Impact of Temperature on Growth, Early Development and Sex Differentiation in Tiger Shrimp (*Penaeus monodon*)

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Session: 2018-2019

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July. 2019

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A Thesis

By

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The Thesis submitted to the Fisheries and Marine Resource Technology Discipline, Khulna University, in partial fulfillment of the requirements for the degree of

Masters of Science

In

Fish Genetics and Biotechnology

July, 2019

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DECLARATION

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The results submitted in this thesis are entirely the candidate's own investigations and no part of the results has been accepted for any degree, nor is it being concurrently submitted for any degree.

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July, 2019

DEDICATED
TO
MY BELOVED FAMILY MEMBERS & MY FRIEND
FORIDA PARVIN PINKY

ACKNOWLEDGEMENT

All praises are due to the almighty "ALLAH ", the great creator, Who enabled me to pursue higher education and also to complete the thesis and to prepare and submit this dissertation for the degree of Msc in Fish Genetics and Biotechnology .

I feel proud to have the privilege to express deep respect and gratitude, heartfelt appreciation, regards and irrepayable indebtness to my supervisor, Associate Professor **Dr. Md. Lifat Rahi**, Fisheries and Marine Resource Technology Discipline Khulna University (KU), Khulna for his constant guidance, affectionate encouragement, scholastic supervision, valuable advice, helpful comments, co-operation, criticism and realistic feelings during conducting thesis work and also completing this dissertation. I am highly grateful to all the respected teachers of the department for their invaluable ad advice, constructive criticism to complete the thesis.

Specially, I express gratitude to my beloved parents, sisters, brother, maternal uncle and grandfather who sacrificed a lot of their happiness for the cause of the higher study.

Md. Shujon Mahmud

July 2019

ABSTRACT

Tiger shrimp (*Penaeus monodon*) is the prime aquaculture species in Bangladesh and provides a huge source for export earnings. Female individuals grow larger and faster than the males and thus production of mono-sex female cohorts will be highly demanding. The present study was conducted to determine the impact of different temperature levels on growth, early development, survival and sex differentiation in tiger shrimp. The experiment individuals were maintained at six different temperature levels (18°C, 22°C, 26°C, 30°C, 34°C and 36°C) with identical stocking density (300 individuals/ temperature) for a period of 60 days. At the end of 60th day, mean body weight of shrimp individuals were found to be 0.6755 ± 0.0513 g at 18°C, 0.922 ± 0.0359 g at 22°C, 1.14 ± 0.2197g at 26°C, 1.421 ± 0.0925 at 30°C, 1.576 ± 0.0676 at 34°C and 1.281 ± 0.1171 at 36°C respectively, which were significantly different ($p<0.05$) from each other. The daily average growth rate at different temperature ranged from 0.8541 ± 0.0777 to 2.355 ± 0.1316. The significantly ($p<0.05$) higher mean growth rate 2.355 ± 0.1316 was found at 34°C while the lowest rate was 0.8541 ± 0.0777 obtained at 18°C. Relative mean growth rate at different temperature ranged from 323.8466 ± 68.2871 to 895.5114 ± 186.7582. The significantly ($p<0.05$) highest mean growth rate 895.5114 ± 186.7582 was found at 34°C while the lowest 323.8466 ± 68.2871 was obtained at 18°C. Specific growth rate at different temperature ranged from 2.2739 ± 0.5087 % to 3.8028 ± 0.3087%. The significantly ($p>0.05$) highest mean growth rate 3.8028 ± 0.3087% was found at 34°C while the lowest 0.2739 ± 0.5087 % was obtained at 18°C. At 36°C, 65% female was produced within 38 days. Survival rate ranged from 32% to 68%, highest at 30°C and 26°C and lowest at 35°C. This study shows that there is a significant impact of temperature on growth, early development and sex differentiation in tiger shrimp (*Penaeus monodon*).

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Chapter One

Introduction

1.1 Introduction

The water bodies of Bangladesh are divided into inland and marine fisheries. Inland fisheries are divided into capture fisheries and culture fisheries (Shamsuzzaman *et al.*, 2017). It has a wide variety of fish species comprising 260 indigenous freshwater fish species, 475 marine fish species, 12 exotic fish species, 24 freshwater prawn and 36 marine shrimp species. The total area of capture fishery is 4.05 million hectares which covers 93.3% and culture fishery is 0.29 million hectares which covers 6.69% of the total inland fisheries, respectively. This total area covers 34% of the total area of Bangladesh. About 12.5 million people are directly or indirectly dependent on fisheries sector for their livelihood (Hossain, 2015). In Bangladesh about 1.28 million fishermen live on fishing as their main occupation in which 7.7 lac people are inland fresh water fisherwomen (DOF, 2011, Shamsuzzaman *et al.*, 2017). The fisheries sector plays a very important role in the national economy, contributing 3.69% to the Gross Domestic Product (GDP) of the country and 22.60% to the agricultural GDP (FRSS, 2016, Shamsuzzaman *et al.*, 2017) including 1.92%, of the country's export earnings, and provides 60% of the national animal protein consumption. The prawn and shrimp sector as a whole is the second largest export industry after readymade garments generating US$460 million annually and 5.6% of the total value of exports (DOF 2016). Approximately 1.2 million people are directly involved with prawn and shrimp production and a further 4.8 million household members are associated with the sector (USAID 2006).

The tiger shrimp (*Penaeus monodon*) is the most important penaeid species for mass hatchery production and land-based aquaculture system in Bangladesh, Thailand and many other countries in Asia. Shrimp farming is affected by physical, chemical, and biological factors (Aktas, *et al.*, and Babu *et al.*, 2012). Salinity and temperature are two of the most important abiotic factors

affecting the growth, survival and sex determination of aquatic organisms. Larval stages of most penaeid shrimp species occur in full strength seawater and at stable water temperatures. In addition, it is also well known that the response to these environmental parameters is species-specific and that salinity and temperature may also interact to influence growth and survival. It is generally agreed that temperature has a more pronounced effect on growth and survival of penaeids (Bett *et al*., 2009).

