic systems [a]

Country/region	Conc. (μg L^{-1})	Source	Sampling period
Taiwan [b]	10 – 1820	Groundwater	NA [c]
Inner Mongolia	1 – 2400	Drinking water	1990s
Xinjiang, China	0.05 – 850	Well water	1983
Shanxi, China	0.03 – 1.41	Well water	Not stated
Bangladesh	<10 – >1000	Well water	1996 – 1997
West Bengal, India [b]	<10 – 3200	Well water	NA
Japan	0.001 – 0.293	Natural	1994
Thailand	1 – 5114	Mining waste	1980s – 1994
Vietnam [b]	1 – 3050	Natural	NA
Nepal	8 – 2660	Drinking water	2001
Cambodia	1 – 1340	Groundwater	2004 – 2006
Ghana [b]	1 – 175	Anthropogenic, natural	NA
Hungary	1 – 174	Deep groundwater	1974
Romania	1 – 176	Drinking water bores	2001
South-west Finland	17 – 980	Well water, natural	1993 –1994
Germany [b]	<10 – 150	Natural, mining	NA
Spain [b]	<1 – 100	Natural	NA
United Kingdom [b]	<1 – 80	Mining	NA
Argentine [b]	<1 – 9000	Natural, thermal spring	NA
Chile	470 – 770	Anthropogenic, natural	NA
Mexico	8 – 624	Well water	Not stated
Peru	500	Drinking water	1984
Western USA	1 – 48000	Drinking water	1988

[a] Adopted from Sharma and Sohn (2009).

[b] Adopted from Nordstrom (2002).

[c] NA: Not available.

3.3. Mathematical Models

3.3.1. Toxicokinetic model

The one-compartment toxicokinetic model depended upon the chemical concentration can be written as (Fig. 3.4),

$$\frac{dC_b}{dt} = k_u C_w - k_e C_b(t), \tag{3.1}$$

where C_w is the chemical concentration in the aquatic ecosystem (mg L^{-1}), C_b is the chemical concentration in aquatic organism (μg g^{-1}), k_u is the uptake rate constant (mL g^{-1} d^{-1}), k_e is the elimination rate constant (d^{-1}), and t is the exposure time (d), respectively.

After the aquatic organism are transferred to clean water, the elimination rate constant can be estimated from the slope of linear regression of log–transformed chemical concentration of tissue in aquatic organism on the elimination phase,

$$\frac{dC_b}{dt} = -k_e C_b(t). \tag{3.2}$$

When the steady–state chemical concentration of tissue in the aquatic organism approached ($t \to \infty$), Eq. (3.1) can be reduced as,

$$C_b = \frac{k_u}{k_e} \cdot C_w. \tag{3.3}$$

Under the steady–state condition, the bioconcentration factor (BCF) of aquatic organism and aquatic ecosystem can be calculated from the ratio of the chemical concentration, or the ratio of the uptake rate constant to that the elimination rate constant,

$$\text{BCF} = \frac{k_u}{k_e} = \frac{C_b}{C_w}. \tag{3.4}$$

BCF can be used to quantify chemical accumulation in the tissue of aquatic organism relative to the chemical concentration in the aquatic ecosystem (USEPA, 2003; Luoma and Rainbow, 2005; Fairbrother et al., 2007).

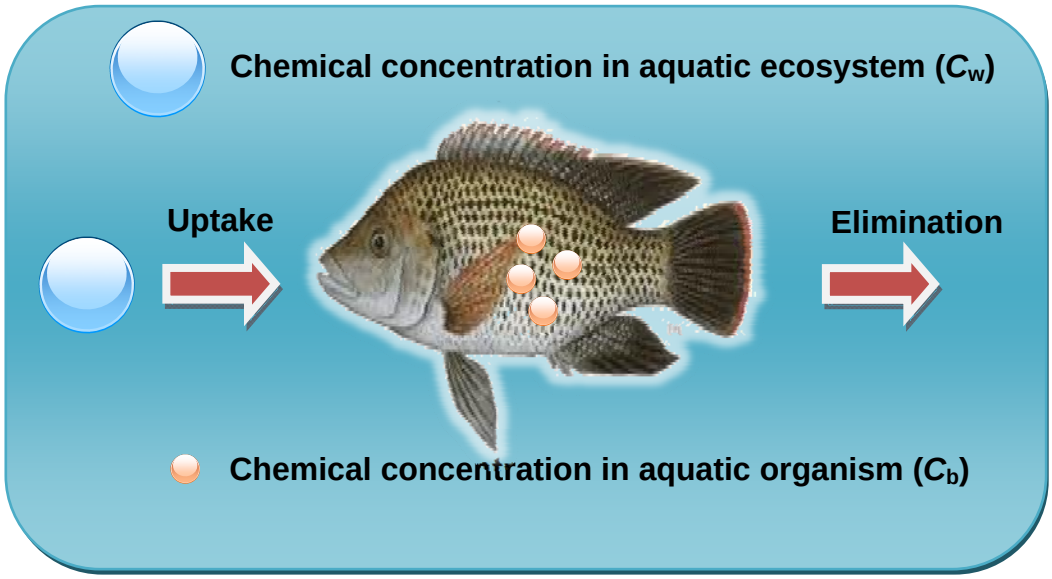


Figure 3.4. Schematic presentation of one–compartment toxicokinetic model.

3.3.2. Toxicodynamic model

Toxicodynamic model is defined as the toxic processes that the quantitative relationship between the chemical concentration in target sites and the magnitude of the toxic effects for aquatic organisms (Rozman and Doull, 2000; Heinrich-Hirsch, 2001). Toxicodynamic model describes the time-course of chemical action in the target site of the aquatic organism, subsequent physiological impairment that affect the compensatory mechanism, and finally the toxic effect will be emerged at the hazard level of the organism, such as mortality. That could be understood to include all physiological mechanisms through which the chemical concentrations in the blood, plasma, or some tissue cause the increasing intensity of chemical effects (Fig. 3.5).

The concentration-response interaction could be represented as the particular affinity between chemical substance and molecules site of action (i.e., biological receptor). In the previous studies, the characterization of dose–response relationship could be expressed as types of linear and nonlinear models (Bellissant et al., 1998). Generally, the sigmoid E_{max} model is commonly used in toxicodynamic model.

Sigmoid E_{max} model is also referred to as the Hill equation model which was proposed to describe the interaction of the dissociation of oxyhemoglobin (Hill, 1910) (Fig. 3.6),

$$E = E_0 + \frac{E_{max} \cdot C^n}{EC50^n + C^n}, \tag{3.5}$$

where E_{max} is the maximum effect, $EC50$ is the chemical concentration that causes the half of maximum effect, and n is the slope factor or is referred to as the Hill coefficient which is a measure of cooperactivity. If $n > 1$ represents positive cooperativity, the relationship is outstanding sigmoid. If $n = 1$, the mode is hyperbolic

and could be expressed another nonlinear equation.

The Hill equation model has been widely applied in the biochemistry, physiology, and pharmacology to investigate the interaction between biological receptor and chemical molecular.

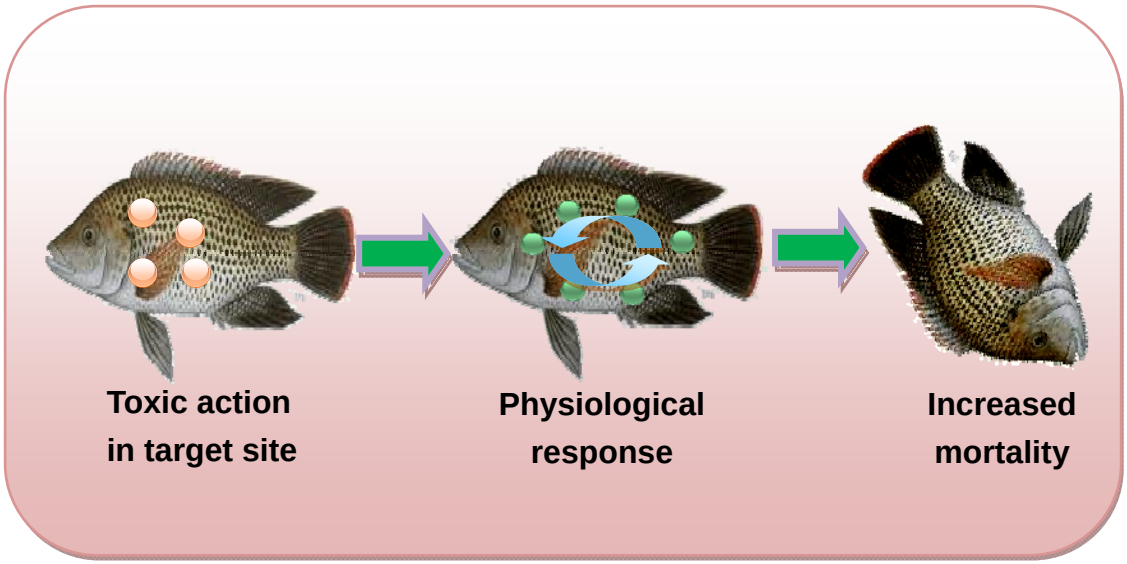


Figure 3.5. Schematic presentation of toxicodynamic model.

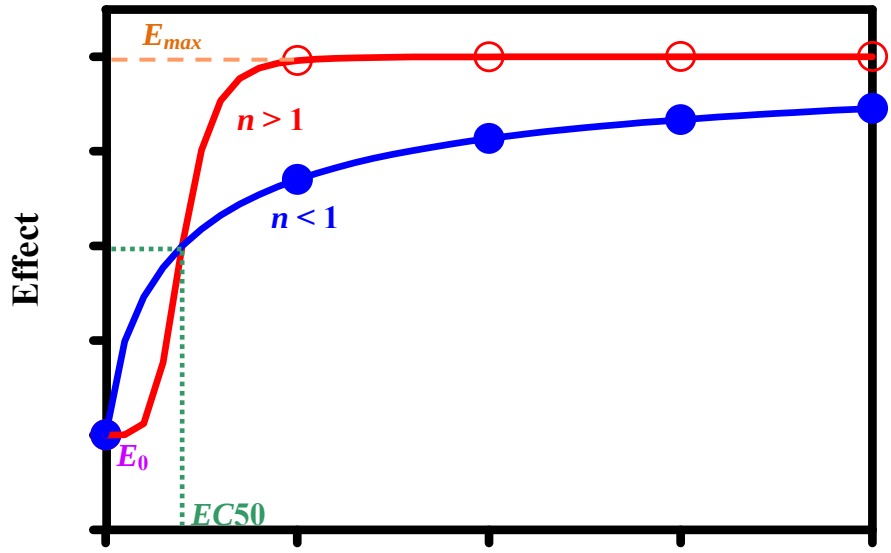


Figure 3.6. Hill-based concentration–effect model.

3.3.3. Biotic ligand model

The biotic ligand model (BLM) is a mechanistic model for considering metal bioavailability that has been developed to quantify water chemistry affecting the speciation and bioavailability of chemical in aquatic systems (Paquin et al., 2002; Niyogi and Wood, 2004; Schwartz and Vigneault, 2007). There is much qualitative evidence indicating that the total metal concentrations are not good predictors of metal bioavailability (Campbell, 1995; Janssen et al., 2003). Specifically, the metal speciation will greatly affect the availability or the bioavailable fraction of chemical to aquatic organisms. The theory of BLM evolved from free ion activity model (FIAM) (Morel, 1983; Campbell, 1995; Brown and Markich, 2000) and gill surface interaction model (GSIM) (Pagenkopf, 1983).

The FIAM concepts describe the variation of toxic effect levels of metal that could be elucidated on the metal speciation and metal interactions with the aquatic organisms. The mechanisms not only take into account the competition among metal ion species and other cations but also consider the binding of free metal ion and metal complexes to the target cellular sites. To induce the biological effects, the metal ion (M) or metal complex (ML) in the external aqueous must be reacted with sensitive site on the biological cellular ligand (BL) of the aquatic organism (Campbell, 1995; Brown and Markich, 2000; Slaveykova and Wilkinson, 2005),

$$M^{+} + L^{-} \xleftrightarrow{K_{ML}} ML, \tag{3.6}$$

$$[ML] = K_{ML} \cdot [M^{+}] \cdot [L^{-}], \tag{3.7}$$

$$M^{+} + BL^{-} \xleftrightarrow{K_{MBL}} MBL, \tag{3.8}$$

$$\{MBL\} = K_{MBL} \cdot [M^{+}] \cdot \{BL^{-}\}, \tag{3.9}$$

where [ ] and { } represent the dissolved ion concentration and free ion activity

concentrations, respectively. The K_{ML} and K_{MBL} are the stability constants for the binding of metal to the ligand and the biotic ligand.

The FIAM indicated that the free metal ions activity is correlated to the toxic effects in aquatic organisms. However, there were still not practically in used and the effects of metal complexation by organic matter were also neglected (Morel, 1983; Campbell, 1995; Paquin et al., 2002).

The framework of GSIM indicates that pH and alkalinity affect the metal speciation and shows that metal toxicity and availability of fish decreased with increasing hardness and protective cations (Ca^{2+}, Mg^{2+}) by competition between cationic metals. Besides, the decreasing toxicity levels include the cations binding at the physiologically active gill surface sites since gill membranes could provide the surface capacities of the negative charged proteins to complexes with metal ions and hydrogen ions that within the aquatic organism is associated with acute toxicity (Pagenkopf, 1983).

The GSIM hypothesizes that the respiratory impairment as the criterion of acute toxicity of all metal to fish and the gill surface are capable of forming complexes with metal species and hydrogen ion in the test aquatic environment. Previous studies indicated that acute Cu toxicity had specific inhibitory effects on ion transport functions in fish gill (Wood, 2001; van Heerden et al., 2004). Specifically, Cu toxicity blocked active Na^+ and Cl^- uptake and transportation in the gills of fish (Laurén and McDonald, 1987; Janes and Playle, 1995). Briefly, the GSIM takes into account the metal binding at target site, stability constant values (binding affinities of free metal

and cation and interacting at site of toxic action on the biotic ligand), and the physiological response to the toxicity.

Fig. 3.7 demonstrates BLM framework based on FIAM and GSIM mechanisms. The toxic effects of metals to aquatic organism depend on water chemical characteristics (such as inorganic composition, organic composition, cation and metal) and physiological mechanism (i.e. acting on the site of action). BLM involves several processes: (i) the metal must first be complexed with organic, inorganic matters and negative biotic ligands inertly during the transport in water that decreases the metal activity, (ii) metal and cation $\{Ca^{2+}, Mg^{2+}, Na^{+}, H^{+}\}$ take together to diffuse to the surface of the aquatic organism with competition interaction that cause diminishing the metal binding to the negative biotic ligand and hence decrease the toxicity, and (iii) the anions (OH^{-}, CO_3^{2-}) could be complexed with metal and immediately binding to negative biotic ligands of aquatic organism to invoke the toxic effect. The metal toxic process must first react on biological membrane and following transport in internal toxicity receptor.

The water chemical equilibrium computer program "Windermere humic aqueous model (WHAM)" (Tipping, 1994) can be used to determine metal and ions interactions accounting for the degree of effective chemical binding at the site of action to reflect the toxic effect level.

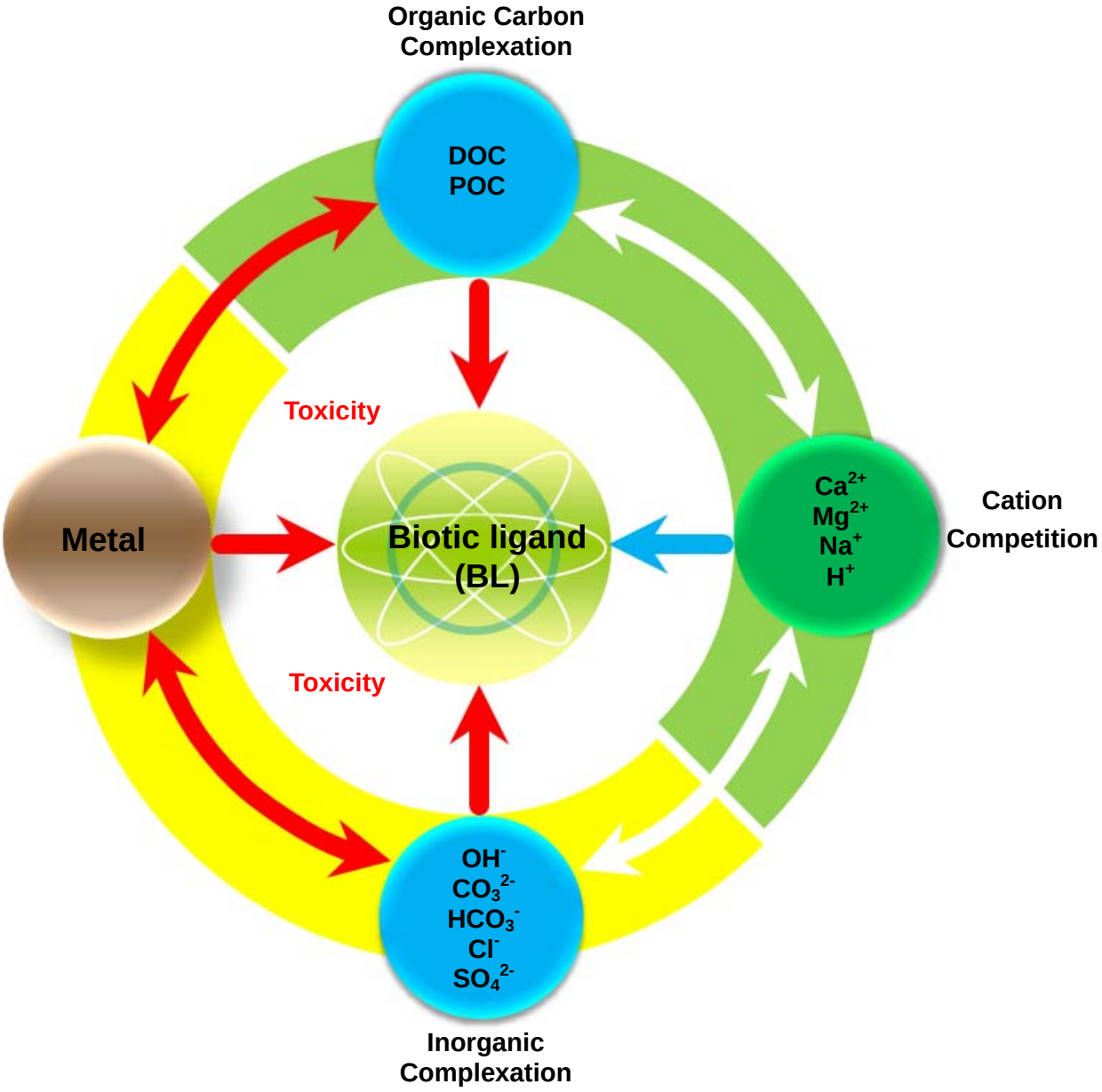


Figure 3.7. Schematic representation of the biotic ligand model for metal bioavailability where DOC: dissolved organic carbon and POC: particular organic carbon.

3.3.4. Threshold damage model

The toxic effect of biological mechanism describes toxic chemical induced-adverse response caused by chemical accumulation within the aquatic organism, indicating that aquatic organism could resist the toxicity invasion and compensates the health. Most methods were used toxicokinetic principle to explore the chemical reactivated the internal adverse effect to aquatic organisms, such as the critical area under the curve and the critical body residue models (Liao et al., 2005; Tsai et al., 2006). There were some unreasonable assumptions that chemicals could reversible binding in the critical burden residue model and irreversible binding in area under the curve model. Besides, Kooijman and Bedaux (1996) proposed the dynamic energy budget theory that could evaluate the effect of chemical and was referred to as the dynamic energy budgets in toxicology (DEBtox) theory. The DEBtox theory assumes that non-detectable effect of aquatic organisms due to the critical internal chemical does not reach the non-effect threshold.

The internal threshold concentration ($C_{i,0}$) links the external threshold concentration ($C_{e,0}$) and toxicokinetics (k_u, k_e), that express as $C_{i,0} = C_{e,0} \cdot k_u / k_e$. Hence, the exceeding internal threshold concentration can kill the tissue and causes the hazard to aquatic organisms. The cumulative hazard function is given by,

$$\frac{dH(t)}{dt} = k_k \left(C_b(t) - C_{i,0} \right), \tag{3.10}$$

where the k_k is the killing rate constant (g μg^{-1} d^{-1}). However, the DEBtox theory does not include the recovery mechanism. It is only related to the toxicokinetics, not to the toxicodynamics. Hence, Lee et al. (2002) proposed damage assessment model (DAM) that could describe the time course of median effect concentration data for chemicals

acting through the reversible interaction between chemicals and receptors. DAM proposes that the hazard occurs based on the irreversible cumulative damage reaching a critical level (Lee et al. 2002).

It could generally illustrate the health state of aquatic organisms with recovery mechanism. The model assumes that chemical accumulates by toxicokinetics of aquatic organisms that could be described by Eq. (3.1). Then the damage accumulation is proportional to the body chemical concentration, and damage recovery is proportional to the cumulative damage (D) that could be expressed as,

$$\frac{dD(t)}{dt} = k_a C_b(t) - k_r D(t), \tag{3.11}$$

where k_a is the damage accumulation rate (g μg^{-1} d^{-1}), k_r is the damage recovery rate constant (d^{-1}). Final, the cumulative hazard is proportional to the cumulative damage,

$$H(t) = k_3 D(t), \tag{3.12}$$

where k_3 is the proportion between damage (D) and hazard (H).

However, there is no critical internal threshold concept in the cumulative hazard function. Ashauer et al. (2007a, b, c) integrated DEBtox theory and DAM to overcome this problem. The refined model was referred to as the threshold damage model (TDM) that have been confirmed to be most suitable mechanism to simulate the survival of aquatic organism after fluctuating and sequential pulsed exposure to chemicals (Ashauer et al., 2007a, b, c, 2010). The TDM includes toxickinetics, reversible-irreversible recovery, toxicodynamics, and critical damage threshold concepts.

3.3.5. West growth model

The ecophysiology of aquatic organism has been widely studied and monitored. Many aquatic organisms such as algae, invertebrates, mussel, and fish are commonly used as bioindicators since their sensitive, the advantage that changes in their behaviors, e.g., swimming, feeding, growth, reproduction, and avoidance response pattern can be measured immediately as responses to the occurrence of contaminants. This behavior could be used as ecophysiological activity parameters for providing ecological relevance to standard toxicity testing, that is suitable used in online biomonitors (van der Schalie et al., 2001; Gerhardt et al., 2005; 2006). Fishes and bivalves are commonly preferred for biomonitoring in aquatic ecosystems because of wild distribution, abundant, and tolerant to various environmental conditions (Zhou et al., 2008). The body biomass measurement was commonly preferred biomonitoring endpoint of fish for the evaluation of metal in aquatic system. The ecophysiological models of the growth for aquatic organism exposed to contaminant stress had been well developed.

Previous studies have been investigated the allometric scaling relationship between the forms and functions of organism (McMahon, 1973; Feldman and McMahon; 1983; West et al., 1997; West and Brown, 2004). The hypothesized mechanism to explain the pattern of body biomass is based on the allocation of metabolic energy rate between body biomass of maintenance and production (West et al., 1997). Many studies have affirmed that an excess of energy acquisition over maintenance energy requirement, which the relationship between the metabolic rate and body size is the exponent 3/4 (Feldman and McMahon; 1983; Savage et al., 2004; West and Brown, 2004), whereas some have argued that it is 2/3 based on a simple

surface area to volume ratio contention (Dodds, et al., 2001; White and Seymour et al., 2003).

West et al. (2001) developed a general model of ontogenetic growth to describe the growth trajectories for organisms, which allometric function of body biomass was well-described by 3/4 power law based on the properties of optimized energy distribution networks from mammals, birds, aquatic organism, and plants. The relationship between metabolic rate and body biomass is characterized by the form,

$$m = m_0 \cdot W^{3/4}, \tag{3.12}$$

where m is the average resting metabolic rate of the whole organism (J d^{-1}), m_0 is the taxon-specific constant (–), and W is the body biomass (g).

When growth for processing, the energy allocation between maintenance of tissue and reproduction of new body biomass could be expressed as,

$$m = Nm_c + E_{c0}\frac{dN}{dt}, \tag{3.13}$$

where N is the total number of cell, m_c is the metabolic rate of a single cell (J d^{-1}), E_{c0} is the metabolic energy required to create a new cell (J), and t is the time (d). Nm_c in Eq.(3.13) indicates the metabolic energy needed to support all of activities in organism, whereas $E_{c0}\frac{dN}{dt}$ indicates energy production to create new cells and further to growth.

The growth of the total body biomass $W=NW_c$ at any time t, where W_c is the biomass of a cell. Hence Eqs. (3.12) and (3.13) could be integrated to rewrite as,

$$\frac{\mathrm{d}W}{\mathrm{d}t} = \frac{W_c}{E_{c0}} m_0 W^{3/4} - \frac{m_c}{E_{c0}} W \,, \tag{3.14}$$

where $A_0 \equiv W_c m_0 / E_{c0}$ ($g^{1/4}$ d^{-1}) and $b \equiv m_c / E_{c0}$ (d^{-1}) in that A_0 represents the biological species-specific growth coefficient the rate of energy allocation to create a cell, and b is the ratio of the metabolic energy required to maintain a cell relative to the energy allocation to create a cell. There are fundamental explanations for the growth trajectory of organisms which are close to an asymptotic maximum body biomass without contaminant exposure, $W_{\max 0}$. Thus A_0 and b are directly related to the growth size, and are calculated from fundamental cellular parameters.

The energy metabolic allocation is not balance between supply and demand to ultimately limited growth. Hence, this model hypothesized that the maximum body biomass occurs when $\mathrm{d}W/\mathrm{d}t = 0$, giving $W_{\max 0} = (A_0/b)^4$, whereas $b = A_0 / W_{\max 0}^{1/4}$. Hence Eq. (3.14) could be rewritten as

$$\frac{\mathrm{d}W}{\mathrm{d}t} = A_0 W^{3/4} \left[1 - \left(\frac{W}{W_{\max 0}} \right)^{1/4} \right], \tag{3.15}$$

the solution of Eq. (3.15) has the form as

$$W(t) = W_{\max 0} \left\{ 1 - \left[1 - \left(\frac{W_0}{W_{\max 0}} \right)^{1/4} \right] e^{-A_0 t / 4 W_{\max 0}^{1/4}} \right\}^4 , \tag{3.16}$$

where W_0 is the body biomass at birth (t = 0) (g). West growth model could well predict the growth biomass in nature.

Tsai and Liao (2006) proposed the integrated West growth model with growth stress of metal toxicity function. The mechanisms based on three intrinsic mode of action that distinguished as: (1) increasing the cost of growth energy, (2) increasing

the cost of maintenance energy, and (3) decreasing feeding. The stress function describing the adverse effect of growth was related to the accumulated chemical concentration and the threshold of internal effect concentration. In order to create a new cell, the organism should switch a great deal of the metabolic energies (E_c) when exposed to chemical stresses.

The cost of growth energy is to increase the metabolic energy (E_c) that can be expressed as $E_c = E_{c0}(1+\text{stress})$. Hence, the species–specific growth coefficient has the form as $A = W_c m_0 E_{c0}(1+\text{stress})^{-1} = A_0(1+\text{stress})^{-1}$. In view of maintenance energy, the organisms increase the cost of maintenance energy to fightback the chemical stress rather than increasing that of growth energy. Previous studies have suggested that multiplied biomass by (1+stress) could account for the cost of maintenance energy, obtaining the reduction of time-dependent biomass of $W(t)(1+\text{stress})$ (Kooijmand and Bedaux, 1996; Tsai and Liao, 2006). On the other hand, when feeding is decreasing, the growth energy income is decreasing, and this will act on growth biomass reduction. Hence, the feeding rate can arrest the maximum biomass (W_{max}) that could defined as $W_{max} = W_{max0}(1+\text{stress})$. Incorporating the above mentioned mode of actions into West growth model could describe the chemical effect on coefficient of growth cost, time-dependent biomass, and ultimate biomass of organism.

CHAPTER 4. MATERIALS AND METHODS

4.1. Pulsed Cu Exposure Experiments

4.1.1. Acute accumulation exposure bioassay

This present laboratory study was to conduct the sequential pulsed Cu experiments to examine the accumulation ability of two-week old larva, one-month old juvenile, and eight-month old mature adult tilapia *O. mossambicus* (Fig. 4.1) with mean length 1.01±0.24 cm (Mean ± SD), 2.03±0.38 cm, and 12 ± 1.44 cm and mean body biomass 8.24±1.58 mg wet wt, 31.24±35.22 mg wet wt, and 26.88±9.29 g wet wt, respectively. Fish were cultured in the Graduate Institute of Ecology and Evolutionary Biology, China Medical University (Taichung, Taiwan). The tilapia were acclimatized for 14 days in the following conditions: water temperature 28 °C, pH 7.8, 12 hours light cycle, DO = 7.5 mg L^{-1}, Ca^{2+} = 59.60 mg L^{-1}, Mg^{2+} = 13.17 mg L^{-1}, Na^{+} = 9.40 mg L^{-1}, and K^{+} = 2.73 mg L^{-1} before they exposed to Cu.

During the acclimation period from February 1st to 14th, 2010, the fish were fed twice per day with commercial fish food. The exposure experiment was carried out with 42 adult fish under static conditions in three aquariums of 81 L (60×30×45 cm^3) volume filled with 70.2 L of exposure solution. Each aquarium contained a stock density of fourteen adult fish. Forty-two larvae and forty-two juveniles were respectively cultivated for one and two aquariums (aquaria measuring 27×21×21 cm^3), containing 9 L of water in static conditions.

The copper sulfate ($CuSO_4 \cdot 5H_2O$) stock solution was prepared with deionized water. The sequential pulsed Cu exposure bioassay was carried out with 10-day exposure periods exposed to 100 μg L^{-1} background exposure concentration and 300

μg L^{-1} pulsed exposure concentration. The sequential pulsed Cu exposure design were accomplished by siphoning the volume of Cu contamination water in the test aquarium from *X* L to 1/3*X* L, and fill water to *X* L in the test aquarium. Later, after water was siphoned from *X* L to 1/3 *X* L, Cu-amended water was added and filled to *X* L in the test aquarium in order to increase the fluctuating/pulses concentration and vice verse.

The pulsed Cu exposure timing were occurred twice time during the exposure periods that was days 1 and 6, respectively. The pulsed exposure duration was carried out 6 hours in each event. For example, to adult exposure bioassays, the sequential pulsed Cu exposure design was accomplished by siphoning the volume of Cu contamination water in the test aquarium from 70.2 L (60×30×39 cm^3) to 23.4 L (60×30×13 cm^3), and filled water to 70.2 L in the test aquarium. Later, after water was siphoned from 70.2 L to 23.4 L, Cu-amended water was added to 70.2 L in the test aquarium to increase the pulses concentration and vice versa (Fig. 4.2).

