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Research paper

Effect of Salinity upon Biology of Copepoda and Cladocera in culture medium under lab conditions

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Abstract: The effect of different salinities (5, 10, 25 and 30 psu) has been investigated with salt-mixed freshwater on population growth and density of Copepoda and Cladocera in freshwater as they are unable to emigrate to escape the problem. The findings showed that difference in salinities give different effects on the population number of selected copepods cultured in the same temperature. The copepoda reared in 25 psu salinity showed the highest population density (mean of 3.7 ind/ml), but the most frequent population growth is showed in 35psu in the laboratory conditions. *Daphnia carinata* is used as a toxicological test species for water systems. The 45h-LC_{50} from studies salinities were 2.99, for sodium chloride solution. A survival and growth rate of daphnid was decreased with increasing salinity. The highest total reproduction (95± 0.1) neonates per female was achieved at 0.44% (LC_{10}) for saline water over a period of 21 days.

Keywords: Zooplanktons, Neonates, Aquaculture, Salinity, Water quality.

Introduction:

Since saline and freshwater environment require completely different adaptations due to cell homeostasis, most aquatic organisms are either lives in one habitat or the other (Young et *al.* 1989), Nevertheless, as salinity varies markedly in many habitats (Hall and Burns, 2002), local populations may be characterized by the presence of micro-evolutionary changes, the widening of tolerance ranges and greater phenotype plasticity occurs. Zooplankton production plays an important role in the functioning of aquatic ecosystem by making part of the production of phytoplankton available to higher trophic levels.

Copepoda and Cladocerans are very important components of zooplankton, usually restricted to freshwater environments (Arner and Koivisto, 1993). The genus *Daphnia* is freshwater in its origin and distribution (Teschner, 1995). The zooplanktons are hypertonic to the medium and the fluxes of water, but they have reduced their osmotic loads through the impermeability of their bodies and the

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low internal concentration of solutes in their body (Peters, 1987). According to Arner and Koivisto (1993), although it is possible to find zooplankton in rock pools with salinity values up to 12.5% in the Baltic sea, they experimentally determined that the best development was achieved at 4%. Schuytema *et al.* (1997) also concluded that the best growth of *D. magna* occurs at salinity values lower than 4%. The freshwater Cladocera that successfully colonize brackish environments are smaller in size and have a reduced reproduction (Arner and Koivisto, 1993).This study was aimed to assess effect of salinity upon freshwater Zooplanktons.

Materials and Methods:

Experiments were carried out in glass Petridishes filled with 15 ml treated freshwater mixed with NaCl to prepare 5, 10, 25 and 35 psu salinity .Salinity in glass Petridishes were measured using a refractometer.

Daphnia carinata and *Mesocyclopus hyalinus* has been cultured in the laboratory for about six months and the species has already adapted to its new environment. One gravid female was placed in each Petridishes. No aeration was given to the culture as aeration will only increase evaporation. Two different sets of culture were maintained in two temperatures (21^0C and 29.5^0C) and a refrigerator temperature for 45 days. The temperature was measured everyday using a mercury thermometer.

Everyday each individual of copepods at all stages counted under a stereo microscope before being fed with Baker's yeast at 0.1 ml. Dead animals, molted exoskeleton and any debris were taken out from the culture using a wire loop. The data collected were analyzed using one way analysis of variance (ANOVA, treatment vs population).

Result and Observations:

As for 5 psu salinity, the population density of copepod reached to 3.5 no./ml at 4th week of culture, whereas 10 psu salinity with 2.9 no./ml in 2nd week and 2.3 no./ml for 35 psu at the 6th week (Figure 1). The Copepoda cultured in 25 psu salinity showed the highest population density with 3 no./ml during the 4th week of culture (Figure 2).