Sex determination is a fundamental biological process. It affects not only the sexual differentiation of gonads, but also the development of most organs, and leads to sex-specific differences in behavior, physiology and morphology. Organisms have evolved a variety of different sex-determining systems (Zarkower D 2001). Sex-determining mechanisms are broadly divided into two major categories: genetic and environmental (Goto-Kazeto *et al*., 2006). Genetic sex determination (GSD) is attributed to the genetic segregation of genes, often residing on sex chromosomes that initiate alternate sex-determining developmental pathways by changing the expression pattern of candidate genes at that particular condition. Environmental sex determination (ESD) is initiated by environmental cues that presumably trigger alternative genetic signals, which regulate male or female sex-determining genes (Crews *et al*., 2009). Environmental cues involved in ESD include temperature, photoperiod, nutrition, and population density (Korpelainen 1990). The most important environmental sex determining factors are temperature and nutrients. These environmental parameters stimulate and maintain gametogenesis and other reproductive processes in aquatic invertebrates (Soomro *et al*., 2011).

Sex determination and differentiation is a fundamental biological process that also determines the suitability of a particular species for aquaculture. This is because farming of mono-sex population has gained a special momentum in recent years due to faster growth (increased

economic return and profitability) and easy management (no risk of reproduction in the wild) of farmed populations. Research investigation on a wide range of aquatic species demonstrated that temperature, salinity and nutrients are the major environmental parameters that have direct influence on sex determination. Out of these three factors, temperature was found to have the most profound effect. For many species, higher temperature from optimum range was found to be male specific while for other species, reverse pattern was observed. Which temperature (high or low from optimum range) is female specific for commercially important crustaceans (i. e., *Penaeus monodon*) still remains unknown. Production of mono-sex female cohorts of *P. monodon* will be of great interest because females grow faster than the males. Many studies have reported the effects of different environmental factors, in particular, salinity and temperature on development, survival and growth of various commercially important penaeid species (Hernandez et al., 2005, Jackson *et al.*, 2003, Kumlu *et al.*, 2000, Lemairea *et al.*, 2002, Palafox *et al.*, 1997) but no study has been found on temperature dependent sex determination issue of *Penaeus monodon*. Therefore, the present study was conducted to investigate the impact of different temperature (18-36 ^{o}C) on the growth, development and sex determination of tiger shrimp (*Penaeus monodon*).

1.2. Literature Review

1.2.1. Geographic Range

Tiger shrimp (*Penaeus monodon*) is native to the coasts of the Arabian peninsula and the Pacific and Indian Ocean coasts of Australia, Indonesia, south and southeast Asia, and South Africa. *P. monodon* was accidentally introduced to the United States off the coast of South Carolina in 1988, by an unexpected release from an aquaculture center. They had spread as far south as Florida's coastline by 1990 and, since 2006, have been found in the Gulf of Mexico; they are found along the coastlines of Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina,

South Carolina and Texas (Duda and Palumbi, 1999; FAO Fisheries and Aquaculture Department, 2012; Knott, et al., 2011; South Carolina Department of Natural Resources, 2008; Xu, et al., 2001).

1.2.2. Economic Importance for Humans: Positive

Farming of Giant tiger prawns constitutes 47% of total world shrimp production giving it significant economic importance, particularly in Asian countries. With a high demand in Asian and international markets, building and running farms to produce these shrimp can be highly profitable and create many jobs (FAO, 2012; Monterey Bay Aquarium, 2012). Tiger shrimp plays a significant role to the economy of Bangladesh. In the last economic year, Bangladesh earned ≈2500 core taka by exporting ≈30,000 metric ton shrimp. Currently 275,000 acre coastal area in under shrimp farming practice that has been providing employment opportunities to more than 1.5 million people.

1.2.3. Economic Importance for Humans: Negative

This species is invasive in waters around the United States. Diseases carried by giant tiger prawns are highly contagious and can infect native shrimp populations, harming local fishing industries (Institute for the Study of Invasive Species, 2011). It has been estimated that up to 38% of native mangrove forests in Asia have been destroyed to be converted into ponds for shrimp farming, triggering erosion and harming habitat for mollusks and many other species, including shorebirds. Farming pools are sprayed with many chemicals and antibiotics to maximize shrimp production and these chemicals can enter natural waterways, harming animals and humans alike. These pools are often abandoned after a few years and there is typically no effort to return these lands to their original conditions (FAO, 2001; GreenPeace, 2012).

1.2.4. Ecosystem Roles

Tiger shrimp is detritovore and consumers of small invertebrates. They also are prey for many species of fishes and invertebrates (FAO, 2001; Johnson, 1995; Motoh, 1981). *P. monondon* is also a host for a variety of viruses, all of which are extremely contagious within populations and cause high mortality rates. The Yellowhead virus, originally isolated from this species, causes the hepatopancreas and cephalothorax to become discoloured and swollen. WSSV (White Spot Syndrome Virus) causes white spot disease, symptoms of which include lesions and white deposits on the skin and connective tissue. There are two types of Baculovirus infections commonly seen in these prawns: Baculoviral Midgut Gland Necrosis, which affects mainly larvae, and Monodon baculovirus disease, which is typically followed by secondary bacterial infections. These diseases are of particular concern in aquaculture environments and in areas where this species has been introduced (Crockford, 2008; FAO, 2001; Nadala, Jr., et al., 1997).

1.2.5. Habitat

Larvae, post-larvae and juveniles are most commonly found in estuaries, lagoons and mangroves; they are very tolerant to a range of salinity levels from 2-30 ppt. Adults move into deeper waters and live on rocky or muddy bottoms, ranging in depth from 0-110 m (most commonly at 20-50 m). These shrimps may bury themselves in the substrate during the day, emerging to feed at night. They live in waters ranging from 28-33°C and are unlikely to survive in waters colder than 13°C (FAO, 2012; Knott et al., 2011).

1.2.6. Physical Description

Penaeus monodon is easily identified by distinct black and white stripes on their backs and tails; on their abdomens, these stripes alternate black/yellow or blue/yellow. Base body color varies from green, brown, red, grey, or blue. Tiger shrimp is large, reaching 330 mm or greater in

length (largest individual found at 336 mm total length) and are sexually dimorphic, with females are larger than males. At sexual maturity, female carapace lengths range from 47-164 mm and their total lengths from 164-190 mm, while male carapace lengths fall between 37 and 71 mm, with total lengths of up to 134 mm. On average, females weigh 200-320 g and males weigh 100-170 g (Dall et al., 1991; Environmental Defense Fund, 2011; FAO Fisheries and Aquaculture Department, 2012; FAO-FIRA, 2010; Knott et al., 2011; Primavera et al., 1998; Vainio and Lagerspetz, 2006).