The entire Cu solution was replaced and collected daily to avoid the regression of water quality and removed feces every 6 hours and collected forage debris after feeding 1 hour in the aquarium. Three fish were sequentially removed from each experimental tank on days 0, 1, 1.25, 2, 3, 6, 6.25, 7, 8, and 10 of exposure. The tilapia was anesthetized with Benzocaine hydrochloride solution during the sampling. The dissected tissues samples were cleaned with double–deionized water (ddH_2O) and freeze dried overnight.

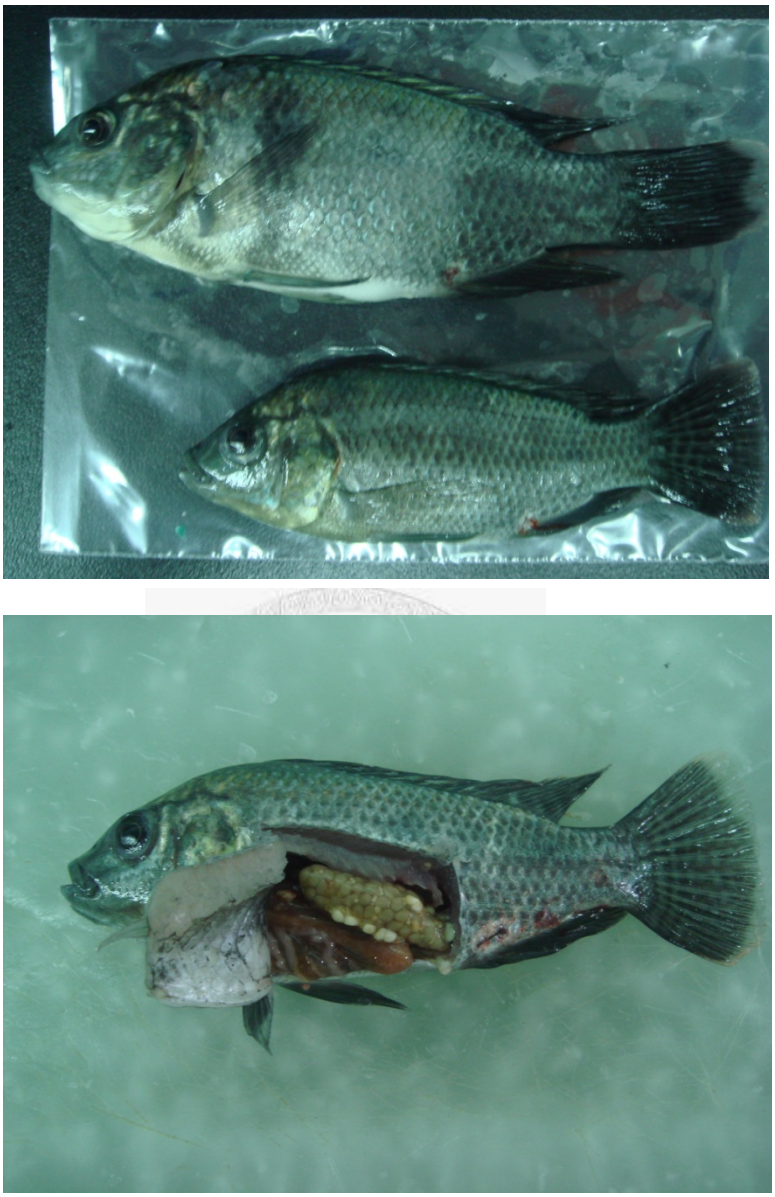


Figure 4.1. Fish samples used in the Cu-tilapia pulsed exposure bioassay.

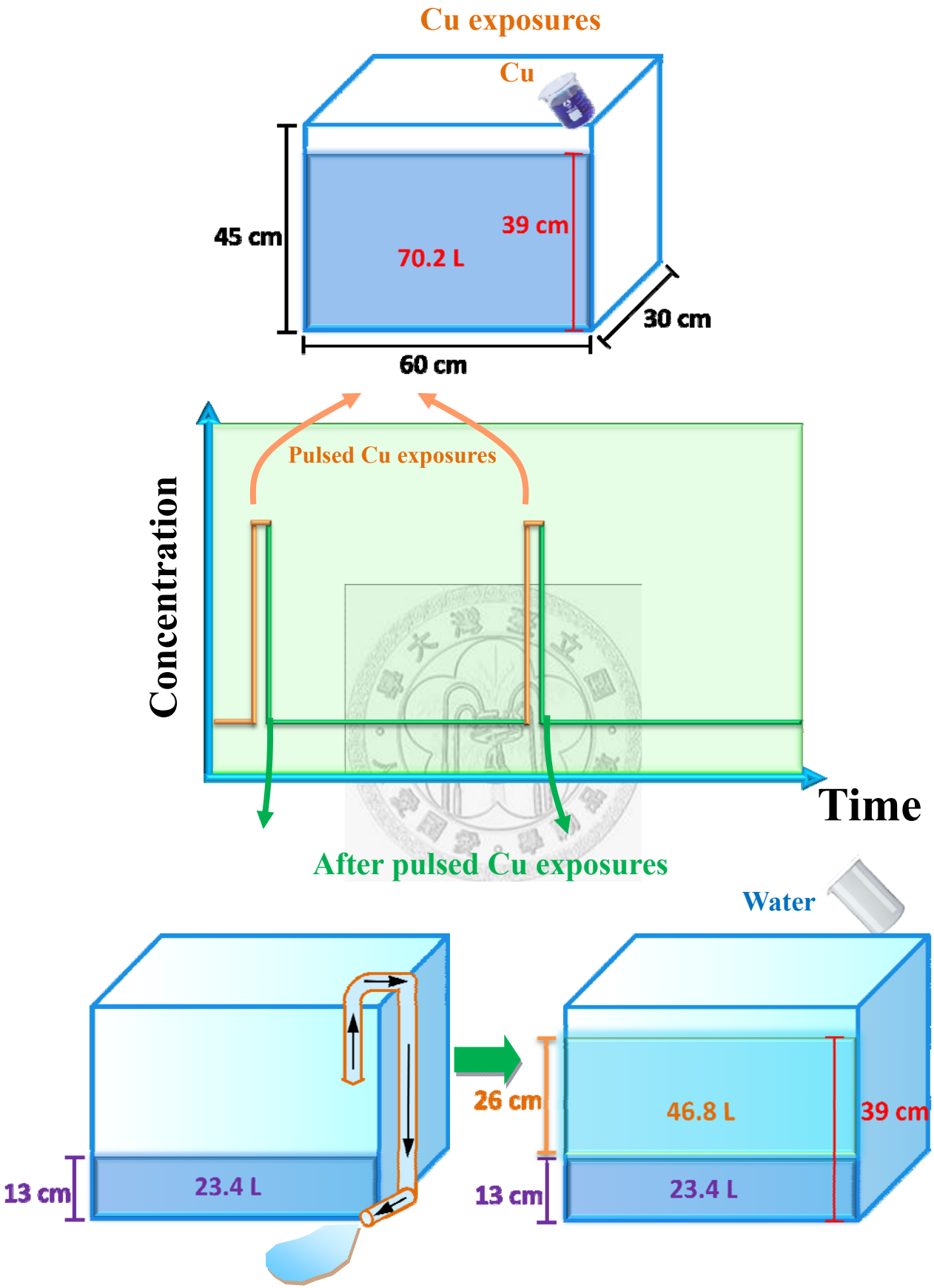


Figure 4.2. Changes of Cu concentration to achieve the sequential pulsed exposure.

4.1.2. Chronic accumulation exposure bioassay

A sequential pulsed chronic Cu exposure experiments were conducted to examine the accumulation ability of 1-month old juvenile and 8-month old mature adult tilapia with mean length 2.43 ± 0.45 cm (Mean ± SD) and 10.09 ± 1.69 cm and mean body biomass 0.25 ± 0.15 g wet wt and 17.02 ± 9.80 g wet wt, respectively. Tilapia were acclimatized for 14 days in the following conditions: water temperature 28 °C, pH 7.8, 12 hours light cycle, and DO = 7.5 mg L^{-1}. The water chemistry characteristics were Ca^{2+} = 66.60 mg L^{-1}, Mg^{2+} = 13.60 mg L^{-1}, Na^{+} = 10.30 mg L^{-1}, and K^{+} = 3.30 mg L^{-1} for juvenile and Ca^{2+} = 59.60 mg L^{-1}, Mg^{2+} = 13.17 mg L^{-1}, Na^{+} = 9.40 mg L^{-1}, and K^{+} = 2.73 mg L^{-1} for adult.

During the acclimation period from August 16th to September 13th, 2010, the fish were fed twice per day with commercial fish food. The exposure experiment was carried out with 52 juvenile fish and 45 adult fish respectively under static conditions in aquaria. The juvenile fish aquarium of 11.9 L (27×21×21 cm^3) volume was filled with 9 L of exposure solution, and the adult fish aquarium of 81 L (60×30×45 cm^3) volume filled with 70.2 L of exposure solution. The aquarium was containing a stock density of 26 juvenile fish, whereas 15 fish for adult tilapia per aquarium.

The copper sulfate ($CuSO_4$•$5H_2O$) stock solution was prepared with deionized water. The sequential pulsed Cu exposure bioassay was carried out with 28-day exposure periods exposed to 30 and 100 μg L^{-1} background exposure concentrations and 100 and 300 μg L^{-1} pulsed exposure concentrations to juveniles and adults, respectively. The sequential pulsed Cu exposure design was the same as the pulsed Cu acute accumulation exposure bioassay (Fig. 4.2). The pulsed Cu exposure timing

was occurred twice during the exposure periods of days 0.5 and 25, respectively. The pulsed exposure duration was carried out 1 day in each event.

The entire Cu solution was replaced and collected daily to avoid the regression of water quality and removed feces every 6 hours and collected forage debris 1 hour after feeding. Three fish were removed from experimental tanks on days 0, 0.5, 1.5, 4, 7, 11, 14, 18, 21, 25, 26, and 28 of exposure. The tilapia was anesthetized with Benzocaine hydrochloride solution during the sampling. The dissected tissues samples were cleaned with double–deionized water (ddH_2O) and freeze dried overnight.

4.1.3. Chronic growth bioassay

The chronic growth treatments on the growth of adult fish were performed with 10 fish to control and pulsed Cu exposures, respectively. Ages of 8 – 9 months old mature adult tilapia with the mean body length 9.2 ± 0.57 cm and mean body weight 13.29 ± 1.83 g wet wt. Tilapia were acclimatized for 14 days before the Cu exposure, and water conditions were as follows: water temperature 28 °C, pH 7.8, 12 hours light cycle, and DO = 7.5 mg L^{-1}. The water chemistry characteristics were Ca^{2+} = 59.60 mg L^{-1}, Mg^{2+} = 13.17 mg L^{-1}, Na^{+} = 9.40 mg L^{-1}, and K^{+} = 2.73 mg L^{-1} for adult, respectively.

The control and exposure treatments were with two replicated tanks for 28 days. The pulsed Cu exposure protocol was the same as the chronic accumulation exposure bioassay. During the exposure period, the tilapia in the both treatments were fed twice per day with fish food at a rate of 4% tilapia body biomass, and the water was renewed twice per day after feeding. The Cu concentrations were monitored every day before feeding to make sure the water quality. In each exposure tank, the mean body biomass and body length of fish were measured at days 0, 0.5, 1.5, 4, 7, 11, 14, 18, 21, 25, 26, and 28.

The daily growth rate (k_g, % d^{-1}) of tilapia was calculated as (Allen et al., 2006),

$$k_g = \ln\left(\frac{W_t}{W_i}\right)/t \times 100, \qquad (4.1)$$

where W_i and W_t (g wet wt) are the fresh body biomass of tilapia at the beginning and at time t (day), respectively. The pulsed Cu exposure induced growth inhibition study was to compare the daily growth rate with and without pulsed Cu exposure to

investigate the safe growth probability that can be calculated by,

$$S = 1 - \left(\frac{k_{g,0} - k_{g,m}}{k_{g,0}} \right), \tag{4.2}$$

where S is the safe growth probability (–) and $k_{g,0}$ and $k_{g,m}$ are the daily growth rate of body biomass of tilapia without pulsed Cu exposure and with pulsed Cu exposure concentration, respectively.

Since the growth toxicity is not followed by an irreversible mechanism for ecophysiology of aquatic organisms, the growth toxicity induced adverse effect was situated at the damage level. Hence, the time-dependent safe growth probability can be expressed as the exponential of time-dependent damage level,

$$S(t) = e^{-D(t)}, \tag{4.3}$$

where the damage level is analogous to threshold damage model-based damage mechanism.

4.1.4. Chemical analysis

The flame atomic absorption spectrometer (Perkin Elmer AA-200, USA) was used to analyze Cu of fish tissues. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated standard reference material (Merck, Darmstadt, Germany). Fish tissues were digested with 2 mL 65% HNO_3 and 1 mL 30% H_2O_2 overnight at 95°C. The 20 mL fish samples were stored at -4°C in the dark until they are analyzed. The 15 mL water sample with 65% HNO_3 was digested for 2 – 3 hours at 95°C, then the water characterizations were analyzed by Inductively Coupled Plasma Mass Spectrometer (Perkin – Elmer ELAN DRC ROMAN II, USA).

4.2. Data Reanalyses

4.2.1. As–tilapia system

4.2.1.1. Exposure data

The valuable bioaccumulation dataset of tilapia exposed to As was provided by Tsai and Liao (2006). Tsai and Liao (2006) carried out the exposure treatment by using forty-two mature adult tilapia (mean body length = 13.9 ± 1.54 cm and mean body biomass =16.8 ± 5.2 g) exposed to 1 mg L^{-1} As (as $NaAsO_2$) concentration for 7 days uptake phase. The tilapia were acclimated in aquaria provided with the Taipei tap water at 27.7 °C ± 0.24°C and pH 7.75 ±0.02 during a light–dark cycle of 16:8 hrs. The tested fish were without feeding during the exposure periods. Three fish were sequentially removed from each tank to determine As accumulation after 0, 1, 2, 4, and 7days of exposure. The bioaccumulation data of tilapia are shown in the Fig. 4.3.

First-order bioaccumulation model can be used to predict the organism body burden followed the exposure to As concentrations. Uptake and elimination rate constants were determined by fitting the following integrated form of the kinetic rate equation to bioaccumulation data for constant arsenic exposure,

$$C_b(t) = C_b(t=0)e^{-k_e t} + \frac{k_u}{k_e} C_w(1 - e^{-k_e t}), \quad (4.4)$$

where $C_b(t = 0)$ is initial dependent concentration of As in aquatic organism tissue (μg g^{-1} dry wt), k_u is the organism uptake rate constant (mL g^{-1} d^{-1}), k_e is the elimination rate constant (d^{-1}), and C_w is the As concentration in the tank (mg L^{-1}).

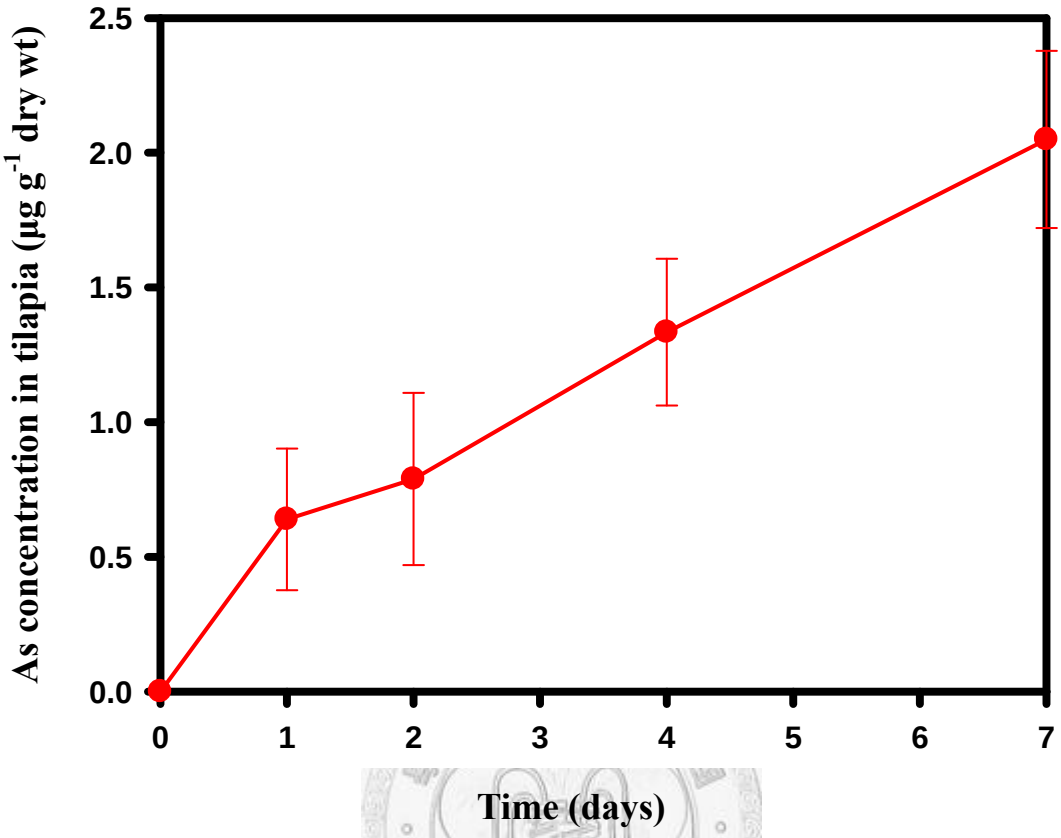


Figure 4.3. As accumulation concentration by tilapia exposed to 1 mg L^{-1} waterborne As for 7 days. Errors bars are standard deviation from mean.

4.2.1.2. Chronic toxicity data

A valuable chronic toxicity dataset of tilapia exposed to As was adopted from Tsai and Liao (2006). The chronic toxicity bioassay was carried out 28 days to assess the correlations between As exposure concentration and bioenergetically growth inhibition. Mature adult tilapia (ages 8 – 9 months) with a mean body biomass of 10.58 ± 1.52 g wet wt and mean body length 12.9 ± 1.54 cm were collected from Taiwan Fisheries Research Institute (Tainan, Taiwan). Fish were acclimated in aquaria provided with Taipei tap water at 27.7 °C ± 0.24°C and pH 7.6 ± 0.2 during a light–dark cycle of 12:12 hrs. The concentrations of As (as $NaAsO_2$) in the tanks were 0, 1, 2, and 4 mg L^{-1}, respectively. Each fish were fed twice per day with commercial fish food (4% of the total fish wet biomass per day). The mean body biomass of each exposed fish was recorded in the exposure tanks weekly. The growth biomass curve of tilapia exposed to 0, 1, 2, and 4 mg L^{-1} As concentrations were shown in Fig. 4.4.

The growth curve of tilapia exposed to As were reanalyzed to construct relationship between concentration and growth inhibition response. The growth rate (G) is estimated as follow,

$$G = \left(\frac{W_{\mathrm{t}} - W_i}{W_i} \right) \times 100, \tag{4.5}$$

where W_i and W_{t} are the fresh body biomass of tilapia at the beginning and at time t in specific As exposure concentration, respectively. The growth inhibition response (GI) is estimated as follow,

$$GI = \left(\frac{G_0 - G_{\mathrm{m}}}{G_0} \right) \times 100, \tag{4.6}$$

where G_0 and G_{m} are the growth rate of body biomass of tilapia without As exposure

and with As exposure, respectively. Hence, the growth inhibition curve with specific time could give the process to determine the median effect concentration (EC50(t)) for tilapia.

By fitting employed Hill based concentration–response function to As concentration-growth inhibition response data, the growth inhibition of tilapia in response to waterborne As can be obtained,

$$E(t, C_w) = \left(\frac{E_{\max} \cdot C_w^{n(t)}}{\mathrm{EC50}(t)^n + C_w^{n(t)}} \right), \tag{4.7}$$

where $E(t, C_w)$ is the time-dependent effect (% response) based on As concentration (mg L^{-1}) at any given time t, $E_{\max}$ is the effect time-specific maximum effect (%), and $n(t)$ is a time-dependent Hill coefficient which is a measure of cooperativity.

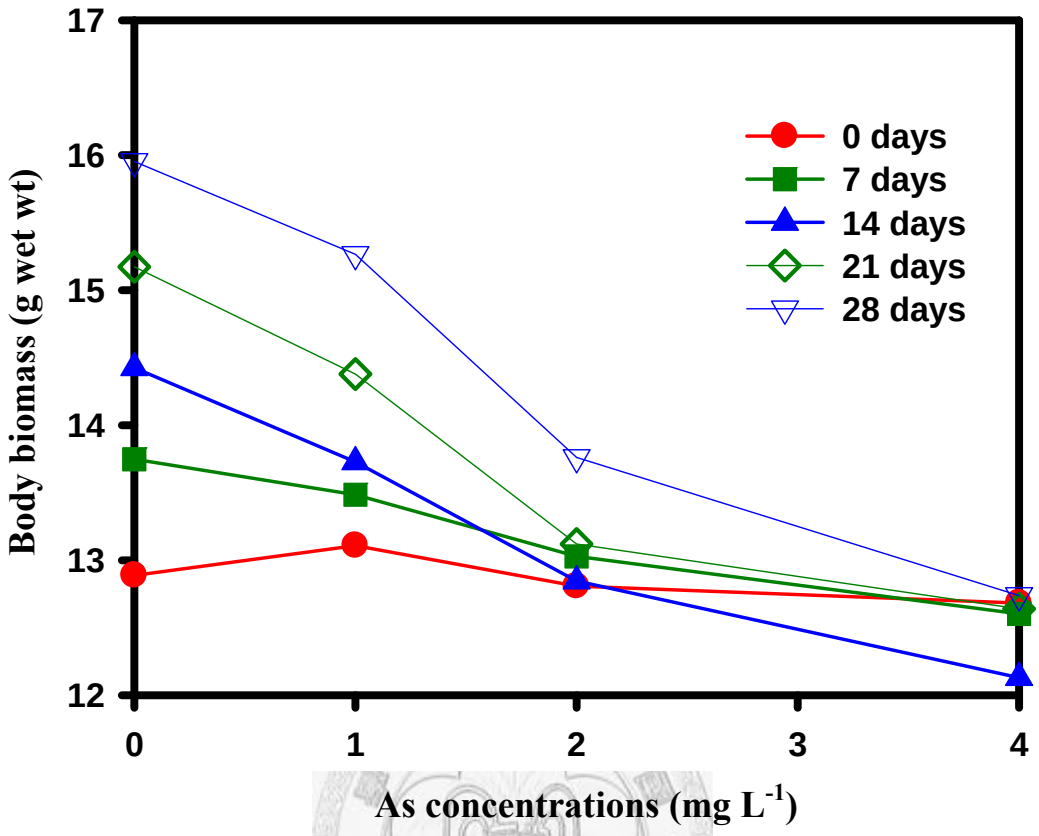


Figure 4.4. Growth biomass data of tilapia exposed to 0, 1, 2, and 4 mg L^{-1} As concentrations during the 28 days.

4.3. Model Development

Waterborne toxic metals rely on passive diffusion from ambient water that could enter aquatic organisms via water and accumulate at tissues control by multitudinous water chemical and physiological elements, such as water ions, ingestion behaviors, and copying mechanism (Rainbow, 2002; Nichols et al., 2006; Pan and Wang, 2008). This study links water chemical and physiological mechanisms to develop the systems-level mechanistic model. The proposed model can describe the toxic chemical induced-adverse response caused by their accumulation within the tilapia, indicating they could resist the toxicity invasion and compensate the health, such as the bioenergetics.

4.3.1. Modeling sequential pulsed and fluctuating exposure patterns

The environmental relevance contaminant exposure patterns may induce hazard and influence the recovery mechanism to aquatic organisms. This study developed a simple mathematical model that links these likely existent exposure patterns in aquatic systems. To investigate the impact of time-varying chemical exposure on the dynamic responses of tilapia in greater detail, this study performed various exposure patterns include the sequential pulsed and fluctuating exposures that are the time-varying exposure patterns.

Generally, the sequential pulsed exposure can be described as Cu/As concentrations maintain at a steady-state, but at a specific timing the Cu/As concentration might increase sharply due to a sudden Cu/As loading and then reduce to the original steady-state in the very short times. On the other hand, the fluctuating exposure that is referred to as the repeated exposure of Cu/As concentrations followed a certain pattern such as a sinuous wave pattern in a time-varying fashion. Hence, this study used Dirac delta function to describe the sequential pulsed exposure patterns and used sine wave to describe the fluctuating exposure patterns.

Dirac delta function can be written as,

$$C_w(t) = C_0 + C_1 \sum_{n_i} \delta(t - n_i T), \tag{4.8}$$

where δ is Dirac delta function, C_0 and C_1 represent background and pulsed concentrations (mg L^{-1}), n_i is the pulsed frequency of this exposure duration, and T is exposure timing (day). The sequential exposure pattern was shown in Fig. 4.5A.

Sine wave function can be written as,

$$C_w(t) = C_0 + C_1 \sin\left(\frac{2\pi t}{T} + d\right), \tag{4.9}$$

where C_0 and C_1 represent base line contaminant concentration and amplitude concentrations (mg L^{-1}), T is exposure periods (day), and d is the phase that without contaminant exposure. The fluctuating exposure pattern was shown in Fig. 4.5B.

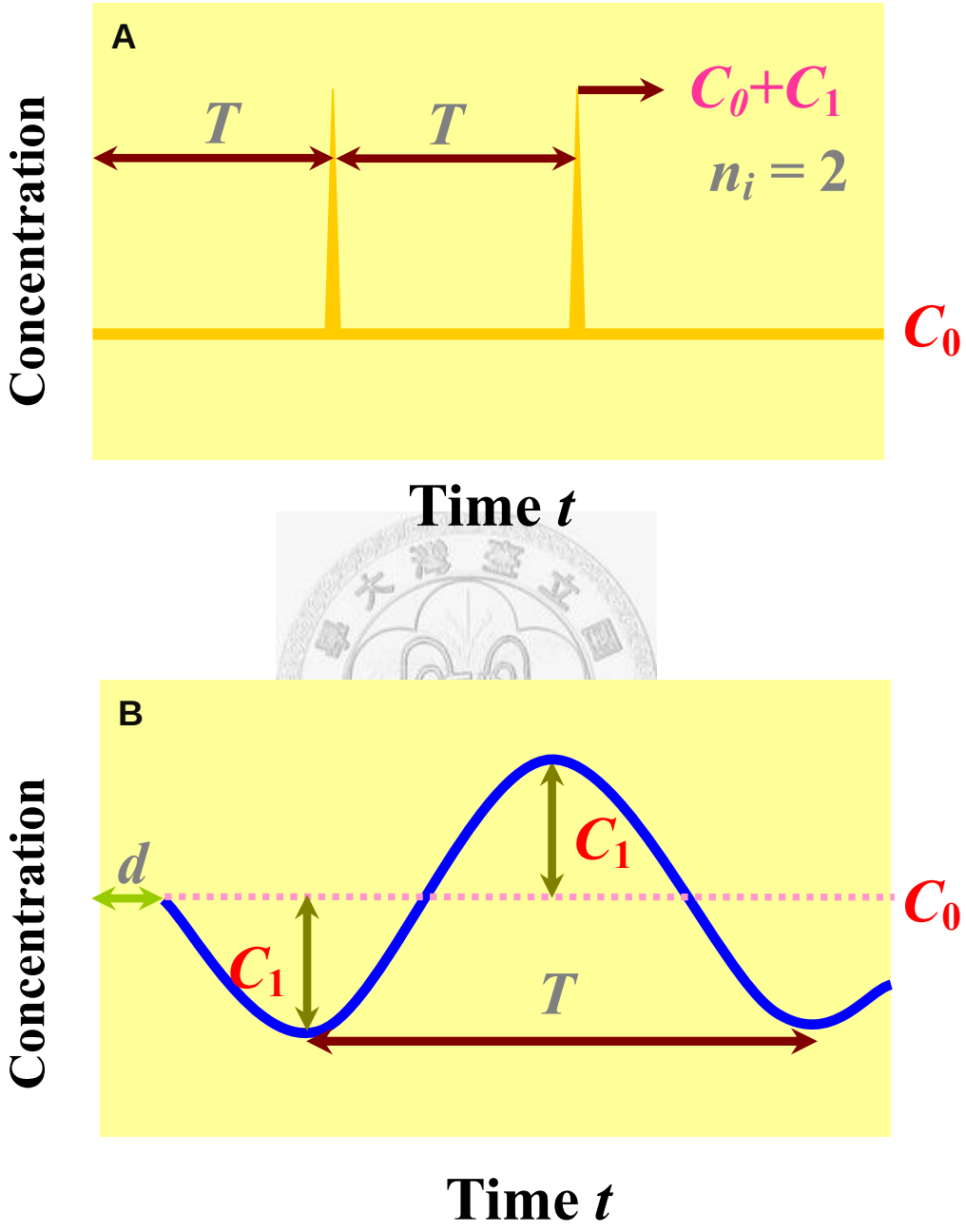


Figure 4.5. Diagram of (A) the sequential pulsed waterborne metal exposure pattern and (B) the fluctuating waterborne metal exposure pattern.

4.3.2. Water chemistry-based toxicokinetic/toxicodynamic model

4.3.2.1. Water chemistry

The sodium arsenite ($NaAsO_2$) stock solution is used in As–tilapia and (Tsai and Liao, 2006). Wang et al. (2007) indicated that the most of arsenicals in fish ponds of tilapia was As(V). Hence this study considers the oxidation and reduction of As, since the bioassays were carried out with oxidation state. The As states can be described as follows,

$$NaAsO_2 \rightarrow Na^+ + AsO_2^- \qquad \text{[As(III)]}$$

With O_2,

$$AsO_2^- + O_2 \rightarrow AsO_4^{3-} \qquad \text{[As(V)]}$$

The effective chemical concentration was calculated with the principles of water chemistry in that, the theoretical expressions was based on Debye-Huckel limiting law. The temperature, pH, measure ion concentration, and ionic strength were employed to calculate the activity coefficient for obtaining the site-specific metallic ionic activity concentration in the aquatic environment. The activity coefficient calculating process can be described as follows (Stumm and Morgan, 1981).

The ionic strength can be written as,

$$I = \frac{1}{2}\sum_{i=1}^{i=i} C_i Z_i^2 , \qquad (4.10)$$

and the activity coefficient can be written as,

$$\gamma = 10^{-AZ^2\sqrt{I}} , \qquad (4.11)$$

with

$$A = 1.82\times10^6 (PT_s)^{-3/2} , \qquad (4.12)$$

where I is the total metal ionic strength (M), C_i is the analytic ion concentration (M) of the each ion, Z_i is the valence charge number of each ion in a solution, and A is a constants depend on the water permittivity constant (P) and solution temperature, T_s (K), where P is 78.3.

