

1        **Physiological tolerance of the early life history stages of fresh water prawn**  
2                **(*Macrobrachium rosenbergii* De Man, 1879) to environmental stress**

3

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10 **Key words:** Prawns, Climate change, Early life history stages, Cardiac performance, Yolk  
11 consumption, larval activity

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14 environmental stress

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**28           Physiological tolerance of the early life history stages of fresh water prawn**  
**29           (***Macrobrachium rosenbergii* **De Man, 1879) to environmental stress**

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36

37 **Abstract**

38 Global climate change is transforming life on earth, causing widespread effects on all  
39 ecosystems. Among marine ecosystems, estuaries are considered as nursery grounds for marine  
40 and fresh water species. *M. rosenbergii*, a euryhaline species, migrate to the estuaries for  
41 breeding and spawning. The subsequent larval rearing takes place by experiencing variations in  
42 temperature and salinity conditions. The present study examines the effect of different  
43 temperature and salinity on the larval development and survival by observations on stored yolk  
44 utilization, cardiac performance, as well as changes in the rate of growth in body appendages and  
45 larval activity. The larvae showed 100% mortality at higher temperature ( $33.5 \pm 0.5$  °C) in all the  
46 salinity conditions (12 PPT, 15 PPT, and 20 PPT). The survival rate varied between 76- 96 % on  
47 exposure to lesser temperature conditions. Likewise, the post-embryonic yolk lasted for 4 days at  
48 ambient temperature (29 °C); whereas, at  $33.5 \pm 0.5$  °C, it lasted only for 2-3 days. There was an  
49 increase in total length of larvae, when exposed to higher temperature and salinity, independently  
50 or in combination, but at  $33.5 \pm 0.5$  °C under all salinity conditions the larvae died on the 5<sup>th</sup> day.  
51 For the cardiac performance, larval heart beat ( $f_H$ ) significantly increased for higher temperature  
52 and salinity conditions (20 PPT; 33.5 °C) and lowered at ambient condition 12 PPT; 29°C.  
53 Larval stroke volume  $V_s$ , Cardiac output  $Q$  were higher in ambient conditions and lowest in  
54 higher temperature and salinity conditions. However, temperature and salinity together did not  
55 show any significant effect on cardiac performance. On the other hand, the larval activity  
56 decreased significantly at higher temperature and salinity conditions, compared to ambient  
57 conditions but the interactive effect did not show any change. Thus, the physiological responses  
58 to temperature and salinity by the early life stages of *M. rosenbergii* could restrain the tolerance

59 capability of the organism, thereby interfering in the successful completion of the larval  
60 development under the altered climatic conditions.

61

62 **Key words:** Prawns, Climate change, Early life history stages, Cardiac performance, Yolk  
63 consumption, larval activity

64

## 65 INTRODUCTION

66 Earth's climate is changing at a rapid pace, mainly because of the increased carbon  
67 dioxide emission caused due to anthropogenic activities (Solomon et al., 2009). Though climate  
68 change is a global phenomenon, its effects on living organisms manifest at very local levels  
69 (Helmuth, 2009), and the magnitudes of these changes/effects could considerably fluctuate from  
70 location to location. Estuaries are one such ecosystem that is influenced by a variety of  
71 anthropogenic stressors (Donders et al., 2008). Estuaries act as a natural shelter for all myriad  
72 forms of aquatic life on earth where the spawning and feeding of early life forms of fish and  
73 shellfish happen (Beck et al., 2001). The diversity, distribution and biological functions of the  
74 organisms living in estuaries are influenced by climate change stressors (Kinne, 1971; Pörtner,  
75 2008; Pörtner, 2005; Portner and Knust, 2007; Widdicombe and Spicer, 2008). Climate change  
76 stressors including, rise in temperature, precipitation, salinity changes, sea level rise and ocean  
77 acidification pose deleterious impact on marine organisms and ecosystems (Brierley and  
78 Kingsford, 2009)

79 The giant fresh water prawn, *Macrobrachium rosenbergii*, is an indigenous species to  
80 south and south-east Asia (Holthuis, 1980). Lately, this prawn has been introduced to several  
81 other countries as commercially important aquaculture species (New, 2002). In their natural  
82 environment, *M. rosenbergii* is inhabited in various environments including fresh water streams,  
83 estuarine waters and canals connected to the sea (Jalihal et al., 1993; Shokita, 1979; Tiwari,  
84 1955). The life history of *M. rosenbergii* is amphidromous in nature. The adults spend their life  
85 in the fresh water; after spawning, brooders migrate to estuarine waters for hatching. The larval  
86 development takes place in estuarine water, and after settling down, it returns to the fresh water  
87 (Nandlal and Pickering, 2005; New, 2002). However, fresh water prawn culture in India  
88 increased steadily since 1999 reaching a peak output of 42,780 t in 2005, but then declined to  
89 6,568 t in 2009–2010 due to poor seed and brood stock quality (Nair and Salin, 2012).

90 In general, elevated temperature and salinity variations affect the metabolic rate, calorific  
91 intake and energy budget of decapods (Anger, 2003). Temperature and salinity are the important  
92 abiotic factors that control the growth and development of decapod crustaceans (Anger, 2003;  
93 Kinne, 1964; Kinne, 1971; Chand et al., 2015; Habashy and Hassan, 2010). Salinity around 12-  
94 15 PPT and temperature range from 28 to 30 °C appeared to be optimal for the adults and larvae  
95 (Ling, 1978). Salinity plays a critical role on egg, embryo and larval development during the life  
96 cycle of *M. rosenbergii*. Yen and Bart (2008) studied the negative effects of elevated salinity on  
97 the reproduction and growth of female *M. rosenbergii*. Salinity influences all aspects of larval  
98 biology including survival, development, morphology, the moulting cycle, growth, feeding,  
99 metabolism, energy partitioning, and behavior (Anger, 2003). Likewise, Guest and Durocher  
100 (1979) reported the necessity of brackish water for the completion of larval development in *M.*  
101 *amazonicum*.

102 Temperature, the other major factor, influences the species distribution, range of thermal  
103 tolerance and acclimatization of ectotherm organisms (Schmidt-Nielsen, 1997). In tropical  
104 environment, ectotherms have narrow range of thermal tolerance due to lack of seasonality in  
105 this region and most of them are living at the verge of their maximum thermal limit, making  
106 them vulnerable under global warming scenarios (Sunday et al., 2012). In crayfish, significant  
107 difference on the gonad development and spawning were observed at different temperatures  
108 (Carmona-Osalde et al., 2004). Similarly, the effect of high temperature showed irregular  
109 patterns of egg development in *M. americanum* (Sainz-Hernández et al., 2016). The growth  
110 pattern of the *M. rosenbergii* adults also changed, as temperature increased from low to  
111 normal/optimum, with the growth declining at the higher temperature (Habashy and Hassan,  
112 2010). Furthermore, synergistic effects in combination with one or more environmental variables  
113 (e.g., temperature and salinity) also play a key role in the ecological and geographical  
114 distribution of a species. Nelson et al. (1977) reported interactive effects of salinity and  
115 temperature on the metabolic rate of juveniles of *M. rosenbergii*.

