ZOOLOGICAL RESEARCH

Effects of osmotic pressure, temperature and stocking density on survival and sexual reproduction of *Craspedacusta sowerbii*

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ABSTRACT

The effects of osmotic pressure, temperature and stocking density on medusae survival of *Craspedacusta sowerbii* were examined. The medusae were shown to be sensitive to the variations of osmotic pressure. And the survival time was <90 h at 34 mOsm/L and it declined rapidly with rising osmotic pressure. The peak survival time of >200 h was recorded at 0.2 mOsm/L. Comparing with 27 °C and 32 °C treatments, 23 °C treatment yielded lower activities at a range of 8-13/min. However, there was a longer survival time. A non-linear relationship existed between survival time and stocking density. Lower density resulted in larger body size. And sexual reproduction resumed after breeding for >22 days. Newly-formed polyps and medusae appeared subsequently but only in the higher-density groups of 10, 14 and 18 ind./L. It suggested that the number of newly-formed polyps and medusae was highly dependent on stocking density. That is, a higher stocking density produced more organisms. However, newly-formed medusae died within one month and none grew a diameter of >5 mm.

Keywords: *Craspedacusta sowerbii*; Osmotic pressure; Temperature; Stocking density

INTRODUCTION

As a freshwater jellyfish, *Craspedacusta sowerbii* is widely distributed domestically in the Yangtze River (Dumont, 1994; Jankowski, 2001). Although several species of *Craspedacusta* have been identified, including *C. chuxiogensis, C. kawaii, C. kiatingi, C. kuoi, C. sichuanensis, C. sinensis* and *C. ziguiensis*, they were considered as the synonyms of *C. sowerbii* Lankester, 1880 (Bouillon & Boero, 2000a; Kramp, 1961; Lankester, 1880). Since the documentation of *C. sowerbii*, uni-sexual medusae organisms had been collected from many foreign habitats (Deacon & Haskell, 1967; Lytle, 1962). All Chinese medusae are composed of an almost equal number of females and males harvested from Zhejiang, Sichuan, Hubei and Yunnan Provinces (He, 2005). And it has survived as a successful invasive species on all continents except for Antarctica (Jankowski et al., 2008).

Previous studies of *C. sowerbii* have concentrated upon the four major aspects, including taxonomy (Bouillon & Boero, 2000b; Kramp, 1950), life cycle (Acker & Muscat, 1976; Lytle, 1959), distribution (Akçaalan et al, 2011; Dumont, 1994; Lytle, 1957) and nutrition relationship & ecological impact (Boothroyd et al., 2002; Dodson & Cooper, 1983; Jankowski et al., 2005; Stanković & Ternjej, 2010). However, few studies have examined its survival rate under different environmental conditions because of its unpredictable occurrence and difficult artificial breeding.

Up to the present, osmotic pressure, temperature and population density have been proposed as three major ecological influencing factors of survival, reproduction and behaviors of some hydrozoan species (Ma & Purcell, 2005; Mills, 1984). And the invasive species have unique patterns of migration and distribution. The present study was intended to elucidate the effects of osmotic pressure, temperature and stocking density on the survival of *C. sowerbii* medusae so as to provide some valuable

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references for preventing its invasion.

MATERIALS AND METHODS

Animal specimens

The specimens of *Craspedacusta sowerbii* were collected from a research pond at Endangered Fish Conservation Center (EFCC), Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS) (Kunming, Yunnan Province of China). And the identity of each organism was confirmed by taxonomists at KIZ. Their morphological characteristics were observed under a dissecting microscope (ZEISS Stemi 2000-c, Edmund Optics, Barrington, NJ, USA). And the confirmation of *C. sowerbii* was based upon the following characteristics: a maximal umbrella diameter of 15 mm, 190-215 tentacles, tentacle order III, annular and papilla-shaped nematocyst warts and approximately 128 tubular statocysts & slightly yellowish gonads. The fishery pond (lenth×width×depth=60.0 m×50.0 m×1.2 m) was used for medusae breeding. Both genders of *C. sowerbii* appeared simultaneously and females slightly predominated.

