MINISTRY OF EDUCATION AND TRAINING

NHA TRANG UNIVERSITY

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DOAN XUAN NAM

STUDY ON SOME BIOLOGICAL CHARACTERISTICS OF GROWTH AND REPRODUCTION OF COPEPOD *Pseudodiaptomus annandalei* (Sewell, 1919) AND MASS CULTURE UNDER CLIMATE CHANGE

Major: Aquaculture

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SUMMARY OF DOCTORAL, THESIS

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LIST OF PUBLICATION

1. **Doan NX**, Vu MTT, Nguyen HT, Tran HTN, Pham HQ, Dinh KV (2018). *Temperature and sex specific grazing rate of a tropical copepod Pseudodiaptomus annandalei to food availability: implications for live feed in aquaculture*. Aquaculture Research, 49, 3864-3873. DOI: 10.1111/are.13854.
2. **Doan NX**, Vu MTT, Pham HQ, Wisz MS, Nielsen TG, Dinh KV (2019). *Extreme temperature impairs growth and productivity in a common tropical marine copepod.* Scientific Reports, 9, 4550.
3. **Doan Xuan Nam**, Bui Van Canh, Pham Quoc Hung, Dinh Van Khuong (2019). *Effect of temperature on growth and reproduction of the copepod Pseudodiaptomus annandalei*. Journal of Fisheries Science and Technology, Volume 3/2019, page 91 - 98.
4. **Doan Xuan Nam**, Pham Quoc Hung, Dinh Van Khuong (2019). *Tolerance to salinity shock and salinity-temperature interaction on survival, biological characteristics and reproduction of the copepod Pseudodiaptomus annandalei*. Journal of Fisheries Science and Technology, Volume 4/2019, page 75 - 87.

INTRODUCTION

Pseudodiaptomus annandalei is an important zooplankton that transfers energy in the food chain of brackish water and mangrove ecosystems (Chew et al, 2012, Dhanker et al, 2012) and is also a good live feed for rearing marine fish larvae (Rayner et al, 2015). Under climate change conditions, water surface temperature increases to the upper thermal limits of copepods (Tewksbury et al, 2008a), so maybe negatively affect to *Pseudodiaptomus annandalei* in nature. The *Pseudodiaptomus annandalei* resource in the mixture of different copepod species, uses as live feed for marine fish larvae, is totally from nature in outdoor aquaculture ponds (Blanda et al,, 2015). This resource has some disadvantages such as seasonal variation and disease infection (Van der Meeren Naas, 1997). Therefore, there is still not safety and pure *P. annandalei* supply for aquaculture. Moreover, there is no study on the effects of warming on *P. annandalei*, as well as, the mass culture of *P. annandalei*. Base on above issues, the topic of "Study on some biological characteristics of growth and reproduction of copepod *Pseudodiaptomus annandalei* (**Swelle, 1919**) and mass culture under climate change".

The main goals of thesis:

- To evaluate of the effects of climate change (temperature at 34^{o}C) on growth and reproduction of *Pseudodiaptomus annandalei.*
- To determine technical measures as a basic information for mass culture of copepod *Pseudodiaptomus annandalei* under 30^{o}C and 34^{o}C.

Main contents of the thesis:

1. Study on the effect of algal concentration (*C. muelleri, I. galbana, T. chuii*) and temperature (25^{o}C, 30^{o}C and 35^{o}C) on the filtration rate of adult *Pseudodiaptomus annandalei*
2. Study on the effect of feeding three different algal species (Chaetoceros muelleri, Isochrysis galbana, Tetraselmis chuii) on the growth and reproduction of P. annandalei at 30^{o}C and 34^{o}C
3. Study on the effect of extreme temperature on growth and reproduction of *Pseudodiaptomus annandalei* over three generations.
4. Study on the effect of temperature on growth and reproduction of *Pseudodiaptomus annandalei*.

5. Research on the tolerance of *P. annandalei* to salinity shock and the interaction of salinity with temperature on the growth and reproduction of this species.
6. Study on the effect of nauplii density and adult density on growth and reproduction of *P. annandalei* in 30^{o}C and 34^{o}C conditions.
7. Study on the mass culture and naupli reproduticon of *Pseudodiaptomus annandalei* in 30^{o}C and 34^{o}C conditions

The scientific and practical significances of the study:

- Scientific significances: The results of the current study provide the knowledge about the effects of the warming of water caused by climate change on some biological characteristics of growth and reproduction of copepod *P. annandalei*. This study contributed the knowledge of effects of climate change on aquatic animal and copepods.

- Practical significances: This is the first study on mass culture and nauplii production of *P. annandalei* in Vietnam. The results of this study help researchers and farmers, who have the basic knowledge to continuously use copepods as live feed organisms to replace for traditional live feeds, such as rotifer and *Artemia*, in rearing aquaculture species to have higher survival rate and better quality fingerlings.

Chapter 1. LITERATURE REVIEW

1.1. Some of biological characteristics of *Pseudodiaptomus annandalei*

1.2.1. Systematic classification

According to Sewell (1919), *Pseudodiaptomus annandalei* belongs to the genus Pseudodiaptomidae, family Pseudodiaptomidae, order Calanoida, class Hexanauplia, phylum Arthropoda.

1.2.2. Distribution

In Vietnam, they distribute in the coastal area (Tran Duc Luong and Ho Thanh Hai, 2013) and dominate in aquaculture ponds (Rayner et al, 2015).

1.2.3. Morphological characteristics

Golez et al (2004) described the morphology of different developmental stages of naupliar and copepodite, as well as, adult males and females (Golez et al, 2004).

1.2.4. Life cycle

Golez et al (2004) showed the life cycle of *P. annandalei* had three main stages: naupliar stage, copepodite stage and adult stage. The lifespan can reach day 40^{th} when cultured in water with salinity 5 – 20 ‰, temperature 25 - 28^{o}C (Chen et al, 2006b).

1.2.5. Reprodutive characteristics

P. annandalei could become the adult stage on day 7^{th} and stopped spawning around day 40^{th}. Mature adults get mating. Female needs males for frequently mate to have high hatching rate of egg batch – 90% (Beyrend-Dur et al, 2011).

1.2.6. Nutritional characteristics

Pseudodiaptomus annandalei feed on plankton and they also eat small zooplankton when lacking food.

1.2. Effects of climate change on copepods

Shallow coastal waters in the tropics are particularly prone to experiencing high-temperature extremes in a warming climate (Stuart-Smith et al, 2017). Such extreme temperatures are expected to impact thermally specialized species occurring in these zones (Tewksbury et al, 2008b, Lough, 2012) and many already approach their upper thermal limits (Wernberg et al, 2012). Therefore, any effects on fitness and productivity of *Pseudodiaptomus annandalei* are expected to have bottom-up controls that reduce food resources for small fish and top-down controls that reduce the grazing rate on phytoplankton, with ecological consequences. (Chew et al, 2012).

1.3. Research on *Pseudodiaptomus annandalei* on the world and Vietnam

1.3.1. Research on effects of temperature and salinity

Copepod *P. annandalei* is tropical and adapts a large range of salinity but they are still affected by variable temperature and salinity (Beyrend-Dur et al, 2011, Chen et al, 2006b). These two factors affect survival rate, lifespan, size of the adult, time to mature, spawning duration, fecundity, fertilization, embryos development, and hatching success (Beyrend-Dur et al, 2011, Chen et al, 2006b). Vu Ngoc Ut (2014) and Truong Si Hai Trinh (2016) also found some biological characteristics of *P. annandalei* at 26 – 28,5^{o}C.

1.3.2. Research on nutritional characteristics

To assess the capability of marine copepod to exploit the food availability the functional response tests are the typical method that has been used for decades (Holling, 1966, Colin Dam, 2007, Sarma et al, 2013, Saiz et al, 2014, Kiørboe et al, 2018). Some studies used algal concentration corresponding to 500 µg C/L (Rayner et al, 2017b) or 500 µg chlorophyll a/L (Lehette et al, 2016) to culture *P. annandalei*. Vu Ngoc Ut (2014) found that *P. annandalei* had the highest feeding rate on *Isochrysis galbana* compared to that fed on *Chaetoceros calcitrans* and *Dunaliella tertiolacta* (Vũ Ngọc Út, 2014).

1.3.3. Research on density

The initial population density of *P. annandalei* adult from 80 to 270 individual/L, had 30 – 40 % of the ovigerous rate. This rate reduced when adult density increase over 270 individual/L and reached lowest at the density of 1,500 individual/L (Rayner et al, 2017b). The presentation of nauplii (density from 200 to 1,600 ind/L) did not affect to ovigerous rate in the population (Rayner et al, 2017b).

1.3.4. Research on mass culture

P. annandalei used to culture successfully in the atifitial condition. The first cultivation was in 1988 by Shiao, *P. annandalei* was cultured by three microalgal species, *Crytomonas sp.*, *Tetraselmis sp.*, and *Isochrysis sp.*. The next study was carried out by Chen (2002). Both studies were conducted in small volume containers about 500 ml (Edward J. Buskey, 2005). Similarly in Vietnam, this species was also cultured in laboratory condition to study some biological characteristics (Truong Si Hai Trinh, 2016, Vu Ngoc Ut, 2014).

Chapter 2. MATERIALS AND METHODS

2.1. Research species

Copepod *Pseudodiaptomus annandalei* (**Sewell**, 1919) was collected in aquaculture pond in Cam Ranh, Khanh Hoa.

2.2. Period and location

The study was conducted from 2017 to 2019 in Cam Ranh station, Institute of Aquaculture, Nha Trang University, Cam Thinh Dong, Cam Ranh, Khanh Hoa province.

2.3. Methodology

2.3.1. Study on the effect of algal concentration (C. muelleri, I. galbana, T. chuii) and temperature (25°C, 30°C and 35°C) on the filtration rate of adult Pseudodiaptomus annandalei – Exp- 1

- We separately conducted three separate functional response experiments for three algal species: *Chaetoceros muelleri, Isochrysis galbana* and *Tetraselmis* chuii: 9 algal concentrations (12.5, 25, 50, 100, 200, 400, 800, 1,600 and 3,200 µg C/L) × 3 temperatures (25, 30 and 35°C) × 2 sex (male, female) × 5 replicates = 270 experimental units.
- Response variables: Pellet production - PP (total number of pellets individual^{-1} day^{-1}) and the specific pellet production - SPP (total volume of pellets corrected for size difference of sexes individual^{-1} day^{-1})

2.3.2. Study on the effect of feeding three different algal species (Chaetoceros muelleri, Isochrysis galbana, Tetraselmis chuii) on the growth and reproduction of P. annandalei at 30°C and 34°C – Exp- 2

- Experimental design: 3 microalgal species (*Chaetoceros muelleri*, *Isochrysis galbana*, *Tetraselmis chuiii*) × 2 temperature (30°C and 34°C) = 6 treatments
- Response variables: the development of population, survival rate until adult stage, fecundity, hatching rate, nauplii/female, number of nauplii per female in 10 days.

2.3.3. Study on the effect of extreme temperature on growth and reproduction of Pseudodiaptomus annandalei over three generations – Exp- 3

- We conducted a multiple generational exposure experiment in which copepods were reared at 30°C and 34°C.

- All Response variables were determined for all three generations F1, F2, F3: the development of population, survival rate until adult stage, clutch size, specific clutch size, hatching rate, nauplii/female.

2.3.4. Study on the effect of temperature on growth and reproduction of Pseudodiaptomus annandalei – Exp- 4

- Experimental design: 3 temperature (25^{o}C, 30^{o}C and 34^{o}C) = 3 treatments
- Response variables: the development of population, size of adults, fecundity, hatching rate, nauplii/female, number of nauplii per female in 10 day and lifespan of male and female.

