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Comparative study of brine shrimp bioassay-based toxic activities of three harmful microalgal species that frequently blooming in aquaculture ponds*

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Abstract Typical harmful micro-algal species constantly occurred in high density in marine aquaculture ponds in Xiangshan and Sanmen Bay, Zhejiang Province. Fates of the microalgal cells influenced by activity of the cultured animals largely determined the effects of the harmful microalgae. However, it is difficult to detect the in situ process. In this paper, toxic activities of three harmful microalga, namely *Prymnesium parvum*, *Pleurochrysis elongata*, *Karlodinium veneficum*, which were isolated from the local ponds, were comparatively studied based on brine shrimp toxic bioassays. Different lethal activities of live cells, cell debris, cellular extracts, and cell free mediums prepared by different process were analyzed. The results showed that, (1) all of the three microalgal species had density and time dependent lethal effects on *Artermia* nauplii, while *P. parvum* was the most toxic one and had acute lethal effects in 5 h. No such acute lethal effects were observed in *P. elongata* or *K. veneficum*; (2) live cells, cell debris and cellular extracts of *P. parvum* had the same lethal pattern. Prymnesins, toxin from *P. parvum*, is probably not exotoxic active; For *P. elongata*, toxic activity mainly came from live cells and cell debris; For *K. veneficum*, toxic activity was relatively lower compared with the other two species. However, Karlotoxin, toxin from *K. veneficum*, is exotoxic active. Physical disturbance triggered *K. veneficum* cells actively releasing toxins, which made it an active predator.

Keyword: *Prymnesium parvum*; *Pleurochrysis elongata*; *Karlodinium veneficum*; *Artemia* nauplii; toxic activity

1 INTRODUCTION

With the problems such as high eutrophication, poor water exchange, low biodiversity, marine aquaculture ponds become areas where some specific notorious harmful microalgal species blooms were most likely triggered (Stanley et al., 2005; Martínez-Porchas et al., 2010; Granéli et al., 2012; Place et al., 2012). In return, the blooms caused tremendous damages to the cultured organisms and economical losses (Deeds et al., 2002; Houdan et al., 2004; Roelke et al., 2011; Martinez-Porchas and Martinez-Cordova, 2012).

Harmful microalgae in aquaculture ponds face complicated interactions with different cultured organisms. During the interactions, toxic materials

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may be released from the algal cell or just keep being content inside the cell. On account of the toxins, harmful effects may be caused by definite algal toxins or normal metabolites that are not generally thought as toxins, such as predation detergents DMS (P) (Kiene et al., 2000). It is difficult to answer the question that how toxic microalgae produce toxin or anti-predation detergent when predator present. Simulation of these processes is necessary to analyze the instant moment when the toxic species are physically disturbed or damaged because of the activities of cultured animals.

Harmful microalgae, such as *Prymnesium parvum*, *Pleurochrysis elongata*, *Karlodinium veneficum*, were usually found in some aquaculture ponds in Sanmen Bay, Zhejiang province, blooming or co-blooming. Cell densities ranged 10^2–10^7 cells/mL occasionally. *Prymnesium parvum* is a wide-spread species and can cause mortality to many cultured animals such as fish, shellfish, shrimp and crab (Edvardsen and Paasche, 1998; Igarashi et al., 1998; Sasaki et al., 2001; Edvardsen and Imai, 2006; Baker et al., 2007). Many substances were thought to be responsible to its toxic potency (Henrikson et al., 2010). However, toxic effect of *P. parvum* was highly labile from case to case (Granéli and Salomon, 2010). Modes of action, potency of toxins or toxic effects may change in response to the ambient physical, chemical and biological factors such as salinity, light and temperature (Larsen et al., 1998; Brooks et al., 2010), pH (Valenti et al., 2010), metal ion availability and photolysis (James et al., 2011). Quantification of prymnesins is not analytically feasible because of different geographical strain of different toxic potency existed (Schug et al., 2010; Manning and La Claire, 2010).

As *P. parvum*, calcified coccolithophorid specie *P. elongata* belong to Prymnesiophyceae. Genus *Pleurochrysis* is drawing much attention because that some species of this Genus typically bloom in aquaculture pools in a way of long lasting notorious foam bloom. *Pleurochrysis elongata* is lethal to aquatic organisms such as *Artemia salina* (Houdan et al., 2004). However, few studies are done to clarify its toxic mechanism. Unlike the former one with some identified toxins, *Pleurochrysis* has no definite chemical compounds that could be classified as toxin(s). Mechanism of the lethal effect of *Pleurochrysis* is still unknown (Not et al., 2012).

