International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

THE IMPACT OF TEMPERATURE, SALINITY LEVELS, AND CHITOSAN CONCENTRATIONS ON THE SURVIVAL AND PRODUCTION RATES OF HARPACTICOID COPEPOD, *TIGRIOPUS* SP.

Kuansiri Seelpinyo*, Supawadee Chullasorn, Thamasak Yeemin

Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok 10240

*Corresponding Author

ABSTRACT

This study aims to observe survival and production rates of harpacticoid copepods cultured at different temperatures, salinity levels, and chitosan concentrations. An ovigerous female harpacticoid copepod *Tigriopus* sp. was collected in *Padina* sp. The first experiment was carried out by culturing the copepods at temperatures of 25∘C and 30∘C, and salinity levels of 5, 10, 20, 30, 40, 45 ppt for 15 days to study the survival rate. The results showed that the temperatures and salinity levels significantly affected survival rates of the copepods ($P<0.05$). The copepods cultured at the temperature of 25∘C and salinity level of 30 ppt had highest a survival rate of 85.04%. At the salinity levels of 5 ppt and 45 ppt, the number of survived copepods was the lowest. It took 11 days for the copepods to develop from nauplius to adult stages (25∘C,30 ppt). The production rate we designed by two temperature (25∘C and 30∘C), salinity levels 30 and 35 ppt for culturing of copepods *Tigriopus* sp. the result confirmed that the optimal condition was 25°C, 30 ppt had maximum population density of *Tigriopus* sp. 1163± 488.74 ind./100 ml after 25 days. The second experiment was conducted to evaluate the effects of chitosan concentration on the production of *Tigriopus* sp. The ovigerous female copepods was fed with different chitosan concentrations (0.01, 0.1 and 1%) of microalgae. After 25 days the group with chitosan concentration of 0.01% yielded the highest production rate (1449.67±51.42 ind./100 ml). The chitosan concentration have significant differences effect ($P<0.05$) compared to the control group.

Keywords: *Tigriopus* sp., temperature, salinity levels, chitosan solution

1. INTRODUCTION

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

Harpacticoid copepods are an important component of freshwater and marine ecosystems. During their lifecycle, harpacticoid copepods consume algae, fungi, protozoa, bacteria, and detritus (Ruppert & Barnes, 1991) and are in turn an important food source for fish and crustacean larvae (Gopakumar & Santhosi, 2009; Zaleha et al., 2012; Jeyaraj et al., 2014).

The nutritionally-essential fatty acids, amino acid and other molecules provided by harpacticoid copepods are important for the growth and survival of fish and crustacean larvae (Miles et al., 2001; Zaleha et al., 2012). Therefore, it is of considerable interest to determine optimal condition for copepod cultivation in order to obtain sufficient nutritional yields for usage in aquatic animal nursery (Fleeger, 2005; Olivotto et al., 2008).

Harpacticoid copepods include a large numbers of species which require different environmental factors for development and reproduction (Carlotti & Paul, 1992). While, the genera Tigriopus, Nitokra, Tisbe, and Pararobersonia are popular for laboratory culture (Miles et al., 2001, Zaleha & Farahiyah, 2010; Punnarak et al., 2017), in Thailand, there are only few reports of such cultivation of copepods. Harpacticoid copepod Tigriopus sp are highly populous and distributed throughout the coastal zones in Thailand; furthermore they possess characteristics which make them suitable for cultivation - such as tolerance to extreme environmental changes (salinities ranging from 15 to 70 ppt and temperatures from 17 to 30 °C) (Aurelyanna & Lília, 201). Due to these considerations, we selected Tigriopus sp for this study.

Chitosan is an important biopolymer with a wide variety of uses; its' main sources for commercial production are crustaceans (shrimp and crabs), the cell walls of fungi of the class Zygomycetes and insects (Marguerite, 2006; Suthida, 2009). It is non-toxic and is biodegradable, and it not known to be causative agent of environmental harm (Alishahi & Aïder, 2012). Chitosan has a wide number of applications in the food, cosmetics, biomedical, pharmaceutical, and agricultural industries (Piyabutr et al., 2004; Marguerite, 2006), In addition, chitosan can be used to treat aquaculture industry wastewater (Chung (2006).

