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8 Evidence of Rapid Transfer and Bioaccumulation of Microcystin-LR Poses 9 Potential Risk to Freshwater Prawn *Macrobrachium rosenbergii* (de Man)

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25

26 **Abstract**

27 Microcystins accumulate in aquatic organisms and can be transferred to higher trophic

28 levels, eventually affecting vector animals and consumers. We examined three levels of

29 an aquatic food chain (*Microcystis aeruginosa*, *Daphnia magna*, and *Macrobrachium*

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30 *rosenbergii*) to identify the transfer efficiency and risk of microcystin on prawns.
31 Samples were analyzed using UPLC-MS/MS and microcystin-LR (MC-LR)
32 distributions in prawn tissues were studied. The results showed that prawns accumulate
33 MC-LR both directly from *M. aeruginosa* and indirectly through *D. magna* which was
34 pre-exposed to *M. aeruginosa*. MC-LR was detected in the gills, digestive tracts, and
35 hepatopancreas of the prawns 2 h after exposure. MC-LR accumulated in prawns to
36 0.49±0.04 μg g^{-1} dry weight in hepatopancreas within 24 h, while it was not detected in
37 muscle samples, and rarely appeared in blood samples in such a short period. Although
38 MC-LR was not detected in muscle, the head including hepatopancreas of the prawns
39 accumulated troublesome amounts of MC-LR. These results demonstrate that
40 microcystis blooms in prawn farming potentially pose a risk to human consumers, even
41 though prawns may be exposed to the bloom for a very short time, hence regular
42 monitoring of blue green algae population is recommended.
43

44 **Introduction**

45 Toxic cyanobacterial blooms are globally problematic as they may produce
46 secondary metabolites such as microcystins (MC) (Vareli et al. 2012; Beaver et al.
47 2014). So far, more than 80 variants have been isolated and identified (Dietrich and
48 Hoeger, 2005). Among these isoforms, Microcystin-LR (MC-LR) is the most common
49 and most toxic to mammals. Studies have demonstrated that MCs can cause toxic effect
50 on various aquatic organisms (Cazenave et al. 2005; Chen & Xie, 2005; Xie et al. 2005,
51 2007; Lance et al. 2006; Deblois et al. 2008; Ortiz-Rodríguez and Wiegand 2010;
52 Deblois et al. 2011; Liu et al. 2011; Ziková et al. 2013; Liu et al. 2014). Aquaculture
53 species are exposed to microcystins through the aquatic food web (Andersen et al. 1993;
54 Ibelings et al. 2005; Smith and Haney 2006). Trophic transfer has also been
55 demonstrated under laboratory conditions in which hepatotoxins were transferred from
56 zooplankton to fish (Engström-Öst et al. 2002; Karjalainen et al. 2005; Smith and
57 Haney 2006).
58

59 MC-LR also poses potential threats to human health (Codd et al. 2005a, b; Ibelings
60 and Chorus 2007). The transfer of MC to higher trophic levels through the food web
61 poses serious health implications (Ozawa et al. 2003). Recently, microcystins were

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62 identified for the first time in the serum (average 0.228 ng mL^{-1}) of fishermen who were
63 chronically exposed to cyanotoxins in Lake Chaohu, China, indicating hepatocellular
64 damage (Chen et al. 2009). An important exposure route for humans is through the
65 ingestion of contaminated aquatic food. The World Health Organization (WHO)
66 published a guideline of 1 μg kg^{-1} of MC in drinking water, and established a tolerable
67 daily intake guideline (TDI) of 0.04 μg kg^{-1} per day (Chorus and Bartram 1999).
68
69 In recent years, studies evaluating MC contamination in aquatic organisms from
70 natural waters with cyanobacterial blooms have increased, but these studies mainly
71 focused on fishes (Mohamed et al. 2003; Ibelings et al. 2005; Xie et al. 2005; Chen et al.
72 2006; Deblois et al. 2008; Wilson et al. 2008). Similar information is relatively rare for
73 freshwater prawns. Freshwater prawns are commercially important because they are
74 used for human consumption worldwide. In both intensive and extensive aquaculture,
75 phytoplankton succession mimics natural systems, with cyanobacterial abundance
76 reaching its maximum in summer (Smith et al. 2008).
77
78 Zooplankton can accumulate MC and therefore may act as vectors of the toxin up
79 the aquatic food web; however, information on the transfer and bioaccumulation
80 efficiency of MC through prawns is lacking. It is likely that consumption of prawns
81 exposed to high MC levels could lead to risk of public health. Quantitative evaluation of
82 the potential risk posed to food safety is needed. Therefore, we monitored transfer and
83 bioaccumulation of MC-LR at three levels of an aquatic food chain, namely blue green
84 alga *Microcystis aeruginosa*, daphnia *Daphnia magna* and freshwater prawns
85 *Macrobrachium rosenbergii* in three experiments so as to assess its potential risk by
86 human consumption of prawns.
87
88 **Materials and methods**
89 *Chemicals and reagents*
90 MC-LR (purity≥95.0%) was purchased from Alexis (Lausen, Switzerland).
91 Standard stock solution was prepared in methanol at 0.50 μg mL^{-1}. HPLC-grade
92 methanol and formic acid were supplied by Merck KGaA (Darmstadt, Germany). All
93 other reagents and chemicals used were of analytical grade. Water was purified from a

