Effects of Water Temperature on Growth Performance and Health of Butter Catfish (*Ompok bimaculatus*)

Pyanuth Rem

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Master of Science in Aquatic Science

Prince of Songkla University

2019

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Major Program Aquatic Science

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ABSTRACT

Increased water temperatures, decreased dissolved oxygen levels, and altered chemical substances in freshwater systems cause stress to fish and increase the likelihood of disease. These issues generate a significant amount of economic losses in aquaculture and clearly represent a normal impact of rapid water temperature changes on fish lives. This study examined growth performance and health conditions of butter catfish culture under elevated water temperature levels. In a 10-week experiment, 15 fingerlings per tank were cultured in 4 different temperature regimes (ambient temperature (25.1-29.5 °C) as the control, 29 °C, 31 °C, and 33 °C) with 4 replications. The initial average weight of the fingerlings was 2.72±0.01 g. Water quality parameters (dissolved oxygen (DO), pH, total ammonia nitrogen (TAN), and nitrite (NO_2–N)) were measured weekly. Water pH, TAN, and NO_2–N were not affected by water temperature, but from waste products in the tank. Except that DO slightly decreased when exposed to higher temperatures, with readings of 8.80, 8.79, 8.69, and 8.27 mg/l in the control, 29 °C, 31 °C, and 33 °C temperature regimes, respectively. Water pH (6.4-6.7) gradually decreased with no significant differences among treatments ($p>0.05$). TAN values in the control and 29 °C, 31 °C, and 33 °C treatments were 0.729, 0.805, 0.765 and 0.389 mg N/l, respectively. NO_2–N values followed a similar pattern to TAN with the control treatment reaching the highest value (0.164 mg N/l) and the 33 °C treatment the lowest (0.103 mg N/l). However, all parameters were within safe levels for fish culture.

The highest temperature (33 °C) negatively affected growth performance and fish health. The fish in this regime had significantly reduced percentages of weight gain, feed intake and specific growth rate (SGR) (479.70%, 194.28 g, and 2.55%/day, respectively). However, the feed conversion ratios (FCR)

and survival rates did not differ by temperatures ($p>0.05$) and their values were positive for the culture. However, the fish immune systems were weakened with white blood cell counts (5.50×10^7 cells/ml) lower and lysozyme activity (8.30 μg/ml) higher in fish held at 33 °C. Even though the hemato–immunological parameters (serum protein, nitroblue tetrazolium (NBT) reduction, hematocrit, and average beads per cell) in all four treatments did not show any variations, other parameters (red blood cells, hemoglobin, and phagocytosis) had higher values, possibly a sign of compensation during the adaptation to their new regimes. Exposure to *Edwardsiella ictaluri* at a concentration of 1.32×10^6 CFU/ml led to a mild virulence which caused a 2.5% accumulated mortality rate in the fish held at 33 °C. This infection decreased with higher water temperatures, as there was a virulence rate of 100% accumulated mortality rate at ambient temperature.

In summary, to accomplish good production in growth performance and maintain good fish health and water quality in a culture system, the optimal temperature range of butter catfish during the fingerling stage is between ambient temperature and 31 °C. However, fish farmers should take precautions during the culture because enteric septicemia of catfish (ESC), caused from *E. ictaluri* infection, is a potentially deadly problem in this thermal range. This study concludes that butter catfish can endure slightly increased temperatures arising from global warming.

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Chapter 1

Introduction

1.1 Research background

Average mean annual temperatures, amounts of precipitation and alterations of weather patterns are occurring worldwide, with changing greenhouse gas emissions in both recent and long term climate studies. By using a model based on doubled atmospheric CO_2, an increase of 1-7 °C in mean global temperature with regional changes in precipitation patterns, storm tracks and sudden irreversible changes are predicted. Increased water temperatures, decreased dissolved oxygen levels, and increased toxicity of pollutants on freshwater systems are expected as impacts of climate change. Over time, global climate change is expected to be catastrophic for many fish populations in both natural and artificial systems (Ficke *et al.*, 2007). A study by Green and Fisher (2004) found that a small variation in temperature resulted in a large variation in growth, development and swimming performance of the tropical reef fish species, *Amphiprion melanopus.* Ambient water temperature regulates growth rates by affecting many physiological processes in fish such as food consumption, metabolic rate, reproduction, activity and survival (Jobling, 1997).

In an aquaculture facility, the maximum temperature for most species during the year differs from their optimum water temperature. Compared to too cold water, providing optimum temperatures and slightly warmer than normal water results in better growth and food conversion as a consequence of higher metabolic rates, which tend to slow as temperature cools below optimum for a particular species and increase as the temperature rises above the optimum level (Stickney, 1979). Virtually all aquaculture candidates are poikilothermic animals, and ambient water temperature is the major factor controlling their metabolic rates (Stickney, 1979). Temperature controls the reactions of enzymes through hormonal and nervous control to digestion, from respiration and osmoregulation to all parts of an organism's performance and behavior (Snyder, 2011); therefore, if the temperature is high for a warm water species (25-32 ^{o}C) (Boyd, 1998), it will tend to double the metabolic rate, as the

reactants in the cells have greater thermal energy, leading in turn to many cellular enzymes becoming more active and leading to faster growth (Fondriest Environmental, Inc., 2014; Pearson Education, Inc., nd). Many aquatic animals experience altered health and growth as a variety of metabolic processes including respiration, feeding, and digestion are affected by fluctuations of temperature. Both temperature and pH affect both the biochemical systems of pathogens and the physiological conditions of fish. As fish growth and health are influenced by temperature (Wedemeyer, 1996), it is important to study the effects of thermal stress on fish and other aquatic organisms (Jobling, 1997).

Butter catfish, also known as two-spot glass catfish, is found from India to Indonesia in streams and rivers of all sizes with currents ranging from slow to moderate, including confinements in the Mekong basin of Cambodia and wide distribution in Pakistan, Sri Lanka, Bangladesh and Myanmar (Rainboth, 1996; Ng *et al*., 2010). In Thailand, this species is commonly found in natural water bodies such as rivers and floodplains (Gomonteir *et al*., 2012). This fish is a favorite food of the Thai people and is an important economic species with good market value (Gomonteir *et al*., 2012). It is also an important and favorite food fish and fishery in the Indian subcontinent. It is also an ornamental fish for export (Ng *et al*., 2010). As an economically important freshwater species with high demand in many countries in Asia, in this period of climate change and warming waters worldwide, this research on the "Effects of water temperature on growth performance and health of butter catfish (*Ompok bimaculatus*)" is seen as useful to determine the optimum temperature level in growth with healthy fish for successful aquaculture outcomes.

1.2 Literature review

1.2.1 Habitat, biology and general characteristics of *Ompok bimaculatus* (Bloch, 1797)

Butter catfish or two-spot glass catfish is a freshwater species which is found from India to Indonesia in streams and rivers of all sizes with currents ranging from slow to moderate as well as in the confinements in the Mekong basin of Cambodia. It is often found near submerged brush piles (Rainboth, 1996). It is widely distributed in Pakistan, Sri Lanka, Bangladesh and Myanmar (Ng *et al*., 2010). Adults are found in quiet, shallow (0.5-1.5 m) often muddy water, in sand by streams, rivers and tanks. Also, they occur in canals, beels and during the flood season they shelter in newly inundated habitats (Rainboth, 1996; Ng and Hadiaty, 2009). In Thailand, this species is commonly found in natural water bodies such as rivers and floodplains (Gomonteir *et al.*, 2012). It is a carnivorous fish (digestive tract shorter than body length). Its stomach is a large bag for food storage and digestion (DOF, nd). It is also a slow-moving and stealthy predator as its feed is crustaceans, fishes and sometimes mollusks (Rainboth, 1996) and according to Pethiyagoda (1991), it feeds on vegetable matter and fish. It feeds all day long and it is usually both a surface and water column feeder.

The preferred water depth of the butter catfish ranges from 0-2 m with a pH range of 6.0-8.0. Its tropical habitation is in the range of water temperature from 20-26 °C (Ng and Hadiaty, 2009). The main identifying marks of this species are 1) its eye is subcutaneous or under the skin, with the orbital rim continuous with the skin covering the eye, 2) mouth short not extending to eye, 3) mouth cleft sharply oblique, having vomerine teeth in two patches, and 4) containing three to four dorsal fin rays. Its maxillary barbel (antennae) extends at most to the anal fin origin and there are between 59 and 74 anal fin rays (Figure 1) (Rainboth, 1996). The body is relatively long and flat with a standard length of 45 cm with a dark gray or brown head. The area under the head and abdomen is lighter in color than the body. Next to the gills above the pectoral fins, there is a black spot and some fish may have a black spot at the caudal fin. At irregular intervals or only in a few places along the body and fins

tiny black spots can sometimes be seen. The edges of the fins are dark, particularly around the caudal and anal fins (DOF, nd).

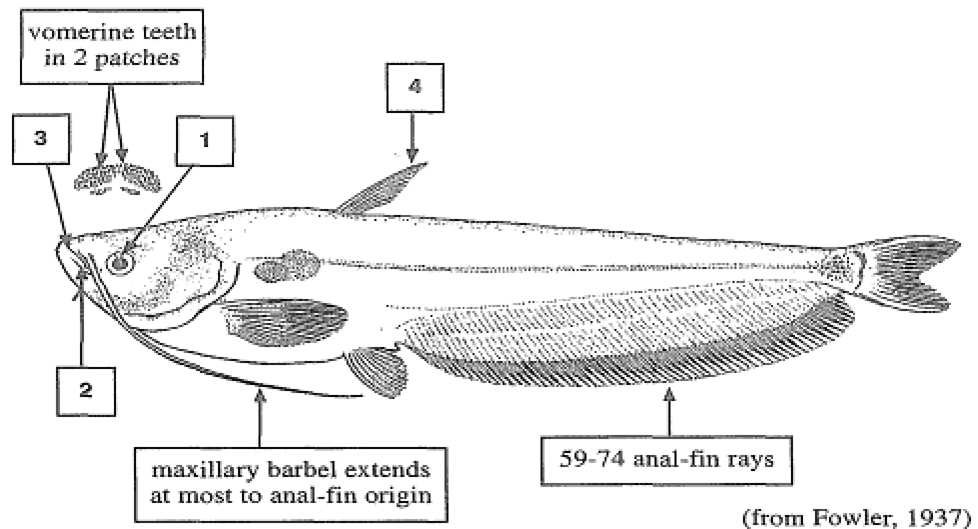

Figure 1 *Ompok bimaculatus* (Bloch, 1797)

Source: Rainboth (1996)

1.2.2 Taxonomy

The taxonomic hierarchy of the butter catfish is as following:

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Siluridae

Genus: *Ompok*

Species: *O. bimaculatus*

Scientific Name: *Ompok bimaculatus*

Synonym: *Silurus bimaculatus*

Common Name: Butter catfish/ two-spot glass catfish (Ng *et al*., 2010)

1.2.3 Major threat(s)

This species population has declined in recent years as a consequence of its overexploitation for food. Habitat destruction and competition from alien species are other anthropogenic threats (Ng *et al*., 2010).

1.2.4 Aquaculture of butter catfish

1. Reproduction

1. In its natural habitat, the spawning season is between May and August. For aquaculture purposes, the parent brood fish should be at least 6 months old.

2. Hormone injections: There are 2 types of hormone injection to stimulate ovulation in female brood fish prior to artificial insemination:

▪ Hormones from the butter catfish pituitary gland: The pituitary gland releases 2 important hormones involving fish spawning – (1) FSH (follicle stimulating hormone) which stimulates egg development in females and sperm cell division in males, and (2) LH (luteinizing hormone), which has a role in ovulation in female and spermatogenesis in male fish (DOF, nd).

The use of hormones from the butter catfish pituitary gland was more popular because of better results. To acquire these hormones, pituitary glands are collected from fish in peak maturation. The most popular fish for collecting these hormones are three species of the Chinese carp, rohu (*Labeo rohita*), common carp (*Cyprinus carpio*) and white or mrigal carp (*Cirrhinus cirrhosus*) because the pituitary gland of these fish contains large amounts of hormones and give a good result after injection into female brood fish (DOF, nd).