2.3.5. Research on the tolerance of P. annandalei to salinity shock and the interaction of salinity with temperature on the growth and reproduction of this species – Exp- 5

- Experiment 5.1: 9 salinity (0, 5, 10, 15, 20, 25, 30, 35, 40 ‰) × 3 replicates = 27 experimental units. The Experiment was designed similarly for four groups of copepods.
- Experiment 5.2: Experiment design: 2 temperatures (30^{o}C and 34^{o}C) × 7 salinities (5, 10, 15, 20, 25, 30, 35 ‰) × 5 replicates = 70 experimental units
- Response variables: The survival rate of copepods at 24h and 48h, size of adults, fecundity, hatching rate, nauplii/female, number of nauplii per female in 10 days.

2.3.6. Study on the effect of nauplii density and adult density on growth and reproduction of P. annandaleim in 30^{o}C and 34^{o}C conditions – Exp- 6

- Experiment 6.1: Effects of interaction between temperature and nauplii density on *P. annandalei*. Experiment design: 4 densities of nauplii (500, 1.000, 1.500, 2.000 ind/L) × 2 temperatures (30^{o}C and 34^{o}C) × 5 replicates = 40 experimental units.
- Experiment 6.2: Effects of interaction between temperature and adult density on reproduction of *P. annandalei*. Experiment design: 6 densities of adult (100, 200, 400, 600, 800, 1.000 ind/L) × 2 temperatures (30^{o}C and 34^{o}C) × 5 replicates = 60 experimental units.
- Response variables: the development of population and survival rate at day 10th, size of adults, fecundity, hatching rate, nauplii/female, number of nauplii per female in 10 days and survival rate of adults.

2.3.7. Study on the mass culture and naupli reproduticon of Pseudodiaptomus annandalei in 30°C and 34°C conditions – Exp- 7

- Experiment 7.1: Mass culture *P. annandalei* at 30°C và 34°C. Experiment design: 2 temperatures (30°C and 34°C) × 3 replicates = 6 experimental units.
- Experiment 7.2: Biomass collection at different ration of cultured volumes from 10% to 50%: 2 temperatures (30°C and 34°C) × 5 volumes (10%, 20%, 30%, 40%, 50%) × 3 replicates = 30 experimental units.
- Experiment 7.3: Nauplii collection at different ratios of cultured volume from 25% to 100%: 2 temperatures (30°C and 34°C) × 4 volumes (25%, 50%, 75%, 100%) × 3 replicates = 24 experimental units.
- Response variables: the density of different stages (ind/liter), total number of copepods/liter, total number of collecting copepods at different volume ratios, total number of collecting nauplii at different volume ratios.

2.4. Data analysis

Statistical analysis was performed using Statistica v.13.1 (StatSoft Inc., Tulsa, OK, USA) in experiment 1 and SAS 9.4 (SAS Institute Inc., Cary, NC, United States) in experiment 3. The others used Microsoft excel 2010 and SPSS (Chicago, IL, USA) 20.0 or 22.0 for Windows with a Ducan test, $P < 0.05$ level of significance.

Chapter 3. RESULTS AND DISCUSSION

3.1. *The effect of algal concentration (C. muelleri, I. galbana, T. chuii) and temperature (25°C, 30°C and 35°C) on the filtration rate of adult P. annandalei*

3.1.1. Chaetoceros muelleri

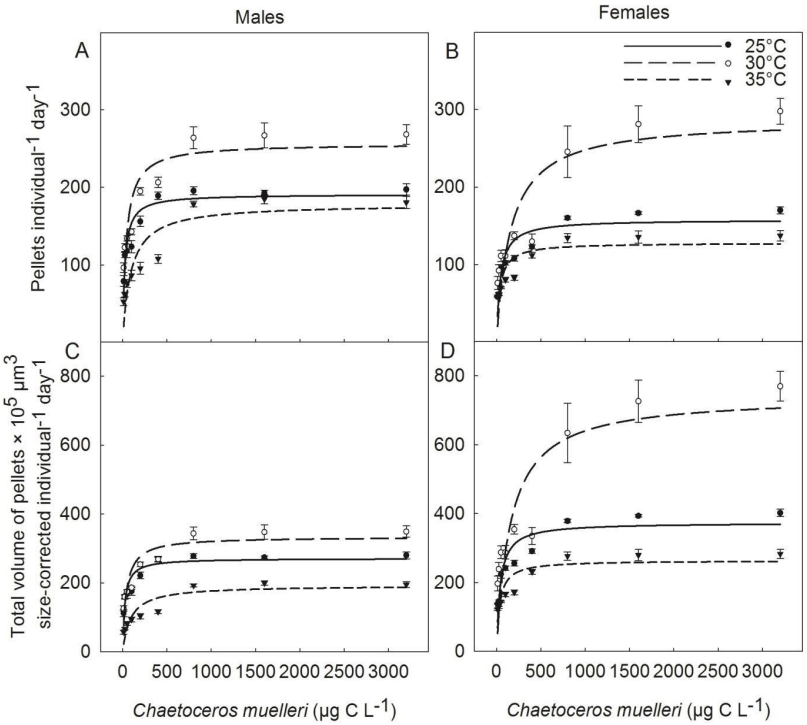


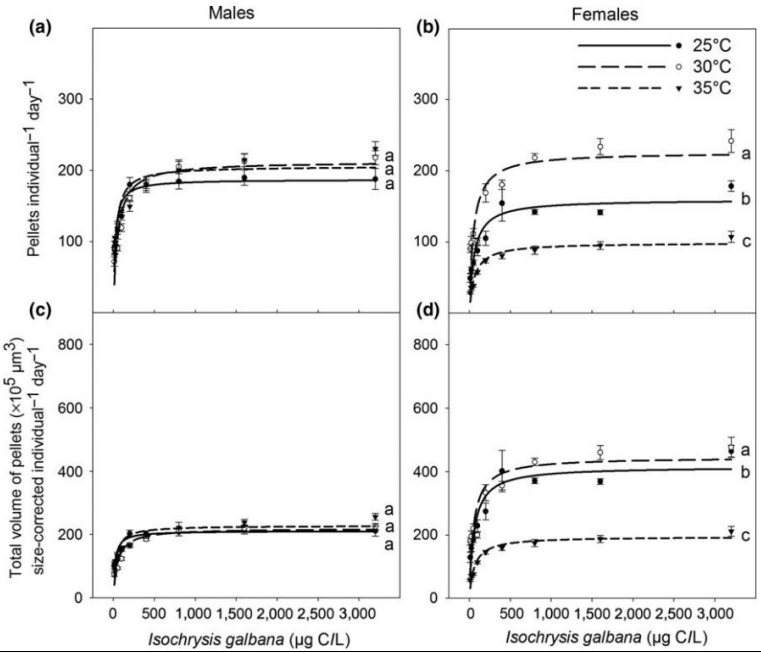
Figure 3. 1. Effects of *Chaetoceros muelleri* concentrations, temperature and sexes on pellet production (PP, SPP) of *P. annandalei*.

Table 3. 1. The effects of *Chaetoceros muelleri* concentrations and temperature on the faecal pellet production of the *Pseudodiaptomus annandalei.*

Chaetoceros muelleri	**Effects on PP**			**Effects on SPP**		
	df1, df2	**F**	**P**	**df1, df2**	**F**	**P**
Food	8,216	227.59	**<0.001**	8,216	161.18	**<0,001**
Temperature	2, 216	310.76	**<0.001**	2,216	387.30	**<0,001**
Sex	1, 216	115.98	**<0.001**	1,216	430.55	**<0,001**
Food × Sex	8, 216	2.88	**0.0046**	8,216	17.27	**<0,001**
Food × Temperature	16,216	11.62	**<0.001**	16,216	14.68	**<0,001**
Sex × Temperature	2,216	5.45	**0.0050**	2,216	54.36	**<0,001**
Food × Temperature × Sex	16,216	4.65	**<0.001**	16,216	7.50	**<0,001**

3.1.2. Isochrysis galbana

Table 3. 2. The effects of *I. galbana* concentrations and temperature on the faecal pellet production of the *Pseudodiaptomus annandalei*.



Isochrysis galbana	Effects on PP			Effects on SPP		
	df1, df2	**F**	**P**	**df1, df2**	**F**	**P**
Food	8,216	180.59	**<0.001**	8,216	144.94	**<0.001**
Temperature	2,216	112.46	**<0.001**	2,216	158.96	**<0.001**
Sex	1,216	305.76	**<0.001**	1,216	358.07	**<0.001**
Food × Sex	8,216	1.42	0.19	8,216	13.23	**<0.001**
Food × Temperature	16,216	4.69	**<0.001**	16,216	4.15	**<0.001**
Sex × Temperature	2,216	161.50	**<0.001**	2,216	224.27	**<0.001**
Food × Temperature × Sex	16,216	2.86	**<0.001**	16,216	4.62	**<0.001**

Figure 3. 2. Effects of *I. galbana* concentrations, temperature and sexes on pellet production (PP, SPP) of *P. annandalei*.

3.1.3. Tetraselmis chuii

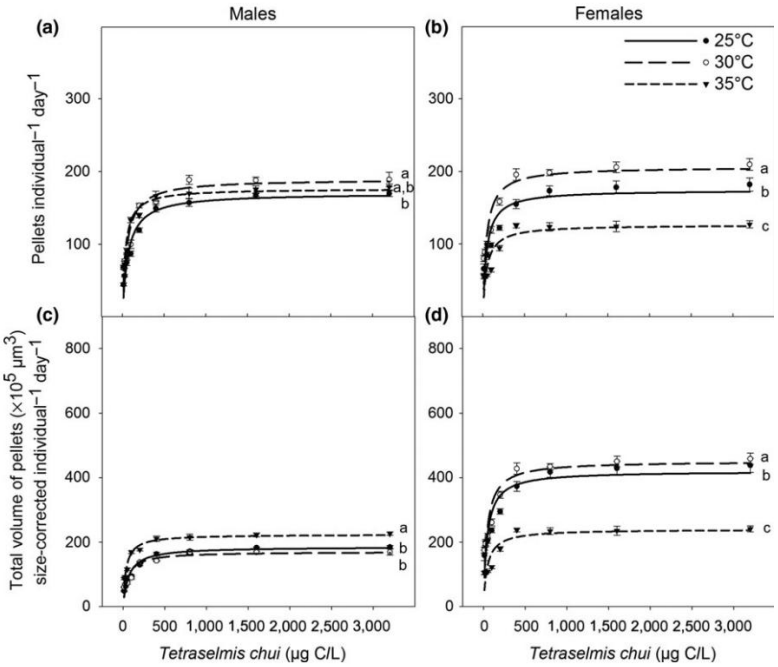


Figure 3. 3. Effects of *T. chuii* concentrations, temperature and sexes on pellet production (PP, SPP) of *P. annandalei*.