This research work is focused to examine the impact of different temperatures, salinity levels, and chitosan concentrations on the survival and production rates of harpacticoid copepods with the goal to inform and guide the development and breeding of harpacticoid copepods to be used as feed in economically important mariculture at present and in the future.

2. MATERIALS AND METHODS

Sample collection, study area and culturing of harpacticoid copepod

The copepod sample was obtained by collecting brown algae, *Padina* sp. on Namsai Beach in Chon Buri Province, Thailand during a period of low-tide in November 2017 and then by

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

washing out the thalli of the algae with seawater. The copepod was then cultured in a laboratory room at Ramkhamhaeng University, Bangkok, Thailand. The copepod was fed with a few drops of microalgae, *Tetraselmis* sp. every other day during a 30-day period, and produced several offspring. Morphological identification was made utilizing a stereo microscope (model SZ30, Olympus Corp., Tokyo; Japan) and light microscope (model CH20 Olympus Corp., Tokyo; Japan) after the copepods were fixed in 10% Neutral buffered formalin and dyed with Bengal's Rose (Sigma Aldrich) with a final concentration of 6-10% for 24 hrs (following the method used by Boxshall & Halsey (2004); Chullasorn (2012, 2013).

Effect of temperature and salinity levels on the survival and Production rate of harpacticoid copepods

The Survival rate

12 ovigerous females were selected and divided into two groups. Each gravid female was placed in a glass petri dish. In Group 1, each individual was cultured at temperature of 25∘C and salinity levels of 5, 10, 20, 30, 40, and 45 ppt, respectively, for 15 days. Meanwhile, each individual in Group 2 was cultured at 30∘C and salinity at 5, 10, 20, 30, 40 and 45 ppt, respectively, for 15 days .The copepods were fed with a few drops of microalgae, *Tetraselmis* sp. every three days for 15 days. The salinity in the petri dish was measured every three days using a refractometer (model MASTER-S/MillM), temperature was measured everyday using a mercury thermometer. Every day the copepods were monitored and counted under a stereo microscope (model SZ30, Olympus Corp., Tokyo; Japan) at all stages; nauplius, copepodid, and adult. Each treatment, data collected is three replicates each treatment of the number of individuals every day.

The production rate

The study of production rate of harpacticoid copepods were designed by each 20 ovigerous female copepods were cultured in 4 treatments , the copepods were cultured at temperature of 25∘C and 30∘C in salinity level of 30 and 35 ppt, respectively, The copepods were fed with microalgae, *Tetraselmis* sp. every 5 days. The temperature and salinity levels were measured as above. The production rates were measured every 5 days for 30 days. Each individual copepod was monitored and counted under a stereo microscope (model SZ30, Olympus Corp., Tokyo; Japan) at all stages (at all stages; nauplius, copepodid, and adult.).

Effect of chitosan on production rate of harpacticoid copepods

The experimental design consisted of three treatment groups and control group. Each 20 ovigerous female copepods was fed with microalgae, *Tetraselmis* sp., and chitosan solution with 0.01, 0.1 and 1% concentrations of 50 ml microalgae and a control group without chitosan, were

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

added to 500 ml seawater. There are all of treatments were cultured at temperature of 25◦C and salinity level of 30 ppt during the 30 day trials. The production rates were measured every 5 days for 30 days, each individual of copepods were monitored and counted under a stereo microscope.

Data analysis

The data of all experiments was collected as means ± SE of three replicates of the individuals number in each treatment. Significant differences in the survival and production rate in different temperature, salinities, and chitosan concentrations were analyzed by using One way-ANOVA with a significance level of 95% (p-value < 0.05) with Minitab Ver. 18.

3. RESULTS

Effect of temperature and salinity levels on survival and production of harpacticoid copepods.

