

Impact of salinity on the grazing rate of a cladocera (*Latonopsis australis*) in a large tropical estuarine system

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Abstract

Cladocerans are ecologically important as active grazers at the secondary trophic level, and they are economically important in aquaculture as potential live feed for many commercially valuable fishes. This study deals with the effect of salinity on grazing of the rare cladocera *Latonopsis australis*. The experimental specimens were collected from the lower reaches of the Kochi backwaters, the largest estuarine system along the west coast of India, during the Pre-Southwest Monsoon (May 2015), and their cultures developed in the laboratory. Eight experimental salinity treatments (Freshwater, 2, 4, 6, 8, 10, 12 and 14 salinity) with three life phases of the specimens (Phase I - neonates with a developed digestive tract, Phase II - adults carrying egg and Phase III - adults carrying developing neonates) were carried out to measure their grazing rates. Two different approaches, namely individual-specific and biovolume-specific grazing measurements were adopted in the study. The results showed a significant influence of salinity on the size and grazing rates of *L. australis*, irrespective of their life phases. Filtration and ingestion rates of the specimens also varied significantly between different life phases with the lowest rates in Phase III. Irrespective of the life phases, grazing rates were the highest in freshwater conditions, which decreased to 25 - 84% in medium salinity (4-8) and 65 - 93 % in high salinity (10-14). Further, the study demonstrated that size/biovolume/weight-based quantification of cladocera grazing provides a more precise method for the estimation of feeding rates compared to the individual-basic approach. This study emerges as the first attempt to quantify the grazing measurements of a cladocera from Indian waters.

Key words: cladocera, *Latonopsis australis*, ingestion rate, salinity, Kochi backwaters

1. Introduction

Estuarine zooplankton communities have heterogeneous composition with diverse feeding capability and they explore a large variety of food available in the environment (Agasild and Noges, 2005). Feeding is the key mode of transfer of energy from primary to the higher trophic levels (Bamsteadt et al., 2000). Smaller crustaceans (copepods and cladocerans) usually dominates the zooplankton in estuaries and play a major role in energy transfer to higher trophic levels (Madhupratap and Haridas, 1975; Sommer et al., 2001; Jyothibabu and Madhu, 2007; Haridevan et al., 2015; Jagadeesan et al., 2017; Arunpandi et al., 2017). Cladocerans have mostly herbivores feeding and their very high abundance during productive periods helps to control phytoplankton bloom formations (Aka et al., 2000; Achuthankutty et al., 2000; Fernando, 2002). *Latonopsis australis* (here after *L.australis*) is a rare cladoceran (Raghunathan and Suresh, 2002) mostly occurring in freshwater situations (Frey, 1993; Halse et al., 1998). They also occur in low saline conditions during the Peak and Post monsoon periods as was observed recently in the Kochi backwaters (Haridevan et al., 2015).

Survival of cladocerans in estuaries is a matter of their acclimation to the prevailing environmental conditions (Haridevan et al., 2015). They exhibits two types of reproduction behavior (parthenogenesis and gamogenetic) during different environmental settings (Egloff et al., 1997; Rivier, 1998). Parthenogenic reproduction is mainly adopted during favorable conditions and gamogenetic reproduction during adverse environmental conditions (Achuthankutty et al., 2000; Pollupuu, 2010). Most of the past studies on cladocerans from Indian waters (Menon et al., 1971; Madhupratap and Haridas, 1975; Haridas et al., 1980; Madhupratap, 1981; Achuthankutty et al., 2000; Haridevan et al., 2015) and the rest of the waters mostly dealt with the reproductive and feeding biology (Burns, 1968; Peters and Downing, 1984; Haney, 1985; Lampert, 1987; Pandian, 1994; Egolf et al., 1997; Nandhini and Sarma, 2000; Pagano, 2008; Sipaúba-Tavare et al., 2014). Studies about the feeding ecology of cladocerans are less in general especially about the cladocera family Sididae in which *L. australis* belongs. Past ecological studies evidenced that cladocera respond rapidly to any significant changes in environmental parameters among which salinity is considered to be the most important factor which affects their distribution and population structure (Halse et al., 1998; Achuthankutty et al., 2000; Haridevan et al., 2015). It is understood that the feeding rates of cladocera differs, in different hydrographical settings as well as between species and life stages (Pollupuu, 2010; Haridevan et al., 2015). Therefore in this study attempts have been made to understand (a) the effect of salinity changes on the grazing rates of *L. australis* in their different life stages and (b) ecological implications- salinity impact on the grazing rate on the

spatiotemporal distribution of *L. australis* in the Kochi backwaters, the largest estuarine system along the west coast of India.

2. Materials and methods

2.1. Study area

The Cochin (Kochi) backwaters, the largest estuarine system along the west coast of India (Figure 1), receives the seasonal highest amount rainfall and riverine influx during the Southwest (summer) Monsoon (June - September). Six rivers (Pamba, Achankovil, Manimala, Meenachil, Periyar and Muvattupuzha) emptied into the central and southern sector of the Kochi backwaters. During the Southwest monsoon, freshwater dominates the entire region of the Kochi backwaters and the entire system carries low saline or freshwater plankton forms (Haridevan et al., 2015). During the Post Monsoon period, as the freshwater influx receded, saline water intrusions make dominations in the Kochi backwaters and as a result a marked gradient in salinity gets formed from the inlet to the upstream locations, Naturally, this causes the cladocerans present in the Kochi backwaters during the Monsoon time to get exposed to different salinity levels from the inlet to the upstream region.