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94 Milli-Q deionization unit (Millipore, Molsheim, France).

95 *M. aeruginosa* was cultured in BG-11 medium in flasks at 25±1 °C, with irradiance
96 at 56 $\mu Em^{-2} S^{-1}$ and a 12 h light:12 h dark photoperiod. *D. magna* was provided by Key
97 Laboratory of Marine Ecology and Environmental Science, Institute of Oceanology,
98 Academy of Science (Qingdao City, China).

99 **Experimental design**

100 Experiment A was an exposure experiment where prawns and *D.magna* were
101 exposed to *M. aeruginosa* water. Prawns with an average weight of 12.92±2.79 g and
102 body length of 8.36±0.57 cm were obtained from a local farm (Shanghai, China),
103 acclimated for 1 week in PVC tanks (150 L) containing de-chlorinated water and fed
104 with commercial prawn feed(Tongwei Group Co.Ltd, China) at a rate of 3% of body
105 weight per day. The water temperature was 25±1 °C, and dissolved oxygen was 5.9 ±
106 0.6 mg L^{-1}. Fresh cells of *M. aeruginosa* were used for this experiment, after being
107 concentrated by centrifugation (4 min, 7000 rpm, 4 °C) and resuspended in water at cell
108 densities of 5×10^7 cells mL^{-1}. *D.magna* at density of 2 individual $mL^{-1}$was maintained in
109 5 glass tanks each with 10 L in volume containing water with *M. aeruginosa* at density
110 of 5×10^7 cells mL^{-1} for 24 h. Each group of prawns (4 groups, n=10) was placed in a 30
111 L PVC tanks containing water with *M. aeruginosa* at the density of 5×10^7 cells mL^{-1} for
112 24 h. During exposure the prawns were fed with commercial feed and aeration was
113 provided. Prawns were sampled from tanks and dissected at 2, 8, 16 and 24 h of
114 exposure. The gills, blood, digestive tract, muscle and hepatopancreas were frozen and
115 freeze-dried separately. *D. magna* was collected and frozen for MC-LR content analysis
116 and feeding experiment B.

117

118 Experiment B was a feeding experiment where prawns were fed with *D. magna*
119 which was exposed to *M. aeruginosa* in experiment A. The prawns (average weight of
120 13.87±2.52 g and body length of 8.65±0.47 cm) were acclimated to laboratory
121 conditions for 1 week in PVC tanks with de-chlorinated water. During acclimation
122 period commercial feed was supplied, while when the experiment began prawns were
123 exclusively fed with frozen *D. magna* exposed to *M. aeruginosa*. Prawns were sampled
124 and dissected at 8, 16 and 24 h post feeding and the gills, blood, digestive tract, muscle

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125 and hepatopancreas were frozen and freeze-dried separately.

126

127 Experiment C was a food chain experiment where prawns (average weight of 128 13.25±2.85 g and body length of 8.43±0.86 cm) were acclimated to laboratory 129 conditions for 1 week in 5 PVC tanks. Prawns along with *D. magna* (2 individuals mL^{-1}) 130 were stocked in 30 L PVC tanks with *M. aeruginosa* at cell densities of 5×10^{7} cells mL^{-} 131 ^{1}. Prawns from each tank were dissected at 2, 8, 16 and 24 h and the gills, blood, 132 digestive tract, muscle and hepatopancreas were frozen and freeze-dried separately.

133

134 MC analyses

135 Extraction of the microcystins was carried out according to the method of Zhang et 136 al. (2009) with some modification. The lyophilized samples (~0.1 g dry weight for each 137 sample) were extracted two times with 25 mL of 0.01 M EDTA-Na_2-5 v/v % formic 138 acid by homogenization with a high-speed blender (PT2000, Polytron, Switzerland) for 139 30 s at 20,000 rpm and followed by 5 min sonication. The samples were then 140 centrifuged at 4500 rpm (Type 3K-18, Sigma, Germany) at 4 ^{o}C. The supernatant was 141 applied to an Oasis HLB cartridge (500 mg, 6 mL, Waters, Milford, MA, USA) which 142 had been preconditioned by washing with 5 mL 100% methanol and 10 mL distilled 143 water. The column containing sample was washed with 5 mL water followed by 3 mL 144 10% methanol, and then eluted with 4 mL 100% methanol. The elutriant was dried 145 under a nitrogen stream in a 45 ^{o}C water bath, and the residue was dissolved in 1.5 mL 146 of 20% methanol with 0.1% formic acid. The resulting solution was filtered through a 147 0.45 μm nylon filter for analysis.

