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STUDIES ON THE LARVICULTURE OF THE FRESHWATER FAIRY SHRIMP *Streptocephalus torvicornis* (ANOSTRACA: CRUSTACEA)

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ABSTRACT

The present study is aimed to standardize the culture conditions of larviculture of *Streptocephalus torvicornis.* Effect of selected physico-chemical parameters such as temperature, salinity, hardness of different culture media on the survival of *S. torvicornis* larvae was studied. The effect of different feeds (baker's yeast, rice bran and spirulina) on the survival was also tested. Furthermore, effect of nitrogenous metabolites (NO_2-N, NO_3-N) on the survival of *S. torvicornis* nauplius was recorded. The morphometric study revealed an overall specific growth rate of 2.3 mm/day. The baker's yeast yielded a significantly highest survival rate (60 to 76%) after 48 hrs. Of the different temperatures tested, 20ºC was found to be optimum for the survival of *S. torvivornis,* whereas the 40º C was found to be lethal. The survival rate of nauplii in different salinities (0.15º/₀₀, 0.3º/₀₀ and 2.5º/₀₀) after 24 hrs ranged between 0 and 100%, whereas it was ranged between 0 and 63% after 48 hrs. Beyond 2.5º/₀₀ salinity, no survival of nauplii was noticed. A maximum survival rate of about 56% was noticed in the very hard medium, whereas it was only 16% in the very soft medium. Among the nitrogen metabolites, NO_2-N was found to be more toxic. The results are discussed in the light of available literature.

KEYWORDS: ***Streptocephalus torvicornis,*** **Baker's yeast, Spirulina, rice bran, NO_2-N, NO_3-N.**

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INTRODUCTION

In recent years there has been an upsurge of interest in the use of fresh water anostracans (fairy shrimps) in various types of applications *eg*., as live food for commercial fishes, ornamental fishes and prawns[1-9] as test organisms in ecotoxicological testing of waste water and inorganic wastes[10&11] and in recycling of organic waste and the tertiary treatment of waste waters[12 &13] Larval nutrition appears to be a major bottleneck for the mass culture of several aquaculturally important crustaceans including anostracans[14]. Since freshwater anostacans begin to feed on exogenous food from the third instar larvae onwards, heavy mortalities are not uncommon[15]. In addition to food, temperature can also be expected to play a major role in larval survival. Much information is available on the effect of food density, physical and chemical properties of the culture medium on adult stages [16, 17&18], but not on larvae of freshwater anostracons[19]. The maintenance of water quality, and sufficient knowledge of tolerance limits of a species regarding water quality are dispensable requirements in any larval rearing system[20&21]. One of the important limiting factors in intensive culture system is the build-up of toxic nitrogenous waste, such as ammonia and nitrite[22]. High concentrations of ammonia and nitrite may result in retardation of growth, and in extreme cases can cause mortality[23]. Though information is available on the effect of ammonia and nitrite on feeding rates of fairy shrimps[17&24], little is known about the sensitivity of larval stages to nitrogenous wastes. The present study was carried out to evaluate the effect of selected physico-chemical parameters (temperature, salinity and hardness) and water quality of different culture media on the growth and survival of *S. torvicornis* larvae. The effect of different food types on the growth and survival of *S. torvicornis* was also recorded. Further the effect of different hatching media on the hatching success of the cysts of *S. torvicornis* was also studied.