2.2. Cultures of *Latonopsis australis* and phytoplankton

Zooplankton/cladocera samples for the present study were collected from 3 locations in the Kochi backwaters (Figure 1). Selected locations represented the entire stretch of the Kochi backwaters, wherein, location 1 (Thevara) was in the downstream, location 2 (Murinjapuzha) in the midstream and location 3 (Muhamma) in the upstream. Hydrographical settings in these locations were measured on a monthly basis for a complete year in 2015. The vertical distribution of salinity in these locations was measured using a conductivity, temperature and depth (CTD) profiler (Seabird-SBE 19plus V2). Zooplankton/cladocera samples for the experiments were collected in May 2015, when low saline conditions (<1 - 8) prevailed in the entire Kochi backwaters (Figure 2). After the zooplankton collection using a ring net (60µm mesh size), samples were transferred to 20 L black cans filled with unfiltered natural water and transported to the laboratory. In the laboratory these samples were acclimatized for 1 hour and 10-15 individuals of *L. australis* from each sampling sites were separated and maintained in separate tanks with 15L G/F filtered insitu water. The salinity and temperature was maintained close to the field conditions. The photoperiod was controlled at 12:12 light and dark cycles with the light intensity of ~1500 Lux. Utmost care was taken while handling the live specimens to avoid any damage. Stock/mass cultures of *L.australis* were developed at six different salinities (Freshwater, 2, 4, 6, 8 and 10) and this salinity range was close to the salinity levels in the sampling locations at the time of plankton collection.

For developing phytoplankton cultures, 20 L of unfiltered natural water samples were collected from all the three locations sampled and the same has been transported to the laboratory. In the laboratory, 10L of the water samples were filtered through sieves of different mesh sizes (50 µm; 20 µm and 10 µm) and the particles collected on the bolting silk were transferred into 1 L F/2 medium for developing the phytoplankton culture. From the mass culture, phytoplankton pure cultures were developed using the serial dilution and plating techniques. The salinity of the culture medium was maintained close to the collection site. Phytoplankton cultures were maintained 12:12 Light and dark cycle with 28 °C temperature (Sanyo Versatile Environmental Test Chamber). Subcultures of phytoplankton were developed once in every 7 days. Three phytoplankton (*Scenedesmus* sp., *Chlorella* sp. and *Skeletonema* sp.) mass cultures were developed (Figure 3). *Scenedesmus* and *Chlorella* mass cultures were developed at freshwater and 5 salinity conditions and *Skeletonema* culture was developed in 10 and 15 salinity conditions. The mixture of *Scenedesmus* and *Chlorella* was used as food in freshwater and 4 PSU to culture *L.australis* and the mixture of *Chlorella* and *Skeletonema* was used in 8 -14 PSU strains. The food concentration was maintained 10^5 - 10^6 cells / ml in the culture medium and the culture medium was changed once in every four days to avoid contamination.

2.3. Grazing experiment of *Latonopsis australis* in different salinities

2.3.1. Experimental setup

In the present study, grazing rate of *L. australis* was measured based on food removal method (Bamsteadt et al., 2000) using 200 ml experimental bottles. All the experimental bottles were acid-washed (10% HCL) and cleaned with mild labolene soap solutions followed by washing with adequate quantity of distilled water just prior to the experiment. The parthenogenesis life cycle of *L. australis* has different life stages, in which stages I, II and III (Figure 4) were used to study the effect of salinity on grazing rate of *L. australis*. To get the same aged individuals, 50 parthenogenesis females from different salinity stock culture tanks were taken and placed in 500 ml beakers and maintained separately and fed with algal mixtures (*Scenedesmus*, *Chlorella* and *Skeletonema*). Before adding the organisms to the experimental jars, the life stages of each individual were rechecked and imaged with an Olympus inverted microscope 1X51. The body dimensions of each individual were measured using image processing software. The measured lengths were converted into bio volume based on their shape.

Each experiment was designed with initial, control and experimental bottles following standard procedure of Bamsteadt et al., (2000). The present experimental approach consisted of eight standard

salinity treatments (freshwater, 2, 4, 6, 8, 10, 12 and 14 salinity), which was adopted from a recent publication (Haridevan et al., 2015). The desired salinity levels were prepared through gradual dilution of high saline estuarine water with G/F filtered low saline estuarine water. The salinity levels in the treatments were grouped into three: low saline (freshwater and 2), moderately saline (4, 6 and 8) and high saline (10-14). The food suspension (algal density was 2×10^8 cells. ml.⁻¹) was prepared using algal mixtures of *Scenedesmus* and *Chlorella* / *Chlorella* and *Skeletonema* in 0-8 /10-14 salinity levels.

15 individuals in each developmental stage were separately introduced into experimental bottle. Triplicates were maintained (i.e., 3 initial; 3 control and 3 treatments) in each salinity level. All bottles were sealed with parafilm and incubated for 24 hrs in the laboratory at near-ambient temperature and the bottles were gently mixed intermittently to avoid the settling of the food. Chlorophyll *a* was used as the index of phytoplankton biomass and grazing rate in the present approach. Chlorophyll *a* concentration in all incubation bottles was measured at the beginning and the termination of the experiments. Chlorophyll *a* was measured by concentrating 100 ml of water samples on Millipore filters (0.2µm) and extracting the pigment using 10 ml 90% acetone. The extracted chlorophyll *a* was measured using a Trilogy fluorometer (TURNER Designs, USA) following the standard procedure (UNESCO, 1994).

