Influence of seawater salinity on the survival, growth, development, and neonate production of *Scapholeberis mucronata* (O. F. Müler) (Crustacea: Cladocera)

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In order to determine the influence of of salinity on the survival, growth, development, and neonate production of the cladoceran *Scapholeberis mucronata* (O. F. Müler), long-term experiments were carried out at four salinity gradients (1, 2, 3, and 4) and a control (freshwater) using *Chlorella pyrenoidesa* as feed. The acute effect of salinity on this species indicated that salinity gradients 4 and 4.5 were its upper tolerance limits for reproduction and survival. The survival and growth rates of individuals reared in salinity gradients 1 and 2 were higher than those reared in other salinity gradients. The mean size of the female adults decreased from 820 to 743 μm when the salinity increased from salinity gradients 1 to 4. For individuals reared in salinity gradients 1 and 2, the r_m of population was 1.021 and 0.903, respectively; the rate of egg production was 1.281 and 1.390, respectively; the cumulative egg production was 83.2 and 106.0 and mean life span was 16.05 and 17.30, respectively. These values of life-history parameters were higher than those of individuals reared in salinity gradient 3. No eggs were produced by females reared in gradient 4 during the entire experiment. Furthermore, individuals reared at gradients 1 and 2 had faster embryonic development. The above results imply that *S. mucronata* prefer an environment with lower salinity (1-2). Resting egg formation and sexual reproduction did not occur in any of the tested salinity gradients.

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17

18 Abstract

19   In order to determine the influence of of salinity on the survival, growth, development, and
20 neonate production of the cladoceran *Scapholeberis mucronata* (O. F. Müler), long-term
21 experiments were carried out at four salinity gradients (1, 2, 3, and 4) and a control (freshwater)
22 using *Chlorella pyrenoidesa* as feed. The acute effect of salinity on this species indicated that
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24 survival and growth rates of individuals reared in salinity gradients 1 and 2 were higher than
25 those reared in other salinity gradients. The mean size of the female adults decreased from 820 to
26 743 μm when the salinity increased from salinity gradients 1 to 4. For individuals reared in
27 salinity gradients 1 and 2, the r_m of population was 1.021 and 0.903, respectively; the rate of egg
28 production was 1.281 and 1.390, respectively; the cumulative egg production was 83.2 and 106.0
29 and mean life span was 16.05 and 17.30, respectively. These values of life-history parameters
30 were higher than those of individuals reared in salinity gradient 3. No eggs were produced by
31 females reared in gradient 4 during the entire experiment. Furthermore, individuals reared at
32 gradients 1 and 2 had faster embryonic development. The above results imply that *S. mucronata*
33 prefer an environment with lower salinity (1-2). Resting egg formation and sexual reproduction
34 did not occur in any of the tested salinity gradients.

35

36 Introduction

37   Cladocera constitute a vital component of most zooplankton communities in the freshwater
38 ecosystem (Dodson and Frey, 1991). Studies have implied the importance of Cladocera in

39 utilizing primary resources which, in turn, influences the turnover of higher trophic levels
40 (Selvakumar, 1970). The functional roles of Cladocerans have been well documented in lakes
41 and seas for their secondary production and energetic associations (Lemly and Dimmick, 1982;
42 Mengestou and Fernando, 1991; Mavuti,1994; Puelles et al, 2003). The effects of certain
43 environmental factors on Cladocera have been studied extensively because of their importance to
44 the aquatic system. These factors include temperature, salinity, light, and the photoperiod..
45 Many studies have been conducted on the effect of salinity on Cladocera (Aladin, 1982; Aladin,
46 1991; Hart et al, 1991; Williams, 1998; Latta et al, 2012;), especially on freshwater species, like
47 *Moina rectirostri* Leydig (He et al, 1986), *Moina mongolica* Daday (He et al 1988, 1990, 1994;
48 Wang and He, 2001，Zhao et al, 2008), *Daphnia magna* Straus (Kikuchi,1983; He et al, 1996;
49 Baillieul et al, 1998), *Moina macrocopa* Straus (He, 1983), *Simoceohalus vetulus* O. F. Müler,
50 *Daphnia pulex* King (Wang et al, 1996), *Daphnia sarsi* Richard (Wang, 1999), *Diaphanosoma*
51 *celebensis* Stingelin (Achuthankutty et al, 2000; Jung et al, 2001) and *Diaphanosoma aspinosum*
52 Chiang (Segawa and Yang, 1987).
53   The present study focuses on the effect of salinity on S*capholeberis mucronata*, which is a
54 cosmopolitan eurythermal cladoceran. Compared with other species, few quantitative studies
55 have been made (Rammner, 1927; Kawabata, 1998; De Meester, 1993). Lemke and Benke
56 (2003) and Huang (1985) studied the biology of *S. mucronata*. Experiments were designed to
57 evaluate the effect of different salinity gradients on parthenogenetic reproduction, growth rates,
58 and the ecological development of this species in the aquatic system in order to learn more about
59 its growth and reproductive behavior and to determine whether it can be used as a live
60 aquaculture food source..
61