Table 3. 3. The effects of *Tetraselmis chuii* concentrations and temperature on the faecal pellet production of the *Pseudodiaptomus annandalei.*

Tetraselmis chuii	**Effects on PP**			**Effects on SPP**		
	df1, df2	**F**	**P**	**df1, df2**	**F**	**P**
Food	8,216	513.80	**<0.001**	8,216	386.03	**<0.001**
Temperature	2,216	171.35	**<0.001**	2,216	169.84	**<0.001**
Sex	1,216	6.72	**0.010**	1,216	276.9	**<0.001**
Food × Sex	8,216	3.34	**0.0013**	8,216	37.40	**<0.001**
Food × Temperature	16,216	5.68	**<0.001**	16,216	5.03	**<0.001**
Sex × Temperature	2,216	188.07	**<0.001**	2,216	639.20	**<0.001**
Food × Temperature × Sex	16,216	2.79	**<0.001**	16,216	7.78	**<0.001**

3.2. *Effects of feeding three different algal species on the growth and reproduction of P. annandalei at 30°C and 34°C*

3.2.1. The development of population and size of adult P. annandalei

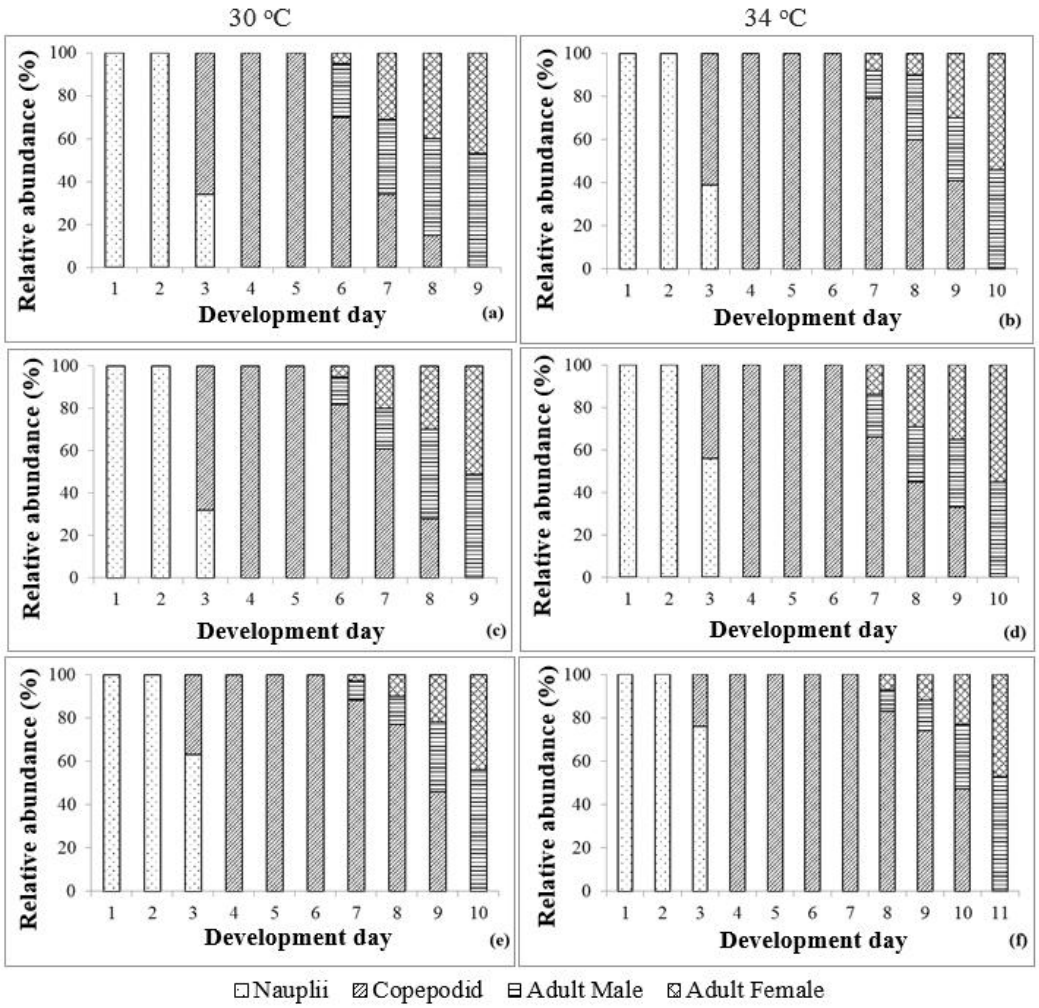


Figure 3. 4. The development of population *P. annandalei* when fed different microalgae species and at different temperatures: *I. galbana* – 30°C (a); *I. galbana* – 34°C (b); *C. muelleri* – 30°C (c); *C. muelleri* – 34°C (d); *T. chuii* và 30°C (e); *T. chuii* – 34°C (f)

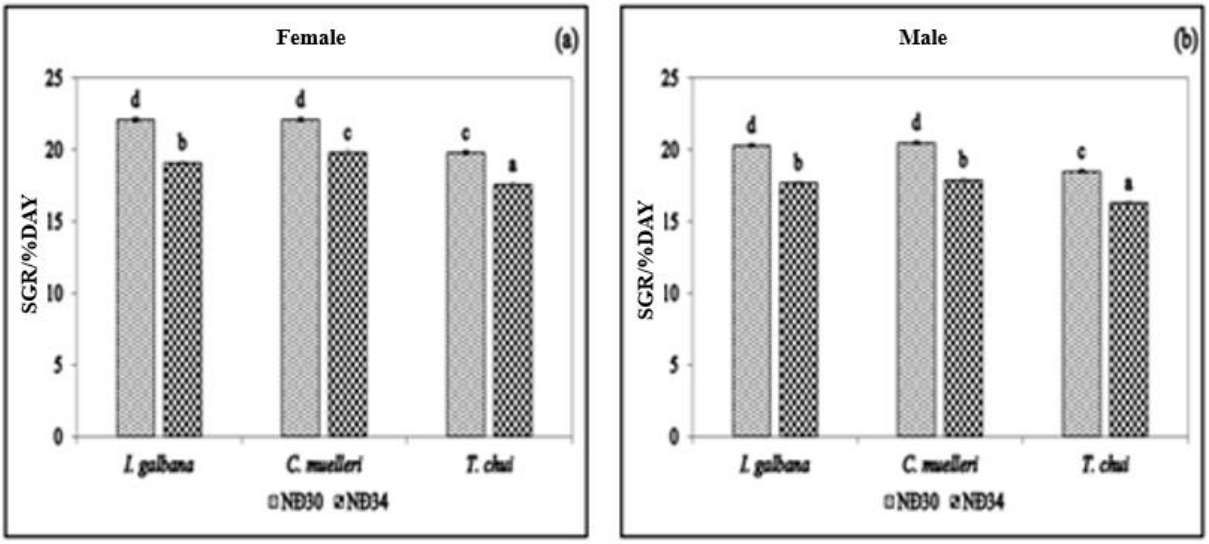


Figure 3. 5. Effects of temperature on typical growth rate of female size (a), male size (b)

3.2.2. Survival rate, clutch size, hatching success, nauplii/female and reproductive ability of female P. annandalei in 10 days

Table 3. 4. The effects of temperatures and microalgal species on survival rate, clutch size, hatching success and nauplii/female and total number of nauplii in 10 day.

Factors	Response variables	*df*	*F*	*P*
Temperature	Survival rate	1	83,516	**<0,001**
	Clutch size	1	91,351	**<0,001**
	Hatching success	1	56,754	**<0,001**
	Nauplii/female	1	152,495	**<0,001**
	Total number of nauplii in 10 day /female	1	143,464	**<0,001**
Microalgal species	Survival rate	2	1,072	0,358
	Clutch size	2	0,721	0,487
	Hatching success	2	0,745	0,486
	Nauplii/female	2	1,258	0,302
	Total number of nauplii in 10 day /female	2	0,968	0,394
Temperature × Microalgal species	Survival rate	2	0,221	0,804
	Clutch size	2	3,342	**0,037**
	Hatching success	2	0,188	0,830
	Nauplii/female	2	0,144	0,866
	Total number of nauplii in 10 day /female	2	0,864	0,441

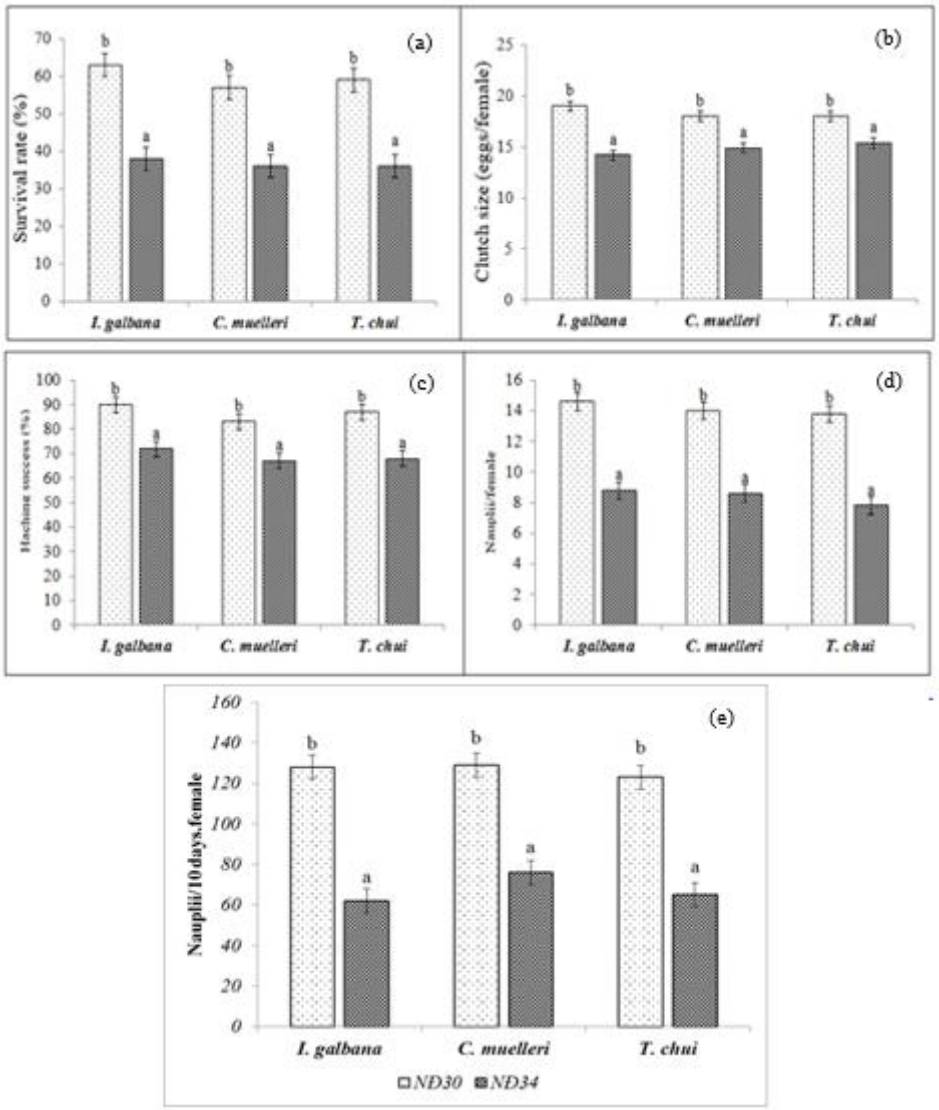


Figure 3. 7. Effects of temperatures and different microalgae species on the survival rate (a), clutch size (b) on the hatching rate (c), nauplii/female (d), on the total number of nauplii/female in 10 days (e). *Data represents the mean ± SE. Different letters indicate the significant difference ($p < 0.05$) between treatments (temperatures-microalgae species).*

3.3. *Effects of extreme temperature on growth and reproduction of Pseudodiaptomus annandalei over three generations*

3.3.1. The development of population Pseudodiaptomus annandalei across 3 generations F1, F2, F3 and adult size at 30°C và 34°C

The development of *P. annandalei* population is shown in Figure 3.8. *P. annandalei* population, was cultured at 34°C, developed slower than that was cultured at 30°C.