Experiment I: Effects of osmotic pressure on different growth stages of *C. sowerbii*

Medusae of three different growth stages (average diameters of 5.0, 8.0 and 15 mm respectively) were collected and then transferred into a 10 L laboratory fish tank filled with pond water for 2 h pre-adaptation. After 2-hour equilibration, each growth stage specimens (including 5 females and 5 males) were independently distributed at 7 gradients containing 1 L solution with duplicates respectively. Seven gradients of osmotic pressure (0.2, 34, 85, 170, 342, 684, 1 709 mOsm/L) were established by adding NaCl to a 6×10^{-4} mol/L $CaCl_2$ solution using underground water and the temperature was maintained at 21±0.5 °C (the same as pond). In each beaker, the diet was composed of ample shrimps *Eubranchipus vernalis* (40 ind./day, body size range of 1.5-2.5 mm). Survival time was recorded immediately after immersion and observation length determined according to osmotic pressure. Dead *C. sowerbii* medusae were removed immediately.

Experiment II: Effect of temperature on *C. sowerbii*

The procedures of specimen collection and pre-adaptation were similar to those of Experiment I. Only mini-sized medusae (average diameter=5.0 mm) were used. Three temperature gradients (23 °C, 27 °C, 32 °C) were established with duplicates (a total of 6 fish tanks). Heating rod was used for stabilizing temperature. Twenty percent of water was replaced every 2 days with pond water. Thirty females and 30 males were placed into each tank after pre-adaptation and fed daily with 100 fairy shrimps (body size=1.5 mm). Survival numbers were recorded once daily and activity was checked twice daily.

Experiment III: Effect of stocking density on *C. sowerbii*

A total of 10 tanks (10 L in volume) were suspended and immobilized by string at the corner of pond. Medium-sized specimens (average diameter=8.0 mm) were collected from pond and transferred into the tanks. And 5 stocking densities (2, 6, 10, 14, 18 ind./L) were established in duplicates (gender ratio=1:1). Ample fairy shrimps (body size=1.8 mm) were provided within each tank. Newly-formed polyps and medusae were checked daily for 76 days and new batches were transferred into a new tank suspended in pond. Average diameter (6 random specimens each time) and survival rate were measured every 3 days. One-third of water was replaced every 3 days by surrounding pond water.

Statistical analyses

The specimens sinking to the bottom of fish tank, without swimming behavior and showing no response to any stimulation were judged as dead *C. sowerbii*. Survival time (T) was calculated by the equation of $T=T_1-T_0$ where T_0 was the starting time of study and T_1 ending time when all specimens died. Activity was measured by recording the rising counts of *C. sowerbii* over half depth of water per minute in triplicates. Activity was calculated by the following formula:

$$\text{Activity}=\text{rising no./min} \qquad (1)$$

All statistical analyses were conducted by SAS statistical software (SAS 9.1). And statistical significance was assessed at a level of P=0.001.

RESULTS

Experiment I: Stress tolerance of osmotic pressure

Osmotic pressure influenced greatly the survival time of *C. sowerbii* medusae (Table 1). There were significant variations of survival time among osmotic pressure gradients in all three growth stages (ANOVA, P<0.001). In 0.2 mOsm/L treatment, medusae could survive over 200 h but activity and feeding rate declined obviously after 150 h. When osmotic pressure rose to 34, 85 and 170 mOsm/L, there was a rapid decline of survival time. At the osmotic pressure of 85 mOsm/L, none could survive over 24 h. At 342 and 684 mOsm/L, there was a quick body contraction and showed non-response to any stimulation after 4 min. At 1 709 mOsm/L, medusae died immediately and body sharply contracted to a minimum volume.

For different growth stages, osmotic pressure also affected its survival time (Duncan statistics based on observed data, P<0.0001). Compared to the diameters of 15.0 and 8.0 mm, the 5.0 mm-diameter specimens were more vulnerable to the variations of osmotic pressure and had a shorter survival time (Table 1). When osmotic pressures rose to 34 and 85 mOsm/L, smaller medusae moved slowly and spent more time on the bottom of beaker than larger ones. When osmotic pressure rose from 342 to 1 709 mOsm/L, the medusae of all growth stages died quickly.