Materials & Methods

Animal incubation and preparation

64   *S. mucronata* were collected from fish ponds at the Biliuhe Reservoir Fisheries Co. and were
65 cultured at the Key Laboratory of Hydrobiology in Liaoning Province. Laboratory-reared
66 parthenogenetic females of *S. mucronata* whose juvenile age was less than 2 hours old (h) were
67 used for the experiment. A monoculture of *Chlorella pyrenoidesa*, taken from Key Laboratory of
68 Hydrobiology in Liaoning Province during the exponential phase of growth, and was offered as
69 food. This source was supplied in sufficient quantity to maintain a slight green hue in the water.
70 Desired salinity gradients were prepared by diluting autoclaved, aerated, and nucleopore-filtered
71 (Millipore, 0.22 μm) sea water (FSW) of salinity at 32 [insert relevant unit of measure] with the
72 required volume of distilled water. The cultured water was changed every other day. All of the
73 experiments were conducted at 25±0.5°C. A photoperiod of light:dark (14 h L:10 h D) was
74 maintained for culturing and testing.
75

Median lethal concentration (LC_{50}) experiment

77   In order to assess the acute effect of salinity on *S. mucronata*, the bioassay consisted of ten
78 newborn juveniles grouped in 6 different salinity gradients (3.79, 4.15, 4.55, 4.99, 5.47, 6.00).

79　The experiment was conducted in 60 ml glass-stoppered bottles, each containing 50 ml of
80　cultured water. The experimental set up consisted of 5 such bottles for each salinity treatment.
81　Juveniles were monitored for mortality over 96 hours periods. Survival data was used to
82　calculate LC_{50}, the salinity resulting in 50% mortality over a given time. LC_{50}s were calculated
83　for 1, 2, 4, 8, 16, 24, 48, 72, and 96 hours using the Probit Method (Zhou and Zhang, 1989).
84　NOAEL (No Observed Adverse Effect Level, which is the highest concentration producing no
85　adverse effects significantly different from the control) can be assessed according to the results.
86

Chronic toxicity studies

87
88　　　One hundred newborn juveniles were reared in a 1000 ml beaker under salinity 1. Seawater
89　was added to the cultured water every day to elevate salinity by 0.125 units. When juveniles
90　were released from the brood chamber of adult *S. mucronata*, they were collected to continue the
91　study using the method mentioned above. After 7 to 8 days of increasing salinity, adults did not
92　produce offspring and the noted value of salinity was deemed the upper limit for the
93　reproduction of *S.mucronata*.The experiment was continued until about 50% specimens died
94　andthis value of salinity was determined to be the upper limit of survival for *S. mucronata*.
95

Life table analysis and reproduction

96
97　　　To determine the influence of salinity on growth, life span, and neonate production, long-term
98　experiments were carried out at four salinity gradients (designated as 1, 2, 3, and 4) and a control
99　(freshwater). 325 neonates (< 2 h) were transferred to each 50 ml bottle and 15 such bottles were
100　maintained for each salinity treatment. Concurrently, ten juveniles were introduced into one
101　bottle and 5 such bottles were prepared for each salinity treatment, which were used to compute
102　the intrinsic increasing rate (r_m) of *S. mucronata*. Survival, growth, maturity instars, and neonate
103　production in each bottle were recorded three times daily and fresh algae liquid bait was
104　supplemented into the freshwater to maintain the stability of salinity. The increase in length (μm)
105　of each live specimen was measured daily with an ocular scale under an Olympus microscope at
106　40×. The experiment lasted until the death of all the animals.
107　　　From the results, life expectancy data under every salinity gradient were tabled and r_m, and
108　other parameters were calculated based on equation $\sum(l_x b_x e^{-r_m x}) =1$. This equation was solved
109　iteratively to obtain a more precise solution for r_m (Gotelli, 1995), where x is the age in days, l_x is
110　the proportion of surviving individuals at the onset of each interval (x), and b_x is the number of
111　juveniles produced per females during interval x.
112　　　About four hundred parthenogenetic females were reared individually in 24-well cell plates in
113　order to study the different embryonic stages under various salinity treatments. It was not
114　possible to clearly observe the details of embryonic development in the brood chamber itself due
115　to its dark color and opacity of the carapace. Therefore, a few parthenogenetic females were
116　killed at fixed time intervals and their brood chamber carefully dissected out to release the
117　developing eggs, revealing the distinct stages of development.
118

Results

Survival

121 During the experiment on the acute effect of salinity on *S. mucronata*, individuals did not die
122 within the first two hours. However, four hours after the onset of the experiment, deaths were
123 observed. The LC_{50}s of juveniles exposed to salinity exhibited at 4, 8, 16, 24, 48, and 72 hours
124 were 13.43, 7.47, 5.60, 4.88, 4.45, and 3.91, respectively. According to the Fig. 1a, it can be
125 assessed that the most vitally protective salinity gradient for *S. mucronata* was less than 4.00.
126 The upper limit for the reproduction and survival of *S. mucronata* were 4 and 4.5, respectively.
127 Under all salinity treatments, the curve of survival rate of *S. mucronata* corresponded with
128 Deevey I's pattern, namely a low death rate in the population at the prophase of the culture and a
129 high death rate at anaphase.