The survival rate

The study focused on uncovering the temperature and salinity levels effect on the survival rate of harpacticoid copepod *Tigriopus* sp. hatching from eggs. The harpacticoid copepods, cultured at 25◦C with salinity level 30 ppt showed the highest survival rate - 85.04% (41.67±4.50 individual from 49±3.74 nauplius), followed by copepods cultured at temperature of 25◦C and salinity level of 20 ppt which had a survival rate of 82.56% (36.33±2.87 individual from 44±2.16 nauplius), Meanwhile, individuals cultured at temperature 30◦C and salinity levels 5 ppt had the lowest survival rate of 26.85 % (3.67±1.70 individual from 13.67±3.86 nauplius) (Tab.1). Development from nauplius to copepodid stages took 6.7 and from copepodid to adult stages took 4.7 days at 25◦C, 30 ppt. These copepods could survive at salinity levels between 5 ppt and 45 ppt but the number of surviving copepods was low (Table 1). One-way ANOVA analysis shows a significant difference (P<0.05) of population density between different condition of cultured (Fig.1).

Table 1: Survival rate of *Tigriopus* sp. from nauplius after the 15 days of the cultivation stage (one gravid female)

25◦C						30◦C					
5 ppt	10 ppt	20 ppt	30 ppt	40 ppt	45 ppt	5 ppt	10 ppt	20 ppt	30 ppt	40 ppt	45 ppt
42.20%	27.52%	82.56%	85.04%	46.84%	15.99%	13.67%	41.78%	53.02%	65.85%	45.78%	34.60%

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

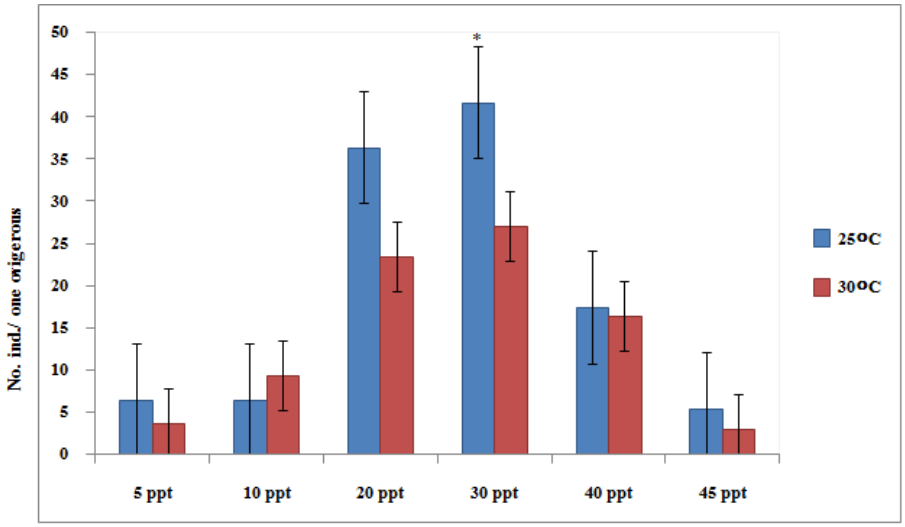

Fig. 1: Individual number of harpacticoid copepod *Tigriopus* sp. from nauplius after the 15 days of the cultivation stage (one ovigerous female) at difference temperature and salinity levels

The production rate

We examined the production rate of harpacticoid copepod *Tigriopus* sp. reared at 25°C and 30°C with salinity levels of 30 ppt and 35 ppt for 30 days. Our results show that the copepods yielded the highest production after being reared for 25 days. Production rate at temperature 25°C with salinity level 30 ppt was significant higher - 1163+ 120.92 ind/100ml (740±425.52 nauplius, 129±81.55 copepodid, 106. 67±52.79 male, 34. 67± 4.71 female, 58. 67± 0.94 ovigerous and 94±38.18 mating); followed by the production rate at temperature of 30°C and salinity level 30 ppt - 949± 120.14 ind/100ml (568.67± 27.05 nauplius, 149±101.69 copepodid , 48.67 ±18.35 male, 66±14.14 female, 51±31.06 ovigerous and 65.67±42.52 mating); then the production rate at temperature of 25°C and salinity level 35 ppt - 789.33+104.6 ind/100ml (482. 67±24.73, nauplius, 97. 67± 44.49 copepodid, 62.67± 8.06 male, 43.33±10.34 female, 51.33± 4.71 ovigerous and 51. 67± 14.52 mating).The group cultured at 30°C, and salinity 35 ppt had very low production rate (461±129.06 ind/100 ml.; 163± 23.93 nauplius, 105.67±67.15 copepodid , 66.33± 19.36 male, 49.67± 27.33 female, 31.67±10.21 ovigerous and 44.33 ± 25.22 mating), however, the results showed that the all of experiments did not have significant difference effect on the production of hapacticoid copepods *Tigriopus* sp. The population of nauplius was at maximum population level at all of the experiments (Fig 2-5).