4.3.2.2. Threshold damage model

In light of a biologically DAM, the relationships between chemical tissue residue and accumulation-induced organism damage can be described by a first-order damage accumulation model and a first-order bioaccumulation model (Lee et al., 2002). Based on DAM, the damage-based EC50 can be derived as (Lee et al., 2002),

$$\mathrm{EC50}(t) = \frac{D_{\mathrm{E,50}}/k_a}{\left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r}\right)} \mathrm{BCF}^{-1}, \tag{4.13}$$

where k_a is the damage accumulation rate (g μg^{-1} d^{-1}), $D_{E,50}/k_a$ is a coefficient which reflects the compound equivalent toxic damage level required for median effect (μg d g^{-1}), k_e is the elimination rate constant (d^{-1}), and k_r is the damage recovery rate constant (d^{-1}).

Recently, Ashauer et al. (2007a, b, c, 2010) proposed a threshold damage model (TDM) that was a modified version of the DAM scheme. TDM can be employed to predict survival/safety of aquatic organism after exposure to sequential pulsed and fluctuating of chemical concentrations. The time course of body metal accumulation in relation to time–dependent external metal concentration to aquatic organism can be expressed as,

$$\frac{dC_b(t)}{dt} = k_u \cdot C_w(t) - k_e \cdot C_b(t), \tag{4.14}$$

where $C_w(t)$ are the sequential pulsed and fluctuating chemical concentrations in water (mg L^{-1}) and can be expressed as Eqs. (4.8) and (4.9).

The accumulation–induced aquatic organism damage is proportional to body chemical concentration, whereas the damage recovery is proportional to the

cumulative damage that can be expressed as,

$$\frac{dD(t)}{dt} = k_k \cdot C_b(t) - k_r \cdot D(t)\,, \tag{4.15}$$

where k_k is the killing rate constant (μg g^{-1} d^{-1}) that are calculated as ln 2/($D_{E,50}/k_a$), and k_r is the recovery rate constant (d^{-1}) that can be used to calculate the recovery time of the aquatic organism for recover damage.

Damage to hazard should be exceeded a threshold for damage to generate the threshold. The hazard rises above zero, the probability of the injure with hazard at give time can be expressed as,

$$\frac{dH(t)}{t} = D(t) - D_0\,. \tag{4.16}$$

Based on Eq. (4.16), the cumulative hazard, $H(t)$, is proportional to the cumulative damage level. Thus, the time-dependent safety probability can be expressed as the exponential of cumulative hazard as,

$$S(t) = e^{-H(t)} \times S_{\text{control}}(t)\,, \tag{4.17}$$

where $S(t)$ is the safety probability (–) and $S_{\text{control}}(t)$ is the safety probability resulting from the control effect (–).

4.2.2. BLM-based toxicokinetic/toxicdynamic model

This study focused on how water quality characteristics affected the Cu toxicity to aquatic organism that could cause adverse effect. Specifically, the BLM does this in a way to consider the significant concern of site–specific water quality and bioavailability and bioreactivity of Cu to regulate the potential adverse effect. The BLM has been developed to quantify water chemistry affecting the speciation and bioavailability of chemical in aquatic systems. The toxic effect level can determine the degree of Cu binding at the site of action ($f_{\mathrm{CuBL}}^{\mathrm{X\%}}$).

De Schamphelaere and Janssen (2002) have developed the BLM–scheme–based median effect Cu concentration model which has the form as,

$$\mathrm{EC50}(t) = \frac{f_{\mathrm{CuBL}}^{50\%}(t) \cdot \left(1 + \sum K_{\mathrm{ionsBL}} \{ions\}\right)}{\left(1 - f_{\mathrm{CuBL}}^{50\%}(t)\right) \cdot [a]}, \tag{4.18}$$

where $\sum K_{\mathrm{ionsBL}} \{ions\} = K_{\mathrm{CaBL}} \{\mathrm{Ca}^{2+}\} + K_{\mathrm{MgBL}} \{\mathrm{Mg}^{2+}\} + K_{\mathrm{NaBL}} \{\mathrm{Na}^{+}\} + K_{\mathrm{HBL}} \{\mathrm{H}^{+}\}$ in that K_{CaBL}, K_{MgBL}, K_{NaBL}, and K_{HBL} represent the stability constants for the binding of these cations to the BL (M^{-1}), $\{ions\}$ denotes the activity of each ion of water chemistry characteristics (M), $[a] = K_{CuBL+} K_{CuOHBL} \{\mathrm{OH}^{-}\} + K_{CuCO_3BL} K_{CuCO_3} \{\mathrm{CO}_3^{2-}\}$ that represents the formation of the Cu complex with inorganic matter and binding to BL (M^{-1}), and $f_{\mathrm{CuBL}}^{50\%}(t)$ is the response time-dependent fraction of the total number of Cu binding site occupied by Cu at median effect.

This present study linked the body metal burden in the BLM scheme and one-compartment bioaccumulation model at steady-state condition (Liao et al. 2007) to estimate the concentration of unoccupied BL sites at the surface membrane of aquaculture species,

$$[\mathrm{CuBL}]_{\mathrm{T}} = [\mathrm{BL}^{-}][a]\{\mathrm{Cu}\} \approx \frac{k_u}{k_e}\{\mathrm{Cu}\}, \tag{4.19}$$

where $[\mathrm{CuBL}]_{\mathrm{T}}$ is the steady-state body Cu burden (μg g^{-1}), $[\mathrm{BL}^{-}]$ is the concentration of unoccupied BL sites (μg g^{-1}).

This study approximated the exponential function $f_{\mathrm{CuBL}}^{50\%}(t) \approx e + f \cdot \exp(-t/g)$ and incorporated $[a] = \mathrm{BCF} \cdot [\mathrm{BL}^{-}]^{-1}$ into Eq. (4.18), and rearranged to an expression as,

$$\mathrm{EC50}(t) = \frac{e + f \cdot \exp(-t/g)}{\left(1 - (e + f \cdot \exp(-t/g))\right)} \times \frac{\left(1 + \sum K_{\mathrm{ionsBL}}\{ions\}\right) \cdot [\mathrm{BL}^{-}]}{\mathrm{BCF}}, \tag{4.20}$$

the coefficients *e*, *f*, and *g* can be estimated by fitting Eq. (4.20) to EC50(*t*) data obtained from the bioassays.

Consideration of both the environmental factors and biological mechanisms, this study linked the DAM and BLM to improve the predictive ability of chemical toxicity to organisms. The proposed median effect concentration over time calculated by the DAM equals to the predicted by the BLM as,

$$\frac{D_{\mathrm{E,50}}/k_a}{\left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r}\right)} \cdot \mathrm{BCF}^{-1}(\{\mathrm{ions}\}, t) = \frac{f_{\mathrm{CuBL}}^{50\%}(t) \cdot \left(1 + \sum K_{\mathrm{ionsBL}}\{ions\}\right)}{\left(1 - f_{\mathrm{CuBL}}^{50\%}(t)\right) \cdot [a]}. \tag{4.21}$$

A two–step approach is used to implement BLM-based DAM. In the first step, we applied the BLM scheme to account for the ions competition with Cu bioavailability in each aquatic organism to obtain a bioavailability–based EC50(*t*) profile. In the second step, we needed to estimate the biophysiological parameters (e.g., recovery rate and killing rate) by using the DAM fitting EC50(*t*).

In view of Eq. (4.21), the BLM–based toxicokinetic model to aquatic organism considering the competition of ions with time–dependent, has the form as,

$$\mathrm{BCF}(\{ions\},t)=\frac{\left(1-f_{\mathrm{CuBL}}^{50\%}(t)\right)\cdot[a]\cdot\left(D_{E,50}/k_a\right)}{f_{\mathrm{CuBL}}^{50\%}(t)\cdot\left(1+\sum K_{\mathrm{ionsBL}}\{ions\}\right)\cdot\left(\frac{e^{-k_r t}-e^{-k_e t}}{k_r-k_e}+\frac{1-e^{-k_r t}}{k_r}\right)}. \tag{4.22}$$

Hence, traditional first–order one–compartmental bioaccumulation model can be refined by BLM concept for linking the relationship between $\mathrm{BCF}(\{ions\},t)$ and $f_{\mathrm{CuBL}}^{50\%}(t)$.

Fig. 4.6 illustrates the block conceptual diagram of the systems-level based TDM.

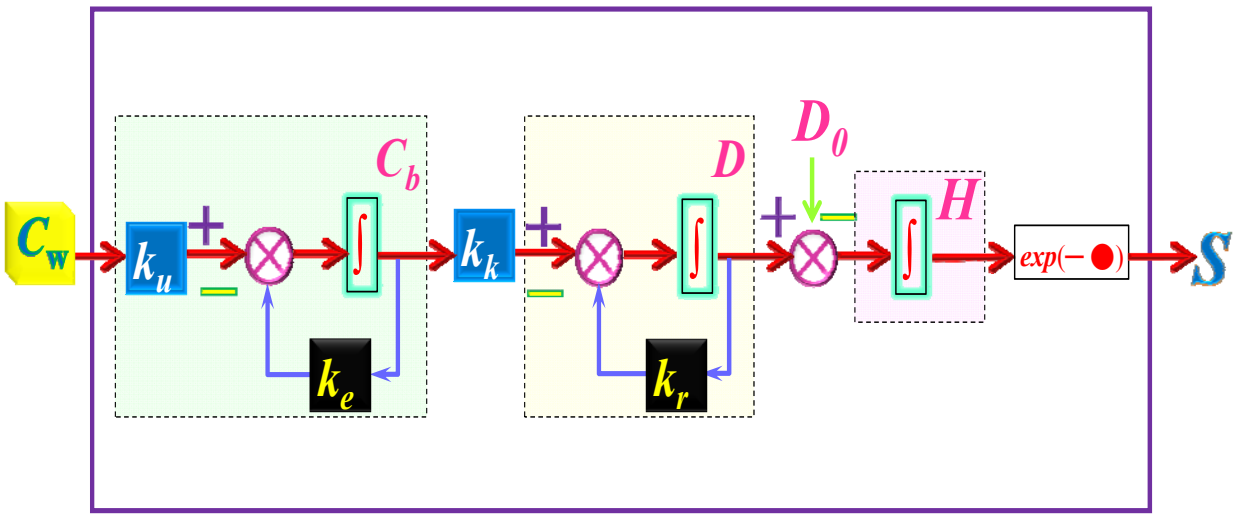


Figure 4.6. Conceptual block diagram of the systems-level based TDM.

4.3.4. Ontogenetic growth-based DEBtox model

All of organisms should fuel ontogenetic growth in the life that energy was allocated to synthesize new biomass and to maintain existing biomass. West growth model (West et al., 2001) describes the organism ontogenetic growth (biomass) trajectory from birth to maturity based on energy allocation without toxicity. The DEBtox describes the mode of action (MOA) of chemical toxicity that alters the energy allocation (Kooijman and Bedauz, 1996; Alunno-Bruscia et al., 2009). Hence, integrated DEBtox theory with West growth model could simulate the chemical toxicity cause growth (biomass) inhibition curve in life span.

Tsai and Liao (2006) revealed that MOA of reducing food assimilation efficiency was well predicted by West growth model,

$$W(t) = \left[W_{\max,0} \cdot S(t)\right] \cdot \left\{1 - \left[1 - \left(\frac{W_0}{W_{\max 0} \cdot S(t)}\right)^{1/4}\right] \exp\left(-\frac{A_0 t}{4\left(W_{\max 0} \cdot S(t)\right)^{1/4}}\right)\right\}^4, \quad (4.23)$$

where $W(t)$ is the time–dependent body biomass (g), W_0 is the body biomass at birth of tilapia that approximately 0.05 g in uncontaminated environment (www.fishbase.org/home.htm), $W_{\max 0} \cdot S(t)$ is the ultimate body biomass of tilapia under the contaminated environment where $W_{\max,0}$ is the maximum body biomass in uncontaminated environment, $S(t)$ is the safe probability and A_0 is a biological species-specific growth coefficient.

In view of Eq. (4.23), a plot of the dimensionless biomass ratio (r) as the function of $W(t)/\left(W_{\max,0} \cdot S(t)\right)^{1/4}$ that was a proportion of total lifetime metabolic power used for maintenance and other activities. When the aquatic organisms exposed to chemical stress, the mechanism can reveal that tilapia increase energy acquisition

to maintain the survival and decrease growth development.

This study employed the TableCurve 2D (Version 5, AISN Software, Mapleton, OR) and 3D (Version 4, AISN Software, Mapleton, OR) to optimal fit the published and experimental data to obtain optimal statistical models. The Crystal Ball® software (Version 2000.2, Decisionerring, Inc., Denver, Colorado, USA) was employed to implement Monte Carlo (MC) simulation. A MC technique was performed 10000 iterations to obtain 2.5^{th}– and 97.5^{th}– percentiles as the 95% confidence interval (CI) for all fitted models that are sufficient to ensure the results. Mathamatica® (Version 5.1, Wolfram Research Inc., Champaign, IL, USA) was used to perform all simulations of the toxicological and ecophysiological responses of aquatic organisms to time-varying metal exposures. WHAM (Windermere humic aqueous model) Version 6 (WHAM VI, Center for Ecology and Hydrology, Lancaster, UK) was performed to calculate the activities of competing and complex ions considered in BLM scheme.

CHAPTER 5. RESULTS AND DISCUSSION

5.1. Sequential Pulsed Cu Toxic Effect on Tilapia

5.1.1. Acute/chronic toxicokinetic parameters

The 10 days (acute) sequential pulsed exposure experiment of Cu in whole body of adult tilapia was best-fitted ($r^2 = 0.79$, $p < 0.05$) by the first–order one compartment bioaccumulation (Eq. (4.14)) with a Dirac delta function (Eq. (4.8)) (Fig. 5.1A). However, the bioaccumulation of larval and juvenile tilapia revealed poor-fitted ($r^2 =$ 0.40 and 0.14) by bioaccumulation model, whereas both *p*-value were less than 0.05 that revealed the significant results (Fig. 5.1B, C). The Cu bioaccumulation data of tilapia showed high values of the standard deviation, due in part to the limited sample size ($n = 3$).

The results indicated that the toxicokinetic parameters of uptake rate constant (k_u) and elimination rate constant (k_e) were estimated to be 223.22 ± 210.13 (Mean ± SE), 239.55 ± 151.69, and 9.40 ± 2.65 mL g^{-1} d^{-1} and 0.20 ± 0.35, 1.06 ±1.11, and 0.10 ± 0.089 d^{-1}, respectively, to larval, juvenile, adult tilapia. Each life-stage tilapia BCF values are above 1. The results revealed that larval tilapia had the higher BCF estimates of 1116.10 mL g^{-1} than that of juveniles (BCF = 225.50 mL g^{-1}) and adults (BCF = 94.00 mL g^{-1}) when exposed to sequential pulsed Cu concentration within 10 days, indicating a high Cu accumulation when tilapia exposed to a given waterborne Cu concentration.

The results showed that time-dependent Cu acute accumulation experimental data increased slightly when tilapia exposed to the sequential pulsed Cu and then decreased with the decreasing waterborne Cu concentration. The results revealed that

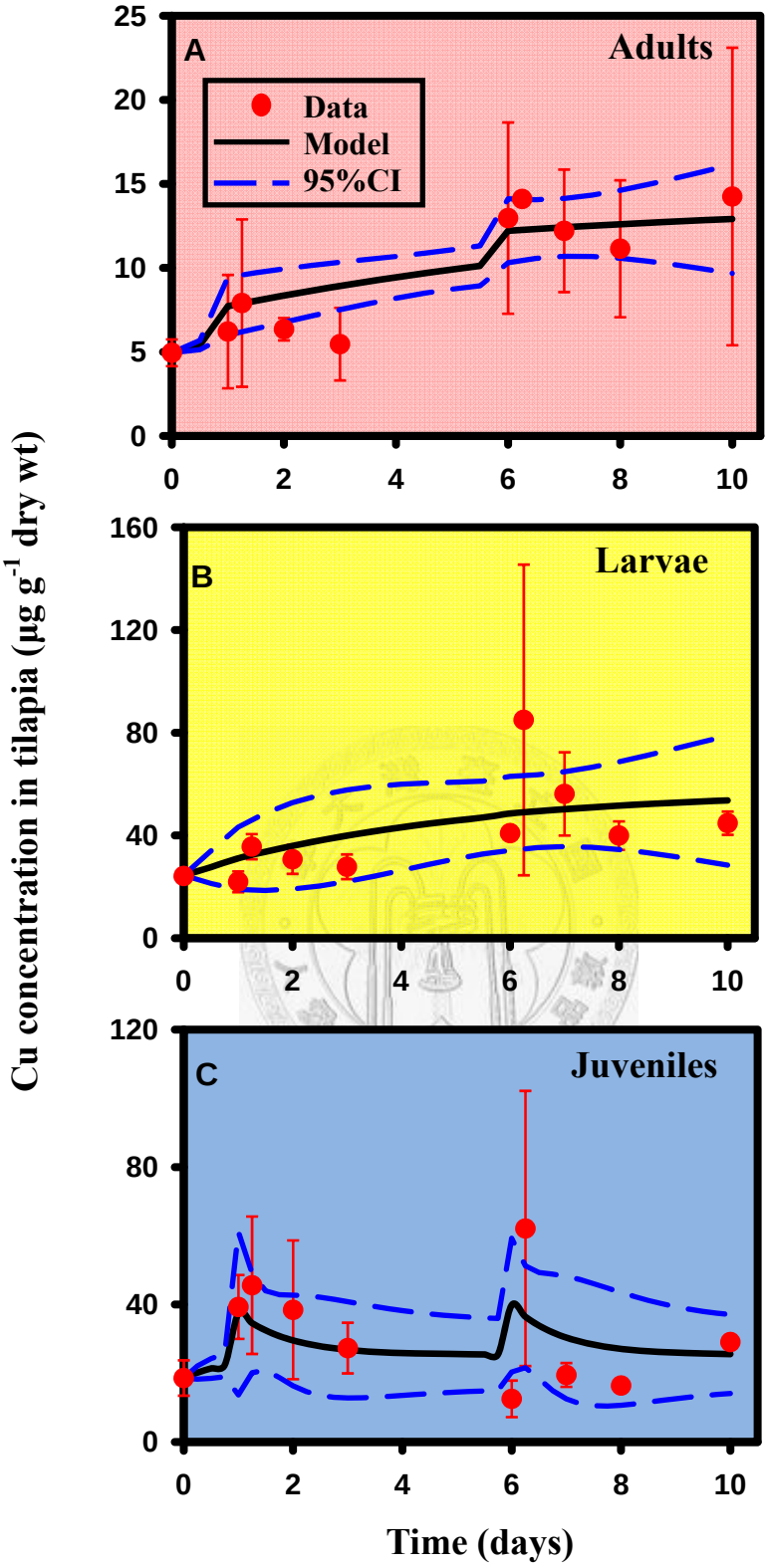


Figure 5.1. Best-fitting regression curves of acute Cu accumulation to (A) adult, (B) larval, and (C) juvenile tilapia from first-order bioaccumulation model. Error bars are standard deviation from mean.

the tilapia may switch the recovery mechanism during the periods of 1st and 2nd sequential pulsed Cu concentrations. Bioaccumulation data of larval and juvenile tilapia revealed that rapidly increasing Cu concentration between days 6 and 6.25, whereas the slightly increasing Cu accumulation was found in adults. However, the fitted curve showed the gradual progress of Cu concentration in larval and adult tilapia, indicating that the predictions could not capture the repair mechanism. However, the repair capacity was found in the model simulation to juvenile accumulations.

This study also carried out 28 days chronic sequential pulsed Cu exposure bioassays to determine the bioaccumulation capacities of juvenile and adult tilapia. Fig. 5.2 showed the best-fitted regression curve of chronic accumulation to pulsed accumulation data of juvenile and adult tilapia, for obtaining the stage-specific toxicokinetic parameters (k_u and k_e). The results revealed that juvenile tilapia had the higher k_u 559.43 ± 226.61 mL g^{-1} d^{-1} (r^2 = 0.36, $p > 0.05$) and k_e 3.62 ± 5.03 d^{-1} than that of adult tilapia k_u 25.18 ± 4.61 mL g^{-1} d^{-1} and k_e 1.09 ± 0.29 d^{-1} (r^2 = 0.61, $p < 0.05$).

Juvenile accumulation data of two hollow circles did not partake in the fitted experimental data to determine the toxicokinetic parameters in the present results. Since the pattern of juvenile chronic accumulation data were disordered in the experimental results, it was difficult to determine the toxicokinetic parameters in original data. The BCF of juvenile tilapia 154.54 mL g^{-1} was greater than that of adult tilapia 23.10 mL g^{-1} during the chronic Cu pulsed exposure. In the experimental bioaccumulation data, the sample sizes were 3 samples before day 21. Hence, the

bioaccumulation data revealed the high values of the standard deviation.

Table 5.1 summarizes the first-order bioaccumulation model determined acute/chronic pulsed Cu toxicokinetic parameters based on experimental accumulation data. The highest k_u and k_e of acute bioaccumulation were compared with chronic bioaccumulation for juveniles and adults, indicating the relative high Cu accumulation capacities in the short-term Cu exposure. Subathra and Karuppasamy (2008) indicated that a high BCF value of acute Cu accumulation exposure than that of chronic Cu accumulation in organs of *Mystus vittatus*. The fingerlings, however, had obvious high Cu accumulation concentration than that of adults.

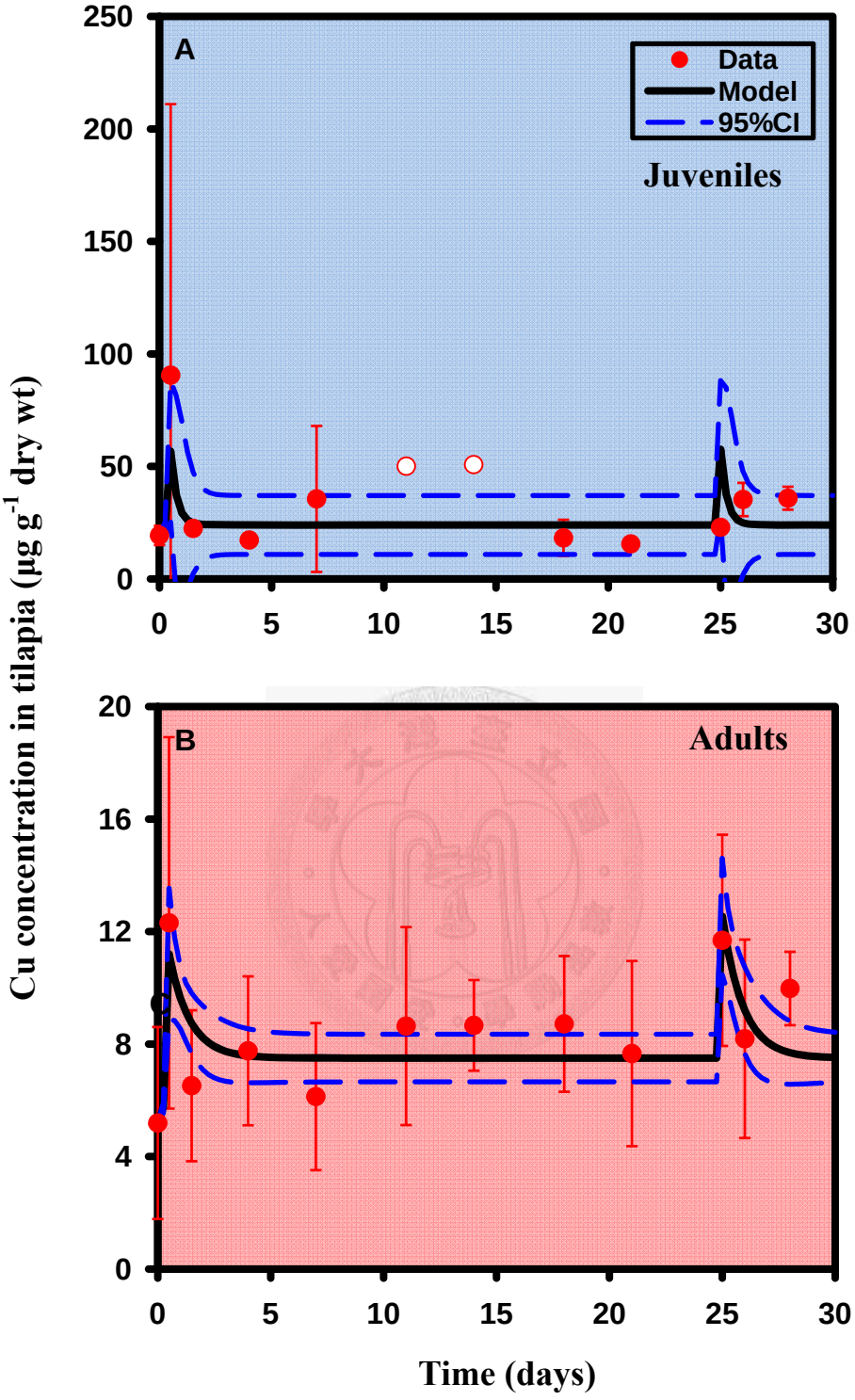


Figure 5.2. Best-fitting regression curves of chronic Cu accumulation to (A) juvenile and (B) adult tilapia from first-order bioaccumulation model. Error bars are standard deviation from mean.

Table 5.1. Parameter estimates of acute/chronic pulsed Cu-tilapia system for bioaccumulation model.

	Larvae	Juveniles	Adults
Acute accumulation			
k_u (mL g^{-1} d^{-1})	232.22±210.13 [a]	239.55±151.69	9.40±2.64
k_e (d^{-1})	0.20±0.35	1.06±1.11	0.10±0.09
BCF (mL g^{-1})	1116.10	225.50	94.00
Chronic accumulation			
k_u (mL g^{-1} d^{-1})	–	559.43±226.61	25.18±4.61
k_e (d^{-1})	–	3.62±5.03	1.09±0.29
BCF (mL g^{-1})	–	154.54	23.10

[a] Mean ± SE.

5.1.2. Cu chronic growth toxicity

The body biomass of tilapia in each control and pulsed Cu exposure groups during 28 day periods were monitored to calculate the daily growth rate (k_g) (Fig 5.3). The excessive high daily growth rates were found on days 0.5 and 1.5 in control group due to tilapia just after feeding no more than 12 hrs. The tilapia did not fully finish the digestion. The growth biomass might be included the weight of forage. The significance of negative daily growth rates of pulsed Cu exposure group were shown in days 1.5 and 4. This result revealed that pulsed Cu concentration induced the lag growth inhibition significantly. The greater part of control group had the higher daily growth rate than that of pulsed exposure group except at day 14. The k_g values of the control group ranged from 0.34 – 13.25 % d^{-1}, whereas pulsed exposure group ranged from 1.28 – 1.77 % d^{-1}. The 28 day chronic growth toxicity bioassays revealed that pulsed Cu concentration positively affected the growth mechanism in tilapia.

This study further employed the daily growth rate of control and the pulsed exposure groups to estimate the relationship between the safe growth probability and the damage level by using Eqs. (4.2) and (4.3). Fig. 5.4 showed the calculated damage data and optimal fitted curves of damage. The original regression models should be included the one-compartment bioaccumulation model (Eq. (4.14)) and damage model (Eq. (4.15)) with the Dirac delta function (describing the sequential pulsed exposure patterns) to estimate the killing rate constant (k_k) and recovery rate constant (k_r) due to growth toxicity. However, the solved damage mechanistic equation was too immense and complex to estimate the toxicodynamic parameters in pulsed Cu exposure bioassays.

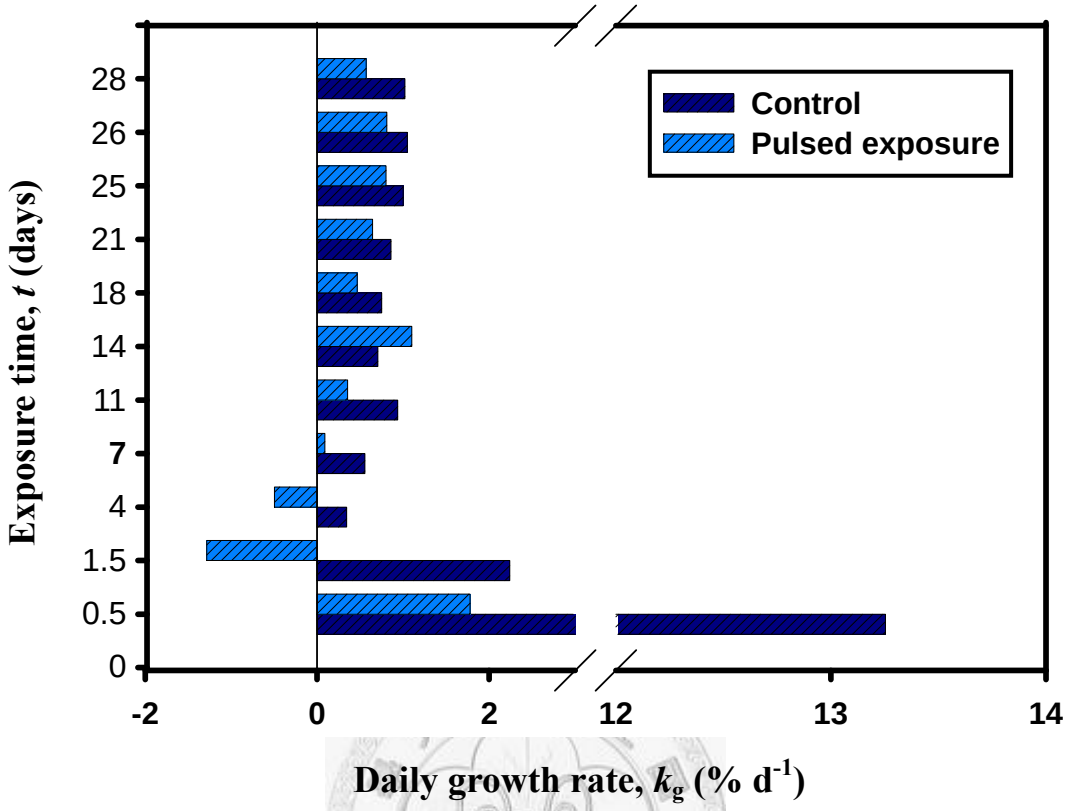


Figure 5.3. The calculated daily growth rate of adult tilapia with/without pulsed Cu exposure during 28 day bioassays.