116 The persistence or the failure of a population is determined by the successful completion  
117 of all larval stages (Byrne, 2011). Even though the impact of varying salinities and temperature  
118 have been extensively studied in adult and juveniles of *M. rosenbergii*, we have poor  
119 understanding on the physiological consequences of individual as well as interactive impacts of  
120 salinity and temperature on the early life stages of this species. Under the predicted climate

121 change scenario, understanding the physiological constrains and energy cost for the completion  
122 of larva stages are vital to know the adaptive capability of successive population. Hence, in the  
123 present study, we used yolk utilization, cardiac performance, larval activity, growth as proxy to  
124 know the physiological fitness of the organism under future climate change condition. These data  
125 may give insight on the impact of climate change stress on early life history stages of this  
126 important aquaculture species.

127

## 128 **MATERIALS AND METHODS**

### 129 **Animal collection and maintenance**

130 The adult male and female of *M. rosenbergii* were procured from a fisherman based at  
131 Cuddalore, Tamil Nadu. The shrimps were transported to the demonstration hatchery at the  
132 Centre for Climate Change Studies, Sathyabama University, Chennai in an oxygen filled poly-  
133 ethylene bags. After transfer, the shrimps were acclimatized for 2-4 hours to the laboratory  
134 condition and shifted to 4×500 l fiber tank filled with the fresh water and fitted with biological  
135 filters. The water temperature was maintained at 29 °C and salinity at 0 PPT. The animals were  
136 fed three times a day with grated potatoes and commercial prawn feeds. Ten to 30% of the water  
137 was exchanged once in 3 days to maintain the quality.

138

### 139 **Experimental set up**

140 Twenty spawned brooders were kept in 4×300 liter fiber tank at 29 °C and salinity 12 PPT, and  
141 observed for the embryonic stages until hatching took place. Organogenesis and developmental  
142 changes were recorded under a light microscope equipped with computer aided software (Nikon  
143 Eclipse E600). On 19<sup>th</sup> day, most of the brooders released embryos which were pooled together  
144 in 20 liter fiber tank. Equal numbers of larvae were distributed among tanks with different  
145 experimental conditions (fig. 1). We chose following combinations of temperature and salinity:  
146 T\*S [29°C/12 PPT] ,T<sub>1</sub>\*S [31°C/12 PPT], T<sub>2</sub>\*S [33.5°C/12 PPT ], T\*S<sub>1</sub> [29°C/15 PPT], T<sub>1</sub>\*S<sub>1</sub>  
147 [31°C/15 PPT], T<sub>2</sub>\*S<sub>1</sub> [33.5°C/15 PPT], T\*S<sub>2</sub> [29°C/20 PPT], T<sub>1</sub>\*S<sub>2</sub> [31°C/20 PPT] and  
148 T<sub>2</sub>\*S<sub>2</sub>[33.5°C/20 PPT]. The desired temperature in the tank was maintained using aquarium  
149 thermostat (Aqua Zonic, Singapore). The desired salinity was achieved by mixing fresh water  
150 with seawater of 35 PPT. The experimental tank consisted of 12 PPT sea water in glass aquaria  
151 with stock of 3540±82.76 larvae in it. During the experiment, larvae were fed with live *Artemia*.

152 The experiment lasted for 5 days from the day of hatching when most of the larvae were at 4<sup>th</sup>  
153 stage. The yolk utilization were measured everyday till fully consumed. Following 5<sup>th</sup> day, larval  
154 survival rate, growth rate, activity, and cardiac performance were measured.

155

### 156 **Yolk utilization**

157 Depletion of yolk in larvae was determined by staining the live larvae with Nile Red. To quantify  
158 the yolk, 10 larvae from each treatment condition were stained with Nile Red (10 mg/ml) for 15  
159 minutes, followed by washing using distilled water. The images were captured using the  
160 Epifluorescence microscope (Nikon Eclipse E600, excitation filter BP 490; barrier filter O515)  
161 equipped with digital camera and computer aided software (NIS-Elements). The images were  
162 analyzed by color threshold function in the image processing software Image J (Abràmoff et al.,  
163 2004). The total area and total intensity were calculated and represented in mm and pixels  
164 respectively. The representative image to show how we measured the area and intensity is shown  
165 in figure 2.

166

### 167 **Larval survival rate**

168 Larval survival rate was estimated after 5 days of incubation at different conditions using the  
169 formulae:

170 Larval survival rate (%) = {initial number - (initial number - final number)/initial number} \* 100.

171

### 172 **Morphometrics of larvae**

173 Morphometric analyses were conducted on the images taken by stereomicroscope equipped  
174 (Motic (Xiamen) Electric Group Co., Ltd, China) with digital camera and computer aided  
175 software (Motic image plus 3.0). Total length was estimated after 5 days of incubation by  
176 subtracting initial length from final length and represented in millimeter (mm). The  
177 representative image for the total length is shown in figure 3.

### 178 **Larval activity**

179 Larval activity, defined as the rate of maxilliped movement, was determined in the video taken  
180 using stereomicroscope (Ceballos-Osuna et al., 2013). Larva was trapped in a drop of water on  
181 cavity slide and covered with a cover slip. The water drop was taken from the respective  
182 experimental tank, and video was recorded at 5X magnification using stereomicroscope (Motic

183 (Xiamen) Electric Group Co., Ltd, China) for 2 minutes. Videos were parsed into 10s segments  
184 (free video cutter v 10.4), and slowed to 25% from original speed (VLC media player V. 2.4.4)  
185 for counting maxilliped movements (first three feeding legs) for at least 3 individuals. The  
186 results were represented in beats per minute (bpm).

187

### 188 **Cardiac performance**

189 Heart rate ( $f_H$ ) and stroke volumes ( $V_S$ ) were determined from the same video recorded for the  
190 larval activity. The videos were slowed down to 25% from original speed (VLC media player V.  
191 2.4.4) and zoomed to count accurate  $f_H$ . A representative video showing the heart beat and  
192 maxilliped movements can be seen in supplementary file video 1. Screen marker (Epic pen V.  
193 3.0) was used to mark end-diastolic and end-systolic perimeter.  $V_S$  were determined by  
194 calculating the difference between the end-diastolic volume (EDV) and end-systolic volume  
195 (ESV), assessed using Image J.