Females have a sperm receptacle (thelycum) located ventrally on the last thoracic segment. After mating, sperm remain in this receptacle until eggs are released. Females have a pair of internal fused ovaries that extend almost the entire length of their bodies, from the cardiac region of the stomach to the anterior portion of the telson (Dall et al., 1991; FAO-FIRA, 2010).

Males have a copulatory organ (petasma, formed by the longitudinally folded endopods of the first pair of pleopods. The presence of an appendix masculina (an oval flap on the second pleopod) can distinguish males from females. Testes are unpigmented/translucent and are found dorsal to the hepatopancreas under the carapace. The vas deferens is also internal, and arises from the posterior margins of the main axis of the testes. Sperm are released through genital pores on the fifth pereopod (Dall et al., 1991; FAO-FIRA, 2010).

1.2.7. Development

Eggs begin to develop by slowly sinking to the bottom of outer littoral areas. Tiger shrimp develop through a complex life cycle beginning with three larval stages. Naupilii hatch out twelve to fifteen hours after spawning is completed and look like tiny spiders. Larvae at this stage do not feed, instead surviving on their yolks as they are carried by tidal currents from Open Ocean towards shore. Naupilii larvae pass through six quick molts, increasing their body size. Individuals

in the next larval stage, called protozoa, are identified by increased body size and length, the appearance of feathery appendages and, though still planktonic, beginning to feed. After molting three more times, protozoa proceed into the Mysis larval stage. At this stage, they begin to have characteristics of adult prawns including segmented bodies, eye stalks, and tails. Mysis larvae molt three more times, becoming post larvae. At this point in the life cycle, they change from planktonic to benthic feeding. This entire process takes two to three weeks. Prawns continue to molt through a juvenile phase, lasting 1-6 months. Juveniles and adults are distinguished mainly by location and carapace length. Carapace lengths of juveniles range from 2.2-11 mm and they are found mainly in estuarine areas located at the mouth or middle of bays and mangroves while adults are found in outer littoral areas of full salinity, and have carapace lengths ranging from 37-81 mm. (Dall et al., 1991; FAO Fisheries and Aquaculture Department, 2012; FAO-FIRA, 2010; Motoh, 1981; State of New South Wales through Department of Industry and Investment, 2010)

1.2.8. Effect of temperature on growth, development and Survival

It is generally agreed that temperature has a more pronounced effect on growth and survival of penaeids (Bett *et al.*, 2009). Water temperature is one of the most important physical factors affecting survival and growth of decapod crustaceans. Growth rate has been shown to increase with increasing temperature to a maximum, before declining near the upper thermal limit of tolerance. However, high temperatures also increase mortality, as demonstrated for penaeid shrimps, possibly because less protein is incorporated into body tissues (O'Brien, 1994; Parado-Estepa, 1998). Water temperature also influences survival and development of embryos and larvae of decapods (Hamasaki, 2003; Anger et al., 2004). A decrease in the incubation period has been reported as a result of increasing temperature for many species (Brillon et al., 2005), and for some of them this has been associated with lower survival, higher energy consumption, and

even serious deformities of embryos (García-Guerrero et al., 2003, 2010). Moreover, the temperature experienced during embryogenesis may influence larval biomass at hatching and subsequent larval development in decapods (Wear, 1974). Therefore, special care must be taken when temperature is manipulated to accelerate embryonic development. This fact confirms the suggestion that high temperatures, to a certain point, increase the moulting frequency and larval growth but reduce the survival of penaeid shrimps (Staples and Heales, 1991; O'Brien, 1994; Parado-Estepa, 1998) possibly because less protein is being incorporated into the body tissues.

A small deviation from the optimal temperature may have pronounced effects on growth; it is thought that a slightly lower temperature than 30°C should be more adequate for the larval culture of penaeids including *P. monodon*, *P. semisulcatus* etc. During the mysis stages, at all salinity levels, the PL1 had the lowest survival 14–21% at 34°C. Regardless of salinity, 26°C consistently gave higher survival 69–77% than 30°C 44–73%. However, growth rate was about 60% faster at 30°C than at 26°C. Staples and Heales (1991) suggested that in order to determine optimum growing conditions; a compromise should be made between growth and survival data. Based on the survival and growth results, the best growth, development and survival larvae of *P. monodon* are grown successfully at extremely high temperatures 32–34°C in commercial hatcheries in Southeast Asia (Chen, 1990; Fagan, 1992).

1.2.9. Effect of temperature on Sex Determination

Sex determination mechanisms produce the sex ratio, a key demographic parameter crucial for population viability. In gonochoristic vertebrates, sex determining mechanisms can broadly be classified as genotypic (GSD) or environment-dependent (ESD) (Bull, 1983, Valenzuela et al., 2003). In species with ESD, there are no consistent genetic differences between sexes. The earliest ontogenetic difference between sexes is an environmental cue because the ambient

temperature during sensitive periods of early development irreversibly determines phenotypic sex and, therefore, the sex ratio (Bull, 1983, Valenzuela et al., 2003). Thus, species with ESD have been proposed to be reliable indicators of the biological impact of global warming, since temperature-induced sex ratio shifts constitute a direct fitness response to thermal fluctuation (Valenzuela et al., 2003).

Luckenbach et al. (2003) conducted a study on southern flounder (*Paralichthys lethostigma*) and found lower ratio of females at low temperature (18°C) while higher ratio of males at high (28°C) temperature. So, temperature affects the sex ratio. Olmstead and LeBlanc (2007) and Sperfeld and Whacker (2009) observed that *Daphnia magna* exhibited phenotypic plasticity in sex ratio and clutch size at different temperatures, e.g. more males occurred at lower temperature (20°C), while more females emerged with smaller clutch size as the temperature was increased (25 and 30°C). It has been widely found that exposure to low temperatures decreases growth rates in poikylo-thermic animals; the increase in males at low temperatures is likely the result of male development according to the threshold model for growth-dependent sex differentiation (Kraak and de Looze, 1992).

