MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

NGUYEN THI THUY

COMBINED EFFECTS OF TEMPERATURE AND SOME ECOLOGICAL FACTORS ON TROPICAL COPEPODS

Major: Aquaculture
Major code: 9620301

SUMMARY OF DOCTORAL THESIS

KHANH HOA – 2022

KEY FINDINGS

Thesis title: *"Combined effects of temperature and some ecological factors on tropical copepods"*

Major:	Aquaculture
Major code:	9620301
PhD Student:	Nguyen Thi Thuy
Period of time:	2015
Science instructor:	1. Assoc. Prof. Dr. Le Minh Hoang
	2. Dr. Dinh Van Khuong
Institution:	Nha Trang University

Key findings:

1. This study provided novel information on the impact of important ecological factors such as salinity, photoperiod, UV combined with temperature on the growth and reproduction of two tropical copepd species *Pseudodiaptomus incisus* and *Oithona rigida*. The results of the study are important for optimizing biomass culture techniques for two copepod species *P. incisus* and *O. rigida*

2. *P. incisus* had a highest growth rate at a range of temperatures 26 – 30^{o}C, salinity 15-20 ppt; photoperiods 12L:12D and 18L:6D. The reproductive efficiency of *P. incisus* was highest at temperature of 30^{o}C, salinity 20ppt, photoperiod 12L:12D and 18L:6D and UV0 (no ultraviolet rays), UVA (wavelength in the range 350-400 nm).

3. *O. rigida* showed the highest growth rate at temperatures 26 – 30^{o}C, salinity 25-30 ppt; photoperiods 12L:12D and 18L:6D. The reproductive efficiency of *O. rigida* was highest at temperature 30^{o}C, salinity 30ppt, photoperiod 12L:12D and 18L:6D and UV0, UVA

4. The study observed the effects of three algae species (*Chaetoceros muelleri*, *Isochrysis galbana* and *Tetraselmis chui*) at three density levels for each algae species (160; 800 and 1,600 µg carbon/L) on the growth and reproductive efficiency of wo tropical copepod species *P. incisus* and *O. rigida*. . Information on this content is important for the biomass culture of two copepod species *P. incisus* and *O. rigida*. *P. incisus* and *O. rigida* species grew and reproduced better when fed with *Isochrysis galbana* and *Chaetoceros muelleri* with the density in the range of 800 - 1600 µgC/L.

Supervisors **Student Ph.D.**

Assoc. Prof. Dr. Le Minh Hoang Dr. Dinh Văn Khuong **Nguyen Thi Thuy**

LIST OF PUBLISHED ARTICLES

1. Nguyen Thuy T., Minh-Hoang Le, Doan Nam X., Nguyen Sy T., Truong Trinh S. H., Vu Minh T. T., Dinh Khuong V., 2020. Salinity and temperature effects on productivity of a tropical calanoid copepod *Pseudodiaptomus incisus*. *Aquaculture Research* 00: pp. 1–12. (Q2, impact factor: 2.184).

2. Nguyễn Thị Thủy, Lê Minh Hoàng, Đoàn Xuân Nam, Bùi Văn Cảnh, Nguyễn Thị Thành, Đinh Văn Khương, 2021. Ảnh hưởng của mật độ vi tảo *Isochrysis galbana* làm thức ăn đến sức sinh sản và tỷ lệ nở của loài copepoda *Apocyclops royi*. *Tạp chí Khoa học - Công nghệ Thủy sản* số 3/2021.

INTRODUCTION

Over the past decades, copepoda has been selected as a food for larvae of many marine fish species of economic value (Burbano et al., 2020; Thuong & Hoang, 2015). Copepoda meets the requirements of good food for marine fish larvae: higher nutritional value than rotifers and artemia (Rasdi and Qin, 2016; Rayner et al., 2017), has a wide size spectrum (Golez et al. ., 2004); The zigzag swimming behavior increases the attractiveness to fish larvae (Ajiboye et al., 2011). However, copepoda is having to adjust to climate change. In tropical marine ecosystems, sea surface temperatures have increased by more than 1°C over the past 100 years and are projected to triple or quadruple in the 21st century (IPCC, 2013). The interaction between climate change and other pollution factors has enhanced the effects on organisms (Dinh ctv., 2016). The response of tropical copepods to increases in temperature combined with factors such as salinity, light, UV and food is still very limited information. Two species of Pseudodiaptomus incisus and Oithona rigida are widely distributed in aquaculture ponds and coastal waters of Central Vietnam. The thesis "Study on the combined effects of temperature with some ecological factors on copepod crustaceans" is very necessary, providing scientific data for biomass farming an important food source for fish. breeding of high-value aquatic species and the basis of management policies in coastal residential areas.

The objective of the study

Clarifying the impact of important ecological factors and food on the growth and reproduction of tropical copeoda, as the basis for biomass culture of two species *P. incisus* and *O. rigida*.

Scientific of the study: The research results are the scientific basis and shed light on the adaptation of copepoda to changes in ecological conditions in the context of climate change.

Practical application of the study: Research results on better growth and reproduction conditions of two copepoda species *P. incisus* and *O. rigida* are the basis for establishing biomass culture techniques for these two copepoda species.

The structure of the dissertation

The dissertation has 151 pages, including 3 pages of introduction, 34 pages of overview, 18 pages of research methods, 94 pages of research results, 2 pages of summary, 12 tables, 69 images, 389 references, 15 appendices (03 Vietnamese documents, 386 English documents).

CHAPTER 1. LITERATURE REVIEW

1.1. The role of copepods in aquaculture

Copepod meets the requirements of natural food for marine fish larvae. Biochemical composition of copepoda meets the needs of marine fish larvae; very wide size spectrum, small enough to fit the mouth size of marine fish larvae; Copepoda's zigzag swimming characteristics stimulated larvae to catch prey. The outstanding feature of copepoda is the ability to synthesize long-chain polyunsaturated fatty acids (HUFAs), their content and ratio meeting the needs of larvae. Micronutrients such as vitamins, carotenoids, chitin, and enzymes of coppeoda have helped marine fish larvae to increase their resistance to adverse factors and improve survival rates.

The value of copepoda has been proven in practice and studies. According to Ananthi et al. (2011), the larvae of black tiger shrimp (*Penaeus monodon*) showed faster growth when fed copepoda than nauplii artemia. The study also showed that the astaxanthin pigment content of black tiger shrimp larvae when fed copepoda was nearly 3 times higher than that of nauplii artemia (9,282 µg/g and 3,5649 µg/g DW, respectively). Similarly, Trochophora larvae of green mussels (Mytilus edulis) improved growth, survival and pigmentation when fed copepoda (Nanton and Castell 1999). Flatfish larvae (Hippoglossus hippoglossus) also had improved pigmentation when fed copepoda compared with artemia (Næss & Lie 1998). Survival and

growth of flounder larvae (Psetta maxima) increased when copepoda was fed in combination with rotifers (Støttrup & Norsker 1997). Growth rate, survival rate and fatty acid biochemistry of body of 14-day-old seabass larvae (Lates calcarifer Bloch) were higher when fed copepoda Acartia clausi compared with artemia and rotifers; The survival rate is 58.13%, respectively; 41.62% and 33.94% (Rajkumar and Vasagam, 2006) A 10-year study by Glenn (2006) showed larvae of snapper (Lutjanus johnii), blue-spotted grouper (Cephalopholis argus), angelfish (Centropyge loricula) for higher growth and survival when fed copepoda.

1.2. Some characteristics of copepods

Copepoda is a small crustacean group with 11,500 species described, including 200 families, 1,650 genera, belonging to the arthropod (Arthroproda), crustacean class (Crustacea) including 10 orders, of which three orders Calanoida, Cyclopoida and Harpacticoida live freely. (Humes 1994). Copepoda is widely distributed in freshwater and brackish water bodies. The genus Pseudodiaptomus is distributed with high density in estuaries and marine areas. They are distributed from latitude 40oN to 40oS (Walter, 1989). The genus Oithona is widely distributed in the seas of the world (Almeda et al., 2011); temperate seas (Castellani et al., 2007); tropical waters (Dahms et al., 2015); the South China Sea (Wang et al., 2017); Nha Trang waters (Truong Si Hai Trinh and Nguyen Tam Vinh, 2015).

The body consists of two parts, the cephalothorax (metasome) and the ventral part (urosome). The head (cephalosome) attached to the cephalothorax has single eyes, antennae and appendages for swimming and eating. The head and thorax are called the prosome. The abdomen is narrow and has no appendages except for the caudal fork. The genital opening is located in the first abdominal segment and the anal opening in the last abdominal segment. The antennae of copepoda vary widely in length in 3 orders, the longest in calanoid and the shortest in Harpacticoida.

Copepod grows and develops through 6 larval stages (nauplii - N1-6); 5 stages of juvenile (copepodait – C1 -5) and adult (Golez et al., 2004). The distinct morphological features of the stages focused on shape, body length, number of abdominal segments, shape of swimming legs 5 and appendages.

Copepod is omnivorous, actively catching prey and has some different characteristics in 3 orders Calanoida, Cyclopoida and Harpacticoida. Nauplii 1 has no mouth, gets its nutrition from the yolk sac, and is not motile (Dussart & Defaye, 2001). Copepoda begins to eat food from N3 (a few can eat outside from N1, N2). The preferred food of most copepods is still algae.

Copepoda's growth is expressed in body length and dry weight. Dry weight is proportional to body length (Mauchline, 1998). When the adult reaches the size of the species, the growth of the female is measured by the number of eggs (Kiørboe et al., 1985). Growth rate depends on temperature, quantity and quality of feed (Mauchline 1998), salinity (Hagiwara et al. 1995) and other ecological factors.

Copepoda differentiates between males and females. The distinguishing features between males and females in Copepoda are usually A1 antennae, enlargement at abdominal segments 1 and 2; body size and slenderness. External sexual characteristics to distinguish males from females are only expressed from stage IV copepodait.

Copepoda reproduce sexually (Gilbert and Williamson, 1998) and fertilize externally. Pairing and mating activities in Copepoda include the following stages: Searching for a mate, Approaching the male's female; Chase and capture the male's female and mate (Dur et al., 2011). Males actively seek mates chemically or mechanically due to the swimming activity of the female (Bagøien and Kiørboe, 2005). Depending on the species of Copepoda, the female may lay eggs in bundles or free-range. The number of eggs/females of each species also varies. Copepoda reproduction is influenced by hydrophysiological, hydrological and aquatic factors.

1.3. Situation of research on Copepods

Cultivation of copepoda biomass remains a major challenge. Research on Copepods began in the 1980s. Research on the biochemical composition of copepoda has focused on lipids

and fatty acids (Gatten et al. 1983; Norsker and Støttrup 1994; Evjemo and Olsen 1997; Morehead et al. 2005); a few amino acids and proteins (Fyhn et al. 1993, 1995; Helland et al. 2003); pigments (Rønnestad et al., 1998) and vitamins (Maeland et al., 2000).

The studies have given basic understanding of the characteristics of Copepoda such as species composition, distribution characteristics; structural morphology, biochemical composition - nutritional value, especially important trace components such as astraxanthin, vitamins, self-synthesis of essential fatty acids (Rayner et al., 2017); effects of some factors (temperature; salinity; light; toxins; heavy metals; food) on growth and reproduction of Copepods.

Information on the development and reproduction of tropical copepoda species under different conditions of temperature and salinity is scarce. This phenomenon may be because each study selected these two factors separately with different levels of testing under different conditions. Does the body size of copepoda change under the influence of temperature and salinity? Answering this question has great significance in raising copepoda as a feed for marine fish larvae. Previous studies focused on evaluating the effect of lighting regime (photoperiod) on reproduction of some copepoda species. The results show that there are influences in different directions in the studies and only concentrated in temperate copepoda species. Therefore, information on body size across stages and reproduction, number of naupli/female with tropical copepoda species under different photoperiods is unknown. Observations have shown copepoda's resistance to ultraviolet (280-400 nm) environmental conditions. However, copepoda species have been studied mainly in temperate regions; Two tropical copepoda species P. incisus and O. rigida have not been reported to respond to ultraviolet light. In the context that the climate fluctuates sharply in the direction of unpredictable solar radiation reaching the Earth, copepoda is expected to have better adaptability. Our study wants to evaluate the impact of high temperature up to 34oC along with the impact of ultraviolet light on two species of tropical copepoda (P. incisus; O. rigida) in a new direction.