In artificial insemination of butter catfish, the female brood fish will be injected with 2 doses of hormone from the common carp pituitary gland combined with 20 μg/kg of synthetic hormone and 10 mg/kg of a synergistic chemical.* 12 hours after hormone injection, eggs are stripped from the female brood fish. 6 hours after injecting the female brood fish, the male brood fish will be injected with 1 dose

of hormone from the common carp pituitary gland combined with 0.5 µg/kg of synthetic hormone and 10 mg/kg of synergistic chemical.

▪ Synthetic hormone is very popular in modern fish raising. The trade name is "Suprefact" and it contains buserelin acetate in solution form. One bottle of suprefact contains 10 cc of solution with 10,000 µg of hormone. It must be diluted before use. To give effective results, it must be combined with a synergistic chemical named "Domperidone", a white tablet containing 10 mg of active ingredient per tablet.

In artificial insemination of butter catfish, female brood fish will be injected with 10 µg/kg of suprefact and 10 mg/kg of domperidone. 8 hours after hormone injection, eggs are stripped from the female brood fish.

2. Artificial fertilization

At a given period, eggs and milt are stripped from the female and male brood fish and mixed together using a modified dry method. Artificial fertilization is undertaken using male and female brood fish in a ratio of 3:5. The fertilized eggs are cast over a net frame hanging in a hatching pond and hatch in 24 hours. The net frame is removed from the pond after the eggs hatch and the larvae will be nursed for further development (DOF, nd).

1.2.5 Growth

1.2.5 Growth

Chawpaknam *et al*. (1993b) studied embryo development of butter catfish and reported that the newly fertilized egg was adhesive with a diameter of 1.2-1.5 mm. It took 2 hours and 10 minutes to develop to cleavage stage (2-64 cells) with a diameter of 2.4 mm. Twenty minutes after that it continued development to the morula stage. Ten minutes later, the embryo developed to the blastula stage. Cell division continued through 2 tissue layers, including the notochord, and then on to the early gastrula stage and the notochord covered the egg yolk in 2 hours, which was the end of the gastrula stage. The embryo then began to form the brain, neural tube, optic cup, and

heart, and the circulatory system began to function and the young fish finally hatched in 11 hours.

It took a total of 18 hours from fertilization to hatching at 27-28°C. The yolk sac of the larvae was totally absorbed in 2 days. The appropriate foods for fish larvae were hard-boiled egg and water fleas. Seven-day-old fish larvae look like their parents.

Chawpaknam *et al*. (2004) studied larval development of butter catfish and found the important characteristics for classification of butter catfish larvae as following:

1. The stage with yolk sac remaining can be classified by 3 pairs of barbels and black spots appearing over the eyes and a black spot on top of the yolk sac.

2. The early larval stage can be classified by 3 pairs of barbels and black spots appear on the head and trunk (over the operculum).

3. The late larval stage can be classified by 50 muscle bands and color spots appearing on the head and upper trunk.

4. The small butter catfish can be classified by an anal fin having 63-69 spines and a large black spot over the pectoral fins.

1.2.6 Nursing of fish larvae

Newly hatched larvae or yolk-sac fry absorb food from the yolk sac. When the yolk sac of the fish larvae is totally absorbed, they need to be fed with rotifers or water flea nauplii which give growth performance and higher survival than feeding with hard-boiled egg yolk because the larvae have better response to a moving diet. After feeding with rotifers or water flea nauplii for 5 days, larger water fleas are given.

Chawpaknam *et al*. (1993a) succeeded in propagating butter catfish by artificial insemination in 1990 at the Chonburi Freshwater Fisheries Development Center in Thailand. They began the feeding of the larvae with rotifers and water flea nauplii. Mass production of fish larvae is crucial for aquaculture development of

butter catfish. The big problem of fish larval production is high mortality in the early stages of larval development due to various problems such as fish health, genetics, water quality, diet quality and digestive system. The given diet must be relevant or aligned with digestive system development because the cells in the stomach that produce the protein-digesting enzymes have not developed yet, and the fish larvae cannot digest the proteins in artificial food. However, they can digest proteins from natural food sources because of enzymes in the natural food such as rotifers, water fleas and artemia.

1.2.7 Interaction with water quality conditions

Water quality in intensive fish culture is an important factor in minimizing disease and stress exposure. The physiological tolerance of fish is affected by many environmental and biological variables. Optimum rearing conditions for fish health differ according to species, age, size, and the exposure to chemical constituents the fish may have been exposed to during their early lives, and thus it is a complex matter to decide on the specific chemical constituents, temperatures, or dissolved gas concentrations for a certain species at a certain time. Water pH, dissolved oxygen and temperature can have extreme impacts on dissolved substances. Identification of acute and chronic toxicity levels of water quality has been reported, but it is difficult to determine the water quality concentrations for optimum rearing conditions. The levels of the major heavy metals that create lethal conditions for fish rearing are well known (e.g., the 96-h LC50). However, there is a lack of information on the maximum safe chronic exposure levels that will cause no harmful effects over the life cycle. Dissolved metal concentration information is necessary in order to promote physiological health and disease resistance. Zinc and copper (toxic heavy metals) are dietary micronutrients for normal growth and development of living organisms, but the optimal dissolved concentrations are still unknown. In spite of these complex issues, there is a consensus view of some of the water quality conditions to promote fish health in intensive culture. The desirable range for both cold and warm water species in regard to dissolved oxygen is 7 mg/l minimum; a pH of 7-8 average and

extremes no more that 6-9; un-ionized ammonia (NH_3) of 0.05 ppm maximum; and total CO_2 <10-15 mg/l (Wedemeyer, 1996).

1.2.8 Relevance of temperature and other water quality parameters

1. Oxygen

Temperature has a conspicuous impact on chemical and biological processes. Normally, the higher the temperature, the greater the requirement for oxygen and food, and the faster the growth rate is (Boyd, 1998; Fondriest Environmental, Inc., 2014). Oxygen requirements will be increased at higher temperatures; raising the water temperature from 10-20 °C at least doubles the oxygen demand because an increase in temperature contributes to a decline in the solubility of gases in water. Warmer water has a lower capacity to hold oxygen (Figure 2), and many fishes are only capable of living in cooler water (Svobodová *et al*., 1993; Hoang and Dvorsky, 2014).

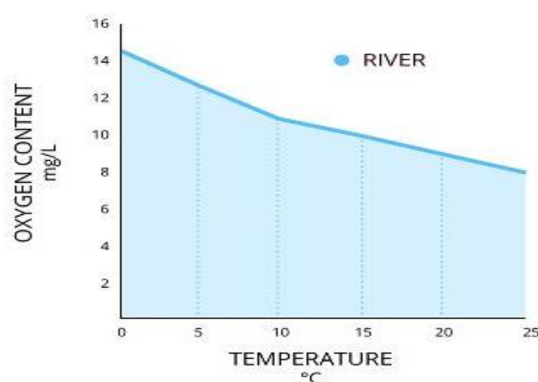

Figure 2 The relationship of temperature and dissolved oxygen concentrations in river water.

Source: Fondriest Environmental, Inc. (2014)

2. Water pH

Both high and low temperatures cause pH shifts, so that the concentrations of ions will also shift. This means that if water temperature rises, the equation will adjust to the left to reach equilibrium again (Figure 3). The ions in the water are decreased by shifting to the left, increasing the pH, and if the temperature decreases, the equation will shift to the right, and increase the concentration of ions

and decrease water pH as well (Fondriest Environmental, Inc., 2014). Noticeably, water pH influences other water factors, leading to harmful changes in other substances in the fish such as ammonia, hydrogen sulfide, cyanide, and heavy metals (Svobodová *et al*., 1993). Alteration of water temperature leads to various hazardous substances in the water shifting simultaneously.

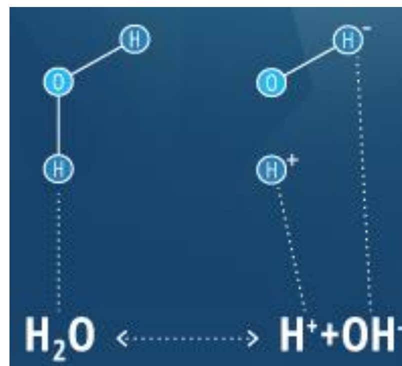

Figure 3 Shifting of the concentration of H^+ and OH^- ions to keep equilibrium in water.

Source: Fondriest Environmental, Inc. (2014)

3. Ammonia (NH_3)

There are two forms of ammonia in water or other biological fluids, a molecular form (NH_3) and an ammonia ion form (NH_4^+). Water pH and temperature determine the ratio of these two forms (Table 1). A concentration gradient occurs then a molecular form diffuses across the tissue barrier which forms the toxicity to fish. But the ammonia ion (NH_4^+) cannot cross cell walls. The dissolved oxygen concentration in water also impacts ammonia toxicity. When the oxygen concentration is low in the water, ammonia toxicity exists. The amount of free CO_2 in water affects the level of toxic ammonia. The extent of low pH in water depends on the amount of the respiratory CO_2 diffusion at the gill surface. When the pH is low, the amount of ammonia ion is reduced but produces more molecular forms in the water. For instance, a case of autointoxication among carp yearlings happened after their transfer from a pond to large aquarium tanks with well water, because the blood serum of the carp contained high ammonia N levels. The mean blood serum level of ammonia N was 3,054 (2,400-3,600) µg per 100 ml of serum in the poisoned fish.

However, the mean blood serum level of ammonia N was 825 (750-900) µg per 100 ml of serum in unpoisoned fish. The reason for the ammonia N poisoning was considered to be the increased level of blood serum ammonia N because of the persistence and absorption of the carp gut contents from natural food and high protein feed pellets which are exposed to environmental stress such as confinement and reduced oxygen levels during transport, and water temperature reduced by about 5 °C (Svobodová *et al.*, 1993).

Table 1 The molecular form [NH_3 as % of total ammonia (TAN)] in water at different pH values and temperatures

pH	T °C					
	0	5	10	15	20	25
7.0	0.082	0.12	0.175	0.26	0.37	0.55
7.2	0.13	0.19	0.28	0.41	0.59	0.86
7.4	0.21	0.30	0.44	0.64	0.94	1.36
7.6	0.33	0.48	0.69	1.01	1.47	2.14
7.8	0.52	0.75	1.09	1.60	2.32	3.35
8.0	0.82	1.19	1.73	2.51	3.62	5.21
8.2	1.29	1.87	2.71	3.91	5.62	8.01
8.4	2.02	2.93	4.23	6.06	8.63	12.13
8.6	3.17	4.57	6.54	9.28	13.02	17.95
8.8	4.93	7.05	9.98	13.95	19.17	25.75
9.0	7.60	10.73	14.95	20.45	27.32	35.46
9.2	11.53	16.00	21.79	28.95	37.33	46.55
9.4	17.12	23.19	30.36	39.23	48.56	57.99
9.6	24.66	32.37	41.17	50.58	59.94	68.63
9.8	34.16	43.14	52.59	61.86	70.34	77.62
10.0	45.12	54.59	63.74	71.99	78.98	84.60
10.2	56.58	65.58	73.59	80.29	85.63	89.70
10.4	67.38	75.12	81.54	86.59	90.42	93.24
11.0	89.16	92.32	94.62	96.26	97.41	98.21

Source: Svobodová *et al.* (1993)

4. Nitrite (NO_2^-)

Nitrites are naturally found in surface waters with nitrates and ammonia nitrogen. Their instability means there are usually only low concentrations in water. The toxic action of nitrite on fish is associated with a number of internal and external factors such as fish species and age and general water quality. Water pH and temperature control the relationship between NO_2^- and non-dissociated HNO_2

(Svobodová et al., 1993). The effect of pH on nitrite toxicity within natural water appears in a short time (Kroupova *et al*., 2005). Dissolved oxygen concentration and water temperature also influence nitrite toxicity. The formation of methemoglobin is from nitrites, which cause a reduction in the oxygen-carrying capacity of hemoglobin and their inability to effectively release oxygen to the body tissues (Greenberg *et al*., 1943). The problem occurs at low oxygen concentrations and the oxygen requirement of fish increases with temperature. Sub-lethal concentrations of nitrites do not cause serious damage to the fish even with long exposure since growth rates are depressed. Fish can tolerate sub-lethal levels up to 20-40% of concentrations of nitrites (Svobodová *et al*., 1993). Hence, the lethal concentrations or safe concentrations of nitrites are difficult to establish for aquaculture (Boyd, 1990).