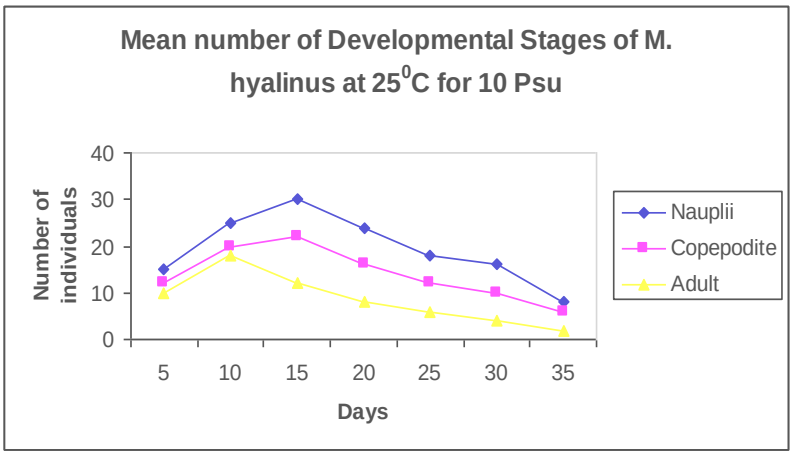

Figure 1: Mean number of *M. hyalinus* at 25^0C in10 psu salinity.

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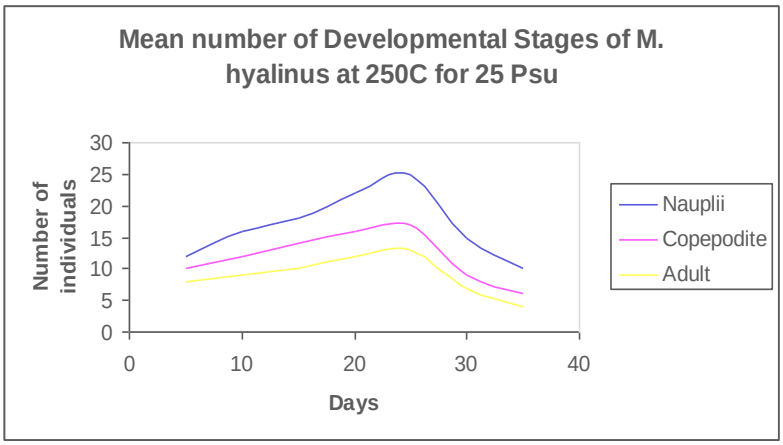

Figure 2: Mean number of *M. hyalinus* stages at 25^{0}C in 25 Psu salinity.

The production of nauplii and gravid females along with other stages, cultured in different salinities at 25^{0}C showed peak of nauplii production for 5 psu, 10 psu, 25 psu, and 35 psu was shown at day 5 and 27, 6 and 19, 7 and 27 and 9 and 37 respectively. Nauplii existed in each treatment until at day 28, 33, 38 and 45 respectively. It is interesting to note that in 35 psu, the nauplii survived until the 45th day and gravid female with frequent occurrence in the culture indicating the occurrence of several new generations throughout the experiment period which again more frequent in 35 psu than other treatments although higher number was in 25 psu. They also occurred in shorter duration during the overall 10 first day of experiment suggesting the more favorable culture condition in the early period of the study. One-way ANOVA analysis showed that there was a significant difference ($p<0.05$) of population density between salinity treatments. Replicate treatments for all salinities (5, 10, 25, and 35 psu) also showed significant difference ($p<0.05$) between the replicates as indicated by the large value of standard deviation. Also, variable salinities have different effects on the population number of the copepod cultured in the same temperature (25^{0}C). Each nauplii and adult stages also significant difference ($p<0.05$) between the treatment indicating that there were different effects on the number of nauplii and adults produced in the different salinity. The effect of different sub-lethal concentrations of NaCl on *Daphnia carinata* showed that the survival rate decreased with increasing the culture period and also with increasing salt concentration. The *Daphnia carinata* cultured in 0% showed the highest significant survival rate ($p<0.01$) represented by 97% after 21 days, while the lowest survival rate ($p<0.001$) for those cultured in the highest concentration, 2.66% represented by 43% after 21 days. There were no significant differences between control and LC_{10} groups. Strong negative correlations was observed from the first 2 days of the experiment r =-0.69 and increased till the end of experiment to reach r = -0.99 at $p<0.005$ (Figure 3). This figure illustrates the effect of different concentrations of NaCl on growth rate of *Daphnia carinata* at the end of experiment (Table 1).