Table 3. 5. Effects temperatures and generations on adult sizes of *P. annandalei*. *Significant p values (p<0.05) are indicated in bold.*

Factors	Male			Female		
	df1, df2	F	P	df1, df2	F	P
Temperature	1,540	32.45	**<0.001**	1,500	65.48	**<0.001**
Generation	2,539	35.29	**<0.001**	2,500	137.17	**<0.001**
Temperature × Generation	2,539	1.64	0.19	2,500	5.82	**0.0032**

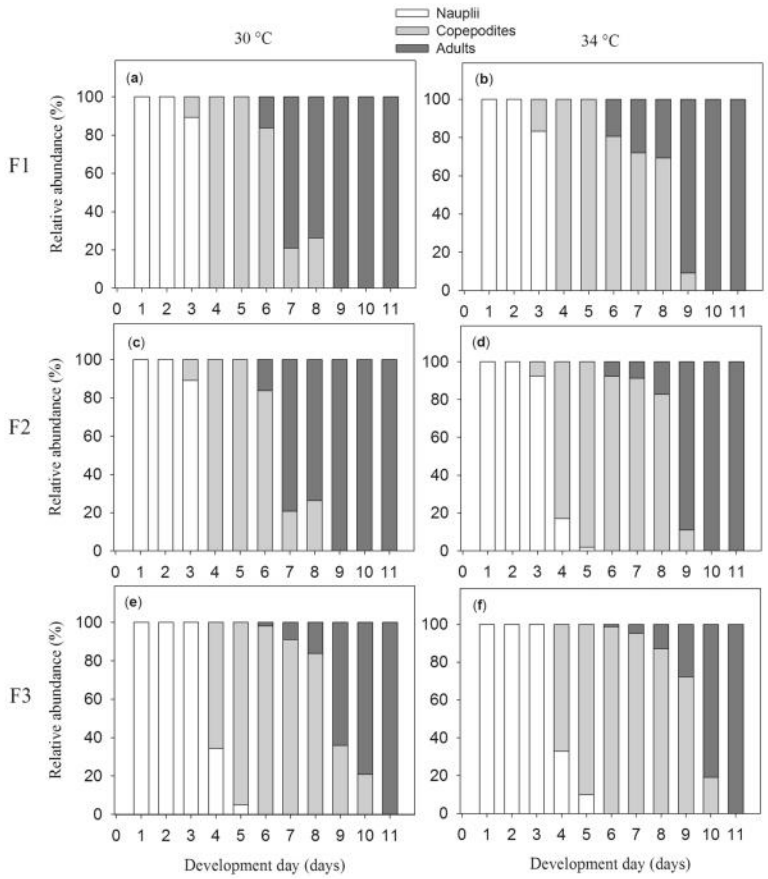


Figure 3. 7. The development of population *Pseudodiaptomus annandalei* at 30°C and

In both sexes, the adult size was smaller when copepods were cultured at 34°C (Figure 3.9, Table 3.5)

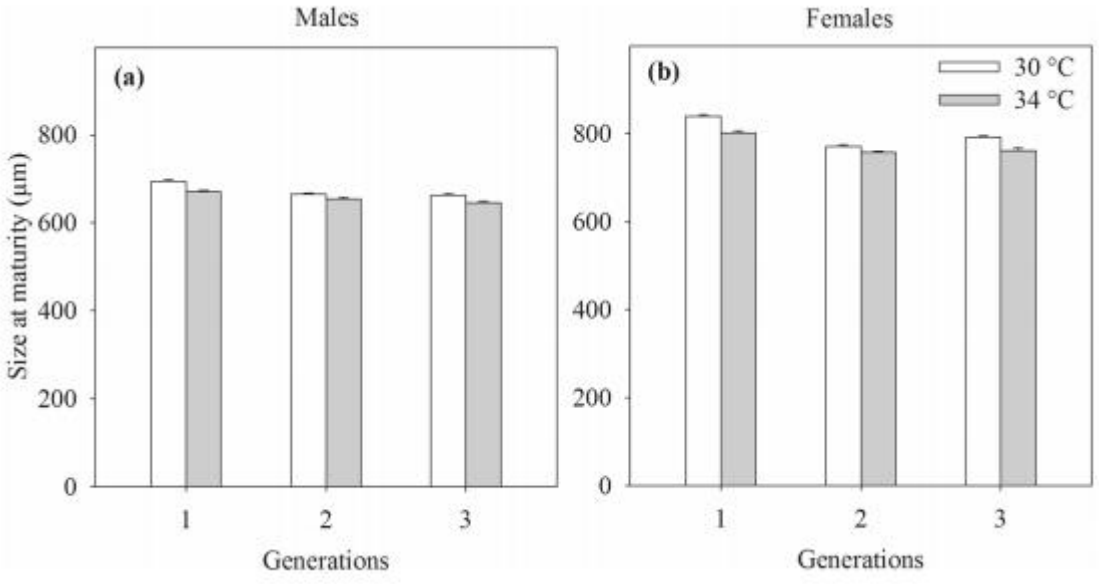


Figure 3. 8. Size at maturity of *P. annandalei* male (a), female (b) at 30 °C and 34 °C across 3 generations. Data represents the mean ± SE.

3.3.2. Clutch size of Pseudodiaptomus annandalei

The effects of temperatures on the clutch size and specific clutch size of *P. annandalei* across 3 generations are shown in Table 3.6 and Figure 3.10. The number of eggs in two egg sacs of females were cultured at 34 °C, were fewer than that at 30 °C.

Table 3. 6. Effects temperatures and generations on clutch size and specific clutch size of *P. annandalei*. *Significant p values (p<0.05) are indicated in bold.*

Factors	**Clutch size**			**Specific clutch size**		
	df1, df2	**F**	**P**	**df1, df2**	**F**	**P**
Temperature	1,191	61.45	**<0.001**	1,191	9.98	**0.0019**
Generation	2,191	54.17	**<0.001**	2,191	3.81	**0.024**
Temperature × Generation	2,191	8.80	**<0.001**	2,191	2.22	0.11

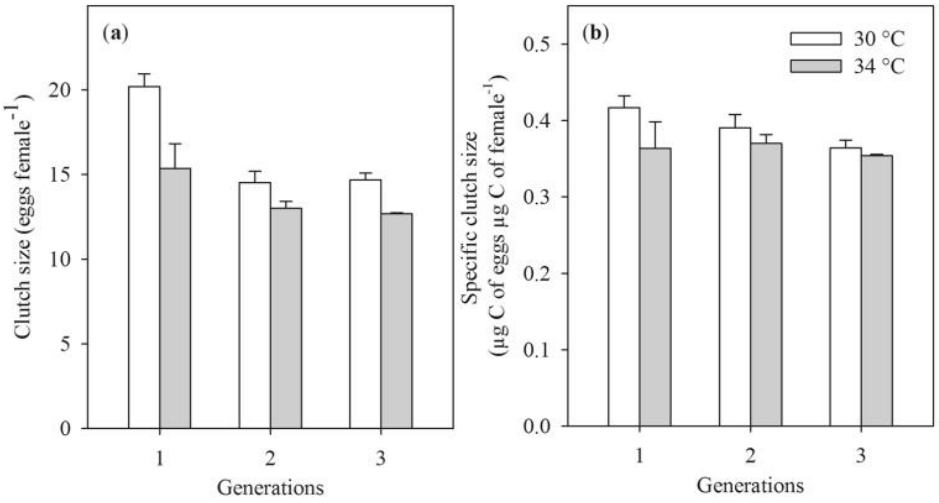


Figure 3. 9. Clutch size (a) and specific clutch size (b) of *P. annandalei* at 30°C and 34°C across 3 generations. *Data represents the mean ± SE.*

3.3.3. Hatching success

The hatching success was 70% at 34ºC (thus approximately 20% lower than that at 30ºC). This pattern was independent of generation (Table 3.7, Figure 3.11).

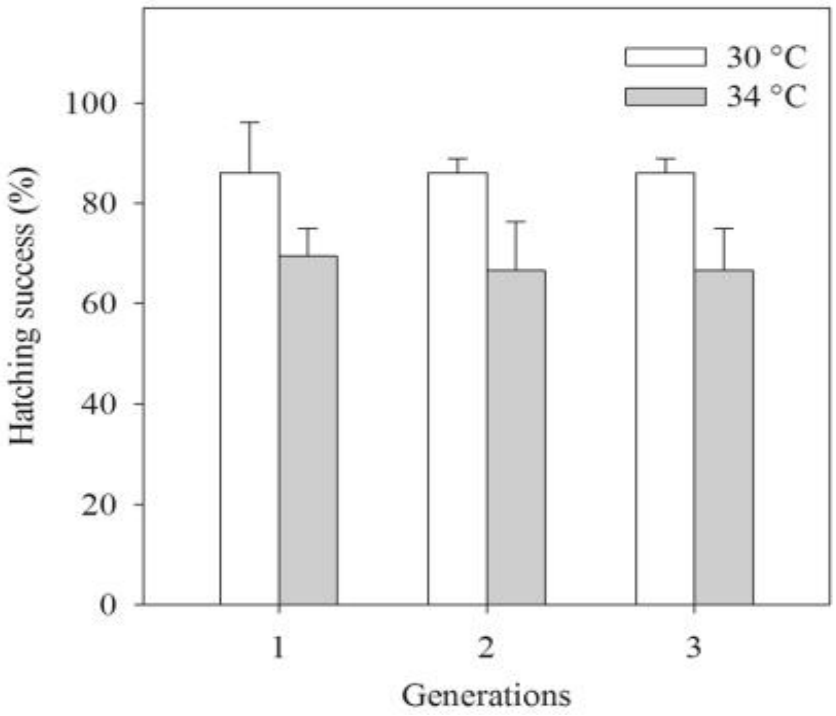


Figure 3. 10. Hatching success of *P. annandalei* at 30°C and 34°C across across 3 generations. *Data represents the mean ± SE.*

Table 3. 7. Effects of temperature and generations on hatching success of *P. annandalei*. *Significant p values (p<0.05) are indicated in bold.*

Factors	Hatching success		
	df1, df2	F	P
Temperature	1,210	9.90	**0.0019**
Generation	2,210	0.02	0.99
Temperature × Generation	2,210	0.02	0.99

3.3.4. Nauplii production

Nauplii production at 34°C was lower than that at 30°C. F1 produced nauplii more than that of F2 and F3 (Table 3.8, Figure 3.12).

Table 3. 8. Effects of temperature and generations on nauplii production and specific nauplii production of *P. annandalei*. *Significant p values (p<0.05) are indicated in bold.*

Factors	Naupli production			Specific nauplii production		
	df1, df2	F	P	df1, df2	F	P
Temperature	1,24	52.79	**<0.001**	1,24	29.21	**<0.001**
Generation	2,24	10.85	**<0.001**	2,24	1.72	0.20
Temperature × Generation	2,24	4.97	**0.016**	2,24	2.17	0.14

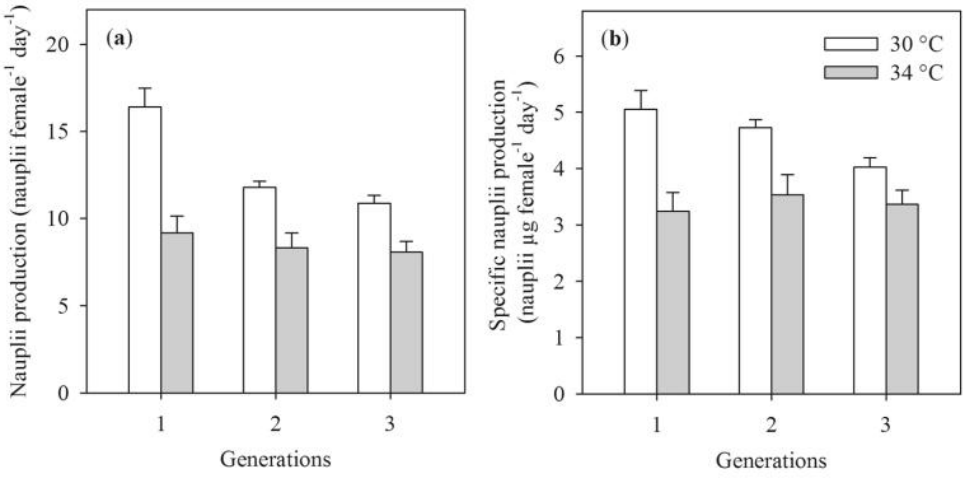


Figure 3. 12. Nauplii production (a) and specific nauplii production (b) at 30°C and 34°C across across 3 generations. *Data represents the mean ± SE.*

3.4. *Effects of temperature on growth and reproduction of P. annandalei*

3.4.1. The development and growth of P. annandalei at 25 °C, 30 °C and 34°C

The effects of temperatures on the development of population *P. annandalei* are shown in Figure 3.13. *P. annandalei* population reached maturity faster than that at 34°C and the lowest was the population at 25°C. Moreover, temperatures also affected on the growth of *P. annandalei* (Table 3.9). Copepods size reduced with increased temperature from 25 to 34°C.

