ORIGINAL PAPER

Effects of salinity levels on survival rates of harpacticoid copepod *Tigriopus* sp. (Copepoda: Harpacticoida)

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Abstract. This research aims to study the survival rates of harpacticoid copepod *Tigriopus* sp. cultured at different temperatures and salinity levels. An ovigerous copepod was obtained by collecting from a brown alga, *Padina* sp., on Namsai sandy Beach in Chon Buri Province, Thailand, and reared at Ramkhamhaeng University. The 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 the survival rates of the copepods ($p<0.05$). The copepods cultured at the temperature of 25°C and salinity level of 30 ppt had a survival rate of 85.04%, and those cultured at the temperature of 25°C and salinity level of 20 ppt, yielded a survival rate of 82.56%. The number of survived copepods was the lowest at the salinity levels of 5 ppt and 45 ppt. It took 11 days for the copepods to develop from nauplius to adult stages, and the number of nauplii produced an average of 49±3.74.

Keywords: *Tigripous* sp., culturing, salinity levels, survival rate

1. Introduction

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 acids and other molecules provided by harpacticoid copepods are important for the growth and survival of fish and crustacean larvae (Miles et al., 2001; Zaleha & Farahiyah, 2010). Therefore, it is of considerable interest to determine optimal conditions for copepod cultivation to obtain sufficient nutritional yields for usage in aquatic animal nurseries (Fleeger, 2005; Olivotto et al., 2008). They include a large number of species that require different environmental factors for development and reproduction (Carlotti & Paul, 1992; Souissi et al., 1997; Santhanam & Perumal, 2012). The growth, reproductive and mortality rates generally are linked directly to external parameters such as food, temperature and salinity. However, the developmental stages are different and are affected differently by the environment. For harpacticoid copepods, the temperature is the major factor controlling the reproductive activity (Zaleha & Busra, 2012). 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 a 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 that make them suitable for cultivation such as tolerance to extreme environmental changes (Aurelyanna & Lília, 2011). Due to these considerations, we selected *Tigriopus* sp. for this study.

2. Materials and Methods

2.1 Sample collection, study area and culturing of the 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 washing out the thalli of the algae with seawater. The copepods were then cultured in a laboratory room at Ramkhamhaeng University, Bangkok, Thailand. The copepods were fed with a few drops of microalgae, *Tetraselmis* sp., every other day during 30 days, and produced several offspring.

2.2 Morphological identification

Morphological identification was made utilizing a stereomicroscope (model SZ30, Olympus Corp., Tokyo; Japan) and light microscope (model CH20 Olympus Corp., Tokyo; Japan) after the copepods were fixed in 6% Neutral buffered formalin and dyed with Bengal's Rose (Sigma Aldrich) for 24 hrs. (following the method used by Ito (1969), Huys et al. (1996), and Chullasorn (2012, 2013).

2.3 Statistical analysis

Twelve ovigerous females were selected and divided into two groups. Each ovigerous female was placed in each glass petri dish. In Group 1, each copepod was cultured at the temperature of 25°C and salinity levels of 5, 10, 20, 30, 40, and 45 ppt, respectively. Meanwhile, each copepod in Group 2 was cultured at 30∘C and salinity at 5, 10, 20, 30, 40, and 45 ppt, respectively. The copepods were fed with a few drops of microalgae, *Tetraselmis* sp., every day for 15 days. Salinity in glass petri dish was measured every three days using a refractometer, the temperature was measured everyday using a mercury thermometer. Everyday, each individual of copepods was monitored and counted under a stereo microscope (at all stages; nauplius, copepodid and adult). Data collected is the means±SE of three replicates of the number of individuals every day. Significant differences in the survival rate in different salinities levels were analyzed by using One Way-ANOVA with a significance level of 95% (*p-value* < 0.05) which Minitab Ver.17 was used.

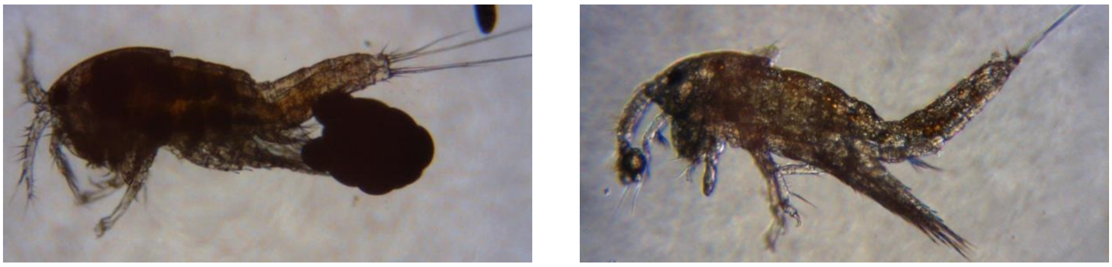
3. Results

3.1 Morphological identification

The morphological identification of harpacticoid copepod following the method used by Ito (1969), Huys et al. (1996), and Chullasorn (2012, 2013) with its closest relative to *Tigriopus japonicus*.