130 The survival rate of *S. mucronata* reared in salinity gradients 1 and 2 was sustained above
131 90% 14 to 15 days after the onset of the experiment, which was obviously higher than those
132 reared in other treatments. However, after days 16 to 17, the survival rate of *S. mucronata* reared
133 in all salinity gradients rapidly declined, ultimately until death (Fig. 1b).

134

Growth and reproduction

136 Mean initial body length (0.28±0.01 mm) did not vary significantly among salinity gradients
137 for *S. mucronata* juveniles. The initial body length increased at a rate that did not correspond
138 with salinity (Fig. 2a). Length was noted to increase more quickly in the initial stages of the
139 experiment at all treatments and slowed more noticeably at higher salinity treatments. The
140 juveniles in salinity gradient 1 grew faster than those in other gradients. There was a significant
141 inverse relationship in the size attained by female adults ($p<0.05$) with salinity (mean size: 820
142 μm at 1 and 743 μm at 4). Females reared in gradient 3 reproduced significantly later than those
143 reared at a salinity ≤2 ($p<0.05$), although the age at first reproduction (A_R) did not differ
144 significantly between the control and salinity gradients 1 and 2 (Tab. 1). Mean intervals between
145 clutches appeared to follow a similar trend. *S. mucronata* individuals reared in salinity gradient 3
146 produced significantly fewer clutches per female, number of eggs per female, and number of
147 eggs per clutch than those reared in other treatments (Tab. 1). These parameters of reproduction
148 were not different among the control, and gradients 1 and 2 (Tab. 1). Egg production was
149 initially high for females reared in salinity gradient 1 but began to level off at 72.3 eggs per
150 female after the 8th maturity instars (Fig. 2b). Females reared at salinity gradient 2 continued to
151 produce eggs and reached a maximum of 106.0 eggs per female. Cumulative egg production
152 corresponded to a maximum of 83.2, 73.1 and 31.7 eggs per female at gradient 1, control, and
153 gradient 3, respectively. The maximum number of eggs per female for individuals reared in
154 salinity gradient 1 was reached at the 5th adult instars, whereas at gradient 2 and control, the
155 maximum value was reached at the 6th. Interestingly, after reaching the maximum value, the
156 number of eggs per female for individuals reared in all treatments presented a wave change until
157 death (Fig. 2c). The cumulative egg production of this species was plotted against adult instar

158 numbers showing the rates of egg production, which was the angle of the slope of the regression
159 line (Murugan and Sivaramakrishnan, 1976).
160   The rates of egg production exhibited 1.281, 1.390, 1.324 and 0.921of salinity gradients 1, 2,
161 control and 3, respectively (Tab. 1, Fig. 2b). The reproductive value (V_X) is important to
162 determine the contribution to the future population that an individual female will make (Krebs,
163 1994). The maximum reproductive value of *S. mucronata* at all treatments was reached 5 to 7
164 days from onset of production. The V_X of specimens reared at gradient 2 was significantly greater
165 than those reared in other treatments (Fig. 2d).
166
167 **Life table**
168   Instar indicates the time of desquamating, while the duration of instar refers to the interval of
169 desquamation. Under all treatments, *S. mucronata* had three consistent pre-adult instars, but had
170 a different number of adult instars. At 2, there were thirteen adult instars, where other treatments
171 had eleven adult instars. The duration of each instar and the cumulative duration of each instar
172 were significantly longer for individuals reared at control compared with those reared at salinity
173 ≥1 (p<0.05), while specimens reared in salinity gradients 1, 2, 3, and 4 did not differ and
174 corresponded to the cumulative duration of each instar of 24.5, 18.4, 23.3, 18.0 and 18.6 days per
175 female for individuals reared at control, and gradients 1, 2, 3, and 4, respectively. As is typical in
176 this stage of development, the first adult instar, during which the females were primiparous, was
177 distinctly longer than the longest pre-adult instar observed in all treatments. Mean lifespan did
178 not differ among individuals reared in all treatments, although those reared in gradient 2
179 exhibited a longer mean lifespan than other groups (Tab. 1). The intrinsic rate of population (r_m)
180 increase was not related to salinity and differed from 0.481-1.021. The value of r_m was highest at
181 1 (Tab. 2). Mean generation time (G) was not related to salinity. *S. mucronata* reared in 1 and 3
182 had a shorter mean generation time than those reared at control and salinity gradient 2. The net
183 reproductive rate (R_0) increased with the salinity from 34.6 at control to 60.6 at 2, but R_0 did not
184 increase with salinity ≥3 (Tab. 2). The finite rate of increase (λ) decreased from 2.78 at 1 to 1.62
185 at 3, while 2.18 at control (Tab. 2).
186
187 **Embryonic development**
188 **Embryonic development process of *S. mucronata***
189   The embryonic development of cladocera was divided into 8, 5, or 4 stages for different
190 cladoceran species (Green, 1956; Murugan, 1975; Gulbrandsen et al, 1990). In the present study,
191 the salient morphological features characteristic of the distinctive embryonic stages of *S.*
192 *mucrunata* were divided into 15 stages (Tab. 3 and Appendix Figure).
193   The time of egg release from the ovaries to the brood chamber was very short. In freshwater at
194 25 °C, this time was about 20 seconds per egg release and typically all eggs were released within
195 2-4 minutes. The intervals between the release of the last juvenile from the brood chamber to the
196 external environment and prior to the discharge of the first egg from the ovaries to the brood
197 chamber is usually short and the eggless periods are only about 1-4 percent of the duration of

198   embryonic development (Huang, 1986, Bottrell, 1975). In the present study the eggless period
199   was approximately 15.5 minutes, which is shorter than other species (Carmouze, 1983).