Table 3. 9. Size of different stages of *P. annandalei* at 25 °C, 30°C and 34°C (µm).

Stages	**25°C**		**30°C**		**30°C**	
N1	118 ± 12,6^{a}	(n=14)	122 ± 13,7^{a}	(n=19)	119 ± 11,8^{a}	(n=16)
N2	170 ± 20,0^{a}	(n=29)	167 ± 17,1^{a}	(n=48)	168 ± 16,3^{a}	(n=32)
N3	207 ± 16,9^{b}	(n=29)	194 ± 16,1^{a}	(n=24)	187 ± 16,8^{a}	(n=24)
N4	260 ± 25,1^{b}	(n=12)	241 ± 13,9^{a}	(n=29)	237 ± 19,4^{a}	(n=37)
N5	285 ± 11,3^{b}	(n=13)	267 ± 17,4^{a}	(n=23)	268 ± 26,1^{a}	(n=33)
N6	294 ± 14,4^{b}	(n=19)	280 ± 23,4^{a}	(n=24)	286 ± 18,8^{a}	(n=15)
C1	316 ± 33,1^{b}	(n=19)	289 ± 17,2^{a}	(n=27)	290 ± 16,3^{a}	(n=11)
C2	402 ± 28,8^{b}	(n=21)	329 ± 19,7^{a}	(n=10)	322 ± 17,5^{a}	(n=11)
C3	490 ± 55,9^{b}	(n=19)	381 ± 23,8^{a}	(n=40)	371 ± 18,9^{a}	(n=27)
C4	556 ± 65,5^{b}	(n=21)	508 ± 37,2^{a}	(n=59)	489 ± 49,1^{a}	(n=57)
C5	635 ± 55,9^{c}	(n=28)	600 ± 32,4^{b}	(n=29)	551 ± 92,5^{a}	(n=53)
Adult Male	695 ± 51,8^{b}	(n=63)	681 ± 42,6^{b}	(n=200)	662 ± 40,1^{a}	(n=214)
Adult Female	877 ± 56,6^{c}	(n=58)	790 ± 70,1^{b}	(n=262)	773 ± 60,6^{a}	(n=193)
SCR_L -Male	20 ± 0,1^{a}		23 ± 0,1^{c}		21 ± 0,1^{b}	
SCR_L - Male	18 ± 0,1^{a}		22 ± 0,1^{c}		19 ± 0,1^{b}	

Data represents the mean ± SD. Different letters on the same row indicate the significant difference ($p < 0.05$).

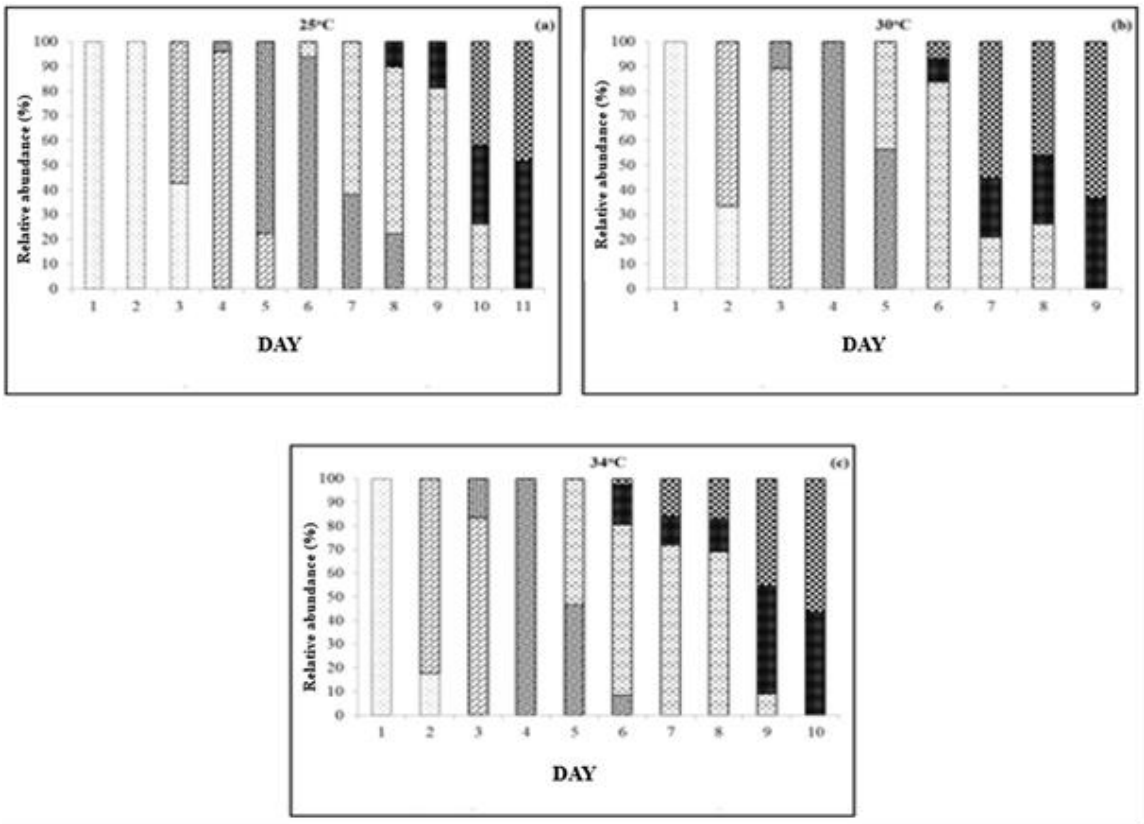


Figure 3. 12. The development of population *P. annandalei* at 25°C (a), 30°C (b) and 34°C (c)

3.4.2. Effects of temperature on clutch size, hatching rate and nauplii/female

The effects of temperatures on survival rate, clutch size, hatching success and nauplii/*P. annandalei* was shown in Table 3.10.

Table 3. 10. Effects of temperature on clutch size, hatching success and nauplii/*P. annandalei*.

Response variables	25°C	30°C	34°C
Survival rate	56,8 ± 1,70^{b}	57,8 ± 3,99^{b}	36,3±4,09^{a}
Clutch size (eggs/female)	22,5 ± 0,97^{a}	19,6 ± 0,44^{b}	14,5 ± 0,61^{c}
Hatching success (%)	83,3 ± 2,64^{a}	91,7 ± 4,56^{a}	63,3 ± 2,04^{b}
Nauplii/female	16,4 ± 2,74^{a}	16,4 ± 1,02^{a}	9,2 ± 0,96^{b}

Data represents the mean ± SE. Different letters on the same row indicate the significant difference ($p < 0.05$).

3.4.3. Effects of temperature on reproduction ability and lifespan of P. annandalei

Effects of temperatures on nauplii/female.10days and lifespan of *P. annandalei* was shown in table 3.11.

Table 3. 11. Effects of temperature on nauplii/female.10days and lifespan of *P. annandalei.*

Response variables	**25°C**	**30°C**	**34°C**
Nauplii/female.10 days	101,6 ± 3,74[b]	178,0 ± 7,90[a]	78,4 ± 4,67[c]
Male lifespan	43,2 ± 0,58[a]	38,8 ± 1,59[b]	32,0 ± 0,71[c]
Female lifespan	44,0 ± 0,55[a]	38,2 ± 1,39[b]	32,0 ± 0,55[c]

Data represents the mean ± SE. Different letters on the same row indicate the significant difference ($p < 0.05$).

3.5. *The tolerance of P. annandalei to salinity shock and the interactive effects of salinity and temperature on its biological and reproductive characteristics*

3.5.1. Effects of salinity shocking on survival rate, hatching success and nauplii/female

The effects of salinity shocking survival rate of different stages, hatching success and nauplii/female of *P. annandalei* were shown in Table 3.12, Figure 3.14 and 3.15.

Table 3. 12. The results of three-way ANOVAs testing for the effects of salinities and shocking period and copepod stages on the survival rate of the *Pseudodiaptomus annandalei. Significant p values ($p < 0.05$) are indicated in bold.*

Factors	df	F	P
Salinity	8	88,069	**<0,001**
Shocking period	1	7,177	**0,008**
Copepod stage	3	229,236	**< 0,001**
Salinity * Shocking period	8	0,256	0,979
Salinity * Copepod stage	24	11,303	**< 0,001**
Shocking period * Copepod stage	3	1,800	0,150
Salinity * Shocking period * Copepod stage	24	0,470	0,983

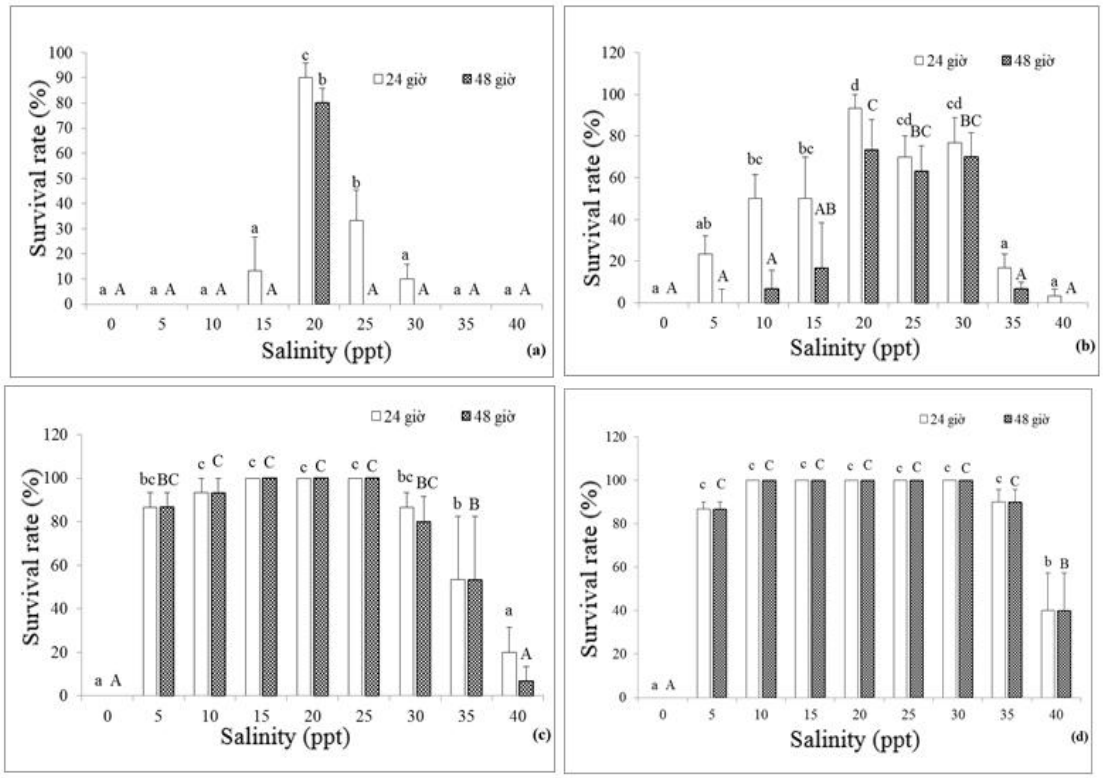


Figure 3. 13. Effects of salinity shocking on the survival rate of different stages of *P. annandalei*: a) nauplii, b) copepodid, c) adult male, d) adult female.