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

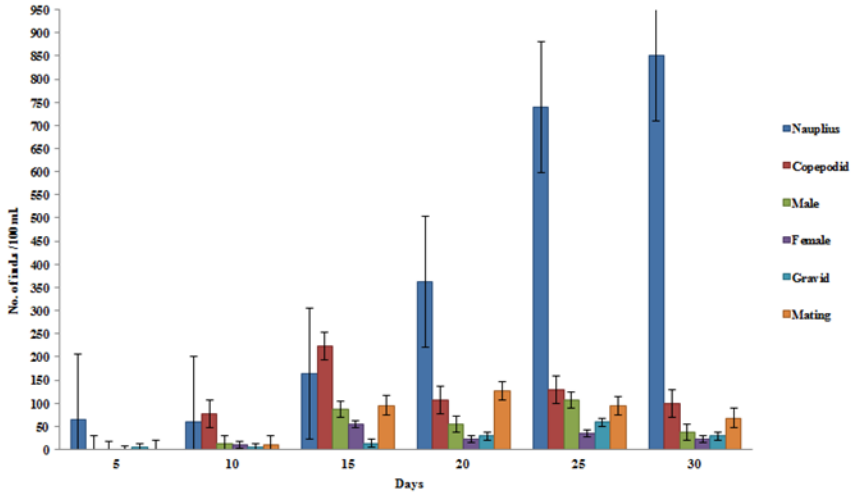

Fig. 2: Population density of harpacticoid copepods *Tigriopus* sp. at 25∘C with salinity of 30 ppt in 30 days (number of individual in 100 ml.).

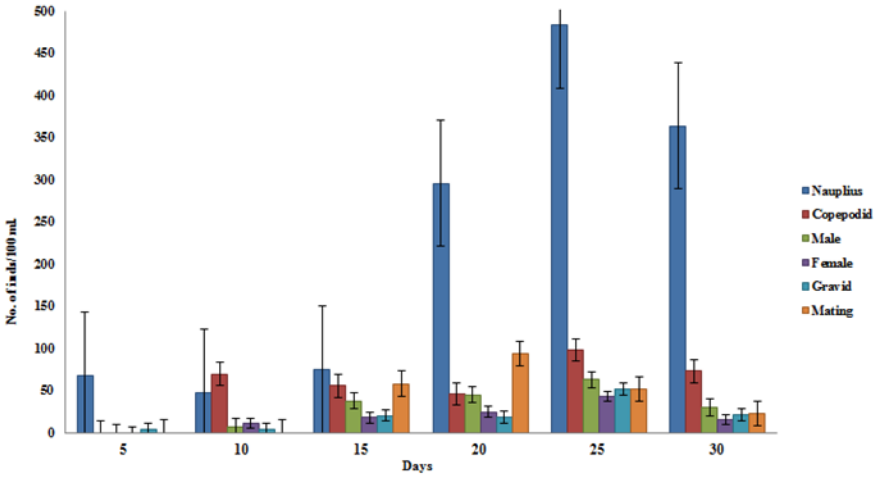

Fig. 3: Population density of harpacticoid copepods *Tigriopus* sp. at 25∘C with salinity of 35 ppt in 30 days (number of individual in 100 ml.).