196

$$V_S = EDV - ESV$$

197 EDV and ESV were assumed as prolate spheroids (Harper and Reiber, 2004; Storch et al., 2009),  
198 so following equation was used to calculate volume.

199

$$\text{Volume} = 4/3\pi ab^2$$

200 Where, a is the radius of major diameter and b is the radius of minor diameter (from image  
201 analysis).

202 Individual cardiac output (Q) was determined as a product of  $V_S$  and  $f_H$ .

203 The representative images showing EDV and ESV and VS are given in figure- 4.

204

### 205 **Statistical analysis**

206 Data were tested for normality and homogeneity using Shapiro–Wilk and variance test. After  
207 successful completion of these parameters, we performed two way ANOVA and post hoc tukey's  
208 tests on the mean values for finding the independent and dependant effect of temperature and  
209 salinity. All the statistical analyses were performed using SPSS v. 22 (Corp, 2013).

### 210 **Results**

#### 211 **Larval survival rate**

212 Immediately after hatching, we collected the larvae and concentrated in 12 PPT seawater with a  
213 density of  $71 \pm 3.7$  individuals  $\text{ml}^{-1}$ . Soon after, equal volume of water assuming equal numbers of

214 larvae (approx.  $3500 \pm 82.76$  individuals) were distributed among all the experimental  
215 conditions. On day 5<sup>th</sup>, we observed 100% mortality at the higher temperature conditions ( $33.5 \pm$   
216  $0.5$  °C) in all the salinity conditions (12 PPT, 15 PPT, and 20 PPT). However, among other  
217 experimental conditions, the survival rate varied between 76-96 % (Table 1). Unfortunately, due  
218 to technical faults, we lost all the larvae in tank with 20 PPT and 31°C on day 1<sup>st</sup>. At the ambient  
219 temperature of 29 °C and different salinity, larvae survival rates were 96 %, while at 31°C; 12  
220 PPT and 31°C; 15 PPT showed 86% and 76% survival rate respectively.

221

### 222 **Yolk consumption**

223 Post-embryonic yolk was depleted at different rates under varying experimental conditions. In  
224 the ambient condition (12 PPT; 29 °C), yolk lasted till day 4<sup>th</sup>. Moderate increase in temperature  
225 (31 °C) and salinity alone did not show any effect on the rate of yolk consumption, whereas  
226 higher temperature (33.5 °C), alone and in combination with increased salinity caused faster  
227 depletion of yolk. In these cases, the yolk was almost depleted either on day 2<sup>nd</sup> or day 3<sup>rd</sup> (fig.  
228 5). The utilization of the yolk under different conditions in terms of total area and intensity has  
229 been shown in figure 6.

230

### 231 **Morphometrics of larvae**

232 Larval morphometrics were analyzed by measuring total length after 4<sup>th</sup> day (fig. 7). In general,  
233 we observed an increase in the total length of larvae upon exposure to higher temperature and  
234 salinity independently or in combination up to 4<sup>th</sup> day ( $p < 0.001(\text{temperature})$ ;  $p < 0.01(\text{salinity})$ ;  
235  $p < 0.05(\text{temperature} * \text{salinity})$ ), however the larvae in the higher temperature (33.5 °C) in all  
236 three salinity (12, 15, and 20 PPT) conditions died on 5<sup>th</sup> day.

237

### 238 **Larval activity**

239 Larval activity was measured in terms of maxilliped movement which ranged from 90 to 250  
240 bpm across different conditions (fig. 8). Mean maxilliped frequency decreased significantly at  
241 higher temperature and salinity conditions than the ambient conditions ( $p < 0.05(\text{temperature})$ ;  
242  $p < 0.001(\text{salinity})$ ), however, the interaction of temperature and salinity did not show any effect  
243 on them ( $p = 0.760$ ).

244

## 245    **Cardiac performance of larvae**

246    Mean cardiac performance in larval stage was significantly affected under the treated conditions.  
247    We found larval heart beat ( $f_H$ ) in the range of 200-300 beats  $\text{min}^{-1}$ , with maximum beating in  
248    higher temperature and salinity conditions (20 ppt; 33.5 °C) and minimum in ambient condition  
249    12 ppt; 29°C. Temperature, salinity, and their interactions showed significant effect on  $f_H$   
250    ( $p < 0.001(\text{temperature})$ ;  $p < 0.01(\text{salinity})$ ;  $p < 0.05(\text{temperature} * \text{salinity})$ ). Larval stroke volumes  
251    ( $V_s$ ) across different conditions were in the range of 0.005-0.020 nl  $\text{beats}^{-1}$ , higher in ambient  
252    conditions and lowest in higher temperature and salinity conditions (fig. 9). Temperature,  
253    salinity, and their interactions showed significant effect on  $V_s$  ( $p < 0.001(\text{temperature})$ ;  
254     $p < 0.001(\text{salinity})$ ;  $p < 0.05(\text{temperature} * \text{salinity})$ ). Cardiac output ( $Q$ ) which is the product of  
255     $V_s * f_H$  was in the range of 2-5 nl  $\text{min}^{-1}$ , with higher in the ambient condition and lowest in the  
256    higher temperature and salinity conditions. Temperature and salinity independently showed  
257    significant effect on  $Q$  ( $p < 0.05(\text{temperature})$ ;  $p < 0.001(\text{salinity})$ ), while their interaction  
258    seemed not to have any impact on  $Q$  ( $p = 0.099$ ).  $V_s$  and  $Q$  were following similar patterns in  
259    wave manner; both of them were inversely related to  $f_H$  across different treatment conditions  
260    (figure 9).

261

## 262    **Discussion**

263    In this study, we assessed physiological performance of *M. rosenbergii* early life stages  
264    following exposure to varying temperature and salinity to understand their response to climate  
265    change stress conditions. Physiological performances are discussed in two broad categories: 1)  
266    survival and growth; 2) larval activity and metabolic performance. Overall, we find a small rise  
267    in temperature and salinity may result in sub lethal physiological rate reductions in *M.*  
268    *rosenbergii* early life stages, but the substantial increase in temperature and salinity may be  
269    detrimental.