Experiment II: Effect of temperature on *C. sowerbii*

The activities of medusae at 27 °C and 32 °C were significantly higher than those at 23 °C (P<0.001) during the first 6 days (Figure 1B). However, more than half specimens died at Day 7 at 32 °C and at Day 9 at 27 °C. By contrast, average activity at 23 °C was the lowest in the first 6 days. However peak survival time was recorded for most specimens and death occurred at Day 13 (Figure 1 A). Prior to death, the non-feeding specimens idled on the bottom of tanks, wriggled bodies and could not

Table 1 Effects of osmotic pressure on survival time of *Craspedacusta sowerbii*

Osmotic pressure (mOsm/L)	Survival time (hour)		
	15.0 mm	8.0 mm	5.0 mm
0.2	216.000±1.414	218.000±2.121	240.000±4.243
34	89.680±0.014	87.680±0.141	47.930±0.339
85	15.500±0.071	19.650±0.042	4.230±0.184
171	0.275±0.007	0.400±0.085	0.230±0.014
342	0.045±0.007	0.055±0.007	0.045±0.007
684	0.015±0.007	0.030±0.000	0.030±0.000
1709	0.000±0.000	0.000±0.000	0.000±0.000

surface. All specimens died within 4 days. The variations of survival time were significant among these temperature treatments (ANOVA, P<0.001). The peak survival time occurred at 23 °C at Day 17 while those at 27 °C and 32 °C were 12 and 13 days respectively. In summary, comparing to 27 °C and 32 °C treatments, 23 °C treatment showed lower activities (range, 8-13/min) with a longer survival time. During the study, no sexual reproduction occurred in all groups and no polyp was observed in each tank.

Experiment III: Effect of stocking density on *C. sowerbii*
Based on daily measurements, the temperature range of pond water was 17 °C to 22 °C. More than half of specimens died at Day 34 in each treatment except for 2 ind./L density group. The mortality time of all individuals in each treatment was as follows: 2 ind./L, 55 days; 6 ind./L, 76 days; 10 ind./L, 76 days; 14 ind./L, 55 days and 18 ind./L, 61 days (Figure 2A). Non-linear relationship existed between survival time and stocking density.

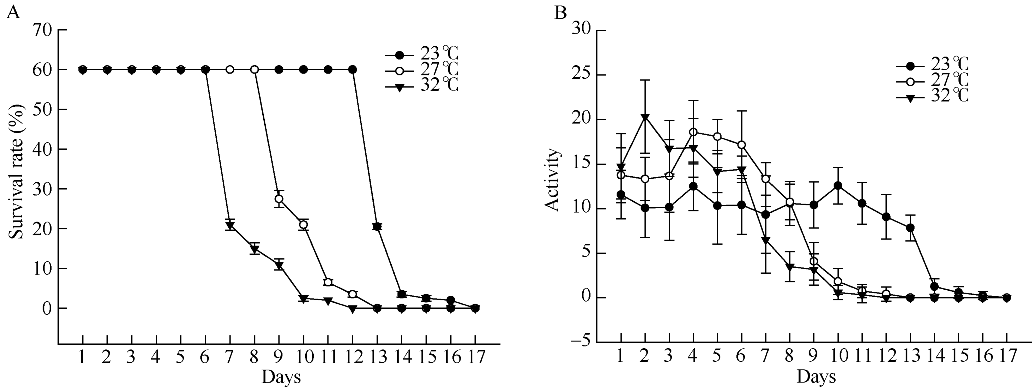

Figure 1 Effects of temperature stress on survival (A) and activity (B) of *Craspedacusta sowerbii*

Sexual reproductive behaviors occurred simultaneously in the groups of 10 ind./L, 14 ind./L and 18 ind./L at Day 22. And the 18 ind./L group reproduced more vigorously while the 10 ind./L group showed the least reproduction. Correspondingly, the numbers of polyps at Day 7 post-breeding showed a similar trend in three treatments (35 in 18 ind./L, 25 in 14 ind./L, 5 in 10 ind./L). At Day 9 post-breeding, a few newly-formed medusae appeared in these groups and often surfaced. And then, the maximal numbers of newly-formed medusae were measured for a few more days and removed gradually. Significant variations existed in the maximal number of newly-formed polyps and medusae between each group (Duncan statistics based on observed data, P<0.0001). All newly-formed specimens died within nearly 1 month and none grew a diameter of >5.0 mm. No reproductive behavior, newly-formed polyps or medusae were observed at the densities of 2 ind./L and 6 ind./L (Figure 2).