3.2 *Effect of salinity levels on the survival rate of harpacticoid copepod Tigriopus sp.*

The study of the effect of temperature and salinity levels on the survival rate of harpacticoid copepod *Tigriopus* sp. that hatching from eggs. The experiments were carried out at salinity levels of 5, 10, 20, 30, 40 and 45 ppt and temperature at 25°C and 30°C for 15 days. The temperature 25°C at salinity level 5 ppt, copepod has survival rate 42.20% (6.33±0.94 individual from 15±2.45 nauplius), salinity 10 ppt has survival rate 27.52% (6.33±2.05 individual from 23±2.16 nauplius), salinity 20 ppt had survival rate 82.56% (36.33±2.87 individual from 44±2.16 nauplius, Table 9), salinity 30 ppt had survival rate 85.04 % (41.67±4.50 individual from 49±3.74 nauplius), salinity 40 ppt had survival rate 46.84% (17.33±6.13 individual from 37±1.41 nauplius) and salinity 45 ppt had survival rate 15.99 % (5.33±1.89 individual from 33.33±3.40 nauplius). The temperature 30∘C at salinity level 5 ppt, copepod had survival rate 13.67% (3.67±1.70 individual from 13.67±3.86 nauplius), salinity 10 ppt had survival rate 41.78% (9.33±1.25 individual from 22.33±2.49 nauplius), salinity 20 ppt had survival rate 53.02% (23.33±4.03 individual from 44.00±4.55 nauplius), salinity 30 ppt had survival rate 65.85 % (27.00±3.27 individual from 41.00+2.16 nauplius), salinity 40 ppt had survival rate 45.78 % (16.33±2.87 individual from 35.67+±5.31 nauplius) and salinity 45 ppt had survival rate 34.60 % (3.00±1.41 individual from 8.67±1.25 nauplius). The harpacticoid copepods of cultured at 25°C with salinity level 30 ppt was showed the highest (mean ±SE) survival rate. Meanwhile, the temperature 30°C salinity levels 5 ppt had a significant lowest survival rate of 13.67%. The development from the nauplius to copepodids stages took 6.7 days and copepodid to adult stages took 4.7 days and the number of nauplii produced an average of 49±3.74 from one egg sac at 25°C, 30 ppt. These copepods could survive at the salinity levels between 5 ppt and 45 ppt, but the number of survived copepods was lowest (Table 1, Figure 2). One-Way ANOVA analysis ($p<0.05$) shows that the different temperatures and salinity levels were significant difference of population density.



(A) (B)

Figure 1. *Tigriopus* sp. (A) female, (B) male (lateral view)

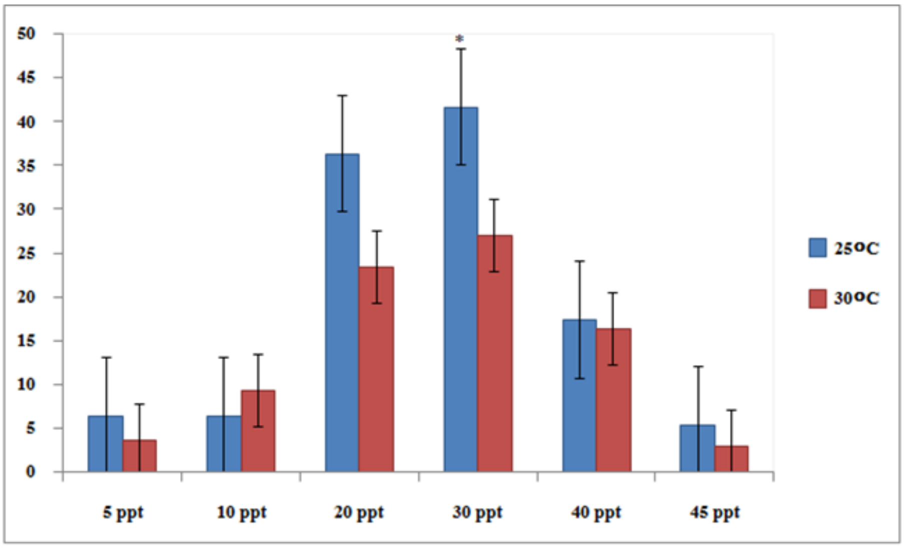


Figure 2. Individual number of harpacticoid copepod *Tigriopus* sp. from nauplius after the 15 days of the cultivation stage (one ovigerous female) at different temperature and salinity levels.

Table 1. The survival rate of *Tigriopus* sp. from nauplius after the 15th day of the cultivation stage (one ovigerous 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%**

4. Discussion

An ovigerous female harpacticoid copepod was collected in Namsai sandy beach, Chon buri Province, Thailand, and reared at Ramkhamhaeng University. The morphology identification of harpacticoid copepod following the method used by Ito (1969), Huys et al. (1996), and Chullasorn (2012, 2013) with its closest relative to *Tigriopus japonicus*.