148

149 An Ultra Performance Liquid Chromatography (UPLC) and Mass Spectrometry 150 system was used consisting of an Acquity UPLC™ system equipped with a XEVO 151 triple quadrupole mass spectrometer (Waters, Milford, USA). The injection volume was 152 full-loop (10 μL) and the chromatographic separation was performed at 40 ^{o}C using a 153 C_{18} column (Waters, 100 mm×2.1 mm internal diameter, 1.8μm particle size), with the 154 flow rate set at 0.35 mL min^{-1}. The mass spectrometer was operated in the positive ESI 155 mode, with a capillary voltage of 3.5 kV. The source and desolvation temperatures were 156 145 and 450 ^{o}C, respectively. Gas desolvation and nebulization were carried out using

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157 nitrogen at flow rates of 850 and 50 L h^{-1}, respectively. The signal acquisition was
158 performed by multiple reactions monitoring mode (MRM). The divert valve was
159 programmed to send the LC flow to waste for the first 2 min after injection and again
160 after the analyte of interest had eluted. The gradient parameters are presented in Table 1.
161 Mass spectrum tuning and optimization were achieved by infusing MC-LR and
162 monitoring the $[M+H]^+$ ion at m/z 995.6. The product ions at m/z 107.1 from the parent
163 ion at m/z 995.6 were used for MC-LR qualitative analysis. For quantification purposes,
164 mass chromatograms monitored the product ions at m/z 135.2 from the parent ion at m/z
165 995.6 (Table 2).

166 Table 1

167 Table 2

168 The method was validated in terms of specificity, limit of detection (LOD), limit of
169 quantification (LOQ), precision and recovery. Linearity of the method was assessed at
170 MC-LR concentration ranging from 1 to 250 ng mL^{-1}. Five calibration curves with six
171 concentration points were constructed. The linearity of the calibration curves was
172 evaluated on the basis of linear regression analysis and the square correlation
173 coefficients (r^2) using SPSS 17.0. A correlation coefficient above 0.99 was desirable for
174 all the calibration curves. LOD and LOQ were defined as concentrations in a sample
175 resulting in signal-to-noise ratios of 3 and 10, respectively. Precision of the assay was
176 expressed by percent relative standard deviation (%RSD).

177 The recoveries were obtained by analyzing MC-LR in prawn tissue at three spiked
178 concentration (0.075, 1.5, 3.75 μg g^{-1} for MC-LR). In all cases, samples were run in
179 sextuplicate. The limit of detection (LOD) and limit of quantification (LOQ) were
180 estimated sextuplicate. The matrix effect was assessed by comparing the peak areas of
181 the neat analyte standards, standards spiked before and after extraction in prawn tissue
182 samples. Mean and standard deviation of MC concentrations between treatments were
183 performed using SPSS 17.0 for windows.

184

185 **Results**

186 MC analyses

187 One possible way to remove the matrix interferences from prawn tissue is sample
188 cleanup using an Oasis HLB extraction cartridge (Waters, Milford, USA). There are

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189 many interfering compositions unclear in complex prawn tissue, which ideally should
190 be washed off before elution. We successfully used 3 mL of 10% methanol in water to
191 wash off the interference of the matrix while leaving the targeted compounds on the
192 cartridge. The washing curve is shown in Fig.1. Pure methanol was chosen for elution
193 because of its high recovery and convenient dryness by nitrogen flush. The volume
194 fraction of pure methanol in water was 4 mL and the elution curve is shown in Fig.2.
195 Figure 1
196 Figure 2
197 According to the analysis of 10 unexposed samples (including gills, blood,
198 digestive tract, muscle and hepatopancreas), this UPLC-MS/MS method provided clean
199 and background-free mass traces for the matrix studied, demonstrating that the method
200 had good selectivity (Fig.3). Quantification matrix-fortified calibration curves were
201 determined to compensate for the matrix effect and loss in sample preparation. Good
202 linearity was obtained for MC-LR, with r^2 ranging from 0.9946 to 0.9990. The LOD and
203 LOQ in this method were 0.0075 μg g^{-1} and 0.01575 μg g^{-1}, respectively. The results are
204 summarized in Table 3.
205 Figure 3
206 Table 3
207
208 Satisfactory MC-LR recoveries were obtained ranging between 84.8±7.6% and
209 128.4±11.3%. The precision was satisfactory since RSD of the mean recovery ranged
210 from 4.2% to 10.9%. The results demonstrated that the accuracy and repeatability of the
211 method were good for quantitative purposes.
212
213 Experimental results
214 MC-LR in the cells of *M. aeruginosa* was 119.67±14.01 μg g^{-1} dry weight and in *D.*
215 *magna* 2.72±0.09 μg g^{-1} dry weight, respectively. The highest MC-LR peaks in the gills
216 of prawns appeared at 8 h (6.08±0.59 μg g^{-1} dry weight) in experiment A. The digestive
217 tracts of prawns showed remarkably high peaks at 16 h (2.19±0.30 μg g^{-1} dry weight),
218 whereas the highest MC-LR content in the hepatopancreas appeared at 24 h (0.49±0.04
219 μg g^{-1} dry weight) in experiment C (Fig.4). MC-LR was not detected in the muscles, and
220 rarely appeared in blood samples in this study (Table 4). Cyanobacteria (*M. aeruginosa*)