The analysis of sex ratio response to temperature, considering the scope of such response as well as the presence or not of sex chromosomes, carried out many species where TSD had been claimed before are in fact GSD species affected by temperature, i.e., cases of GSD+TE. In GSD+TE species, temperature rather than being the external environmental factor controlling sex determination is capable of affecting the process of gonadal sex differentiation under some circumstances. This distinction is neither trivial nor semantic since, according to the canonical definition (Bull, 1983), in TSD species the first ontogenetic difference between sexes is an environmental one (temperature), whereas in GSD+TE species sex determination remains under

genotypic control. The other effect, the increase in males at high temperatures in species previously assigned to pattern 3, is likely the result of sex-reversal of females as a consequence of the inhibition of aromatase, the enzyme that produces estrogens essential for female sex differentiation in fish (Piferrer et al., 1994).

1.3. Objectives of the Study

The specific objectives of the present study include:

- To investigate the impacts of different temperatures on growth of *P. monodon*.
- To observe temperature dependent changes in duration of larval developmental stages.
- To investigate the impact of temperatures on sex determination in *P. monodon*.
- To observe the survival performance of *P. monodon* under different temperature.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1 Shrimp Collection

Tiger shrimp individuals (10 days old larvae) of the same mother and cohort were collected from SPF Panna hatchery Katinagla, Botiaghata, Khulna. In total, 2100 individuals were collected from the hatchery for this study. Shrimp larvae were transported in 3 different plastic bags (700 individuals in each bag) with supplied Oxygen. Water salinity was 20‰ in the transportation bags.

2.2 Experimental Tank Setting

In total, 18 glass tanks (30 L) were used for this study. Prior to the stocking of larvae, the experimental tanks were washed with bleaching powder and then kept under the sunlight for 4 hours to make the tanks sterile. Experimental tanks were then wiped with 50% ethanol. The experimental tanks were filled with 20‰ water and maintained with continuous aeration for 3 days. The experimental trials were performed in the Water Quality Laboratory of the Fisheries and Marine Resource Technology Discipline, Khulna University.

2.3 Experimental Design

Table 1: Different experimental temperature levels with stocking density.

Temperature	Replications per Temperature	Stocking Density	Application of Feed	Sampling Time
18°C	3	100	Twice daily at morning and evening Feeding rate: 10% of total body weight	10 days interval
22°C	3	100		
26°C	3	100		
30°C	3	100		
34°C	3	100		
36°C	3	100		

2.4. Stocking Shrimp Larvae in the Experimental Tanks

Collected *P. monodon* larvae were transferred in 18 different glass tanks in the Water Quality Laboratory of FMRT Discipline. Identical stocking density (100 individuals/ tank) was maintained in each tank. Individuals were maintained in experimental tanks for 5 days to acclimate well with the tanks environments. Digital thermostats were placed in each experimental tank to maintain specific stable temperature in the tanks. Six different temperature regimes were maintained from 18 to 36°C with 4°C interval (i. e., 18°C, 22°C, 26°C, 30°C, 34°C and 36°C). 26°C and 30°C temperatures were considered as the controls while the other four temperature levels (18°C, 22°C, 34°C and 36°C) were considered as the treatments. Initially, attempts were made to maintain 300 individuals at 38°C temperature but none of the larvae survived at this temperature. As a result, the highest level of temperature was maintained at 36 °C. Temperature was increased and decreased by 1°C at every 2 hours interval from control temperatures to achieve the treatment conditions. Once the target temperature levels have been achieved, experimental individuals were maintained under that constant water temperature by the digital thermostats until the end of the experiment. Continuous aeration was also maintained in each tank.

2.5. Feeding and Water Quality Management

The same supplementary feed (Sano S-PAK 8/12) was applied twice daily (morning and evening) at a rate of 10% of total biomass. Protein content of the feed was 40%. The experimental tanks were cleaned by siphoning to remove unused food particles daily (at evening) for maintaining optimum water quality in the experimental tanks. Moreover, half of the water was exchanged at every 3 days interval to maintain healthy water quality.

2.6. Growth, survival and developmental study

Individuals were sampled at every 10 days interval to measure body weight (for checking growth performance). Individuals were checked every day to observe the temperature induced developmental changes (particularly for sex determination). Survival rates were measured by counting the number of individuals at the end of the experimental trial. The data was recorded during the experiment and collected data was accumulated, grouped and interpreted according to the objectives. Collected data will be stored, explored and analyzed using Microsoft Excel, SPSS and Microsoft Word program to present result and discussion. Then these data will be processed through statistical treatments. Twenty individuals were sampled for each sampling. Weight was measured by using electric balance (CSC Balance, YP10002B). After finishing experimental trial all recording data were used for measuring survival rate. The growth performance were calculated according to following formula

2.6.1. Weight gain

$\boldsymbol{WG = Wt - W0}$ (Biswas et al., 2012)

Where WG, Weight gained; Wt, Final weight; W0, Initial weight

2.6.2. Daily weight gain (g/day), DWG

$\boldsymbol{DWG = \frac{Wt-W0}{t}}$ (Ghosh et al., 2016)

Where, DWG, Daily weight gain (g/day); Wt, Final weight; W0, Initial weight; t, the duration of the growth interval in days

2.6.3. DGR, Daily growth rate

$DGR = \frac{Wt-W0}{t} \times 100$ (Rebecca & Bhavan, 2014)

Where, DGR, Daily growth rate; Wt, Final weight; W0, Initial weight; t, the duration of the growth interval in days.

2.6.4. Relative growth rate or Weight gain

$RGR = \frac{Wt-W0}{W0} \times 100$ (Thimmurugan & Subramanian, 2004)

Where, RGR, Relative growth rate or Weight gain (%); Wt, Final weight; W0, Initial weight

2.6.5. Specific growth rate (in % Body weight per day)

$SGR = \frac{Ln\,(Wt) - Ln(W0)}{t} \times 100$ (Thimmurugan & Subramanian, 2004)

Where, SGR, Specific growth rate (in % Body weight per day); Wt, Final weight; W0, Initial weight; t, the duration of the experiment in days.