270

## 271    **Survival and growth**

272    Salinity and temperature are the important environmental factors affecting survival, growth and  
273    distribution of many aquatic organisms (Habashy and Hassan 2010; Kumlu et al. 2000). In the  
274    adult *M. rosenbergii* the survival rate varied between 91% (at 0 PPT) and 78% (at 20 PPT), and  
275    the prawn exhibited lowest final average weight at 20 ppt seawater and higher at 10 PPT salinity

276 (Chand et al., 2015). Similarly, Habashy et al (2010) reared the juvenile prawns for eight months  
277 in different salinity and temperature conditions revealing that growth of the prawn increased as  
278 temperature increased from 24 to 29 °C, but declined at the higher temperature (34 °C). Also,  
279 with increase in salinity from 0 to 16 PPT, growth of female prawn decreased at all temperatures  
280 tested (Habashy and Hassan, 2010). Recently, Mohanty et al. (2016), conducted survival  
281 experiments on zoeae and post larvae of *M. rosenbergii* for combined effects of salinity and  
282 temperature, in which, post larvae showed maximum survival at 31 °C which declined both at  
283 lower and higher temperature of 25 °C and 35 °C, respectively. In the line of these previous  
284 results on adults and post larvae, we also found that larval survival rates were higher at optimum  
285 temperature of 29 °C in 12 PPT, 15 PPT, and 20 PPT. However, survival rate decreased with  
286 increase in temperature at all tested salinity conditions. For the higher temperature conditions  
287 (33.5 ± 0.5 °C) with all salinity conditions (12 PPT, 15 PPT and 20 PPT) no survival was  
288 recorded. Similar to our results, Mohanty et al. (2016) reported lower survival rate for *M.*  
289 *rosenbergii* zoeae (Z1-Z5) at 35 °C and 15-18 PPT salinity.

290 For larval growth, there was an increase in total length of larvae up to 4<sup>th</sup> day for salinity  
291 (p<0.01), temperature and combined temperature and salinity (p<0.05). Although temperature  
292 was major determining factor for growth (Kumlu et al., 2000), the quality of larvae was  
293 compromised and died in 5<sup>th</sup> day for the higher temperature (33.5°C) in all salinity conditions.

294

### 295 **Larval activity and metabolic performance**

296 Lipid in yolk acts as energy sources for the early stages of larvae, ensuring the first successful  
297 molt and supporting the survival of early larvae before it started feeding (Yao et al., 2006). The  
298 effects of temperature on metabolic and developmental rates are expressed through changes in  
299 the consumption speed of reserves (García-Guerrero, 2010). In the present study, larval yolk in  
300 both ambient temperature and 31°C lasted for 4 days, but for the higher temperature, the yolk  
301 depletion was completed either on day 2<sup>nd</sup> or day 3<sup>rd</sup> (figure 5, 6). Under the increased  
302 temperature, the rate of metabolic processes hiked, demanding more energy, and causes the early  
303 depletion of energy reserve. The influence of temperature on the utilization of yolk content was  
304 reported in several aquatic species ((Evjemo et al., 2001; García-Guerrero, 2010; Holland, 1978).

305

306 During the stressful condition, organisms try to maintain its homeostasis. The capacity of  
307 controlling cardiovascular function is one such function to maintain the organism's oxygen  
308 consumption rate and activity of the organism (Ern et al., 2015). Temperature alters cardiac  
309 performance in crustaceans, as has been reported in several other organisms (Goudkamp et al.,  
310 2004; Jury and Watson, 2000; Morris and Taylor, 1985). Salinity also affects the sensitivity of  
311 organism; thereby affecting their oxygen consumption (Barton and Barton, 1987). In the present  
312 study, both salinity and temperature are shown to influence stroke volume and cardiac output,  
313 ultimately affecting the oxygen transport capacity of the animal. Larval heart beat ( $f_H$ ) in *M.*  
314 *rosenbergii* larvae increased significantly with elevated temperature and salinity, but at the same  
315 time, stroke volume ( $V_s$ ) decreased, that reduced cardiac output ( $Q$ ) as well as oxygen transport  
316 capacity. Ern et al. (2014) showed that heart rates and ventilation rates increased and stroke  
317 volume decreased with increasing temperature in adult *M. rosenbergii*. They also showed that  
318 the animals retained their 76% of aerobic scope at 30°C. We observed the lower stroke volume  
319 and cardiac output in the present study, which may have affected the oxygen consumption rate of  
320 organism. Hence, the temperature and salinity beyond tolerance level could initiate anaerobic  
321 metabolism with detrimental effects.

322 Further, the decrease in aerobic scope could affect the function and behavior of larval  
323 activity in order to maintain pejus temperatures for long term survival (Portner and Knust, 2007;  
324 Wang and Overgaard, 2007). For example, the rise of temperature above 15°C in the kelp crab  
325 *Taliepus dentatus* constrained the aerobic scope and affected the level of maxilliped activity  
326 (Storch et al., 2009). This was observed in the present study as well, in which the mean  
327 maxilliped frequency was significantly lowered in elevated temperature and salinity conditions.  
328 However, under the combined conditions of salinity and temperature, larval activity did not show  
329 significant effect as similar to our cardiac output results.

330 Corollary, this study shows that substantial increase of temperature and salinity may  
331 result in negative impact on the survival, growth, cardiac performance and activity of the early  
332 life stages of *M. rosenbergii*. The effect of climate change stressors thus could restrain the  
333 tolerance capability and physical fitness of the early life stages of this freshwater prawn, thereby  
334 affecting the successful persistence of the population.

335

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341 microscope.

342

### 343 **Competing interests**

344 The authors declare no competing or financial interest.

345

### 346 **Author contributions**

347 VSS, JJ conceptualized the work with assistance from AK, VE, TS and SP. VSS, JJ, AK, PR,  
348 and VE performed sampling campaign and laboratory experiments. JJ analyzed the results and  
349 VSS, AK, TS, VE, UK helped her in interpreting results. VSS and AK wrote the first draft which  
350 was subsequently corrected by all authors.