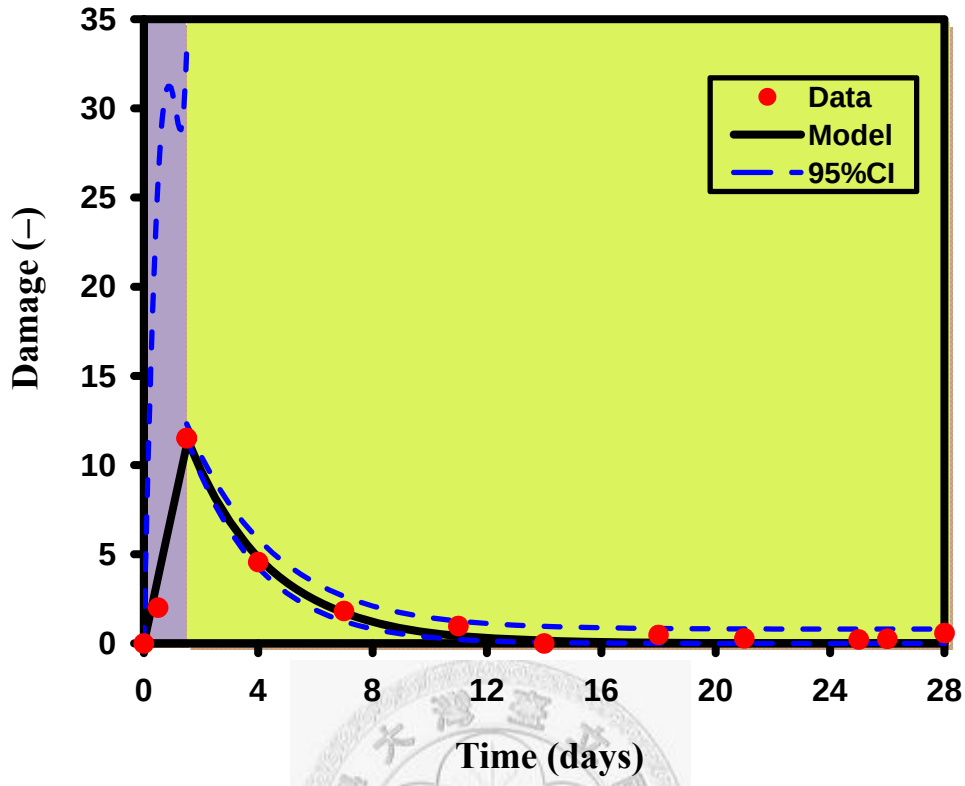


Figure 5.4. Best-fitting regression curves of pulsed Cu damage to tilapia from first-order damage model (purple shadow is killing phase and green shadow is recovery phase).

Hence, this study utilized the approximate equation based on the one-compartment damage model with constant Cu body burden. The body burden calculation was based on the area under the curve (AUC) of body Cu concentration data adopted from Fig. 5.2B. The damage phase could separate killing and recovery phases. The damage data revealed the gradually increased damage before 1.5 days, whereas the decreased damage after 1.5 days. The fitted equation can be expressed by the killing and recovery phases, respectively, as

Killing phase:

$$\frac{dD(t)}{dt} = k_k \cdot C_b - k_r \cdot D(t), \tag{5.1}$$

Recovery phase:

$$\ln D(t) = \ln D(t = T) - k_r t. \tag{5.2}$$

Hence, the estimated toxicodynamic parameters of growth toxicity can be obtained to be k_k = 0.83 g μg^{-1} d^{-1} and k_r = 8.94×10^{-6} d^{-1} in killing phase (r^2 = 0.96). The recovery phased revealed the k_r=0.35 d^{-1} (r^2 = 0.99). Therefore, the further model prediction used the k_k value from killing phase and k_r value from recovery phase.

5.1.3. Bioavailability and bioaccumulation of Cu

This study used damage assessment model (Eq. (4.13)) to predict the time-dependent EC50 by the estimated model parameters (k_u, k_e, k_k, and k_r) from pulsed Cu bioaccumulation and growth toxicity data (Fig. 5.5). Then the $EC50_{DAM}$ was predicted based on accumulative burden of tilapia in the chronic growth bioassay. Therefore, the $EC50_{DAM}$ equation equal to the $EC50_{BLM}$ equation (Eq. (4.22)). To estimate the BLM parameters (the toxic effect level can determine the 50% of Cu binding at the site of action ($f_{CuBL}^{50\%}$), [BL^-]) and predict $EC50_{BLM}$, the first step was needed to take to obtain the values of binding constants in BLM equation in that the stability constant were adopted from De Schamphelaere and Janssen (2002), Hollis et al. (1997), Macrae et al. (1999), Tao et al. (2002), Niyogi and Wood (2004), and Hatano and Shoji (2010) estimated the uncertainties of log*K* in various Cu exposure bioassays.

This study used the lognormal distribution to determine log*K*s. The distributions and point values of log*K* (biotic ligand-cation and inorganic complexes) were given in Table 5.2. The available water chemistry data were used to calculate the activities of the competing cation and complex anion by WHAM software. Hence, Eq. (4.20) together with the time-dependent $EC50_{DAM}$ value could be used to calculate the time-dependent $f_{CuBL}^{50\%}$ values. The $f_{CuBL}^{50\%}(t)$ values were selected for exposure time 0.5, 1.5, 4, 7, 11, 14, 18, 21, 25, 26, and 28 days.

Fig. 5.6A demonstrates the relationship between predicted chronic $f_{CuBL}^{50\%}(t)$ and response time in that the predicted $f_{CuBL}^{50\%}(t)$ has the form as $f_{CuBL}^{50\%}(t) = 0.77 + 0.20\exp(-t/1.59)$ ($r^2 = 0.99$). Here a best regression model of

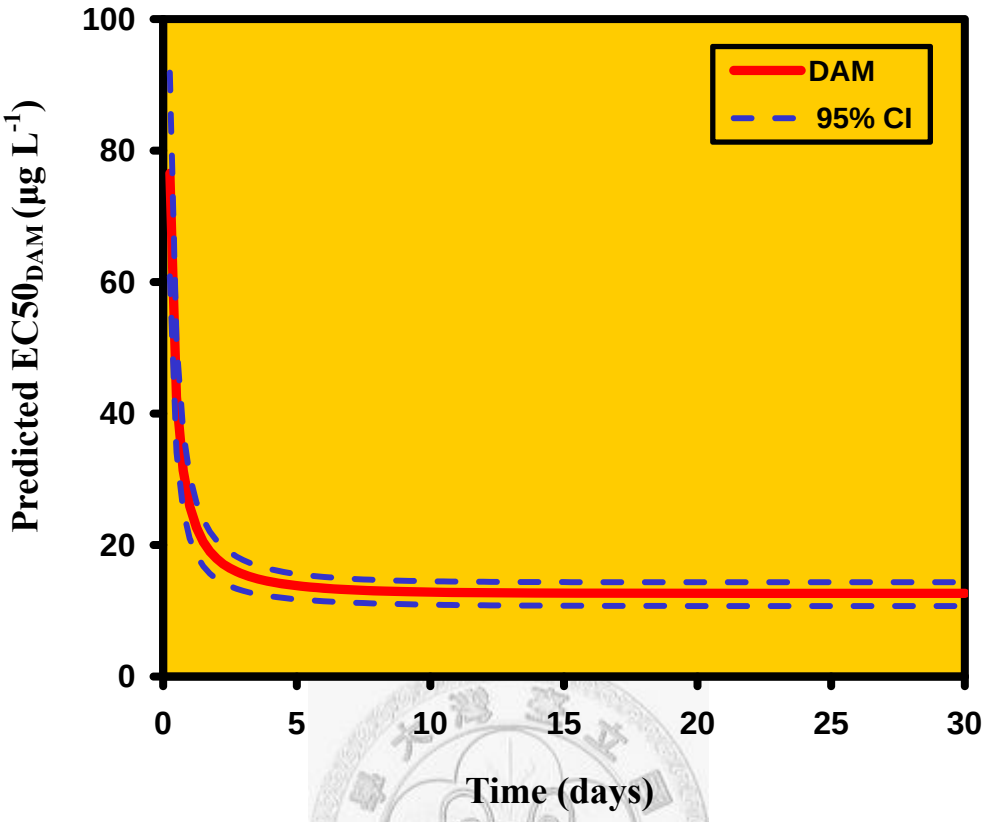


Fig. 5.5. Using the estimated toxicokinetic and toxicodynamic parameters to predict DAM-based EC50 in adult tilapia.

Table 5.2. Point values and distribution of stability constant (log K, M^{-1}) used in Cu-tilapia system.

log K	Point value [a]	log K	Distribution [b]
$\log K_{NaBL}$	3	$\log K_{CuBL}$	LN(7.86,1.08)
$\log K_{CuOHBL}$	7.45	$\log K_{CaBL}$	LN(3.79,1.16)
$\log K_{CuCO_3BL}$	7.01	$\log K_{MgBL}$	LN(3.79,1.08)
$\log K_{CuOH}$	6.48	$\log K_{HBL}$	LN(5.19,1.06)
		$\log K_{CuCO_3}$	LN(6.68,1.02)

[a] Adopted from Niyogi and Wood (2004).

[b] Lognormal distribution with a geometric mean and a geometric standard deviation.

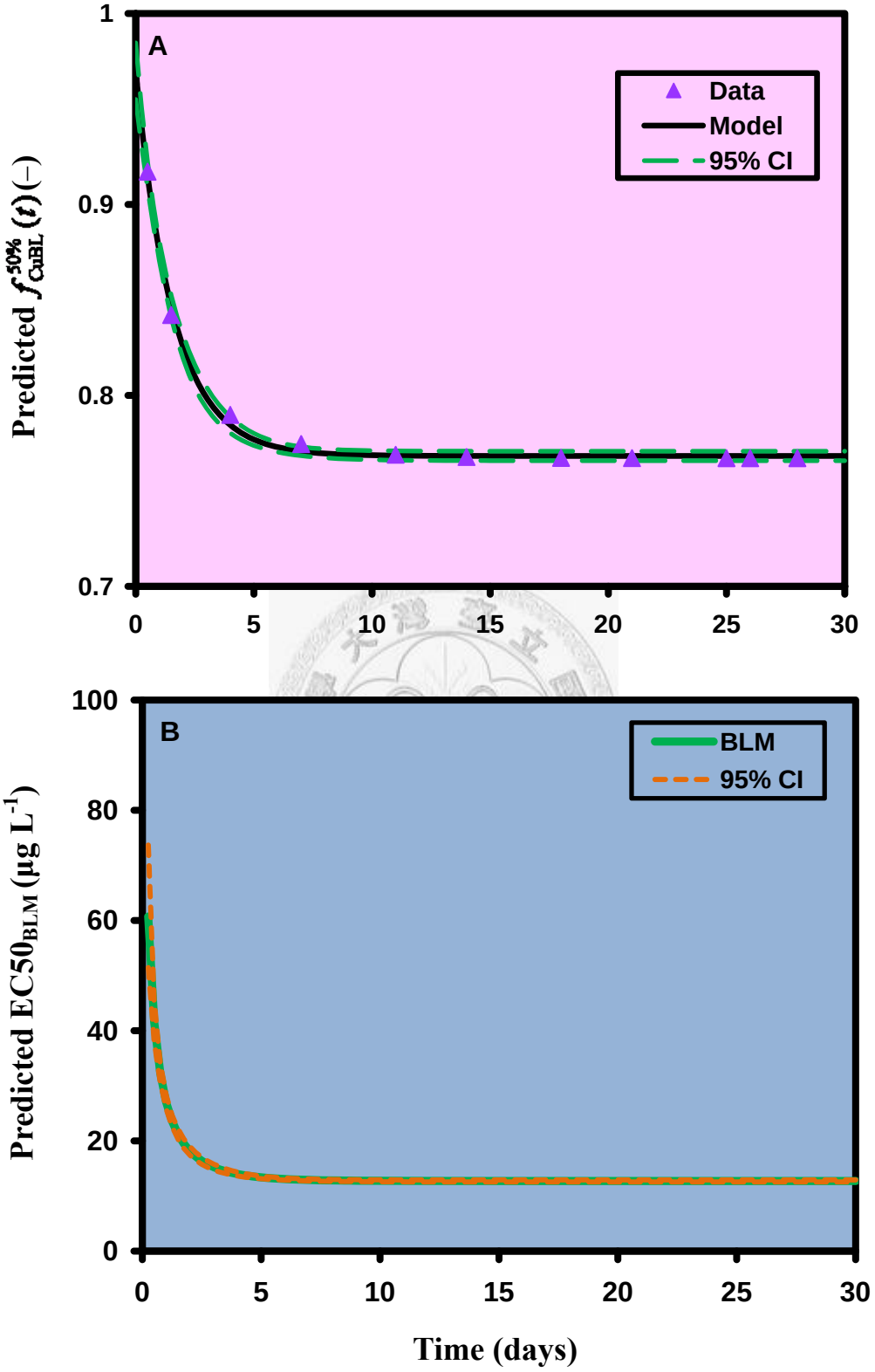


Fig. 5.6. (A) Predicted time-dependent $f_{CuBL}^{50\%}$ by the best-fitting model to estimate (B) BLM-based EC50.

$f_{\mathrm{CuBL}}^{50\%}(t)$ could be used to estimate $EC50_{BLM}$ that was shown in Fig. 5.6B. The concentration of unoccupied biotic ligand site of tilapia [BL^-] could be estimated from the relationship of $[a] = \mathrm{BCF} \cdot [\mathrm{BL}^-]^{-1}$ with the estimated with BCF = 23.10 mL g^{-1} and calculated $[a] = K_{CuBL+} K_{CuOHBL} \{\mathrm{OH}^-\} + K_{CuCO_3BL} K_{CuCO_3} \{\mathrm{CO}_3^{2-}\} = 1.81 \times 108\ \mathrm{M}^{-1}$, resulting in [$BL^-$] = 1.28×10^{-10} mol g^{-1}.

This study used the function of $EC50_{DAM}(t)$ to fit $EC50_{BLM}(t)$ data to obtain the BLM-based DAM key parameters of $D_{E,50}/k_a$ and k_r. The regression curve of $EC50_{BLM\text{-}DAM}$ (r^2 = 0.89) was shown in Fig. 5.7A. The estimated BLM-based DAM parameters could enhance the real metal toxic effect assessment to tilapia. The resulted $D_{E,50}/k_a$ and k_r parameters estimates were 0.04±0.015 μg d g^{-1} and 7.91±3.02 d^{-1}, respectively. The k_k value was 17.33 g μg^{-1} d^{-1} that calculated as ln 2/($D_{E,50}/k_a$). Hence, linkages between BLM and DAM mechanistic concepts that can develop the time-course BCF including site-specific water chemistry and physiological mechanisms. Fig. 5.7B showed the time-profiles of BLM-based BCF (BCF({*ions*}, *t*)) which were predicted by the input of toxicodynamic parameters (i.e. $D_{E,50}/k_a$ and k_r), BLM parameters (i.e. $\sum K_{\mathrm{ionsBL}} \{ions\}$ and [*a*]), and predicted $f_{\mathrm{CuBL}}^{50\%}(t)$. The predicted BCF({*ions*}, *t*) revealed a dramatic decreasing from nearly 114.8 (95%CI: 109.00 – 233.84) mL g^{-1} at day 0.1 and then slowly increased to a steady-state value of 25.30 (95%CI: 3.35 – 47.87) mL g^{-1}. The lowest BCF value of this time course (BCF({*ions*}, *t*)) was 19.42 (95%CI: 4.50 – 36.77) mL g^{-1} at day 1.25.

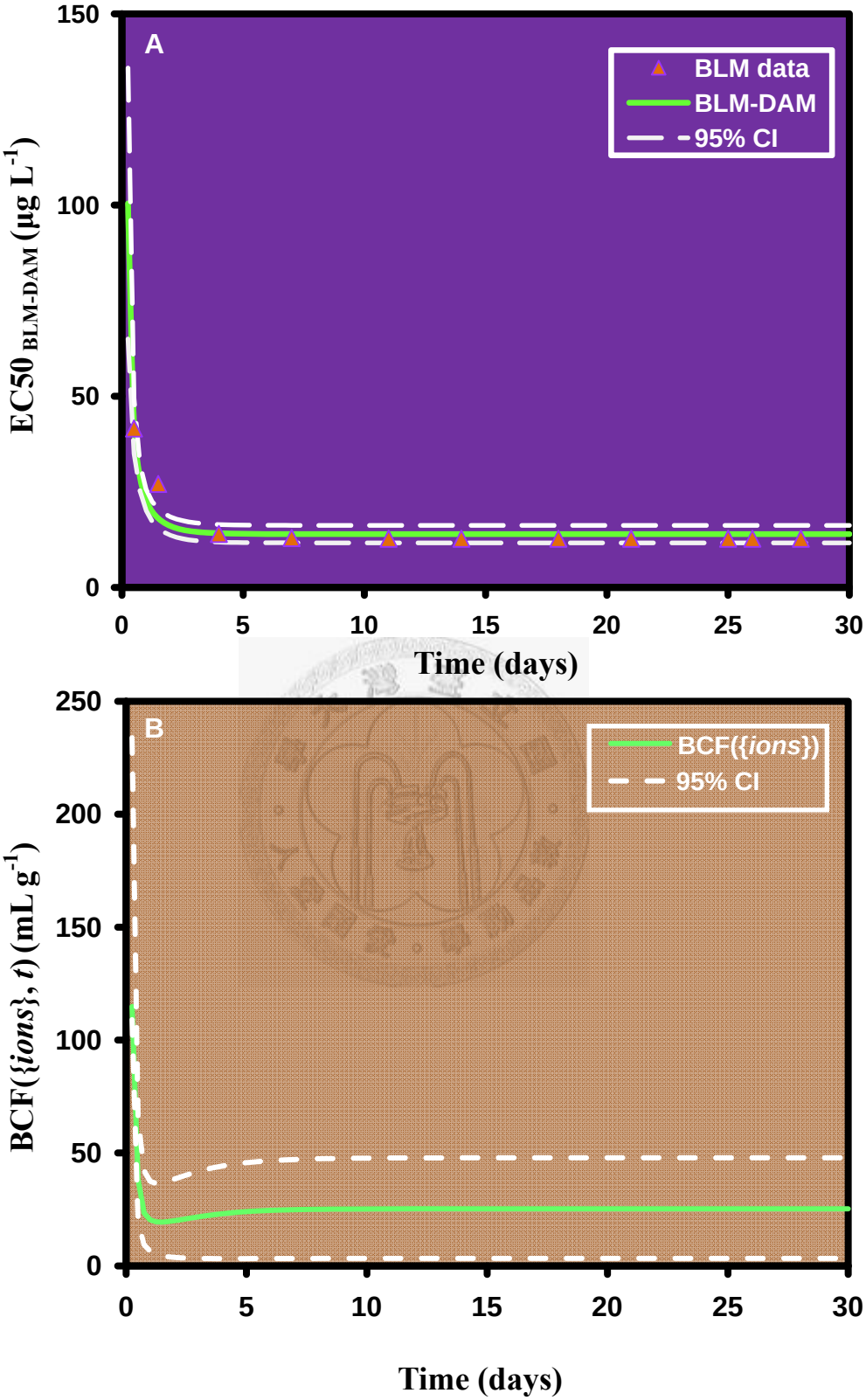


Figure 5.7. (A) Using DAM to fit BLM-based EC50 to estimate DAM parameters. (B) The time series of BLM-based bioconcentration factor predicted by Eq. (4.22).

5.1.4. Internal effects with different Cu exposure patterns

This study was adopted the exposure condition of water chemistry from Liao et al. (2008) to simulate the internal effect of tilapia in response to pulsed and fluctuating Cu. The water chemistry characteristics as follows: Ca^{2+} = 24.8 mg L^{-1}, Mg^{2+} = 1.0 mg L^{-1}, Na^{+} = 4.9 mg L^{-1}, K^{+} = 2.7 mg L^{-1}, H^{+} = 7.21 mg L^{-1}, NH_4^{+} = 0.26 mg L^{-1}, Cl^{-} = 7.6 mg L^{-1}, NO_2^{-} = 0.047 mg L^{-1}, and NO_3^{-} = 0.318 mg L^{-1}.

5.1.4.1. Dynamic effect of sequential pulsed exposure

Fig. 5.8 shows the dynamics of body burden, internal damage, internal hazard rate, cumulative hazard, and safe probability for tilapia depended on high frequency (T_1 at day 5, T_2 at day 10) and low frequency (T_1 at day 0.5, T_2 at day 25) pulse period-specific sequential pulsed exposure in Cu concentrations 0.03 – 0.63 mg L^{-1} (Cu activity: 0.0013 – 0.0278 mg L^{-1}). The used pulsed period-specific Cu sequential pulsed exposures have the forms of $\{C_w(t)\} = C_0 + C_1 \sum_{n=1}^{2} \delta(t - n \cdot T)$ with T=5 days (Fig. 5.8A) and $\{C_w(t)\} = C_0 + C_1 \cdot (\delta(t - T_1) + \delta(t - T_2))$ with T_1=0.5 and T_2=25 days (Fig. 5.8B) varying with C_0=0.0013 and C_1=0.0265 mg L^{-1}.

Fig. 5.8C and D showed that Cu body burdens of tilapia were rapidly increased with increasing external Cu concentration, and were rapidly decreased with decreasing of Cu concentration except at the 1^{st} pulsed timing of low frequency exposure. Owing to the prior short exposure duration (0.5 day), the body burden of Cu was slightly decreased after the 1^{st} pulsed timing. On the 1^{st} pulsed Cu exposure timing, the Cu accumulative burden of tilapia were 0.0586 and 0.0407 μg g^{-1}, with the 2^{nd} pulsed exposure timing were 0.0588 and 0.0587 μg g^{-1}, respectively, to high and low frequency patterns. The results found that the pulsed accumulative burden was not

significant differences between 1st and 2nd pulse exposure timings in high frequency exposure, whereas the significant differences in low frequency exposure.

The internal damage of tilapia was proportional to the body burden. Hence, the damage curve was similar to burden curve at high and low frequency patterns (Fig. 5.8E and F). The pulsed exposure induced damage level at pulse timing were nearly 0.136 in the 1st and 2nd pulsed timings of high frequency and the 2nd pulsed timing of low frequency. Specifically, this study used {EC5} = 0.936 μg L^{-1}, the effect activity that caused 5% effect, to calculate the damage threshold (D_0) via DAM, resulted in D_0 = 0.051. When damage exceeded the damage threshold, the time-course hazard rate rose above zero (Fig. 5.8G and H).

The cumulative hazard of both sequential pulsed frequency exposures showed the similar levels of 0.4922 and 0.4919 on day 30 for high and low frequency patterns, respectively (Fig. 5.8I and J). Hence, the results showed that cluster pulsed exposure have identical median safe time (ST50 = 43.28 days) with that of less cluster pulsed exposure (Fig. 5.8K and L). In the low Cu concentration exposure, the dynamic response level of tilapia was similar despite the difference of pulsed exposure intervals.

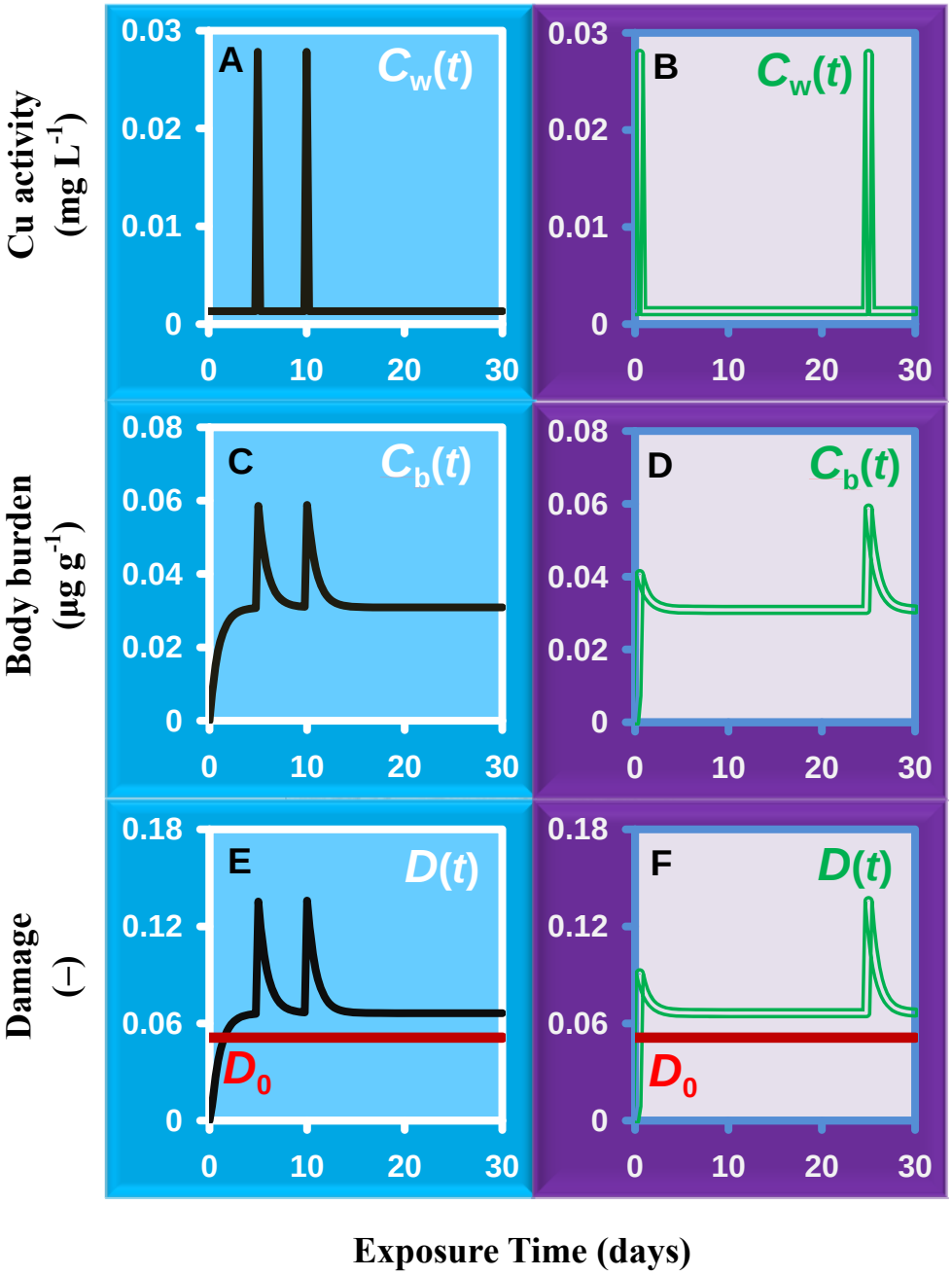


Figure 5.8. Simulations of the sequential pulsed Cu exposure with 5 days pulse periods (left), and 0.5 and 25 days pulse periods (right) for tilapia. (A, B) Sequential pulsed Cu activity range from 0.0013 – 0.0278 mg L^{-1}. (C, D) Body burdens. (E, F) Time course of the damage. (G, H) Hazard rate. (I, J) Cumulative hazard. (K, L) Safe probabilities.

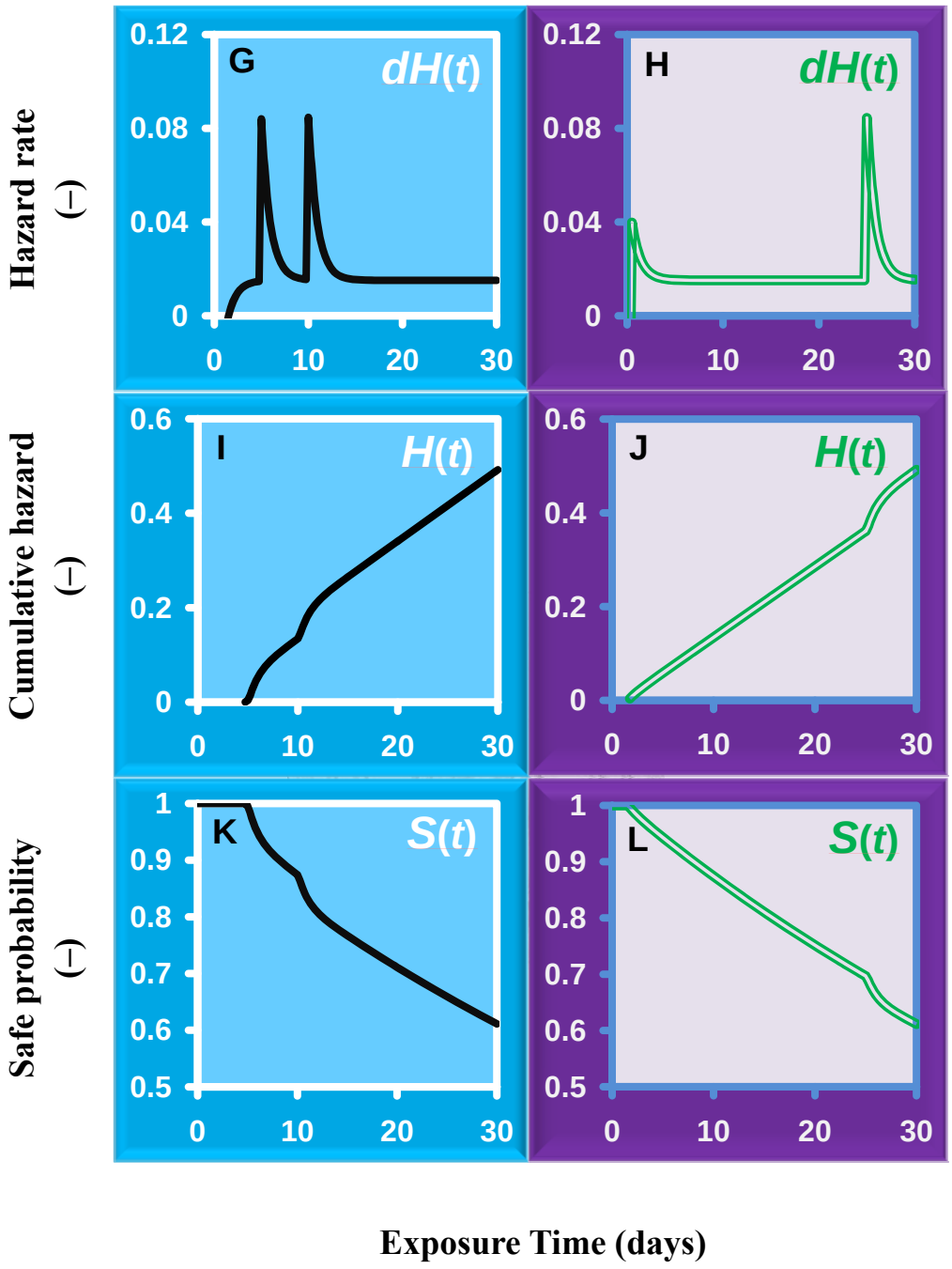


Figure 5.8. Continued.

This study used the higher pulsed concentration with high and low pulsed frequent patterns to investigate the internal effect in that the exposure concentration ranged from 0.03 – 6 mg L^{-1} of total Cu concentration (i.e., Cu activity: 0.0013 – 0.2664 mg L^{-1}) (Fig. 5.9A and B). Fig. 5.9C and D show that Cu body burden of tilapia were increased with increasing external Cu concentration, and were decreased with decreasing of Cu concentration. On the 1st pulsed Cu exposure timing, the Cu accumulative body burdens of tilapia were 0.3088 and 0.2910 μg g^{-1}, respectively, to high and low frequency patterns, whereas the Cu accumulative burdens of the 2nd pulsed exposure timing were 0.3102 and 0.3090 μg g^{-1}, respectively.