2.7. Temperature dependent sex determination

Individuals were checked regularly (daily) to observe the onset of sex organs or gonads using magnifying glass. Number of males and females were counted to determine effectiveness of different temperature regimes on sex differentiation.

2.8. Data analysis

Various experimental data (both qualitative and quantitative) were analyzed using different software packages including: SPSS, R-package and MS Excel. Growth data were analyzed using One-Way ANOVA at 5% level of significance.

Chapter Three

Result

3. Results

3.1.1. Growth performance of *Paneaus monodon* larvae at different temperature levels

Significant differences ($P<0.05$) were observed for the growth performance between individuals from different temperature levels. Higher growth rates were observed with increasing temperatures. At the end of the experimental period, the highest growth performance (mean body weight 1.58 g) was observed at 34°C while the lowest growth was found at 18°C. For all other growth parameters (weight gain, daily weight gain, daily growth rate and specific growth rate), experimental *P. monodon* individuals showed the best performance at 34°C (Table 2).

Table 2: Rearing performance of *P. monodon* larvae at different temperature levels with different growth parameters. ± indicates standard deviation (SD), WG= weight gain, DWG= daily weight gain, DGR= daily growth rate, RGR= relative growth rate, SGR= specific growth rate, different letters in the same row indicate significant difference between temperature levels

Temperature	18°C	22°C	26°C	30°C	34°C	36°C
Initial weight (g)	0.163 ± 0.0273	0.1775 ± 0.0133	0.15 ± 0.0235	0.1775 ± 0.0133	0.163 ± 0.0273	0.163 ± 0.0273
Final Weight (g)	0.6755 ± 0.0513	0.922 ± 0.0359	1.14 ± 0.2197	1.421 ± 0.0925	1.576 ± 0.0676	1.281 ± 0.1171
WG	0.5125^{a} ± 0.0466	0.7445^{b} ± 0.0350	0.98^{c} ± 0.2155	1.2435^{d} ± 0.0922	1.413^{d} ± 0.0790	1.118^{d} ± 0.1152
DWG	0.0085^{a} ± 0.0007	0.0124^{b} ± 0.0005	0.02^{c} ± 0.0035	0.0207^{d} ± 0.0015	0.0235^{e} ± 0.0013	0.0186^{f} ± 0.0019
DGR	0.8541^{a} ± 0.0777	1.2408^{b} ± 0.0583	1.64^{c} ± 0.3592	2.0725^{d} ± 0.1538	2.355^{e} ± 0.1316	1.8633^{f} ± 0.1920
RGR	323.846^{a} ± 68.2871	421.7816^{b} ± 38.2404	649.19^{c} ± 156.3582	704.4030^{c} ± 74.1541	895.5114^{d} ± 186.7582	705.1739^{c} ± 139.7679
SGR	2.2739^{a} ± 0.5087	2.7491^{b} ± 0.1236	3.33^{c} ± 0.3122	3.4678^{c} ± 0.1586	3.8028^{d} ± 0.3087	3.4522^{c} ± 0.2929

Figure 1 shows the mean body weight of experimental individuals for a period of 40 days (from 20th day to 60th day). There was no significant difference in mean body weight between

individuals at the start of the experimental trial (on day 20). Temperature specific significant differences were observed from 40th day to the end of the experiment (60th day). Significant differences were not observed between 26°C, 30°C, 34°C and 36°C temperature levels from day 30 to day 40, following which significant differences were observed between these temperature levels until the end of the experiment. On 60th day, all the experimental individuals showed temperature induced significant differences in mean body weight between individuals from different temperature levels; clearly indicating a stronger effect of temperature on growth performance. Of note, significantly lower growth performance was observed at 36°C compared to 34°C and 30°C at the end of the experimental trial (on 60th day). Significant differences were observed between individuals from lower temperature levels (18°C, 22°C and 26°C) from Day 40 to the end. But significant differences between individuals from higher temperature levels (30°C, 34°C and 36°C) were observed only at the end (60th day) of the experiment.

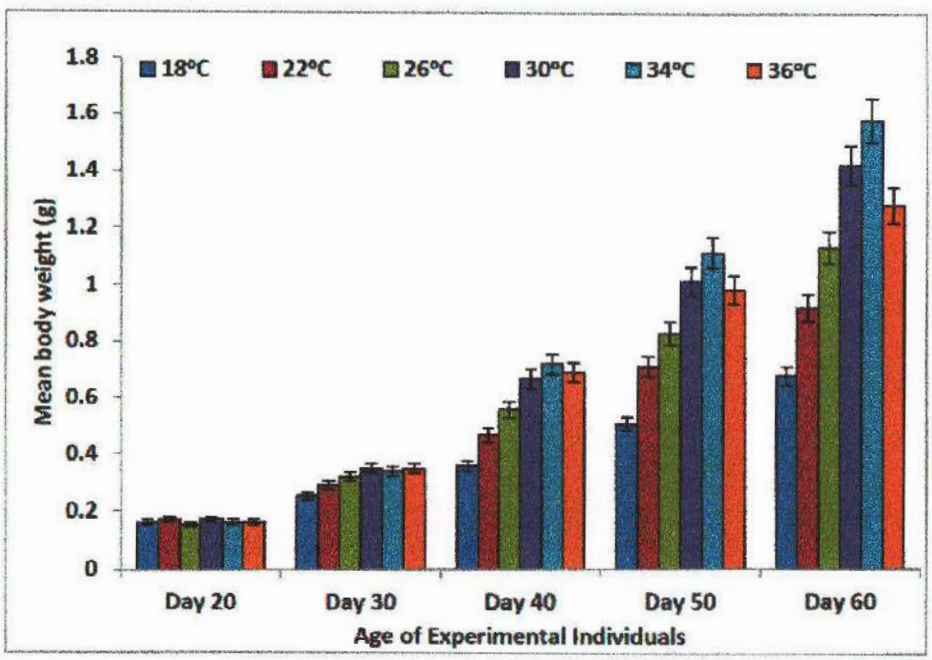

Figure 1: Mean body weight of *P. monodon* individuals between different temperature levels. Error bars indicate ±SD, N = 20 individuals during each sampling for each temperature.