Copepoda biomass culture began to be studied in 1972 in Japan, with the aim of feeding marine fish larvae. Initially, 13 copepoda species were studied, including: *Acartia clausi*, *A. longiremis*, *Eurytemora pacifica*, *Euterpina acutifrons*, *Microsetella norvegica*, *Oithona brevicornis* (*O. davisae*), *O. nana*, *O. similis*, *Pseudodiaptomusinopinus*, *P. marinus*, and *Tigriopus japonicus*. However, only *T. japonicus*, *Acartia tsuensis* are mass cultured and fed in marine fish hatcheries. Copepoda biomass farming has faced difficulties such as unstable and slow population growth; requires large amounts of algae and frequent and frequent water changes.

CHAPTER 2. MATERIALS AND METHODOLOGY

2.1. Subject and scope of the study

Subject and scope of the study: Development time, body size of all stages, reproductive capacity, successful hatching rate and naupli production capacity of the species *Pseudodiaptomus incisus, Oithona rigida*

Study period and sites:

+ The study period runs from 5/2019 to 12/2021

+ Location:

- Sampling location and experimental arrangement: Cam Ranh aquaculture research farm, Nha Trang University, Khanh Hoa province (11^{o}49’25.59’’N, 109^{o}07’29.73’’E)

2.2. Contents

Experiment 1: Effect of temperature and salinity on growth time, body size of all stages, fertility, hatching success rate and naupli production of *Pseudodiaptomus incisus*, *Oithona rigida*

Experiment 2: Effect of temperature and photoperiod on development time, body size of all stages, reproductive power, successful hatching rate and naupli production of *Pseudodiaptomus incisus, Oithona rigida*

Experiment 3: Effect of temperature and UV light on development time, body size of all stages, reproductive power, successful hatching rate and naupli production ability of *Pseudodiaptomus incisus*, *Oithona rigida*

Experiment 4: Effect of temperature and food on development time, body size of all stages, reproductive power, successful hatching rate and naupli production of *Pseudodiaptomus incisus*, *Oithona rigida*

2.3. Some general preparations for experiments

2.3.1. Water

The water used for the experiment was mechanically filtered and salinity phased according to the design. Sea water from Cam Ranh Bay is pumped into a settling pond with a volume of 3000 m^3, then filtered through sand in a tank with a volume of 200 m^3 and then filtered through a 0.5 µm filter core. Fresh water used to mix salinity is tap water that has been dried and aerated for at least 48 hours before use. Salinity was prepared using the diagonal formula. Salinity is measured with a hydrometer.

2.3.2. Algae

Algae *Isochloropsis galbana* as feed for copepoda is sourced from Toan Hung Co., Ltd., Nha Trang. Algae were grown semi-continuously under laboratory conditions at 25°C (controlled by air conditioning). Algae were grown in 5 liter glass jars. The light uses algae farming from 2 - 60W LEDs (1.2 m long) with 24-hour continuous lighting mode. The medium used for algae culture was F/2 medium. The water used to grow algae is treated like that of Copepoda, but with a salinity of 25 ppt. Algae density was determined before feeding Copepoda. Experiments under content 1, 2 and 3 fed Copepoda with I. galbana with density of 30 000 – 35 000 cells/ml according to Doan et al. (2018). Experiment 4 used 3 species of algae *Isochloropsis galbana*, *Chaetoceros muelleri* and *Tetrselmis chui* in the experimental design in section 2.4.4.

Method to determine the density of algae

Using microscopy counting with erythrocyte counting chamber.

Take 100 ml of algae water into a beaker and fix it with 1 drop of Lugol, then pipette 1 ml into the red blood cell counting chamber and count at objective 4 -10, repeat 5 times.

Formula: Algae density = N (total number of cells in 5 cells) $*5*10^4$

2.3.3. Experimental system

The design includes 3 separate experimental systems corresponding to 3 temperature levels of 26ºC, 30ºC and 34ºC. Experimental system of 26ºC temperature, including 8 composite tanks (40 x 80 x 50cm) arranged in 2 rows in a closed room controlled by air conditioner. Experimental systems of 30 and 34ºC are 2 recirculating systems with 8 composite tanks (40 x 80 x 50cm) and 1 total tank for each system; The temperature is adjusted by the heater in the total tank and the water that has reached the test temperature is pumped to the tanks of the system by the pump. The culture system was operated stably for two days before the experiment.

The experimental system and the instruments used in the experiment were cleaned, labeled with full information about the treatment, the number of repetitions.

2.4. Experimental

2.4.1. Experiment 1: Effect of temperature and salinity on growth time, body size of all stages, fertility, hatching success rate and naupli production of *Pseudodiaptomus incisus*, *Oithona rigida*

Experimental water: The water used for raising copepods in experiment 1 was mixed and maintained at 8 salinity levels (5, 10, 15, 20, 25, 30, 35 and 40 ppt) throughout the experiment period.

Experiment 1.1. Experiment with species *P.incisus*

Copepod: Collecting copepoda in shrimp ponds in Cam Ranh (+11°49'N; +109°07'E) by a mesh of 200 µm mesh size, in July 2019, consisting of a mixture of 3 species *Pseudodiaptomus incisus*, *Oithona rigida* và *Eudactylopus latipes*. Environmental conditions of copepod ponds: temperature 28 – 29ºC, salinity 30 ppt. Copepoda was transferred to the wet

laboratory of Cam Ranh Aquaculture Research Center. Here, Copepoda is purified to 3 temperatures 26, 30 và 34ºC as described by Doan et al. (2019). Copepoda was grown under conditions of dissolved oxygen maintained at 5 – 6 mg/L; photoperiod 12 light:12 dark. Copepoda's food is algae *I. galbana* (30 000 – 35 000 cells/ml, theo Doan et al. (2018)). Simultaneously, pure salinity was carried out to experimental levels (5, 10, 15, 20, 25, 30, 35 và 40 ppt). The amplitude of salinity fluctuations during the purification process is from 2 to 3 ppt/time, with the interval between 2 times being 2 hours. Thereafter, the females bore eggs (3 600 individuals) of species *P. incisus* were selected based on the description by Mulyadi (2004) and they produced experimental nauplii corresponding to the treatments.

Experimental design and setup

Evaluation of the influence of each factor and the combination of salinity and temperature on the growth and reproduction of *P. incisus*, we conducted experiments with 8 levels of salinity (5, 10, 15, 20, 25, 30, 35 và 40 ppt) and 3 temperature levels 26ºC, 30ºC và 34ºC. The number of treatments is 24, with 5 replicates for each treatment, the total number of experimental units was 120. Each experimental unit is 1 plastic bottle (polyethylene) 1L. The levels of temperature (Doan et al., 2018) and salinity (Lund-Hansen et al., 2018) have shown the range of variation in coastal areas with a high distribution of *P. incisus*.

At the beginning of the experiment, 3,600 egg-bearing *P. incisus* females were randomly placed in 120 1L plastic bottles (30 females/bottle). Each glass is supplied with 800ml of sea water in 8 levels of salinity and 3 levels of temperature. They were incubated for 30 h according to the treatments (Doan et al., 2018, 2019; Grønning et al., 2019). Then, about 600 - 700 nauplii/bottle were collected for the experiment, the females were removed using a 200 µm mesh strainer. Nauplii were placed in glasses containing clean seawater, with the same temperature and salinity as each treatment and the experiment started.

During the whole culture period, *P. incisus* was fed with the same algae species and density as the domestication period; copepoda was fed 3 times a day at 6, 14 and 22 o'clock. Daily, water is added or renewed to ensure salinity. Temperature and salinity were checked daily at copepoda feeding. All bottles are lightly aerated to keep algae suspended and maintain dissolved oxygen 5 – 6 mg/L.

Methods of sample collection

Determining the development time and the size of the stages: Every day, we collected 30 ml of samples in each experimental unit (120 samples/day). The amount of copepoda was filtered using a 50 µm mesh mesh and transferred to a petri dish (labeled with treatment, repeat order and isolation order) and fixed with 4% formalin.

- Development time of the stages: monitor daily and record the composition of the stages in the sample for each experimental unit. Ratio of nauplii, copepodite, and adult stages used to determine developmental time of nauplii, copepodites and time to adulthood.

- Stage size: The copepods of the stages (720 nauplii, 600 copepodites, 120 males and 120 females) in the samples were measured for length using a stereomicroscope (SZ51, Olympus, Japan). The length of the nauplii was measured from the head to the end but excluding the antennae (Golez et al., 2004). The length of copepodites and adults was measured from the cephalothorax to the terminal abdominal segment (Doan et al., 2019; Grønning et al., 2019).

Determination of fecundity, successful hatching rate and 30 hr nauplii production

When 100% copepoda of each experimental unit was mature, copepoda was collected to determine 3 parameters of fertility, successful hatching rate and number of naupli/female after 30 h.

- Fertility: is the total number of eggs in the egg sac in the female. Females carrying 4 to 13 eggs in each experimental unit were collected (number dependent on abundance) and fixed with 4% formalin. Use a needle to separate and count the number of eggs in the egg sac under the stereo microscope (SZ51, Olympus, Japan).

- Hatching success rate: 10 egg-bearing females were randomly collected from each experimental unit. Each female was transferred to a well in a 12-well plate containing 3 ml of seawater with the corresponding salinity of the treatment and the algae I. galbana. The plates were placed in the respective temperature and salinity for 30 h. The offspring and occurrence of nauplii in the wells (small 4% lugol) were observed under a stereomicroscope (SZ51, Olympus, Japan) (Doan et al., 2019; Grønning et al., 2019).

- Number of nauplii/female after 30 h: Collect 4-15 egg-bearing females in each experimental unit, transfer to a glass with seawater and algae corresponding to the experimental conditions of each treatment. The number of replicates of each treatment was 5. After 30 h, nauplii were collected and counted under a stereo microscope (SZ51, Olympus, Japan) (Doan et al., 2019).

Experiment 1.2. Experiment with species *O. rigida*

Collected Copepoda similar to *P. incisus* in November 2019, including a mixture of 3 species Oithona rigida, Apocyclop royi and Eudactylopus latipes. Environmental conditions of copepoda ponds: temperature 28 – 29^{o}C, salinity 30 ppt. The process of temperature domestication is similar to that of *P. incisus*. Females of species *O. rigida* were selected according to the description of Radhika et al. (2016) to produce nauplii for the experiment.

Experimental design: Similar to experiment 1.1. The difference from experiment 1.1 is that the number of females carrying eggs/bottle (experimental unit) is 50 and the number of repetitions of each treatment is 10.

Method of collecting parameters: Similar to experiment 1.1

2.4.2. Experiment 2: Effect of temperature and photoperiod on development time, body size of all stages, fecundity, hatching success rate and naupli production of *Pseudodiaptomus incisus, Oithona rigida*

Experimental water: Water used for farming copepoda was mixed and maintained at salinity of 20 ppt with experiment 2.1 and 30 ppt with experiment 2.2 throughout the experimental period.

Experiment 2.1. Experiment with species *P.incisus*

Copepod: ollecting and purifying copepoda was similar to experiment 1.1. Environmental conditions of copepoda ponds: temperature 28 – 29^{o}C, salinity 30 ppt. Puree *P. incisus* to a salinity of 20 ppt. Proceed to produce nauplii for the same experiment as experiment 1.1.