5. Hydrogen sulfide (H_2S)

Hydrogen sulfide is a toxic soluble gas which arises from organic pollution due to the decomposition of proteins. Svobodová *et al*. (1993) reported that 0.4 mg/l (salmonids) to 4 mg/l (crucian carp, tench and eel) of H_2S is the harmful range of concentrations for various fish species. Additionally, H_2S is toxic in hypoxic conditions (Boyd, 1990; Wedemeyer, 1996). Reduced water pH and colder water temperatures also lead to increases in the toxic form (H_2S); therefore, acidic environments are dangerous for aquatic organisms (Boyd, 1990). According to Wedemeyer (1996), at pH 7 and above, total sulfide will be converted to the less toxic (HS^-). Higher than optimal levels of temperatures and water pH in fish culture trigger the dissociation of an equilibrium mixture of HS^- and H^+ ($H_2S \leftrightarrows HS^- + H^+$) (Wedemeyer, 1996).

6. Carbon dioxide (CO_2)

The major natural CO_2 sources are from the atmosphere, which contains a small amount of carbon dioxide, from the respiration of microorganisms, algae and other aquatic plants, and from the microbial decomposition of organic matter in the bottom sediments of water bodies. But in fish rearing units, the main source of CO_2 is from fish metabolism (Wedemeyer, 1996). A narcotic effect on fish, up to and including death, appears at high concentrations of carbon dioxide

(exceeding about 100 ppm). The environmental sources of CO_2 average only 1-2 mg/l which is too small to have any detrimental effect on the fish, and the most common source large enough to have an effect on the fish is from fish respiration at ambient CO_2 levels >40 mg/l (Boyd, 1990; Wedemeyer, 1996). Higher water temperatures result in lower dissolved oxygen concentrations which also contribute to the toxicity of CO_2. Dissolved oxygen has an adverse effect on CO_2 concentrations; for example, the hemoglobin loading of oxygen becomes interrupted at high CO_2 concentrations. Since the accumulation of CO_2 happens in fish blood, it can deteriorate blood pH and trigger serious effects (Boyd, 1990). More importantly, water pH is associated with the toxicity of dissolved CO_2; bicarbonate (non-toxic HCO_3^-) and carbonate ions (CO_3^{2-}) act as buffers at between pH 7-9 and above pH 11, respectively. Hence, dissolved CO_2 reduces fish distress in alkaline water. The presence of carbonic acid (H_2CO_3) (only 10%, less severe) is usually ignored (Svobodová *et al.*, 1993; Wedemeyer, 1996). Mostly dissolved CO_2 presents as CO_2 at below pH 5 (Wedemeyer, 1996).

$$CO_2 + H_2O \leftrightarrows H_2CO_3 \leftrightarrows H^+ + HCO_3^- \leftrightarrows H^+ + CO_3^{2-}$$

1.2.9 Temperature limits

Fluctuation and extremes in the temperature of the water impact fish health in intensive culture. Every fish species thrives in its own water temperature range, while a normal temperature or a sudden temperature change within this range (25-32 °C) can lead to stressful or lethal conditions (Wedemeyer,1996).

Many alterations are caused to fish by increasing water temperatures, e.g. dissolved contaminants distress, growth promotion, fish pathogens invasion, lessening DO concentrations, and maximizing of oxygen consumption due to a boost in body temperature leading to enhanced metabolic rates, while body temperatures, immune response, feeding, activity, and growth become lower during decreasing water temperatures. Overall, fish health and physiological conditions are strongly associated with water temperature. And water temperature is only one amongst other important variables in the environment with the possible exception of dissolved oxygen. The

channel catfish is a eurythermal species which is capable of adapting to a broad range of temperatures. By contrast, salmonids and other cold-water species are stenothermal and have a smaller thermal limit, making them slightly sensitive to sudden changes of water temperature. Tropical fish such as tilapia are stenothermal but have a smaller tolerance to cold temperatures. Typically, both stenothermal and eurythermal species are satisfactory for pond culture. However, to evaluate temperature effects on fish in a pond is difficult because the ecology of the pond produces more complexities along with changes of water temperature. This is because every aquatic organism develops and proliferates within an individual temperature limit. If a change of a few degrees happens, some species decline and some increase (Wedemeyer, 1996).

The highest or lowest temperatures at which fish can survive are known, and these temperatures affect some variables such as alteration of acclimation extent, DO concentration, and the amount and type of dissolved ions which are present in the water. Therefore, the lethal temperature levels of fish are not a stable value and lethality can change dramatically with only a few degrees of temperature change. The most important consideration of lethal temperatures is in hatchery work and the lethal temperature ranges of several popular market fish species are known, such as channel catfish (4 and 35 °C), rainbow trout (0 and 26 °C), tilapia spp. (10 and 38 °C), carp (0 and 30 °C) (Wedemeyer, 1996).

The reasons for fish death at high temperatures are complicated. However, the effect of temperature is connected with other variables. Temperature affects the fishes at the interaction of the body temperature. Instantly, oxygen consumption rises when temperature increases, but the solubility in water of DO declines simultaneously by at least half by at least half with a tiny temperature increase. Serum electrolyte concentrations and energy supplies of whole body lipid content also decline as the water rises. Blood oxygen levels and oxygen transportation to the tissues in the fish body consistently lessen at higher temperatures. The ultimate cause is a broken osmoregulation system that may cause the fish to die. To mitigate the problem of high temperatures, essential ions (sodium, magnesium, calcium) can be added to the water (Wedemeyer, 1996).

1.2.10 Tolerance to temperature changes

The lethal temperature extremes are of less concern than temperature changes which affect individual fish physiology during culture. Too rapid change of water temperature is a well-known issue, resulting in temperature shock syndrome, which fish farmers have to deal with during cultivation (Wedemeyer, 1996). Sudden changes in temperature of as little as 3-4 °C can stress aquatic animals or even cause death. This often happens when moving fish from colder water to warmer water. Water temperature changes have caused the adjustment of aquatic animals' life history and behavior (Boyd, 1998). For instance, one study found that threadfin shad survived less than 24 hours as a result of becoming acclimated to 15 °C and then immediately having the water chilled to 5 °C. Rapid temperature changes of 10 °C or more can also activate infections and often cause temperature shock mortality (Wedemeyer, 1996). Likewise, studies in catfish found that fertilized eggs of *Clarias gariepinus* died at 15 °C, and 16 °C was below the lower thermal tolerance limit in channel catfish (*Ictalurus punctatus*) and was harmful to scale fish as well (Haylor and Mollah, 1995; Small and Bates, 2001).

The degree of sudden temperature that any individual species can tolerate is not known, thus gradually acclimating fish (two or more hours) to warmer or colder water is necessary if the temperature difference is higher than 10 °C (Wedemeyer, 1996).

1.2.11 Effects of temperature on fish growth and fish health

Fish can easily tolerate the seasonal changes in temperature in their natural environment. In a new environment, fish are susceptible to temperature shock when the temperature is 12 °C colder or warmer (8 °C in the case of salmonids) than the original water. Fish can die in conditions of abrupt temp change, showing symptoms of paralysis in the respiratory and cardiac muscles. When young fry adapt to the different water temperature from 1.5-3 °C, various problems can happen. For example, their digestive processes will slow down or stop unless the fish are transferred immediately after feeding to water that is colder by 8 °C or more. When

the digestive tract retains undigested or half-digested food and produces gases, the fish become bloated, lose balance control, and finally die. The level of ammonia nitrogen in the blood serum will considerably increase when the fish keep consuming high-nitrogen feed (natural food or high-protein pellets) and fish are abruptly transferred to much colder water. This is because the decreasing metabolic rate reduces the diffusion of ammonia from the gills. Thus, ammonia autointoxication and death can occur (Svobodová *et al*., 1993).

1.2.12 Temperature manipulation in aquaculture

Knowledge of the temperature requirements for each fish species is necessary for selecting aquaculture species, not just survival criteria. There are three basic groups of optimum temperatures for aquatic animals, warmwater (tropical species), coldwater (temperate species) and midrange. Warmwater species have temperature optima at or above 25 °C, coldwater species at or below about 15 °C and midrange species between the other two. Some species such as the American oyster (*Crassostrea virginica*) and channel catfish can be cultured in either warm or relatively cool environments. These two areas are considered as the extremes for suitable aquatic animal cultivation. The culturists must also consider the required time to produce a marketable animal (Stickney, 1979). The daily growth of the particular species controls the production in the aquatic farming industry, the animal husbandry sector, agriculture, and forestry (Hernández *et al*., 2007). Growth in a tank or cage depends on the ration size. It is necessary to consider internal and external factors such as density, environmental conditions (water composition, water temperature) or fish social behavior, which influence growth rate and production outcomes. Hernández *et al*. (2007) reported that in seabream culture in Mediterranean countries, harvesting time and ration size were dependent on water temperature and stocking date.

In general terms, the temperature ranges suitable for fish in aquaculture systems are often restricted. But in natural water fishes have the capability of seeking out the preferred temperature zones. Each species has a characteristic temperature

range. In northern temperature regions, the production cycle may occur under ice or at the near zero temperatures in which marine fish species and salmonids are cultured, while fish and shrimp cultured along the equator pass through their growth cycle at temperatures exceeding 30 °C for most of the year (Goddard, 1996).

1.2.13 Effects of climate change on aquaculture

The increasing water temperatures have altered fundamental ecological processes and the geographic distribution of aquatic species (Poff *et al.*, 2002). Higher inland water temperatures have produced wild and cultured stock extinction by declining water quality, worsening of dry season mortality, and introduction of new predators and pathogens (Worldfish Center, 2007). These variations have not only contributed to the deterioration of productiveness and the distribution of marine and freshwater species in aquaculture activities but also have affected biological processes and the alteration of food webs (Cochrane *et al.*, 2009; Yazdi and Shakouri, 2010).

However, rising temperatures also provide new opportunities in some aquaculture areas, as new areas become viable for new aquaculture operations due to rising temperatures and sea levels. Thus, aqua-farmers may benefit from the expansion of aqua-areas. On the other hand, reduced water availability and water quality, increasing disease incidence, and reduced inland water aquaculture due to modified groundwater salinization have become important issues affecting aquatic cultivation (De Silva and Soto, 2009; Yazdi and Shakouri, 2010). However, increasing water temperatures have less negative effects on warm water species than cold water species (Ficke *et al.*, 2005; De Silva and Soto, 2009). For example, trout and salmonids are in danger from their current geographic range in the continental United States due to warming that goes beyond their thermal tolerance limits (Poff *et al.*, 2002), while on the other hand warm water species such as channel catfish, tra catfish, spotted tilapia, common carp, and rohu cultures benefit from higher water temperatures (Ficke *et al.*, 2005; Suja *et al.*, 2009; Phuc, 2015). However, the changes that will result from climate change in Asian tropical and subtropical regions on freshwater aquaculture are still in question (De Silva and Soto, 2009; Yazdi and

Shakouri, 2010). The 2019 NOAA global climate report reported that the average global land and ocean surface temperature in May 2019 was +0.85 °C above the 20th century average (NOAA National Centers for Environmental Information, 2019).