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

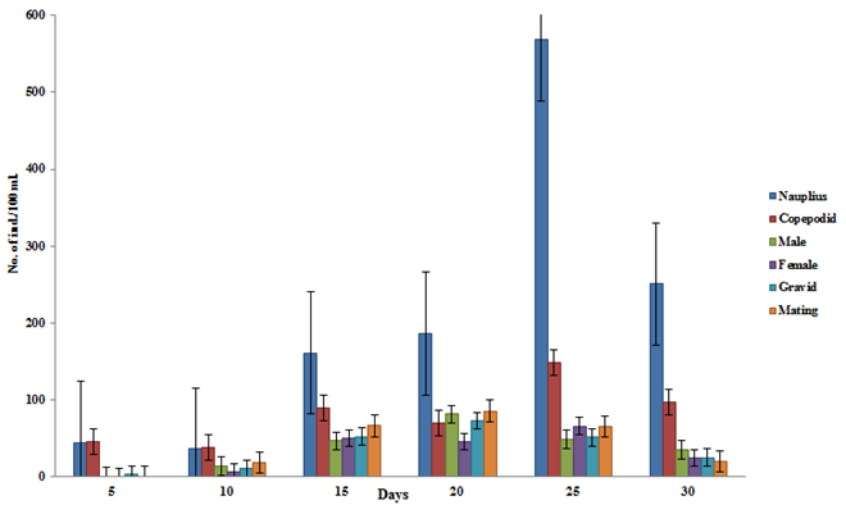

Fig. 4: Population density of harpacticoid copepods *Tigriopus* sp. at 30∘C with salinity of 30 ppt in 30 days (number of individual in 100 ml.).

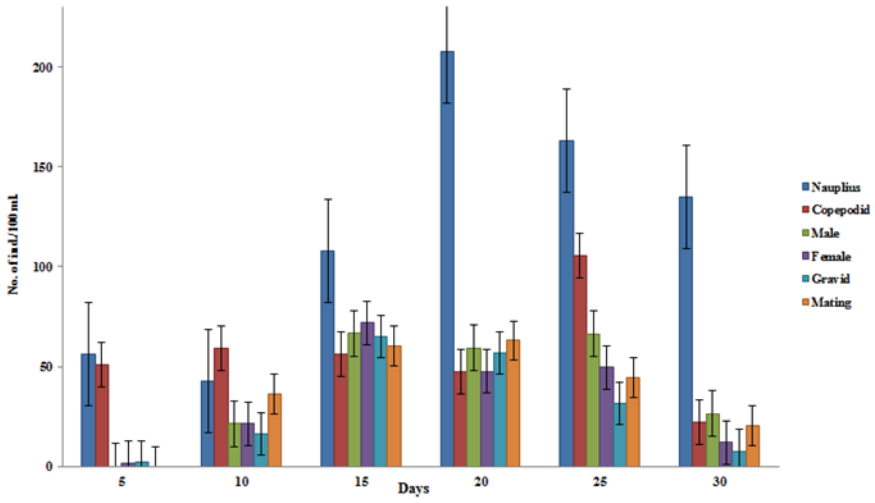

Fig. 5: Population density of harpacticoid copepods *Tigriopus* sp. at 30∘C with salinity of 35 ppt in 30 days (number of individual in 100 ml.).

Effect of chitosan concentration on the production of *Tigriopus* sp.

We performed then studied the effect of chitosan on the production of harpacticoid copepods *Tigriopus* sp. A design of three treatments was realized where each treatment had a different level of chitosan concentration (0.01, 0.1, and 1 and without), with microalgae *Tetraselmis* sp . The production rates were measured every 5 days for 30 days. After 25 days the group with