200   Stages of the embryonic development of *S. mucronata* are briefly described as followed:

201   I. Stages 1-2: Eggs changed from elongate columniform to oval and ovoplasm changed from
202   even to translucent with a transparent peripheral zone. The central region of the egg had fat cells
203   surrounded by cleaved peripheral granulated cells. At this stage, both the outer egg membrane as
204   well as the inner naupliar membrane were seen (Appendix Fig.1-2).

205   II. Stages 3-6: Eggs divided from one cell to multiple cells. Vegetal and animal poles appeared
206   and cell differentiation began (AdditiveFig.3-6).

207   III. Stages 7-8: The blastula and gastrula orderly formed (Appendix Fig.7-8).

208   IV. Stages 9-11: The antennae elongated and formed a "T" type embryo. The head rudiment
209   was formed and a distinct head and limb rudiment were formed but eyes were not yet apparent
210   (Appendix Fig.9-11).

211   V. Stages 12-13: Embryo had two very small pink eyes, which rapidly increased in size and
212   became two large black eyes. A distinct single compound eye was formed and the embryonic
213   heart began to beat. Juveniles in the brood chamber were able to turn around (Appendix Fig.12-
214   13).

215   VI. Stages 14-15: The egg membrane was cast off and characters of adult morphology such as
216   the straight ventral margin of the shell ending posteriorly in a spine, the quadrate shape, the fine
217   short setae on the ventral margin of the shell, and the reddish color were developed. Then
218   juveniles were released from the brood chamber and moved freely (Appendix Fig.14-16).

219   Parthenogenetic and ephippium females of *S. mucronata* are shown in additive (Appendix
220   Fig.17-18).

221

222   **Embryonic development at different salinities**

223   The speed of embryonic development was significantly greater for females reared at 2
224   compared with those reared at other treatments ($p<0.05$), and the cumulative duration of each
225   phase for individuals reared in salinity gradient3 was longer than those reared at other treatments
226   ($p<0.05$). The cumulative duration of each phase corresponded to the mean of 25.4, 24.8, 23.2,
227   and 28.0 hours per female for individuals reared in the freshwater control, and gradients 1, 2, and
228   3, respectively (Tab. 3).

229

230   # Discussion

231   Many of studies looking at the effect of salinity on Cladocerans provide important
232   physiological information and insight into their ecological differences. The present study
233   identified *Scapholeberis mucronata* O. F. Müler as a freshwater zooplankton with a higher
234   tolerance to low salinity concentrations. However, they vary from other geographically diverse
235   populations such as *Daphnia magna* (Straus) (He et al, 1996). A study of some salt lakes in the
236   JinNan and YinChuan regions, found *S. mucronata* in a small wetland with a salinity
237   concentration 1.73. According to Alonso et al (1990), *S. mucronata* appeared and formed