Experimental design and setup

To test how extending artificial light time at night may alter the sensitivity of tropical zooplankton to extreme temperatures from marine heatwaves, we exposed a coastal calanoid copepod P. incisus to one of four photoperiods (0L:24D, 12L:12D, 18L:6D, and 24L:0D) and three temperatures (26, 30 and 34◦C). It resulted in a total of 12 treatment combinations. Each treatment had 10 replicates which were 1-L plastic bottles (polypropylene). The photoperiods were chosen to simulate different levels of lighting hours at night in the shallow coastal water from human activities. To have a full picture of how light may modulate the sensitivity of copepods to temperature, we also included the treatment 0L:24D, which had no light during the experimental period. However, we only discussed the effect of this light condition when it is relevant to the focus of our study. We simulated artificial light pollution using 12 LED lights (Model 12150079E, 220V/ 50 Hz and 20 W, EUROSUPER Inc., Vietnam) mounting 30 cm above the water surface. This is a widely used light source in coastal aquaculture farms along more than 2000 km of the coastal line in Vietnam. It has a peak wavelength of 455 nm and has the dominant spectrum from 430–630 nm (technical information from the led light), similar to the range of wavelengths in the study of Kim et al. (2019). In the inner Cam Ranh Bay, the seawater temperature of 26–34 ◦C occurs 334–338 days per year (Doan X.N., Dinh K.V., and Pham Q.H., unpublished data). The temperature of 34◦C is an extreme temperature that often occurs from spring to autumn (Doan et al., 2018).

To start the experiment, 3600 egg-carrying females were isolated and randomly allocated in 120 1-L bottles (30 females per bottle). Each bottle was filled with 720 ml of seawater with a salinity of 30 ppt. They were incubated at 26, 30 and 34◦C and the photoperiod of 12L:12D for 30 h for nauplii production (Doan et al., 2018, 2019; Grønning et al., 2019). Subsequently, nauplii in each bottle (ca. 1000 individuals) were collected; all females were removed from the bottle. The bottle was refilled with clean seawater and algae. All nauplii were returned to the bottle, and the experiment started at the designated temperatures and photoperiods.

During the whole rearing process, *P. incisus* was cared for and managed similarly to the experiment 1.1.

Method of collecting parameters: Proceed in the same way as experiment 1.

Experiment 2.2. Experiment with species *O. rigida*

Collecting copepod similar to P. incisus species in February 2020, including a mixture of 3 species *Oithona rigida*, *Apocyclop royi* and *Eudactylopus latipes*. Environmental conditions of copepoda ponds: temperature 28 – 29^{o}C, salinity 30 ppt. The process of temperature domestication is similar to that of *P. incisus*. Females of species *O. rigida* were selected according to the description of Radhika et al. (2016) to produce nauplii for the experiment.

Experimental design and setup: Similar to experiment 2.1. The difference from experiment 2.1 is that the number of females carrying eggs/bottle (experimental unit) was 50.

Method of collecting parameters: Similar to experiment 2.1

2.4.3. Experiment 3: Effect of temperature and UV light on development time, body size of all stages, reproductive power, successful hatching rate and naupli production ability of *Pseudodiaptomus incisus, Oithona rigida*

Experimental water: Water used for raising copepods was mixed and maintained at a salinity of 20 ppt with experiment 3.1 and 30 ppt with experiment 3.2 throughout the experiment period.

Experiment 3.1. Experiment with species *P.incisus*

Copepod: Collecting copepoda in shrimp ponds in Cam Ranh (+11◦49′24.74 N; +109◦07′25.34 E) by a mesh of 200 m mesh size, in May 2019, consisting of a mixture of 3 species *Pseudodiaptomus incisus*, *Oithona rigida* and *Eudactylopus latipes*. Environmental conditions of copepoda ponds: temperature 28 – 29^{o}C, salinity 30 ppt. Copepoda was domesticated and cared for similar to experiment 2.1.

Experimental design and setup: To test for the single and combined effects of ultraviolet ray and temperature on the productivity of a coastal calanoid copepod *P. incisus*, we exposed them to one of 3 ultraviolet ray (UV0; UVA và UVB) and three temperatures (26, 30 and 34°C). In total,we had 9 treatments, and each 10 replicates each (90 experimental units). Experimental units were 1-L plastic bottles (polypropylene). The UV modes are selected to simulate the UV situations present in the climate change reality in the coastal areas of Vietnam. To fully observe the sensitivity of copepoda to ultraviolet light and temperature, we also included the treatment UV0, i.e. no UV light throughout the experiment. Simulation of UV pollution using emitting LEDs UVA (Model TL-K 40W/10R ACTINIC BL, PHILIPS), UVB (Model PL-S9W/01/2P, PHILIPS), mounting 30 cm above the water surface, light on time was 8h, from 8h to 16h daily (reflects the actual lighting time of the weather). This is a type of UV lamp that is commonly used in Vietnam. UVA emitters have a maximum wavelength of 370 nm, in the range of 350-400 nm (according to lamp specifications), UVB emitters have a wavelength of 311 nm.

To start the experiment, 3600 egg-carrying females were isolated and randomly allocated in 120 1-L bottles (30 females per bottle). Each bottle was filled with 720 ml of seawater with a salinity of 20 ppt. They were incubated at 26^{o}C, 30^{o}C and 34^{o}C and 12L:12D lighting conditions for 30h (Doan et al., 2018, 2019; Grønning et al., 2019). Then, about 1,000 nauplii/bottle were collected for the experiment, the females were removed using a 200 µm mesh filter. Nauplii were placed in glasses containing clean seawater, with the same temperature and salinity as each treatment and the experiment started.

During the whole rearing process, *P. incisus* was cared for and managed similarly to the experiment 1.1.

Method of collecting parameters

Determining the development time and the size of the stages: Every 24 hours, we collect 30 ml of samples in each experimental unit (90 samples/day) to determine the development time and the size of the stages. The method of sampling and preserving samples is similar to the experiment 1.1.

Parameters were collected, preserved and observed similar to the experiment 1.1.

Experiment 3.2. Experiment with species *O. rigida*

Collecting copepoda was similar to *P. incisus* species but different from shrimp pond locations (+11°49'N; +109°07'E) in July 2020, a mixture of 3 species *Oithona rigida*, *Apocyclop royi* và *Eudactylopus latipes*. At the sampling site, temperature and salinity in the pond were 28–29°C and 30 ppt respectively. The process of temperature domestication is similar to species *P. incisus*. Females of species *O. rigida* were selected according to the description of Radhika et al. (2016) to produce nauplii for the experiment.

Experimental design and setup: Similar to the experiment 3.1. The difference from experiment 3.1 is that the number of females carrying eggs/bottle (experimental unit) was 50.

Method of collecting parameters: Similar to experiment 3.1

2.4.4. Experiment 4: Effect of temperature and feed on growth time, body size of all stages, reproductive strength, successful hatching rate and naupli production of *Pseudodiaptomus incisus, Oithona rigida*

Experimental water: Water used for farming copepoda was mixed and maintained at a salinity of 20 ppt with experiment 4.1 and 30 ppt with experiment 4.2 throughout the experimental period.

Experiment 4.1. Experiment with species *P.incisus*

Copepod: Collecting copepoda in shrimp ponds in Cam Ranh (+11◦49′24.74 N; +109◦07′25.34 E) similar to the experiment 1.1. At the sampling site, temperature and salinity in the pond were 28–29°C and 30 ppt respectively. *P. incisus* was cared for and bred similar to the experiment 1.1. After purifying temperature and salinity, *P. incisus* not fed for 1 day (to limit the effect of algae treatment *I. galbana* compared to the other two species of algae). Then, egg-bearing females (3 600 individuals) of *P. incisus* species were selected based on the description of Mulyadi (2004) and they produced experimental nauplii corresponding to the treatments.

Experimental design and setup

To test for the single and combined effects of food and temperature on the productivity of a coastal calanoid copepod *P. incisus*, we exposed them to one of three commonly used microalgal species in aquaculture: *Chaetoceros muelleri*, *Isochrysis galbana* và *Tetraselmis chui* at concentrations of 160; 800 và 1600 µgC/L and three temperatures (26, 30 and 34°C). In total, we had 27 treatments, and each 10 replicates each (270 experimental units). Experimental units were 1-L plastic bottles (polypropylene).

At the beginning of the experiment, 8,100 egg-bearing *P. incisus* females were randomly assigned to 270 1-L bottles (30 females per bottle). Each bottle was filled with 720 ml of seawater with a salinity of 20 ppt. They were incubated at 26^{o}C, 30^{o}C and 34^{o}C and 12L:12D lighting conditions for 30h (Doan et al., 2018, 2019; Grønning et al., 2019). Then, about 900 - 1,000 nauplii/bottle were collected for the experiment, the females were removed using a 200 µm mesh strainer. Nauplii were placed in bottles containing clean seawater, with the same temperature and density of algae according to each treatment and the experiment started.

During the whole rearing process, *P. incisus* was cared for and managed similarly to experiment 1.1.

Method of collecting parameters: similar to experiment 1.1

Nauplii production: Ten oviparous females were randomly collected from each experimental unit, transferred to a glass containing seawater and algae corresponding to the

experimental conditions of each treatment. The number of replicates of each treatment was 10. Monitor the number of nauplii for the entire life cycle of female Copepoda. Daily, nauplii was collected and counted under the stereo microscope (SZ51, Olympus, Japan) (Doan et al., 2019); female copepda was cared for similar to during the experiment. Follow-up stops when the female dies; If a male dies, another male will be replaced. The number of nauplii/female is the mean total number of nauplii of each female under the treatment.

Experiment 4.2. Experiment with species *O. rigida*

Collecting copepoda was similar to *P. incisus* species but different from shrimp pond locations (+11°49'N; +109°07'E) in February 2020, a mixture of 3 species *Oithona rigida*, *Apocyclop royi* và *Eudactylopus latipes*. At the sampling site, temperature and salinity in the pond were 28–29°C and 30 ppt respectively. The process of temperature domestication is similar to species *P. incisus*. Females of species *O. rigida* were selected according to the description of Radhika et al. (2016) to produce nauplii for the experiment. *O. rigida* not fed for 1 day (to limit the effect of algae treatment *I. galbana* compared to the other two species of algae). Then, egg-bearing females (13,500 individuals) of O. rigida were selected based on the description of Radhika et al. (2016) and they produced experimental nauplii corresponding to the treatments.

Method of collecting parameters: similar to experiment 4.1

At the beginning of the experiment, 13,500 egg-bearing *O. rigida* females were randomly assigned to 270 1-L bottles (50 females per bottle). Each bottle was filled with 720 ml of seawater with a salinity of 30 ppt. They were incubated at 26ºC, 30ºC and 34ºC and 12L:12D lighting conditions for 30h (Doan et al., 2018, 2019; Grønning et al., 2019). Then, about 900 - 1,000 nauplii/bottle were collected for the experiment, the females were removed using a 200 µm mesh strainer. Nauplii were placed in bottles containing clean seawater, with the same temperature and density of algae according to each treatment and the experiment started.

During the whole rearing process, *O. rigida* was cared for and managed similarly to experiment 1.1.

Method of collecting parameters: Similar to experiment 4.1. *O. rigida*'s stages based on the description by Takahashi and Uchiyama (2007); Santanam and Perumal (2013).

2.5. Statistical analyses

Data were stored and edited initially using Microsoft Excel 2016. Data were checked for normal distribution and homogeneity of variance. Analysis of linear model (GLM) to evaluate the effect of temperature with either salinity or photoperiod or UV light or feed and their interactions on growth time, stage size and fertility. Data on hatching success rate and number of 30 hr nauplii production in experiments 1 and 2 were converted to log(x+1) before ANOVA analysis. In all treatment models, temperature along with either salinity or photoperiod or UV light or feed were the two main influencing factors. Statistical significance level is $p < 0.05$. The data of experiments 1.1 and 2.1 were analyzed on Statistica v12 (StatSoft Inc., Tulsa, OK, USA). All remaining experiments were analyzed on Minitab®19 (Minitab Inc, USA). The graphs were plotted with SigmaPlot 14 software for Windows (Systat Software, Inc., Germany). Data are expressed as mean ± standard error (SE) for 10 replicates of 1 treatment (for experiment 1 it was 5 replicates for 1 treatment).