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## 481 **Figure legends**

482 **Figure 1: Experimental set up.** The different experimental conditions. Where T\*S [29°C/12  
483 PPT] ,T<sub>1</sub>\*S [31°C/12 PPT], T<sub>2</sub>\*S [33.5°C/12 PPT ], T\*S<sub>1</sub> [29°C/15 PPT], T<sub>1</sub>\*S<sub>1</sub> [31°C/15 PPT],  
484 T<sub>2</sub>\*S<sub>1</sub> [33.5°C/15 PPT], T\*S<sub>2</sub> [29°C/20 PPT], T<sub>1</sub>\*S<sub>2</sub> [31°C/20 PPT] and T<sub>2</sub>\*S<sub>2</sub>[33.5°C/20 PPT]

485 **Figure 2: Yolk Utilization.** Determination of depleted yolk volume in larvae treated under  
486 different conditions. A: Total yolk area marked by using image J; B- Total yolk Intensity  
487 analyzed by color threshold function by using image J

488 **Figure 3: Morphometrics.** Total length measured by using stereo dissection microscope.  
489

490 **Figure 4: Cardiac performance.** A marked picture of larval heart showed EDV (maximal area)  
491 and ESV (minimal area).  $V_s$  was calculated as the difference between EDV and ESV

492  
493 **Figure 5: Utilization of yolk.** Depletion of yolk in different conditions T\*S-29°C/12 PPT;  
494 T1\*S-31°C/12 PPT; T2\*S-33.5°C/12 PPT; T\*S1-29°C/15 PPT; T1\*S1-31°C/15 PPT; T2\*S1-  
495 33.5°C/15 PPT; T\*S2-29°C/20 PPT;T2\*S2-33.5°C/20 PPT

496  
497 **Figure 6: Yolk utilization in total area and intensity.** A- Total yolk area in mm and B- Total  
498 yolk Intensity in pixel T\*S-29°C/12 PPT;T1\*S-31°C/12 PPT;T2\*S-33.5°C/12 PPT; T\*S1-  
499 29°C/15 PPT;T1\*S1-31°C/15 PPT;T2\*S1-33.5°C/15 PPT;T\*S2-29°C/20 PPT;T2\*S2-33.5°C/20  
500 PPT

501 **Figure 7: Growth rate of larvae.** Growth rate of larvae on 4th day was calculated by  
502 subtracting the growth in mm on 1st day from 3rd day. Where T\*S-29°C/12 PPT; T1\*S-31°C/12  
503 PPT; T2\*S-33.5°C/12 PPT; T\*S1-29°C/15 PPT; T1\*S1-31°C/15 PPT; T2\*S1-33.5°C/15 PPT;  
504 T\*S2-29°C/20 PPT;T2\*S2-33.5°C/20 PPT

505 **Figure 8: Larval activity.** Maxilliped activity of larvae on 4th day under different conditions.  
506 Where, T\*S-29°C/12 PPT; T1\*S-31°C/12 PPT; T2\*S-33.5°C/12 PPT; T\*S1-29°C/15 PPT;  
507 T1\*S1-31°C/15 PPT; T2\*S1-33.5°C/15 PPT;T\*S2-29°C/20 PPT;T2\*S2-33.5°C/20 PPT

508  
509 **Figure 9: Cardiac performance.** Cardiac performance of larvae on 4<sup>th</sup> day. Where, T\*S-  
510 29°C/12 PPT; T1\*S-31°C/12 PPT; T2\*S-33.5°C/12 PPT; T\*S1-29°C/15 PPT; T1\*S1-31°C/15  
511 PPT; T2\*S1-33.5°C/15 PPT;T\*S2-29°C/20 PPT;T2\*S2-33.5°C/20 PPT

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517 **Table**

518 Table 1: Survival rate of larvae exposed under different conditions

Condition	Rate of survival (in %)
T*S	95.8
T1*S	87.6
T2*S	0
T*S1	96.8
T1*S1	76
T2*S1	0
T*S2	95.3
T2*S2	0

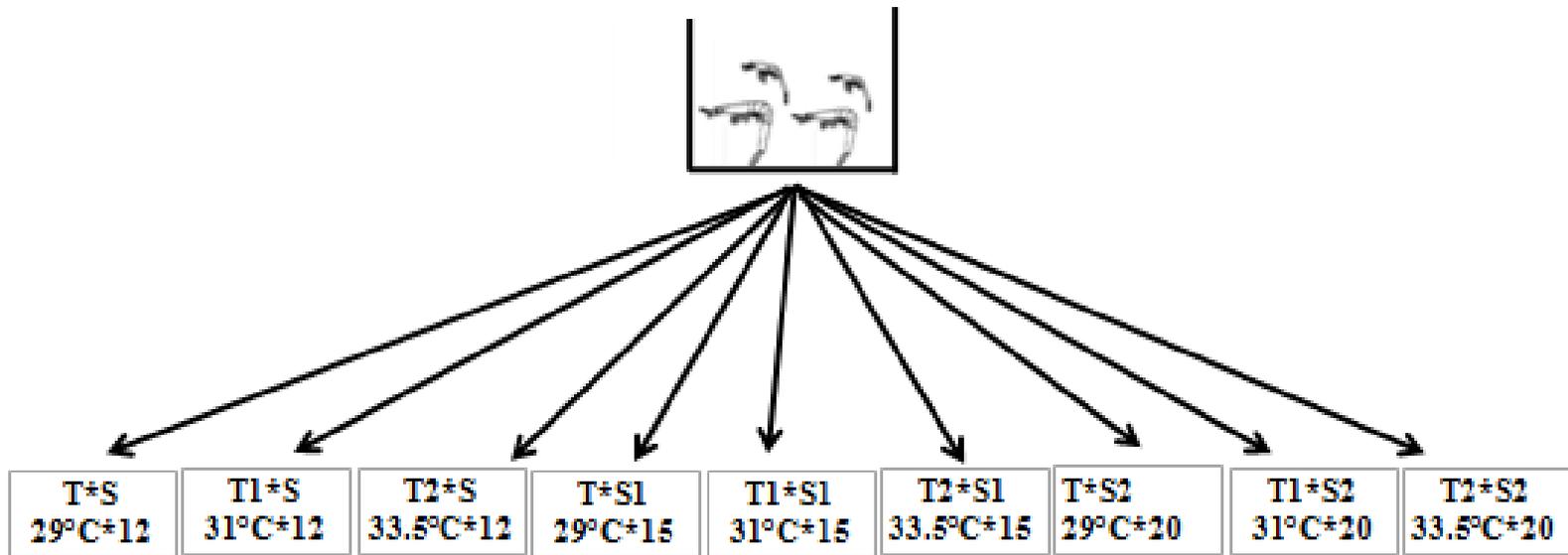
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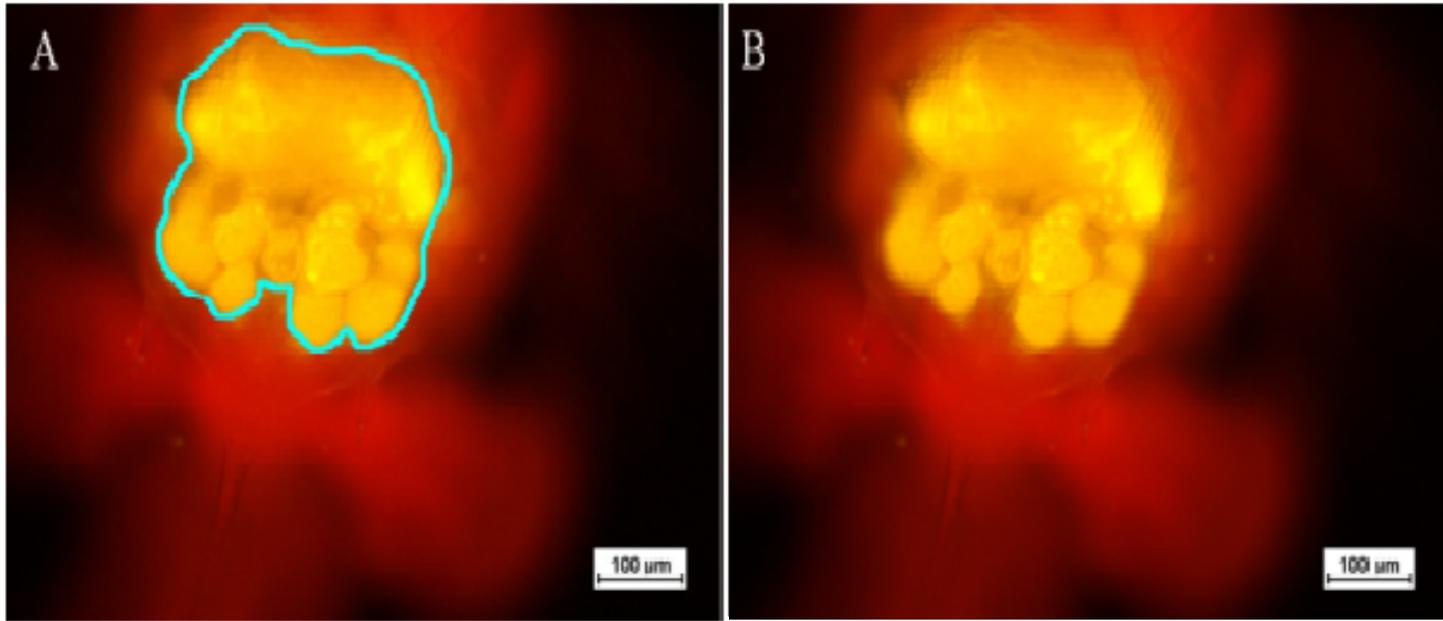
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**Figure-1**