The damages of tilapia on the 1st pulsed exposure timing were 0.7586 and 0.7143, respectively, to high and low frequency patterns, whereas the damages of the 2nd pulsed exposure timing were 0.7620 and 0.7589, respectively (Fig. 5.9E and F). When the damage exceeded the damage threshold of 0.051, the hazard rate will be triggered (Fig. 5.9G and H). The cumulative hazard reflects the toxic potency of the Cu to tilapia, indicating that the high frequency exposure resulted in 1.4888 of cumulative hazard, whereas 1.4859 on day 30 for the low frequency exposure (Fig. 5.9I and J). Hence, the results of safe probability showed that cluster pulsed exposure had shorter ST50 (10.21 days) than that of less cluster pulsed exposure (ST50 = 13.96 days) (Fig 5.9K and L), indicating that the pulsed timing and sequence for tilapia exposed Cu could be mattered in the high pulsed concentration level.

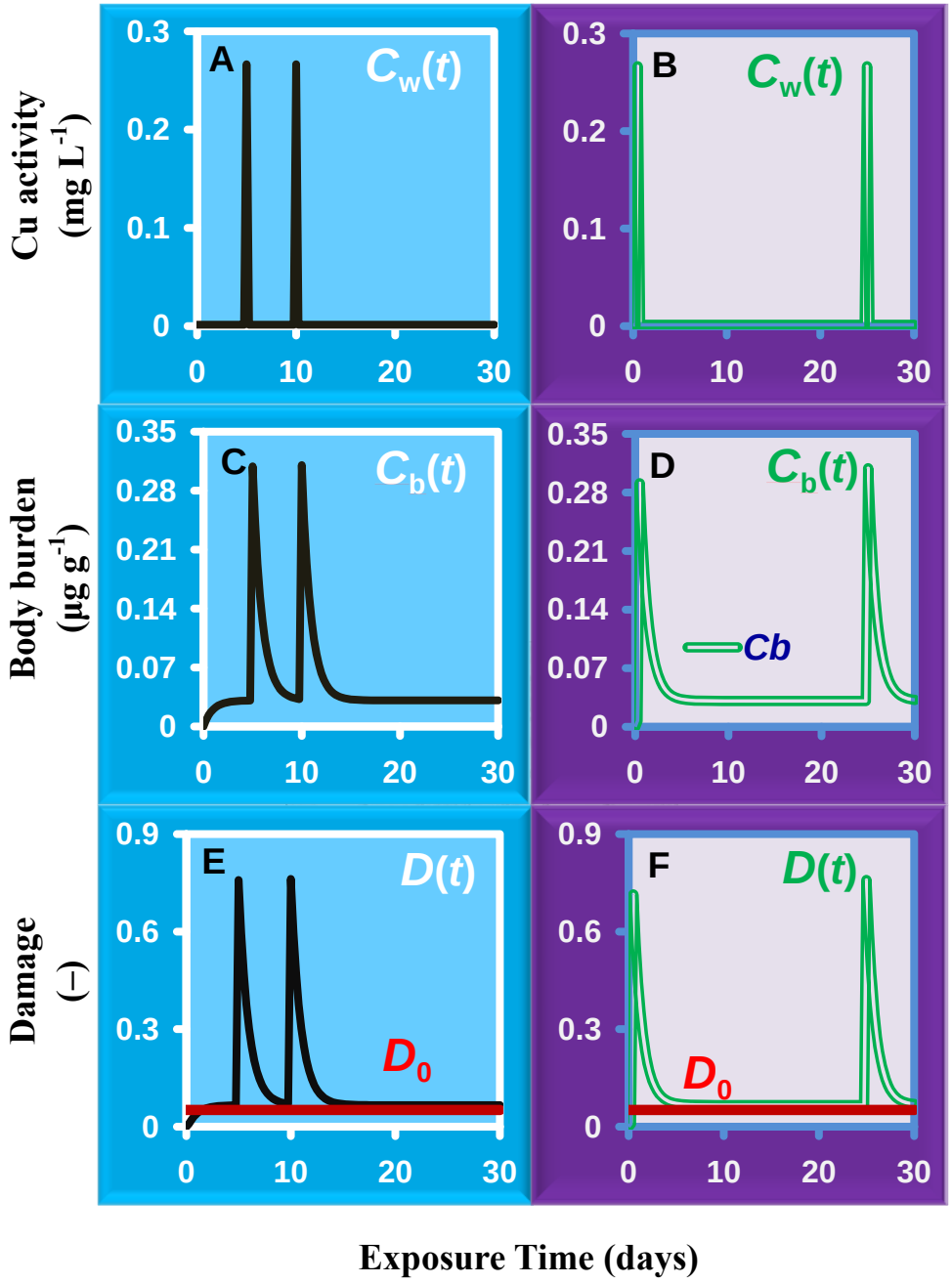


Figure 5.9. Simulations of the sequential pulsed Cu exposure with 5 days pulse periods (left), and 0.5 and 25 days pulse periods (right) for tilapia. (A, B) Sequential pulsed Cu activity range from 0.0013 – 0.2664 mg L^{-1}. (C, D) Body burdens. (E, F) Time course of the damage. (G, H) Hazard rate. (I, J) Cumulative hazard. (K, L) Safe probabilities.

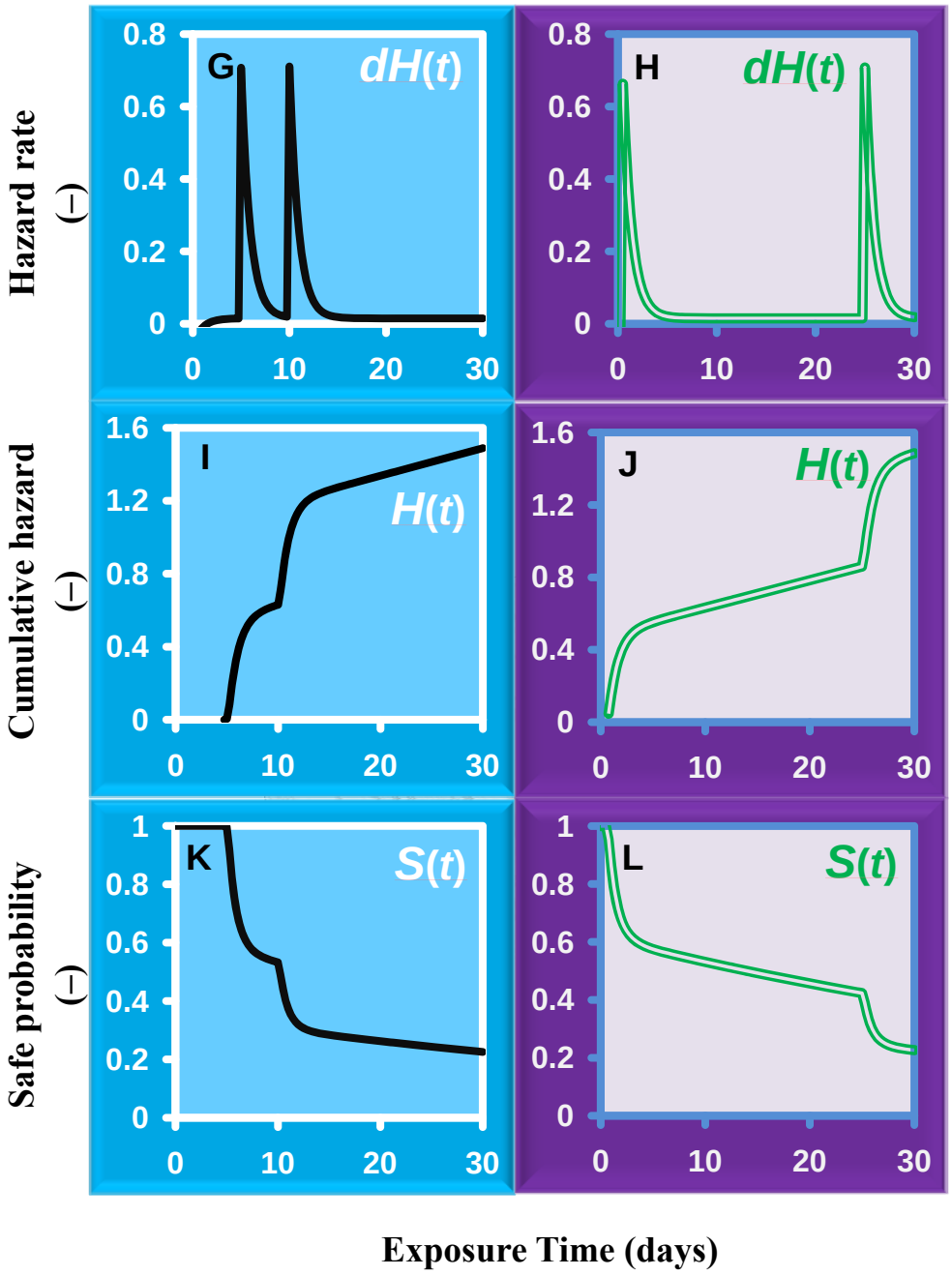


Figure 5.9. Continued.

5.1.4.2. Dynamic effect of fluctuating exposure

The study used the Cu concentrations varying sinusoidally over a range of periods $\{C_w(t)\} = C_0 + C_1 \sin(2\pi t / T + d)$ with low Cu concentration 0.03 – 0.15 mg L^{-1} (Cu activity: C_0 = 0.0040 and C_1 = 0.0027 mg L^{-1}) to examine the underlying dynamic responses of tilapia in response to the periodic patterns.

Tilapia in response to sine-wave patterns between 0.0013 and 0.0067 mg L^{-1} Cu activity was simulated with the period, T=3 days (Fig. 5.10A) and 15 days (Fig. 5.10B), respectively. The Cu body burdens of tilapia were 0.121 and 0.131 μg g^{-1}, respectively, to high and low frequency on day 30 (Fig. 5.10C and D). The highest and the lowest accumulative burden of tilapia were found in the low frequency exposure while the gradually increasing or decreasing Cu activities are continued for a long period. In light of the body burden pattern, the damages of tilapia were 0.258 and 0.285 respectively to high and low frequency patterns on day 30 (Fig. 5.10E and F) .

The hazard rates with sine-wave exposure to tilapia were shown in Fig. 5.10G and H. The cumulative hazard reflects the toxic potency of the Cu to tilapia, indicating that the high frequency exposure resulted in 4.117 and 4.094 on day 30, respectively to high and low frequency patterns (Fig. 5.10I and J). There are no significant differences to cumulative hazard between low and high frequency exposure on day 30.

However, the results indicated that the ST50 = 6.42 days tilapia in response to smaller periodic change (i.e., high frequency) experienced shorter ST50 than that of greater periodic fluctuation (i.e. low frequency) ST50 = 10.08 days (Fig. 5.10K and

L). The results revealed that low frequency Cu exposure could provide more suitable environment than high frequency exposure to safe living even it induced high accumulative Cu burden.

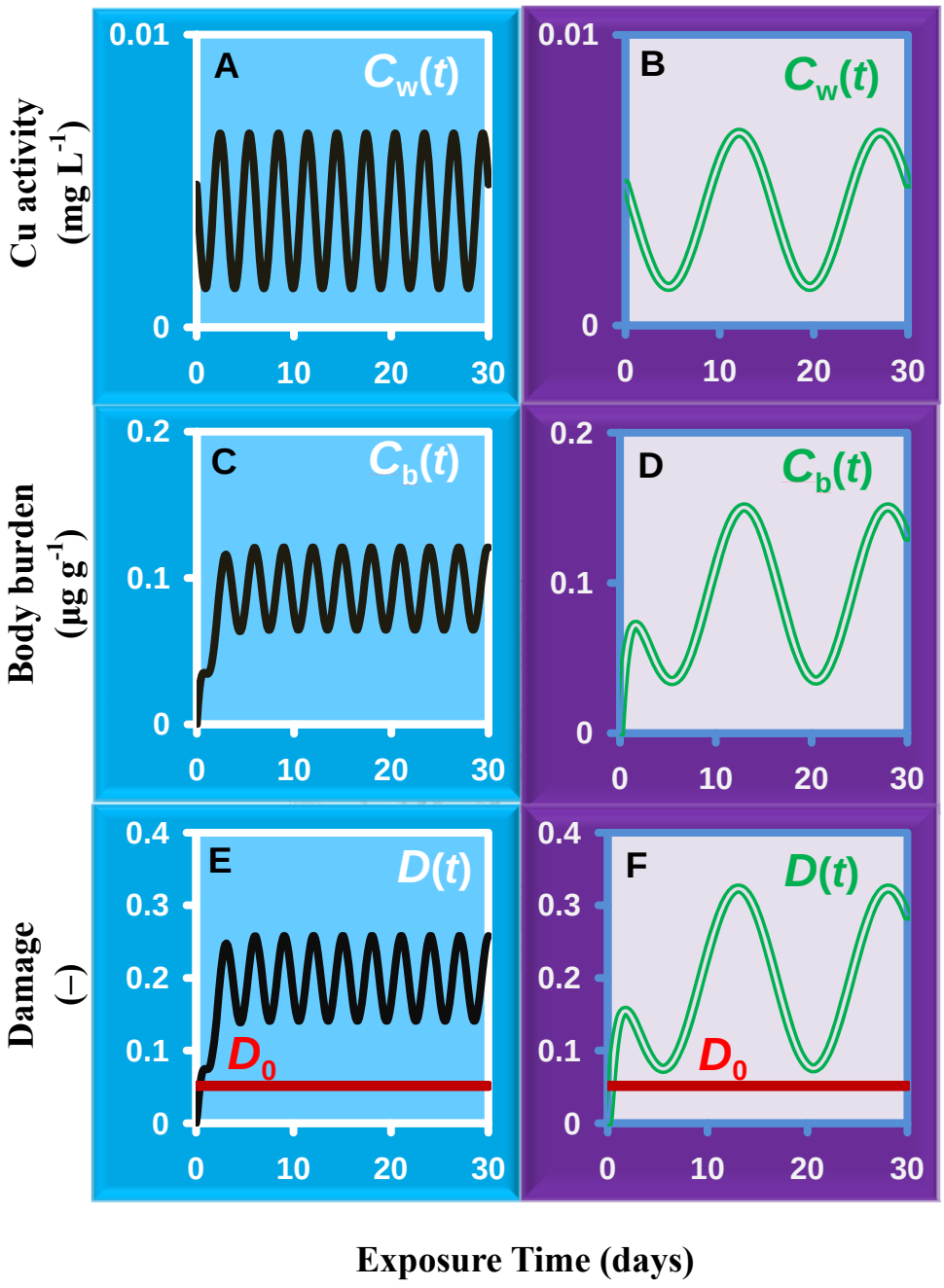


Figure 5.10. Simulations of the sine-wave Cu exposure with 3 days (left) and 15 days periods (right) for tilapia. (A, B) Sine-wave Cu activity range from 0.0013 – 0.0067 mg L^{-1}. (C, D) Body burdens. (E, F) Time course of the damage. (G, H) Hazard rate. (I, J) Cumulative hazard. (K, L) Safe probabilities.

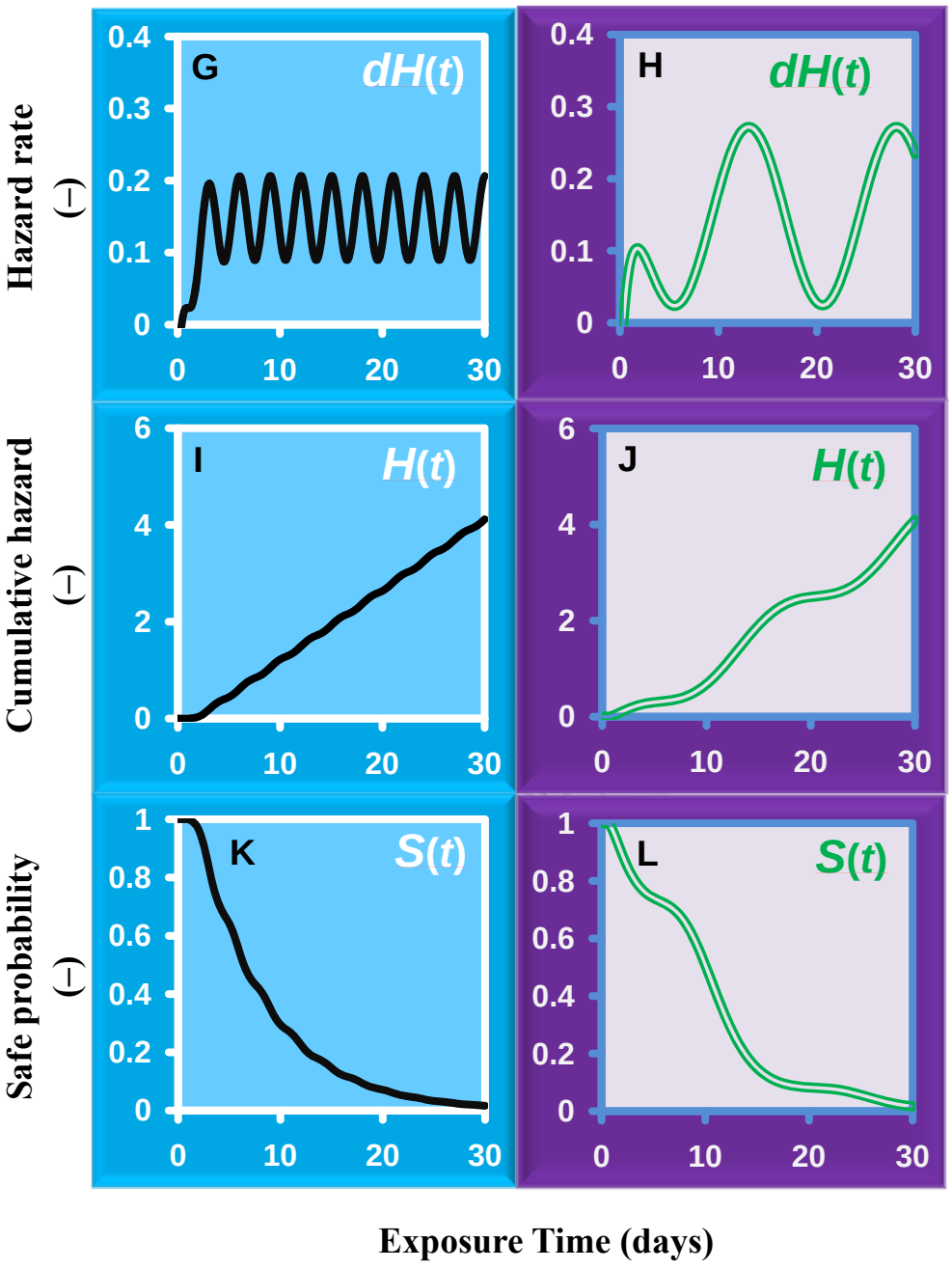


Figure 5.10. Continued.

The study also performed the high concentrations of total Cu 0.03 – 0.6 mg L^{-1} (Cu activity 0.0013 – 0.0265 mg L^{-1}) varying sinusoidally over a range of periods to investigate the dynamics of internal effect of tilapia in response to the periodic patterns. Tilapia in response to sine-wave patterns in the base line Cu activity (C_0) 0.0139 mg L^{-1} and amplitude Cu activity 0.0126 mg L^{-1} were simulated with the period, T = 3 days and 15 days (Fig. 5.11A and B), respectively.

The Cu body burdens of tilapia were 0.458 and 0.504 μg g^{-1}, respectively, to high and low frequency patterns on day 30 (Fig. 5.11C and D). When tilapia exposed to high Cu activity, Fig. 5.11E and F reveal that damage of tilapia were 0.975 and 1.107, respectively, to high and low frequency patterns. The triggered hazard rates with sine-wave exposure to tilapia were shown in Fig. 5.11G and H. The cumulative hazards of tilapia were 18.179 and 18.071, respectively, to high and low frequency patterns (Fig. 5.11I and J).

Finally, tilapia in response to periodic sine-wave patterns with high Cu concentration revealed smaller periodic change ST50 = 2.71 days experienced much longer ST50 than that of greater periodic ST50 = 2.04 days fluctuation (Fig. 5.11K and L). According to safe probabilities of tilapia exposed to sine-wave exposure patterns revealed that toxic effect and living physiological mechanism will be triggered based on the different Cu exposure frequency and timing.

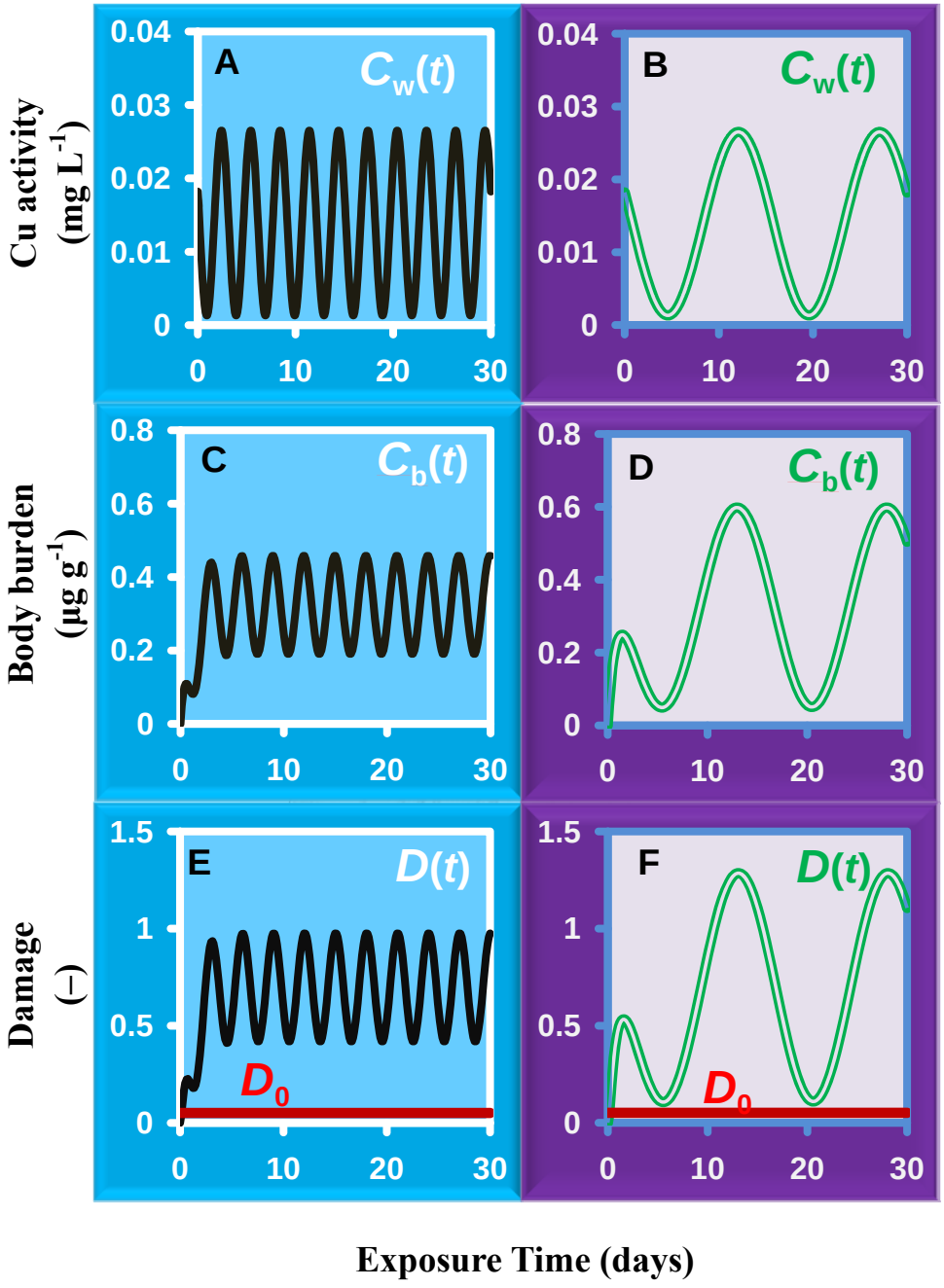


Figure 5.11. Simulations of the sine-wave Cu exposure with 3 days (left) and 15 days periods (right) for tilapia. (A, B) Sine-wave Cu activity range from 0.0013 – 0.0265 mg L^{-1}. (C, D) Body burdens. (E, F) Time course of the damage. (G, H) Hazard rate. (I, J) Cumulative hazard. (K, L) Safe probabilities.

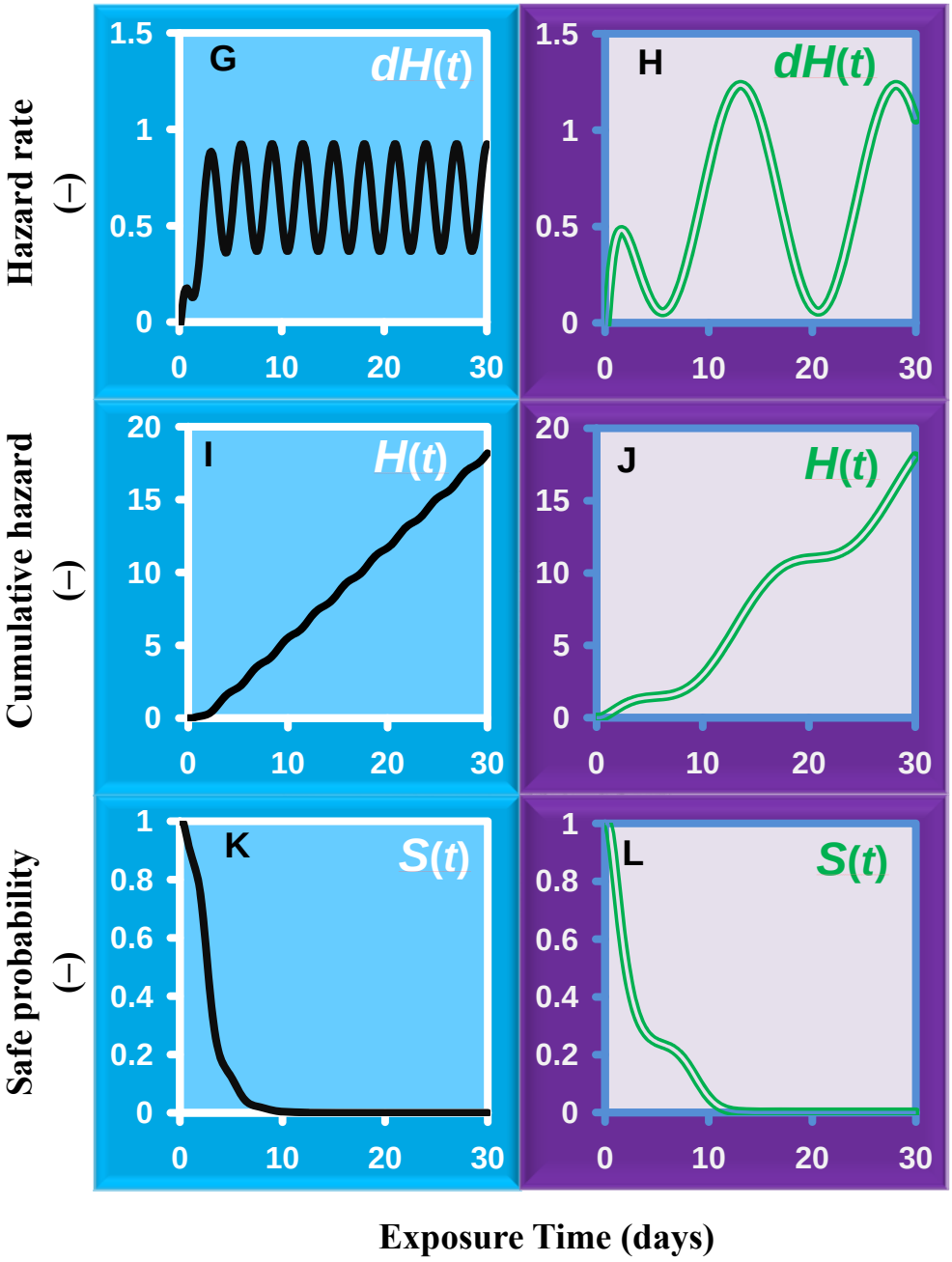


Figure 5.11. Continued.

5.1.5. Ontogenetic growth toxicity of Cu

A best-fitting of the West growth model (Eq. (3.16) could demonstrate biomass growth effect of tilapia under control and Cu exposure scenarios based on chronic growth bioassay and to estimate the model-specific parameters (growth coefficient). Fig. 15A and B showed the tilapia grow progressively body biomass in the control and pulsed Cu exposure groups. The biological species-specific growth coefficients (A_0) were estimated to be 0.029± 0.0015 $g^{1/4}$ d^{-1} (Mean±SE) for control (r^2 = 0.92) and 0.019±0.0017 $g^{1/4}$ d^{-1} for pulsed Cu exposures 100 – 300 μg L^{-1} (r^2 = 0.83). The results indicated that growth coefficient positively depends on the exposure concentrations, revealing that Cu concentration inhibited much more growth energy and affected the growth of tilapia.

To assess the growth trajectories of tilapia in various sequential pulsed and fluctuating Cu exposure scenarios, the estimated growth parameters were adopted from Tsai and Liao (2006), including the maximum body biomass in uncontaminated environment ($W_{max,0}$) of 1130 g and initial birth body biomass of 0.05 g. The biological species-specific growth coefficient A_0 of 0.029 $g^{1/4}$ d^{-1} that was estimated from chronic growth bioassay (control group) in this study.