CHAPTER 3. RESULTS AND DISCUSSIONS

3.1. Effect of temperature and salinity on copepods

3.1.1. *Pseudodiaptomus incisus*

Development of *Pseudodiaptomus incisus*: The development time of nauplii and copepodite stages was shorter at high temperature ($P<0.001$), the effect of temperature was dependent on salinity ($P<0.001$), the results were evident in the treatments of 15 and 20 ppt ($P<0.001$). Two treatments with salinity of 15 and 20 ppt had 100% copepodite transition to maturity at day 8, 9 and 10, respectively, at 34ºC, 30ºC and 26ºC, respectively. The development time of copepodite stage was longest in low (5ppt) and high (35ppt, 40 ppt) salinity treatments at 26ºC, 100% adult copepodide at day 14 (Figure 3.1).

The biomass of development stages: N2 -N6 had the largest biomass at the treatment temperature of 26ºC and salinity of 15 ppt. Stages C3 and C5 had the highest biomass at 15 – 20 ppt salinity, smaller in both low and high salinity. At salinities, the biomass of C3 and C5 was smaller than at higher temperatures (Figure 3.2). The biomass of males and females was greatest in the treatments at 26ºC and 15 - 20 ppt (interaction of temperature × salinity, Figure 3.3) ($P<0.001$).

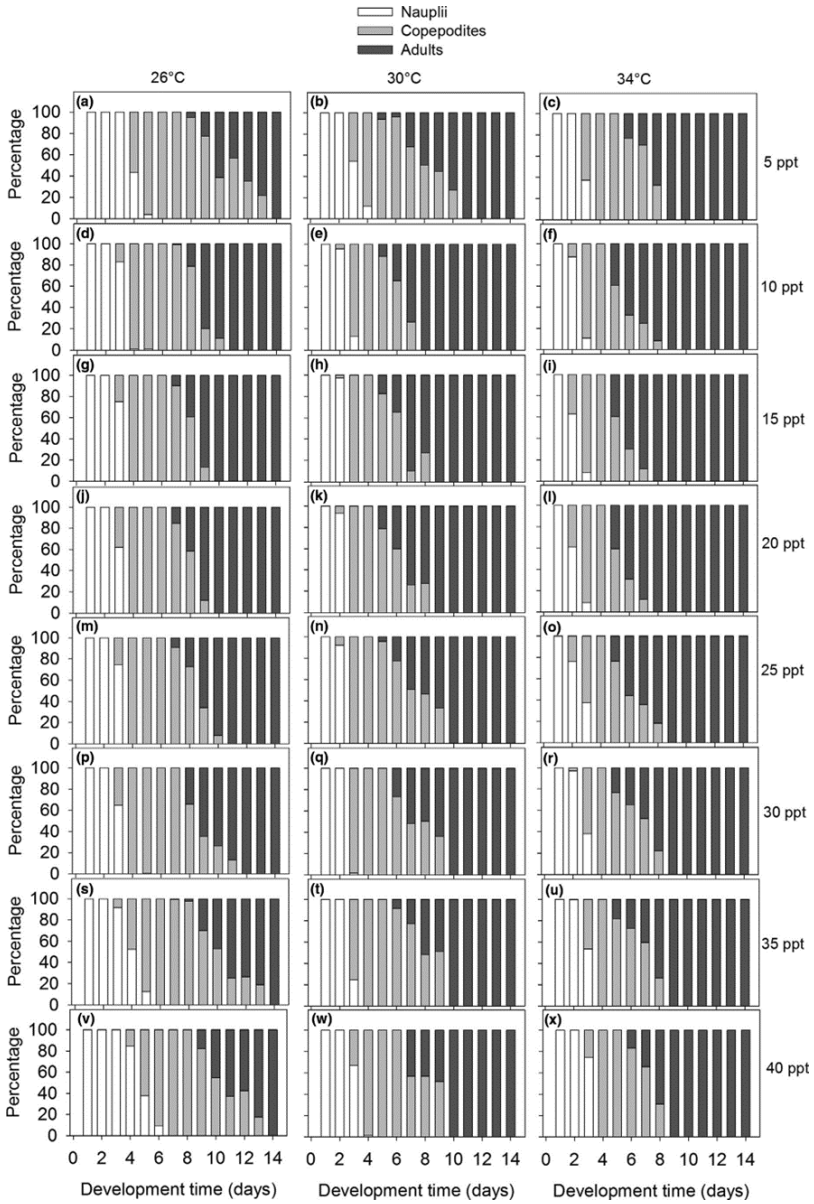


Figure 3.1. The percentages of nauplii, copepodites and adult of the calanoid copepod *P. incisus* in response to temperature and salinity

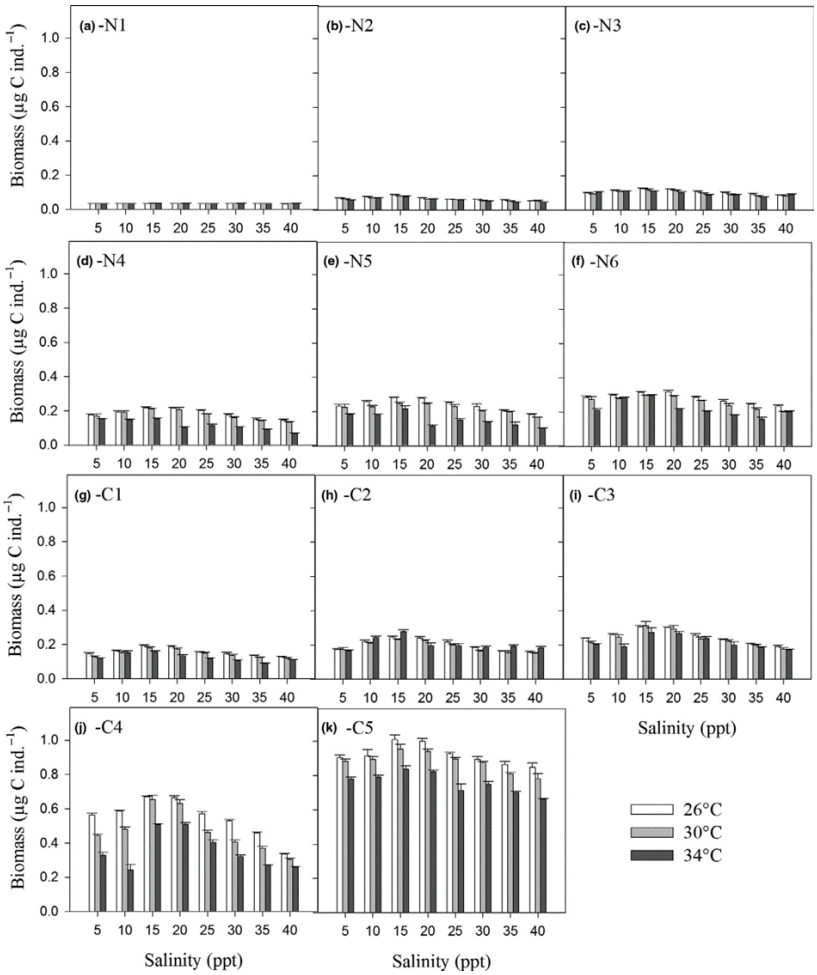


Figure 3.2. Biomass in carbon content (µg C) of nauplii stages I-VI (N1–N6) and copepodite stages I-V (C1–C5) of the calanoid copepod *P. incisus* in response to temperature and salinity. Data are means + 1 SE

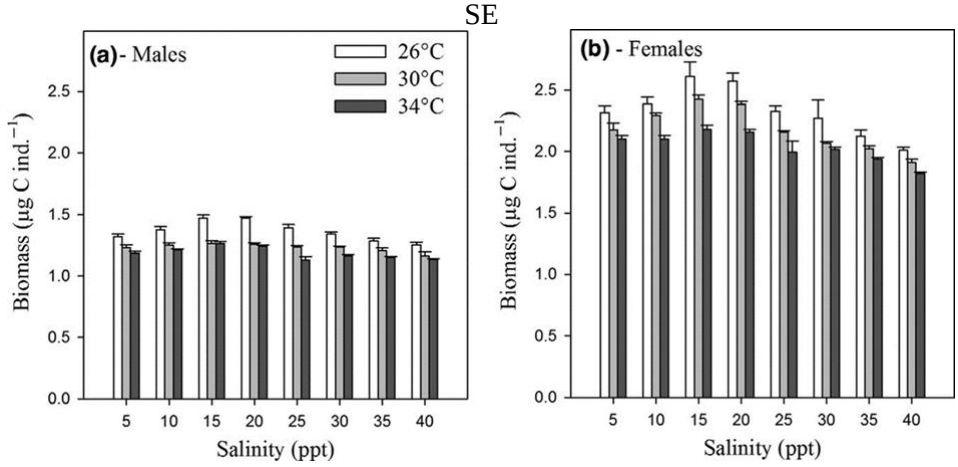


Figure 3.3. Biomass in carbon content (µg C) at maturity of males (a) and females (b) of the calanoid copepod *P. incisus* in response to temperature and salinity. Data are means + 1 SE.

Clutch size: The treatment of 26°C and 10 ppt showed that *P. incisus* had the greatest fertility, this parameter decreased by 10% in the treatment with salinity of 5 ppt (Figure 3.4). At 30°C, fecundity was greatest in the treatment with salinity of 5-15 ppt and decreased with increasing salinity. At 34°C, the fertility of *P. incisus* decreased by 10-20% at 5-15 ppt salinity compared to 20 ppt salinity and less than 30% for the same salinity treatment at 26°C and 30°C (main effect of temperature, Figure 3.4). In the salinity range of 25-40 ppt, a decrease in fertility with increasing salinity was observed at all three experimental temperatures. The 40 ppt salinity

treatment showed a 45% decrease in *P. incisus* fertility compared with the 20 ppt treatment (main effect of salinity, Figure 3.4).

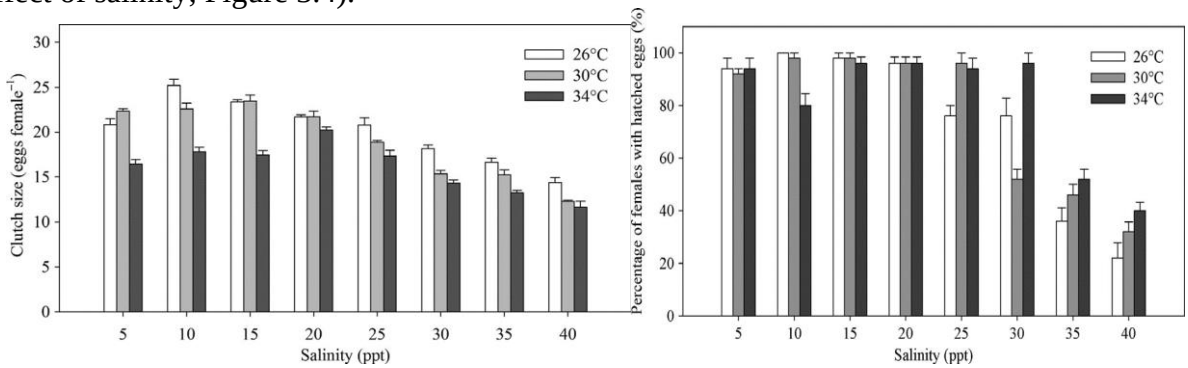


Figure 3.4. The clutch size and hatching success of the calanoid copepod *P. incisus* as a function of temperature and salinity. Data are means + 1 SEs

Percentage of females with hatched eggs: At all experimental salinities, the hatching success rate was higher with increasing temperature ($P<0.001$) (main effect of temperature; Figure 3.4). Over 90% of females hatched eggs and were not statistically different in treatments with salinity from 5 to 25 ppt. The hatching success rate decreased with increasing salinity, observed clearly at the salinity of 35-40 ppt and more clearly at low temperature ($P<0.001$) (interaction temperature x salinity). ; Figure 3.4). This parameter was lowest (22%) in the treatment of 26^{o}C and 40 ppt.