238 populations in a salty lake in Spain in which the salinity was 40. Zhao (1992) suggested that *S.*
239 *mucronata* belongs to the halophile species, in which the appearance of amplitude is affected by
240 salinity levels from 10 to 60 (Williams, 1983). However, Pennak (1989) pointed out that this
241 species is broadly distributed throughout the USA but that they only inhabit freshwater
242 sources.The differences in habitat are influenced by geographical isolation and by the genetic
243 makeup of the organism.
244   Reproduction is a major physiological activity for any living organism and is influenced by the
245 prevailing environmental conditions (Vernberg and Vernberg, 1972). The review of the
246 reproductive capacity of Cladocera in relation to the environment by Zheng (1990) provided
247 information on various environmental factors affecting parthenogenetic reproductive behavior
248 for many species of Cladocera. However, he did not mention the studies on the effect of salinity
249 on growth or parthenogenetic reproduction. In this study, salinity was the only variable factor in
250 the experiment and obviously had a direct impact on growth and neonate production. Females
251 reared at lower salinity (1-2) had a longer lifespan; higher r_m, R_0 values, and neonate production,
252 and a faster rate of growth. These observations were in close agreement with the earlier studies
253 of Segawa and Yang (1987) and Achuthankutty, et al (2000). Age at first reproduction and the
254 size of the first clutch were two determining factors affecting the r_m value (Cole, 1954; Meats,
255 1971; Snell, 1978; Lynch, 1980; He, 1983).
256   The individuals of *S. mucronata* reared in all treatments began with the same number of pre-
257 adults, but a different number of eggs were produced in the first clutch. *S. mucronata* reared at
258 salinity gradients 1 and 2 reproduced with 12.57±0.74 and 12.43±0.27 eggs in the first clutch,
259 which was more than other treatments. Population at gradients 1 and 2 had a higher r_m value. The
260 rate of egg production (*a* value) is an index of many synthetic factors, which strongly relates
261 with adult instar numbers and number of eggs per clutch (Huang, 1983). Because of the greater
262 number of eggs per clutch, individuals reared at salinity gradients 1 and 2 had higher overall
263 rates of egg production. The results of the present study reveal that salinity gradients 1 and
264 2could be considered the optimum salinity amplitude for the population of *S. mucronata.*
265   Few studies regarding the effect of salinity on the embryonic development of Cladocera have
266 been published (Wang et al, 2000). The present study indicates that there were variations in the
267 duration of the embryonic period with different treatments. The rate of development was
268 relatively faster for individuals reared at 1 and 2 than those reared at other salinity gradients,
269 which prove that this species is suitable to live in low salinity water.
270   It is known that a small increase of salinity in freshwater can stimulate metabolism, growth,
271 and reproduction of freshwater animals. *Daphnia magna* reared at 2-3 had the highest feeding
272 rate and rate of food assimilation, while the rate of oxygen consumption was lowest for
273 individuals reared in salinity gradient 2 (Wang, 1991; Yang, 1997). These results indicated that
274 the physiological activity of *D. magna* was stimulated by the environment conditions of 2-3. In
275 addition, in a study on the effect of salinity on standard metabolism and the energy budget of
276 carp, it was found that the feeding rate, rate of growth, and rate of energy metabolism for
277 individuals reared at salinity between 3 to 7 were all higher than those reared in freshwater,

278   while standard metabolism, rates of oxygen consumption were lowest at 3. In the present study,
279   the rate of growth and embryonic development were faster for *S. mucronata* reared at 1-2 than
280   those reared in other salinities. These findings were in keeping with the results mentioned above.
281   Such a stimulative effect can be explained by two aspects of bioenergetics: first, a small
282   increase of salinity in freshwater can accelerate the capacity for assimilation and absorption in
283   freshwater animals, which enhances the efficiency of energy assimilation. Secondly, many of
284   freshwater animals must maintain the stability of ion concentration within the cells through the
285   active uptake of ions and this osmotic regulation requires the consumption of energy. Increasing
286   salinity in freshwater reduces ion gradients inside and outside of cells which causes freshwater
287   animals to decrease their energy consumption in order to osmoregulate and increase energy
288   consumption for growth reproduction.
289   Females with resting eggs were not observed in the present study. Production of resting eggs
290   was probably a genetically acquired trait to overcome the extreme temperature changes
291   experienced during glacial periods (Madhupratap et al, 1996). No study has yet yielded any
292   evidence to show that salinity extremes induce resting egg formation in invertebrates.
293   From present study, we know that population of *S. mucronata* have higher r_m and tolerance to
294   low salinity and that the adult body length is less than 1mm. Therefore, this species can be
295   considered as a potential resource for using as a live aquaculture feed.
296

Conclusions

298   The acute effects of salinity on *S. mucronata* indicate that 4 and 4.5 its upper limit for
299   reproduction and survival. The survival and growth rate of individuals reared at gradients 1 and 2
300   were higher than those reared in other salinity gradients. The mean size of adult females
301   decreased from 820 to 743 μm when the salinity was increased from 1 to 4. To individuals reared
302   in gradients 1 and 2, the r_m of population was 1.021 and 0.903; the rate of egg production was
303   1.281 and 1.390; the cumulative egg production was 83.2 and 106.0 and mean life span was
304   16.05 and 17.30, respectively. These values of life-history parameters were higher than those of
305   individuals reared at 3. No eggs were produced by females reared at 4 during the entirety of the
306   experiment. Furthermore, specimens reared at gradients 1 and 2 had a more rapid rate of
307   embryonic development. The above results show that *S. mucronata* prefer an environment with
308   lower salinity (1-2). Resting egg formation and sexual reproduction did not occur in any of the
309   tested salinity gradients.
310

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314

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457

Figure 1(on next page)

LC$_{50}$s (a) between salinity and time and survivorship (b) of *S. mucronata* reaed at different salinity gradients.