This study did not use A_0 0.019±0.0017 $g^{1/4}$ d^{-1} from pulsed Cu exposures due to MOA of reducing food assimilation efficiency was well predicted by West growth model (Tsai and Liao, 2006). This model considers that the chemical stress will be affected by the maximum body biomass, hence the safe growth probability could provide the relationship between chemical stress and growth body biomass. Otherwise, this study was interested in the adult tilapia population. The growth cost coefficient is

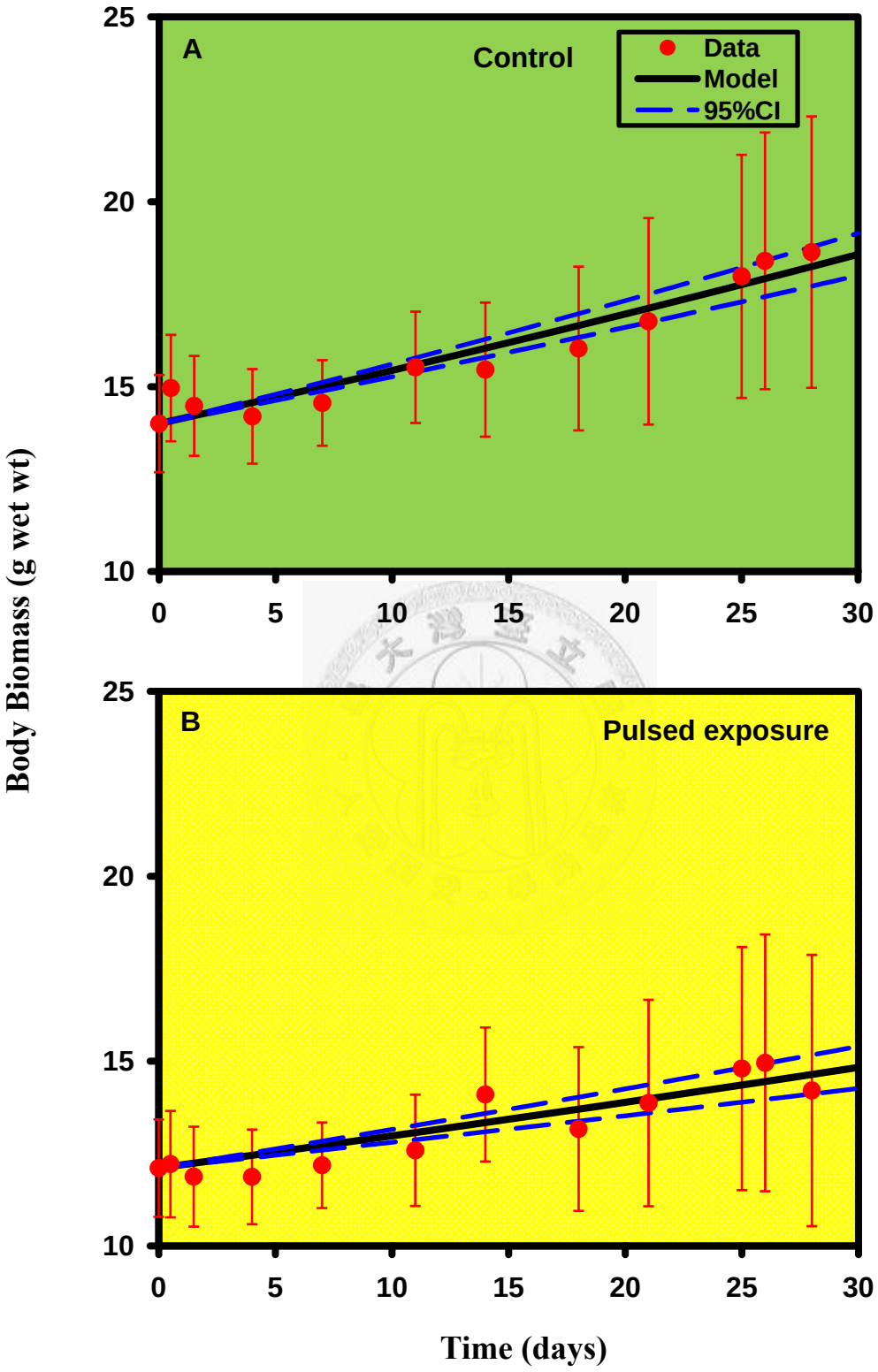


Figure 5.12. Best-fitting regression curves of body biomass to tilapia in (A) control and (B) pulsed Cu exposure groups.

not the most important factor to adult tilapia population based on the MOA and DEBtox concepts. The most energy budget is used to maintain and survive life in adult tilapia populations.

This study applied West growth model with reducing food assimilation efficiency (Eq. (4.23)) to predict the potential growth inhibition of body biomass for tilapia at life cycle day 600 (age) started to subject two period-specific sequential pulsed patterns and sine-wave Cu exposure patterns with 30 days. For pulsed and sine-wave exposure, the growth curve of body biomass revealed low Cu activity induced less body biomass losing than that of high Cu activity. In the unexposure environment, the body weight of tilapia is 129.87 g at age 630 days. The high frequency exposures (Pulsed: T_1 = day 5, T_2= day 10; Sine-wave: T = 3 days) induced more body biomass losing than that of low frequency exposures (Pulsed: T_1 = day 0.5, T_2 = day 25; Sine-wave: T = 5 days). For low Cu activity pulsed exposures, the growth body biomass at age 630 days were 107.14 g and 107.1 g, respectively, to high and low frequencies, whereas for high activity pulsed exposure, the body biomass were 68.10 g and 68.19 g, respectively, to high and low frequencies (Fig. 5.13A).

For low Cu activity sine-wave exposures, the body biomass were 12.90 g and 13.13 g, respectively, to high and low frequencies, whereas high Cu activity sine-wave exposures, the body biomass at age 630 days were lower than body biomass at birth of tilapia (0.05 g) that implied death (Fig. 5.13B). However, the high Cu activity with high frequency revealed the low growth body biomass losing than that of high Cu activity with low frequency before age 610 days. After age 610 days, the body biomass of high Cu activity with high frequency exposure was rapidly losing

than that of high Cu activity with low frequency exposure. Similarly, the same growth body biomass situation was showed in sine-wave exposures. Yet, growth body biomass of high Cu activity with high frequency exposure lower than that of high Cu activity with low frequency was after age 604 days. Overall, the results of body biomass losing revealed that there are insignificant differences between exposure frequencies.

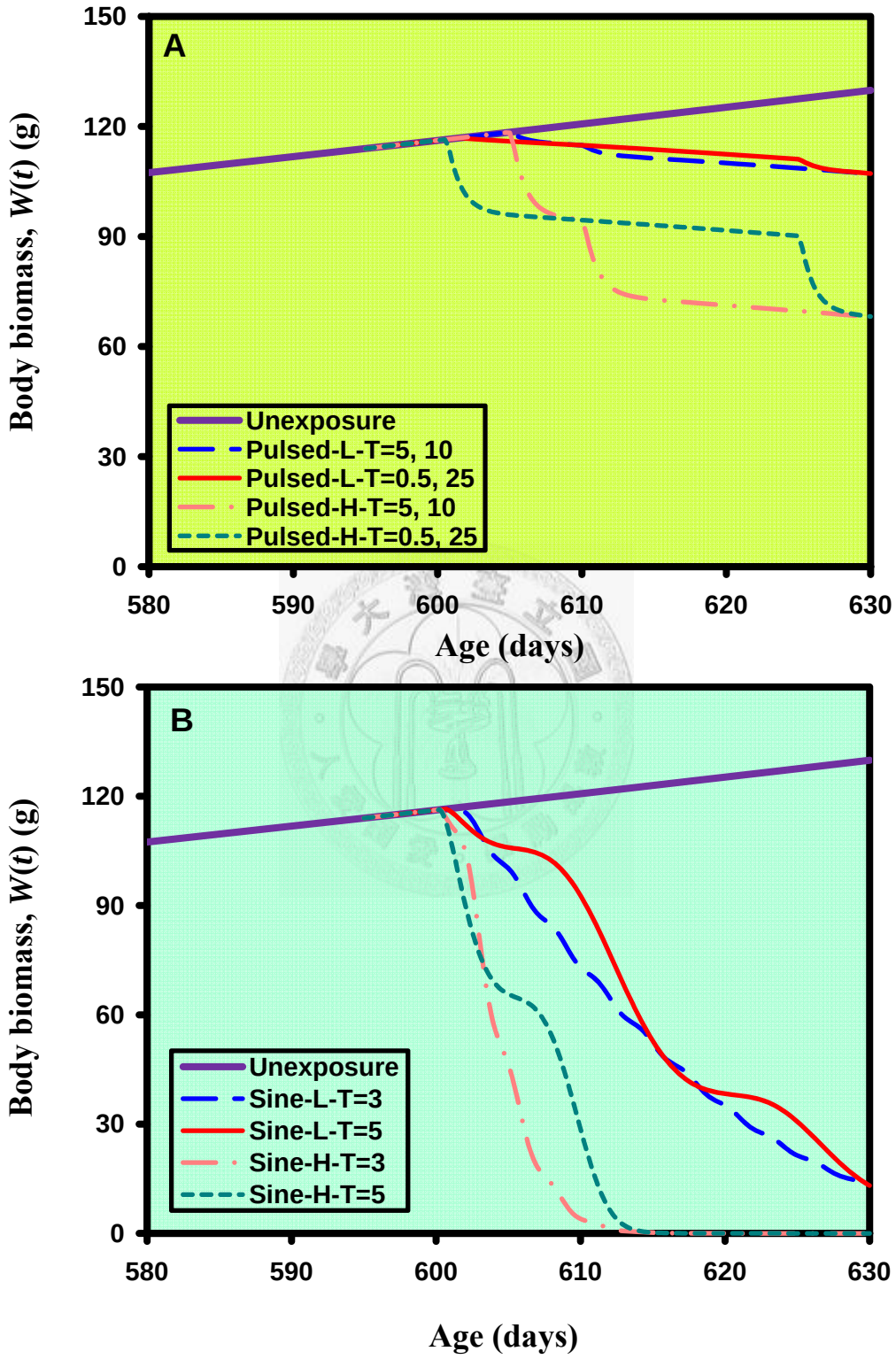


Figure 5.13. The time courses of the body biomass of tilapia with the (A) sequential pulsed and (B) sine-wave Cu patterns.

The dimensionless biomass ratio (r) was a proportion of total lifetime metabolic power used for maintenance and other activities. When the dimensionless biomass ratio value arrived at 1 means that the life span of tilapia is ended. The pulsed and sine-wave exposures revealed that the dimensionless biomass ratio of low frequency exposures were lower than that of high frequency exposures (Fig. 5.14). The allocation of metabolic energy rate to maintain survival and life, the high frequency exposures were more than that of low frequency exposures. Otherwise, the above-mentioned high Cu activity sine-wave exposures induced the mortality of tilapia, the dimensionless biomass ratio of high Cu activity sine-wave exposures could responded to these results.

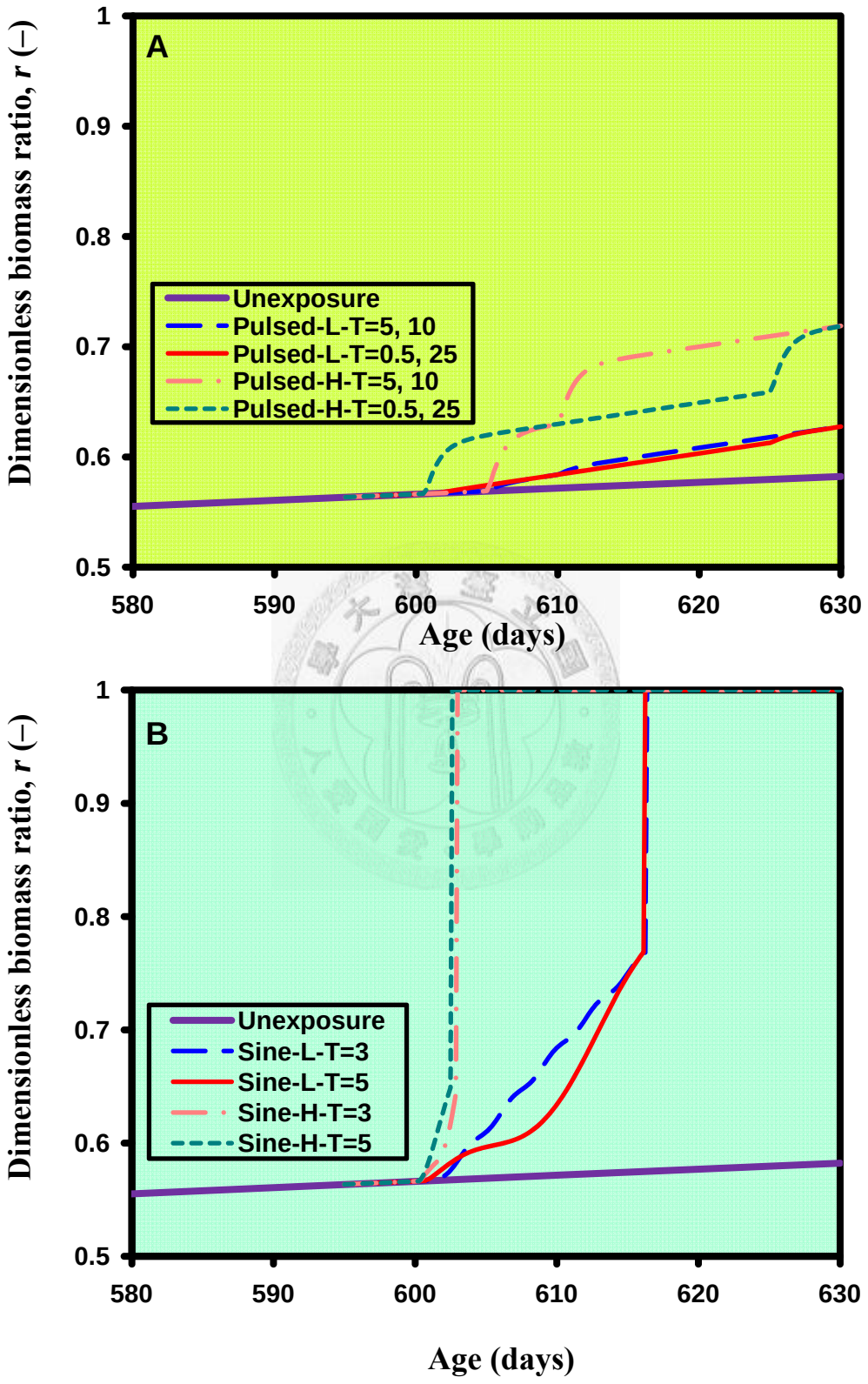


Figure 5.14. A plot of the dimensionless mass ratio for tilapia with the (A) sequential pulsed and (B) sine-wave Cu patterns.

5.2. Sequential Pulsed As Toxic Effect on Tilapia

5.2.1. Parameter estimates

5.2.1.1. Bioaccumulation factor

The rapid accumulation fashion was found in tilapia exposed to 1 mg L^{-1} As in the course of 7 days uptake phase (Fig. 5.15). The toxicokinetic rate equation in Eq. (4.4) was fitted to As accumulation data of tilapia to obtain the estimated uptake rate constant k_u of 0.489 ± 0.077 mL g^{-1} d^{-1} and elimination rate constant k_e of 0.169 ± 0.066 d^{-1} (r^2 = 0.98). The BCF values of As in tilapia was 2.893 mL g^{-1}. The results revealed that BCF values were above 1, indicating the potential As accumulation capacity when tilapia exposed to waterborne As concentration. The appearance of uptake curve implied that As did not reach steady-state in tilapia after 7 days exposure.

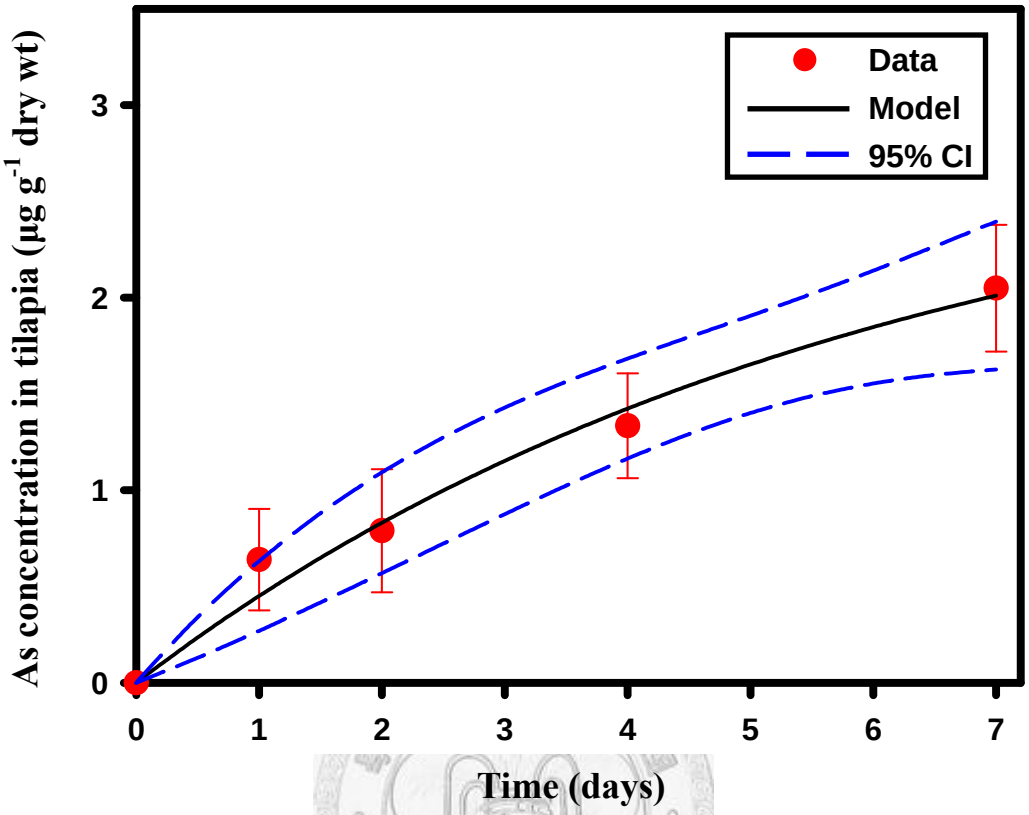


Figure 5.15. Best-fitting regression curves of As accumulation to tilapia from first-order bioaccumulation model. Error bars are standard deviation from mean.

5.2.1.2. External median effect concentration (EC50)

A Hill equation was used to best fit the growth inhibition data with the selected times of days 7, 14, 21, and 28 to determine 50% growth inhibition As activity (Fig. 5.16). The response curve relationship between growth inhibition and waterborne As activity (r^2 = 0.86 – 0.99), can be used to estimate the Hill coefficient $n(t)$, $E_{max}(t)$, and EC50(t) values. The results showed that $n(t)$s was to be 1.513, 4.800, 1.758, and 1.052 and 0.900, 0.738, 0.700, 0.680 mg L^{-1} for EC50(t) at the response times of 7, 14, 21, and 28 days, respectively.

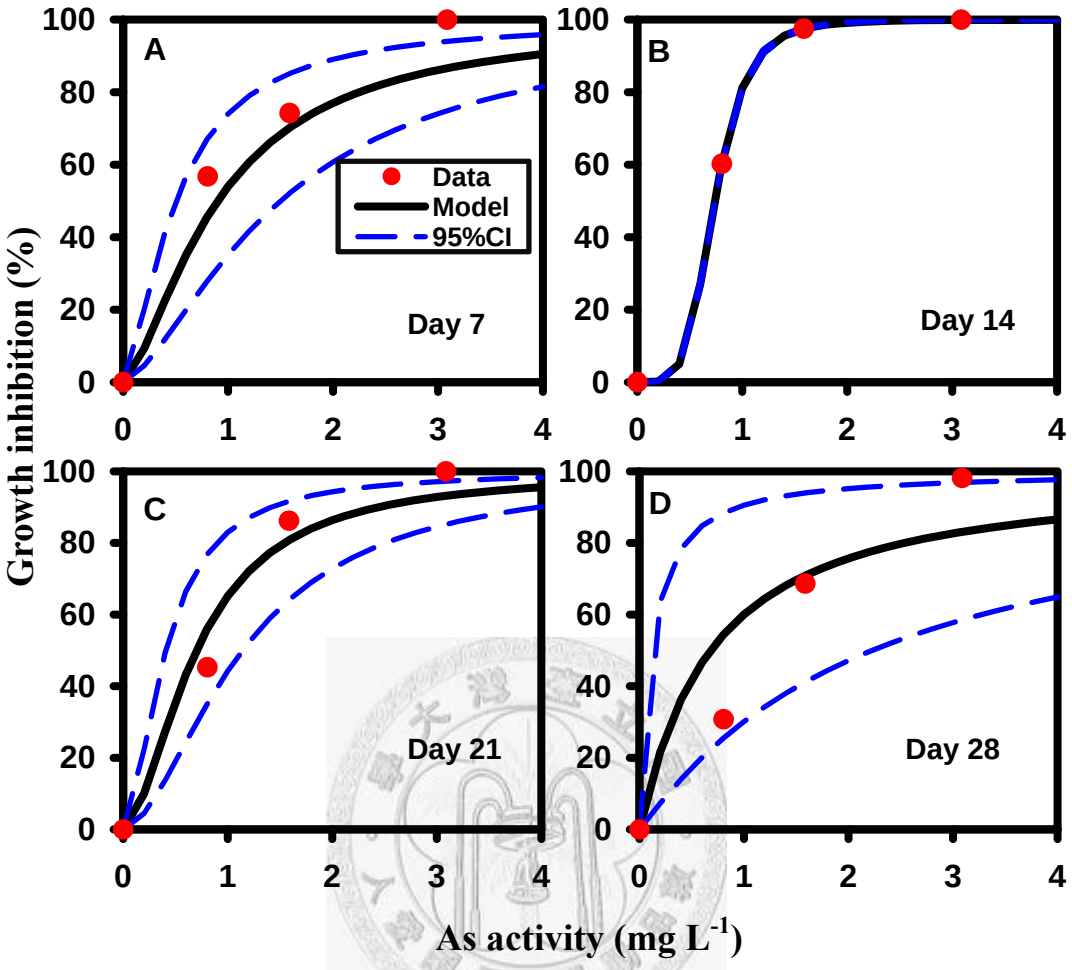


Figure 5.16. Predictions of dose-response profiles of tilapia by a Hill based model fitting to measured data varied with different integrated response time of (A) day 1, (B) day 2, (C) day 3, and (D) day 4, respectively.

5.2.1.3. Model prediction of EC50(t) data

The time-dependent EC50(t) could be estimated by fitting DAM scheme-based EC50 model (Eq.(4.13)) to Hill-based EC50(t) data of tilapia (r^2 = 0.87) (Figs 5.17). The results revealed that damage-based model was capable of describing the EC50(t). The estimated parameters compound equivalent toxic damage level for 50% effect $D_{E,50}/k_a$ of 0.199 μg g^{-1} d and recovery rate constant k_r of 9.519 d^{-1} for tilapia. The killing rate constant k_k were calculated to be 3.495 g μg^{-1} d^{-1} via ln 2/($D_{E,50}/k_a$). Table 5.3 summarizes the experimentally determined bioaccumulation (toxicokinetics) and DAM parameters for As from tilapia data.

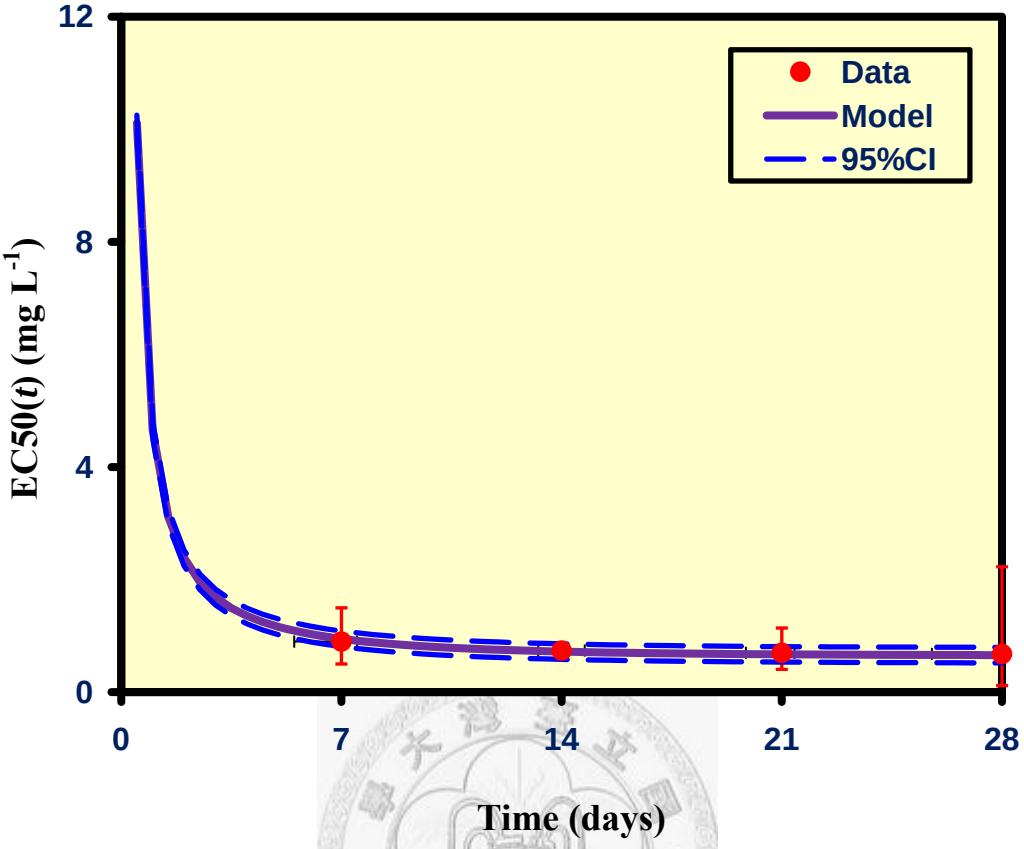


Figure 5.17. Fitting proposed DAM-EC50 equation to experimental EC50(*t*) data for tilapia.

Table 5.3. Parameter estimates of As-tilapia system for bioaccumulation and damage assessment models.

Bioaccumulation parameters [a]	
k_u (mL g^{-1} d^{-1})	0.489±0.077
k_e (d^{-1})	0.169±0.066
BCF (mL g^{-1})	2.893
r^2	0.98
DAM parameters [b]	
k_k (g μg^{-1} d^{-1})	3.495
k_r (d^{-1})	9.519
r^2	0.87

[a] Determined from uptake phase bioaccumulation experiment.

[b] Estimated from EC50(t) data.

5.2.2. Internal effects with different As exposure patterns

This study was adopted the exposure condition of water chemistry from Liao et al. (2008) to simulate the internal effect of tilapia in response to pulsed and fluctuating As.

5.2.2.1. Dynamic effect of sequential pulsed exposure

Fig. 5.18 shows the dynamics of body burden, internal damage, internal hazard rate, cumulative hazard, and safe probability for tilapia depended on high frequency (T_1 at day 5, T_2 at day 10) and low frequency (T_1 at day 0.5, T_2 at day 25) pulse period-specific sequential pulsed exposure in As concentration 0.1 – 2.1 mg L^{-1} (As activity: 0.061 – 1.270 mg L^{-1}). The used pulsed period-specific As sequential pulsed exposures have the forms of $\{C_w(t)\} = C_0 + C_1 \sum_{n=1}^{2} \delta(t - n \cdot T)$ with T=5 days (Fig. 5.18A) and $\{C_w(t)\} = C_0 + C_1 \cdot (\delta(t - T_1) + \delta(t - T_2))$ with T_1 = 0.5 and T_2 = 25 days (Fig. 5.18B) varying with C_0 = 0.061 and C_1 = 1.209 mg L^{-1}.

Fig. 5.18C and D showed that tilapia continued to increase body burden of As after the 1st As pulsed exposure, even tilapia were at the low external As activity environment. However, As body burden of tilapia gradually increases after the tilapia in response to the 2nd As pulsed exposure. On the 1st pulsed As exposure timing, the As accumulative burden of tilapia was 0.126 and 0.039 μg g^{-1}, respectively, to high and low frequency patterns, whereas the As accumulative burden of the 2nd pulsed exposure timing were 0.179 and 0.199 μg g^{-1}, respectively, to high and low frequency patterns.

The internal damage was proportional to the body burden. Hence, the damage

curve was similar to body burden curve at high and low frequency patterns (Fig. 5.18E and F). This study used {EC5} = 48 μg L^{-1}, the effect activity that caused 5% effect, to calculate the damage threshold (D_0), resulted in D_0 = 0.051. When damage exceeded the damage threshold, the time-course hazard rate rises above zero (Fig. 5.18G and H). The cumulative hazard of both sequential pulsed frequency exposures shows the similar levels of 0.123 and 0.101 on day 30 for high and low frequency patterns, respectively (Fig. 5.18I and J). Hence, the results showed that cluster pulsed exposure have similar median safe time (ST50 = 72.33 days) with that of less cluster pulsed exposure (ST50 = 72.35 days) (Fig. 5.18K and L).

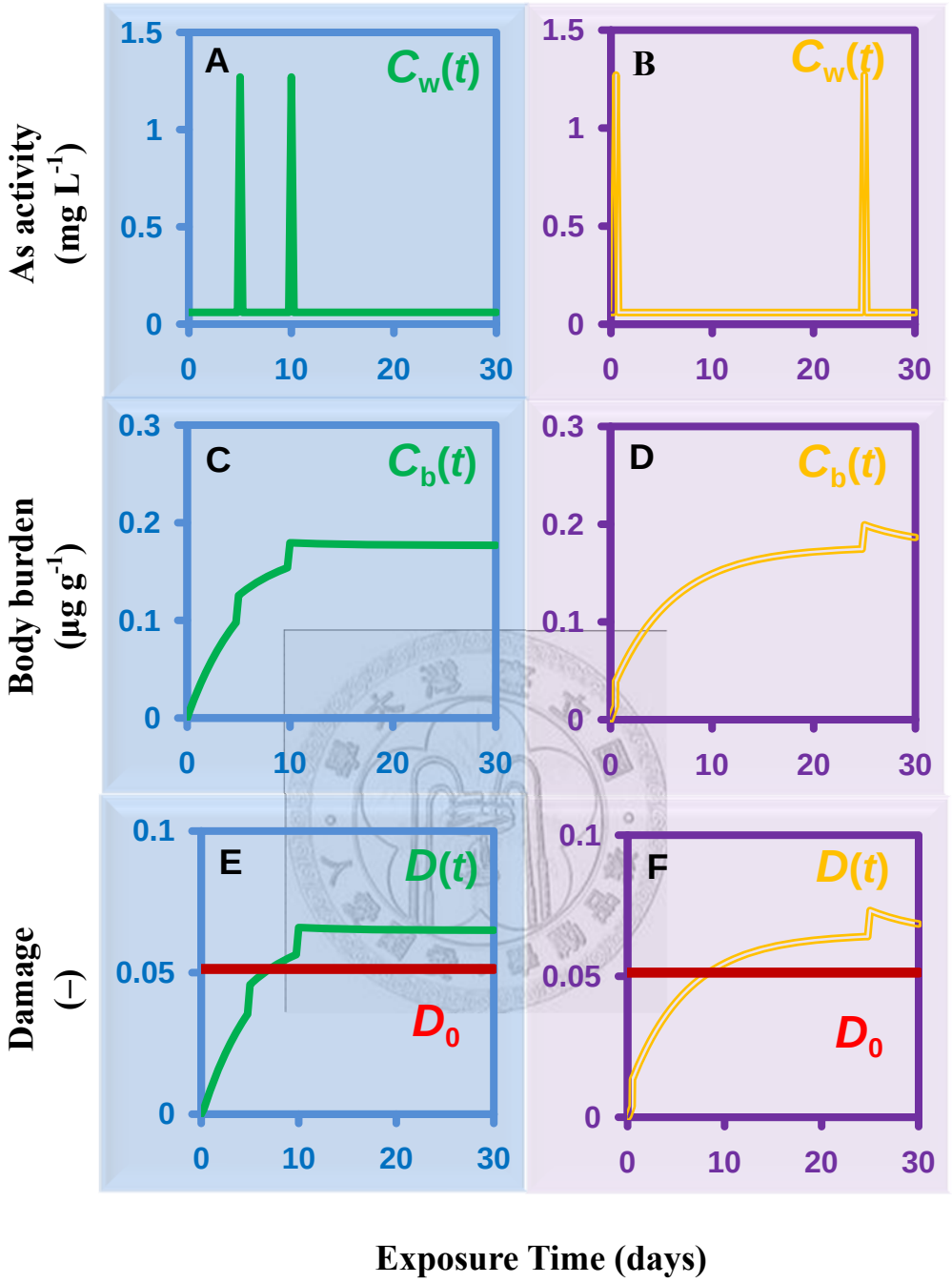


Figure 5.18. Simulations of the sequential pulsed As exposure with 5 days pulse periods (left), and 0.5 and 25 days pulse periods (right) for tilapia. (A, B) Sequential pulsed As activity range from 0.061 – 1.270 mg L^{-1}. (C, D) Body burdens. (E, F) Time course of the damage. (G, H) Hazard rate. (I, J) Cumulative hazard. (K, L) Safe probabilities.