MATERIALS AND METHODS

Streptocephalus torvicornis cysts were procured from the Laboratory of Animal Ecology, University of Ghent, Belgium and mass cultured in the laboratory. All experiments were carried out in 150ml beakers containing 100ml aerated tap water at room temperature with continuous illumination. Ten nauplii were used for all experiments except for the mixed diet experiment in which twenty nauplii were used. Healthy and free swimming nauplii were separated by attraction to a fibre light source. For all the experiments except the feeding experiment, Baker's yeast was used as food. Morphometry experiment was carried out by fixing larvae in 5% formalin and then size was measured using calibrated micrometer (from the tip of the head to posterior margin of the telson). The effect of food types were studied by using Baker's yeast, rice bran and *Spirulina* powder. A microalga (*Scenedesmus* sp*)* was used as control (*ad libitum*). For hatching experiments, the following five different hatching media were used. 1. Distilled water; 2. 75% distilled water (75:25; distilled water: aged tap water): 3. 50% distilled water (50:50 aged tap water: distilled water), 4. 25% distilled water (75:25 aged tap water: distilled water) and 5. 0% distilled water (100% aged tap water) on the hatchability of *S. torvicornis* cysts. For temperature experiments, ten nauplii were released into 100ml beaker kept at 20± 1.00, 30±1.00, 40±1.00ºC and the animals were fed with Baker's yeast. Survival of the nauplii was monitored after 24 & 48 hrs. Effect of salinity was tested using culture media of 0.15º/₀₀ to 2.5º/₀₀. The effect of different properties of culture medium such as hardness and pH were studied. The composition of different test media is shown in Table -1. The effect of NO_2-N (added as Na NO_2) and NH_3-N (added as $(NH_4)_2$ SO_4) was tested at concentrations of 0.2, 0.4, 0.6, 0.8 and 1mg/l. survival after 24 and 48 hrs was recorded. One way analysis of variance (ANOVA) and the multiple comparisons (Least Significant Difference) was used to compare the survival rates of larval stages between different treatments were analyzed using SPSS 11.5[25].

Table 1
Composition of Different medium

Types of water hardness	Salts Required per mg/l				Hardness[a]	Hardness[b]	pH	Alkalinity
	$CaSO_4 . 2H_2O$	$MgSO_4$	$NaHCO_3$	KCl				
Very soft	12	7.5	7.5	0.5	10-13	8-10	6.4-6.6	13-13.5
Soft	48	30.0	30.0	2.0	40-48	35-41	7.1-7.3	30-35
Moderately Soft	96	60.0	60.0	4.0	80.-100	71-110	7.6-7.8	58.5-59.5
Hard	192	120.0	120.0	8.0	160-180	135-170	7.8-8.0	110-120
Very Hard	384	240.0	240.0	16.0	280-320	250-327	7.9-8.2	225-245

RESULTS

The growth (length-mm) of the *S. torvicornis* larvae as a function of time is shown in Fig. 1.The size of the larvae gradually increased from 3.04 ± 0.14mm at day 1 to 16.03±0.4mm at day 7. A maximum specific growth rate of 3.15mm was noticed at day 4.The growth was rapid after 3rd day.

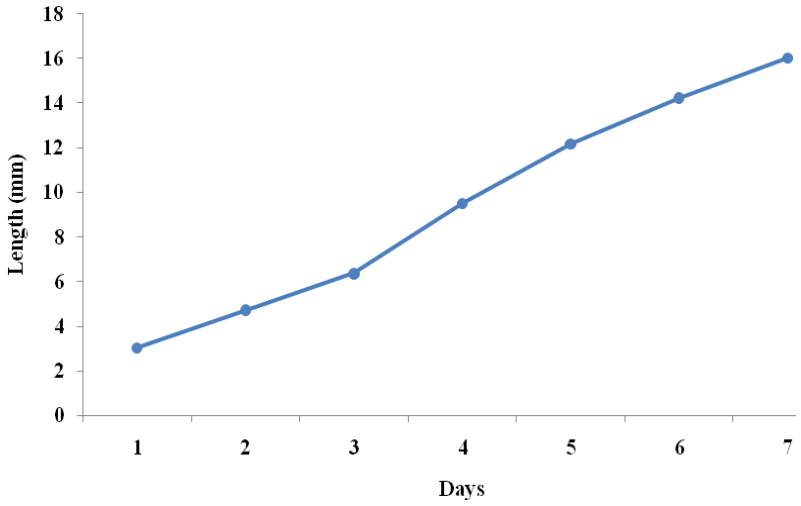

FIGURE 1
MORPHMOETRY: GROWTH (LENGTH) OF S.TORVICORNIS AS A FUNCTION OF TIME (N = 10)

Effect of different hatching medium on the hatchability of S. torvicornis cysts

A significant effect of hatching medium on the hatchability of the cysts of *S. torvicornis* was observed ($P<0.05$), Cysts incubated in the distilled water showed a cumulative hatching of about 65% whereas it was reduced to 10% at 75% distilled water. In all other media, no hatching was noticed (Fig.2).