Small dinoflagellate *K. veneficum* contains toxins (karlotoxins) of multiple toxic characteristic, such as hemolytic, ichthyotoxic, and cytotoxic activities (Deeds et al., 2002; Kempton et al., 2002; Zhou et al., 2011). Feature and activity model of the toxins differ from different strains and have not been completely characterized (Bachvaroff et al., 2009; Calbet et al., 2011). The content and structure of karlotoxins vary significantly with different strains, locations, environmental conditions (Place et al., 2012).

It is difficult to detect instant interaction between prey and predator in situ. In order to simulate the possible origination of the harmful effects, in this study, different algal samples were prepared to analysis the toxic potency based on brine shrimp toxicological bioassay. Toxic assessment based on crustaceans *Artemia* nauplii has been widely used for long time (Michael et al., 1956; Vanhaecke et al., 1981; Nunes et al., 2005; Hameed et al., 2009). When simultaneous assessment of multiple ecotoxicological significance of harmful species with multiple toxic characteristics is needed, *Artemia* nauplii are perfect model candidates (Nunes et al., 2006; Waggett et al., 2008; Faimali et al., 2012).

2 MATERIAL AND METHOD

2.1 Microalgal cultures and culture conditions

Three harmful microalgal species, namely *P. parvum*, *P. elongata*, *K. veneficum*, were isolated from aquaculture pond in Sanmen Bay, typical aquaculture area in Zhejiang Province, in the coastal area of the East China Sea. Identification was done through analyses of morphology and Nucleic acid sequencing and SEM. Pure isolates were kept in Microalgae Collection Center in Ningbo University, China. Accession code in NCBI for *P. parvum* is MF039887. Strain number of *P. elongate* and *K. veneficum* are NMBjih026-1 and NMBjah047-1 respectively in the Collection Center. *Isochrysis galbana* (strain NMBjih021-1) was used as non-toxic species control.

The cultures were grown in sterile natural seawater (salinity 25) enriched with NMB3 medium (Xu et al., 2012). Conditions of culture room were 20°C, 40 μmol photons/(m^2·s) illumination with 12 h:12 h L:D cycle.

2.2 *Artemia* nauplii preparation

Artemia cysts preserved in 4°C were warmed up to room temperature before use. The cysts were incubated at 30°C in an incubator with continuous light of 50 μmol photons/(m^2·s). The cysts hatched

gradually after 24 h. Active nauplii were picked and placed into fresh seawater to keep for an additional 24 h to make sure all the nauplii moulted into instar II–III stages. These nauplii were prepared in the study.

2.3 Mortalities of *Artemia* as a function of microalgal cell densities

Experiment was performed using 24-well plate. Cell densities of the pre-cultures were 1.1×10^6, 2.8×10^6 and 9.8×10^5 cells/mL for *P. parvum*, *P. elongata* and *K. veneficum* respectively at their early stationary growth phase. The cultures were 10 fold serially diluted with fresh culture medium. Five groups of different densities were prepared for each species. Ten nauplii together with 10 μL seawater were added to each well with micropipette (Eppendorf, Germany). Two milliliter algal cultures were put into each well. Nauplii in fresh culture medium were used as starving control. All treatments were replicated four times. The plates were placed in the culture room of 20°C with continuous illumination of 15 μmol photons/(m^2·s). Conditions of nauplii were documented and the dead ones were counted under inverted microscope (Nikon Ti-U Microscope Type 108) at 24 and 48 h. Still nauplii on the bottom without any kind of moving were considered dead.

2.4 Lethal effects of different algal samples on *Artemia*

Algal samples preparation. Various samples were prepared after the cultures grew to late exponential phase. For each microalgae strain, totally 5 sorts of samples from 4 aliquots of each culture were prepared for the bioassay:

Cell free mediums (**CFM**). There were two ways to get CFM: cell free supernatants of the cultures centrifuged at 4°C by 4 700×*g* for 10 min (CR11GIII, Hitachi, Japan), CFMcf; cell free filtrates by natural gravity filtration through 0.3 μm GF/C film (Whatman, USA), CFMgf.

Cell extracts (**CEcf**). Cell sediments by centrifugation were re-suspended in fresh culture medium to original volume. The cells were ultrasonic ruptured for 10–30 min (20 kHz, 400 W, with 5 s interval at 4°C). Cells totally ruptured were confirmed by observing under microscope. The suspensions were centrifuged at 42 300×*g* (4°C) for 10 min. The supernatants were used as cell extracts, CEcf.

Cell debris (**CD**). Ultrasonic ruptured cell debris was got from sediments while CEcf was prepared. The debris was re-suspended to original volume with fresh culture medium before use.