3.1.2 Growth in weight

The initial average weight of larvae was 0.163 ± 0.0273 in all different temperatures. There were no significant differences for initial weights of larvae under different temperature. The mean final weight of larvae were 0.6755 ± 0.0513, 0.922 ± 0.0359, 1.14 ± 0.2197, 1.421 ± 0.0925, 1.576 ± 0.0676 and 1.281 ± 0.1171 respectively at 18°C, 22°C, 26°C, 30°C, 34°C, and 36°C temperature (table 2). The maximum and minimum final weights were 1.576 ± 0.0676 and 0.6755 ± 0.0513 at 34°C and 18°C respectively. The statistical data shows that final weight at different temperature are significantly difference ($p<0.05$). In case of weight gain, the lowest mean weight gain of larvae *Paneaus monodon* were 0.5125 ± 0.0466 at 18°C and the highest value was 1.413 ± 0.0790 at 34°C.

3.1.3. Daily weight gain

Daily mean weight gain in different temperature ranged from 0.0085 ± 0.0007 to 0.0235 ± 0.0013 (Table 2). Significant differences were observed for DWG between all different temperature levels throughout the experimental period. The highest level of daily weight gain was observed as 0.0235 ± 0.0013 at 34°C while the lowest level was 0.0085 ± 0.0007 at 18°C.

3.1.4. Daily growth rate

Daily mean growth rate in different temperature ranged from 0.8541 ± 0.0777 to 2.355 ± 0.1316 (Table 2). The highest level of daily growth rate was found to be 2.355 ± 0.1316 at 34°C while the lowest level was found as 0.8541 ± 0.0777 at 18°C. The DGR was also found to be significantly different ($p<0.05$) between different temperature levels.

3.1.5. Relative growth rate

Relative mean growth rate in different temperature ranged from 323.8466 ± 68.2871 to 895.5114 ± 186.7582 (Table 2). Significant differences ($p<0.05$) for RGR were observed between 18°C,

22°C and 34°C temperatures while there was no significant differences between 26°C, 30°C and 36°C. The highest mean growth rate (895.5114 ± 186.7582) was found at 34°C and the lowest (323.8466 ± 68.2871) was obtained at 18°C.

3.1.6. Specific growth rate

Specific growth rate in different temperature ranged from 2.2739 ± 0.5087% to 3.8028 ± 0.3087% (Table 2). The same pattern of statistical differences was observed for SGR as it was observed for RGR (significant differences between 18°C, 22°C and 34°C while no significant differences between 26°C, 30°C and 36°C). The highest mean growth rate (3.8028 ± 0.3087%) was found at 34°C while the lowest (2.2739 ± 0.5087 %) was obtained at 18°C.

3.2. Development Pattern and Sex Determination

Table 3: Temperature induced sex determination pattern and developmental time for tiger shrimp (*Penaeus monodon*).

Temperature	18°C	22°C	26°C	30°C	34°C	36°C
Male	62%	57%	51%	46%	37%	35%
Female	38%	43%	49%	54%	63%	65%
Time Required for Sex Determination	53 day	47 day	45 day	43 day	40 day	38 day

Different temperature levels showed a clear impact on the sex determination and developmental duration for tiger shrimp (Table 3 and Figure 2). Temperature levels significantly ($p<0.05$) affected developmental time as well as sex differentiation/determination (formation of male or female). The control temperature levels (26°C and 30°C) produced almost similar proportions of males and females. There was no significant differences ($p>0.05$) for developmental time and sex ratios between 26°C and 30°C. But significant differences ($p<0.05$) were observed for larval developmental time for the remaining other temperature levels: 18°C, 22°C, 34°C and 36°C. Developmental time for sex determination was found to be the highest (53 days) at 18°C while

the lowest time (38 days) was required at 36°C. No significant difference was observed for larval developmental period between 34°C and 36°C temperature levels. Significant differences for sex ratios were observed between lower and higher temperature levels (18°C, 22°C, 34°C and 36°C). There was no significant difference in sex ratios between control temperatures (26°C and 30°C). Higher proportions of females (63-65%) were observed at higher temperatures (34°C and 36°C) while a higher proportion of males (57-62%) were obtained at lower temperature (18°C and 22°C) (Table 3 and Figure 2). The highest amount of time (53 days) was required at 18°C for sex differentiation while the lowest amount of time was required (38 day) at 36°C (Table 2).

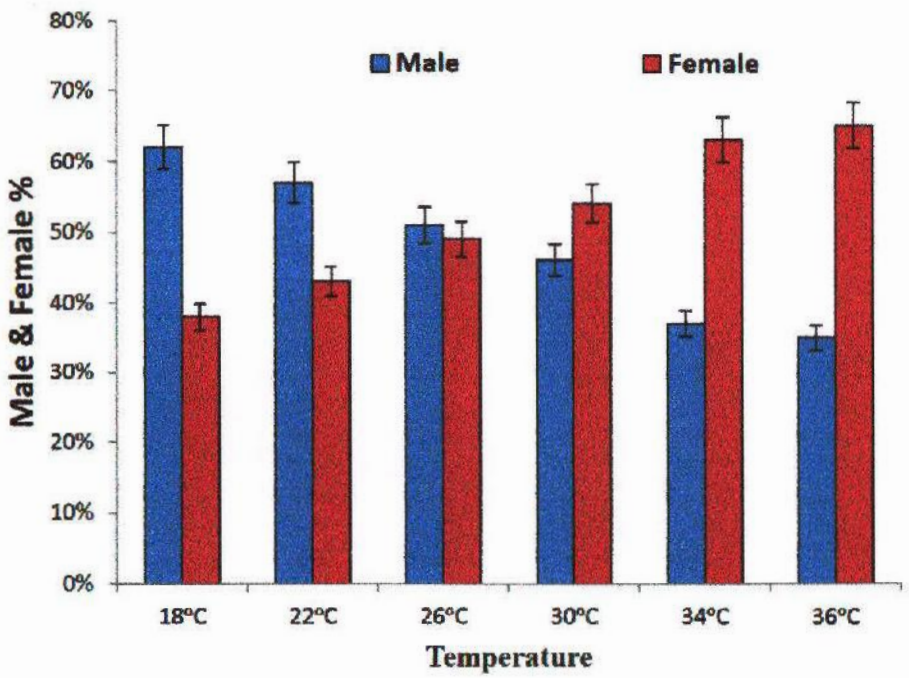

Figure 2: Temperature induced sex differentiation of tiger shrimp (*Penaeus monodon*). Error bars indicate ±SD, N= 300 individuals per treatment.