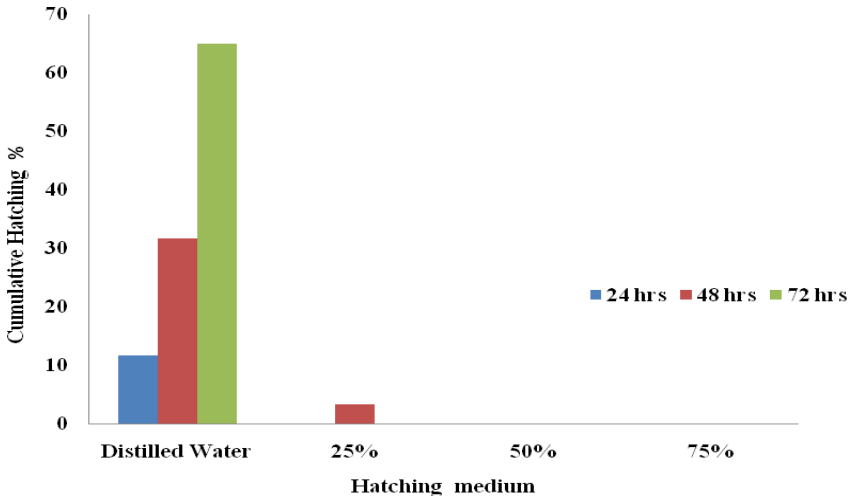

FIGURE 2
EFFECT OF HATCHING MEDIUM ON THE HATCHING SUCCESS OF S. TORVICORNIS CYST

Effect of food types on survival

The survival rate of larvae fed with different food types is shown Fig.3 and Table 2. The rate of survival (%) of the *S. torvicornis* fed different food types was ranged between 77and 87% after 24 hrs, whereas it was 60-76% after 48 hrs. Though animals fed yeast showed better survival rate, it is not statistically significant when compared to other food types both after 24 and 48 hours (*P*>0.05).

Table 2
Effect of Food types on the Survival of S. torvicornis (24 hrs and 48hrs) ANOVA

Variable		Sum of Squares	df	Mean Square	F	Sig.
Food types Vs Survival (24hrs)	Between Groups	158.33	3	52.778	1.583	0.268
	Within Groups	266.667	8	33.333		
	Total	425.00	11			
Food types Vs Survival (48hrs)	Between Groups	491.667	3	163.889	3.278	0.080
	Within Groups	400.000	8	50.00		
	Total	891.667	11			

Significant at P > 0.05

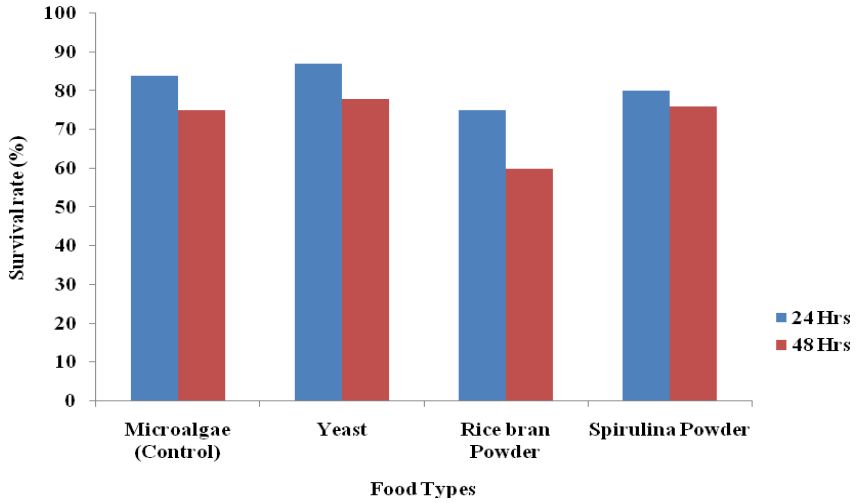

FIGURE 3
EFFECT OF FOOD TYPES ON SURVIVAL OF S. TORVICORNIS

Effect of temperature

An overall significant effect of temperature on the survival rate of the *S. torvicornis* was revealed (P<0.001). Among the three temperatures tested, survival rate was almost identical at 20ºand 30º C whereas at the highest temperature (40º C), 100% mortality of the nauplii was observed both after 24 and 48hrs. Though the highest survival rate was noticed at the lowest temperature (20 ˚C), it was not significant with that of 30 ˚C (Fig. 4 & Table 3).