1.2.14 Effects of temperature on fish hemato-immunology

The environment (extrinsic factor: temperature) is one of the three broad factors affecting the immune response of fish (Roberts, 2012). Fish are poikilothermic animals whose body temperature is similar to the environment. Below or above the thermal tolerance can generate anomalous conditions or deleterious fish immune responses and lead to increased susceptibility to infections (Bly and Clem, 1992; Martins *et al*., 2011). Environmental temperatures below 11 °C, at 15 °C constant temperature and 15-20 °C cycling temperature are a factor of immunosuppression in channel catfish (Bly and Clem, 1992; Martins *et al*., 2011). Not only low temperatures, but also elevated temperatures are able to strengthen certain pathogens' abilities (highest outbreak of enteric septicemia of catfish (ESC) at 29 °C in channel catfish) (Johnson and Hinck, 1985) and impair the immune response and other mechanisms of the fish (Harrahy, 2000). Available data on the thermal influence on non-specific defenses in orange-spotted grouper (*Epinephelus coioides*) are with regard to the decreasing of respiratory burst and phagocytic activity at the lower and upper thermal limits (Cheng *et al*., 2009). Channel catfish show enhanced lysozyme activity at above the optimal range (Suja *et al*., 2009). The impacts of temperature on immune function vary and depend on the species and life stage of the fish, genetic strain, pathogen species, previous pathogen exposure, previous acclimation, and general health of the fish such as physiological and nutritional status (Harrahy, 2000).

Hematological tests are valuable as indicators of fish health status (disease or stress, physiological and metabolic alterations). Especially, the effect of temperature on hematological parameters is an important diagnostic tool to determine any variations in fish health (Bansal *et al*., 1979). Hemoglobin and red blood cell counts are altered by the alteration of water temperature, and the oxygen supply then varies dependent on these factors. For instance, *Channa punctatus* (Ravichandra, 2012) and *Shizothorax richardsonii* (Tyagi *et al*., 2013) had smaller red and white blood cells

and lower hemoglobin content at low temperatures. Hemoglobin concentration and hematocrit decreased in common carp culture after acclimation from 24 °C to 4 °C in acute and chronic tests (Chen *et al*. 1996). Low hematocrit, red blood cell counts, and hemoglobin cause health problem like anemia to both teleosts and humans (Billett, 1990; Witeska, 2015).

1.2.15 Innate immune system

The immune system of fish is physiologically different from the higher vertebrates. Fish openly live dependent on their innate immune system from the early stages of embryogenesis (Uribe *et al*., 2011). The immune system is an essential mechanism comprising two main defense weapons (cellular and humoral components) which destroy invading foreign substances and prevent disease outbreak (Biller-Takahashi and Urbinati, 2014). The cellular and humoral components exist in two categories of fish immune system, the innate or nonspecific and adaptive or specific immune systems (memory). The adaptive system is a secondary defense which occurs after the invading pathogens successfully enter through the innate immune system (Uribe *et al*., 2011). Innate immunity is a rapid response, a natural inborn resistance to infectious agents, and non-selective (attempts to kill all pathogen types). The innate immune system is commonly divided into 3 main components (Magnadóttir, 2006; Roberts, 2012):

a) First line of defense or surface barriers: The epidermis (skin) of a fish is a barrier to invading pathogens. The external parts, e.g. scales, spines or toxic secretions, also help to protect against strange agents or microorganisms from entering the body. The mucus of the skin, gills and gut plays a role in preventing unwanted material (Roberts, 2012). The mucus contains beneficial substances such as lectins, lysozymes, pentraxins, complement proteins, antibacterial peptides, and immunoglobulin M (IgM), which have a fundamental function for inhibiting the entry of pathogens (Magnadóttir, 2006; Uribe *et al*., 2011).

b) Humoral factors: These are an array of soluble substances within the body fluids which present as an army to restrain the growth of microorganisms or to neutralize the enzymes in accordance with the pathogens. Nonspecific humoral factors are classified by their activity as growth inhibitors (transferrin and interferon), inhibitors of pathogenic enzymes, lysins (complement proteins, antimicrobial peptides, and lysozymes) and precipitins and agglutinins (pentraxins and lectins) (Roberts, 2012).
c) Cellular factors: The widespread macrophages or monocytes are keenly phagocytic for the destruction of invading agents (Roberts, 2012). Monocytes, macrophages, dendritic cells, and granulocytes are proficient phagocytes that are inducible at the inflammation site when injured tissue releases molecular signals of inflammation (cytokines) (Stuart and Ezekowitz, 2005; Biller-Takahashi and Urbinati, 2014). Neutrophils and macrophages specialize in phagocytosis, chemotaxis, and bactericidal activity. During phagocytosis, these defense cells destroy the microorganisms with hydrolytic enzymes and reactive oxygen species (Biller-Takahashi and Urbinati, 2014).

1.2.16 *Edwardsiella ictaluri*

1. History of *E. ictaluri* and its distribution

E. ictaluri is known as a gram-negative bacterium and the causative agent of ESC, which is a pathogen of fish and a zoonotic disease. This disease was initially identified in 1976 from a specimen examination in the Southeastern Cooperative Fish Disease Laboratory (SECFDL) at Auburn University, Alabama. The specimens were initially collected from pond-reared fingerlings and yearling fish in Alabama and Georgia, USA, and recognized as a new species in 1981. Mortality reports of up to 50% have been recorded for catfish in Mississippi, Arkansas, Idaho, Colorado, Indiana and Maryland. About 2% of total bacterial cases caused from other genera, *Edwardsiella tarda* and the newly discovered *Edwardsiella piscicida,* have also been identified. Ultimately, the catfish industries decided to downsize their culture quantities with the result of millions of dollars in losses yearly. Its geographic range has extended in the catfish industry and it has become one of the top two

bacterial columnaris diseases (Johnson and Hinck, 1985; Austin and Austin, 1987; Noga, 2010; Hawke *et al*., 2013; Esmail *et al*., 2015; Hawke, 2015).

Channel catfish in the US are the main host of this infection, but other species of catfish such as the white catfish and the brown bullhead are vulnerable to *E. ictaluri* infection. Similarly, blue catfish and blue x channel catfish hybrids have little resistance and are fairly vulnerable. In recent years this infection has expanded to countries in Asia such as Japan, Vietnam, Thailand, and China in cultured Ayu, *Pangasius* “basa”, Clarias “Walking catfish” and yellow catfish, respectively. The strains from Asia and from tropical Western Hemisphere tilapia farms vary from the U.S. catfish strains and they have been isolated from some non-catfish species such as rosy barb, green knife fish, and devario. In recent years zebrafish have been found to be markedly vulnerable to ESC, and the survival rates of this fish decreased dramatically after bacterial infection both in the laboratory and in pond populations. Hence, *E. ictaluri* is now recognized as an international bacterial disease (Noga, 2010; Hawke *et al*., 2013; Esmail *et al*., 2015; Hawke, 2015).

2. Temperature of disease outbreak

ESC outbreaks occur at a temperature range of 20-28 °C in both the spring and fall seasons. After the spawning season in summer, the culture of channel catfish fingerlings in the southeast United States encountered high disease outbreaks from early fall and spring (Francis-Floyd *et al*., 1987; Noga, 2010; Hawke, 2015). Beyond the peak of temperature level (20-28 °C), the mortality was lower and it was a chronic infection (Francis-Floyd *et al*., 1987; Noga, 2010; Hawke, 2015). In spite of a study saying they survived only a short time in water, another study found them surviving after 3 months in the sterile pond mud at 25 °C (Hawke, 2015).

3. Characteristics of the disease

There are two specific forms of ESC in channel catfish such as acute and chronic. In the acute (septicemia) form, bacteria are ingested through the gut, pass into the blood stream through the intestine and then colonize into diverse organs, leading to necrosis and ulceration. Acute mortality normally happens with few

external signs (Noga, 2010). The clinical signs of *E. ictaluri* in channel catfish include the fish hanging at the water surface, spinning rapidly in circles and swimming spirally just prior to death. The external signs include the presence of petechial hemorrhages (pinpoint red spots) on the skin in the throat and mouth areas. The gills become pale and exophthalmia may occur. Internally, the kidney and spleen become swollen, with liver hemorrhage and necrosis, the peritoneum contains bloody or ascitic fluid and the muscles have petechial hemorrhages (Austin and Austin, 1987; Noga, 2010). The chronic (encephalitic) form occurs via the nervous system. The bacteria enter the olfactory organ through the nasal opening and move to the olfactory nerve then to the brain. The infection proliferates from the meninges to the skull and eventually to the skin, leading to open lesions on the frontal bone of the skull (Noga, 2010).

1.3 Objectives of the study

1. To evaluate water quality in butter catfish cultured under temperature manipulation.
2. To evaluate fish growth performance and health conditions under temperature manipulation.

Chapter 2

Materials and methods

2.1 Materials

1. Butter catfish (*Ompok bimaculatus*)
2. Floating pelleted, commercial frog feed (Charoen Pokphand Foods PCL., Thailand)

2.2 Equipment and apparatus

1. Equipment for preparation of circulation system in culture: fiberglass tanks (200 L), bio-filters, heaters, air bubble stones, aquarium airline tubing, air pump, plug sockets, electric wire with power cable leads, hand nets, PVC clear plastic sheets, black sunshade nets
2. Immuno-hematological apparatus:
    - 2.1 Blood drawing apparatus: 1 ml syringes with needles (25 G; 2.54 cm), plastic tubes (1.5 ml), gloves
    - 2.2 Blood analysis apparatus: hemacytometer, light microscope, RBC diluting pipette
    - 2.3 Total hemoglobin measuring apparatus: test tubes (20 ml), micropipette, spectrophotometer
    - 2.4 Hematocrit measuring apparatus: microhematocrit capillary tubes, hematocrit centrifuge (TTE 4203, Biozen.co. LTD), ruler
    - 2.5 Serum lysozyme activity measuring apparatus: micropipette, 96-well plate, Microplate Reader [(Microplate) BIOTEK PowerWaveX]
    - 2.6 Serum protein measuring apparatus: micropipette, plastic tube (1.5 ml), vortex mixer, centrifuge, spectrophotometer
    - 2.7 Phagocytic activity measuring apparatus: centrifuge tube (10 ml), plastic tube (1.5 ml), petri dish, test sieve, forceps, tweezers, slides, centrifuge

2.8 Nitroblue tetrazolium measuring apparatus: 96-well plate, micropipette, plastic tube (1.5 ml), and Microplate Reader

2.3 Methods

2.3.1 Experimental location, fish, and design

The experiment was carried out by using a complete randomized design with four replicates for ten weeks at the Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Thailand. In this experiment, the fish were supplied by Inland Aquaculture Research and Development Regional Center 12 (Songkhla) (IARDRC 12). The average initial fish weight was 2.72$\pm$0.01 g (1.52-4.71 g). Two hundred and forty fingerlings (15 fish/tank) were randomly stocked in each tap-water aquarium. There were four treatments with various temperature ranges: ambient temperature as the control temperature (25.1-29.5 °C), and 29, 31 and 33 °C. Sixteen fiberglass tanks (40x50x110 cm^3) were used to rear the fish with 150 liters in each tank (Chawpaknam and Bowonsupakijkul, 2003). Each tank was equipped with individual biological filtration, two air stones for aeration, and an individual heater unit to maintain water temperature. Except for the control treatments, heaters were not installed, and the temperature in the tanks altered through the day and night. The fish were acclimated to the trial temperatures from ambient water temperature by increasing 1 °C/day until reaching the experimental treatment temperatures.

2.3.2 Water samples

Water samples were collected weekly for laboratory analysis according to the method of Boyd and Tucker (1992). Ammonia was determined by the indophenol method or phenate method and measured spectrophotometrically at 630 nm. Colorimetric methods were used for nitrite measurements using diazotizing reagents and a spectrophotometer operating at 543 nm. Dissolved oxygen was measured by the Winkler method (Boyd and Tucker, 1992) and pH was measured by a pH meter (Cyberscan pH 510). Water temperatures were checked between 12:30 p.m. and 1:00 p.m. with a glass thermometer.