Original cultures of live cells (LC) were used to compare the effects of the algal samples. Original cell densities of the cultures were: 3.3×10^6, 5.3×10^5, 7.5×10^5, 6.4×10^6 cells/mL for *P. parvum*, *P. caterae*, *K. veneficum* and *I. galbana* respectively.

Toxicity detection process was the same as described in Section 2.3. Effects on *Artemia* were observed at time point of 0, 5, 12, 24, 36, 48 and 72 h. Dead nauplii were counted at each time.

Artemia in fresh culture medium (DSW) was set as starving control in all the tests.

2.5 Data handling, statistical analyses, LD_{50} and LT_{50}

Data are present as means ±SD.

Mortality of *Artemia* nauplii was calculated as:

$$R_t=(N_t/10)\times100\%,$$

where N_t represents dead nauplii at time t.

Median lethal density (LD_{50}) or median lethal time (LT_{50}) was obtained by applying a linear regression between cell concentration (or time) and mortality of *Artemia* nauplii according the method of Hewlett and Plackett (1979). LD_{50} in 24 h and 48 h were analyzed in Section 2.3. LT_{50}, the time when 50% mortality of *Artemia* nauplii occurred, was analyzed in 2.4.

Statistical analyses were conducted with Statistical Package for the Social Sciences (SPSS). Significant differences were determined by one-way ANOVA test.

3 RESULT

3.1 Mortality of *Artemia* nauplii as a function of microalgal cell density

All the three cultures had density dependent lethal effects on *Artemia* nauplii. Linear regression relationship existed in 24 h and 48 h (Fig.1). For *P. parvm*, linear relationship between cell density and mortality of *Artemia* nauplii was the most significant (R^2=0.954 79 and 0.970 71 in 24 h and 48 h respectively). LD_{50} were 1.1×10^4 cells/mL in 24 h and 8 cells/mL in 48 h. For *P. elongate*, linear relationship was more significant in 48 h (R^2=0.979 4) than in 24 h (R^2=0.870 55). LD_{50} was 7.2×10^5 and 2.9×10^3 cells/mL in 24 h and 48 h respectively. At the early time of exposure, there was no significant difference among the mortalities in groups of cell density of 10^2–10^4 cells/mL (P>0.05, ANOVA). Not until the cell density rise to magnitude of 10^5 did the mortality increase significantly. In 48 h, however, mortality increased with rising culture density in a way of significant linear relationship. For *K. veneficum*, LD_{50}