Table 3
Effect of Temperature on the Survival of S. torvicornis (24 hrs and 48hrs) ANOVA

Variable		Sum of Squares	df	Mean Square	F	Sig.
Temperature Vs Survival (24hrs)	Between Groups	14466.67	2	7233.333	130.2	0.000***
	Within Groups	333.33	6	55.556		
	Total	14800.00	8			
Temperature Vs Survival (48hrs)	Between Groups	11266.67	2	5633.333	253.5	0.000***
	Within Groups	133.333	6			
	Total	11400.003	8			

*** ***Significant at P< 0.001***

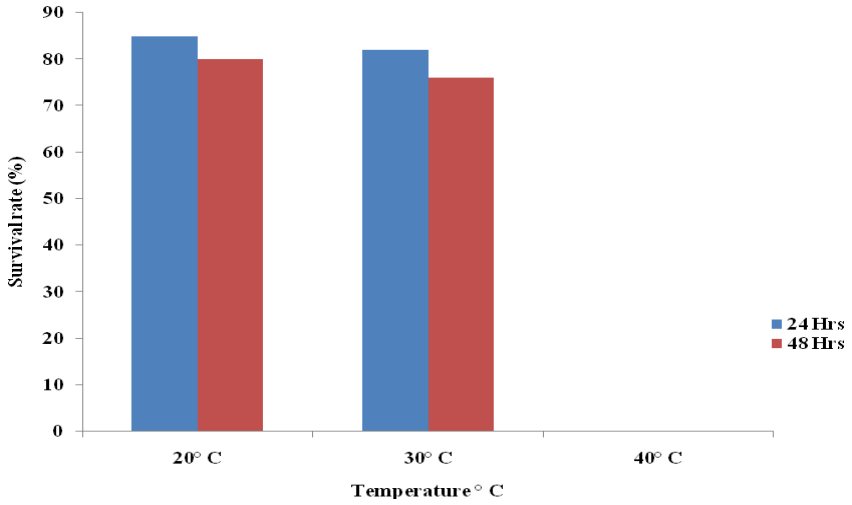

FIGURE 4
EFFECT OF TEMPERATURE ON SURVIVAL OF S. TORVICORNIS

Effect of salinity and hardness of the culture medium

After 24 hrs almost 100% of the nauplii survived at the lowest salinities (0.15‰). About 60% of the nauplii survived in the 2.5‰ salinity. An overall significant effect of water hardness of the culture medium on the survival rate of *S. torvicornis* nauplii was revealed after 24 hrs (P<0.001). However it was not significant after 48 hours (P>0.001). Nevertheless, multiple comparisons revealed a significant difference in survival rate between different media at 0.05 levels. An increase in water hardness yielded an increase in the survival rate of *S. torvicornis* nauplii. A maximum survival rate of about 56% was noticed in the very hard medium, whereas it was only 16% in the very soft medium. After 48 hours, the survival rate ranged between 3 and 27% (Figs. 5 & 6; Table 4&5).