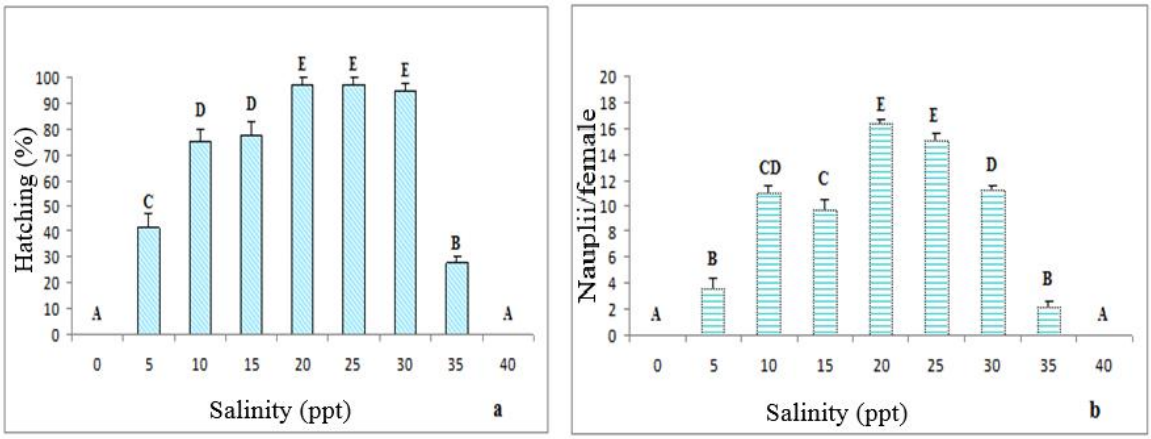


Figure 3. 14. Effects of salinity shocking on the hatching rate (a) and nauplii/female (b). *Data represents the mean ± SE.*

3.5.2. Effects of temperature and salinity on size, clutch size, hatching, naupli/female and number of nauplii/female.10 days

The effects of of salinities and temperatures on the size, hatching rate, nauplii/female and number of nauplii/female.10days of *P. annandalei* were shown in Table 3.13, Table 3.14.

Table 3. 13. The results of three-way ANOVAs testing for the effects of salinities and temperatures on the size, hatching rate, nauplii/female and number of nauplii/female.10days. *Significant p values (p < 0.05) are indicated in bold.*

Factors	Response variables	df	F	P
Temperature	Male size	1	427.428	**<0.001**
	Female size	1	179.347	**<0.001**
	Clutch size	1	325.507	**<0.001**
	Hatching success	1	13.567	**0.01**
	Nauplii/female	1	40.111	**<0.001**
	Nauplii/female.10days	1	1489.649	**<0.001**
Salinity	Male size	6	22.960	**<0.001**
	Female size	6	6.115	**<0.001**
	Clutch size	6	13.049	**<0.001**
	Hatching success	6	5.493	**0,01**
	Nauplii/female	6	20.898	**<0.001**
	Nauplii/female.10days	6	82.031	**<0.001**
Temperature * Salinity	Male size	6	4.393	**<0.001**
	Female size	6	3.410	**0.03**
	Clutch size	6	10.822	**<0.001**
	Hatching success	6	0.378	0.887
	Nauplii/female	6	2.843	**<0.001**
	Nauplii/female.10days	6	3.218	**<0.001**

Table 3. 14. The results of one-way ANOVAs testing for the effects of salinities on size female and male, hatching success, nauplii/female and number of nauplii/female.10days at 30°C and 34°C.

Salinity	**5 ‰**	**10 ‰**	**15 ‰**	**20 ‰**	**25 ‰**	**30 ‰**	**35 ‰**
Male 30°C	742±3,1^{ab}	747±4,3^{b}	752±4,1^{b}	754±5,3^{b}	772±5,2^{c}	738±7,5^{ab}	725±5,9^{a}
Male 34°C	693±2,4^{b}	720±3,6^{e}	712±4,3^{de}	699±2,7^{bc}	707±4,0^{cd}	678±4,3^{a}	670±3,3^{a}
Female 30°C	842±7,1^{ab}	832±6,5^{a}	871±8,8^{c}	832±5,6^{a}	860±8,8^{bc}	847±8,1^{bc}	823±7,3^{a}
Female 34°C	801±6,3^{c}	790±3,8^{bc}	802±3,1^{c}	799±6,1^{c}	775±7,0^{ab}	807±9,1^{c}	769±10,1^{a}
F 30°C	14±0,2^{a}	16±0,3^{c}	17±0,4^{d}	15±0,3^{b}	15±0,3^{bc}	15±0,3^{b}	13±0,3^{a}
F 34°C	12±0,2^{ab}	12±0,2^{ab}	13±0,2^{ab}	13±0,2^{b}	13±0,2^{ab}	12±0,2^{ab}	12±0,2^{a}
HS 30°C	83±0,0^{ab}	83±4,9^{ab}	97±2,7^{c}	89±5,7^{bc}	92±4,9^{bc}	83±4,9^{ab}	78±2,7^{a}
HS 34°C	78±2,7^{ab}	78±2,7^{ab}	86±3,0^{b}	89±3,0^{b}	80±2,7^{ab}	80±2,7^{ab}	69±5,7^{a}
N 30°C	6,3±0.33^{ab}	10±1,00^{c}	10,7±0,33^{c}	10±0,58^{c}	8±0,58^{b}	6,3±0,67^{ab}	5,3±0,33^{a}
N 34°C	5±0,00^{ab}	6±0,58^{bc}	8±0,58^{d}	7,7±0,67^{d}	7,3±0,33^{cd}	6±0,58^{bc}	4±0,00^{a}
N10-30°C	125±2,4^{c}	152±1,4^{ef}	157±3,0^{f}	146±1,9^{e}	138±3,8^{d}	110±2,7^{b}	92±3,6^{a}
N10-34 °C	60±3,2^{b}	87±5,9^{c}	85±1,8^{c}	68±4,8^{b}	70±2,0^{b}	48±2,6^{a}	40±2,2^{a}

Data represents the mean ± SE. Leter F is clutch size; HS is hatching rate; N is nauplii/female, N10 is total number of nauplii/female.10days.

3.6. *Effects of nauplii density and adult density on growth and reproduction of Pseudodiaptomus annandalei*

3.6.1. Survival rate and adult ratio in population P. annandalei

The effects of temperature and nauplii density on the survival rate and adult ratio on day 10 were shown in Table 3.15, Figure 3.15, Figure 3.16.

Table 3. 15. The results of three-way ANOVAs testing for the effects of temperature and nauplii density on the survival rate and adult ratio at day 10.

Factors	**Response variables**	***df***	***F***	***P***
Temperature	Survival rate (%)	1	94.170	**<0.001**
	Adult ratio (%)	1	78.478	**<0.001**
Nauplii density	Survival rate (%)	3	12.239	**<0.001**
	Adult ratio (%)	3	228.898	**<0.001**
Temperature * Nauplii density	Survival rate (%)	3	8.091	**<0.001**
	Adult ratio (%)	3	26.673	**<0.001**

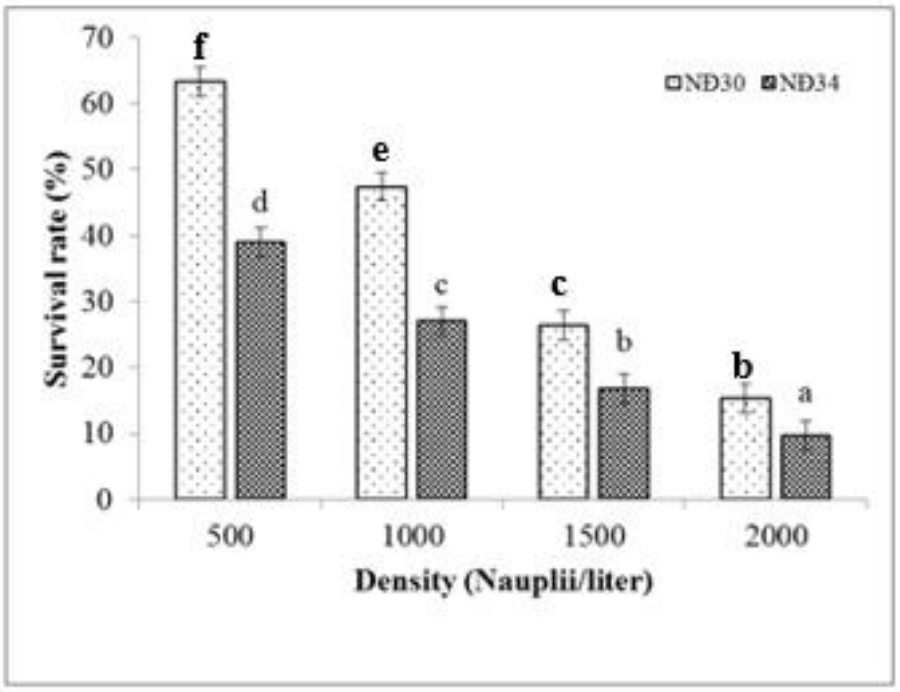


Figure 3. 15. Effects of temperature and nauplii density on the survival rate of *P. annandalei* at day 10th.

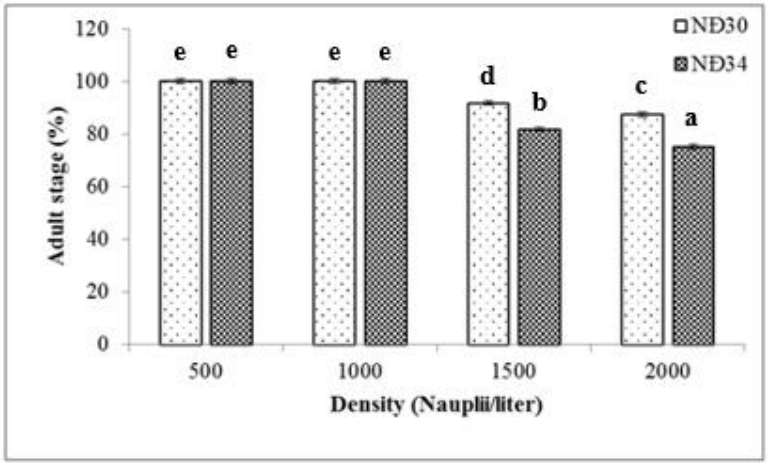


Figure 3. 16. Effects of temperature and nauplii density on the adult ratio of *P. annandalei* at day 10th.

3.6.2. Adult size and clutch size of P. annandalei

The effects of temperature and nauplii density on the adult size and clutch size of *P. annandalei* were shown in Table 3.16, Figure 3.17, Figure 3.18.

Table 3. 16. The results of three-way ANOVAs testing for the effects of temperature and nauplii density on the adult size and clutch size of *P. annandalei*.