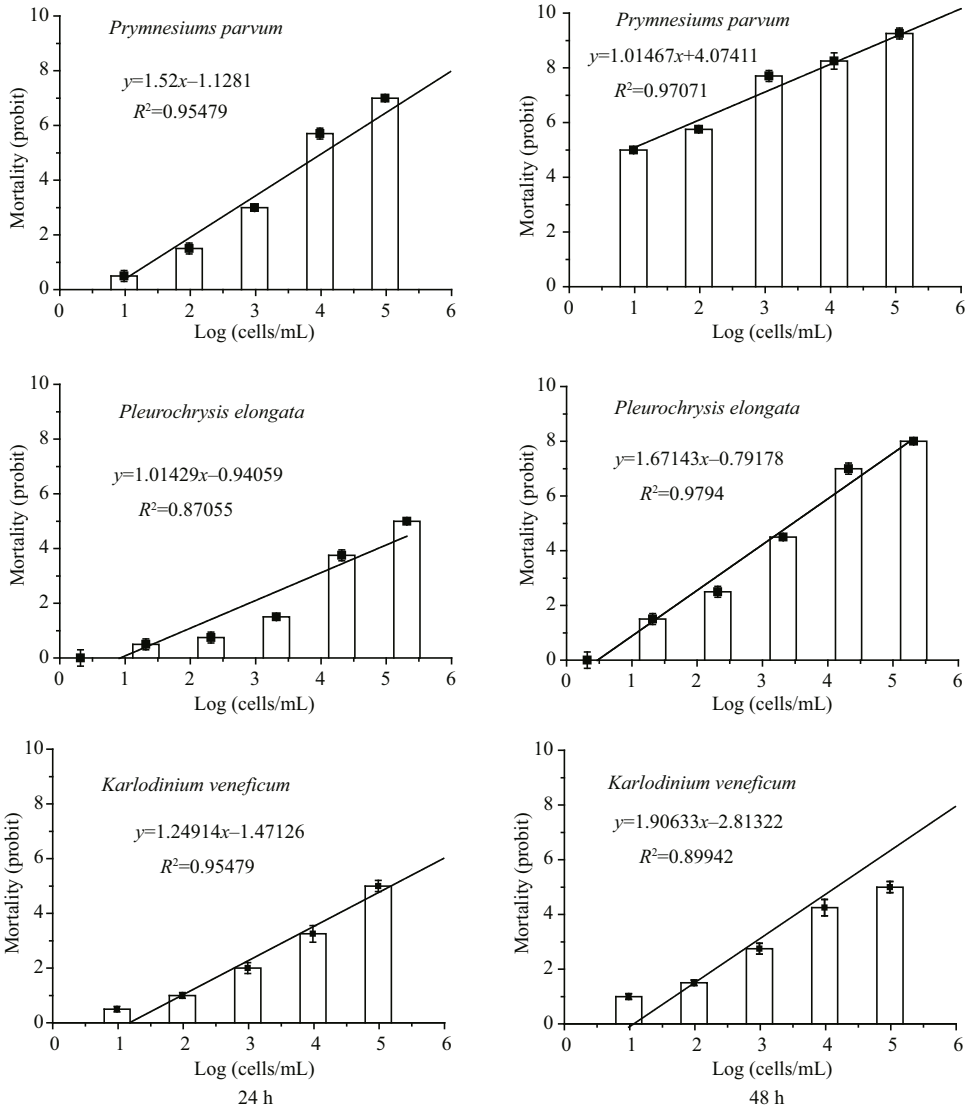


Fig.1 Mortality of *Artemia* nauplii as a function of microalgal cell density in 24 h and 48 h

Error bars: standard deviation.

Table 1 Median lethal density (LD_{50}) of *Prymnesium parvum*, *Pleurochrysis elongata* and *Karlodinium veneficum* on *Artemia* nauplii in 24 h and 48 h

	LD50 (cells/mL, 24 h)	LD50 (cells/mL, 48 h)
Prymnesium parvum	$1.1\times10^4\pm19.0$	8.0 ± 1.1
Pleurochrysis elongata	$7.2\times10^5\pm14.8$	$2.92\times10^3\pm2.6$
Karlodinium veneficum	$1.5\times10^5\pm66.9$	$1.25\times10^4\pm16.5$

in 24 h was 1.5×10^5 cells/mL. It was 1.3×10^4 cells/mL in 48 h. Linear regression relationship in both 24 h and 48 h were significant.

Table 1 compared toxic potency of the three harmful species based on simultaneously assessment of mortalities of *Artemia* in 24 h and 48 h. *P. parvm* had the highest lethal activities than the other two. In 24 h, *K. veneficum* showed higher toxic activity than *P. elongata*. In 48 h, *P. elongata* showed higher toxic activity than *K. veneficum*. No dead nauplii were observed in feed or starving controls.

3.2 Lethal effects of different algal samples on *Artermia* nauplii

3.2.1 Effects of the cellular extracts and cell debris

By comparing with the effects of live cells (LC) of each culture, mortality of *Artermia* as function of cell extracts (CEcf) and cell debris (CD) of the three species were studied (Fig.2).

For *P. parvm*, acute effects were observed in 5 h from live cells, cell extracts and cell debris groups,

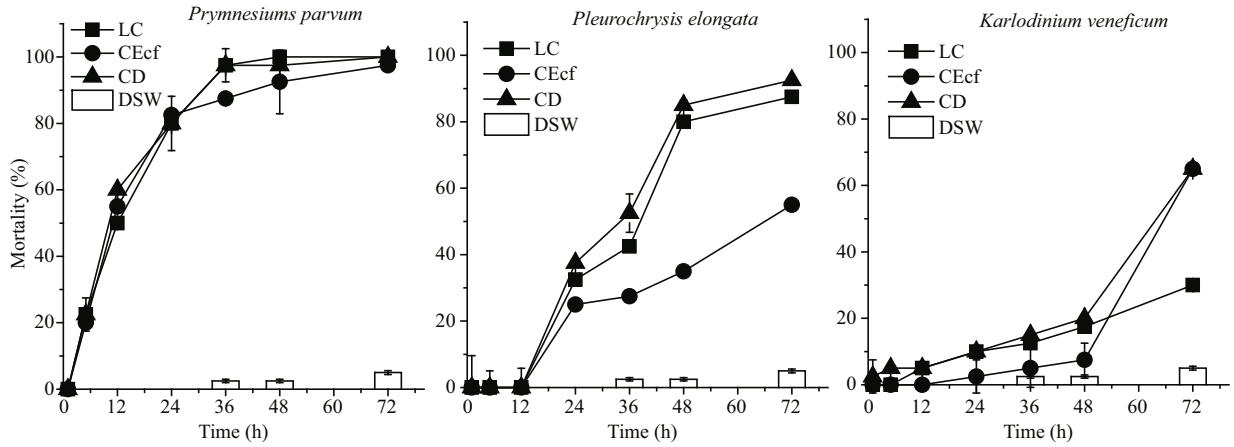


Fig.2 Lethal effects of living cells (LC), cellular extracts (CEcf) and cell debris (CD) of the three microalgal species on *Artemia* nauplii

DSW: fresh culture medium. Error bars: standard deviation.