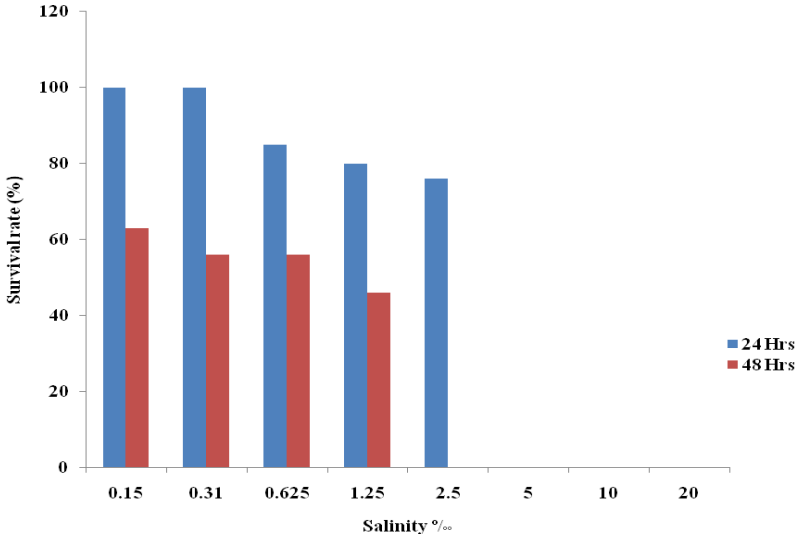

FIGURE 5
EFFECT OF SALINITY ON SURVIVAL OF S. TORVICORNIS

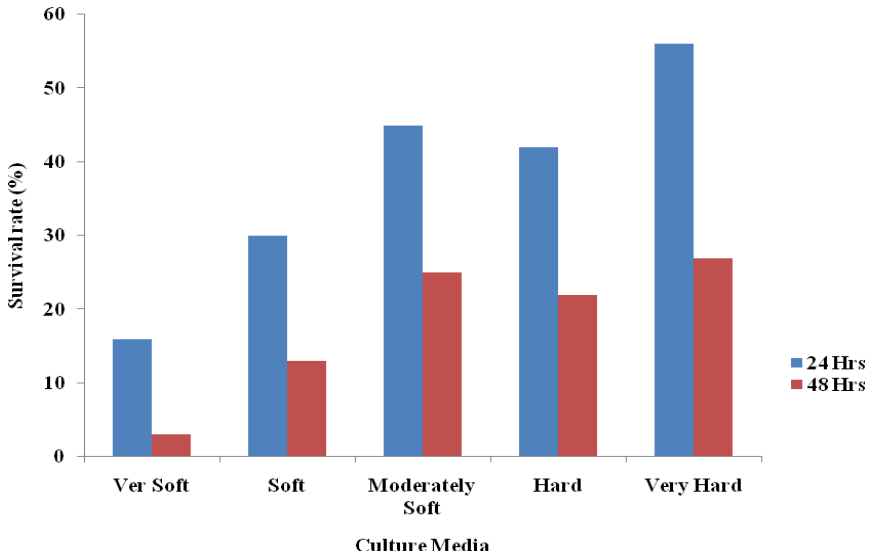

FIGURE 6
EFFECT OF HARDNESS OF THE CULTURE MEDIUM ON SURVIVAL OF S. TORVICORNIS

Table 4
Effect of Salinity on the Survival of S. torvicornis (24 hrs and 48hrs) ANOVA

Variable		Sum of Squares	df	Mean Square	F	Sig.
Salinity Vs Survival (24 hrs)	Between Groups	2573.33	4	643.333	32.167	0.000***
	Within Groups	200	10	20		
	Total	2773.33	14			
Salinity Vs Survival (48 hrs)	Between Groups	7906.667	4	1976.667	74.125	0.000***
	Within Groups	266.667	10	26.667		
	Total	8173.334	14			

*** ***Significant at P< 0.001***

Table 5
Effect of Hardness on the Survival of S. torvicornis (24 hrs and 48hrs) ANOVA

Variable		Sum of Squares	df	Mean Square	F	Sig.
Hardness Vs Survival (24 hrs)	Between Groups	2693.333	4	673.333	6.733	.007
	Within Groups	1000.000	10	100		
	Total	3693.33	14			
Hardness Vs Survival (48 hrs)	Between Groups	1240.00	4	310.000	2.325	0.127
	Within Groups	1333.333	10	133.333		
	Total	2573.333	14			

Significant at P > 0.001

Effect of water quality

More than 50% of the nauplii survived at 0.4 mg/l of NO_2-N after 24 hrs, whereas after 48 hrs 50% mortality was noticed at 0.2 mg/l of NO_2-N. However, 50% of the mortality of the nauplii was observed at 0.8 mg/l of NO_3-N after 24 hours, and after 48 hours 50% of nauplii died at 0.4 mg/l (Fig.7& 8).