**Figure -2**



**Figure - 3**



**Figure -4**

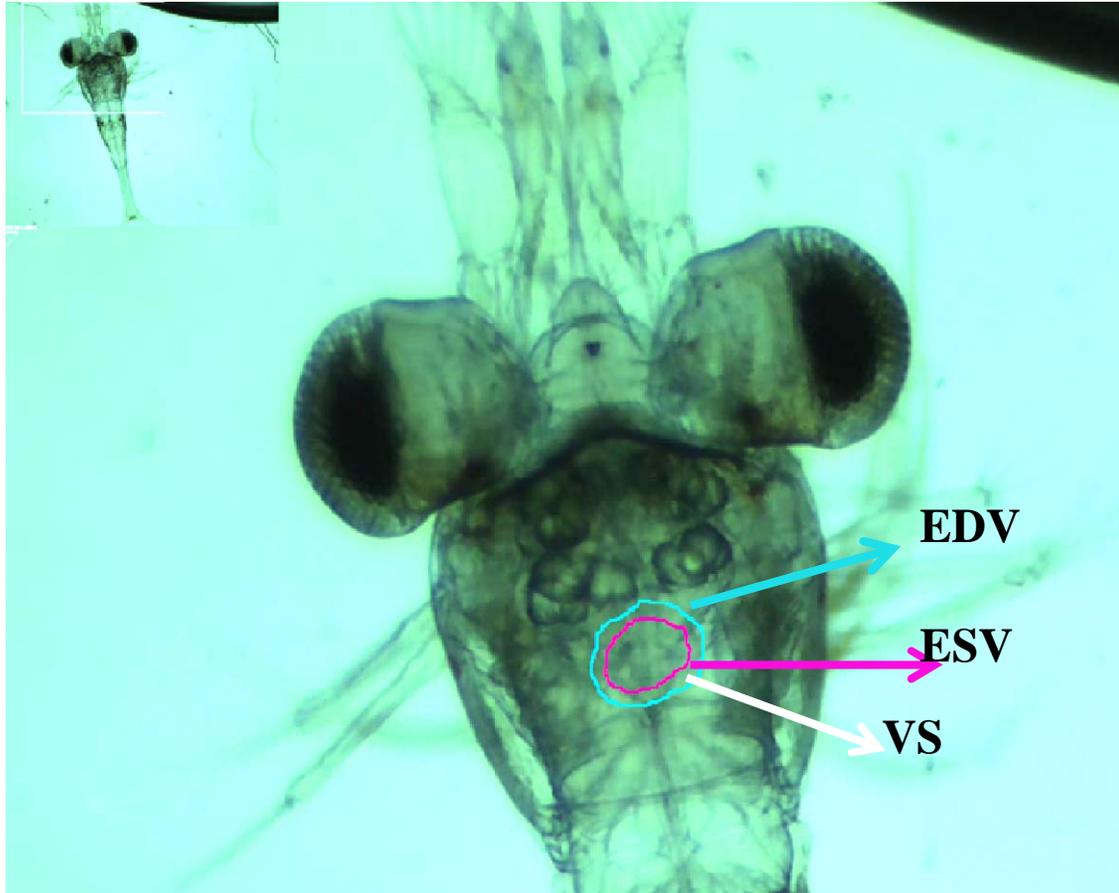