Based on the experimental data of the first 43 days, the average diameters of medusae changed marginally (Figure 3). Low-density group attained a faster growth speed of average diameter than high-density group in the first 7 days. And then there was a general decrease of growth speed, the average diameter at a low stocking density was longer than that at a high stocking density.

DISCUSSION

One of the most prominent features for *C. sowerbii* lied in its high water content. The medusae of freshwater jellyfish was presumed to have a water content of >98% of total body weight (Fleming & Hazelwood, 1971). Though hyper-osmotic to a pond water environment, the medusae had an appreciable permeability to sodium and water (Fleming & Hazelwood, 1967; Hazelwood et al., 1970) and could survive >200 h at 0.2 mOsm/L in underground water according to our study. However, under the stress concentration of 34 mOsm/L, its life span could not surpass 90 h. And its tolerability of osmotic pressure

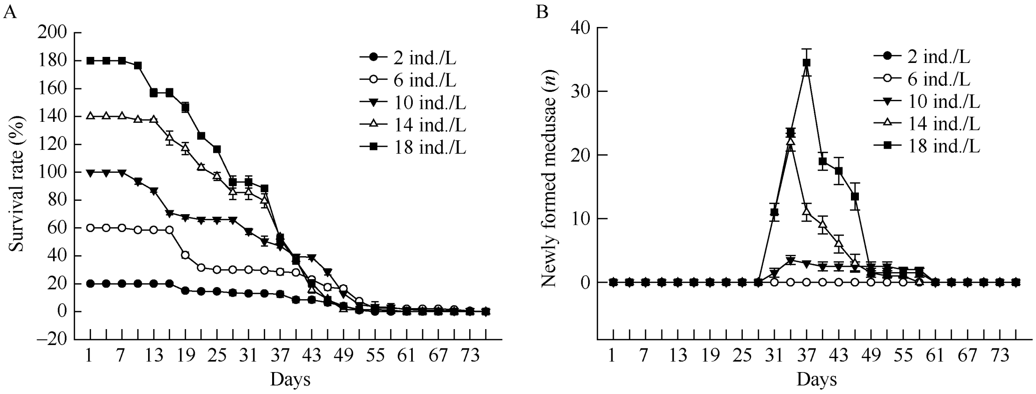

Figure 2 Effects of stocking density on survival (A) and sexual reproduction (B) of *Craspedacusta sowerbii*

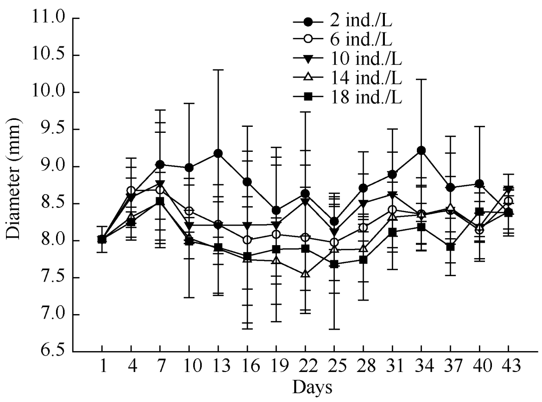

Figure 3 Effect of stocking density on diameter of *Craspedacusta sowerbii*

Up to Day 43, >6 specimens survived in each treatment.