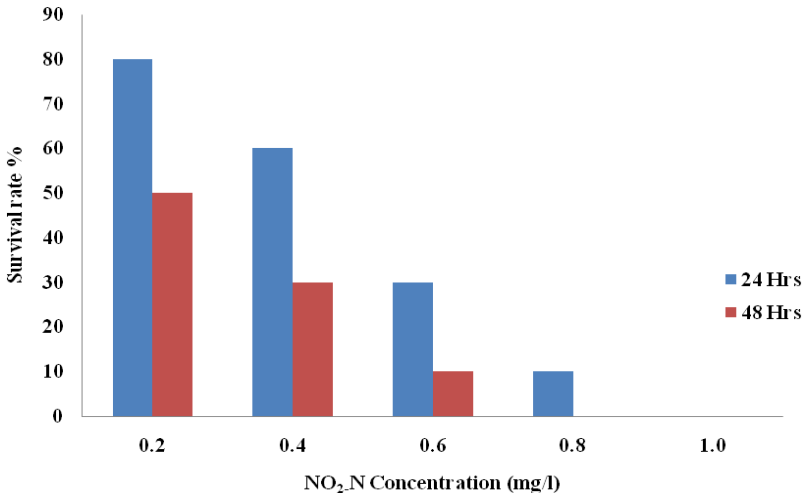

FIGURE 7
THE EFFECT OF NO_2-N ON THE SURVIVAL OF S.TORVICORNIS

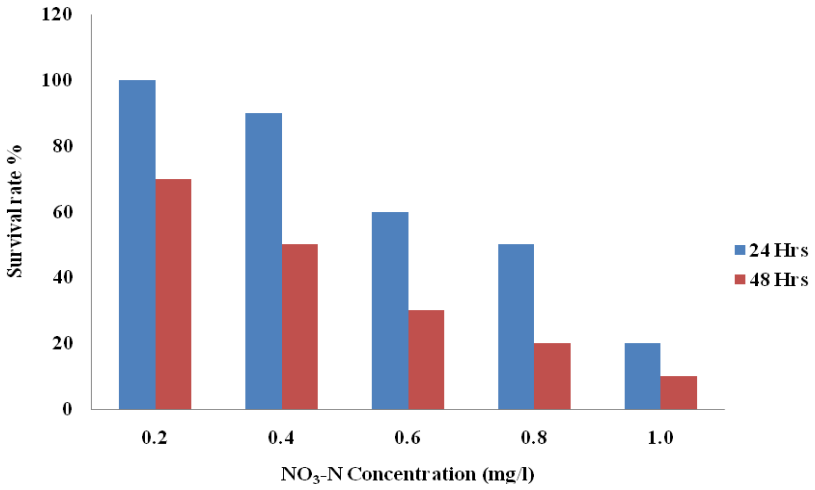

FIGURE 8
THE EFFECT OF NO_3-N ON THE SURVIVAL OF S.TORVICORNIS

DISCUSSION

In the present study, a preliminary attempt has been made to standardize the culture conditions of the fresh water fairy shrimp, *S. torvicornis*, for their eventual use in aquaculture as a potential live food for the freshwater fish and shrimp. A steady increase in the growth (length) of the *S. torvicornis* with an increment of about 2.4mm per day was noticed for duration of 7 days. When compared to later phase, the growth rate in the initial phase was relatively slow. It may be due to the acclimatization to the culture conditions. In general, other species of fresh water anostracan such as *S. proboscideus*[3]. *Thamnocephalus platyurus* [26]also showed a steady and rapid growth. [3] noticed a specific growth rate of 1.7mm/day whereas[26] reported that individuals of *Thamnocephalus platyurus* had a mean size of 0.88 mm at day 1 and 11.7 mm at day 9. In general, unlike its

halophilic counterpart *Artemia*, hatching in freshwater anostracans is highly variable. The mechanism of hatching also very poorly understood. An erratic and less hatching success of freshwater anostracan was reported in earlier studies[4]. In the present study, a hatching success of about 60% was obtained after 2 days using distilled water. Hatching in freshwater anostracan species can be invoked or augmented by renewal or dilution medium. Usually, cysts did not hatch in the habitat water or original culture medium, but did so after dilution or transfer into distilled water[17]. Better hatching rate of *S. dichotomus* cysts was also recorded in the distilled water. It may be attributed to the low osmotic pressure of the hatching medium, which believed to facilitate the breaking of the cyst wall. In the larviculture, it is very important to feed larval stage right from the beginning with the suitable food types. [26] reported better survival of *T. platyurus* and *B. lindali* using yeast. The success of yeast as diet solely depends upon the ability of larvae to digest yeast cell wall. In contrast to[17], *Spirulina* resulted relatively better survival. Temperature is one of the most important ecological factors affecting growth and survival of fairy shrimps[16]. The study of [27] indicated that growth in *S. seali* was strongly correlated with temperature and found an optimum range of temperature between 27 and 31º C for *S. proboscideus.* However this species reared at 35º C did not survive for more than 3-4 days, whereas at lower temperature (20, 25 and 30º C) better survival rate was noticed[3]. Nitrate and ammonia are generally considered to be toxic metabolic wastes to aquatic organisms [28&29]. The present study confirms that the nauplii of *S. torvicornis* are quite sensitive to NO_2-N and NO_3-N. [24]noticed 50% reduction in ingestion rate of *S. proboscideus* adults at a concentration of 4mg/l and 60mg/l of NH_3-N and NO_2-N, respectively. [4]recorded the 24 hrs LC_{50} values for NO_2-N and NH_3-N (0.46 mg/l and 0.68 mg/l, respectively), and 48 hrs LC_{50} values for these metabolites were 0.25 mg/l and 0.39 mg/l, respectively. According to [30]*Artemia* is more sensitive to nitrate than ammonium. However [31]reported that *Artemia* nauplii are more sensitive (above 2 times) to NH_3-N than NO_2-N. [32] noticed a 17% depression of rotifer (*Brachionus plicatilis*) activity at a concentration as low as 0.32mg/l of ammonia.