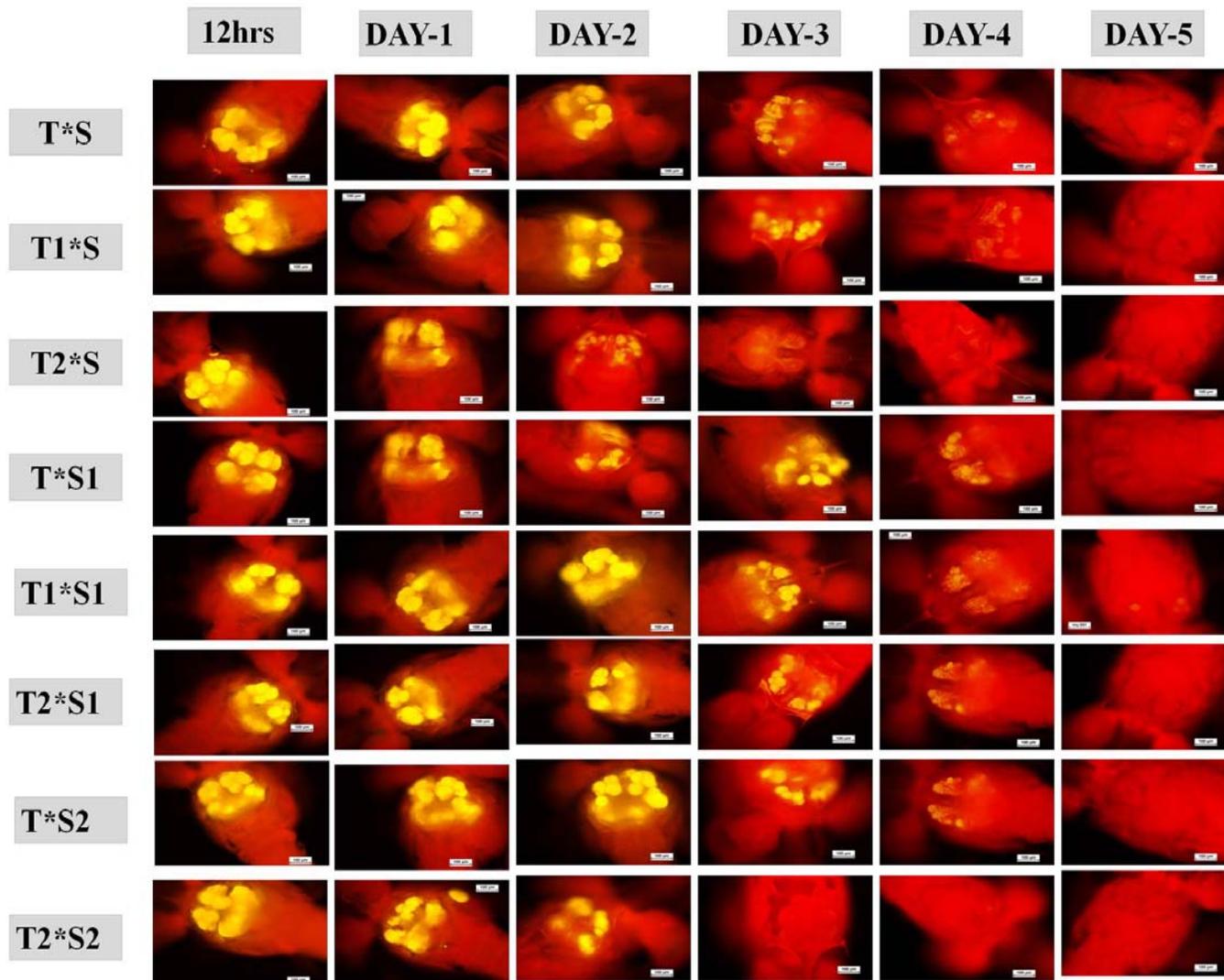
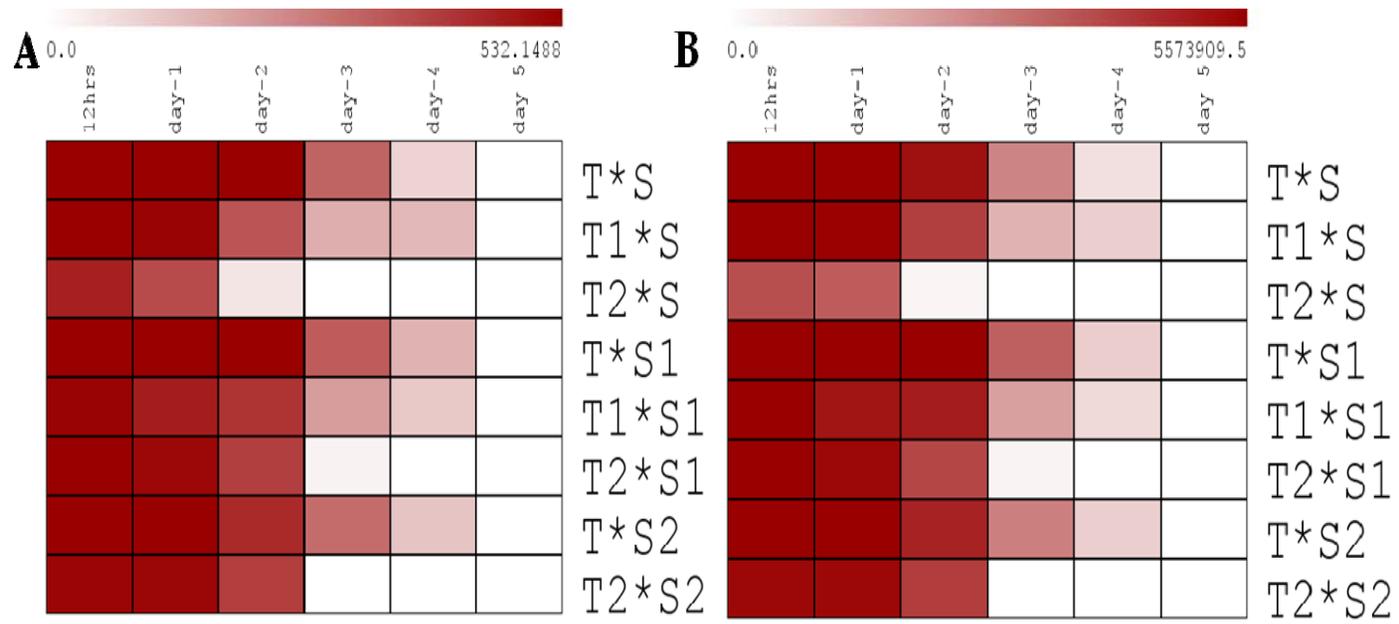