2.4. Calculation

Filtration and Ingestion rates (I) was calculated using the formula of Frost (1972).

$$I = F \times C$$

Clearance rate (F) - is the volume of food suspension from which *L.australis* would have to remove in unit of time to provide its measured ingestion; C - mean concentrations of the prey

Clearance rate (F) was calculated by the equation:

$$F = [\ln(C_0 - C_c) - \ln(C_0/C_e)] \times V / (t \times N)$$

C₀ - initial concentrations of the Chl. *a*; C_c and C_e - Chl. *a* in control and experimental bottles respectively; V - Volume of the incubation bottle in litres; N - number of organisms added to the incubation bottle and t - incubation time.

$$C = C_0 [e^{Kt} - 1] / K$$

Growth rate of phytoplankton (K):

$$K = \ln(C_0/C_e)$$

For expressing the result in terms of carbon units, standard carbon: Chl. *a* ratio (50) was used for converting the chlorophyll *a* into carbon (Anjusha et al., 2013). *L. australis* filtration and ingestion rates in three life stages, and in different the salinity treatments were analysed between and within different stages of *L. australis* through parametric ANOVA with Dunns post hoc test. Parametric ANOVA and the Dunns posthoc tests were performed using XL-Stat pro software package.

3. Results

L. australis abundance in the study site varied from 20 to 100 ind/m³. The size and bio volume of various life stages of *L. australis* in different salinity conditions are presented in Table 1. Overall size pattern shows that irrespective of the life stages, *L. australis* are larger in size in low saline conditions and their sizes were decreased in moderate saline. The size differences between the low saline and high saline treatments (10, 12 and 14) were 15-22% smaller size in the latter. The results of the size measurements indicates, irrespective of life cycle, salinity influenced the size structure of *L. australis*.

Gut fluorescence in various life stages (I, II and III) of *L. australis* showed that the given algae feed was grazed by all the life stages (Figure 5). The filtration rate of *L. australis* was found to vary significantly between life stages and salinity treatments. In life stage I, the filtration rate was $173.2 \pm 24.5 \mu\text{l ind}^{-1} \text{h}^{-1}$ and $153.6 \pm 24.2 \mu\text{l ind}^{-1} \text{h}^{-1}$ in freshwater condition; while it was $122.6 \pm 37.3 \mu\text{l ind}^{-1} \text{h}^{-1}$, $112.3 \pm 33.6 \mu\text{l ind}^{-1} \text{h}^{-1}$ and 107.1 ± 14.9 respectively in salinity treatments 4, 6 and 8 (Figure 6a). In higher salinity levels the filtration rate decreased 62-75% of the low saline conditions and 57-69% than the moderate saline treatments. The filtration rate in higher salinity treatments showed large and significant decline from the freshwater and moderate saline treatments (ANOVA, $P < 0.05$).

In life stage II, filtration rate of *L. australis* was higher than during the life stage I. The filtration rate in stage II was 269.20 - 338.96 $\mu\text{l ind}^{-1} \text{h}^{-1}$ in freshwater; while it was 198.37 - 251.53 $\mu\text{l ind}^{-1} \text{h}^{-1}$ in moderate saline conditions and 54.75 to 105 $\mu\text{l ind}^{-1} \text{h}^{-1}$ in high saline treatments (Figure 6a). In life stage II, filtration rate of *L. australis* decreased by 25 - 41% in medium saline conditions and by 64-83% in high saline conditions from that of the freshwater conditions. Also, the filtration rate of *L. australis* in freshwater treatment during this situation was large significantly high as compared to the moderate and high saline conditions (ANOVA, $P < 0.05$).

Interestingly, it was observed that filtration rate of *L. australis* was noticeably low during life stage III when compared with life stages I and II. The filtration rate in life stage III was 105-134 $\mu\text{l ind}^{-1} \text{h}^{-1}$ in low saline treatments, 21-66.5 $\mu\text{l ind}^{-1} \text{h}^{-1}$ in medium saline treatments and 8.2-9.6 $\mu\text{l ind}^{-1} \text{h}^{-1}$ in high saline treatments (Figure 6a). In life stage III also, ingestion rate of

L. australis was significantly high in freshwater compared to other two more saline treatments (ANOVA, $P < 0.05$).

The ingestion rate of *L. australis* significantly varied in different salinity treatments. In life stage I, ingestion rate ranged from 0.39 - 3.5 $\mu\text{g C ind}^{-1} \text{d}^{-1}$ between different salinity treatments (Figure 6b); in life stage II, ingestion varied between 1.14-4.86 $\mu\text{g C ind}^{-1} \text{d}^{-1}$ and in life stage III, ingestion rate ranged from 0.11 - 2.64. In life stage I, high ingestion rate was found in the freshwater (3.51 $\mu\text{g C ind}^{-1} \text{d}^{-1}$) and 2 PSU (3.12 $\mu\text{g C ind}^{-1} \text{d}^{-1}$), which declined by 19 - 43% in moderate saline and 82 - 84% in high saline conditions (Figure 6b). In life stage II, individual specific ingestion was 4.69 and 4.86 $\mu\text{g C ind}^{-1} \text{d}^{-1}$ in low saline conditions which decreased by 18 - 31% in moderate saline conditions and by 64-75% in high saline conditions. In stage III, the ingestion rate was 2.1 and 2.6 $\mu\text{g C ind}^{-1} \text{d}^{-1}$ in freshwater, which declined by 43-81% in moderate saline conditions and by 91-95% in high saline conditions. The ANOVA showed that irrespective of the life stages, *L. australis* ingestion rates significantly declined during high saline conditions (ANOVA, $P < 0.05$).