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221 were observed in the gills of prawns in both experiments A and C.

222 Figure 4

223 Table 4

224

225 In all treated prawns, the average MC-LR content in the hepatopancreas rapidly
226 increased during the experimental period (Figs. 4, 5). The highest MC-LR levels were
227 recorded in the gills and digestive tracts of the prawns (Fig. 6). The average MC-LR
228 content of experiment A in the gills and digestive tracts increased initially (around 8 h),
229 then decreased until the end of the test (Fig. 4). However, the average MC-LR content
230 in the gills and digestive tracts in experiment C increased initially then decreased
231 after16 h. Prawns accumulated a maximum of 6.08±0.59 μg g^{-1} dry weight in the gills in
232 experiment A.

233 Figure 5

234 Figure 6

235

236 **Discussion**

237 Aquatic organisms are generally considered more tolerant of cyanobacteria toxins
238 than mammals as a result of their co-evolutionary history, which can reduce the
239 likelihood for catastrophic losses of the cultured species but increase the potential for
240 damaging human exposure to these toxins (Smith et al. 2008). Bioaccumulation of MCs
241 in crustaceans has been reported in several publications. Vasconcelos et al (2001)
242 detected a concentration of 2.9 μg g^{-1} dry weight in crayfish *Procambarus clarkii* under
243 laboratory conditions. The seasonal changes of mixed hepatotoxins (MC-LR, MC-LA,
244 MC-RR, MC-YR, and NODLN) in the marine prawn *Penaeus monodon* was measured
245 through the enzyme linked immunosorbent assays (ELISA) method and the total
246 concentration of hepatotoxins in *P. monodon* hepatopancreas varied between 0.0064 and
247 0.0816 μg g^{-1} DW (Kankaanpää et al. 2005). Zhang et al. (2009) studied seasonal
248 variations of MC-LR contents in shrimp (*Macrobrachium nipponensis*) from Lake Taihu
249 through liquid chromatography electrospray ionization mass spectrum (LC–ESI-MS),
250 and found that the MC-LR concentrations ranged from 0.031±0.004 to 0.605±0.179 μg
251 g^{-1} DW with averaging 0.244 ± 0.220 μg g^{-1} DW. In our study, the toxin concentration
252 shown by freshwater prawns is consistent with the reported cases in the hepatopancreas

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253 (maximum 0.49 μg g^{-1} DW). Nevertheless, Zimba et al (2006) reported a case of co-
254 occurrence of white shrimp, *Litopenaeus vannamei*, mortalities and microcystin toxin in
255 ponds, where water samples from the affected pond contained high levels of
256 microcystin-LR (45 μg g^{-1}), and free microcystin-LR concentrations in dead shrimp
257 hepatopancreas determined by HPLC were 55 μg g^{-1} total shrimp weight. However, we
258 did not determine the total MC load in prawn. The common results from the above
259 references demonstrated that crustacean muscle was not the primary organ for
260 hepatotoxin bioaccumulation.

261

262 In this study, MC-LR was detected in the gills, digestive tracts, and hepatopancreas of
263 the prawns after 2 h of exposure. The prawns accumulated MC-LR both directly from *M.*
264 *aeruginosa* (Experiment A) and indirectly through *D. magna* (Experiment B) which was
265 preexposed to *M. aeruginosa*. To our best knowledge, this might be the first evidence
266 that freshwater prawns accumulate MC-LR in very short time, as well as the direct
267 transfer of MC-LR from zooplankton to prawns and the subsequent accumulation of
268 toxin in the hepatopancreas and digestive tract.