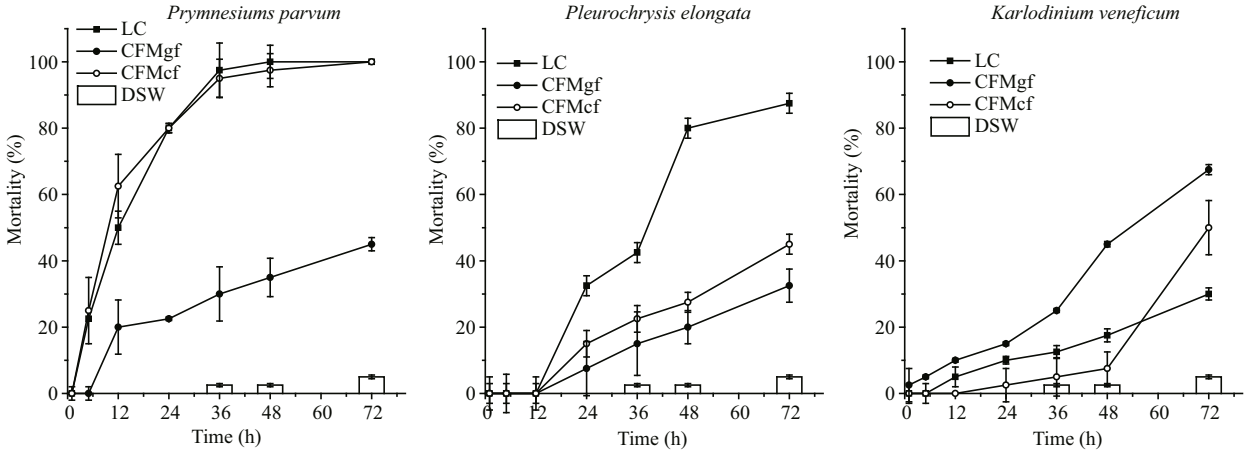


Fig.3 Lethal effects of living cells (LC) and cell free mediums of the three harmful species on *Artermia* nauplii

CFMgf: cell free culture medium prepared by way of gravity filtration; CFMcf: cell free medium prepared by way of centrifuge; DSW: fresh culture medium. Error bars: standard deviation.

mortality rates were 20%–22% without significant difference. In 36 h, the mortality reached 100%.

For *P. elongata*, no lethal effect was observed in 5 h or 12 h. Lethal effects showed no significant difference in 24 h. From 36 to 72 h, cell debris and live cells groups had similar lethal activities without significant differences. *Artemia* mortality was 2 times higher than that of the cells extracts in 48 h. It reached 90% in 72 h.

For *K. veneficum*, no acute effect occurred in 5 h. Mortalities, as low as 2% to 10%, were no significant differences in 24 h. Similar mortality in live cell and cell debris groups ranged 15%–18%. That of cell extracts was the lowest (5%). However, from 48 to 72 h, mortalities in cell extracts and cell debris groups increased significantly, averaged 60%, which was 2 times higher than that in live cells.

3.2.2 Toxic activities of cell free medium of the harmful microalgae

By comparing with effects of live cells (LC), mortalities of *Artermia* as function of cell free mediums were studied. Cell free process changed the toxic activities with species specific characteristics as showed in Fig.3.

Cell free process caused significant different results of toxic activities of the medium. For *P. parvum*, cell free medium prepared by the way of gravity filtration, CFMgf, had significant lower lethal effects on *Artermia*. Mortalities of *Artermia* in CFMgf decreased 60%–80%. And no acute mortality occurred in 5 h. However, cell free medium prepared by way of centrifuge, CFMcf, presented the same characteristics of toxic activities exactly as that of the live cells.

Table 2 Median lethal time (LT_{50}) of *Artemia* nauplii under the effects of samples from *Prymnesium parvum*, *Pleurochrysis elongata*, *Karlodinium veneficum*

	LC (live cells)	CEcf (cell extract)	CD (cell debris)	CFMcf (medium)	CFMgf (medium)
Prymnesium parvum	10.9±1.8	11.2±0.9	10.2±0.9	9.6±1.4	138.6±8.5
Pleurochrysis elongata	36.6±5.0	66.8±4.0	31.0±7.7	92.3±6.0	161.6±2.5
Karlodinium veneficum	125.6±4.3	74.3±2.7	60.0±2.8	90.9±5.3	55.7±6.3

LT_{50} was not supposed to be compared between different species.