Factor	**Response variables**	***df***	***F***	***P***
Temperature	Adult male	1	44.651	**<0.001**
	Adult female	1	168.462	**<0.001**
	Clutch size	1	264.191	**<0.001**
Nauplii density	Adult male	3	0.420	0.739
	Adult female	3	0.477	0.698
	Clutch size	3	0.091	0.965
Temperature * Nauplii density	Adult male	3	0.772	0.510
	Adult female	3	1.290	0.278
	Clutch size	3	0.533	0.660

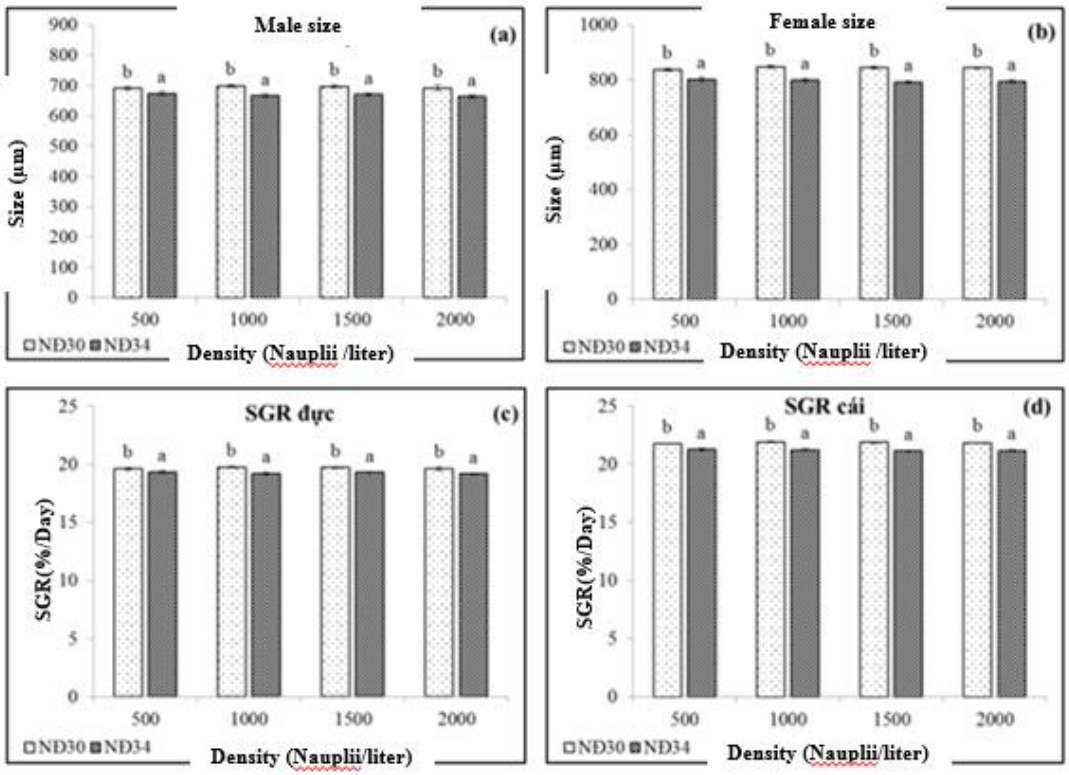


Figure 3. 17. Effects of temperature and nauplii density on male size (a), female size (b), SGR (c, d). *Data represents the mean ± SE.*

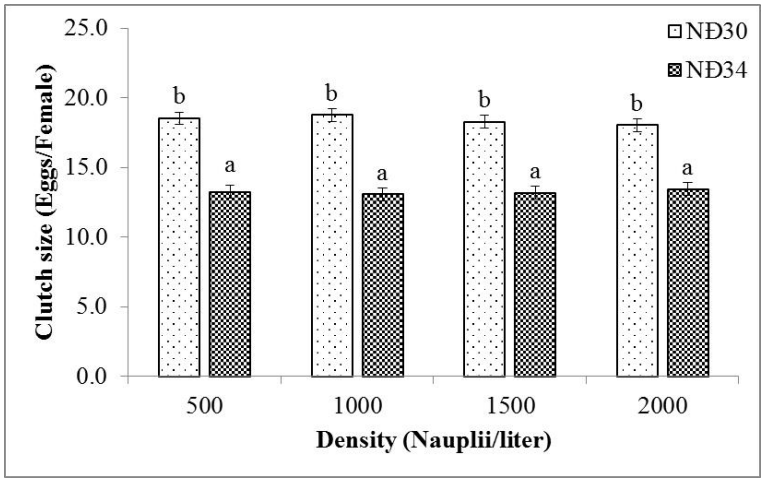


Figure 3. 18. Effects of temperature and nauplii density on clutch size of *P. annandalei.*

3.6.3. Effects of adult density on number of nauplii and survival rate of adult

The effects of density and cultured day on the number of nauplii in day 1st, 10th; the effects of temperature and adult density on the total number of nauplii in 10 days and survival rate of *P. annandalei* at day 10th were shown in Table 3.17, Figure 3.19, Figure 3.20.

Table 3. 17. The effects of density and cultured day on the number of nauplii in day 1st, 10th; the effects of temperature and adult density on total number of nauplii in 10 days and survival rate of *P. annandalei* at day 10th.

Factor	**Response variables**	***df***	***F***	***P***
Density	Nauplii/female at 30 °C	5	14.151	**<0.001**
	Nauplii/female at 34 °C	5	8.450	**<0.001**
Cultured day	Nauplii/female at 30 °C	1	172.429	**<0.001**
	Nauplii/female at 34 °C	1	224.645	**<0.001**
Density * Cultured day	Nauplii/female at 30 °C	5	11.054	**<0.001**
	Nauplii/female at 34 °C	5	12.987	**<0.001**
Temperature	Total nauplii/10 days	1	202.933	**<0.001**
	Adult survival rate	1	197.171	**<0.001**
Density	Total nauplii/10 days	5	481.442	**<0.001**
	Adult survival rate	5	142.870	**<0.001**
Temperature * Density	Total nauplii/10 days	5	7.909	**<0.001**
	Adult survival rate	5	3.170	**0.015**

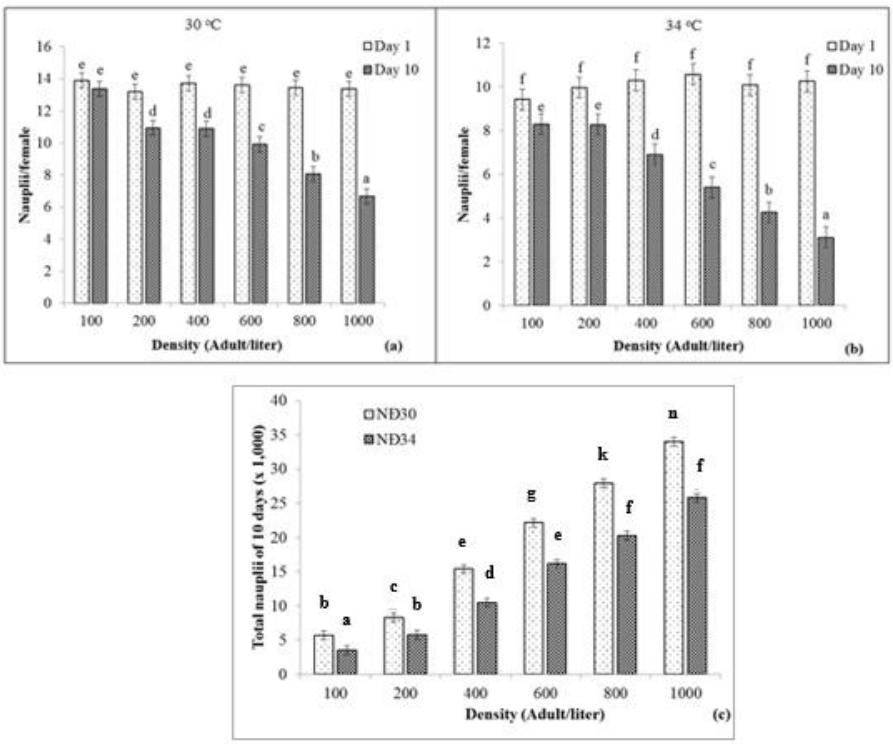


Figure 3. 19. Effects of temperature and adult density on nauplii/female at day 1st and day 10th at 30°C (a), 34°C (b) and total number of nauplii of polulation in 10 days (c).

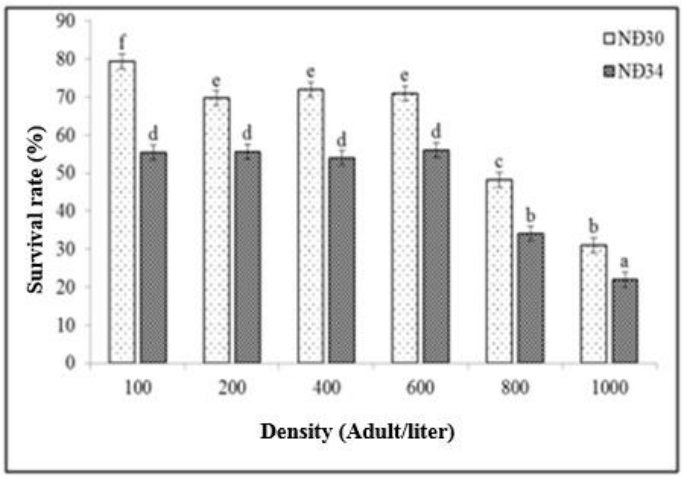


Figure 3. 20. Effects of temperature and adult density on adult survival rate at day 10th.

3.7. *Mass culture and naupli reproduticon of Pseudodiaptomus annandalei at 30°C and 34°C*

2.7.1. P. annandalei density

The density of different stages and total density of *P. annandalei* in the mass culture at 30°C and 34 °C were shown in Figure 3.21, Figure 3.22.

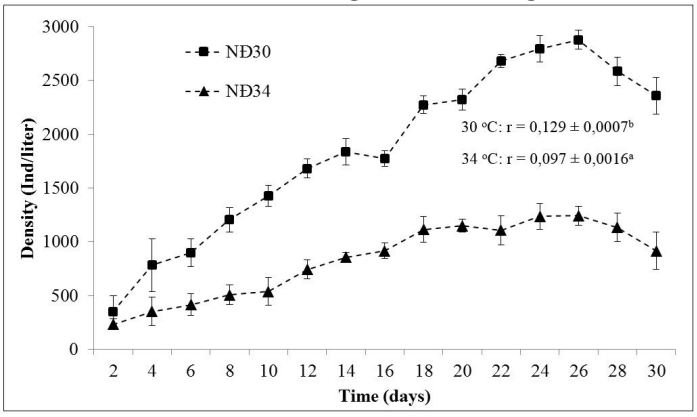


Figure 3. 21. *P. annandalei* density during culture period at 30 and 34 °C.

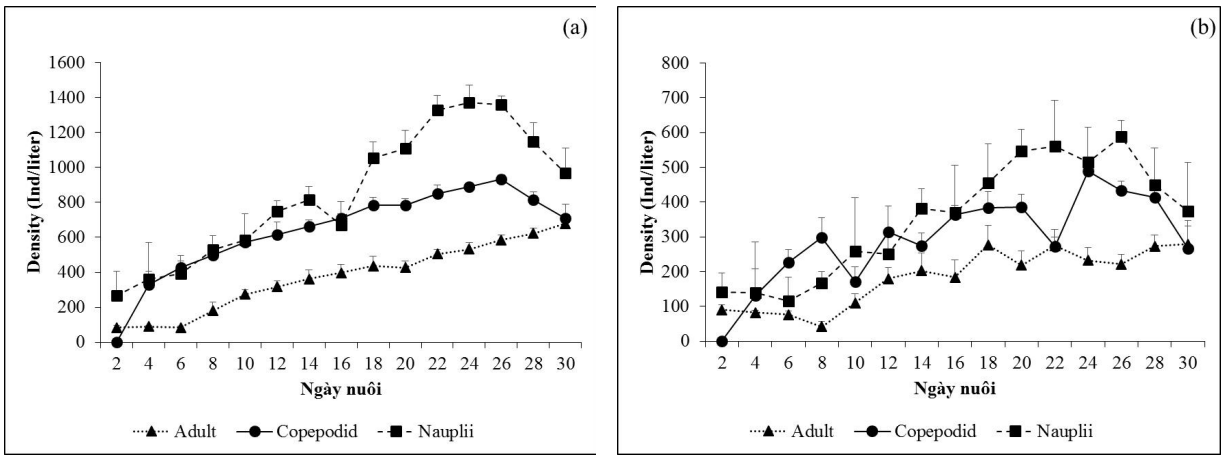


Figure 3. 22. Density of different stages in population during culture period at 30°C (a) and 34°C (b). *Data represents the mean ± SD.*

2.7.2. Total number of copepods of different dilution ratios

The effect of temperatures and dilution ratios on the total number of copepods that were collected in total culturing days was shown in Figure 3.23, Table 3.18. The total number of copepods during the culturing period was shown in Figure 3.24.