30 hr nauplii production: At all 3 temperatures, 30 hr nauplii production of *P. incisus* was highest at a salinity 20 ppt ($P<0.001$). 30 hr nauplii production of was lower at both low and high salinity (Figure 3.5).

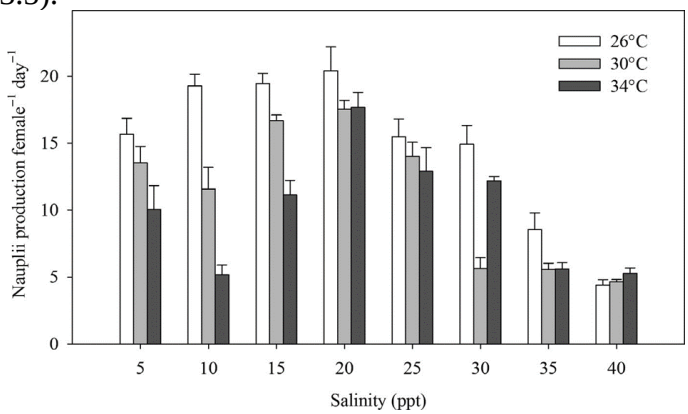


Figure 3.5. The nauplii production of the calanoid copepod *P. incisus* as a function of temperature and salinity. Data are means + 1 SEs

3.1.2. *O. rigida*

Development of *O. rigida*: The development time at the nauplii and copepodites stages was faster than at 30^{o}C, the results were clearly shown in the two salinity treatments of 25 and 30 ppt ($P<0.001$). The development time of copepodite stage was longest in the low salinity treatment (5ppt and 10ppt) at 26^{o}C, 100% of copepodide matured on 13th and 12th day.

Body size: The body size of *O. rigida* was smaller at higher temperatures (main effect of temperature, Figure 3.8). The effect of temperature on body size of *O. rigida* was strongest at 34^{o}C and 15 and 40 ppt. Accordingly, O. rigida had the smallest body size in the treatment of 34^{o}C and salinity of 15 and 40 ppt (interaction temperature x salinity).

Clutch size: In group 1, the fertility of *O. rigida* did not differ between salinities but *O. rigida* had the greatest fertility at 30^{o}C. In group 2, treatment at 30^{o}C and 25-30 ppt showed the greatest fertility of *O. rigida*.

Percentage of females with hatched eggs: In group 1, the hatching success rate of *O. rigida* did not differ between salinities but *O. rigida* had the greatest success rate at 30^{o}C. In

group 2, all experimental salinities, the hatching success rate was higher at 26 and 30ºC and lowest at 34ºC (P<0.001). The success rate of hatching increased with increasing salinity, observed clearly at salinity 25-35 ppt and more clearly at low temperature (P<0.001) (interaction of temperature x salinity). This parameter was lowest in the treatment of temperature 34ºC and salinity of 15-20 ppt.

30 hr nauplii production: In group 1, 30 hr nauplii production of *O. rigida* did not differ between temperatures, but *O. rigida* had the largest 30 hr nauplii production at 10 ppt. In group 2, at all 3 temperatures, 30 hr nauplii production of *O. rigida* was highest at salinity of 30 ppt (P<0.001). 30 hr nauplii production of *O. rigida* was lower at both low and high salinity. 30 hr nauplii production *O. rigida* was highest at 26ºC, lower at 30ºC and lowest at 34ºC (main influence of temperature).

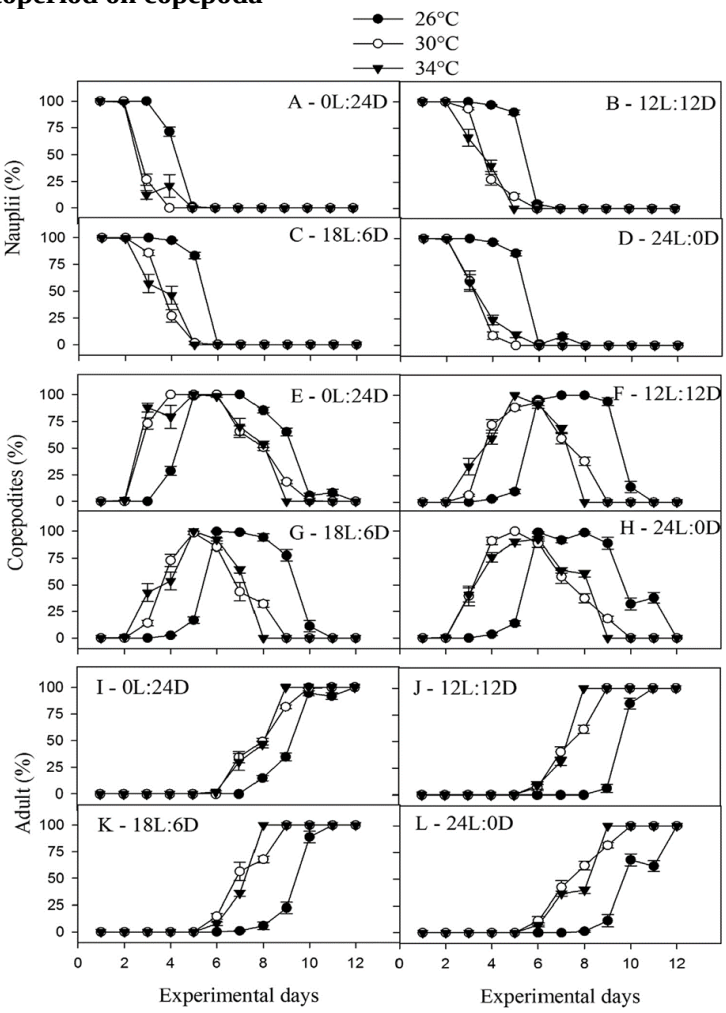
3.2. Effect of temperature and photoperiod on copepoda

3.2.1. *P. incisus*

Development of *P. incisus*: The development time of the nauplii stage of *P. incisus* was faster in the absence of light (0L:24D) and high temperature (30 and 34ºC). Nauplii (100%) switched to copepodites stage on day 6 in all treatments. The copepodites stage grew faster in 12L: 12D and 18L: 6D light conditions than in no light and 24L:0D. Under continuous lighting conditions (24L:0D), the development time of nauplii of *P. incisus* did not increase but was longer in the copepodite stage. The development time of *P. incisus* was shortest at 34ºC, followed by 30ºC and slowest at 26ºC. The development time from nauplii to adults of *P. incisus* was longest in treatment 24L:0D (Figure 3.6).

Body size: The body size of *P. incisus* from nauplii 2, copepodite and adults was larger in 12L:12D and 18L:6D regimes, the smallest in 24L:0D treatment. This parameter is smaller at high temperature, most evident in the treatment of 24L:0D and 34ºC (Figure 3.7).

Clutch size: This parameter of *P. incisus* is highest at 26ºC, smaller at 30ºC and lowest at 34ºC.

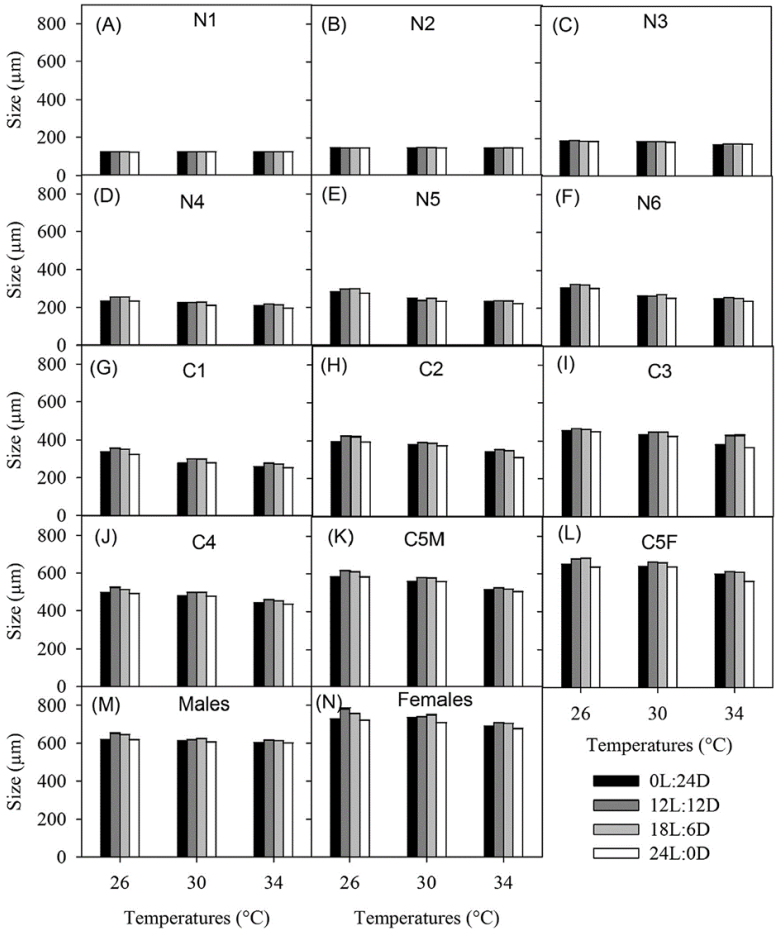


Hình 3.6. Development of nauplii, copepodites, and adult of the calanoid copepod *P. incisus* in response to the photoperiod and temperature

Fertility was lowest in 0L:24D and highest in 12L:12D at 26ºC (Figure 3.8).

Percentage of females with hatched eggs: The successful hatching rate of *P. incisus* was high (98.75%) at 26ºC and did not differ between photoperiods (Figure 3.13). The hatching success rate was similar at 30ºC and 34ºC in 3 photoperiods 0L:24D, 12L:12D, 18l:6D. However, the hatching success rate decreased sharply in 24L:0D, namely 74% and 86% at 30ºC and 34ºC respectively. This has reflected the effects of temperature, photoperiod and the interaction of these two factors (P<0.001, Figure 3.8).

30 hr nauplii production: 30 hr nauplii production decreased by 12% and 30% respectively at 30ºC and 34ºC compared to 26ºC and higher in 12L:12D and 18L:6D. Increased temperature reduced 30 hr nauplii production of *P. incisus* and was more strongly expressed in 24L:0D and 0L:24D than in 12L:12D (Figure 3.9).



Hình 3.7. Size of all developmental stages of the calanoid copepod *P. incisus* in response to the photoperiod and temperature. N1–N6: nauplii stages I–VI; C1–C4 = copepodite stages I–IV. C5M = male copepodite stage 5, C5F = female copepodite stage 5. Data are means + standard errors of 10 replications per experimental treatment.