3.3. Survivability

The highest survival rate (66%) was obtained at 30°C while the lowest rate (32%) was obtained at 36°C. There was no significant difference in survival rate between control temperatures (26°C

and 30°C). Significant differences ($p<0.05$) were observed for survival rate between all other experimental temperature levels (Figure 3).

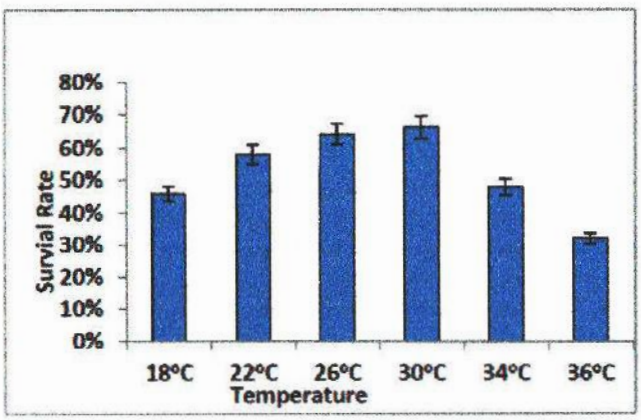

Figure 3: Survival rate of *P. monodon* individuals at different temperature levels Error bars indicate ±SD, N= 300 individuals per temperature.

Chapter Four

Discussion

4. Discussion

The present study showed that temperature had a clear effect on larval developmental time, growth and survival, and sex differentiation/determination of *P. monodon*. Higher temperature (>30°C) produced higher proportions of female than male. On the other hand, reverse pattern was observed at lower temperature (<22°C) that provided higher proportions of male. So, it is clear that temperature has a significant role on the sex determination process of *P. monodon*.

4.1. Effect of different temperature levels on the growth of *Penaeus monodon*

At the beginning of the study all experimental individuals had almost similar body weight as they were from the same mother and same age group (Table 2 and Figure 1). No significant differences were observed during the 1st sampling (Day 30) between individuals from different temperature levels (Figure 1). This is because of the temperature stress; all experimental individuals were under stressful conditions due to temperature change. As a result, more energy was required for acclimation at differential temperature levels instead of growth. From the 2nd sampling (Day 40), a clear effect of temperature was observed on the growth performance between individuals from different temperature where higher temperature levels (30-36°C) initiated faster growth and lower temperature levels (18-26°C) provided slower growth. In the current study, significant effect of temperature was observed on all other growth parameters as well including weight gain (WG), daily weight gain (DWG), daily growth rate (DGR), relative growth rate (RGR) and specific growth rate (SGR).

It is well known that 1°C increase in water temperature can increase the metabolic rate by 10% in aquatic organisms (Campos-Ramos et al., 2006; Goto-Kazeto et al., 2006). Therefore, increase in metabolic rate can in turn provide, faster weight gain (growth) to individuals exposed to higher temperatures within individual's natural tolerance limit (Kumlu et al., 2000; Jackson and

Burford, 2003). Higher temperature levels (30°C, 34°C and 36°C) in the current study likely increased the metabolic activity in the experimental individuals and provided faster growth. This can explain the reason for higher growth of individuals at higher temperature levels in the present study. Lower growth of individuals at the highest temperature level (36°C) in the current study was probably due to higher level of temperature stress imposed. Thus, experimental individuals at 36°C likely diverted a significant amount of energy from growth to acclimation to temperature stress. Such type of temperature dependent differential growth pattern was observed on a number of other aquatic species including *Metapenaeus monoceros* (Aktas and Nurun, 2012), *Macrobrachium rosenbergii* (Babu et al., 2012), *Litopenaeus vannamei* (Bett and Vinatea, 2009), goldfish (Goto-Kazeto et al., 2006), *Penaeus semisulcatus* (Kumlu et al., 2000; Hussain et al., 2015), *Penaeus stylorstris* (Lemairea et al., 2002) etc.

4.2. Effect of different temperature levels on development and survivability of *P. monodon*

Different temperature levels significantly ($p<0.05$) affected the larval development time period and survivability of experimental *Penaeus monodon* individuals (Table 3 and Figure 3). The lowest temperature (18°C) significantly delayed the developmental time to maturity (formation of sex organs: petasma or thelycum) and also negatively affected the survival rate (lower survival rate was observed at this temperature). Although the highest temperature level (36°C) severely affected the survival rate (lowest survivability as 32% was obtained at this temperature), this temperature provided the quickest development to sexual maturity (within 38 days). As stated previously that the higher temperature can rapidly increase the metabolic rates, it provides faster developmental pattern (shorten the developmental stages). The reverse pattern can happen at lower temperature. Thus, increased developmental time to maturity was obtained at lower temperatures. This is because lower temperature reduces the metabolic rate and consequently

growth and developmental stages (Angilletta, 2001). High temperature specific faster development and low temperature specific slower developmental processes were observed in other crustaceans including: *Metapenaeus monoceros* (Aktas and Nurun, 2012), *Macrobrachium rosenbergii* (Babu et al., 2012), *Litopenaeus vannamei* (Campos-Ramon et al., 2006) etc.

The highest levels of survivability (64% and 66%) were obtained at control temperatures (26°C and 30°C respectively) (Figure 3). No significant difference was observed for survival rates between these two temperature levels. In hatchery condition, 26-30°C is normally considered as the optimum temperature level for larval rearing/production. Higher survival performance of the experimental individuals also support that this temperature range could preferably optimum. Significantly lower ($p<0.05$) survival rates were obtained for the other temperature levels (18°C, 22°C, 34°C and 36°C). Any deviation from the optimum temperature range can significantly affect the survival performance of aquatic crustaceans but the extent of mortality rate depends on intensity of deviation (how large is the deviation?) (Jackson and Burford, 2003; Wacker and Sperfeld, 2009). Larger deviation from optimum temperature range can impose more stress (with very high levels of mortality) while smaller deviation can impose little stress with lower rates of mortality. At 18°C, 46% survivability was obtained while at 36°C the survival rate was found to be only 32% (Figure 3). Both high and low temperatures can increase and decrease metabolic rates at a greater extent to adversely affect survivability of aquatic crustaceans. Large changes in metabolic activities within individual's body due to larger temperature fluctuations can impose severe biological changes in all aspects that in turn, put organismal cell and body under tremendous risk. As a result, very lower survivability is normally observed. It was found that a vast majority of aquatic crustaceans do not perform well (in terms of survivability) below 20°C

and above 34°C (Zarkower, 2001; Wacker and Sperfeld, 2009). A very similar pattern of result was observed in the current study.