2.3.3 Fish culture

The two hundred and forty butter catfish were released into the tanks one week before the trial began. Commercial floating frog feed pellets (Charoen Pokphand Foods PCL., Thailand) (protein not less than 40%, lipids not less than 4%, moisture not more than 12% and crude fiber not more than 4%) were used as their feed, as suggested by a fisheries biologist at IARDRC 12 who raised this fish commercially. The fish were fed two times a day at 9:30 a.m. and 4:00 p.m. Feeding was provided to satiation and any uneaten food was removed from each tank about 30 minutes after feeding. The filters were turned off for 30 minutes during feeding and waste was siphoned from the tanks as necessary. In order to record the food intake, the fish food was weighed in two ways - first the dry food distributed in each tank was weighed before feeding, and then the unconsumed food in the tanks was kept until the end of experiment and then dried and weighed. Dead fish were removed from the tanks and fish mortality recorded. The fish gut has to be empty before weighing, so at the end of the ten weeks the fish were starved for 24 h before weighing.

The amount of the supplied ration was weighed to determine the apparent feed conversion. The feed conversion ratio (FCR) was assessed by the standard method, the relation between total feed intake and weight gain over the total 10-week period:

$$FCR = \frac{\text{Total feed intake}}{\text{Total weight gain of fish}}$$

Performance measurements, such as fish weight, length, specific growth rate (SGR) and survival rate were then determined.

The SGR (% x d^{-1}) was calculated as:

$$SGR = [(\ln W_f - \ln W_i)\ d^{-1}] \times 100$$

Where W_f and W_i were the final and initial weight, respectively, and "d" the days of cultivation:

$$\text{Percentage weight gained (\%)} = \frac{(W_f - W_i) \times 100}{W_i}$$

$$\text{Survival Rate (SR \%)} = \frac{\text{Number of surviving fish x100}}{\text{Number of stocked fish}}$$

2.3.4 Hemato-immunological parameters

Three of the fish from each tank were used for blood sampling after ten weeks. The health of the fish was assessed through several hemato-immunological parameters, i.e., total red and white blood cell counts, hematocrit, total hemoglobin, serum protein, serum lysozyme activity, respiratory burst activity and phagocytic activity. The fish were euthanized with clove oil and blood samples were collected at the caudal vein from twelve fish randomly selected from each treatment group using a 1-ml syringe. The hemato-immunological measurements were determined as follows:

2.3.4.1 Total red and white blood cell counts (RBC, WBC)

The blood samples were diluted with Yokoyama's fluid in a red blood cell diluting pipette and counted under a light microscope at 40X using a hemacytometer (Supamattaya, 1995).

2.3.4.2 Hematocrit

The hematocrits of the fish were determined using the method of Blaxhall and Daisley (1973). The blood was initially drawn into an Eppendorf tube, and immediately two capillary tubes were filled from a 1.5 ml Eppendorf tube and sealed with play clay. Then they were centrifuged in an ALC hematocrit centrifugette 4203 (A.L.C International S.r.l., Italy) and the hematocrit was calculated as:

$$\text{Hematocrit (\%)} = \frac{\text{Packed red blood cells volume (mm)}}{\text{Total blood volume (mm)}} \text{ x 100}$$

2.3.4.3 Total hemoglobin

The cyanmet-hemoglobin method was used to measure total hemoglobin (Larsen and Snieszko, 1961). A 20 µl blood sample was reacted with 5 ml of Drabkin's solution, then mixed and kept at room temperature for

20 minutes, then read with a UV-1201 spectrophotometer (Shimadzu Corporation, Japan) at an optical density of 540 nm and compared with hemoglobin standards.

2.3.4.4 Serum protein

To determine serum protein, the method from Lowry *et al.* (1951 as cited in Supamattaya *et al.*, 2000) was used. Each blood sample was centrifuged at 5,000 rpm for 10 minutes at 4 °C, after which the supernatant (serum) was transferred into a new microcentrifuge tube. 5 µl of the serum was diluted with 995 µl of distilled water and then 2 ml of alkaline copper solution was added. The sample was well mixed and left at room temperature for 10 minutes for reaction. 3 ml of Folin reagent (1:10) was then added, and the sample again mixed well and left at room temperature for 10 minutes for reaction. After transferring to a 96-well plate, serum protein was measured by a microplate reader at an optical density of 640 nm and compared to the graph of bovine serum albumin standards (Sigma, USA).

2.3.4.5 Serum lysozyme activity

A turbidimetric assay was applied to assess serum lysozyme activity according to Demers and Bayne (1997). 25 µl of serum was transferred to each well of a 96-well plate and then 175 µl of *Micrococcus lysodeikticus* (Sigma) suspension was also added to each well (the concentration 0.075% into Phosphate Citrate buffer solution 0.1 M with pH 5.8). The plate was read at a wavelength of 450 nm with a microplate reader. The reduction rate in the absorbance of the samples was converted to lysozyme concentration (µg/ml) using a standard curve. Hen egg white lysozyme was used as an external standard.

2.3.4.6 Respiratory burst activity

The respiratory burst activity of the leucocytes was determined using the reduction of nitroblue tetrazolium (NBT) to formozan as a measure of superoxide anion (O_2^-) production. 50 µl of blood was mixed with 30 µl of ACD (anticoagulant acid citrate dextrose) and was transferred into each well of a 96-well plate. The 96-well plate was incubated for 1 hour at 37 °C. Then the clear fluid was taken away and washed with PBS (pH 7.2) three times (100 µl/time). 50 µl of NBT at a concentration of 0.2% was added into each 96-well plate which was incubated again for 1 hour at

37 °C. After that 50 µl of 100% methanol was added and the 96-well plate was kept at room temperature for 2-3 minutes. It was washed three times with 50 µl/time of 70% methanol, let dry at room temperature then 60 µl of 2 M of KOH and 70 µl of DMSO were added into each well of a 96-well plate as a blank. The solution was mixed gently and the 96-well plate was measured spectrophotometrically in triplicate with a microplate reader (Model PowerWaveX, Biotek) at an absorbance of 630 nm (Stasiak and Baumann, 1996).

2.3.4.7 Head kidney leucocytes

Head-kidney leucocytes were isolated under sterile conditions following Chung and Secombes (1988). After isolation, 400 µl of leucocytes at a density of 2×10^6 cells/ml were suspended with latex beads (density: 2×10^8 latex beads/cell) in 100 µl of L-15 solution. Then 200 µl of this new solution was dropped on a circular cover slip. The cover slip was incubated at 25 °C for 2 hours and then stained with Diff Quick and prepared as a permanent slide with permount. Finally, it was examined under a light microscope at 40x magnification. Leucocyte phagocytic activity, phagocytic index, and average number of beads ingested per cell were measured following the method of Thuvander *et al.* (1987) according to these formulae:

$$\text{Phagocytosis (\%)} = \frac{\text{No. of cells ingesting beads}}{\text{Total number of WBCs}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{No. of cells ingesting beads}}{\text{Total number of WBCs}} \times \frac{\text{No. of beads ingested}}{\text{Total number of WBCs}} \times 100$$

$$\text{Average number of beads ingested per cell} = \frac{\text{No. of beads ingested}}{\text{No. of cells ingesting beads}}$$

2.3.5 Effect of water temperature on the susceptibility of butter catfish to *E. ictaluri*

At the end of the study, four treatments with four replicates (ten fish per tank, except for the 29 °C trial which had 38 fish in 4 tanks) were studied to examine the susceptibility of the butter catfish to *E. ictaluri.* Each fish in each treatment was injected intraperitoneally with 0.1 ml of *E. ictaluri* suspension containing 1.32×10^6 CFU/ml. The fish were not fed during this challenge test and mortality was recorded twice per day for 14 days. Clinical signs of the infected fish were recorded. To confirm the infection, tryptic soy agar (TSA: Difco) was used for isolation of *E. ictaluri* from freshly infected dead or almost dead fish by using the brain tissue for inoculation of TSA. The tryptic soy agar plates which stored the brain tissue were incubated at 30 °C for 24–48 h.

2.3.6 Data analysis

To study the differences in growth performance against water quality and hemato-immunological parameters, and the relation of these factors with fish survival in the challenge test, one way ANOVA was applied individually to each performance indicator with the SPSS program (version 23.0). The comparisons among the treatment means were made with a 95% confidence level (Duncan's Multiple Range test).

Chapter 3

Results

3.1 Water quality parameters

Only the control tanks were not equipped with a heater unit, meaning the temperature fluctuated throughout the whole day (Figure 4A) during the 10 weeks of the experiment. The water quality parameters studied, DO, pH, TAN and NO_2-N, changed among all 4 treatments in similar patterns. Some of the overall average values of the studied water quality parameters were significantly different among the different temperature trials ($p<0.05$) with the exception of pH, which did differ among the treatments from 6.4 to 6.7, but not significantly (Figure 4B). Increased temperatures led to decreased DO concentrations (8.27–8.80 mg/l) with the lowest DO concentration found in the highest temperature trial, the 33 °C water (Figure 4C). The TAN concentrations in the control, 29 °C, and 31 °C tanks/trials were 0.729, 0.805 and 0.765 mg N/l, respectively ($p>0.05$), with the least toxic TAN level recorded in the 33 °C water (0.389 mg N/l) (Figure 4D). Similarly, NO_2-N concentrations were significantly lower in the 33 °C water and the NO_2-N was less toxic than in the other treatments (0.103 mg N/l) (Figure 4E and Table 2).

3.2 Growth performance of butter catfish

The average initial weight of the fish in the four trials (2.71-2.72 g) was non-significantly different ($p>0.05$) (Table 3). However, after the ten-week trial, the final weights and percentages weight gained were significantly different among the treatments ($p<0.05$). The average final weight (16.34 ± 2.91 g) and percentage weight gained (479.70 ± 86.64%) of the fish at 33 °C were significantly lower than the fish reared in the control and other treatments (Table 3). The lowest average SGR (2.55 ± 0.26 %/day) and feed intake (194.28 ± 42.40 g) ($p<0.01$) were also found in the fish reared at 33 °C. The average FCR (1.0 ± 0.1) and survival rate (95.0-98.3%) were not significantly different among all treatments ($p>0.05$) (Table 3).