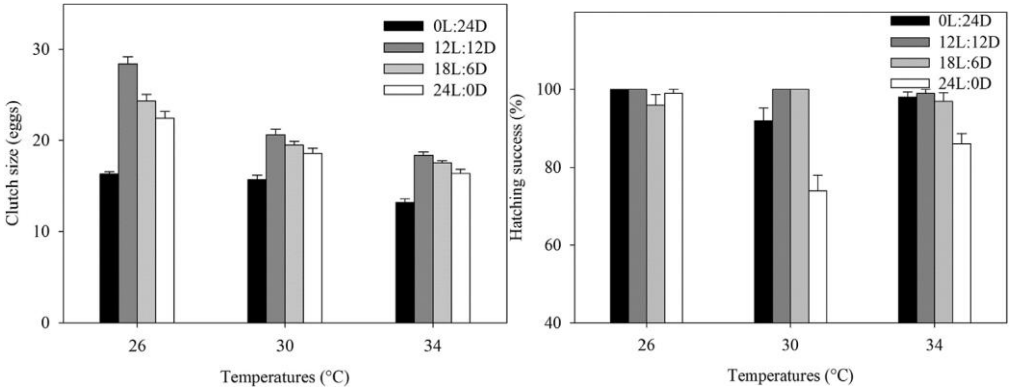


Figure 3.8. The clutch size and hatching success of the calanoid copepod *P. incisus* as a function of photoperiod and temperature. Data are means + 1 SEs

3.2.2. *O. rigida*

Development of *O. rigida*: The development of the nauplii stage was faster in the high-temperature salinity treatments (30 and 34ºC), the nauplii stage development time was about 4 days; about 6-7 days at 26ºC at all 4 photoperiod levels. *O. rigida* changed 100% to copepodite the slowest at 26ºC (8th day) and 5th day at high temperature (30 and 34ºC). *O. rigida* is 100% mature by day 8, 9 and 10

at 34ºC, 30ºC and 26ºC respectively. Growth in the *O. rigida* phases is faster than in the 0L:24D photoperiod; 12L:12D and 18L:6D.

Body size: Stage N3 – N6, copepodites and adults had the largest body size in treatment 12L:12D and the smallest in treatment 24L:0D. The body size of *O. rigida* was smaller at higher temperatures (main effect of temperature).

Clutch size: Clutch size of *O. rigida* decreased when the temperature increased from 26 to 34ºC. The maximum fertility was in the treatment 26ºC and 18L:6D, smaller than in the treatment 24L:0D and 0L:24D, the lowest in treatment 12L:12D.

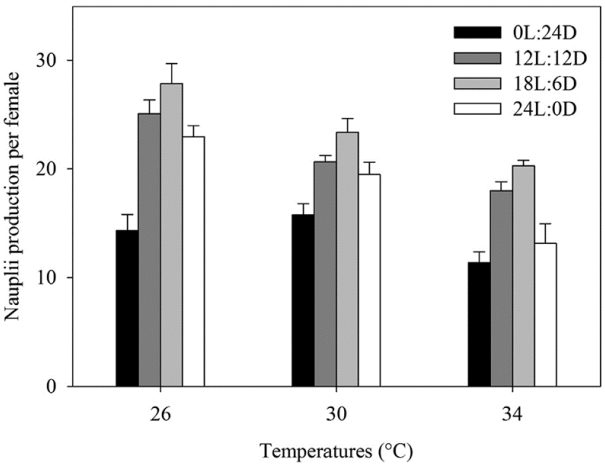


Figure 3.9. The nauplii production of the cyclopoid copepod *P. incisus* as a function of photoperiod and temperature.

Percentage of females with hatched eggs: The hatching success rate of *O. rigida* was high (90.75%) at 30ºC and four photoperiods (Figure 3.17). The hatching success rate no difference at 30ºC and 34ºC at three photoperiods 0L:24D, 12L:12D, 18l:6D. However, the hatching success rate decreased sharply in 24L:0D, namely 82.25% and 71.75% at 26ºC and 34ºC respectively. This reflected the impact of temperature, photoperiod and the interaction of these two factors on the hatching success rate (P<0.001).

30 hr nauplii production: 30 hr nauplii production was higher than in photoperiod 0L:24D, maller in the 12L:12D and 18L:6D, the smallest in the 24L:0D treatment and the highest results at 30ºC.

3.3. Effect of temperature and ultraviolet on copepoda

3.3.1. *P. incisus*

Development of *P. incisus*: The development time of the nauplii stage of *P. incisus* was faster when the temperature was high (34ºC). *P. incisus* turned 100% to the adult stage on days 9 and 10, respectively, at 34 and 30ºC of UVB treatment; In the treatment of 26ºC and UVB 100% copepodite died (Figure 3.10).

Body size: This parameter did not show a clear difference between treatments at nauplii stage, but to copepodite and adults, body size was larger at 26ºC, highest in UVA, very small in UV0 treatment, the lowest in the UVB treatment (Figure 3.11).

Clutch size: Treatment of 26ºC and UV0 showed the greatest fertility of *P. incisus*, this parameter decreased by 12 and 34% in UVA and UVB treatments compared to UV0 (influence of UV, Figure 3.21). The fertility of *P. incisus* was greatest at 26ºC, smaller at 30ºC and 34ºC, this parameter decreased by 34% at 30 and 34ºC compared to 26ºC (main effect of temperature, Figure 3.12).

Hatching success: In all 3 experimental UV, the hatching success rate decreased when the temperature increased from 30 to 34ºC and at low temperature (26ºC) (P<0.001). The hatching success rate was highest in the UV0 treatment, smaller in UVA and lowest in UVB (P<0.001) (Figure 3.12).

30 hr nauplii production: At all 3 temperatures, 30 hr nauplii production of *P. incisus* was highest at 26ºC (P<0.001). 30 hr nauplii production of P. incisus was lower than at high temperature. Effect of temperature on 30 hr nauplii production of *P. incisus* depends on UV. The

results showed that 30 hr nauplii production of *P. incisus* varied between temperatures and was clearly expressed in the UVB and the temperature of 34ºC. At the temperature of 26ºC, 30 hr nauplii production of *P. incisus* only obtained data of UV0 and UVA, in the UVB treatment *P. incisus* died, showing the strongest interaction between temperature and UV (Figure 3.13).

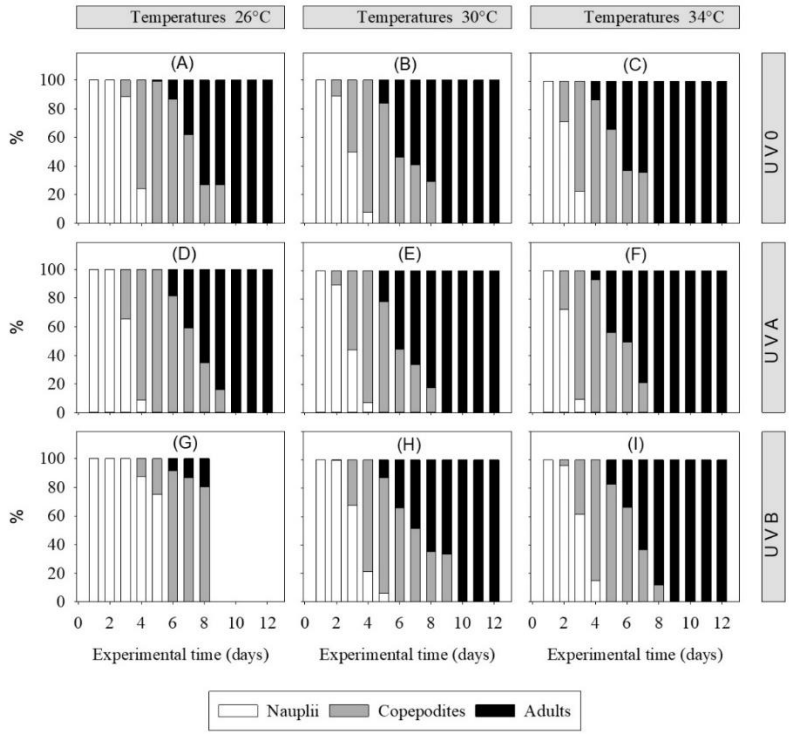


Figure 3.10. The percentages of nauplii, copepodites and adult of the calanoid copepod *P. incisus* in response to temperature and ultraviolet

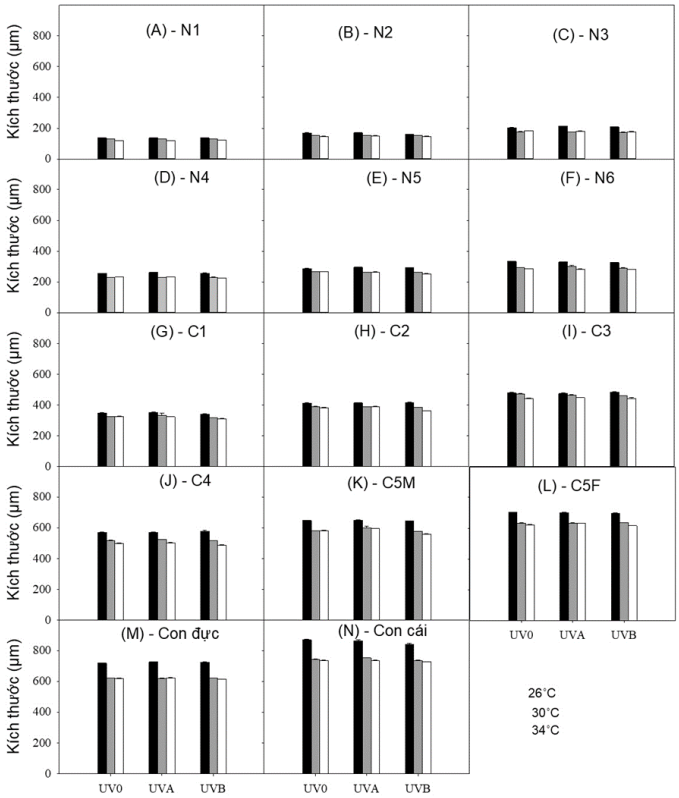


Figure 3.11. Size of all developmental stages of the calanoid copepod *P. incisus* in response to the temperature and ultraviolet. N1–N6: nauplii stages I–VI; C1–C4 = copepodite stages I–IV. C5M = male copepodite stage 5, C5F = female copepodite stage 5

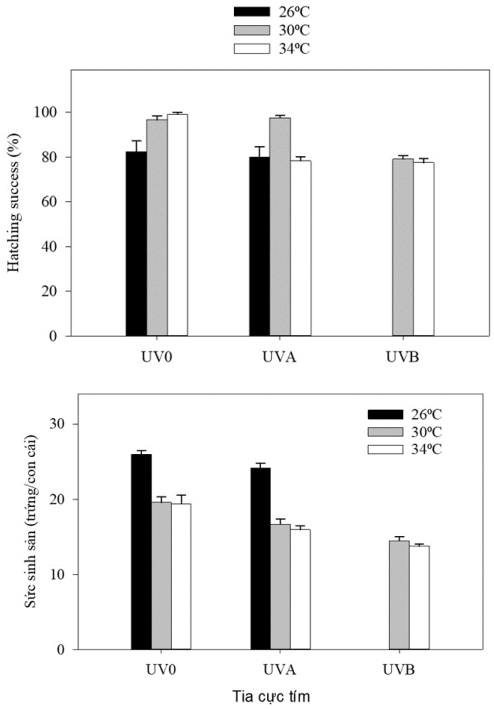


Figure 3.12. The hatching success and clutch size of the calanoid copepod *P. incisus* as a function of temperature and ultraviolet

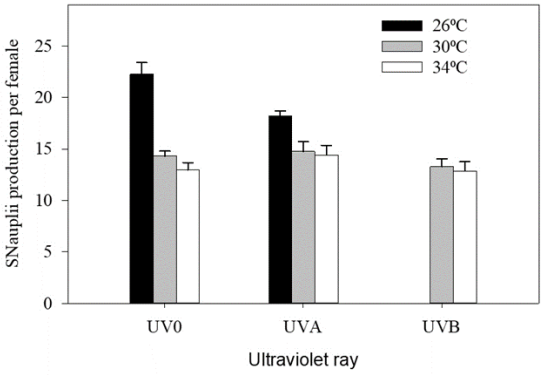


Figure 3.13. The nauplii production of the calanoid copepod *P. incisus* as a function of temperature and ultraviolet. Data are means + 1 SEs

3.3.2. *O. rigida*

Development of *O. rigida* : The development time of the nauplii stage of *O. rigida* was faster than in the UVA treatment at 30 and 34ºC. This parameter was significantly different in the UVB treatment compared with UVA and the control (UV0), specifically, *O. rigida* switched to the copepodite stage 2-3 days earlier in the UV0 and UVA treatments than in UVB. *O. rigida* changed 100% to the adult stage on days 8, 10 and 11 respectively with the temperature of 34, 30 and 26ºC of UVB treatment.