269

270 According to our results, MC appears to be absorbed through the digestive tract of
271 prawns at a higher rate when the toxin is administered through a vector, *D. magna*,
272 rather than through toxic cyanobacteria directly. In experiment C, as prawns and *D.*
273 *magna* were exposed to *M. aeruginosa* together, *D. magna* ingested toxic cyanobacteria
274 cells and prawns appeared to consume these toxic *D. magna*, based on the decrease of
275 MC-LR content in prawn gills and the increase of MC-LR concentrations in
276 hepatopancreas and digestive tracts. Thus, other aquaculture species may also be
277 exposed to microcystins through the ingestion of cyanobacteria (Ortiz-Rodríguez and
278 Wiegand 2010), consumption of contaminated food items (e.g., prey or detritus)
279 (Deblois et al. 2011), and absorption of dissolved microcystins from the water column
280 (e.g., after leakage from cells or cell lysis) (Vasconcelos et al. 2001).

281

282 Microcystins are usually taken up from intestine and transferred to liver tissue
283 (Fischer and Dietrick 2000). In this study, we found that prawns ingested *M. aeruginosa*
284 cells directly. We also observed that algal cells adhered to the gills of prawns. There was

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285 no evidence that the gills absorbed microcystin through algae lysis, nevertheless, the
286 possibility of this pathway should not be disregarded.
287
288 The UPLC-MS/MS analysis method used herein shortened analysis time
289 significantly, with peak time for MC-LR appearing at 4.5-4.8 min. Comparatively, it
290 took 18.51 minutes to analyze MC-LR content in *Litopenaeus vannamei* samples using
291 HPLC/MS method (Zimba et al. 2006), while similar retention time (~21min) was
292 recorded for two tilapia species *Oreochromis niloticus* and *Tilapia rendalli* using HPLC
293 method (Deblois et al 2008).
294 The 24-h test was not sufficient to promote accumulation of MC-LR in the muscle,
295 which is the edible part of the prawn for human consumption. Future studies should take
296 this into consideration. The provisional tolerable daily intake (TDI) suggested by WHO
297 is 0.04 μg kg^{-1} of body weight or 2.4 μg for an adult human weighing 60 kg (Kuiper-
298 Goodman et al. 1999). Assuming an adult human ingests 100 g of whole prawns per day
299 from our experiment A and C, then daily uptake of MC-LR would range from 116 μg to
300 706 μg, much higher than TDI value proposed by WHO, which was unsafe for human
301 consumption. Therefore, the risk of consuming prawns from aquaculture ponds and
302 lakes during toxic cyanobacteria blooms cannot be overlooked, and regular monitoring
303 of MC levels in prawns should be conducted to protect health of consumers. We
304 recommend that future studies evaluate the total MC tissue load and the potentially
305 harmful effects of MC on human health by multiple exposure routes through aquatic
306 food.
307

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440 Table 1. UPLC Gradient parameters
441 Table 2. UPLC-MS/MS acquisition parameter for MC-LR analysis
442 Table 3. The calibration curve of MC-LR and recovery tests of HPLC-MS/MS method
443 (n=6)
444 Table 4. MC-LR content accumulated in different prawn tissues within 24h in the three
445 experiments (n=10) [a]
446 Figure 1. The loss rate of MC-LR in different volume fractions of methanol in water.

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450 real hepatopancreas samples; c. the blank digestive tract samples spiked at 100
451 ng/g MC-LR and real digestive tract samples (up to down); d. the blank muscle
452 sample spiked at 100 ng/g MC-LR and real muscle samples (up to down); e. the
453 blank gills sample spiked at 100 ng/g MC-LR and real gills samples (up to
454 down); f. the blank blood sample spiked at 100 ng/g MC-LR and real blood
455 samples (up to down)).

456 Figure 4. MC-LR contents in gills, hepatopancreas and digestive tract of prawns from
457 experiments A and C. Bars represent means ± SD of four replicates.

458 Figure 5. MC-LR concentrations in hepatopancreas and digestive tract of prawns from
459 experiment B. Bars represent means ± SD of four replicates.

460

461 Figure 6. Distribution of MC-LR (%) in the prawn tissues at different times in
462 experiments A, B and C.

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Table 1. UPLC Gradient parameters

Time (min)	A[a]	B[b]	Curve
0.00	100	0	0
5.00	0	100	6
6.00	0	100	6
6.10	100	0	1
7.60	100	0	1

[a] water containing 0.1% (v/v) formic acid, [b] methanol containing 0.1% (v/v) formic acid.