The overall individual specific ingestion and filtration rates *L. australis* were higher in life stage II compared to life stages I and III, however the detailed bio volume based approach of the present study clearly showed that pattern of ingestion and filtration trend was differed between the life stages of the *L. australis*. It was clear in both methods (individual and biovolume specific ingestion and filtration rates) that ingestion was lesser in life stage III compared to the other two life stages considered. It was noticed during the experiment that free swimming time in life stage III of *L. australis* was less than during life stages I and II. Mostly during life stage III, experimental specimens remained close to the bottom of the experimental jars.

In low saline conditions, biovolume specific filtration was 3.47 - 3.68 $\text{ml mm}^{-3} \text{h}^{-1}$ in life stage I; 0.8 - 1.0 $\text{ml mm}^{-3} \text{h}^{-1}$ in life stage II; 0.65 - 0.75 $\text{ml mm}^{-3} \text{h}^{-1}$ in life stage III (Figure 7a). In medium saline conditions, bio volume specific ingestion was 2.6-2.8 $\text{ml mm}^{-3} \text{h}^{-1}$ in life stage I; 0.74-0.79 $\text{ml mm}^{-3} \text{h}^{-1}$ in life stage II; 0.17 - 0.47 $\text{ml mm}^{-3} \text{h}^{-1}$ in life stage III. In high saline conditions, biovolume specific filtration rate was 1.41 - 2.17 $\text{ml mm}^{-3} \text{h}^{-1}$ life stage I; 0.29 - 0.55 $\text{ml mm}^{-3} \text{h}^{-1}$ in life stage II and 0.12-0.15 $\text{ml mm}^{-3} \text{h}^{-1}$ in life stage III (Figure 7a). Similarly, in low saline conditions, biovolume specific ingestion was 70.46 - 74.62 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage I; 14.3 - 14.5 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage II and 5.3 - 6.28 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage III (Figure 6b). In medium saline conditions, biovolume specific ingestion was 49.55-64.87 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage I; 12.18 - 12.56 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage II and 1.39-3.79 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage III. In high saline conditions, biovolume specific ingestion was 14.24 - 20.43 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage I; 6.11 - 10.25 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage II and 0.46-0.89 $\mu\text{g C mm}^{-3} \text{d}^{-1}$ in life stage III (Figure 7b). The bio-volume specific filtration and ingestion rate was high in

life stage I compared to life stages II and III. Overall, the volume specific filtration and ingestion was found to be least in high saline treatment of stage III and the maximum volume specific filtration rate was found in low saline treatment of life stage I.

4. Discussion

Salinity tolerance and acclimation varies in estuarine organisms; the successful survival and production in saline waters requires physiological mechanisms to acclimate/counter balance the ionic composition of the surroundings. The salinity tolerance response in cladocera mainly depends upon life stages and duration of the exposure (Loureiro et al., 2013; Haridevan et al., 2015). The occurrence of Sididae members were reported in different salinity ranges (Negrea, 1982), but usually they occur up to hypohaline waters (salinity limit 13 - 15). It was observed in the present study that irrespective of life stages, the size of *L. australis* was comparatively less in high saline conditions compared to the moderate and low saline conditions. Similar observation is reported earlier, where in, size reduction in freshwater cladocerans during their intrusion into the brackish water conditions was recorded (Achuthankutty et al., 2000). It was also noticed that in higher salinities, members of Sididae family show failure in the osmoregulatory mechanism that causes a significant reduction in their growth rate (Achuthankutty et al., 2000). Cladoceran of Sididae grow slowly compared to the other related families (Haridevan et al., 2015). It was noticed in the present study that the size differences between life stages II and III was less but the size differences between life stage I and life stage II was significant, which could be attributed to the differential growth and development of the parthenogenetic eggs at different stages.

In this study, the reported filtrations rates were ranged between 8 and 338 $\mu\text{l ind}^{-1} \text{h}^{-1}$, and these results are in general agreement with the past studies on the filtration rates of some other cladocerans (Bodgan and Gilbert, 1987; Rothhaupt, 1990; Kankaala, 1988). It was found in this study that the grazing rates of *L. australis* significantly varies in different salinity levels. Grazing rate was high in low saline conditions indicating the suitability of such salinity levels for the mass production of *L. australis* (Haridevan et al., 2015). It was also evident that grazing rates decreased by 25 - 84% in moderate salinity levels and 65- 93% in high saline conditions. All these indicate the fact that increasing salinity has noticeable negative impacts on the survival of *L. australis* in the Kochi backwaters. The overall grazing rate was found less in the life stage III in this study and it was the result of the behavior of the specimens to decrease the movement/swimming and remain at the bottom of the experimental vessels.

The ingestion of *L. australis* varied from 0.12 to 4.9 $\mu\text{g C ind}^{-1} \text{d}^{-1}$, which is comparable with the ingestion rates of a few other cladocera experimented in some other parts of the world (Haney, 1985; Bodgan and Gilbert, 1987; Rothhaupt, 1990; Lair, 1991; Kankaala, 1988; Wallace and Snell, 2001). Similar to the filtration rates, ingestion rates also varied significantly with different salinity treatments. Irrespective of the stages, ingestion was less in the high saline conditions and this was related with the inability of *L. australis* to acclimate well to the increased salinity. In high saline conditions, less filtration and ingestion rates in *L. australis* can cause lack of sufficient energy for the regular activities, which eventually cause failure in growth and new production (Haridevan et al., 2015).