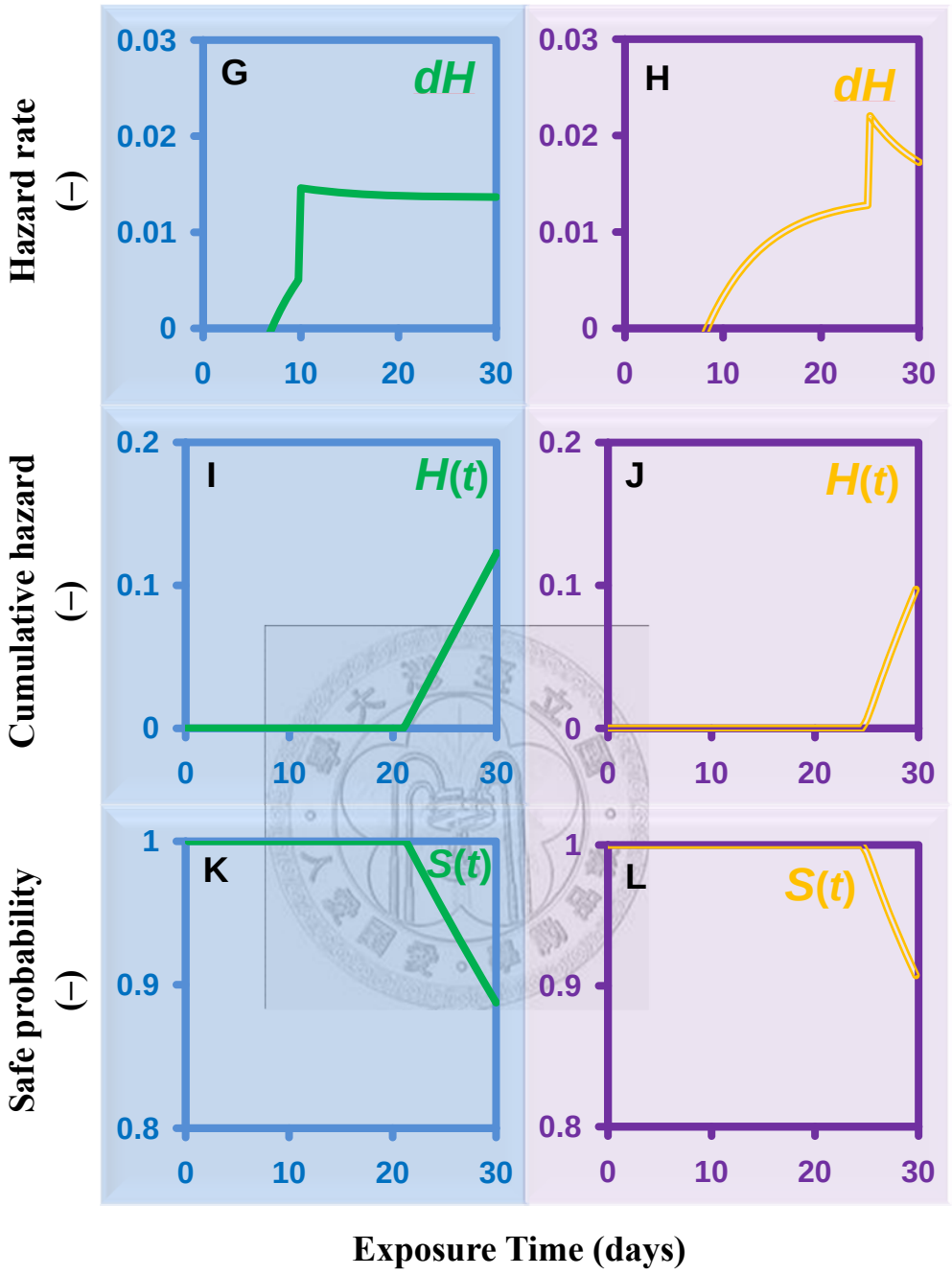


Figure 5.18. Continued.

The study used the higher pulsed concentration with high and low pulsed frequent patterns (C_1 = 11.054 mg L^{-1}) to investigate the internal effect in that the exposure concentration ranged from 0.1 – 20.1 mg L^{-1} of total As concentration (i.e., As activity: 0.061 – 11.115 mg L^{-1}) (Fig. 5.19A and B). Fig. 5.19C and D show that As body burden of tilapia were increased with increasing external As concentration, and were decreased with decreasing of As concentration. On the 1st pulsed As exposure timing, the As accumulative body burdens of tilapia were 0.326 and 0.240 μg g^{-1}, respectively, to high and low frequency patterns, whereas the As accumulative burdens of the 2nd pulsed exposure timing were 0.466 and 0.403 μg g^{-1}, respectively.

The damages of tilapia on the 1st pulsed exposure timing were 0.121 and 0.088, respectively, to high and low frequency patterns, whereas the damages of the 2nd pulsed exposure timing were 0.173 and 0.149, respectively (Fig. 5.19E and F). When the damage exceeded the damage threshold of 0.051, the hazard rate will be triggered (Fig. 5.19G and H). The cumulative hazard reflects the toxic potency of the As to tilapia, indicating that the high frequency exposure resulted in 0.973 of cumulative hazard, whereas 0.779 on day 30 for the low frequency exposure (Fig. 5.19I and J). Hence, the results of safe probability showed that cluster pulsed exposure had shorter ST50 (18.75 days) than that of less cluster pulsed exposure (ST50 = 28.50 days) (Fig 5.19K and L), indicating that the pulsed timing and sequence for tilapia exposed to As could be mattered.

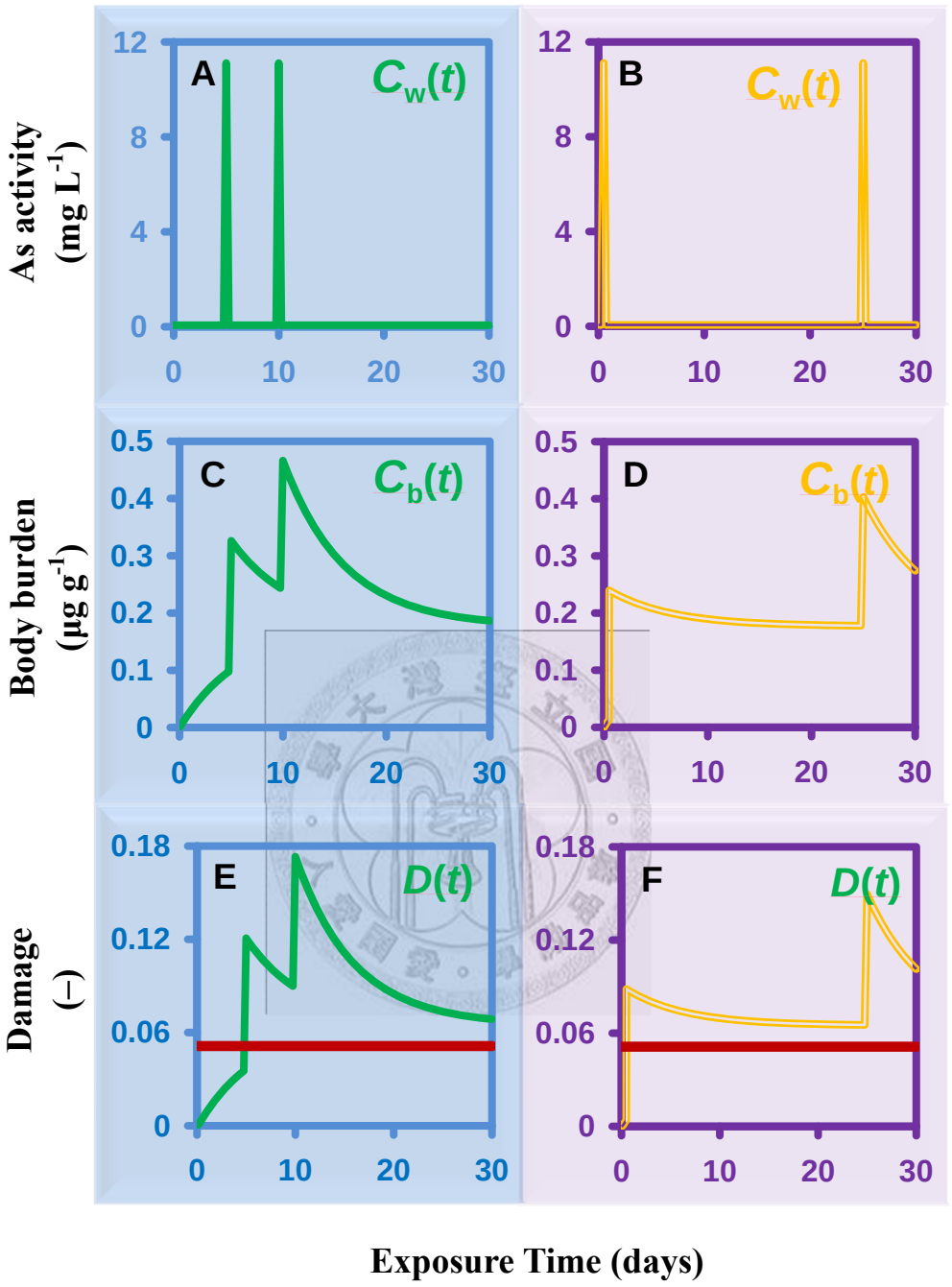


Figure 5.19. Simulations of the sequential pulsed As exposure with 5 days pulse periods (left), and 0.5 and 25 days pulse periods (right) for tilapia. (A, B) Sequential pulsed As activity range from 0.061 – 11.115 mg L^{-1}. (C, D) Body burdens. (E, F) Time course of the damage. (G, H) Hazard rate. (I, J) Cumulative hazard. (K, L) Safe probabilities.

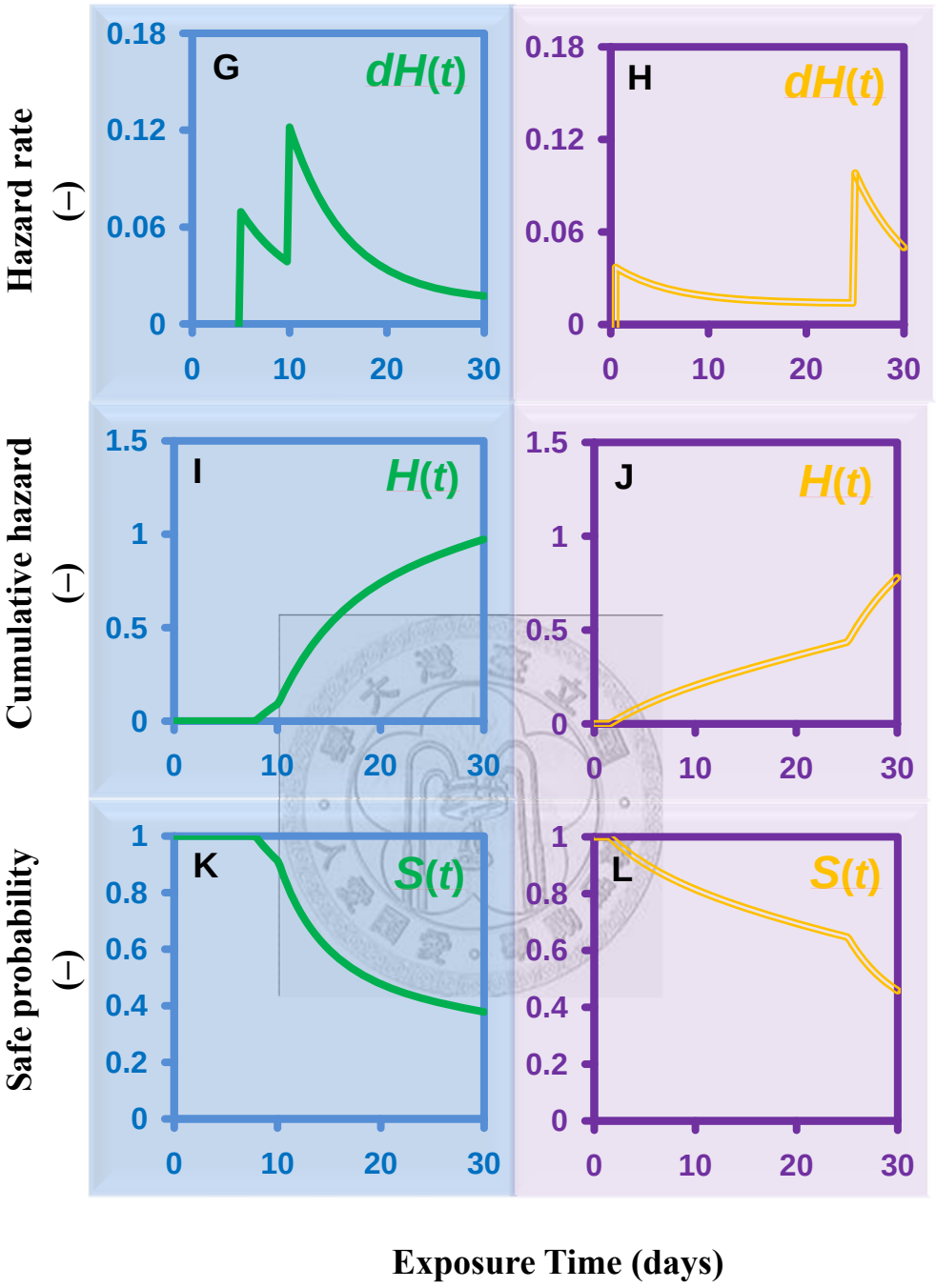


Figure 5.19. Continued.

5.2.2.2. Dynamic effect of fluctuating exposure

The study used the As activity varying sinusoidally over a range of periods $\{C_w(t)\} = C_0 + C_1 \sin(2\pi t / T + d)$ with low (C_0 = 0.183 and C_1 = 0.122 mg L^{-1}) and high (C_0 = 0.638 and C_1 = 0.577 mg L^{-1}) activities to examine the underlying dynamic responses of tilapia in response to the periodic patterns. The sine-wave exposure pattern was chosen so that input As loading would mimic the cycling changes in As concentration observed in the As-rich stream and pond system (Gammons et al., 2007).

Tilapia in response to sine-wave patterns between 0.061 – 0.305 mg L^{-1} and 0.061 – 1.215 mg L^{-1} As activity were simulated with the period, T = 3 days (Figs. 5.20A and 5.21A) and 15 days (Figs. 5.20B and 5.21B), respectively. The As body burdens of tilapia were 0.553 and 0.657 μg g^{-1}, respectively, to high and low frequencies when exposed to 0.061 – 0.305 mg L^{-1} As activities on day 30 (Fig. 5.20C and D), whereas that of exposed to 0.061 – 1.215 mg L^{-1} As activities were 1.963 and 2.424 μg g^{-1}, respectively, to high and low frequency patterns (Fig. 5.21C and D).

When tilapia exposed to high As activity, Figs. 5.21E and F reveal that damage of tilapia were 0.707 and 0.890, and that of tilapia were 40.504 and 49.877, respectively, to high and low frequency patterns. The hazard rates with sine-wave exposure to tilapia were shown in Figs. 5.20G, H and 5.21G, H. The cumulative hazards of tilapia were 3.069 and 2.839 (low concentration), and 14.251 and 13.177 (high concentration), respectively, to high and low frequency (Figs. 5.20I, J and 5.21 I, J).

Finally, the results indicated that tilapia in response to smaller periodic change (i.e., high frequency, low concentration: ST50 = 12.37 days, high concentration: ST50 = 5.20 days) experienced much shorter ST50 than that of greater periodic (i.e. low frequency, low concentration: ST50 = 11.45 days, high concentration: ST50 = 5.12 days) fluctuation (Figs. 5.20K and 5.21K).

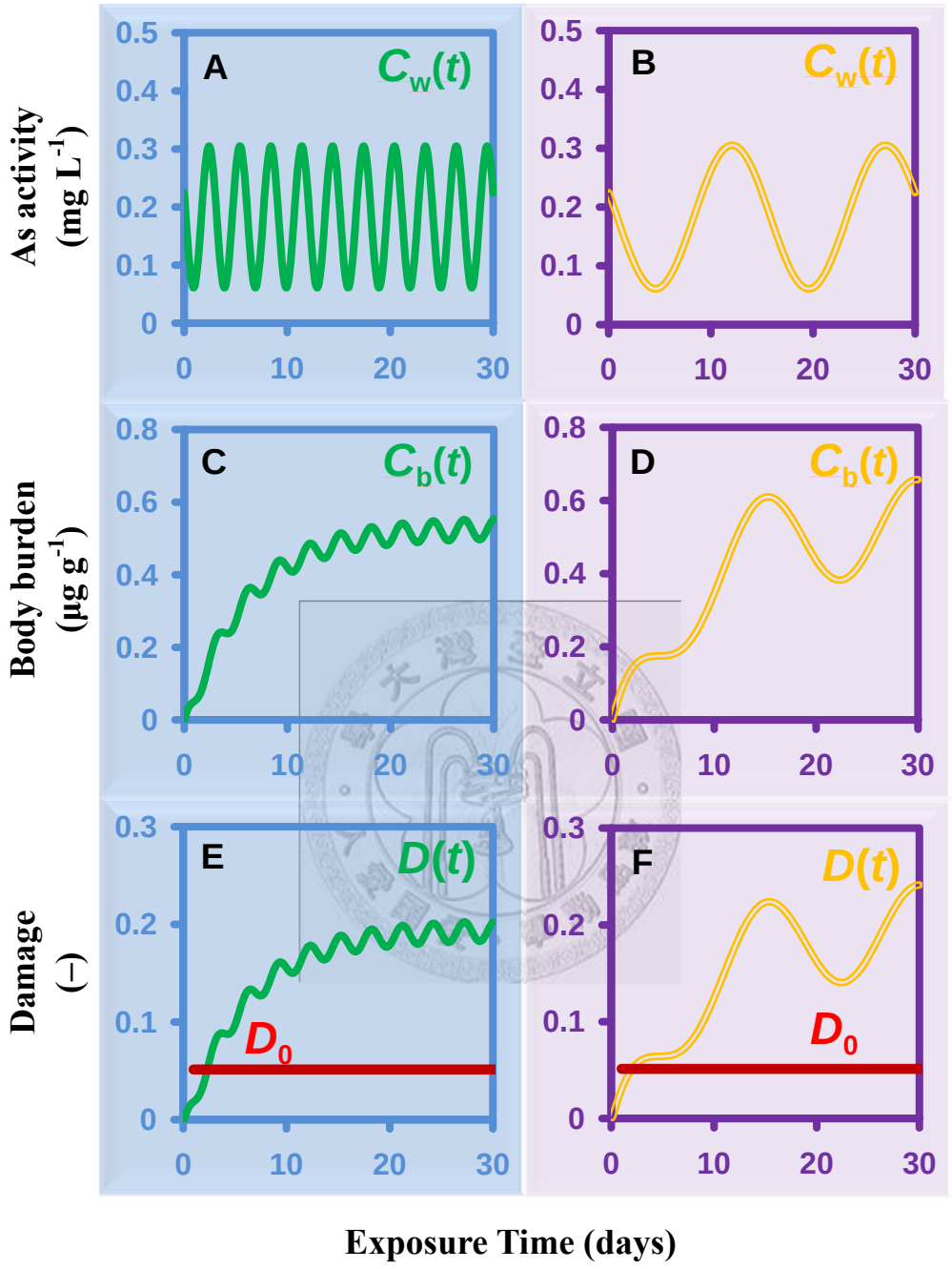


Figure 5.20. Simulations of the sine-wave As exposure with 3 days (left) and 15 days periods (right) for tilapia. (A, B) Sine-wave As activity range from 0.061 – 0.305 mg L^{-1}. (C, D) Body burdens. (E, F) Time course of the damage. (G, H) Hazard rate. (I, J) Cumulative hazard. (K, L) Safe probabilities.

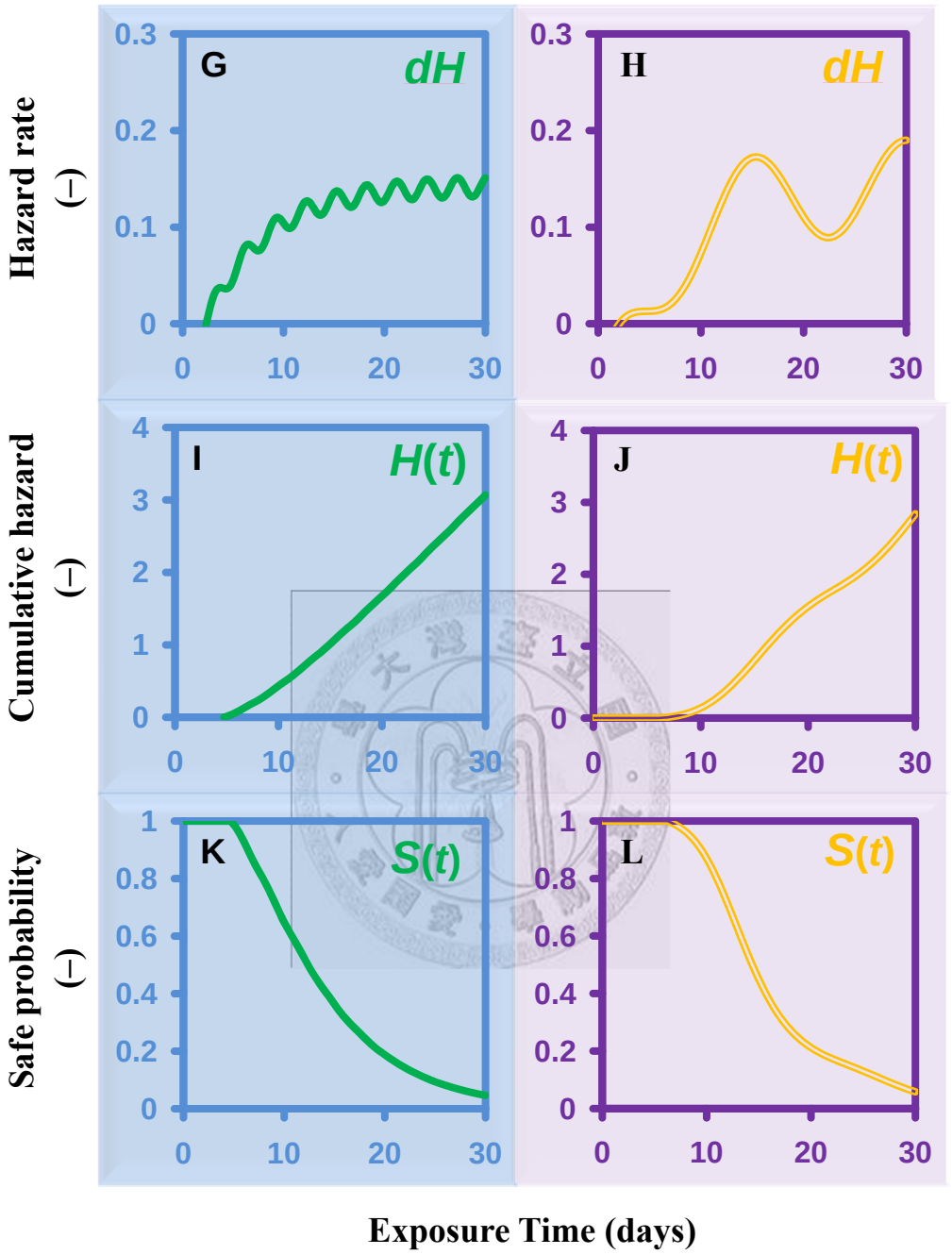


Figure 5.20. Continued.

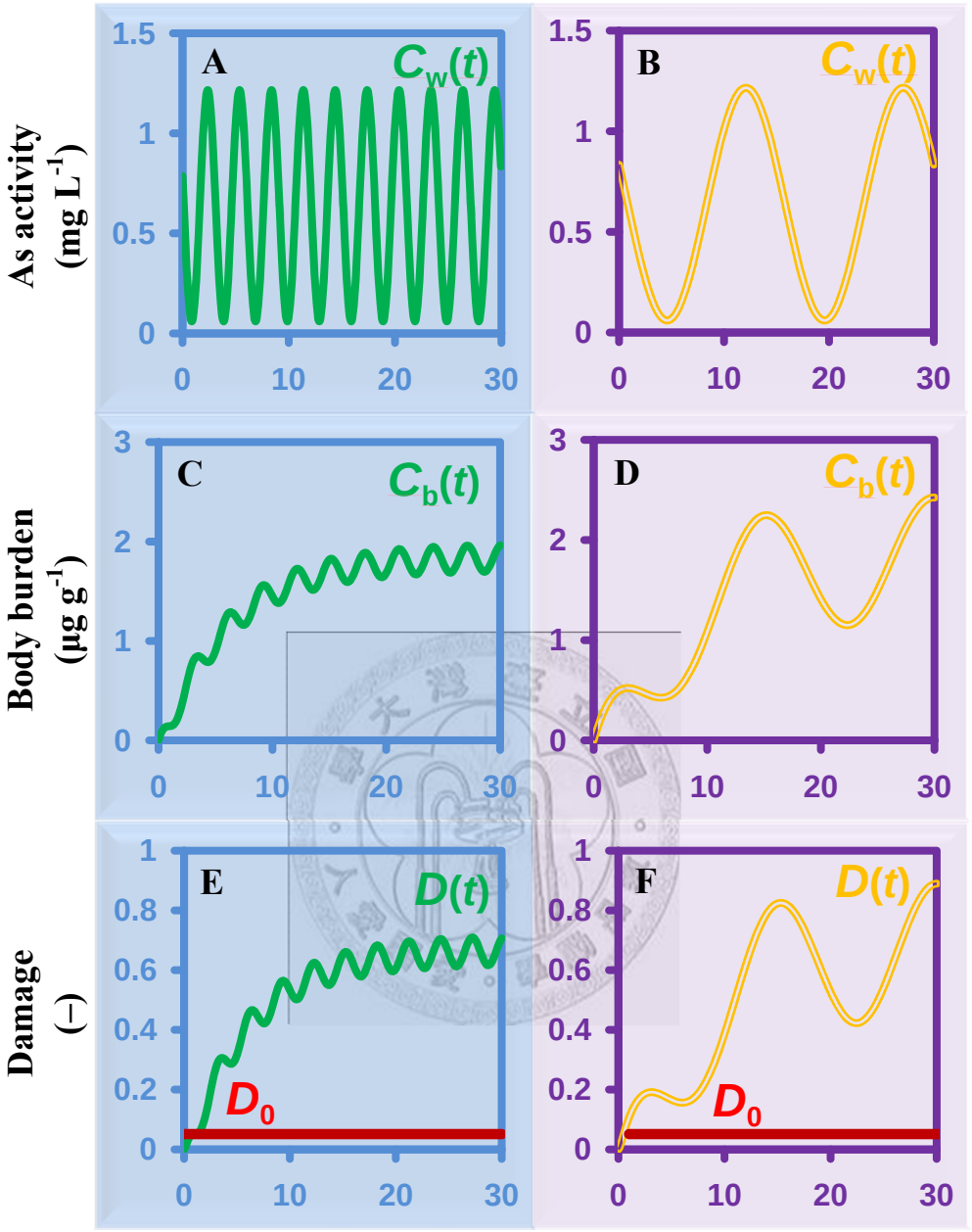


Figure 5.21. Simulations of the sine-wave As exposure with 3 days (left) and 15 days periods (right) for tilapia. (A, B) Sine-wave As activity range from 0.061 – 1.215 mg L^{-1}. (C, D) Body burdens. (E, F) Time course of the damage. (G, H) Hazard rate. (I, J) Cumulative hazard. (K, L) Safe probabilities.

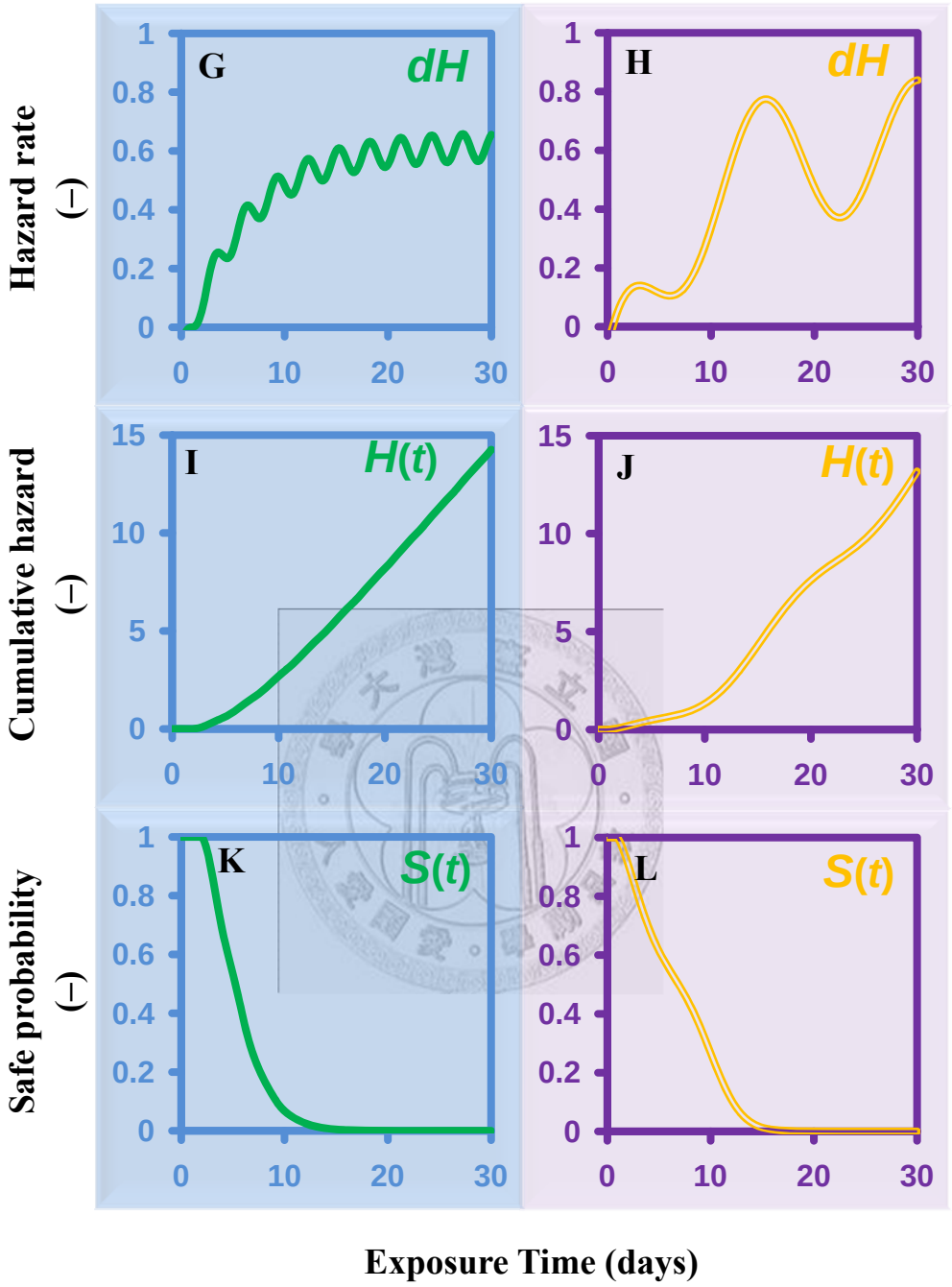


Figure 5.21. Continued.