Table 3 Characteristics of toxic potency of different algal samples of the three harmful species

Algal species	Acute (Y/N)	Toxic potency of different samples			Toxin(s) related
		24 h	48 h	72 h	
Prymnesium parvum	Y	LC=CEcf=CD LC=CFMcf>>CFMgf	LC=CEcf=CD LC=CFMcf>>CFMgf	LC=CEcf=CD LC=CFMcf>>CFMgf	Cell contents
Pleurochrysis elongata	N	LC=CEcf=CD LC>>CFMcf=CFMgf	LC=CD>>CEcf LC>>CFMcf=CFMgf	LC=CD>>CEcf LC>>CFMcf>CFMgf	?
Karlodinium veneficum	N	LC=CEcf=CD CFMgf>LC>CFMcf	LC=CD>CEcf CFMgf>LC>CFMcf	CD= CEcf >>LC CFMgf>>CFMcf>LC	Cell released

LC: live cells; CEcf: cell extracts; CD: cell debris; CFMgf: cell free medium by the way of gravity filtration; CFMcf: cell free medium by the way of centrifuge. The arrow refers to the increase of toxic potency with the time.

For *P. elongata*, mortality in cell free mediums decreased compared with LC group. However, there was no significant differences between CFMgf and CFMcf in 24 h–48 h (P>0.05). In 72 h, toxic activity of CFMcf was higher than that of CFMgf (P<0.05).

For *K. veneficum*, toxic activity of CFMgf significantly increased compared with that of live cells and CFMcf. In 72 h, both cell free culture mediums reached higher toxic activities than live cells. Still, mortality in the CFMgf was the highest (70% at 72 h).

Tables 2 and 3 summarized the different median lethal times (LT_{50}) and different toxic potency of the algal samples. Cell debris, cellular extracts and live cells of *P. parvum* had the same toxic activity, LT_{50} averaged 10.8 h. Cell debris and cell extracts of *K. veneficum* showed higher toxicity than that of living cells. Similar higher toxicities were observed both in debris and live cells of *P. elongate*.

On account of the cell free culture medium, toxic activities were affected by the process of cell free especially when *P. parvum* and *K. veneficum* were compared. Process of gravity filtration significantly decreased the toxic activities of cell free medium of *P. parvum*. However, for *K. veneficum*, LT_{50} of gravity filtrates was 55.7 h, which showed significantly higher toxic activity than that of live cells (LT_{50}=125.6 h) and that of supernatants by centrifugation (90.9 h).

Microalgae feed *I. galbana* was control in all the algal sampling process. The results showed that no lethal effects ever occurred in any of the algal sampling treatment in *I. galbana*.

3.3 Different conditions of dead *Artermia* nauplii showed different damage origination

Conditions of dead *Artermia* nauplii showed different characteristics in cultures of the three harmful species (Fig.4). Dead nauplii in *P. parvum* culture showed no abnormal structure (Fig.4a). Dead nauplii in *P. elongata* culture entwined in small groups with some broken appendages littered in water. The dying individuals were observed struggling with their limb that looked like been stuck (Fig.4b). In *K. veneficum* culture, the algal cells were actively aggregating around the tips of the appendages (Fig.4c). This phenomenon was mostly found in *K. veneficum* cultures but never found in the other two. This phenomenon suggested different active interaction between harmful microalgae and victim.

4 DISCUSSION

Prymnesins, toxins of *P. parvum*, was hemolytic, ichthyotoxic and cytotoxic (Igarashi et al., 1998). In the present study, toxic activities of *P. parvum* medium significantly decreased when cell integrity