Accept the fact that use of the laboratory measured ingestion and filtration rates to interpret field scenario have limitations due to various other environmental factors involved in the natural environment. However, the present study provides clear indication why *L. australis* occurrence significantly decreases towards upstream (high saline) regions of the Kochi backwaters. In the present approach we interpreted the experimental results in both the individual specific and bio volume specific to see the results of both methods, which have similar trends. It was observed that in individual specific approach the ingestion and filtration rate of *L. australis* was high in life stage II compared to life stage I, but the bio-volume specific rate measurements showed high filtration and ingestion rates in life stages I and II.

The individual specific grazing measurements assume that the animals in the experimental jars are in the representative size distribution of species, however in the natural environment all the life stages of the organisms are not in uniform size and life stage as several authors have pointed out the zooplankton filtering rates were differed with the size of the organisms (Peters and Downing, 1984; Haney, 1985; Hart and Jarvis, 1993). Therefore, we too consider that individual specific measurement of filtrations rates and its interpolation with the insitu measurements seems to be less accurate. Instead, the bio-volume/ weight specific ingestion rate measurements can be better used for the field interpretation of *L. australis* grazing according to their life stages and surrounding salinity.

5. Conclusion

This study showed that salinity has a major role in the size and grazing rates of *L. australis* irrespective of their life stages. Their filtration and ingestion were high in low saline treatments, which decreased by 25 - 84%/ 65-93% in the moderate/high saline conditions. The less food consumption in high and moderate saline conditions compared to low saline condition, cause suppressed growth and decreased new production. It was also observed that the grazing rates of *L. australis* vary significantly

between their life stages; grazing and ingestion rates were found less in life stage III compared to other two life stages. Among the two approaches adopted to measure the grazing rate of *L. australis*, biovolume / weight based measurements of the grazing can provide more accurate results, which is really useful to interpret the influence of salinity on the distribution of the selected organism in the natural environment.

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Fig.1- Study area, red dots indicates as sampling stations. Station 1(Thevara) was in the downstream, station 2 (Murinjapuzha) in the midstream and station 3 (Muhamma) in the upstream

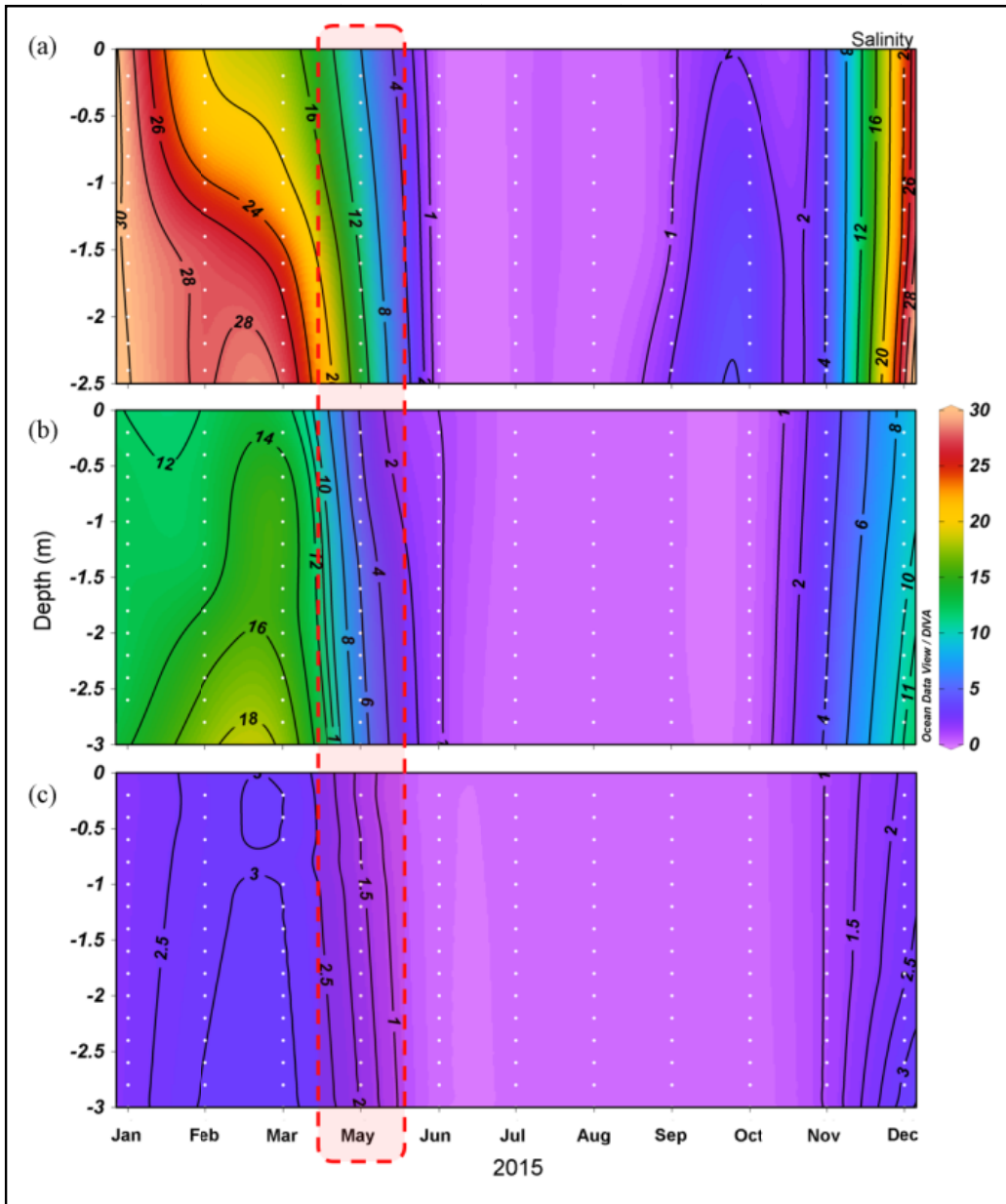


Fig.2-Temporal change in the vertical distribution of salinity in the study sites, (a) location 1 (Thevara) and (b) location 2 (Murinjapuzha) and location 3 (Muhamma). The red discontinuous rectangle shows the time when water samples were collected for conducting the grazing experiments.