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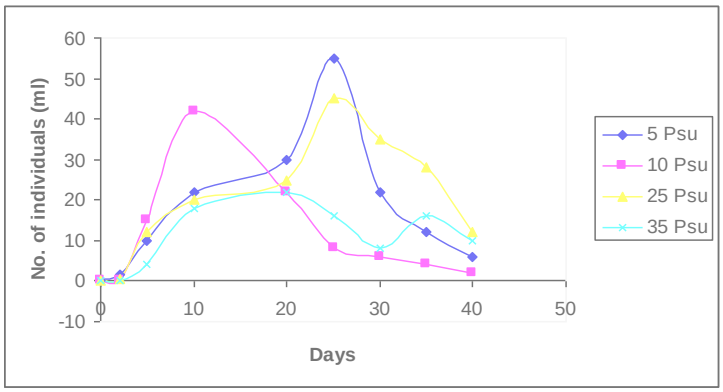

Figure 3: Survival and reproduction of *D. carinata* in different salinity.

Table 1: Effect of salinity as sodium chloride, NaCl on % survival, growth and reproduction rate of D.carinata cultured in static renewal system for 21 days.

NaCl to medium (%)	% survival at 21st day	First brood (days)	progeny/Female at 21st day (mean±SD)	Female length at 21st day (mm/mean)
Control	97^{a}	7	$(70\pm24)^{a}$	8.7
$1.67LC_{10}$	$90^{a,b}$	9	$(54\pm9)^{b}$	8.3
$1.86LC_{15}$	$83^{b,c}$	9	$(49\pm6)^{a}$	7.6
$2.04LC_{20}$	77^{c}	9	$(49\pm7)^{a}$	7.5
$2.20LC_{25}$	70^{c}	9	$(48\pm8)^{a}$	7.5
$2.35LC_{30}$	60^{d}	9	$(45\pm9)^{a}$	7.2
$2.51LC_{35}$	$50^{e,f}$	9	$(33\pm7)^{c}$	6.4
$2.66LC_{40}$	43^{f}	9	$(32\pm3)^{c}$	6.2

Generally it was found that the length of *D.carinata* increased with increasing period of culture for all concentrations. At the end of experiment, control groups (0% salinity) showed the highest significant ($p<.001$) lengths which represented by 3.74 mm, while the lowest significant ($p<0.001$) lengths were observed for groups cultured in the highest NaCl concentrations (2.66%) corresponding to LC_{40}, which represented by 3.38 mm after 21 days. There were no significant differences ($p<0.001$) between control group and groups cultured in concentrations 1.67, 1.86 and 2.04% corresponding to LC_{10}, Lc_{15}, LC_{20}, respectively (Fig. 4).

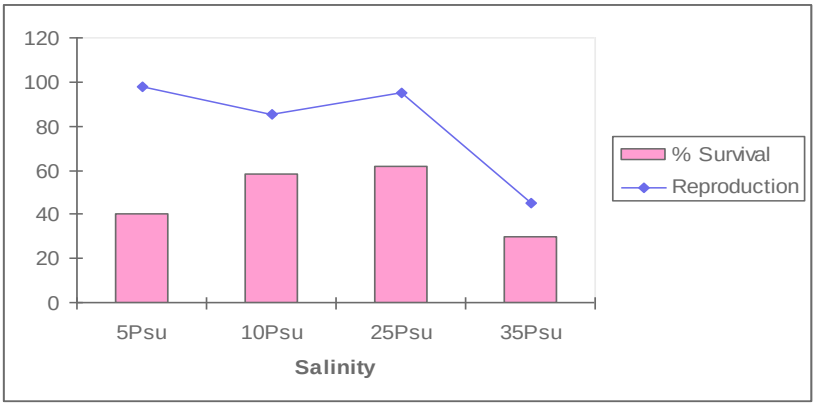

Figure 4: Population density of *D. carinata* under varied psu in terms of time in days.