Value of LC$_{50}$s represent mean (±SE) value. S0, S1, S2 and S3 expressed control, 1, 2 and 3, respectively.
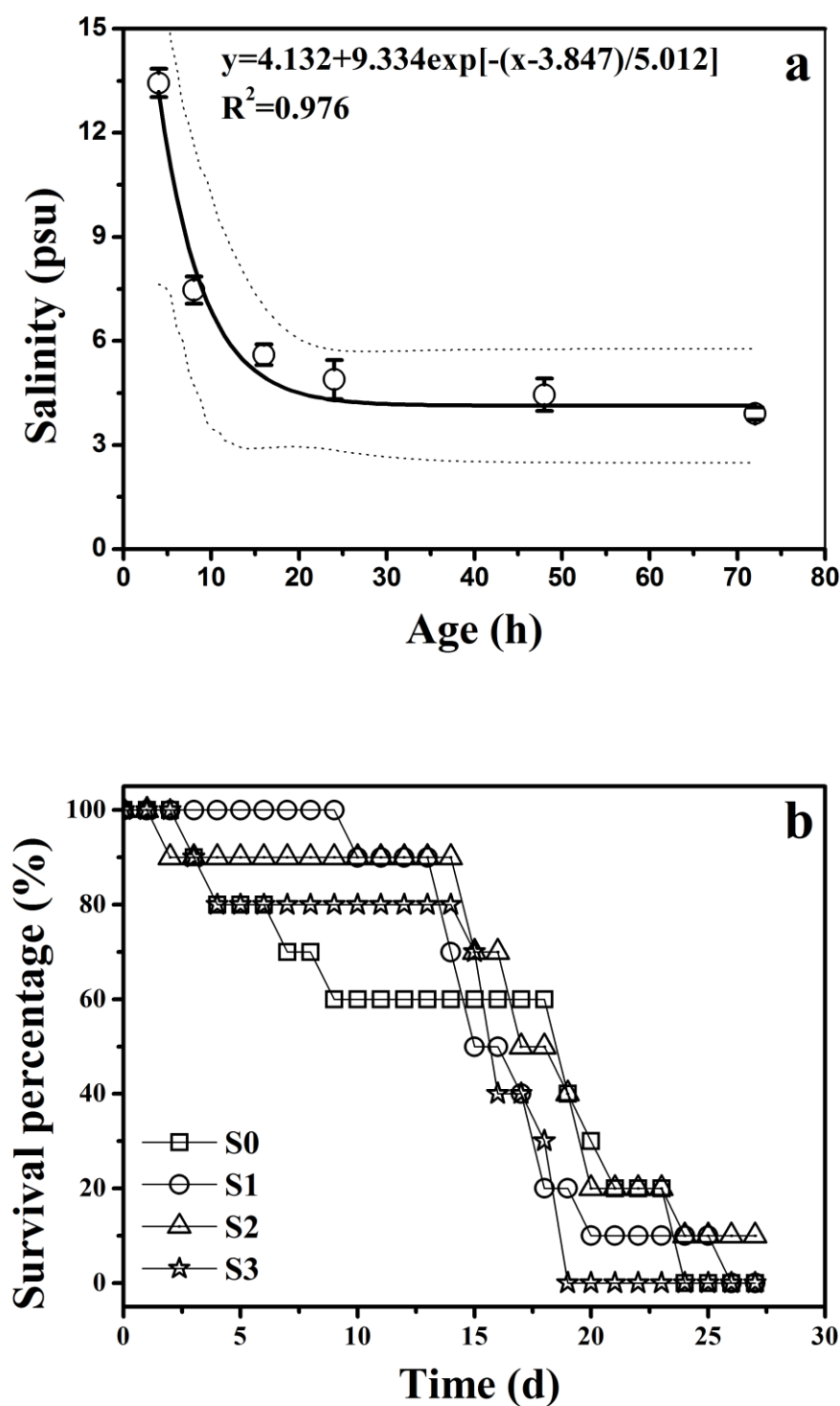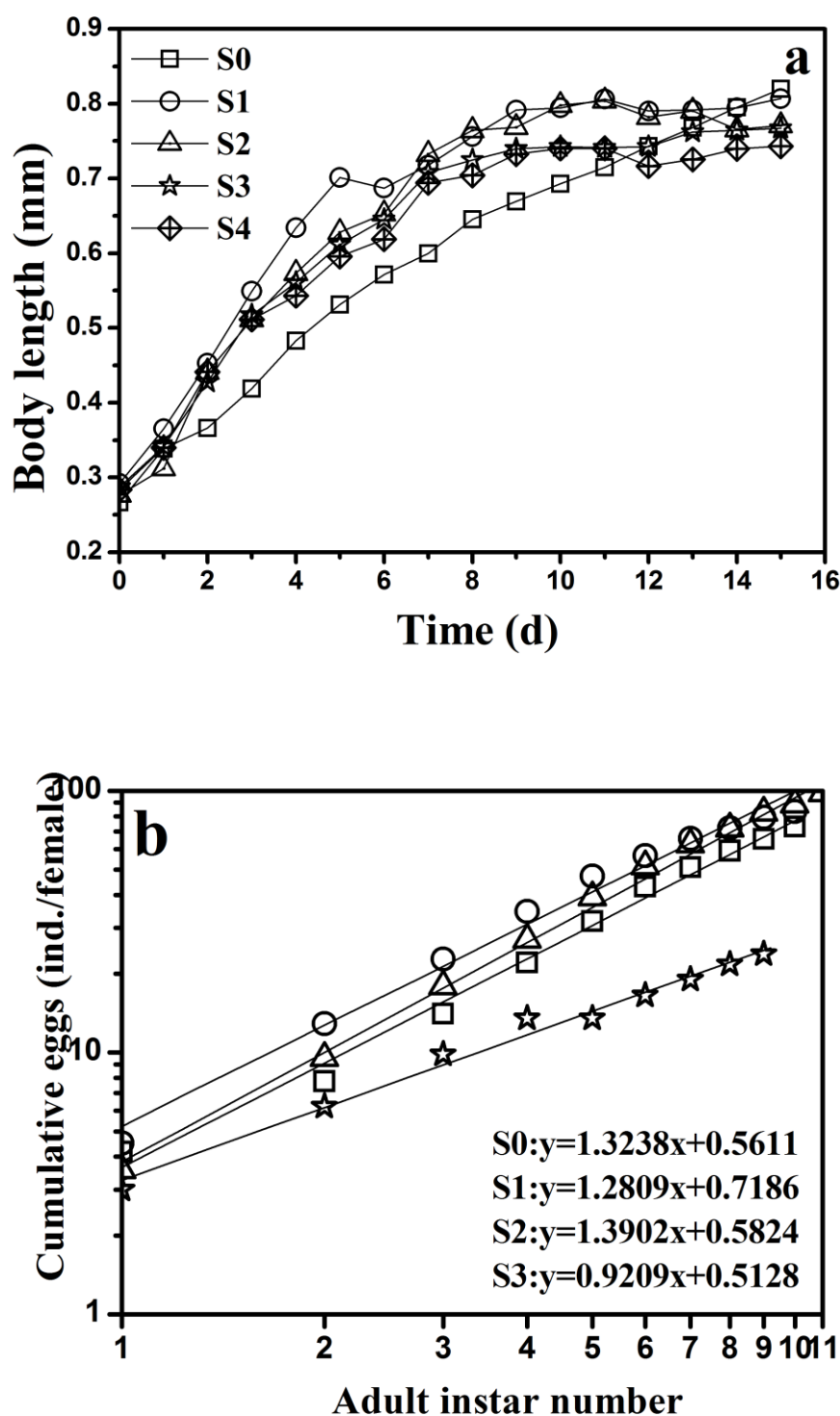
Fig. 1