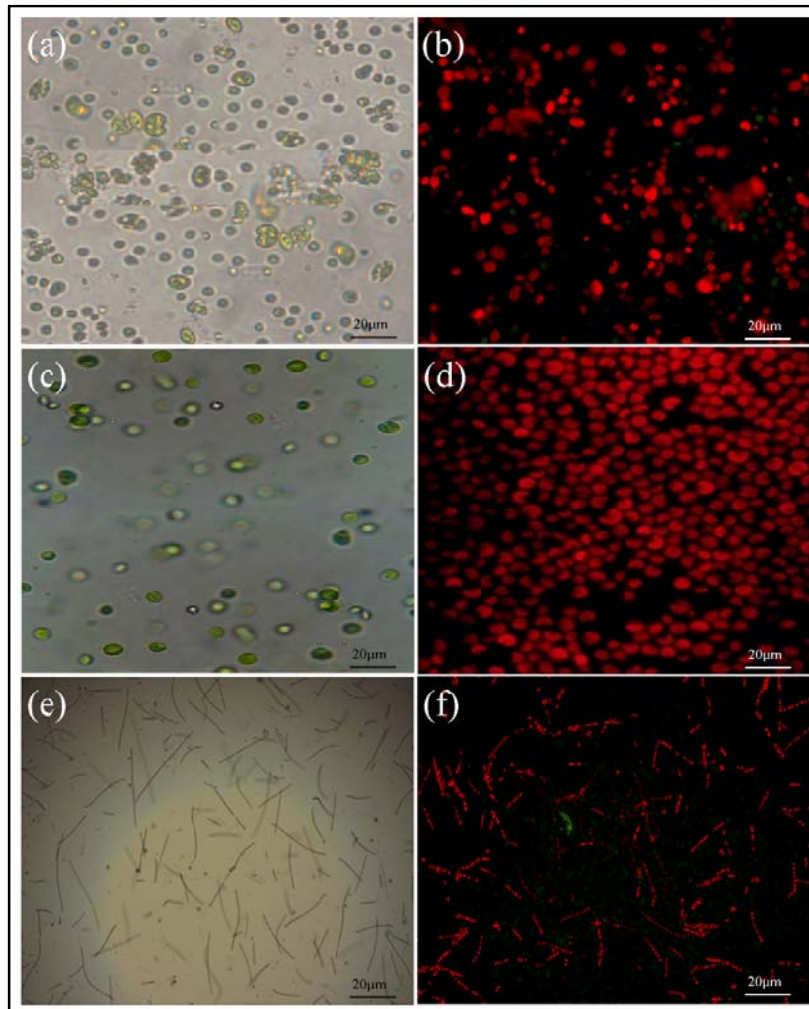


Fig.3-Panels (a-f) represent the phytoplankton culture, which one used for the experiments. Left panels represent the microscope image and right panels are the fluorescence microscope images of (a and b) *Scenedesmus* (c and d) *Chlorella* and (e and f) *Skeletonema*.