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Discussion: The female zooplanktons are fertilized after the entry into adulthood and can produce several brood from one fertilization event. According to Hicks and Coull (1983), harpacticoid copepods need male once to produce multiple of egg. Therefore for every female individual, the tendency of producing eggs is difference of population density between replica samples. This significant difference could potentially due to the cannibalism activity performed by certain nauplii, copepodite and adult stages (Schipp et *al.*, 1999). Cannus and Zeng (2009) found that cannibalism rate on naupliar stage increases with increasing adult stocking density of *Acartia sinjiensis*.

The sharp decline of the number of nauplii could also be due to the number of nauplii that decreased drastically during the culture time. Williams and Jones (1999) noted that in laboratory studies, offspring production generally declines with reduction in food supply below the optimal levels. Salinity level of 35 psu was found to provide the best condition for the development of *Pararobertsonia* species from nauplii to adult, and it shows that this cultures conditions is the most stable among the others. This result is comparable to a study done by Matias-Feraita et *al*. (2005), where a tropical harpactiecoid species, *Nittocra affinis* showed the maximum growth when cultured in 35 psu salinity compared to lower salinities. A sudden decrease in salinity and temperature do affect the survival. The results of this study show that *M. hyalinus* species is more sensitive to the sudden change of temperature rather than salinity. The physiological response and tolerance towards temperature and salinity might be the reason for the different sensitivity to the two parameters. The different tolerant level towards temperature and salinity in culture vessel was also reported for a calanoid species *Eurytemora affinis* (Devreker et *al*., 2009). They also found that the calanoid was more sensitive to temperature change than salinity.

The different species might have different thermal limit in term of reproductive response. This study confirmed the important role of temperature in copepod culture. We found that 26-30^{0}C was the best range for nauplii production while 28-32^{0}C was the best for fast maturation rate of nauplii. A study by Williams and Jones (1999) also noted that a benthic herpacticoid, *Tisbe battaglial* has their best temperature at 20^{0}C, and increasing of temperature towards 25^{0}C decreased the production rate in the case of salinity effect.

This study revealed that there is a non-linear survival response of *Mesocyclops hyalinus* to short term immersion of 24 hours in 3, 12 and 35 psu. Copepods that were transferred in the 12 psu showed the lowest survival rate. It is also significant that exposure of low salinity in more than 24 hours for this species will only cause death, as what happened to *Euterina acutifrons* which survived only for 24 hours when transferred directly from 35 to 5psu seawater (William and Zones, 1999).

The results of salinity effects upon *D. carinata* might be useful for a better understanding of how salinity affected the survival, growth and reproductive responses. It also support that *D. carinata* may capable to endure salinity upto slight level. Casey et *al.* (2000) stated that the LC 50 for *D. magna* varies from 5491 to 5736mg Nacl/l^{-1} and Schuytema et *al* (1997) determined a median LC50 at 6.6 gl^{-1} concentration.

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The present study reveals LC 50 as 3.99 gl^{-1} indicated greater sensitivity of *D. carinata* with salinity in tropical freshwater ecosystem. Arner and Koivisto (1993) reported that *D.magna* grew in salinities of 4% and 8%. Kikuchi (1983) stated that the gills and digestive tracts of crustaceans are their basic osmoregulatory organs, with changes in salinity capable of modifying gill morphology in Daphnia, for example. These changes affect the so-called dark-cell in particular, these being rich in mitochondria and possessed of an elaborate tubular system and modified cell membrane. It is probable that they play an important role in osmoregulation. Aladin (1991) described round nuchal organs in *D. Magna* embryos, whose cytoplasm is capable of intensive cellular absorption of salt on account of high permeability to ions. The behavioral response to salinity in freshwater crustaceans was studied. Grzesiuk and Mikulski (2006) stated that the effect of salinity can be modified by other abiotic factors, about with the pattern of these modification varying. A strong interaction between effects of temperatures and salinity on survival of *Daphnia magna* has been demonstrated, a high temperature compounding the harmful effect on the salinity (Casey et *al*., 2000). Even where it does not reduce lifespan, salinity may limit individuals growth rates, with freshwater animals transferred to a brackish environment found to grow more slowly: as in agreement with *Daphnia carinata* (Hall and Burns, 2002) and *D.magna* (Teschner, 1995; Arner and Koivisto, 1993).

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