Figure 2(on next page)

Body length (a), cumulative egg production (b), number of egg per clutch (c) and reproductive value (d) of *S. mucronata* reared at different salinity gradients.

Body length and number of eggs per clutch curves represent mean (±SE) value. S0, S1, S2, S3 and S4 expressed control, 1, 2, 3 and 4, respectively.
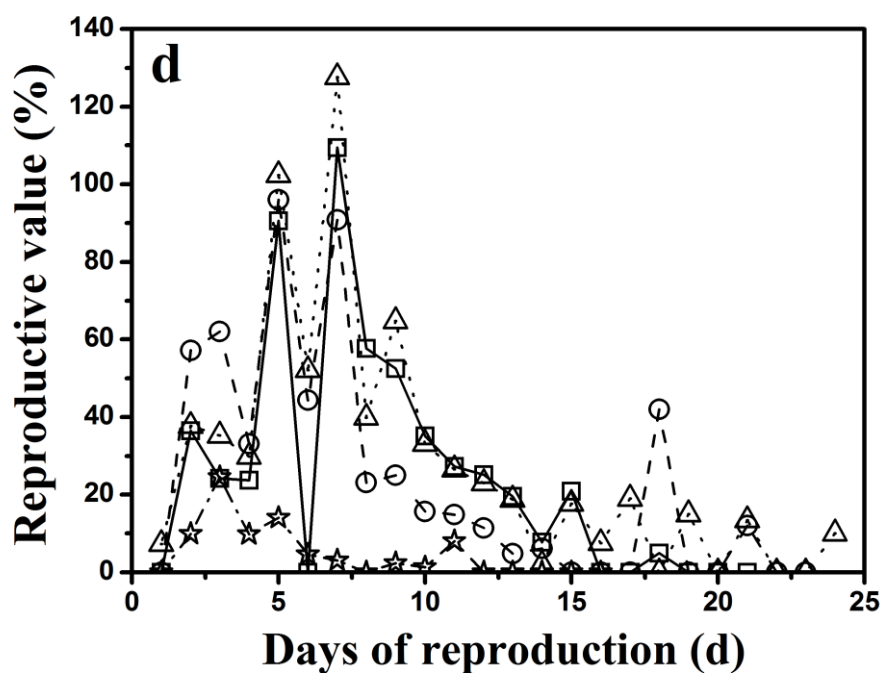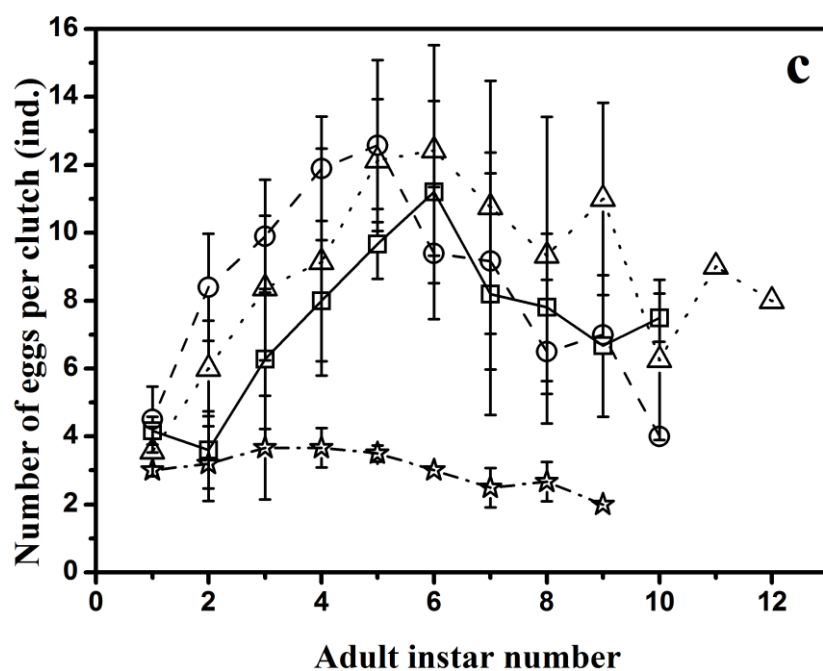
Fig. 2