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Table 2．UPLC-MS/MS acquisition parameter for MC-LR analysis

Parent ion/Product ion	Cone voltage(v)	Collision energy(v)	Dwell time(s)
995.6/107.1[a]	42	70	0.2
995.6/135.2	42	76	0.2

[a]quantification transition

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Table 3. The calibration curve of MC-LR and recovery tests of HPLC-MS/MS method (n=6)

Tissue	Standard curve	r^2	MC-LR (μg/g)	Recovery (%)	RSD (%)
Blood	Y = 259.83X -2.53	0.9990	0.075	91.7±5.2	5.7
			1.5	99.2±10.5	10.5
			3.75	103.7±4.3	4.2
Muscle	Y = 262.45X -2.71	0.9975	0.075	91.5±10.5	10.9
			1.5	84.8±7.6	9.0
			3.75	111.4±6.5	5.8
Gills	Y = 301.00X -2.83	0.9952	0.075	125.2±12.2	9.7
			1.5	99.7±4.7	4.7
			3.75	100.7±5.0	5.0
Hepatopancreas	Y = 451.70X -2.84	0.9968	0.075	104.3±4.8	4.6
			1.5	88.6±8.6	9.8
			3.75	99.2±5.8	5.8
Digestive tract	Y = 652.27X -2.72	0.9946	0.075	128.4±11.3	8.8
			1.5	96.2±6.1	6.3

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Table 4 MC-LR content accumulated in different prawn tissues within 24h in the three experiments (n=10) [a]

Experiment/Time (h)		MC-LR content (μg/g dry weight)				
		Blood	Muscle	Gills	Hepatopancreas	Digestive tract
A	2	ND	ND	2.16±0.19[a]	0.02±0.00[a]	0.35±0.02[a]
	8	0.15±0.004	ND	6.08±0.59[b]	0.06±0.01[a]	0.77±0.07[b]
	16	ND	ND	3.57±0.44[a]	0.19±0.03[b]	0.69±0.08[b]
	24	ND	ND	0.89±0.08[c]	0.20±0.04[b]	0.56±0.02[ab]
B	8	ND	ND	0.05±0.005	0.04±0.02[a]	0.05±0.01[a]
	16	ND	ND	ND	0.05±0.01[a]	0.14±0.01[b]
	24	0.02±0.005	ND	ND	0.10±0.02[b]	0.40±0.04[c]
C	2	ND	ND	0.37±0.04[a]	0.17±0.02[a]	0.62±0.16[a]
	8	ND	ND	1.59±0.09[b]	0.18±0.01[a]	1.34±0.08[b]
	16	ND	ND	1.66±0.18[b]	0.40±0.04[b]	2.19±0.30[c]
	24	0.03±0.005	ND	1.11±0.12[b]	0.49±0.04[b]	1.29±0.11[b]

[a] Values represent means ± SD of four replicates. Means of each experiment in the same column with different superscript letters are significantly different ($P<0.05$).

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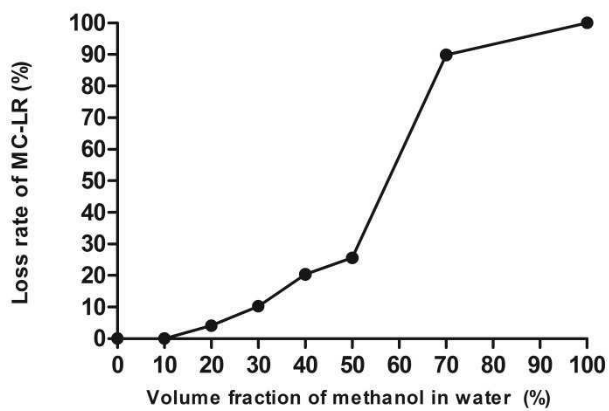

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Figure 1. The loss rate of MC-LR in different volume fractions of methanol in water.

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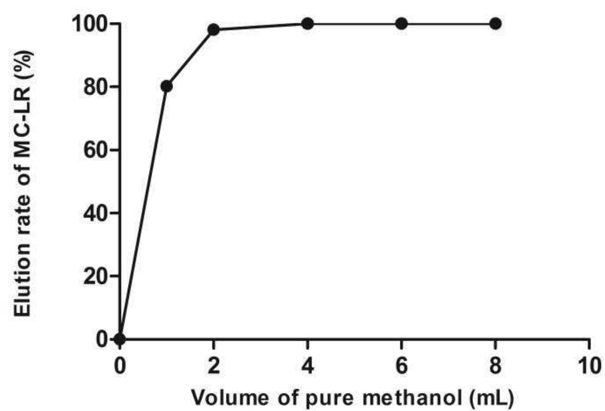

Figure 2.Curve of elution rate of MC-LR against volume of pure methanol.