was less than >120 hr at 92 mOsm/L in sea water as reported by Fleming. In our study, the tolerated osmotic concentration was <34 mOsm/L in pond water. It was lower than that of *Chlorohydra viridissimus* with a tolerated osmotic concentration of 40-50 mOsm/L (Lilly, 1955). At low osmotic pressures, younger specimens were more vulnerable to the variations of osmotic pressure. It suggested that younger medusae had not fully developed an osmoregulation system. Furthermore, for both old and young organisms, their volumes decreased with rising osmotic pressures. According to a previous study, gut acted as an osmoregulatory organ as well as a digestive system (Hazelwood et al., 1970). Thus medusae continued to excrete free water at a faster rate than an osmotic inflow of water through gut. Ultimately massive water loss disrupted the structure of gut and caused the death of organisms. In our non-published study, the organisms were lethal to larval fish through stinging. Therefore a slight elevation of osmotic pressure may be employed for preventing bloom of medusae and averting a depletion of fishery in pond.

On one hand, *C. sowerbii*, a globally distributed species, usually triggered the bloom of medusae at a temperatures range of 21 °C to 24°C (Acker & Muscat, 1976; Fish, 1971; Kimmel et al., 1980; Stefani et al., 2010; Tresselt, 1950). On the other hand, due to a larger adaptive scope of temperature variations, polyps could live within a short temperature range (Acker & Muscat, 1976; Dunham, 1941; Kramp, 1950). In the present study, medusae could survive at a temperature range of 15 °C to 24 °C in pond, but disappeared under 15 °C. Some organisms of *C. sowerbii* bloomed at 22 °C. In the laboratory, warmer temperature promoted the activity of medusae but shortened their survival time (<10 days). Only at a temperature similar to pond water, a longer survival time was obtained. But the mechanism has remained illusive. Low-temperature treatments were not applied due to the difficulties of conditioning by pond water. However, at <17 °C, low activities reappeared and the organisms died quickly. So low temperature decreased activity while high temperature increased activity. However, there was a shorter life span. Based on the previous distribution studies (Dumont, 1994) and our results, it was assumed that the medusae of *C. sowerbii* failed to flourish under the temperature of 15 °C.

Acting as preys to polyps and medusae, zooplankton impacted greatly the life history of *C. sowerbii* (Jankowski & Ratte, 2002; Spadinger & Maier, 1999). In Experiment III, an ample supply of food was provided for eliminating the effect of prey deficiency. Initially the organisms grew normally in all groups but then death occurred gradually. Massive death was not found within a short period. Several specimens survived for up to 73 days in two treatments. A previous report had the similar findings (Pennak, 1956). However, the survival time was much longer in the present study. Except for particular cases, a downward trend of survival number was present among all treatments. Under our experimental conditions, the effect of stocking density on survival rate was not obvious under these settings. However, sexual reproduction and diameter were obviously influenced evidently in each treatment.

Sexual reproduction occurred at a stocking density of 10 ind./L. As shown in Figure 2, the number of polyps and newly-formed medusae increased with rising stocking density. Polyps tended to adhere to the tank bottom and some alga were difficult to discover. However, no polyp was found in each group at the end. We surmised that temperature decrease might explain the disappearance of polyps. The peak number of newly-formed medusae occurred at around 21 °C while there was an onset of death at <19 °C. Thus younger organisms probably required a higher temperature for body growth. Besides, the population quantity of small dying zooplankton was low under a dissecting microscope. Previous study also showed mini-sized body of zooplankton was consumed that larger individuals were spared (Dodson & Cooper, 1983; Spadinger & Maier, 1999). No polyps was found in the groups of 2 and 6 ind./L. So only a high stocking density could induce sexual reproduction.

The diameter variations of medusae have been previously reported. However quantitative study has been scarcely performed (Fish, 1971; Pennak, 1956). The growth of medusae was negatively correlated with stocking density. After initial growth, morphological degeneration appeared after each treatment. So far the underlying reasons for morphological degeneration are still unclear. After the last treatment, secondary growth of medusae was probably due to the expansion of environmental space. In summary, low density enhanced growth but it had no effect on reproduction. By contrast, a high stocking density resulted in a lower average diameter. Yet medusae of a larger average diameter also exist and sexual reproduction may be triggered.

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