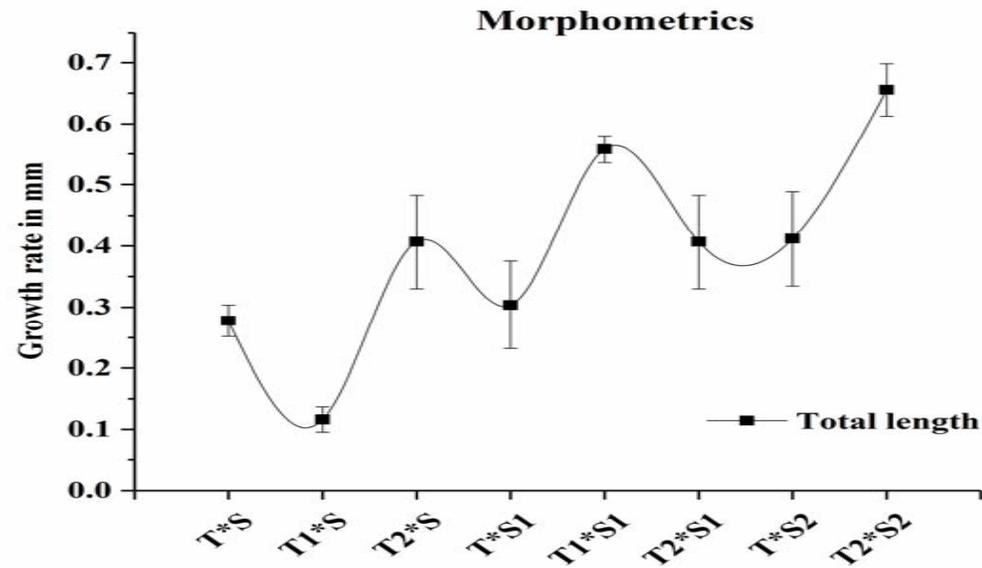
Figure -5



**Figure -6**



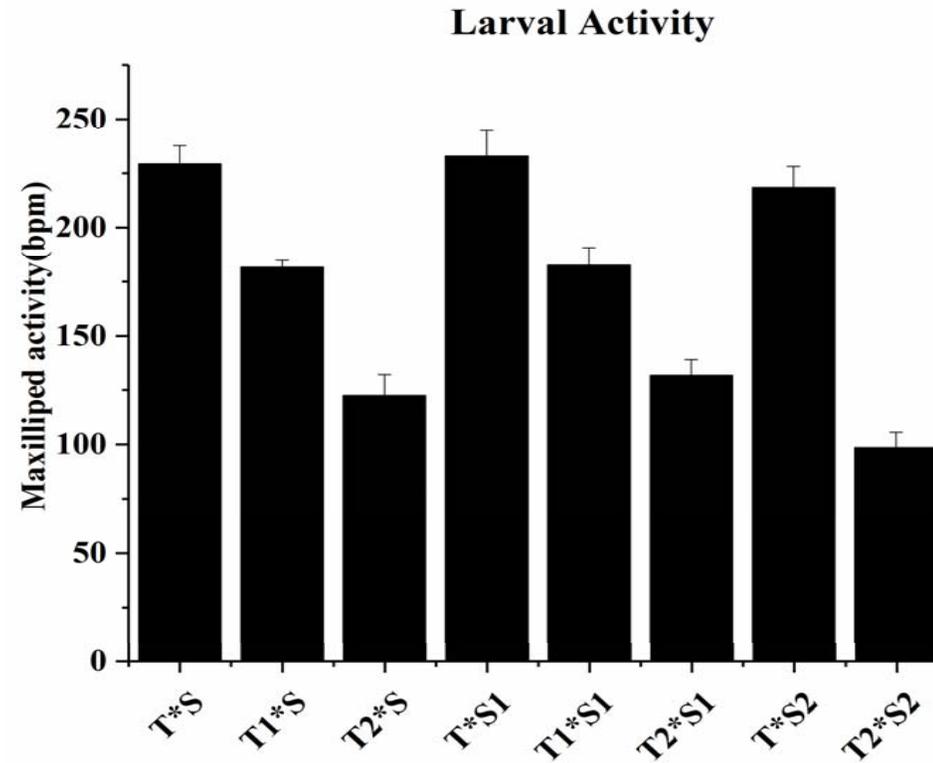
**Figure -7**



**ANOVA results**

Total length	d.f.	F	P
Temperature	2	5.713	.005
Salinity	2	10.658	.000
Temp*Salinity	3	5.024	.003

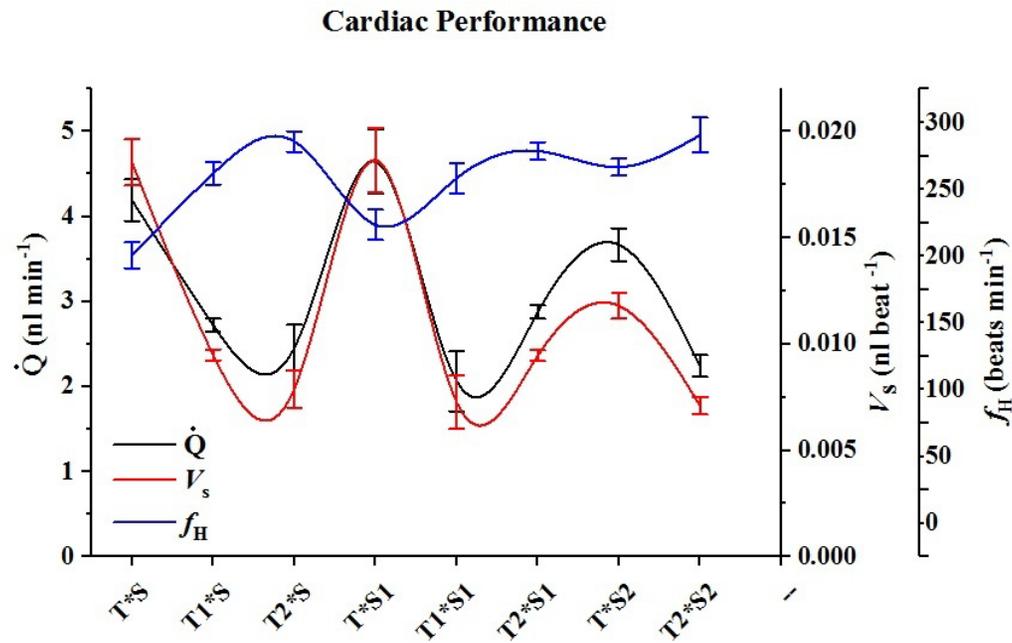
**Figure -8**



**ANOVA results**

Larval activity	d.f.	F	P
Temperature	2	108.842	.000
Salinity	2	3.899	.031
Temp*Salinity	3	.392	.760

Figure -9



ANOVA results

<b>Heart beat</b>	d.f.	F	P
Temperature	2	25.844	.000
Salinity	2	7.365	.002
Temp*Salinity	3	3.572	.025
<b>Stroke volume</b>	d.f.	F	P
Temperature	2	84.282	.000
Salinity	2	14.404	.000
Temp*Salinity	3	5.202	.011
<b>Cardiac output</b>	d.f.	F	P
Temperature	2	49.507	.000
Salinity	2	3.965	.040
Temp*Salinity	3	2.475	.099