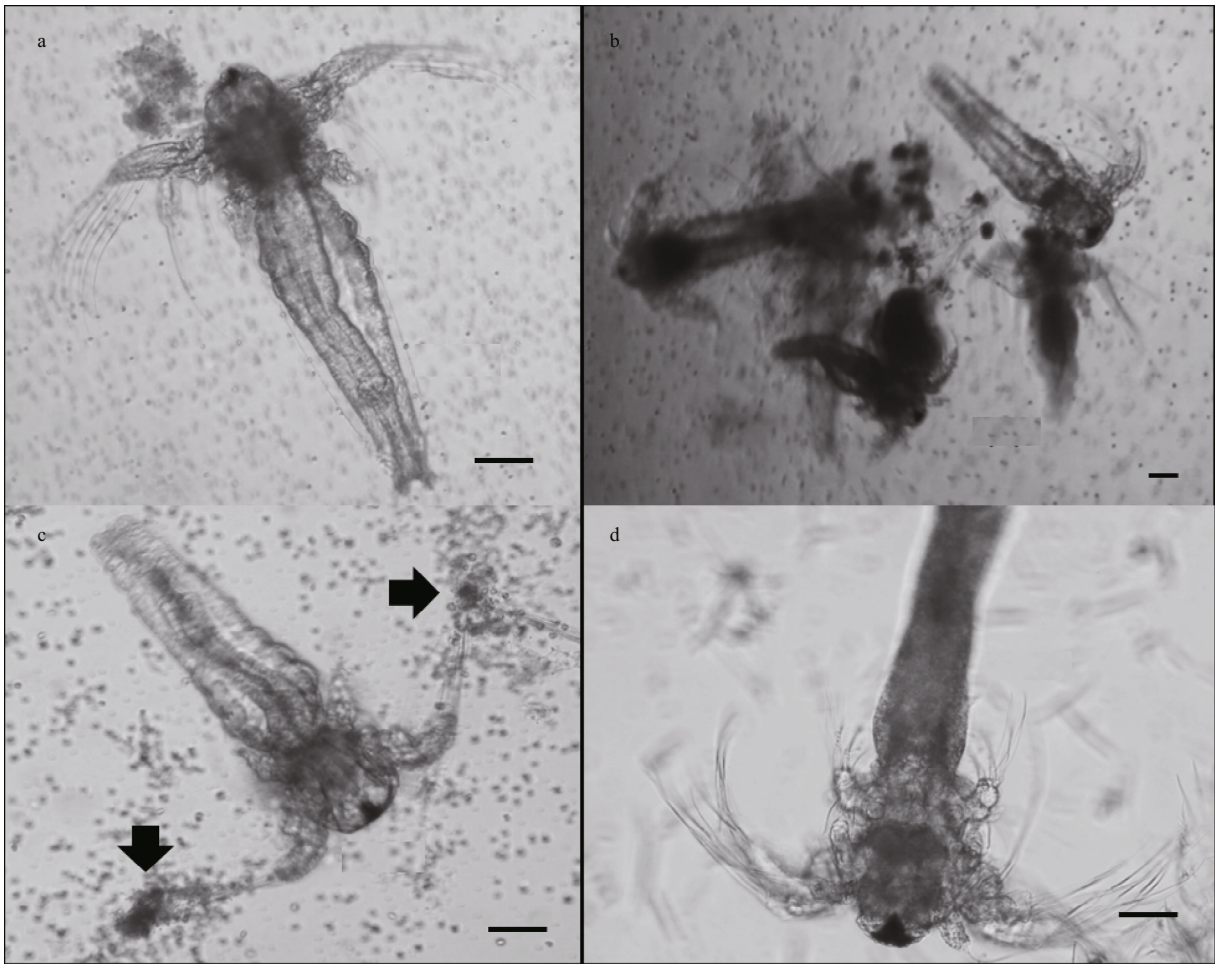


Fig.4 Condition of dead *Artemia* nauplii affected by harmful microalgal species

a. in *Prymnesium parvum* culture. The nauplii showed normal structure; b. in *Pleurochrysis elongata* culture. The nauplii aggregated in small groups, and some broken appendages littered in water; c. in *Karlodinium veneficum* culture. *Karlodinium veneficum* actively aggregated around the tips of the appendages (arrow); d. dead nauplii in filtrates of the harmful species showed common condition as that of live individuals. Bar=50 μm.

was maintained by gentle gravity filtration. Cell debris had the same toxic characteristic as that of live cells. This phenomenon agreed with the study of Remmel and Hambright (2012) that direct contact between predator and prey contributed a lot to the toxic activity. By separating algal cell and tested animals in a system that medium could exchange freely and algal cell integrity maintained, the authors demonstrated that toxic effects of *P. parvum* on micropredators occurred only if the microalgae and the predator contacted with each other, rather than exotoxicity.

Toxicity potency of *P. parvum* may not be dependent on prymnesins only, but on ambient nutrient conditions (Granéli and Johansson, 2003), growth conditions (Fresnel et al., 2001), laboratory-grown or natural field samples (Henrikson et al., 2010; James et al., 2011), and the activity and stages of tested animals. Different geographical strains may also show different toxic activities. In the study of Houdan et al. (2004), algal growth phase affected the toxicity of *P. parvum*. The highest toxicity came from the cultures of stationary phase. In their study, *P. parvum* was not the most toxic one to *A. salina* nauplii. LD_{50} in 24 h and 48 h were at the order of magnitude of 10^5 cells/mL, which was much larger than that in our study. Simonsen and Moestrup (1997) found that toxicity of *P. parvum* on *A. salina* was not significantly. According the supplier of *Artemia* nauplii in our study, the tested animals were originally collected from Qinghai Lake, a big salt lake in China. Differences of *Artemia* intoxication might be due to different strain of tested animals as well. So, even multiple toxicity characteristics can be obtained, the quantitative parameters of toxic activity based on *Artemia* lethality was not supposed to be compared among different studies, especially for those whose toxicity potency is dependent on various conditions.