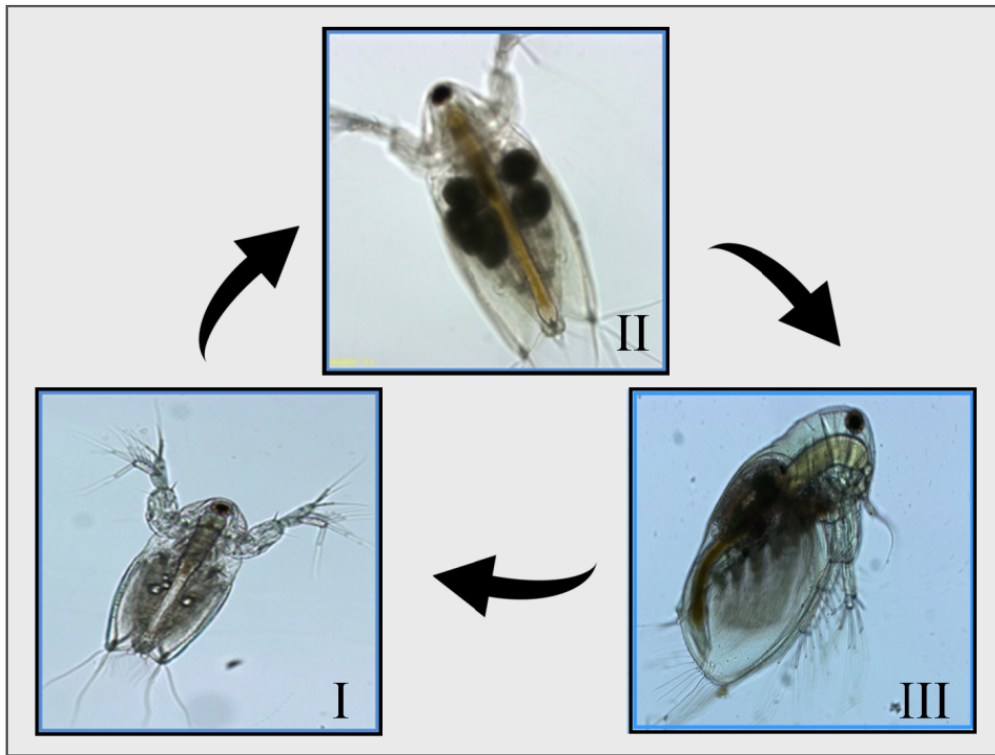


Fig.4-Three different life stages (I, II and III) of *Latonopsis australis*, which were used for the experimental studies. I- digestive systems activated stage, II- Organisms with developed parthenogenetic eggs. III- developed neonates

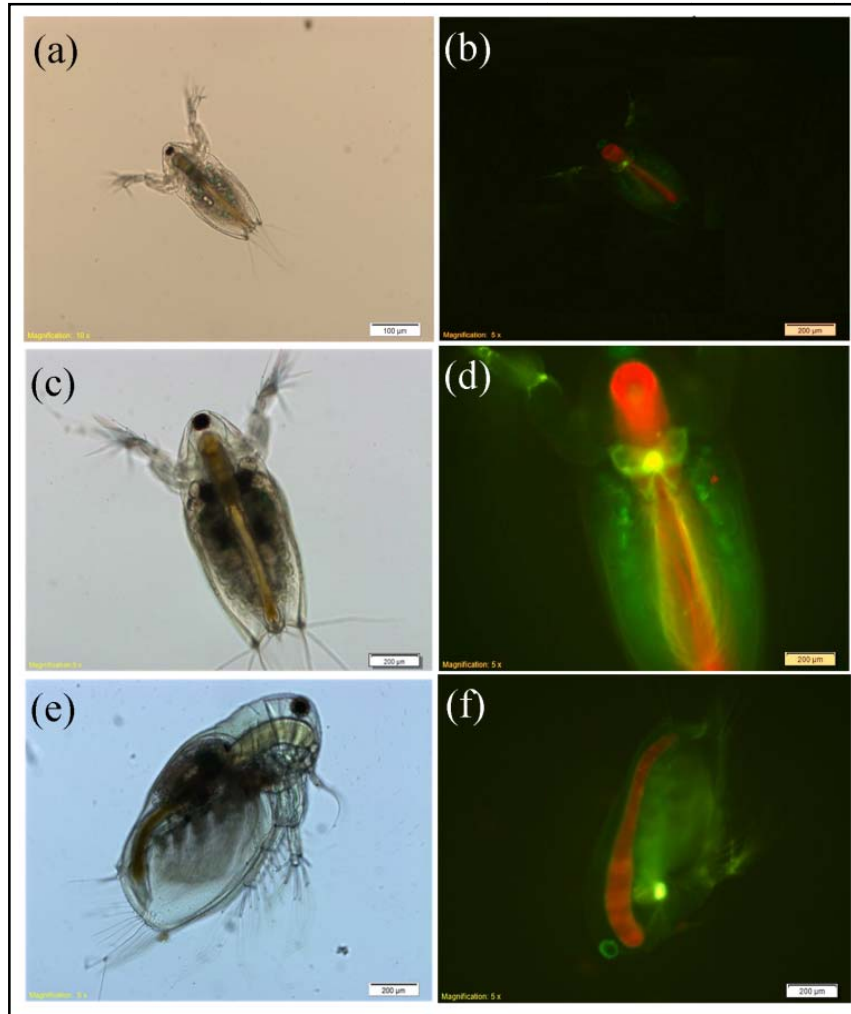


Fig.5- Panels (a –f) present the qualitative observational evidences of *L. australis* grazing on phytoplankton (*Scenedesmus*, *Chlorella* and *Skeletonema*). (b, d and f) Fluorescence microscope images showing the red chlorophyll *a* gut fluorescence in all the life stages (I, II and III).