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REFERENCES

1. Meade, JW, Bulkowsk, Cummings L., 1987. T1: Acceptability of fairy shrimp (*Streptocephalus seali*) as a diet for larval fish. To Progressive – fish-culturist. 49:3, 217 – 219.
2. Prasath, E.B., Natesan-Munuswamy., Nasar, A.K.A 1994. Perliminary studies on the suitability of a fairy shrimp *Streptocephalus dichotomus* as live food in aquaculture. Journal of the World–Aquaculture society 25: 2, 204-207.
3. Ali, A.J. and H.J.Dumont, 1995. Suitability of decapsulated cysts and nauplii of *Strptocephalus proboscidus* (Crustacea; Anostraca) as food for *Tilapia, Oreochromis aureus* larvae. In Larvi – 95. EAS special Publication.
4. Ali, A. J., 1995. Aspects of the biology of the freshwater fairy shrimp, *Streptocephalus proboscideus* (Frauenfeld) (Crustacea: Anostraca), Ph. D thesis. pp, 101-128. State University of Ghent, Belgium, pp1-261.
5. Sanoamuang, L., P. Pakmaluk and W. Sirisan, 2006. The use of fairy shrimp *Streptocephalus sirindhornae* as a supplementary food for enhancing skin pigmentation of flower horn fish. Burapha Sci. J., 14: 19-28.
6. Imanpour Namin, J Uma Arshad and Zohreh Ramezanpoor. 2007. Mass