Mechanism of lethal effects of *Pleurochrysis* is still a mystery. No toxin was found in *Pleurochrysis* genus even though obvious lethal effects on *Artemia* had been demonstrated (Houdan et al., 2004). Reifel et al. (2001) found that *P. pseudoroscoffensis* had a moderately toxic effect on *A. salina*, but was not toxic to vertebrates using mouse bioassay. In the present study, exposure time related mortalities were found and linear relationship depended on different time period. No acute toxic effects in 5 h or 12 h were observed in *P. elongata*. Linearity between mortality and algal cell density was less significant in 24 h than in 48 h. Mortality in control of the culture medium increased from 0 at 24 h to detectable at 48 h. At this time, probably the nauplii began to feed. Algae culture and cell debris of *Pleurochrysis caterea* were lethal to *Artermia* nauplii only when nauplii begin to feed (Jiang et al., 2009). The reason that entwined dead nauplii and broken appendages existed in *P. elongata* culture probably because of direct contact between prey and predator. In study of Houdan (2004), not all the calcified coccolithophorids was lethal to *A. salina*. Obviously, both the algae and the predator need further studies.

Compared with the former two species, toxic activities of *K. veneficum* of different samples were relatively lower in 72 h. However, species specific character was observed in *K. veneficum*. Firstly, it actively aggregated at the tip of the appendages of *Artermia* nauplii. Secondly, cell free filtrate of gentle gravity filtration was significantly higher than that of live cells and of cell free supernatant by centrifugation. To some extent, these two features are correlated because of one important fact that, *K. veneficum* is an active predator armed with specific toxin, karlotoxins. *K. veneficum* is mixotrophic dinoflagellate with ability of phagotrophy. It actively feed on various microalgae such as cryptophytes, diatoms, dinoflagellates and zooplankton such as *Acartia tonsa* (Place et al., 2012; Zhou et al., 2015). Karlotoxins help *K. veneficum* in predation (Deeds et al., 2002; Van Wagoner et al., 2010). Not only it can penetrate through cell membrane and makes prey's cell leaking, it can immobilize smaller prey for *K. venefivum* to catch the prey during predation (Sheng et al., 2010). Sterol composition have been demonstrated playing a role in sensitivity to the effects of karlotoxin (Deeds and Place, 2006; Place et al., 2006). Cholesterol is an important sterol component of *Artermia* (Payne and Kuwahara, 1972). Respiration apparatus of *Artermia* nauplii locates on the fibrous, feather like plates (Hickman, 1967). The relationship needs further demonstration.

Karlodinium venificum cells actively release karlotoxins either by filtration or by centrifugation (Deeds et al., 2002). This present study demonstrated that different toxic activity occurred during different cell free processes and *K. venificum* actively enhance toxin releasing when the cell experiences external force disturbance. For *K. veneficum* cell, both gravity filtration and centrifugation are stresses. Gentle gravity filtration process might mimic a kind of mechanical stimulation to the mciroalgae. Possibly karlotoxin release in respond to the mechanical stimulation such as shear stress, or to some kind of water current stirred by interaction between prey and predator in a micro scale (Adolf et al., 2007). Compared gravity filtration, centrifugation process gave *K. venificum* cells much stronger force. However, it is also a much rapid process. During this process, *K. venificum* cells had been captured before they were able to release any further toxins. It is reasonable that toxic activity was lower than gravity filtration. This phenomenon strongly demonstrated that *K. venificum* is an active toxin releaser. Gravity filtration may be applied in further studies on how and why *K. venificum* enhances toxin releasing.

5 CONCLUSION

Among the three harmful species that frequently occurred in aquaculture ponds, *P. parvum* is the most toxic one and acute toxic. Prymnesins, toxin of *P. parvum*, is possibly not exotoxic active. Lethal effects of *P. elongata* on *Artermia* mainly originated from cell's structural materials, suggesting harmful effects occurred when predator directly contact with the algal cells. Physical turbulence will significantly trigger *K. veneficum* to release more toxins into medium and increase its toxic activity. Karlotoxin is exotoxic active.

6 DATA AVAILABILITY STATEMENT

The authors declare that the data supporting the findings of this study are available within the article.

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