Table 1(on next page)

The effect of salinity on the population increase parameters in *S. mucronata*.

1   Tab.1   The effect of salinity on the population increase parameters in *S. mucronata*

2

Salinity Treatments	A_R (days)	No. clutch per female	No. neonates per female	No. neonates per clutch	Mean lifespan (days)	Mean intervals between clutch (days)	a value
control	4.28[a] (0.76)	7.14[a] (2.16)	52.00[a] (22.46)	8.37[a] (1.77)	14.30[a] (8.19)	1.75[a] (0.18)	1.3238
1 psu	4.00[a] (0.00)	6.20[b] (1.69)	54.90[a] (16.23)	8.84[a] (0.85)	16.05[a] (4.31)	1.66[a] (0.37)	1.2809
2 psu	4.00[a] (0.00)	7.67[a] (1.94)	67.33[a] (17.78)	8.79[a] (0.90)	17.30[a] (6.72)	1.76[a] (0.35)	1.3902
3 psu	5.25[b] (1.04)	2.75[c] (2.75)	9.38[b] (4.17)	3.38[b] (0.83)	14.20[a] (6.01)	4.48[b] (2.23)	0.9209
4 psu	-	-	-	-	14.55[a] (5.31)	-	-

3

4

Table 2(on next page)

The effect of salinity on intrinsic rate of increase, generation time, net reproductive rate, and finite rate of increase in *S. mucronata*

The effect of salinity on intrinsic rate of increase, generation time, net reproductive rate, and finite rate of increase in *S. mucronata*

1   Tab.2   The effect of salinity on intrinsic rate of increase, generation time, net reproductive rate,

2   and finite rate of increase in *S. mucronata*

Salinity Treatments	r_m	G days	R_0	λ
control	0.777	4.56	34.60	2.175
1 psu	1.021	3.94	55.80	2.776
2 psu	0.903	4.55	60.60	2.467
3 psu	0.481	3.95	6.70	1.617
4 psu	-	-	-	-

3

4

Table 3(on next page)

The effect of salinity on duration of each phase of *S. mucronata*

1   Tab.3   The effect of salinity on duration of each phase of *S. mucronata*

Development phase	Duration of each phase				Additive figure ordinal (Notes)
	control	1psu	2psu	3psu	
One-cell stage	2~4 min	2~4min	2~4min	2~4min	1
	0.5~1min	0.5~1min	0.5~1min	0.5~1min	2
Membrane lift	2.1±0.4h	2.1±0.6h	1.9±0.7h	2.4±0.8h	3
2-cell stage	1.8±0.4h	1.9±0.4h	1.6±0.5h	1.9±0.4h	4
4-cell stage	2.4±0.5h	2.3±0.5h	2.3±0.8h	2.8±0.7h	5
8-cell stage	1.6±0.8h	1.5±0.6h	1.5±0.5h	1.8±0.6h	6
Mang-cell stage	2.5±1.0h	2.4±0.9h	2.2±1.0h	2.8±1.0h	7
Blastula stage	2.0±0.6h	1.9±0.5h	1.8±0.8h	2.2±0.3h	8
Gastrula stage	1.7±0.5h	1.6±0.3h	1.5±0.4h	1.6±0.4h	9
Formation of "T"type embryo	1.8±1.1h	1.7±1.0h	1.5±0.9h	2.0±0.8h	10
Formation of antenna rudiment	2.0±0.8h	2.0±0.8h	2.0±0.6h	2.5±0.8h	11
Formation of pereiopod rudiment	1.7±0.3h	1.8±0.4h	1.6±0.5h	1.8±0.5h	12
Formation of 2-compound eye	3.6±1.1h	3.4±1.3h	3.4±1.2h	4.0±0.5h	13
Single compound eye stage	1.4±0.3h	1.4±0.4h	1.3±0.4h	1.3±0.5h	14
Membrane rive	0.8±0.4h	0.8±0.3h	0.6±0.5h	0.9±0.8h	15~16
Expel from matrix	2~4min	2~4min	2~4min	2~4min	16
Cumulative duration of each phase	25.4h[a]	24.8h[a]	23.2h[b]	28.0h[c]	-

2