Body size: The body size of *O. rigida* at the N4 - N6 stage, copepodite and adults decreased with increasing temperature and showed the strongest expression in the treatment of 34ºC and UVB. This parameter was highest in UV0, very small in UVA treatment, and lowest in UVB treatment (P<0.001).

Clutch size: Treatment of 26ºC and UV0 showed the highest fertility of *O. rigida*, this parameter decreased by 10 and 35% in UVA and UVB treatments compared with UV0 (influence of UV, Figure 3.25). Clutch size of *O. rigida* was highest at 26ºC, smaller at 30ºC and 34ºC. Temperature reduced the fertility of *O. rigida*, the strongest expression in UVB and 34ºC treatment, the results showed that fertility in this condition, the number of eggs/female was 26 at UV0, 23 eggs /female in UVA compared with 16 eggs/female in UVB (interaction temperature x UV).

Hatching success: In all 3 experimental UV, the hatching success rate decreased when the temperature increased from 26 to 34ºC (P<0.001) and was highest in UV0 treatment, smaller in UVA and lowest in UVB (P<0.001).).

30 hr nauplii production: In all 3 UV, 30 hr nauplii production of *O. rigida* was highest at 26ºC (P<0.001). The results showed that the number of nauplii/female was highest in UV0 treatment, smaller in UVA and smallest in UVB.

3.4. Effect of temperature and food on copepods

3.4.1. *P. incisus*

Development of *P. incisus*: In all 3 experimental algae species (*C.muelleri, I. galbana, T. chui*), the development time of the nauplii and copepodites stages of *P. incisus* was 1 - 3 days faster at the algae density of 800 and 1600 µgC/L than at the density of 160 at all 3 temperatures

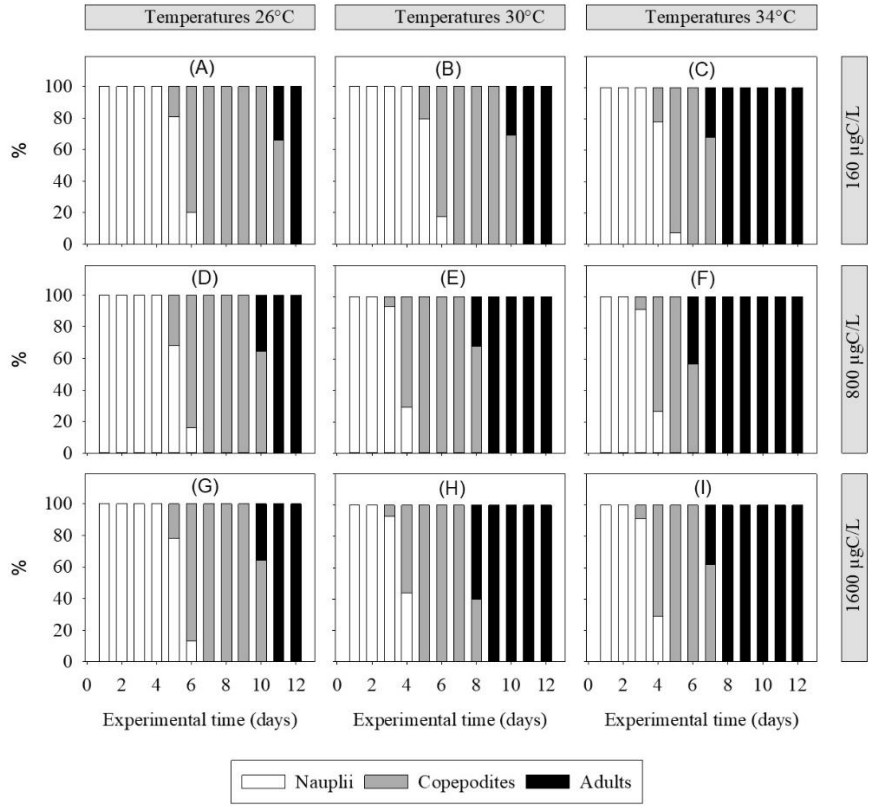
of 26, 30 and 34ºC. *P. incisus* had the fastest 100% maturation rate in the 34ºC treatment and the algae density of 800, 1600 µgC/L, the slowest at 26ºC and the density of 160 µgC/L (Figure 3.14).

Body size: In all 3 experimental algae, the body size of all stages of *P. incisus* decreased when the temperature increased from 26 to 34ºC and was larger at the food density of 800 and 1600 µgC/L (Figure 3.28).

Clutch size: In all three experimental species of algae, the treatment of 26ºC and 1600µgC/L was the greatest reproductive power of *P. incisus*. The fertility of *P. incisus* was greatest at 26ºC, smaller at 30ºC and lowest at 34ºC.

Hatching success: In all 3 algae species, the successful hatching rate was highest in the treatment of 800 µgC/L. This parameter was highest at 30ºC and lowest at 26ºC, observing *P. incisus* eating algae *I. galbana* and *T. chui*, the results were not different at three temperatures in *C.muelleri* (Figure 3.15).

30 hr nauplii production: In all three experimental algae species, 30 hr nauplii production of *P. incisus* was highest at 26ºC and density 800 - 1600µgC/L when eating *C.muelleri* and *I. galbana*, but the highest at 30ºC when observed with *T. chui* (Figure 3.16).



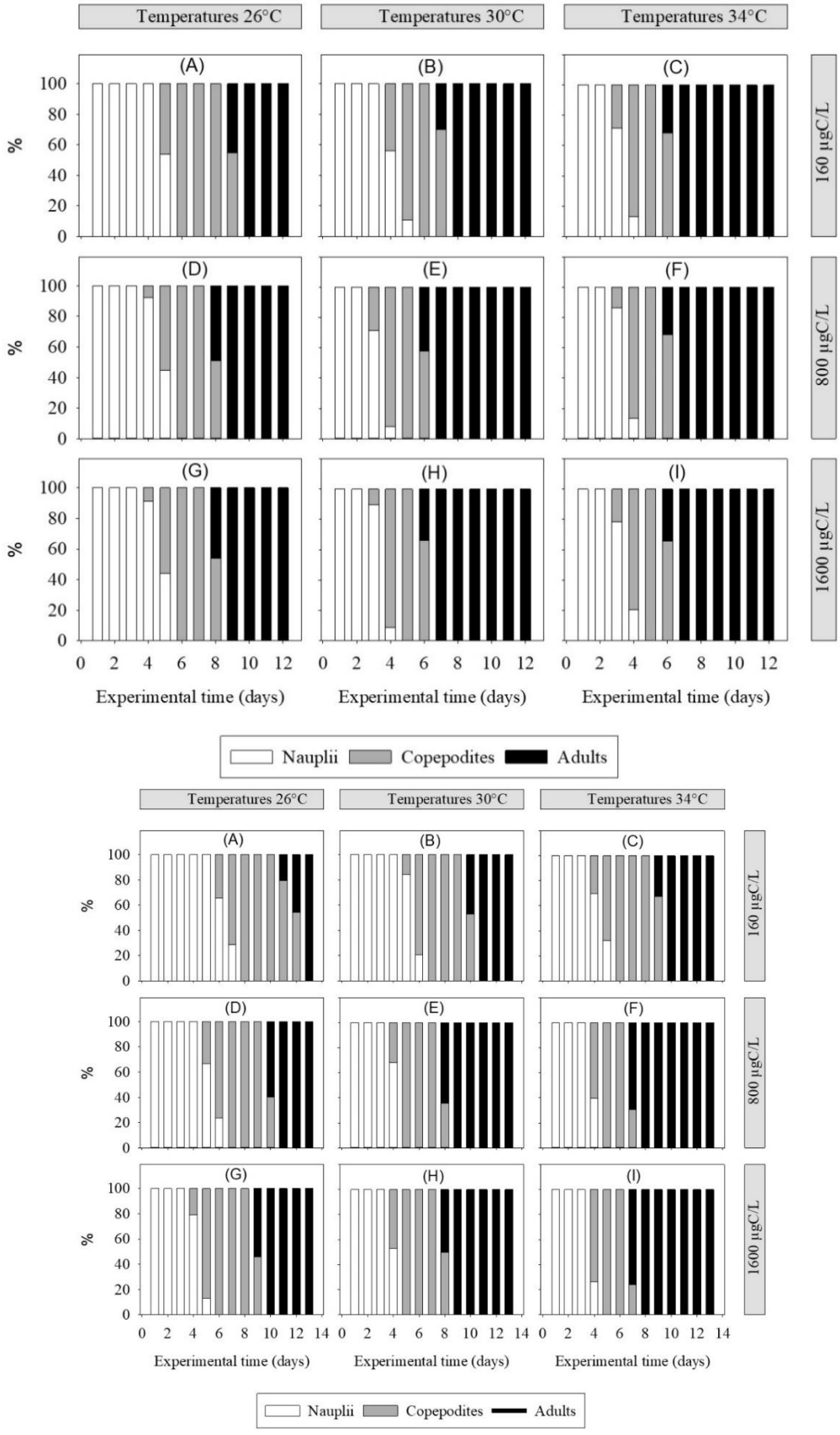


Figure 3.14. The percentages of nauplii, copepodites and adult of the calanoid copepod *P. incisus* in response to temperature and food. A – *C. muelleri*; B – *I. galbana*; C – *T. chui*

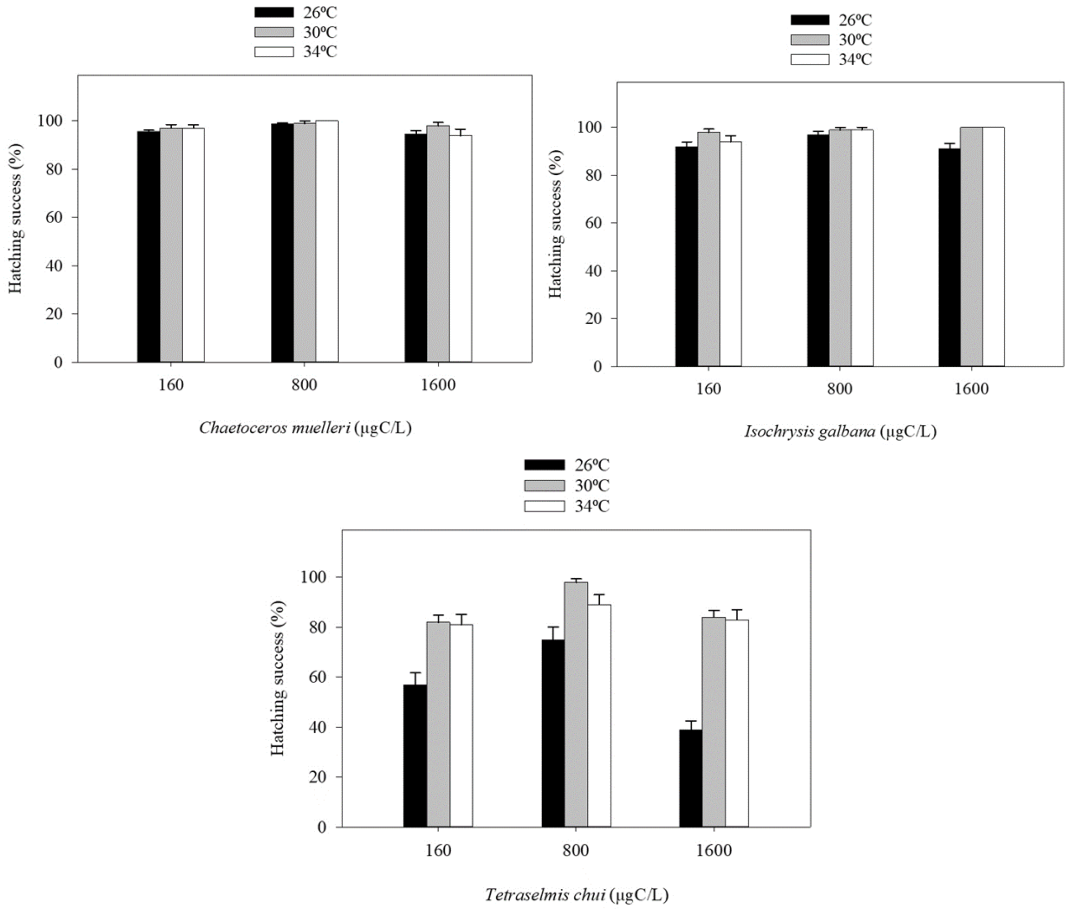


Figure 3.15. The hatching success of the calanoid copepod *P. incisus* as a function of temperature and food. A – *C. muelleri*; B – *I. galbana*; C – *T. chui*