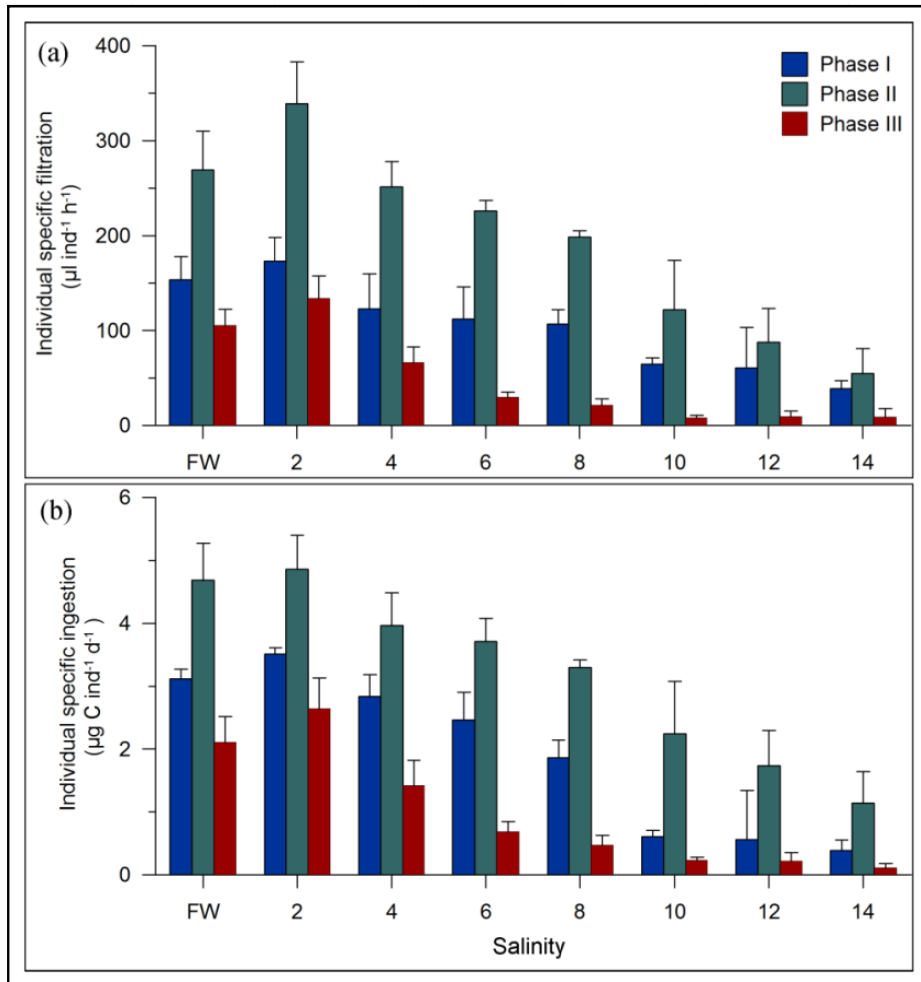


Fig.6- (a) Filtration (mean \pm SD) and (b) ingestion rates (mean \pm SD) of *L. australis*

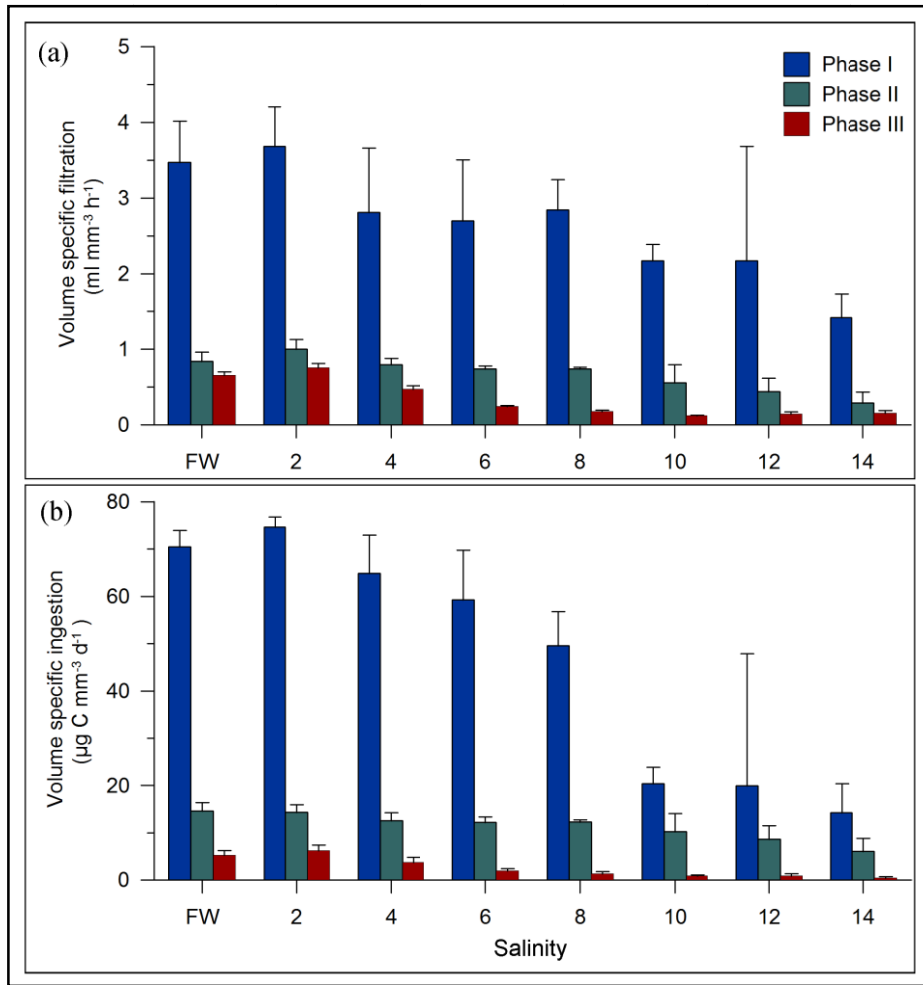


Fig.7-(a) Bio-volume specific filtration (mean \pm SD) and (b) ingestion rates (mean \pm SD) of *L. australis*

Salinity	Stage I		Stage II		Stage III	
	Length (µm)	Volume (x10 ⁻² mm ³)	Length (µm)	Volume (x10 ⁻² mm ³)	Length (µm)	Volume (x10 ⁻² mm ³)
Fresh water	439	4.43	850	32.14	915	40.09
2	448	4.71	865	33.87	930	42.09
4	437	4.37	845	31.56	895	37.52
6	430	4.16	835	30.47	875	35.06
8	416	3.77	802	26.79	870	34.46
10	385	2.99	748	21.90	793	26.10
12	377	2.80	725	19.94	787	25.51
14	374	2.74	710	18.73	775	24.36

Table 1 - Detail about the length and bio volume (mean) of different life stages of laboratory cultured *L. australis* in various salinity treatments