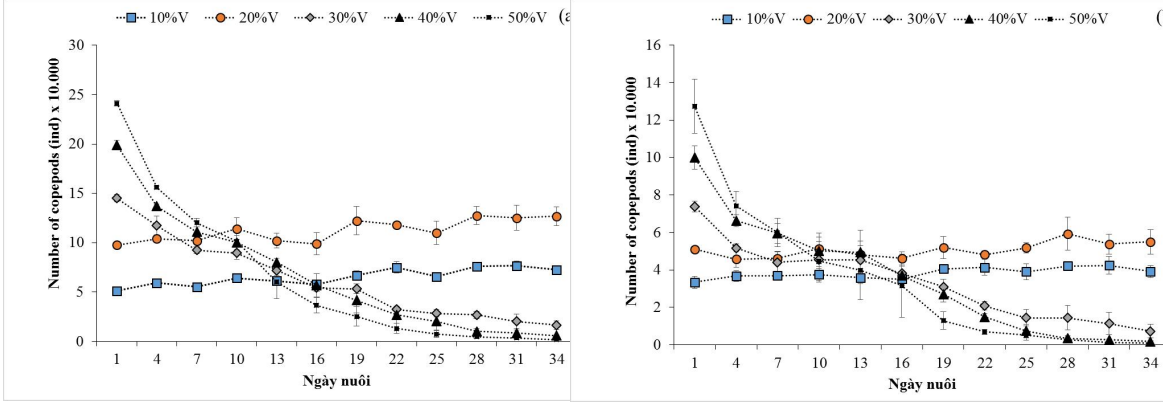


Figure 3. 23. Total number of copepods during culturing period of different dilution ratios from 10% to 50% at 30°C (a) and 34°C (b). *Data represents the mean ± SD.*

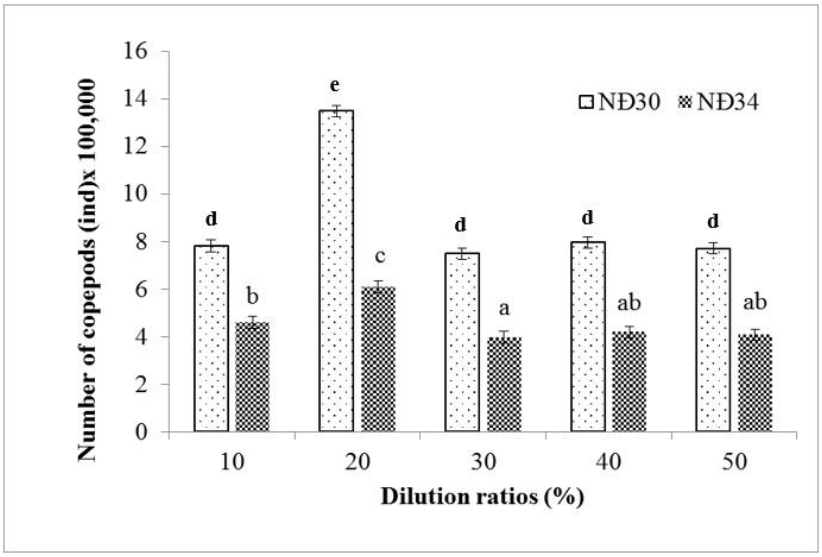


Figure 3. 24. Total number of copepods of different dilution ratios from 10% to 50% at 30°C and 34°C.

Table 3. 18. The results of three-way ANOVAs testing for the effects of temperatures and dilution ratios on the total number of copepods during culturing period. *Significant p values (p < 0.05) are indicated in bold.*

Factors	df	F	P
Temperature (°C)	1	798.356	**<0.001**
Dilution ratio (%)	4	101.117	**<0.001**
Temperature * Dilution ratio	4	26.123	**<0.001**

2.7.3. Total number of nauplii of different dilution ratios

Number of nauplii during the culturing period of different dilution ratios at 30°C, 34°C was shown in Figure 3.26. Moreover, the effects of temperature and dilution ratios on the total number of nauplii during 30 days also were shown in Figure 3.26 and Table 3.19.

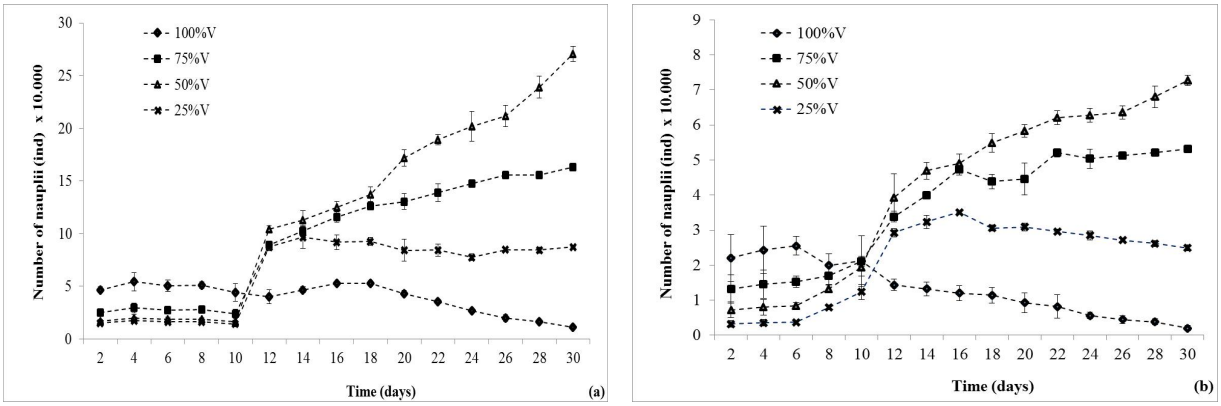


Figure 3. 25. Number of nauplii during culturing period of different dilution ratios at 30°C (a), 34°C (b). *Data represents the mean ± SD.*

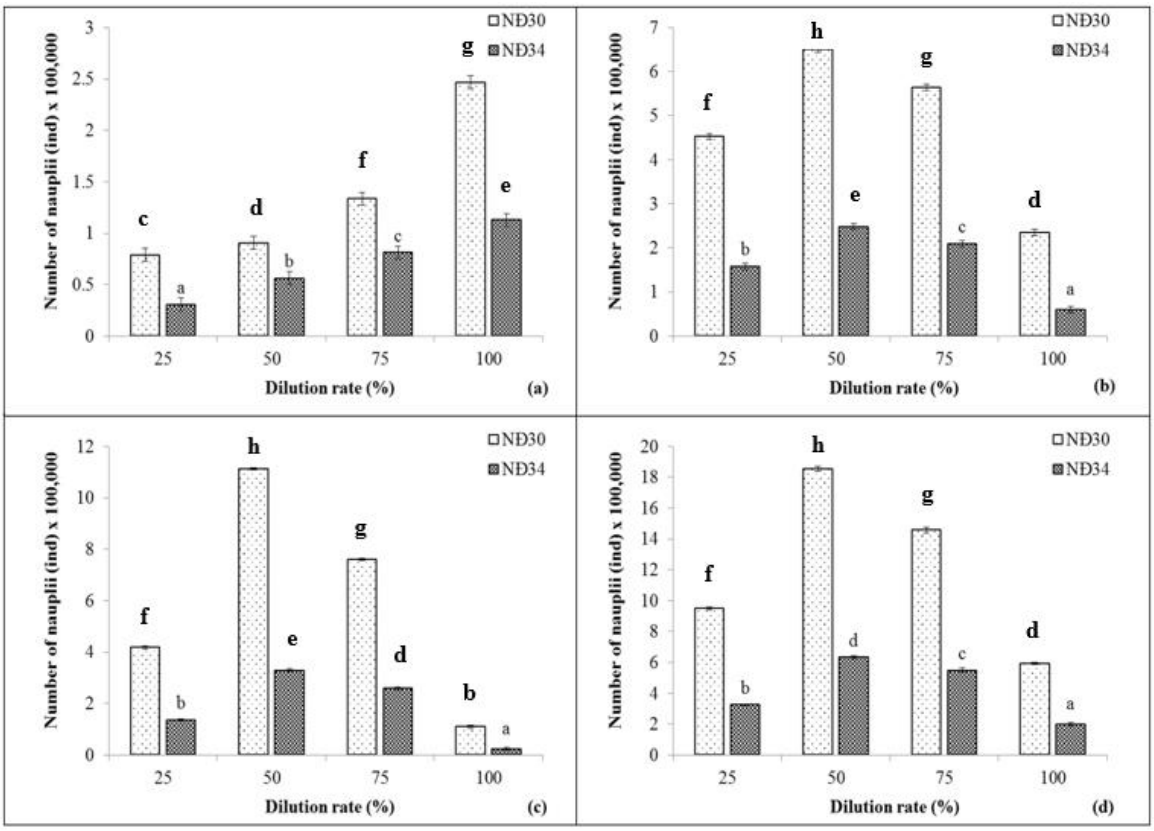


Figure 3. 26. Effects of temperature and dilution ratios on total number of nauplii in 30 days: (a) first five times, (b) second five times, (c) third five times, (d) total of fifteen times. Data represents the mean ± *SE. Different letters indicate the significant difference ($p < 0.05$).*

Table 3. 19. The results of three-way ANOVAs testing for the effects of temperatures and dilution ratios on the total number of nauplii during culturing period. *Significant p values ($p < 0.05$) are indicated in bold.*

Factors		***df***	***F***	***P***
Temperature (°C)	First five collecting times	1	235.378	**<0.001**
	Second five collecting times	1	3924.278	**<0.001**
	Third five collecting times	1	14107.042	**<0.001**
	Fifteen collecting times	1	8570.374	**<0.001**
Dilution ratio (%V)	First five collecting times	3	158.704	**<0.001**
	Second five collecting times	3	710.858	**<0.001**
	Third five collecting times	3	6633.597	**<0.001**
	Fifteen collecting times	3	1972.350	**<0.001**
Temperature * Dilution ratio	First five collecting times	3	26.514	**<0.001**
	Second five collecting times	3	100.740	**<0.001**
	Third five collecting times	3	1854.441	**<0.001**
	Fifteen collecting times	3	441.403	**<0.001**

CONCLUSION AND RECOMMENDATION

1. CONCLUSION

1) At 25°C - 35°C, the saturation level of adult P. annandalei is 400 µg C/L with *Isochrysis galbana* or *Tetraselmis chuii*. Saturation levels were 400 µg C/L for males and 800 µg C/L for females with *Chaetoceros muelle*ri.
2) When fed with *I. galbana* or *C. muelleri*, populations of *P. annandalei* exhibited faster growth and higher growth rates than when fed with *T. chuii*. In an experiment comparing the effect of feeding *P. annandalei* with three different algal species at 30°C and 34°C, the survival and reproductive parameters of the species were found to be similar across all treatments.
3) Exposure to a temperature of 34°C slows down population growth, reduces size, growth rate, hatching rate, fertility, larval reproduction, survival rate, and lifespan of *P. annandalei* compared to individuals maintained at a temperature of 30°C.
4) A temperature of 30°C is more conducive to the growth and reproduction of *P. annandalei* compared to temperatures of 25°C and 34°C.
5) All larvae died at salinities of 0, 5, 10, 35, 40‰ (at 24 h) and salinity of 15, 25, 30‰ (at 48 h). At a salinity of 20‰, larvae have a high survival rate (80 - 90%). All juveniles died at salinity 0‰ (at 24 h) and salinities 5‰ and 40‰ (at 48 h). When adults were shocked at salinities from 10‰ to 30‰, the success rate of hatching, larval hatching and survival was higher. A salinity of 15‰ is suitable for *P. annandalei* to grow and reproduce.
6) An initial stocking density of 500 larvae/L is suitable for biomass culture; 100 adults/L is suitable for *P. annandalei* biomass culture.
7) The copepod density was higher at 30°C than at 34°C, and the population growth rate was also higher at 30°C. A harvest rate of 20% is suitable for biomass culture, while a harvest rate of 50% is suitable for larval rearing of *P. annandalei* species.

2. RECOMMENDATION

1) Further study on the effect of extreme temperature (34°C) on more than three generations to investigate the adaptive ability of this species under warming.
2) We need to further study on the saturation level of food for the naupliar stage and the copepodid stage to have enough basic knowdege to culture this species in all stages.

3) Further study on suitable culture system to mass culture and nauplii production. Moreover, need to study on a larger volume of mass culture and nauplii production.