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

chitosan concentration of 0.01% yielded the highest production rate of 1449.67± 51.42 ind./100 ml (803.67±64.31 nauplius, 415.67±21.01 copepodid, 61± 6.16 male, 47.67± 2.87 female, 56.67± 4.12 ovigerous and 48.33±9.74 mating), while the one with chitosan concentration of 1% had the production rate of 1033.33±29.95 ind./100 ml (708.67+ 37.60 nauplius, 107.67±7.72 copepodid, 100±29.03 male, 37.67±1.25 female, 31.67± 2.87 ovigerous and 47.67±0.92 mating) when compared with the group without chitosan (control group) and control group has 1194.3± 51.04 inds./100 ml (828±80.75 nauplius, 202.67±46.92 copepodid, 57±7.79 male, 45±2.16 female, 18.33±3.10 ovigerous, 43.33±12.68 mating), but that the group with 0.1% chitosan had showed the maximum growth rate of copepods after 20 days (804.67±11.32 ind./100ml; 533.67±28.45 nauplius, 101.33±9.98 copepodid, 23.67±3.68 male, 38±4.55 female, 40.33±1.25 ovigerous and 67.67±7.85 mating)(Fig. 6). The concentration of chitosan 0.01% have significant differences effect on production of hapacticoid copepods *Tigriopus* sp. ($P<0.05$)

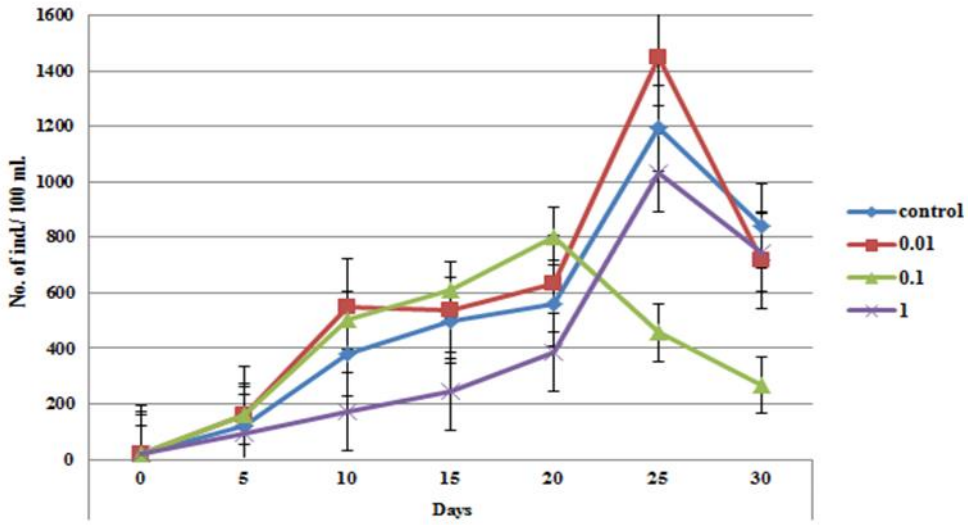

Fig. 6: Population density of harpacticoid copepods *Tigriopus* sp. at different chitosan concentration in 30 days (number of individual in 100 ml.)

4. DISCUSSION

In this study we explored the effect of temperature, salinities, and chitosan concentration on survival and production rate of harpacticoid copepod *Tigriopus* sp. Our results show that salinity levels (5, 10, 20, 30, 40, and 45 ppt) have a significant difference ($p<0.05$) on survival and production rate but the temperature (25∘C and 30∘C) do not have significant difference on survival and production rate.

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

Recent work by Chinery and Williams (2004) reported that salinity level have impact on the survival rate of calanoid copepos *Acartia* spp. Santos et al., (2006) confirmed the salinity level effect on harpacticoid copepos *Tisbe biminiensis* nauplii hatching and development, showing that at 34 ppt salinity there is maximum hatching with 300 nauplius/ day (10 ovigerous). Moreover, Zaleha and Farahiyah (2010) reported that for marine hapacticoid *Pararoberisonia* sp reared in 25°C salinity levels (5, 10, 25, and 35 psu) have a significantly different effect on population density. At salinity level 25 psu population density is highest and at salinity level 35 psu maximum specific growth rate shows a high value. The copepods did not survive at temperature 5°C. Our study establishes the limit of tolerance of harpacticoid copepod *Tigriopus* sp. salinity levels of 5 ppt (minimum limit) to 45 ppt salinity (maximum limits). Rhodes (2003) report that the harpacticoid copepods *Nitrokra lacustris* can tolerate salinity levels of 10-40 ppt and are still found to be reproductive. Punnarak et al., (2016) also found that the highest survival rate of copepods at salinity level 27 and 30 psu, the harpacticoid copepods could survive in between 10 psu and 40 psu, but not in freshwater.