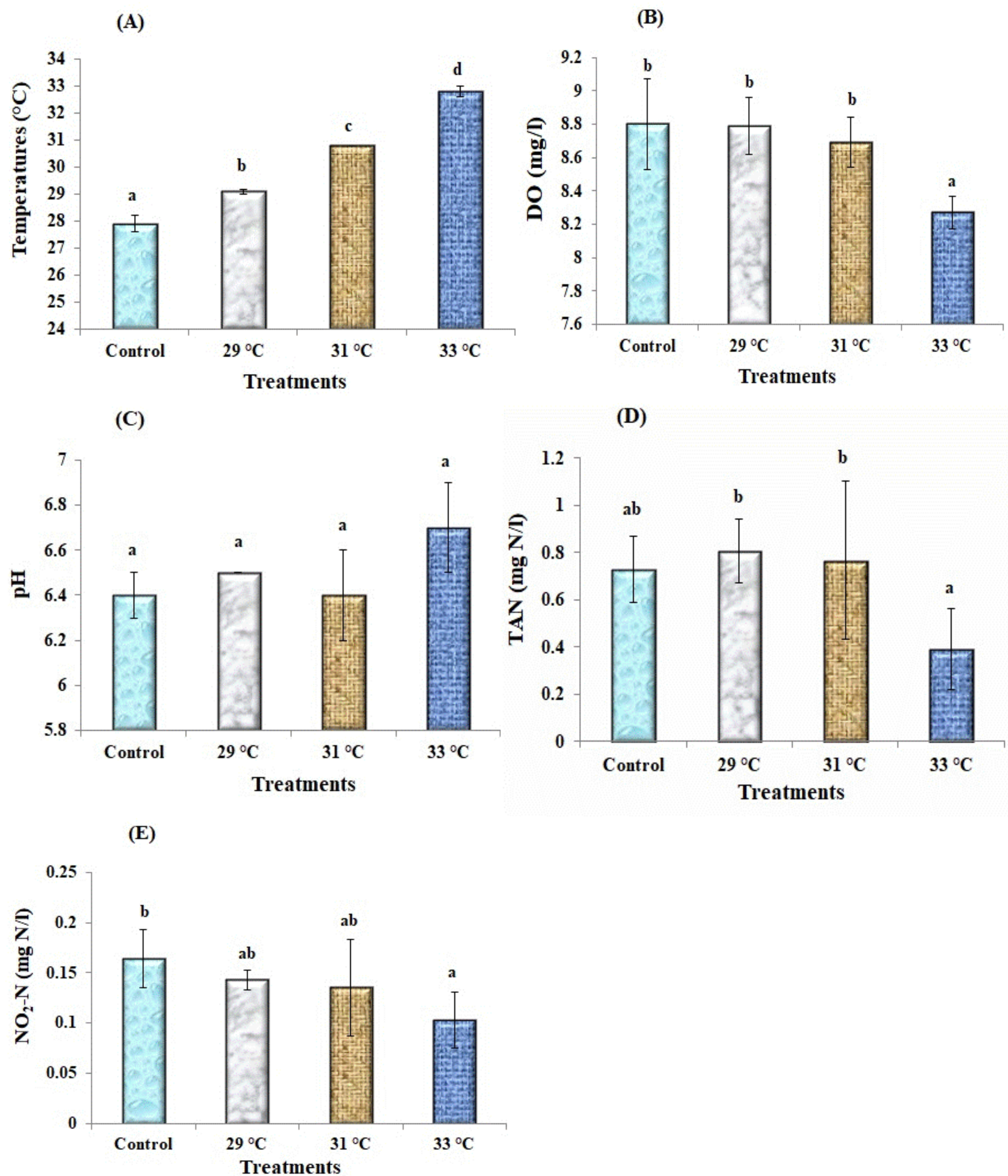

Figure 4 Water qualities at different temperatures (A), DO (B), pH (C), TAN (D), NO_2-N (E) in culture of butter catfish for 10 weeks under temperature manipulation (control treatment without heater unit). Each mean is the average of four replications in a treatment (only three replications averaged in the 29 °C trial as a result of a heater malfunction). The treatment means with different letters represent a significant difference among treatments ($p<0.05$).

Table 2 Average of water quality parameters in 10 weeks under temperature manipulation (min-max (mean±SD))*.

Parameter	Control	29°C	31°C	33°C
Temperature (°C)	25.1-29.5 (27.9±0.3)[a]	27.0-31.2 (29.1±0.1)[b]	28.0-32.0 (30.8±0.0)[c]	30.0-35.0 (32.8±0.2)[d]
DO (mg/l)	5.83-9.63 (8.80±0.27)[b]	7.86-9.38 (8.79±0.17)[b]	8.11-12.17 (8.69±0.15)[b]	7.10-9.12 (8.27±0.10)[a]
pH	5.4-7.8 (6.4±0.1)[a]	5.6-7.9 (6.5±0.0)[a]	5.6-7.9 (6.4±0.2)[a]	5.7-7.9 (6.7±0.2)[a]
Total ammonia nitrogen (mg N/l)	0.023-2.575 (0.729±0.140)[ab]	0.008-2.226 (0.805±0.136)[b]	0.023-2.457 (0.765±0.335)[b]	0.000-1.388 (0.389±0.171)[a]
Nitrite (mg N/l)	0.001-0.778 (0.164±0.029)[b]	0.001-0.760 (0.143±0.010)[ab]	0.000-0.682 (0.135±0.048)[ab]	0.000-0.856 (0.103±0.028)[a]

* Each mean is the average of four replications in a treatment (control treatment without heater unit) (only three replications in the 29 °C treatment as a result of a heater malfunction). In the same row, means sharing the same letters are not significantly different (p>0.05).

Table 3 Average of growth performance in 10 weeks under temperature manipulation (mean±SD)*.

Parameter	Treatments			
	Control	**29 °C**	**31 °C**	**33 °C**
Initial Weight (g)	2.72±0.01^{a}	2.72±0.00^{a}	2.72±0.00^{a}	2.71±0.01^{a}
Final Weight (g)	26.95±4.21^{b}	25.30+1.84^{b}	22.62±4.69^{b}	16.34±2.91^{a}
Percentage Weight Gained (%)	839.22±132.74^{b}	787.34±35.21^{b}	716.44±161.60^{b}	479.70±86.64^{a}
SGR (%/day)	3.26±0.21^{b}	3.18±0.11^{b}	3.00±0.33^{b}	2.55±0.26^{a}
Feed Intake (g)	336.24±51.69^{b}	315.94±49.15^{b}	305.53±69.86^{b}	194.28±42.40^{a}
FCR	1.0±0.0^{a}	1.0±0.1^{a}	1.0±0.0^{a}	1.0±0.1^{a}
Survival Rate (%)	95.0±3.3^{a}	95.6±3.8^{a}	98.3±3.3^{a}	96.7±6.7^{a}

* Each mean is the average of four replications in a treatment (control treatment without heater unit). But only three replications are in treatment with 29 °C as a result of a heater malfunction. In the same row, means sharing the same letters are not significantly different ($p>0.05$).

3.3 Effects of temperature on hemato-immunological parameters

After ten weeks at the trial temperatures, the higher the temperatures, the higher the increase of red blood cells (2.85-3.34 x 10^9 cells/ml) and hemoglobin (7.29-9.52 g/dl) in 29 °C, 31 °C and 33 °C trials ($p<0.05$). However, hematocrit was the same in all treatments (28.84-29.25%) ($p>0.05$). The white blood cells did not increase at the higher water temperatures, as the white blood cells from the control treatment (7.47$\pm$1.46 x 10^7 cells/ml) were higher than from the 31 °C and 33 °C treatments (5.90$\pm$2.49 and 5.50$\pm$1.24 x 10^7 cells/ml, respectively) ($p<0.05$) (Table 4).

The serum protein (44.35-47.11 mg/ml) and NBT reduction (0.23-0.25 OD) were not influenced by higher temperatures ($p>0.05$). Lysozyme activity exhibited an increasing trend during culture. The highest lysozyme activity (8.30$\pm$2.00 μg/ml) was found in the fish reared at 33 °C ($p<0.05$). Though phagocytic activity (28.94-40.44%) and indexes (14.53-28.70) showed enhanced capacities at the elevated temperatures of 29 and 31 °C ($p<0.05$), the capacities of ingesting inert particles (latex beads) did not differ among treatment groups ($p>0.05$) (Table 4).

Table 4 Hemato-immunological parameters under temperature manipulation (mean±SD)*.

Parameter	Treatment			
	Control	29 °C	31 °C	33 °C
Red blood cells ($\times 10^9$ cells/ml)	2.85 ± 0.36^{a}	2.91 ± 0.33^{ab}	3.22 ± 0.37^{bc}	3.34 ± 0.48^{c}
White blood cells ($\times 10^7$ cells/ml)	7.47 ± 1.46^{b}	6.36 ± 1.45^{ab}	5.90 ± 2.49^{a}	5.50 ± 1.24^{a}
Hemoglobin (g/dl)	7.29 ± 0.64^{a}	8.30 ± 1.21^{b}	9.52 ± 2.13^{b}	9.30 ± 1.51^{b}
Hematocrit (%)	28.84 ± 5.53^{a}	29.25 ± 4.53^{a}	29.00 ± 3.41^{a}	29.15 ± 3.18^{a}
Lysozyme activity (µg/ml)	5.46 ± 0.96^{a}	6.35 ± 1.66^{a}	6.67 ± 1.80^{a}	8.30 ± 2.00^{b}
Serum protein (mg/ml)	44.35 ± 4.96^{a}	47.11 ± 4.95^{a}	45.50 ± 5.79^{a}	46.72 ± 4.94^{a}
NBT reduction (OD)	0.23 ± 0.06^{a}	0.24 ± 0.09^{a}	0.25 ± 0.03^{a}	0.23 ± 0.04^{a}
Phagocytic activity (%)	28.94 ± 5.80^{a}	40.44 ± 2.62^{b}	38.33 ± 1.89^{b}	34.00 ± 4.77^{ab}
Phagocytic index	14.53 ± 3.44^{a}	28.70 ± 2.83^{c}	17.42 ± 0.82^{bc}	15.07 ± 2.61^{ab}
Average beads per cell (bead/cell)	1.35 ± 0.25^{a}	1.76 ± 0.09^{a}	1.46 ± 0.36^{a}	1.31 ± 0.14^{a}

* In the same row, means sharing the same letters are not significantly different ($p > 0.05$).

3.4 Effects of water temperature on the susceptibility of butter catfish to *E. ictaluri*

The accumulated mortality rate of the butter catfish exposed to *E. ictaluri* during the 14-day trial was inversely related to the water temperature, with the controls, 29 °C, 31 °C, and 33 °C trials having 100%, 76.7%, 55%, and 2.5% mortality, respectively. The bacterial outbreaks began 2 days following the injections at ambient water temperature and at 31°C, and on day 3 in the 29 °C and 33 °C trials. At ambient temperature all fish died after six days, while at higher temperatures the mortality rate slowed, to zero at day 4 in the 33 °C trial and by the end of the trial on day 14 in the other two trials (Figure 5).

The physical symptoms and behavior of the infected fish were similar in all of the trials. The fish would swim in tight circles, spiraling and spinning (Figure 6A). They had swollen abdomens (Figure 6B), red skin (indicating hemorrhage) appearing under the lower jaw or belly region (Figure 6C), and the appearance of a cranial foramen at the top of the skull or open lesions on the head (Figure 6D).

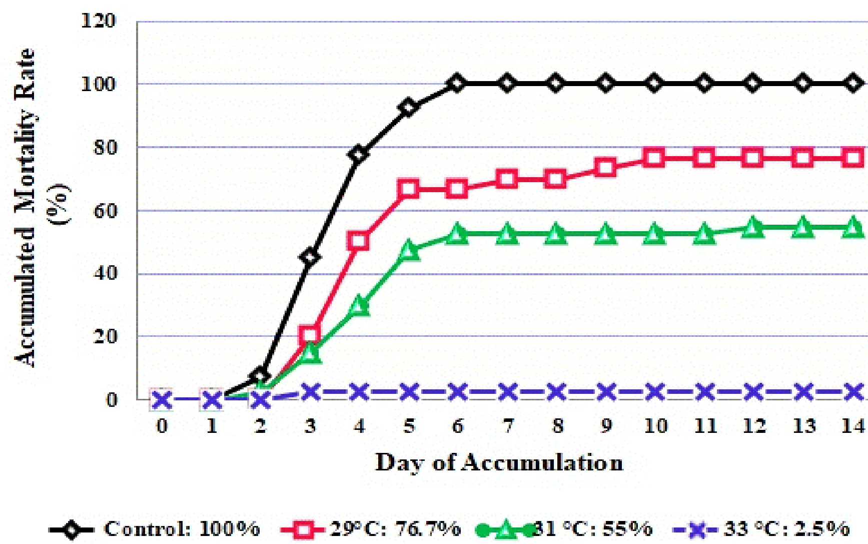

Figure 5 Cumulative mortality rate of butter catfish susceptible to *E. ictaluri* at 1.32×10^{6} CFU/ml under the manipulation of water temperature ($p<0.05$). Each mean is the average of four replications of each treatment (only three replications in the 29 °C treatment due to a heater malfunction).