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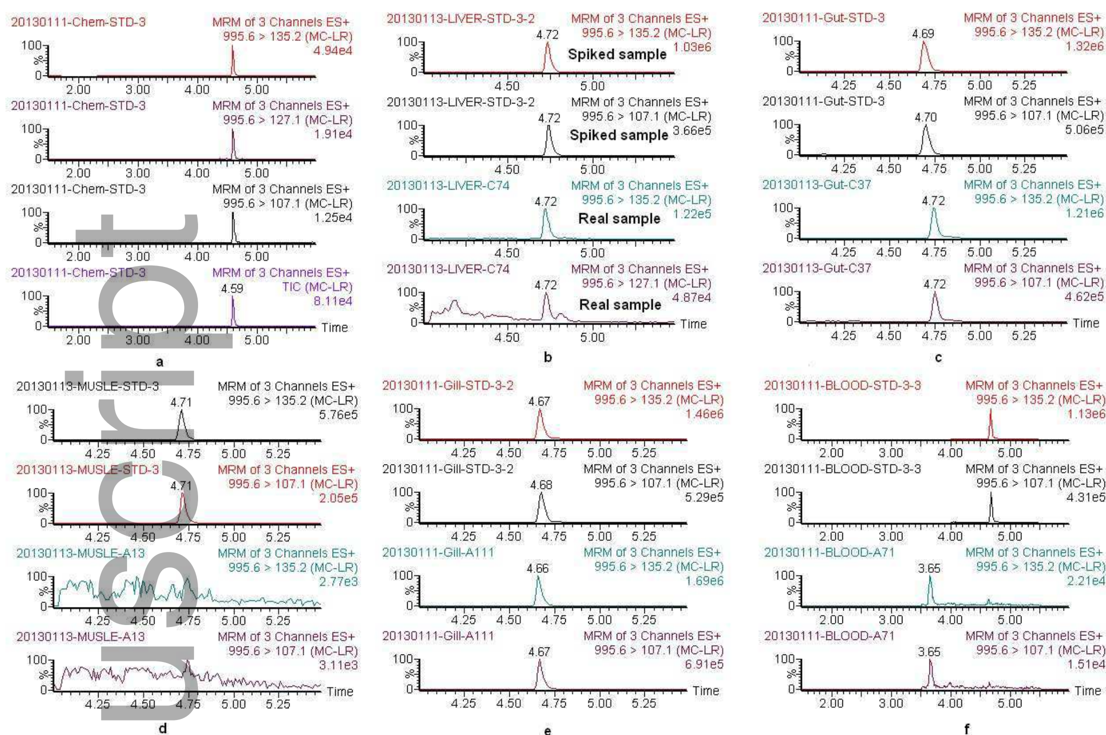

Figure 3. Chromatograms of the MC-LR in the samples (a. chemical standard MC-LR at 100 ng/g; b. the blank hepatopancreas sample spiked at 100 ng/g MC-LR and real hepatopancreas samples; c. the blank digestive tract samples spiked at 100 ng/g MC-LR and real digestive tract samples (up to down); d. the blank muscle sample spiked at 100 ng/g MC-LR and real muscle samples (up to down); e. the blank gills sample spiked at 100 ng/g MC-LR and real gills samples (up to down); f. the blank blood sample spiked at 100 ng/g MC-LR and real blood samples (up to down)).