The temperature effect in this study showed no significant difference ($P<0.05$) on the survival and production rate which may be because the temperature range used in this experiment was in a narrow range of 25°C and 30∘C. In contrast, Punnarak et al., (2016) report that the hapacticoid families Harpacticidae and Laophontidae reared in temperature of 30°C on after 7 days have a maximum population. Josef et al., (2016) cultured *Nitocra spinipes* at different temperature (15, 20, and 25°C); their study showed that the mortality was lowest in the temperature at 20°C, indicating a narrow temperature optimum for this species, however, Zaleha and Farahiyah (2010) reported that different species of copepods might have different thermal limit on their reproductive yield.

Although the chitosan treatment groups were not significantly different, it is notable that treatment group with 0.01% chitosan showed the highest production rate after 25 days while the treatment group with 0.1% chitosan showed the highest increase of population density after 20 days.

There is currently no report of the use of chitosan for copepod rearing, but for other organisms it is commonly used e.g. Piyabutr et al., (2009) studied the chitosan-coating of the shrimp feed, and the results showed significantly increasing average daily gain (ADG) and feed conversion rate (FCR) of shrimp. Rodolfo et al., (2017) studied of effect of dietary chitin on digestive enzyme activity, growth, and survival of *Macrobrachium tenellum* juvenile prawns, the results showed that highest final weight and specific growth rate resulted from diets with 20% chitin but chitin does not have significant difference effect on survival of juvenile prawns. Zaki et al., (2015) used different concentrations of chitosan (0.5, 1.0,2.0, 3.0, and 4.0) incorporated into feed

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

formulation on the growth in sea bass for 75 days, the result showed average final weight (FW) and specific growth rate (SGR) were significant at chitosan concentration 3 and the lowest in control fish group. Temperature, salinity, and concentration of chitosan (food) are all abiotic limiting factor controlling the development of copepod populations (Ibon and Fernando, 2005) namely, the copepods require a suitable living environment and limit of tolerance.

Overall

In the present study the salinity had the most effect on survival and production of *Tigriopus* sp. The lower and higher limits of salinity in which harpacticoid copepod *Tigriopus* sp. can survive were 5 and 45 ppt and the optimal salinity was 30 ppt. Optimal chitosan concentration was 0.01% and chitosan concentration 1% was the maximum tolerated. The environment factors for each species are different; and in environmental conditions with concentrations or temperatures above the minimum and maximum limits the organisms cannot survive. Moreover, we found that the most increase of population density to occur after 25 day.

5. CONCLUSION

In this study we establish that temperature of 25°C and salinity levels of 30 ppt are the optimum conditions for the culturing of harpacticoid copepod *Tigriopus* sp. The organisms do survive in brackish water and hyper-saline water (5 ppt and 45 ppt) but the number of surviving copepods was low. Development from the nauplius to adult stages took 11 days at 25°C and salinity of 30 ppt. The chitosan had not have not significant differences effect on production of hapacticoid copepods *Tigriopus* sp. However, environmental factors such as temperature, salinity levels, and chitosan concentration (food) have significant impact on the survival, production rates, and development of *Tigriopus* sp.

6. ACKNOWLEDGMENT

Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand.

REFERENCES

Alishahi, A., & Aïder, M. 2012. Applications of chitosan in the seafood industry and aquaculture: A review. Food and Bioprocess Technology, 5, 817-830.

Carlotti, F., & Paul, N. (1992). Model of copepod growth and development: moulting and mortality in relation to physiological processes during an individual molt cycle. Marine Ecology Progress Series, 84, 219-233.

Chinnery, F. E. & Williams, J. A. (2004). The influence of temperature and salinity on *Acartia (*Copepoda: Calanoida) nauplii survival. Marine biology, 145, 733-738.