4.3. Effect of different temperature levels on sex determination of *Penaeus monodon*

Temperature and photoperiod are the two most important factors affecting gametogenesis. The temperature is also effective in the formation of gametes or sex cells. A number of studies on different species already showed a clear effect of temperature on sex determination (Luckenbach et al., 2003; Dhraief et al., 2008; Gao et al., 2014). In the previous studies, sex ratio changes were found to be temperature dependent and there were clear effects of different temperatures on the formation of female/male ratio. However, there was no available information on *P. monodon*. In the present study, a clear effect of temperature was observed on the sex determination process of the tiger shrimp (*Penaeus monodon*). A higher proportion of female individuals (63-65%) were obtained at high temperature levels (34-36°C) while a reverse pattern (higher proportion of males: 57-62%) was observed at lower temperature (18-22°C) levels (Table 3 and Figure 2). Therefore, it can be inferred that higher temperature from the optimum level can promote formation of female cohorts whereas lower temperature can increase male populations for *P. monodon*. As in *Penaeus monodon* females grow faster than males, higher temperature has the influential ability to produce more females at high temperatures by manipulating biological properties (metabolism, enzyme activity etc.) of the individuals.

Chapter Five

Conclusion

5. Conclusion

Effect of different temperature levels on the growth, survivability, larval development pattern and sex ratios (sex determination process) were successfully investigated in the present study. Higher temperature levels (34-36°C) provided the best growth performance while lower temperature (18-22°C) exhibited slower growth pattern. Moreover, the highest level of survivability (64% and 66%) was observed at control temperatures (26°C and 30°C). In addition, higher temperature made the sexual developmental processes faster and lower temperature delayed the process of sex determination. Finally, it was found that high temperature was female specific (higher proportions of females were obtained at higher temperatures) while the lower temperature levels were found to be male specific. Therefore, this study provides a clear indication and enough clues to manipulate sex ratios of *Penaeus monodon* by altering temperature in hatchery conditions. This will help to produce higher proportions of female individuals of tiger shrimp to facilitate fast growing mono-sex female aquaculture in the coastal areas of Bangladesh. Further study is required to investigate these effects with more temperature levels at every 1°C or 2°C temperature intervals.

Chapter Six

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6. References

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Appendix

Development stage of Tiger shrimp (*Penaeus monodon*)

Figure 2: Petasma (Male Genital Organ)

Figure 1: Thelycum (Female Genital Organ)

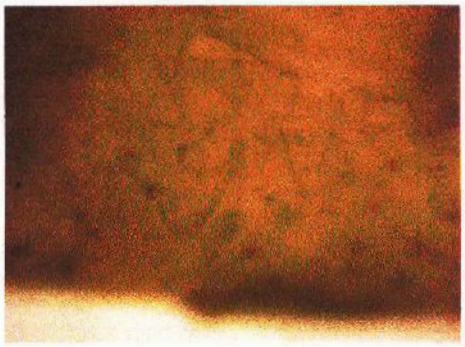

Figure 4 No Genital Organ is found

Figure 3 One set of Thelycum is found

Figure 5 One set of Petasma is found

Homogeneous Subsets

WG

	Sl	N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Tukey HSD[a]	18	20	.512500					
	22	20		.744500				
	26	20			.984500			
	36	20				1.118000E0		
	30	20					1.243500E0	
	34	20						1.413000E0
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Tukey B[a]	18	20	.512500					
	22	20		.744500				
	26	20			.984500			
	36	20				1.118000E0		
	30	20					1.243500E0	
	34	20						1.413000E0

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

Homogeneous Subsets

DG

	Sl	N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Tukey HSD[a]	18	20	.008542					
	22	20		.012408				
	26	20			.016408			
	36	20				.018633		
	30	20					.020725	
	34	20						.023550
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Tukey B[a]	18	20	.008542					
	22	20		.012408				
	26	20			.016408			
	36	20				.018633		
	30	20					.020725	
	34	20						.023550

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

Homogeneous Subsets

DGR

	sl	N	Subset for alpha = 0.05					
			1	2	3	4	5	6
Tukey HSD[a]	18	20	.854167					
	22	20		1.240833E0				
	26	20			1.640833E0			
	36	20				1.863333E0		
	30	20					2.072500E0	
	34	20						2.355000E0
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000
Tukey B[a]	18	20	.854167					
	22	20		1.240833E0				
	26	20			1.640833E0			
	36	20				1.863333E0		
	30	20					2.072500E0	
	34	20						2.355000E0

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

Homogeneous Subsets

RGR

	Sl	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	18	20	3.238467 E2			
	22	20	4.217817 E2			
	26	20		6.491892 E2		
	30	20		7.044030 E2		
	36	20		7.051739 E2		
	34	20			8.955115 E2	
	Sig.		.126	.702	1.000	
Tukey B[a]	18	20	3.238467 E2			
	22	20		4.217817 E2		
	26	20			6.491892 E2	
	30	20			7.044030 E2	
	36	20			7.051739 E2	
	34	20				8.955115 E2

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.

Homogeneous Subsets

SGR

	Sl	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	18	20	2.387600 E0			
	22	20		2.749149 E0		
	26	20			3.326657 E0	
	36	20			3.452274 E0	
	30	20			3.467870 E0	
	34	20				3.802800 E0
	Sig.		1.000	1.000	.494	1.000
Tukey B[a]	18	20	2.387600 E0			
	22	20		2.749149 E0		
	26	20			3.326657 E0	
	36	20			3.452274 E0	
	30	20			3.467870 E0	
	34	20				3.802800 E0

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 20.000.