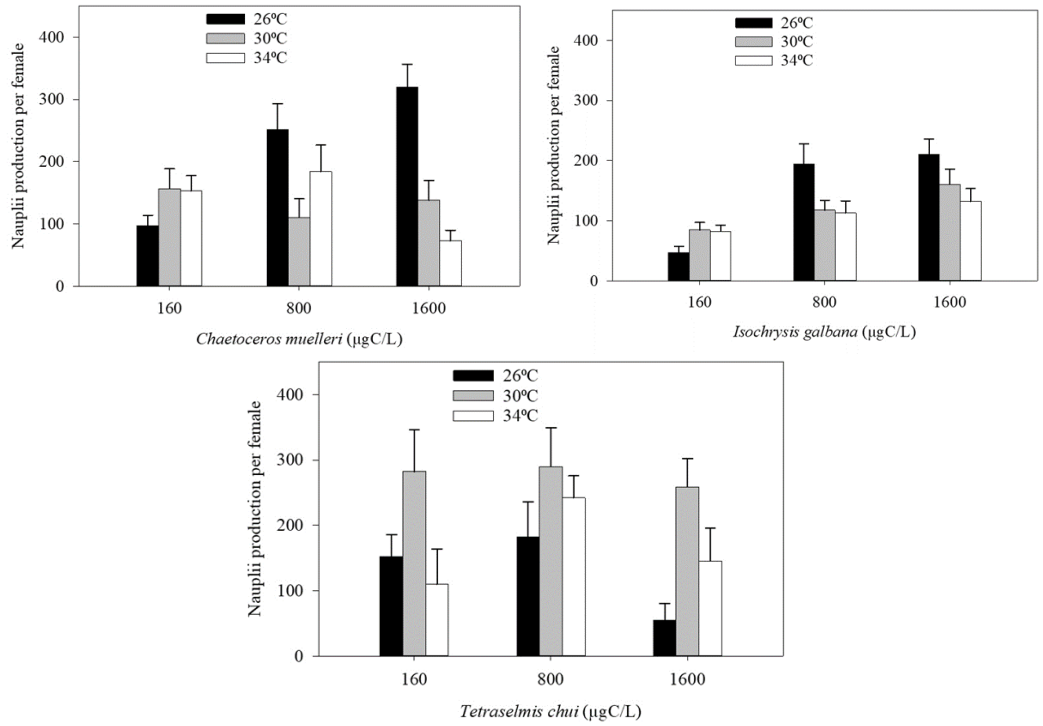


Figure 3.16. The nauplii production of the calanoid copepod *P. incisus* as a function of temperature and food. A – *C. muelleri*; B – *I. galbana*; C – *T. chui*

3.4.2. *O. rigida*

Development of *O. rigida*: In all 3 experimental algae species (*C.muelleri, I. galbana, T. chui*), the development time of the nauplii, copepodites stages of *O. rigida* was faster by 1-2 days at the density of 800 and 1600 µgC/L compared with the density of 160 at all 3 temperatures of 26, 30 and 34ºC. *O. rigida* had the fastest maturation rate of 100% in the 34ºC treatment and the algae density of 800, 1600 µgC/L, the slowest at 26ºC and the density of 160 µgC/L.

Body size: In all three experimental species of algae, the body size of the stages of *O. rigida* decreased when the temperature increased from 26 to 34ºC and was larger at the food density of 800 and 1600 µgC/L.

Clutch size: Clutch size of *O. rigida* was greatest in the treatment of 26ºC and 1600µgC/L (observed on *C. muelleri*), treatment at 30ºC and 1600µgC/L (observed on *I. galbana*), treatment at 26ºC and 800µgC/L (observed on *T. chui*). In general, clutch size of *O. rigida* was higher at low temperature (26 - 30ºC) and lowest at 34ºC.

Hatching success: In all 3 tested algae species, the highest successful hatching rate was in the treatment of 800 – 1600 µgC/L. This parameter was highest at 30ºC and lowest at 26ºC when *O. rigida* eating *C.muelleri* and *T. chui*, respectively at 34ºC and 30ºC when observed with *I. galbana*.

30 hr nauplii production: Nauplii production of *O. rigida* was highest at 26ºC and density 160 - 800µgC/L when eating *T. chui* but highest at 30ºC and density 800µgC/L when observed with algae *C.muelleri* and *I. galbana* (Figure 3.17).

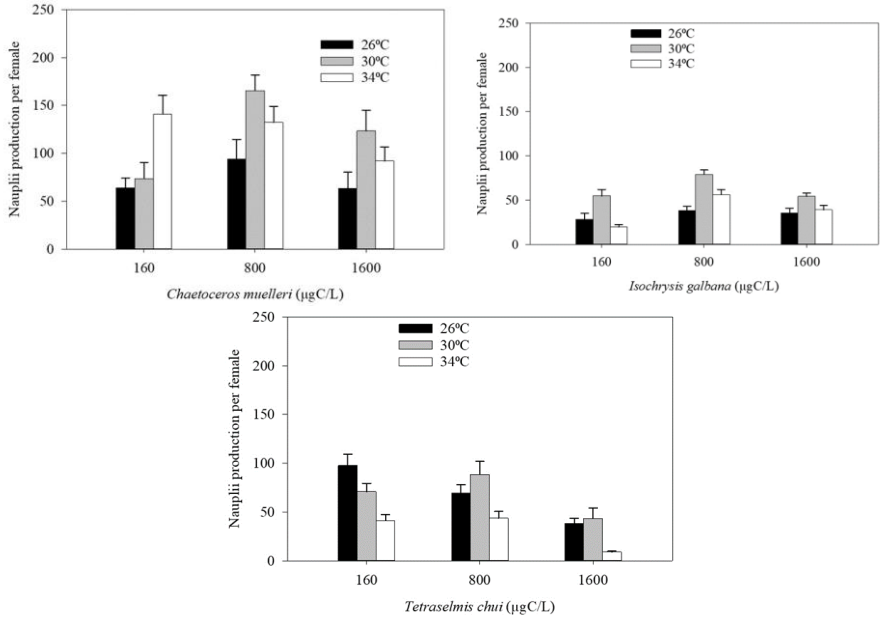


Figure 3.17. The nauplii production of the cyclopoid copepod *O. rigida* as a function of temperature and food. A – *C. muelleri*; B – *I. galbana*; C – *T. chui*

3.5. Effect of temperature, algae species and density on *P. incisus* và *O. rigida*

Body size: Size of nauplii, copepodite and adult stages of both *P. incisus* and *O. rigida* were larger at 800 and 1600 gC/L, with all three species of algae *C. muelleri, T. chui* và *I. galbana*. The results showed that this parameter is highest at 26ºC, the lowest at 34ºC. *P. incisus*, this parameter was higher at low temperature (26ºC) with treatment *C. muelleri* and *I. galbana* and at high temperature with algae *T. chui*. *O. rigida* showed higher results in treatments than at high temperature (30 và 34ºC) and low temperature (26ºC) with *T. chui*.

Clutch size: Clutch size of *P. incisus* and *O. rigida* in this study showed the highest results at the density of 1600 µgC/L in three algae species (except for the case of *T. chui* that observed *O. rigida* at 800 µgC/L). The fertility of *P. incisus* and *O. rigida* gave the best results when fed with algae *T. chui*.

Hatching success: The successful hatching rate of *P. incisus* and *O. rigida* in this study showed the highest results at the density of 800 µgC/L in three algae species (except for the case

of *T. chui* that observed *O. rigida* in level 1600 µgC/L). The hatching hatching rate of *P. incisus* and *O. rigida* gave the best results when fed with algae *C. muelleri*.

30 hr nauplii production: 30 hr nauplii production of *P. incisus* and *O. rigida* species in this study showed the highest results at the density of 800 µgC/L in three algae species (except for the case of *I. galbana* on *P. incisus* at 1600 µgC/L). 30 hr nauplii production was different in *P. incisus* and *O. rigida*; 30 hr nauplii production was with *P. incisus* when eating *T. chui*, while *O. rigida* ate *C. muelleri*.

Our observations had three important findings that provide information for biomass culture of two tropical copepoda species *P. incisus* and *O. rigida*. Firstly, the best feeding density of algae is in the range of 800 - 1600 µgC/L. Second, about the suitable algae for biomass culture of both copepods is *I. galbana*. Third, in general, in terms of reproductive efficiency, *P. incisus* species is more suitable for *T. chui*, while *O. rigida* is more suitable for *C. muelleri*.

CONCLUSIONS AND RECOMMENDATIONS

1. CONCLUSION

Salinity and temperature had negative effects on growth and reproduction of *P. incisus* and *O. rigida* if too high or low. The most suitable salinity for *P. incisus* culture in the range of 15-20ppt and 25-30ppt for *O. rigida*. The suitable temperature for both copepoda species to grow and reproduce is in the range of 26 – 30^{o}C.

Photoperiod and temperature had negative effects on the growth and reproduction of *P. incisus* and *O. rigida* if the lighting time was continuous 24h/day. The most suitable photoperiod for growing *P. incisus* in 12L:12D and 18L:6D. The suitable temperature for both copepoda species to grow and reproduce is in the range of 26 – 30^{o}C.

All 5 observed parameters including development time, size of stages, fecundity, successful hatching rate and 30hr naupius production in both *P. incisus* and *O. rigida* species were highest in UV0 and UVA. UVB strongly affects the growth and reproduction of *P. incisus* and *O. rigida*. The interaction between temperature and UV was very clear on *P. incisus* (100% of *P. incisus* died at 26^{o}C and UVB).

The best density of algae fed with *P. incisus* and *O. rigida* ranged from 800 - 1600 µgC/L. The suitable algae for biomass culture of both copepods is *I. galbana*. In general, the reproductive efficiency showed that *P. incisus* was suitable for *T. chui* and *O. rigida* was suitable for *C. muelleri*. Basically there is an interaction between temperature, density and algae species in the survey parameters.

The size of all stages of both experimental copepoda species was largest at 26^{o}C and decreased with increasing temperature..

The fecundity of both *P. incisus* and *O. rigida* species was highest in the 26^{o}C treatment and 1600 µgC/L and smaller at high temperature..

Hatching success rate in both copepoda species tends to increase with increasing temperature (in the range of 26 – 34^{o}C). Nauplii production also tended to decrease with increasing temperature (in the range of 26 – 34^{o}C).

2. RECOMMENDATION

Ecological factors such as salinity, photoperiod, ultraviolet have the impact on the growth and reproduction of *P. incisus* and *O. rigida*. Therefore, it is necessary to conduct applied research in biomass culture of *P. incisus* and *O. rigida*.

Feed affected the growth and reproduction of *P. incisus* and *O. rigida*. Therefore, it is necessary to conduct research to discover alternative and more proactive algae foods. The effect of combined feed on copepoda. To be proactive in culturing biomass copepod, it is necessary to test dry food in copepods farming.