Chung, Y. C. 2006. Improvement of aquaculture wastewater using chitosan of different degrees of deacetylation. Environmental Technology, 27(11), 1199-208.

Fleeger, J. W. (2005). The potential to mass-culture harpacticoid copepod for use as food for larval fish. In Lee, C. S., O'Beyen, P.,Marcus, N. H. (2005). Copepod in aquaculture. Des Moines, IA: Blackwell Publishing.

Gopakumar, G., & Santhosi, I. (.(2009Use of copepods as live feed for larviculture of Damsel fishes. Asian Fisheries Science 22, .6 -1

Ibon, U, & Fernando, V. (2005). Differences in the abundance and distribution of copepods in two estuaries of the Basque coast (Bay of Biscay) inrelation to pollution. Journal of plankton research, 27(9), 863–874.

Jeyaraj, N., Santhanam, P., Raju, P., Ananth, S., & Jothiraj, K. (2014). Alternative methods for marine harpacticoid copepod, *Macrosetella gracilis* production in marine fish larviculture. International Journal of Zoology Research, 10(1), 1-8.

Josef, K., Thuy, T. B., Elin, L., & Markus, B. (2017). Temperature and food quantity effects on the harpacticoid copepod *Nitocra spinipes*: Combining in vivo bioassays with population modeling. PLOS ONE, 1-18.

Miles, D., Auchterlonie, N., Cutts, C., & Mazorra de Quero, C. (2001). Rearing of the harpacticoid copepod *Tisbe holothuriae* and its application of hatchery production of atlantic halibut *Hippoglossus hippoglossus*. *Fish Development aquaculture, 23*, 1-41.

Olivotto, I., Capriotti, F., Buttino, I., Avella, A. M., Vitiello, V., Maradonna, V., & Carnevali, O. (2008). The use of harpacticoid copepod as live prey for *Amphiprion clarkia* larviculture: Effects on larval survival and growth. *Aquaculture, 274*, 347-352.

Piyabutr, W. , Nopchanok, T.,& Suwalee, C) .2001). Application of chitin-chitosan as giant black tiger shrimp growth promoter .(Proceedings of The 39th Kasetsart University annual conference pp. 30-37). Bangkok, Thailand: Kasetsart University.

Punnarak, P., Jarayabhand, P., & Piumsomboon, A. (.(2017Cultivation of harpacticoid copepods (families harpacticidae and laophontidae) under selected environmental conditions. *Agriculture and Natural Resources, (4)51,* .285-278

ISSN: 2455-6939

Volume: 05, Issue: 06 "November-December 2019"

Rhodes, A. (2003). The big fish bang. (Proceedings of the 26th annual larval fish conference). Bergen, Norway: Institute of Marine Research.

Rodolfo B. D. S. R., Marcelo, G. G., Fernando, V. V. & Héctor, N. S. (2017). Effect of dietary chitin on digestive enzyme activity, growth and survival of *Macrobrachium tenellum* juvenile prawns. *Latin American Journal of Aquatic Research, 45*(1), 130-138.

Ruppert, E. E. & Barnes, R. D. (1991). Invertibrate zoology (6th ed.). New York: Saunders collage publication.

Santos, L. S., Pastor, J., Ferreira, N., Costa, W., Castro, C., & Santos, P. J.(2006). Developing the harpacticoid copepod *Tisbe biminiensis* culture: Testing for salinity tolerance, ration levels, presence of sediment and density dependent analyses. *Aquaculture Reseaech, 37*, 1516-1523.

Suthida, K. (2009). Chitin-Chitosan. *Journal of Industrial Education, 3*, 1-7.

Zaki1,M. A.,. Salem, M. El-S., Gaber, M. M., & Nour1, A. M. (2015). Effect of chitosan supplemented diet on survival, growth, feed utilization, body composition & histology of sea bass (*Dicentrarchus labrax*). *World Journal of Engineering and Technology, 3*, 38-47.

Zaleha, K., & Farahiyah, I. J. (2010). Culture and growth of a marine harpacticoid, *Pararobertsonia* sp.in different salinity and temperature. *Sains Malaysiana, 39*(1), 135-140.