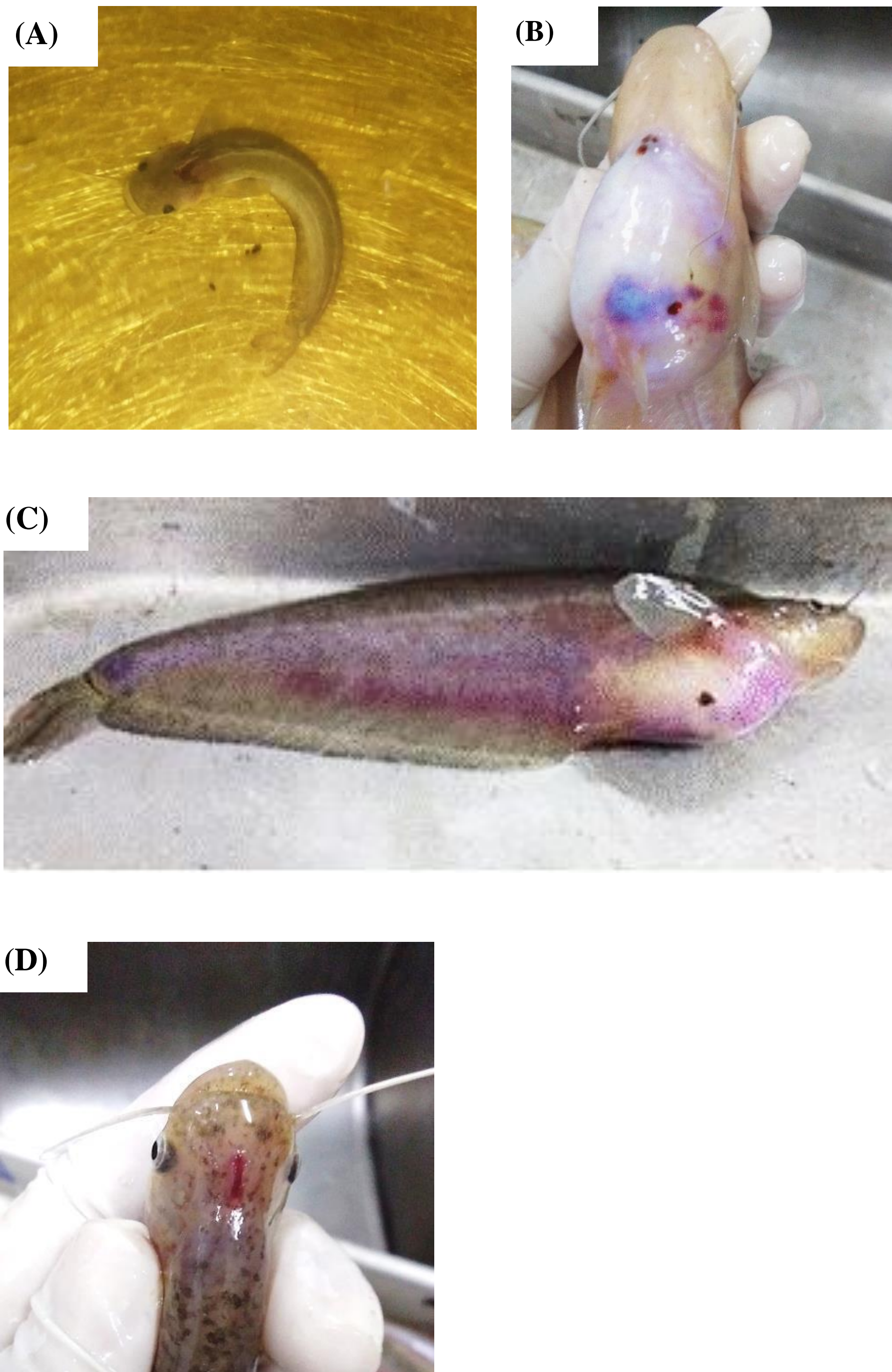

Figure 6 Signs of enteric septicemia of catfish on butter catfish swimming in tight circles (A), swollen abdomen (ascites) (B), hemorrhage appearing under the lower jaw or belly region (C), and the appearance of a cranial foramen at the top of the skull (D).

Chapter 4

Discussion

Ambient water temperature regulates growth rate by affecting many physiological processes in fish (food consumption, metabolic rate, reproduction, activity, and survival). In case of any alterations of water temperature, the fish's environment will change and affect the biochemical characteristics of pathogens, the physiological condition and health of the fish, and alter the bio-availabilities of hazardous substances in the water (Svobodová *et al.*, 1993; Wedemeyer, 1996; Jobling, 1997).

Recent studies have found that water quality parameters are not affected by water temperature except for DO, which gradually decreases at elevated temperatures. In a study by Phuc (2015), DO was low at higher temperatures. However, in the present study, variations in DO were not harmful during culture as the values from all treatments were above 5 mg/l (Figure 4B). In general, warm water is less capable of holding oxygen at saturation in water while increased oxygen consumption is necessary for fish digestion and to meet the demand of amino acids in metabolic processes and other nutrients (Boyd, 1990; Stickney, 1994; Wedemeyer, 1996). A report from Wedemeyer (1996) stated that to support the digestion and the metabolic processes of salmonids in intensive culture it was necessary to increase the oxygen supply at temperatures higher than 27 °C. DO concentrations in this study were above the freshwater saturated DO concentration of 25-35 °C (8.24-6.93 mg/l, respectively) (Colt, 1984). This is probably because of aeration from a pair of air stones producing a strong movement of air bubbles in each tank.

According to Ng *et al.* (2010), the optimal pH level for butter catfish is 6.0-8.0. In the current study, the culture pH in every treatment slightly decreased (pH $\leq$ 6) over time (Figure 4C), so calcium hydroxide (lime) was added every week to maintain the desired pH during culture. Fish cultured at 33 °C obtained better water pH quality than the other trials. Boyd (1990) reported that fish mortality appeared in water at pH lower than 4 and higher than 11. The desirable pH is in the range of 6.5-9.5 and the

acceptable pH for aquatic animals is in the range of 5.5-10.0 (Boyd, 1990; Stone and Thomforde, 2004). According to Ndubuisi *et al.* (2015), low pH had a high impact which reduced the growth rate of *C. gariepinus* fry at an average constant temperature of 26.5 °C. Another study found that when silver catfish (*Rhamdia quelen*) larvae were exposed to low pH (5.5-6.0), both length and weight were reduced at 25 °C (Lopes *et al*., 2001). However, pH varies and depends on water temperature, plus its effect is different depending on fish age, stage of development, and fish species (Svobodová *et al*., 1993; Sapkale *et al*., 2011).

TAN and NO_2-N fluctuated during the study. The TAN concentration pattern was the reverse of the NO_2-N pattern (Figures 4D and 4E). In general, the efficient operation of bacteria conversion in culture systems occurs when the proportion of TAN works to reverse the proportion of NO_2-N. So, when the concentration of TAN is high, the concentration of NO_2-N is low in fish tanks then the process of the nitrogen carries on to the next conversion in the nitrogen cycle. Durborow *et al.* (1997) found that the proportion of TAN in un-ionized ammonia (NH_3) became higher in water at higher temperatures and pH, i.e. for each unit of increase of pH, the amount of NH_3 increased approximately 10 times. In the ammonia cycle, various bacteria generate excess hydroniums (H_3O^+) in the water at low pH and adjust to ionized ammonia (NH_4^+), which is relatively nontoxic to aquatic animals (Boyd, 1990; Hargreaves and Tucker, 2004; Strange, 2004). Our study results also corresponded with a study by Stone and Thomforde (2004), who recommended levels of 0-2 mg N/l of TAN and 0-1 mg N/l of NO_2-N. Boyd (1990) stated that the safe and lethal concentrations of NO_2-N are difficult to determine for aquaculture since they depend on several factors such as chloride concentration in the water, the size of the animals, pH, DO concentration, feed and nutrition, infection, and previous exposure. In our study, the DO concentrations in all treatments were ample and even if the pH was slightly low, the TAN and NO_2-N were within safe levels. Stickney (1994) found that fish reduce feed intake lead to minimize waste products and the toxicity in water, as a consequence of better pH, TAN and NO_2-N concentrations in the 33 °C water (Figure 4).

The temperature range of butter catfish in their natural habitat is 20-26 °C (Ng *et al*., 2010). In our study, the growth performance of the fish in higher temperatures was lower in terms of percentage of weight gain, feed intake, and SGR (Table 3). Stickney (1994) reported that fish cultured in upper or lower thermal limits (≤25-≥32 °C) had reduced feed intake and a lower growth rate. Warmer temperatures increase the metabolic demands and increase fish growth but if fish increase feed consumption for body maintenance without growth, it means that the temperatures are at abnormal levels or at or over the temperatures which fish can adapt to and still grow (Stickney, 1994). That is one reason why our cultivation of butter catfish in ambient water temperatures (control), 29 °C and 31 °C water obtained more satisfactory results than the fish raised at 33 °C (Table 3). Likewise, water temperatures ranging from 24-26 °C for rearing *Labeo rohita* produced the best growth rates and FCR compared to 22-24 °C and 20-22 °C trials. The optimum temperature range of *L. rohita* is between 25-30 °C (Kausar and Salim, 2006). In blackchin tilapia (*Sarotherodon melanotheron*) and Mozambique tilapia (*Oreochromis mossambicus*) juveniles cultured at various temperatures (25, 30 and 35 °C), both species showed significantly decreased growth rates at 35 °C and around 30 °C was their optimum temperature (Price *et al*., 1985).

FCRs and survival rates in this study were good at all temperatures (Table 3). Other studies found that FCR was better at 27 °C than at 32 °C in channel catfish culture, and growth of channel catfish was better at 27 °C similarly to butter catfish (Suja *et al*., 2009). A study by Phuc (2015) found showed that the FCRs for tra catfish (*Pangasianodon hypophthalmus*) at various temperatures, 27, 30, 32, 34, and 36 °C, all had positive results. The survival rates in this study were better at ambient temperature and those less than 33 °C, a similar finding to the studies of Suja *et al*. (2009) and Phuc *et al*. (2017). The best growth and survival rates of tra catfish were at 34 °C according to Phuc (2015), and another study by Phuc *et al*. (2017) reported that at a water temperature of 35 °C and 0 ppt salinity tra catfish still had good weight gain.

The utilization of food consumption for metabolism or growth or for excretion as waste is temperature dependent and high temperatures damage the assimilation of the nutrients in formulated foods (Wedemeyer, 1996). The thermal limits of aquatic

organisms essentially affect feeding rates since a decrease in feeding rates happens at below or above their optimum temperatures (Stickney, 1994). For instance, one study found the feeding rate of channel catfish was 3% of body weight daily when they were raised in water at 21 to 32 °C, with a reduction to 2% of body weight at temperatures less than 21 °C, and at temperatures more than 32 °C lessened to 1% of body weight. At less than 7 °C no feed was accepted (Stickney, 1994). Another study reported that tra catfish had decreased growth, survival, and FCR at 36 °C, and surmised that the energy of the fish was transferred from metabolic activities into the synthesis of new tissues to cope with temperature stress (Phuc, 2015). In the current study, the FCR and survival rate did not show any differences during culture under temperatures up to 33 °C. Fish declined growth rate at 33 °C. Even though the fish in the current study did not show efficient growth at 33 °C, other studies have reported that the higher temperatures within the optimal temperature range (26-33 °C) provided benefits during the early life stage of warm water fish species such as superior development of the embryo, rapid hatching time, and high hatching and survival rates (Haylor and Mollah, 1995; Small and Bates, 2001; Hallare *et al*., 2005).

Temperature is considered as a vital element of fish health assessment along with immune system and fish blood parameters, which reflect conditions that cause stress to the fish and, ultimately, disease. Our study on the hemato-immunological changes of butter catfish exposed to various temperatures indicated that red blood cell counts and hemoglobin increased at elevated temperatures in the experiment but hematocrit in all treatments was stable during the trial (Table 4). Red blood cells (an indicator of the fish's capacity for oxygen transport) are directly linked to the amount of hemoglobin (oxygen-binding abilities). Higher temperatures increase the rate of growth, and thus the need for more red blood cells to provide sufficient oxygenation (Stickney, 1994; Health, 1995). Ravichandra (2012) suggested that high temperatures depressed the oxygen capacity in red blood cells leading to the need for more red blood cells and hemoglobin to compensate for the abnormal condition. Hematocrit refers to the volume percentage of red blood cells in whole blood as well as being a common measure of oxygen carrying capacity, which responds to variations of temperature (Muñoz *et al*., 2018). A similar observation was made in African

sharptooth catfish (*C. gariepinus*) culture in which the hemoglobin content of the fish increased at 29 °C and 35 °C compared to fish held at 23 °C, but red blood cell count values only slightly increased and hematocrit differed among fish cultured at 23 °C, 29 °C, and 41 °C (Adeyemo *et al*., 2003). Phuc (2015) reported that tra catfish had increased red blood cells, hemoglobin and hemotocrit values at higher temperatures (30 °C, 32 °C, and 34 °C compared to 27 °C). Another study found that the hemoglobin content of snakehead fish (*Channa punctatus*) was lower at 15 °C and 20 °C and higher at 30 °C and 35 °C compared to the control treatment (26 °C) (Ravichandra, 2012). Verma *et al*. (2007) found that common carp red blood cell counts and hemoglobin were higher at temperatures up to 33 °C.