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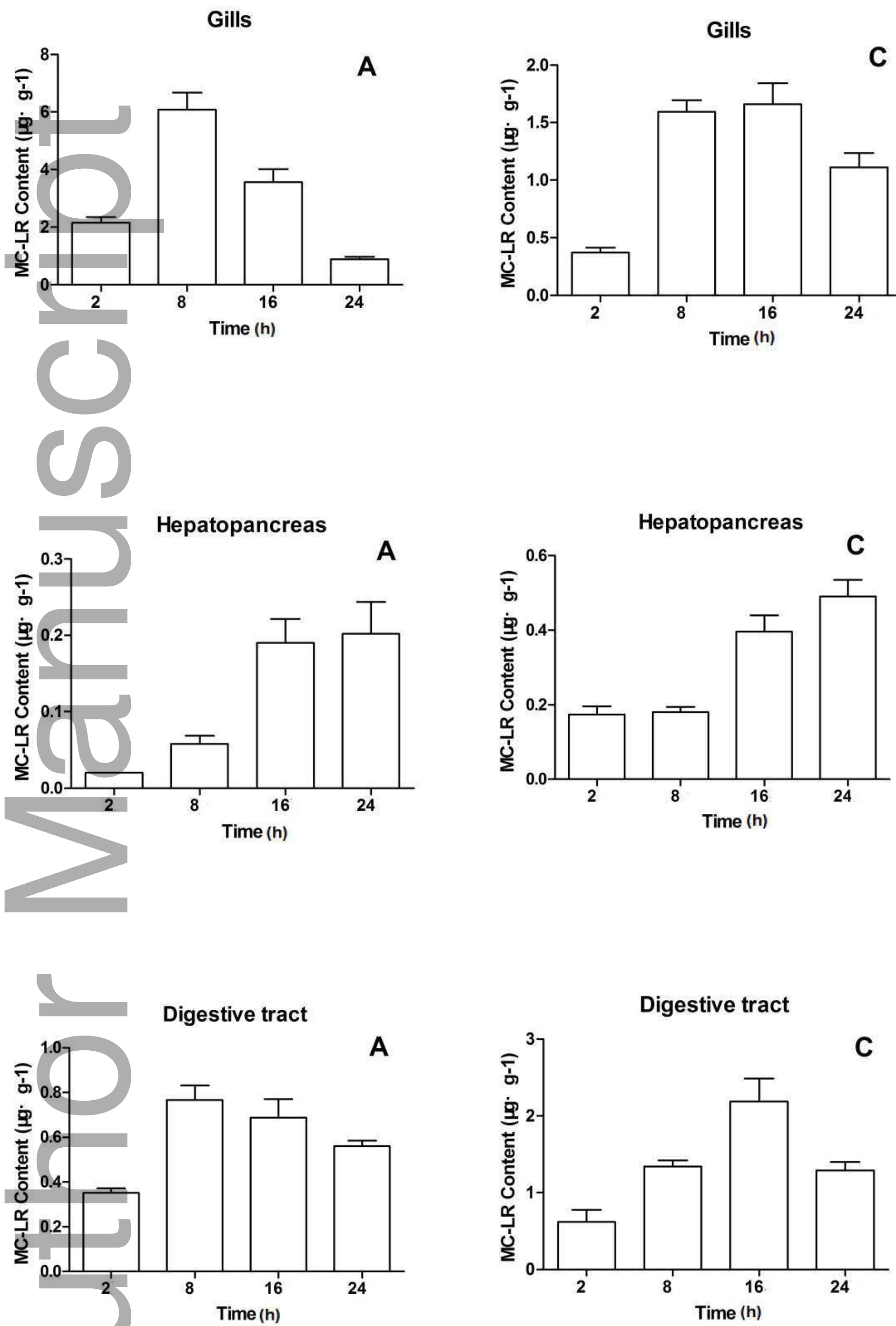

Figure 4. MC-LR contents in gills, hepatopancreas and digestive tract of prawns from experiments A and C. Bars represent means ± SD of four replicates.

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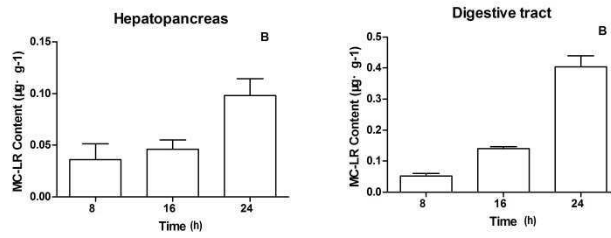

Figure 5. MC-LR concentrations in hepatopancreas and digestive tract of prawns from experiment B. Bars represent means ± SD of four replicates.

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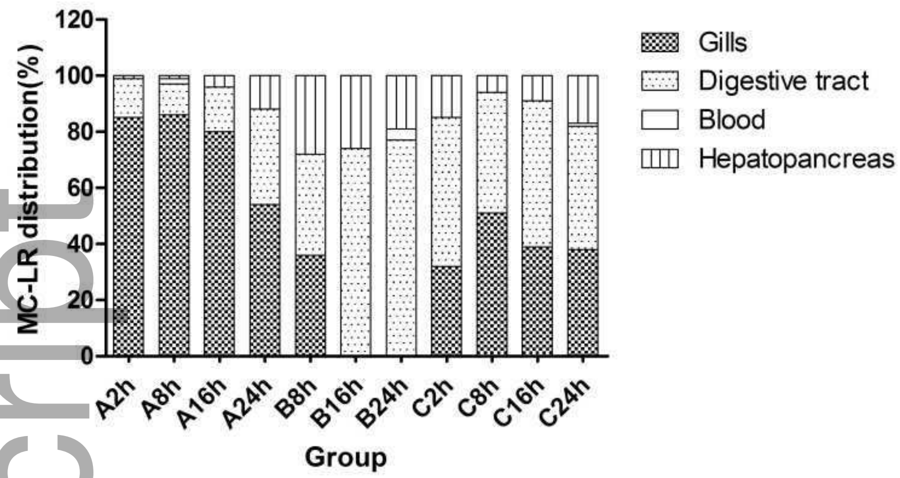

Figure 6. Distribution of MC-LR (%) in the prawn tissues at different times in experiments A, B and C.

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