culture of fairy shrimp *Streptocephalus proboscideus* (Crustacea-Anostraca) and its use in larviculture of the Persian sturgeon, *Acipenser persicus*. Aquaculture Research, 38, 1088-1092.
7. Velu, C.S and N. Munuswamy, 2007. Composition and nutritional efficacy of adult fairy shrimp *Streptocephalus dichotomus* as live feed. Food Chem., 100: 1435-1445.
8. Sriputhorn, K. & L. Sanoamuang. 2011. Fairy shrimp (*Streptocephalus sirindhornae*) as live feed to improve growth and carotenoid contents of giant freshwater prawn *Macrobrachium rosenbergii*. Int. J. Zoo. Res. 7(2): 138-146.
9. Salma, D., R. Davoodi., M. Shamsaei and A. Kamali. 2013. Comparative effect of fairy shrimp and *Artemia* in the rearing of Blue Gourami, *Trichogaster trichopterus* Larvae. Annual Review & Research in Biology: 3(2): 70-75.
10. Brendonck, L & G.Persoone, 1993. Biological / Ecological characteristics of large freshwater branchipods from endorheic regions and consequences for their use in cyst based toxicity tests. In Soares, A.M.VM and P. Calow (eds.). Progress in standardization of aquatic toxicity tests, 7-35.
11. Centeno M Brendonck L and Persoone G 1993. Cyst based toxicity tests III. Development and standardization of an acute toxicity tests with the freshwater anostracan Crustacean *Streptocephalus proboscideus*. In soares, A.M.V.M and P.Calow (Eds) progress in standardization of aquatic toxicity tests. Lewis publication U.S.A 35-55.
12. Mitchell, S.A., 1990. Algae produce stock feed supplements from waste waters. S. Afr. Water. Bull. 16: 4-7.
13. Mitchell, S.A., 1991. The growth and growth efficiency of *Streptocephalus macrourus* Daday (Crustacea:Ansotraca) cultured on microalgae. In D. Belk, H.J. Dumont and N. Munuswamy (eds.) Studies on large Branchiopod Biology and Aquaculture, Hydrobiologia 212:1-10.
14. Sorgeloss, P., P. Lavens, P. Legaer and W. Tackaert, 1991. State of the art in larviculture of fish and shell fish. In P. Lavens, P. Sorgeloss, E. Jaspers & F. Ollivier (eds.). Larvi' 91. Fish & Crustacean larviculture symposium, special publication 15: 3-5.
15. Anderson, D.T., 1961. Larval development and segment formation in the branchiopod crustaceans *Limnadia staleyana* King (Conhostraca) and Aremia salina (L.) (Anostraca). Aust.J.Zool. 15: 47-91.
16. Sluzhevskaya-Drobysheva, E.B., 1981. Effect of temperature and feed on growth, maturation and survival rate of *Streptocephalus torvicornis*. Hydobiol.J. (Washington) 18: 95-98.
17. De Walsche, C., J.Mertens and H.J.Dumont, 1991. Observations on temperature optimum, cyst production and survival of *Streptocephalus proboscideus* (Frauen feld, (Crustacea: Anostraca) fed different diets. In Belk, D, H.J. Dumont and N.Munswamy (eds.,). Studies on large Branchipod Biology and aquaculture. *Hydrobiologia*, 212; 21-26.
18. Brendonck L, 1993a. Feeding in the fairy shrimp *Streptocephalus proboscides* (Frauenfeld) (Branchiopoda: Anostraca) I. Aspects of the feeding biology. Journal of Crustacean Biology 13. 224 - 235.
19. Anderson, G and S. Yu Hsu, 1990. Growth and maturation of North American fairy shrimp. *Streptocephalus seali* (Crustacea: Anostraca): a laboratory study. Freshwat. Biol. 24: 429-442.
20. Spotte, S., 1970. Fish and invertebrate culture. Wiley- interscience, New York, USA.
21. Kinne, O., 1976. Marine Ecology vol. III. Part 1. John Wiley & Sons, New York, NY, 557 pp.
22. Osterensky, A. and W. Wasielesky Jr. 1995. Acute toxicity of ammonia to various life stages of the Sao Paulo shrimp *Penaeus paulensis* Perez-Farfante, 1967. Aquaculture132:339-347.
23. Zen, K.Y and C.J. Chu, 1991. Acute toxicity of ammonia to *Penaeus japonicas* Bate juveniles. Aquacult. Fish. Manage. 22: 259-263.

24. Brendonck L, 1993b. Feeding in the fairy shrimp *Streptocephalus proboscides* (Frauenfeld) (Branchiopoda: Anostraca). II Influence of environmental conditions on feeding rate Journal of Crustacean Biology 13: 236-244.
25. Zar, J H 1984. Biostatistical analysis. Prentice Hall Englewood Cliffs, N J. 718.
26. Alejandro M. Maeda Martinez, H. Obregon, Barboza, H.J Dumont. 1995. Laboratory cultures of fairy shrimps using baker's yeast as basic food in a flow throw system. *Hydrobiologia*, 298:141-157.
27. Moore, W.G. 1957. Studies on the laboratory culture of Anostraca. Trans. Am. Microsc. Soc. 76: 159-173.
28. Wheaton, F.W.1977. Aquacuture engineering wiley & Sons, New York, U.S.A.
29. Spotte, S. 1979. Fish and invertebrate culture – water management in closed system. Wiley and Sons, New York, U.S.A.
30. Le Roy D. 1988. Waterguivering bij intensive Artemia-opkweek, Thesis. State University of Ghent, Belgium. 69 p.
31. Chen, J. C., K. J. Chen, and J. M. Liao. 1989. Joint action of ammonia and nitrite on Artemia nauplii. Aquaculture 77~329-336
32. Snell, T.W., M.J. Childress E.M. Boyer and F.H. Hoff. 1987. Assessing the status of rotifer mass cultures. J. World Aquacult. Soc. 18: 270-277.