The effects of temperature and salinity levels on the survival rate of harpacticoid copepod, our results show that salinity levels (5, 10, 20, 30, 40, and 45 ppt) have a significant difference (p<0.05) on survival rate. Chinnery and Williams (2004) reported that salinity level have impact on the survival rate of calanoid copepod *Acartia* spp. Santos et al. (2006) confirmed the salinity level effect on harpacticoid copepods *Tisbe biminiensis* nauplius 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 harpacticoid *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 levels 27 and 30 psu, the harpacticoid copepods could survive in between 10 psu and 40 psu, but not in freshwater. The number of nauplius in this study produced an average of 49±3.74 at 25°C in salinity30 ppt from one egg sac. According to Hagiwara et al. (1995) reported the *T. japonicus* produces nauplius an average 52.2 at salinity level 32 ppt per egg sac.

Overall, in the present study the salinity had the most effect on survival rate of harpacticoid copepod *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.

5. Conclusions

This research aims to studied survival rates of harpacticoid copepods cultured at different salinity levels the conclusions followed by items below:

1. The morphology is closest to *Tigriopus japonicus* Mori.

2. The temperature of 25°C and salinity levels of 30 ppt are the optimum conditions for the culturing of harpacticoid copepod *Tigriopus* sp.

3. The harpacticoid copepod *Tigriopus* sp. can survive in brackish water and hyper-saline water (5 ppt and 45 ppt), but the number of surviving copepods was low.

4. Development from the nauplius to adult stages took 11 days at 25°C and salinity of 30 ppt, and the number of nauplius produced an average of 49±3.74 from one egg sac.

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References

Aurelyanna CBR, Lília PSS (2011) Mass culture and offspring production of marine harpacticoid copepod Tisbe biminiensis. Aquaculture 321(2011):280-288

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 FE, Williams JA (2004) The influence of temperature and salinity on Acartia (Copepoda: Calanoida) nauplii survival. Marine Biology 145(4):733-738

Chullasorn S, Ivanenko VN, Dahms HU, Kangtia P, Yang WX (2012) A new species of *Tigriopus* (Copepoda, Harpacticoida, Harpacticidae) from Thailand with the description of its naupliar development. Helgoland Marine Research 66:139-151

Chullasorn S, Dahms HU, Klangsin P (2013) A new species of *Tigriopus* (Copepoda: Harpacticoida: Harpacticidae) from Thailand with a key to the species of the genus. Journal of Natural History 47(5-12):427-447

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

Gopakumar G, Santhosi I (2009) Use of Copepods as Live Feed for Larviculture of Damselfishes. Asian Fisheries Science 22:1- 6

Hagiwara A, Kim HJ, Matsumoto H, Ohta Y, Morita T, Hatanaka A, Ishizuka R, Sakakura Y (2017) Production and use of two marine zooplanktons, *Tigriopus japonicus* and *Diaphanosoma celebensis*, as live food for red sea bream *Pagrus major* larvae. Fisheries Science 82:799–809

Huys R, Gee JM, Moore CG, Hamond R (1996) Marine and brackish water harpacticoid copepods part 1. In: Kermark DM, Barnes RSK, Crothers JH (eds.) Synopses of the British Fauna (New Series). Dorset Press, Dorchester, Great Britain, pp 1-353

Ito T (1969) Descriptions and records of marine harpacticoid copepods from Hokkaido, II. Journal of the Faculty of Science, Hokkaido University 17(1):58-77

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

Miles D, Auchterlonie N, Cutts C, de Quero CM (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 AM, 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

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

Rhodes A (2003) Methods for high density batch culture of *Nitokra lacustris*, a marine harpacticoid copepod. Proceedings of the 26th Annual Larval Fish Conference: The big fish bang. Institute of Marine Research, Bergen, Norway, pp 449-465

Ruppert EE, Barnes RD (1991) Invertebrate zoology (6th ed) Saunders collage publication, New York

Santhanam P, Perumal P (2012) Feeding, survival, egg production and hatching rate of the marine copepod *Oithona rigida* Giesbrecht (Copepoda: Cyclopoida) under experimental conditions. Journal of the Marine Biological Association of India 54(1):38-44

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

Souissi S, Carlotti F, Nival P (1997) Food- and temperature-dependent function of moulting rate in copepods: An example of parameterization for population dynamics models. Journal of Plankton Research 19(9):1331-1346

Zaleha K, Busra I (2012) Culture of harpacticoid copepods: understanding the reproduction and effect of environmental factors: Aquaculture 343-360

Zaleha K, Farahiyah IJ (2010) Culture and Growth of a Marine Harpacticoid, *Pararobertsonia* sp. in Different Salinity and Temperature. Sains Malaysiana 39(1):135-140

Zaleha K, Ibrahim B, John BA, Kamaruzzaman BY (2012) Generation time of some marine harpacticoid species in laboratory condition. Journal of Biological Science 12(8):433-437