5.2.3. Ontogenetic growth toxicity of As

To assess the growth trajectories of tilapia in different fluctuating As exposure scenarios, the valuable estimated growth parameters were adopted from Tsai and Liao (2006). The maximum body biomass in uncontaminated environment, $W_{max,0}$ is 1130 g and a biological species-specific growth coefficient A_0 is 0.029 $g^{1/4}\ d^{-1}$ that were estimated from chronic growth bioassay (control growth) in this study. The estimated critical parameters were based on the resembling aquacultural manipulation of tilapia farms. This study applied West growth model with reducing food assimilation efficiency (Eq. (4.23)) to predict the potential growth inhibition of body biomass for tilapia at life cycle day 600 (age) started to subject two period-specific sequential pulsed patterns and sine-wave As exposure patterns with 30 days.

For pulsed and sine-wave exposure, the growth curve of body biomass revealed low As activity induced less body biomass losing than that of high As activity. In the unexposure environment, the body weight of tilapia is 129.87 g at age 630 days. The high frequency exposures (Pulsed: T_1 = day 5, T_2 = day 10; Sine-wave: T = 3 days) induced more body biomass losing that that of low frequency exposures (Pulsed: T_1 = day 0.5, T_2 = day 25; Sine-wave: T = 5 days). For low As activity pulsed exposures, the growth body biomass at day 630 were 124.02 g and 125.02 g respectively to high and low frequencies, whereas for high activity pulsed exposure, the body biomass were 87.08 g and 94.88 g respectively to high and low frequencies (Fig. 5.22A).

For low As activity sine-wave exposures, the body biomass were 27.27 g and 31.65 g respectively to high and low frequencies, whereas high As activity sine-wave exposures, the body biomass at age 630 days were lower than body biomass at birth of

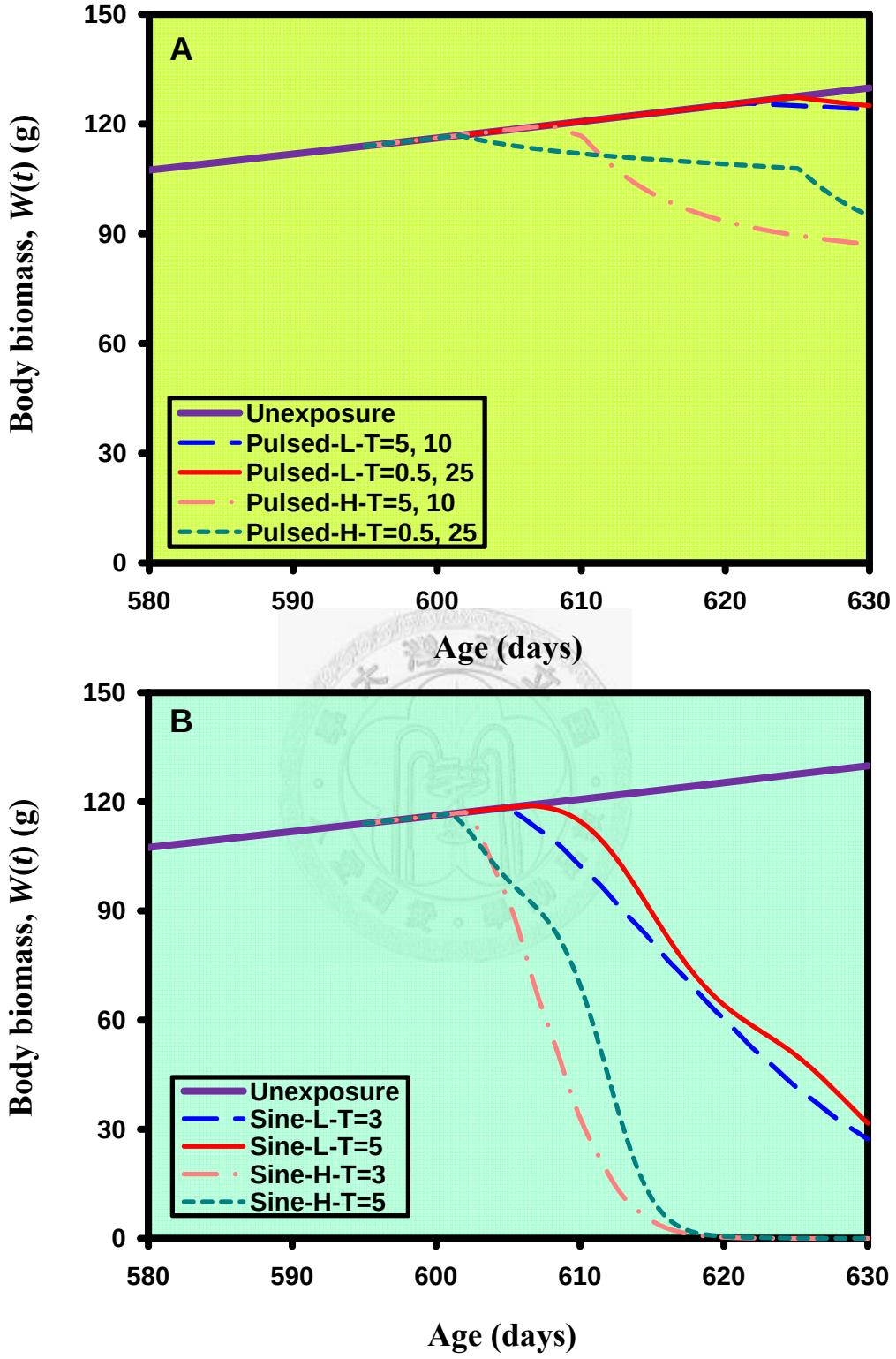


Figure 5.22. The time courses of the body biomass of tilapia with the (A) sequential pulsed and (B) sine-wave As patterns.

tilapia (0.05 g) that implied death (Fig. 5.22B). However, the high As activity with revealed the low growth body biomass losing than that of high As activity with low frequency before day 610. After day 610, the body biomass of high As activity with high frequency exposure was rapidly losing than that of high As activity with low frequency exposure. Similarly, the same growth body biomass situation was showed in sine-wave exposures. Yet, growth body biomass t of high As activity with high frequency exposure lower than that of high As activity with low frequency was after day 604.

The pulsed and sine-wave exposures revealed that the dimensionless biomass ratio (r) of low frequency exposures were lower than that of high frequency exposures (Fig. 5.23). The allocation of metabolic energy rate to maintain survival and life, the high frequency exposures were more than that of low frequency exposures. Otherwise, the above-mentioned high As activity sine-wave exposures induced the mortality of tilapia, the dimensionless biomass ratio of high As activity sine-wave exposures could be responded to these results.

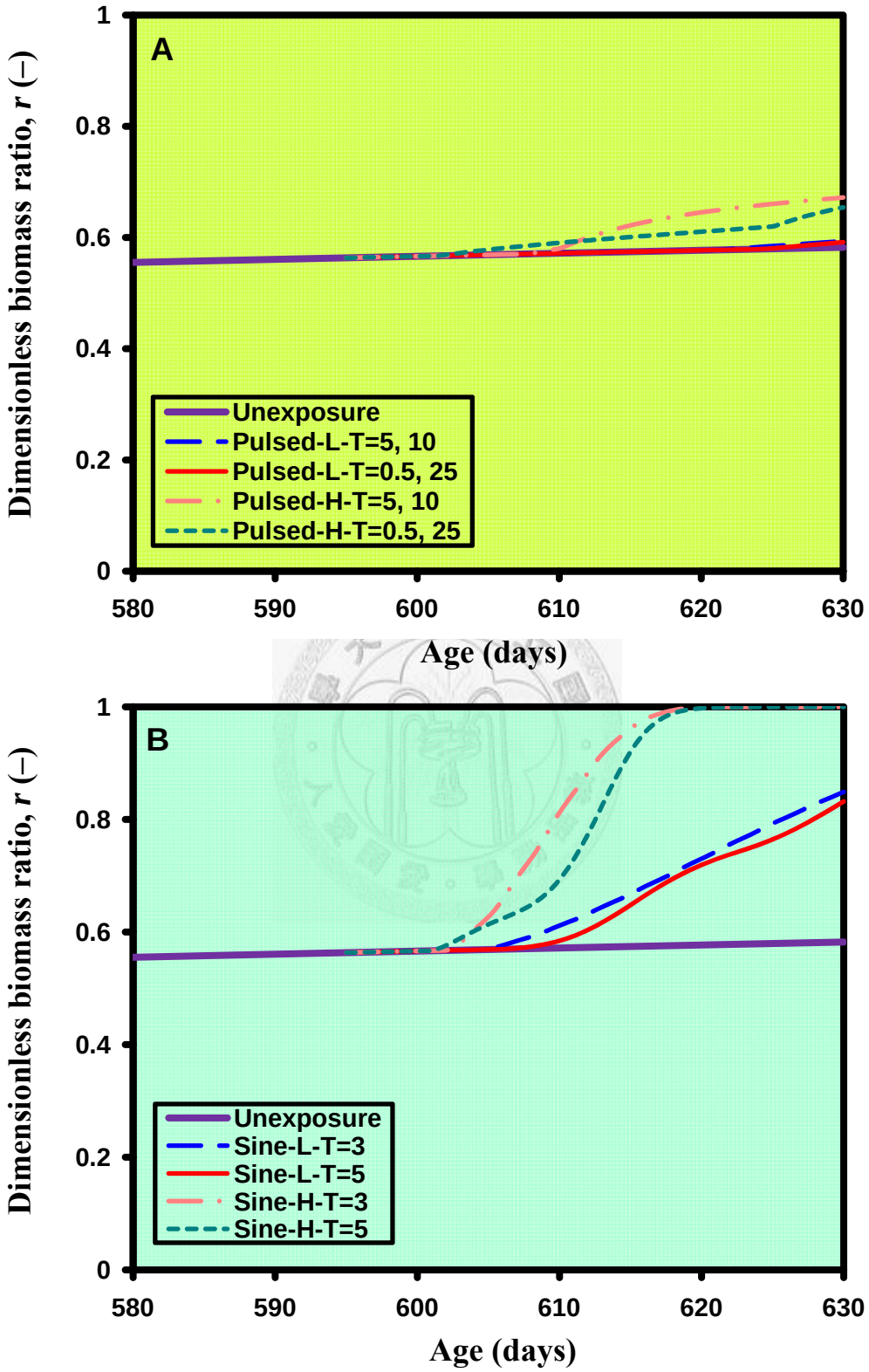


Figure 5.23. A plot of the dimensionless mass ratio for tilapia with the (A) sequential pulsed and (B) sine-wave As patterns.

5.3. Discussion

The pulsed Cu acute accumulation of tilapia in whole body increased significantly between the first pulsed timing and second timing under the 300 μg L^{-1} pulsed Cu concentration for juvenile and adult tilapia population. This result showed that Cu accumulative capacity depends on the ambient Cu concentration. Furthermore, the tilapia accumulated high Cu concentration in the body at the second pulsed timing than that at the first pulsed timing in acute exposure bioassays, whereas the chronic accumulation exposure bioassay cannot find this trend. Hoang et al. (2007) indicated that the toxicity of second pulsed exposure was positively influenced by the first pulsed exposure, depending on the pulsed concentration, duration, and the interval between the pulsed exposures.

The result of bioconcentration factor was agreed with the result from Pelgrom et al. (1994) in the short-term Cu exposure. Pelgrom et al. (1994) pointed out that BCF values of Cu for feeding tilapia ranged from 63.544 to 107.536 mL g^{-1} with Cu concentrations of 100 – 400 μg L^{-1} exposures at 96-h, respectively. In this study, the tilapia were exposed to background concentration of 100 μg L^{-1} and pulsed concentration of 300 μg L^{-1} Cu, resulting in the BCF value was 94 mL g^{-1} that fell within the range of 63.544 to 107.536 mL g^{-1}.

This thesis proposed BLM-based toxicokinetic model to estimate time- and ions-dependent BCF({*ions*}, *t*), the result found that BCF({*ions*}, t = 28 days) value 25.3 mL g^{-1} was approximate to the experimental result of pulsed Cu chronic accumulation bioassay (BCF = 23 mL g^{-1}). Otherwise, BCF of the different exposure periods revealed that the short-term BCF 94 mL g^{-1} was higher than that of long-term

23 mL g^{-1}. The results were consistent with BCF decreased with the time from BLM-based toxicokinetic model prediction.

Although this study was focused on the BCF of waterborne Cu to tilapia, tilapia was fed with fish food daily during the exposure period. The fish with feeding food or without food were different in Cu accumulative capacities. The reason may due to the physiological consequence of fish food limited that determines the ability to regulate external Cu (Pelgrom et al., 1994). The variation of accumulative level of this study may exist, since the fish food may contain Cu residue. Hence, the accumulation results of this study were compared with that of feeding tilapia in Pelgrom et al. (1994). However, the accumulation capacity in feeding fish was similar to that of without feeding when tilapia exposed to 100, 200, and 400 μg L^{-1} Cu (Pelgrom et al., 1994).

The results of this study indicated that predominant exposure frequencies of Cu and As pulses may switch the safe probability for tilapia. In accordance with Diamond et al. (2006), frequency and recovery time between pulses have significant effects on the responses of aquatic organisms. The contrast in physiological response suggests that during the pulsed/fluctuating Cu and As exposure, the recovery time that affects tilapia safety may reflect different fashions. The recovery time can be estimated from recovery rate constant estimates (i.e., recovery time = $1/k_r$) that obtained by fitting the first-order damage model (Eq. (5.1)) to time-dependent in each sequential pulsed and sine-wave Cu and As exposures.

The findings show that damage recovery times of nearly 1.76 – 2.50 days and

0.167 – 0.17 days for tilapia exposed to cluster and less cluster sequential pulsed Cu exposures, respectively; whereas for sequential pulsed As exposures, the recovery times were estimated to be 0.83 – 3.06 days and 0.71 – 0.74 days for high and low frequency sequential pulsed patterns, respectively (Table 5.4).

For period-specific fluctuating Cu exposures, recovery times increased with the increasing of period: 1.69 – 1.85 days and 6.59 – 6.77 days for tilapia after sine-wave exposures with T = 3 and 15 days, respectively; whereas for fluctuating As exposures, recovery times were estimated to be 7.48 – 9.95 days and 14.08 – 19.10 days for high and low frequency fluctuating patterns, respectively (Table 5.4). The low frequency fluctuating exposures (T = 15) need more recovery time due to the high concentration exposure for a long period. The cumulative exposure concentrations of low frequency fluctuating exposures were higher than that of high frequency fluctuating exposures in the initial exposure time.

In the results of tilapia internal responses, the long duration between pulses had higher safe probability than that of short duration. Previous study indicated that some level of elimination or detoxification to chemical during the chemical-free period may decrease the toxic effect, depending on the duration between pulses (Reinert et al., 2002; Zhao and Newman, 2006). The recovery time was a critical factor to influence the safe/survival capacities to aquatic organisms for chemical pulsed exposures. Diamond et al. (2006) demonstrated that the longer recovery times were associated with diminished hazardous effect of sequential chemical pulses, whereas the more significant hazard effects were observed at the more closely spaced pulses of exposure.

Table 5.4. Recovery time estimates (mean with 95% CI) for tilapia after sequential pulsed and fluctuating Cu and As exposures.

		Recovery time (d)
		Cu-Tilapia
Sequential pulsed		
0.03 – 0.6 mg L^{-1}	High frequency	1.76 (0.76 – 5.32)
	Low frequency	0.17 (0.04 – 2.48)
0.03 – 6 mg L^{-1}	High frequency	2.50 (0.60 – 29.28)
	Low frequency	0.17 (0.02 – 36.36)
Fluctuating		
0.03 – 0.15 mg L^{-1}	High frequency	1.85 (1.41 – 2.68)
	Low frequency	6.77 (4.81 – 11.42)
0.03 – 0.6 mg L^{-1}	High frequency	1.69 (1.26 – 2.60)
	Low frequency	6.56 (4.64 – 11.17)
		As-Tilapia
Sequential pulsed		
0.1 – 2.1 mg L^{-1}	High frequency	0.83 (0.30 – 3.12)
	Low frequency	0.74 (0.39 – 7.72)
0.1 – 20.1 mg L^{-1}	High frequency	3.06 (1.71 – 14.97)
	Low frequency	0.71 (0.40 – 3.25)
Fluctuating		
0.1– 0.5 mg L^{-1}	High frequency	9.95 (9.26 – 10.75)
	Low frequency	19.10 (14.15 – 29.39)
0.1 – 2 mg L^{-1}	High frequency	7.48 (7.07 – 7.94)
	Low frequency	14.08 (10.93 – 19.76)

The above results of internal responses for tilapia exposed to pulsed/fluctuating exposure support this finding. The internal responses of second Cu/As pulse for healthy aquatic organisms were similar to that of the primary Cu/As pulse. Besides, the adaptive mechanisms are triggered if the plenty of recovery time existed between pulses (Fig. 5.2). However, if effective pulse concentrations occur, the aquatic organisms will respond to the toxic effect associated with previous pulses situation. According the results of internal effect and growth response, this study found that Cu had a higher toxic effect for tilapia than that of As. Owing to the tilapia had high ST50, elimination rate constant, and damage recovery rate and low accumulative capacity and killing rate constant for As exposures than that of Cu exposures.

This study chose the water chemistry characteristics of groundwater located at Hsinchu, Yilan, Hualien, and Tainan area to implement the proposed model to predict the site-specific BCF($\{ions\},t$), $f_{\mathrm{CuBL}}^{50\%}(t)$, and the damage risk of Cu exposures for tilapia population. The water chemistry characteristics were different to each other (Table 5.5). This study used the base Cu concentration 0.1 mg L^{-1} and the sequential pulsed concentration 2.4 mg L^{-1} to evaluate aquatic Cu exposure risk. The Cu activity depends on the abundance of the water chemistry, and therefore revealed the different Cu activities in each location. The estimated $f_{\mathrm{CuBL}}^{50\%}(t)$ was decreasing from 0.94 – 0.53, 0.95 – 0.75, 0.94 – 0.55, and 0.52 – 0.07, respectively, for Hsinchu, Yilan, Hualien, and Tainan at the same median effect level (Fig. 24A, D, G, and J).

The maximum BCF($\{ions\},t$) estimates were 39.51, 106.56, 42.25, and 2.636 for Hsinchu, Yilan, Hualien, and Tainan, respectively (Fig. 24B, E, H, and K). According to the estimated site-specific $f_{\mathrm{CuBL}}^{50\%}(t)$ and BCF($\{ions\},t$), this study found that the

lowest damage risk for tilapia population appeared in Tainan, whereas the highest damage risk was found in Yilan. However, the similar fashions of damage risks were found in Hsinchu and Hualien due to the similar water chemistry characteristic in those locations. The lowest $f_{\mathrm{CuBL}}^{50\%}(t)$ and BCF($\{ions\}$, t) were found in Tainan due to the highest abundant ion concentration processed competition and complex effect on Cu decreasing, whereas the deficient in ions increased Cu bioavailability that induced highest $f_{\mathrm{CuBL}}^{50\%}(t)$, BCF($\{ions\}$, t), and damage for tilapia populations (Fig. 24).

Furthermore, this study adopted a 28 day-growth bioassay data of adult tilapia to determine the growth inhibition in whole life span of tilapia to sequential pulsed and fluctuating Cu/As stressors. A merit of West growth model was used to elucidate the growth inhibition over the entire life cycle based upon the limited growth information at adult stage for tilapia exposed to Cu/As concentrations. Although, the metal susceptibility is specific to difference life-stage of tilapia, that should be further considered if the available specific life-stage toxic effects were well established. Previous studies showed that a serious consequence for population living was the influence of chemicals on juveniles (Kammenga et al. 1996; Ramskov and Forbes 2008).

Table 5.5. Site-specific temperament, pH, and water chemistry characteristics from published measured ion concentrations in Hsinchu, Yilan, Hualien, and Tainan area.

Location	Temp.(°C)	pH	Ion concentrations (mg L^{-1})			
			Na^+	K^+	Ca^{2+}	Mg^{2+}
Hsinchu	25.84±1.39[a]	6.53±0.48	40.55±18.57	5.97±8.59	51.81±29.04	24.92±9.32
Yilan	24.16±0.84	6.28±0.13	7.71±0.44	1.52±0.25	16.8±0.34	5.58±0.43
Hualien	24.70±0.76	7.21±0.14	7.79±4.40	3.29±1.15	87.42±11.97	18.10±2.86
Tainan	27.39±1.01	7.06±0.16	5283±4266	187±157	256±152	556±462

[a] Mean ± SD.

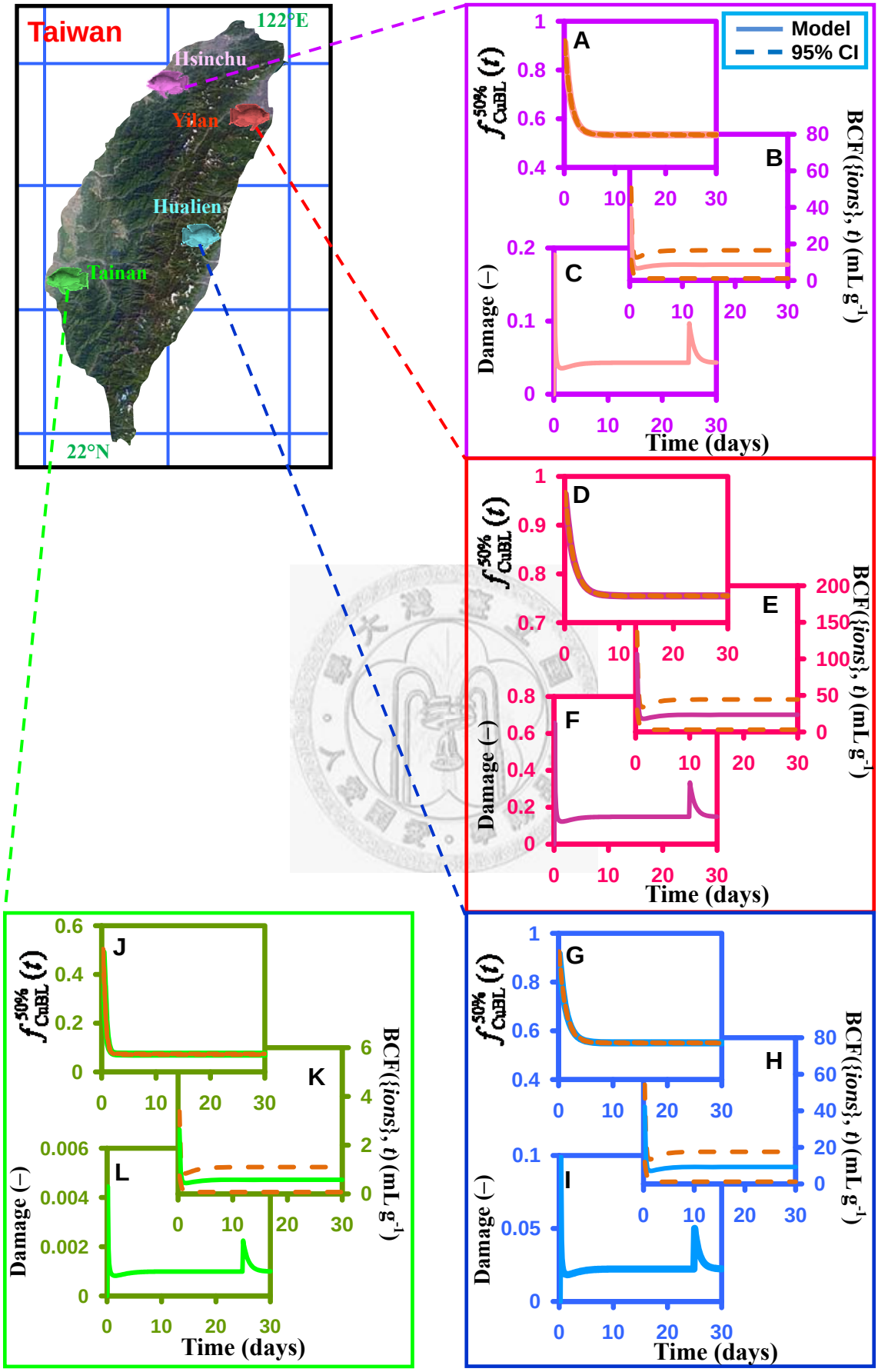


Figure 5.24. Site-specific ecotoxicological risk assessment of tilapia in Taiwan.

CHAPTER 6. CONCLUSIONS

This dissertation presents the systems-level dynamics framework that describes ecophysiological response of tilapia to pulse and fluctuating Cu/As exposures based on the essential features of the underlying damage mechanism. The following major conclusions could be drawn.

1. The first-order one-compartment toxicokinetic model can successfully determine the toxicokinetic parameters (i.e., uptake rate constant, elimination rate constant, and bioconcentration factor) for tilapia exposed to pulsed Cu concentrations.

2. Tilapia populations are capable of accumulating waterborne Cu. A highest BCF of 1116.10 mL g^{-1} for larval tilapia was greater than that of juveniles 225.50 mL g^{-1} and adults 94.00 mL g^{-1} in acute pulsed Cu exposure. Juveniles have the highest BCF 154.54 mL g^{-1} than that of adults 23.10 mL g^{-1} in chronic pulsed Cu exposure. The tilapia has the higher Cu accumulation capacity than that of As.

3. BCF values were dependent on the exposure time and site-specific water chemistry conditions. The acute and chronic pulsed Cu exposure bioassays provide this information in pulsed Cu–tilapia system.

4. High frequency exposure patterns induced shorter the median safe time than those of low frequency in pulsed Cu and As exposures for tilapia due to pulses interval. High frequency exposure patterns induced higher median safe time than those of low frequency in fluctuating (sine-wave) Cu and As exposures owing to high concentration exposure timings and durations.

5. The highest Cu killing rate constant (k_k) of 17.33 g μg^{-1} d^{-1} was greater than that of As of 3.50 g μg^{-1} d^{-1}. On the other hand, the Cu recovery rate constant (k_r) of 7.91 d^{-1} was lower than that of As of 9.52 d^{-1}.

6. The bioenergetics-based ontogenetic growth model could demonstrate biomass growth effect of tilapia under the chemical exposure scenarios. Growth cost coefficients A_0 of tilapia were estimated to be 0.029 $g^{1/4}$ d^{-1} in uncontaminated environment and 0.019 $g^{1/4}$ d^{-1} in the pulsed Cu exposure environment.

7. The accumulation of second Cu pulsed exposure was positively influenced by the first Cu pulsed exposure. The recovery mechanisms are triggered between the intervals of pulsed exposures.

8. The dynamics of physiological responses were dependent on the pulsed and fluctuating concentrations, duration, frequency, and different chemical exposure characters in tilapia.

9. The time and ions-dependent BCF (BCF{*ions*}, *t*) could provide a tool to assess toxic effect in the real field situation.

10. A systems-level based toxicokinetic/toxicodynamic model captures the essential features of internal damage responses for tilapia subjected to pulsed and fluctuating Cu and As exposures. The computational study in this dissertation may provide a framework in assisting the understanding of interactions among

toxicokinetic, toxicodynamic, recovery, and coping mechanisms that reflect the mode of action in physiological response processes for aquatic organisms exposed to environmental metal stressors.

CHAPTER 7. SUGGESTIONS FOR FUTURE RESEARCHES

1. This study provides an approach to predict the site-specific and time-dependent biococentration factor of adults tilapia based on the laboratory chronic experimental condition and stability constant of non-tilapia species in the present dissertation. This study recommend that Cu-tilapia BLM system should be constructed to obtain the stability constants for the binding of Cu to the biotic ligand of tilapia and to validate the site-specific and time-dependent bioconcentration factor of tilapia in the future work.

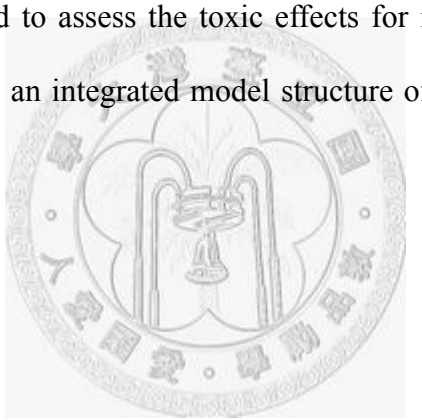
2. The study investigated the metal accumulation capacity of whole body for tilapia populations. The trade-off of organ-specific accumulations may exist in the aquatic organisms. This is an important mechanism to understand the metal toxic transfer and detoxification mechanisms in the tilapia population. This study suggested that trade-off of organs accumulations may be incorporated in the future study.

3. The study investigated the metal toxicity based on the metal ions binding to biotic ligand (whole body) of tilapia populations. Recent studies indicated that subcellular partitioning model can be used to describe the complex binding of chemical ion in different subcellular compartments with different chemical ion-binding ligands to reflect the actual metal toxicity. Future study may focus the critical sites of toxic action and detoxification in the subcellular fraction.

4. This study designed two pulsed and fluctuating frequencies (interval) exposure

patterns, respectively, indicating that the dynamics of physiological response were dependent on pulsed intervals and fluctuating period. Hence, a fully fluctuating exposure environment should be taken into account the exposure frequency, interval, duration, and timing to capture the authentic dynamic responses of aquatic organisms.

5. This study integrated the toxicokinetics/toxicodynamics, bioavailability, and bioenergetics to provide a more reliable prediction for a long-term exposure risk assessment of adult tilapia. A stage-structure of tilapia populations (embryo, larva, juvenile, and adult) should be incorporated the metal toxicity with matrix population model and to assess the toxic effects for individual life-stage. That could further provide an integrated model structure of metal toxicity for tilapia population dynamics.



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