In the current study, the lysozyme contents of the fish increased with rising water temperatures, although the control, 29 °C and 31°C trials were not statistically different; with the highest lysozyme content at the highest temperature (33 °C) (Table 4). This finding is similar to the study of Suja *et al*. (2009), which reported that channel catfish reared at 32 °C had higher lysozyme counts than those reared at 27 °C and 22 °C. Increases in the serum lysozyme levels of the Icelandic and Norwegian strains of juvenile Atlantic halibut (*Hippoglossus hippoglossus*) were also found at elevated temperatures (8 °C < 12 °C < 15 °C < 18 °C) (Langston *et al*., 2002). Notably, the lysozyme counts of gilthead sea bream (*Sparus aurata*) were depressed at a low temperature (11 °C) either immediately upon reduction of water temperature or in long term culture at low temperature (Tort *et al*., 2004). At the cool temperatures for the species of 19 °C and 23 °C, tilapia showed lower lysozyme measurements than in warm temperatures of 31 °C and 35 °C in terms of initial acclimation at 27 °C (Ndong *et al*., 2007). Due to serum lysozymes serving as one of the innate humoral defense factors (lytic-against gram-positive and bacteriostatic-against gram-negative bacteria) (Schäperclaus, 1992), Langston *et al*. (2002) predicted that with rising lysozyme levels at increasing temperatures, fish would encounter the abnormal condition which lysozymes would have to increase to battle an invader.

The serum protein assays in this study in which the fish were exposed to rising temperatures did not show any significant differences between the treatments (Table 4). In earlier studies, Langston *et al*. (2002) and Singh *et al*. (2013) found that

serum protein was sensitive to low temperatures. On the other hand, another study reported that the enhancement of metabolic rates or the elevation of temperatures also promoted the transmitting of serum protein to tissues as a consequence of declining serum protein in fish blood (Taylor, 1977). Changes in total serum protein are possibly related to stress, size, sex, effect of seasons on temperature and metabolic activity, nutrition status, and feed composition (Hersey-Benner, 2013). Ideally, fishes have their acclimation tolerance limits and any changes in their environment naturally enable them to perform compensatory physiological responses such as the primary response of the endocrine system, the secondary response of blood and tissue alterations, the tertiary response of whole-animal changes, and quaternary responses involving whole populations or ecosystems (Wedemeyer, 1996). Basically, serum proteins (albumin and most globulins) synthesize in the liver. It was suggested that there was something wrong with serum protein when the liver function became abnormal (Meisner and Hickman, 1962; Francesco *et al*., 2012; Hersey-Benner, 2013). In this study, at the elevated temperature (33 °C) all the fish had normal serum protein results that showed about normal functions of fish serum protein (control oncotic pressure, transport substances such as hemoglobin, lipids, and calcium, and promote inflammation and the complement cascade) (Hersey-Benner, 2013).

White blood cell counts were highest for the butter catfish reared in the control treatment, with reduction of white blood cell counts at the higher temperatures (29 °C, 31 °C, and 33 °C) (Table 4). This finding is consistent with a Verma *et al*. (2007) study in which white blood cell counts of common carp decreased after thermal acclimation from 26 °C to 36 °C. Another study reported that the volume percentage of white blood cells in whole blood (leukocrit) of common carp (*Cyprinus carpio*) decreased at low temperatures (Chen *et al*., 1996). Additionally, white blood cell counts of tilapia (*O. mossambicus*) dropped at both 35 °C and 19 °C after 24 h compared to the control (27 °C) (Ndong *et al*., 2007). Conversely, there are several fish species which show elevated white blood cells at higher temperatures, e.g. sea bass (*Dicentrarchus labrax*) (Pascoli *et al*., 2011) and tench (*Tinca tinca*), which were found to have higher white blood cell counts in summer than in winter (De Pedro *et al*., 2005), and rainbow trout (*Oncorhynchus mykiss*), which showed significantly

elevated WBCs at higher temperatures in spring and summer compared to winter (Houston *et al*., 1996). White blood cell counts are altered by various non-living and living factors such as water temperature, environmental stressors, age and sex (Modrá *et al*., 1998; Rohlenová1 *et al*., 2011). Typically, white blood cells have a major role in repelling invaders (cell-mediated immune response and phagocytosis) (Secombes, 1996); the reaction of either higher or lower white blood cells more than the normal range is considered as a natural response to any type of interior or environmental stressor (Health, 1995; Chabot-Richards and George, 2014).

In general, the phagocytosis test is a test of functional leucocytes which provide specific antibodies to promote phagocytosis in order to combat infectious diseases and stimulate the immunization processes (Schäperclaus, 1992). NBT reduction is a measure of bactericidal phagocytes by oxygen-dependent utilization (Collazos *et al*., 1994). This study showed a rise in phagocytic activity and increases in phagocytic indexes at higher temperatures (29 °C and 31 °C). Despite the fact that lower white blood cell counts occurred at higher temperatures, NBT reductions and the capability of engulfing inert particles (latex beads) did not undergo any alterations among all of the treatments (Table 4). A previous study found that the phagocytic function test in channel catfish culture that compared increased temperatures at 24-27 °C and 10-27 °C to a low temperature (10 °C) promoted rising trends of both phagocytosis and the phagocytic index along with a similar rising trend of NBT reductions and bacterial killing (Ainsworth *et al*., 1991). Various studies concluded that phagocytosis was important for disease prevention at low temperatures (Ainsworth *et al*., 1991; Bly and Clem, 1991; Collazos *et al*., 1994), and the innate immunity effectively responds to low environmental temperatures. However, adaptive immunity may also respond to some changes at higher temperatures (Bly and Clem, 1992; Morvan *et al*., 1998; Martinez *et al*., 2018). Phagocytosis in cultured tilapia (*Oreochromis mossambicus* and *O. aureus*), decreased at the lower temperatures of 19 °C and 23 °C in comparison to 27 °C (Ndong *et al*., 2007) and at 12 °C compared to 25 °C (Chen *et al*., 2002). Butter catfish in this study reared at 33 °C and the control treatment had a similar result of both mechanisms (phagocytic index and phagocytic activity). Likewise, a study by Ndong *et al*. (2007) which found that phagocytosis,

white blood cells, and respiratory burst in mozambique tilapia at 31 °C and 35 °C water were low after 24 h. Phagocyte counts and phagocytic activity may be different in different fish species, and also affected by seasonality, specific type of leucocytes, experimental set-up and duration, and severity of the stressor (Houston *et al*., 1996; Engelsma *et al*., 2003). The low number of white blood cells of the butter catfish in this study reared at high temperatures was probably balanced by stronger abilities to defeat invaders. Hence, the phagocytic abilities of the fish enabled them to adapt to higher temperatures in this experiment while having a normal NBT reduction at low white blood cell counts.

In aquaculture, fish diseases are responsible for very large economic losses and exposure to abiotic stresses (i.e. high water temperatures and low dissolved oxygen concentrations) significantly contributes to such disease problems by making the fish more susceptible to various diseases (Meyer, 1991; Zhou *et al*., 2018). The primary disease concern in catfish species is ESC, known as hole-in-the-head disease, and is caused by the gram-negative bacterium *E. ictaluri* (Johnson and Hinck, 1985; Austin and Austin, 1987; Noga, 2010; Hawke *et al*., 2013; Esmail *et al*., 2015; Hawke, 2015). In this study, butter catfish were sensitive to this bacterium, especially in ambient temperature exposure (25.1-29.5 °C) with a 100% mortality rate. Higher temperatures (29 °C, 31 °C, and 33 °C) had sequentially decreased mortality rates with the lowest virulence (2.5%) at 33 °C. Likewise, other experimental results of channel catfish after either injection or immersion exposure to *E. ictaluri* found the highest mortality rate at 25 °C (Francis-Floyd *et al*., 1987; Baxa-Antonio *et al*., 1992). ESC outbreaks typically occur between 20 °C and 28 °C (Hawke, 2015). In ayu culture, an increase in water temperature to 20-27 °C resulted in enhanced virulence of *E. ictaluri* infection with the highest virulence at 24 °C (Hassan *et al*., 2012; Nagai and Nakai, 2014). A study by Francis-Floyd *et al*. (1987) found that the losses from a bacterial outbreak were reduced by increasing the water temperatures which resulted in a slightly lower mortality rate of channel catfish at 28 °C, with no deaths at 32 °C. According to Baxa-Antonio *et al*. (1992), only a 4% mortality rate was found at 35 °C in the cultivation of channel catfish. Also, fish raised in temperatures outside of the tolerance levels of the bacteria may suffer low or chronic infections (Francis-Floyd *et*

al., 1987; Noga, 2010; Hawke, 2015). Hence, the susceptibility of butter catfish to *E. ictaluri* infection is lower when fish and the bacteria are at exceeding ambient water temperatures.

Chapter 5

Conclusion

This study found that the thermal temperature limit for butter catfish growth during the fingerling stage was 31 °C and these fish could grow suitably as long as there was acceptable water quality in the culture system. Weight, SGR, and feed intake showed decreasing patterns at 33 °C. TAN and NO_2-N qualities were better at 33 °C. Even though the DO concentration was slightly lower at 33 °C than the other treatments, it was in the desirable range for fish culture. The study on the hemato-immunological parameters found that red blood cell counts and hemoglobin increased to balance the oxygen demands in a compensatory process when the fish were reared under higher temperatures. However, the hematocrits remained at normal values in all temperatures. Fish serum proteins, reduction of nitroblue tetrazolium, and average beads per cell ingested functioned normally in spite of the fact that the fish cultured at 29 °C and 31 °C gained the mechanisms of phagocytosis. The fish reared at 33 °C showed slightly weakened immune systems in which white blood cell counts decreased and lysozyme activity increased at high temperatures after culture for 10 weeks. A challenge test with *E. ictaluri* infection found that fish held at 33 °C had the lowest accumulated mortality rate even though overall their health and growth performance were weaker than in the other treatments. This is due to the fact that the *E. ictaluri* infection disease outbreaks were much milder and easier to suppress at warmer temperatures, and were much worse at lower water temperatures. In conclusion, the study indicates that cultivation of butter catfish during the fingerling stage would be feasible at water temperature ranges between ambient temperature and 31 °C, a range within which hemato-immunological parameters retain their normal functions to support fish health. Fish farmers should watch carefully for signs of ESC outbreaks, however, which are more dangerous within this thermal range.

Finally, addressing the purpose of the study, we found that butter catfish culture can endure the global warming problem at this time.

References

Adeyemo, O. K., Agbede, S. A., Olaniyan, A. O. and Shoaga, O. A. 2003. The haematological response of *Clarias gariepinus* to changes in acclimation temperature. African Journal of Biomedical Research 6: 105–108.

Ainsworth, A. J., Dexiang, C., Waterstrat, P. R. and Greenway, T. 1991. Effect of temperature on the immune system of channel catfish (*Ictalurus punctatus*) -I. Leukocyte distribution and phagocyte function in the anterior kidney at 10 °C. Comparative Biochemistry and Physiology 100: 907–912.

Austin, B. and Austin, D. A. 1987. Bacterial fish pathogens: Disease in farmed and wild fish. New York: John Wiley and Sons, Inc.

Bansal, S. K., Verma, S. R., Gupta, A. K. and Dalela, R. C. 1979. Physiological dysfunction of the haemopoietic system in a fresh water teleost, *Labeo rohita*, following chronic chlordane exposure. Part I - alterations in certain haematological parameters. Bulletin of Environmental Contamination and Toxicology 22: 666–673.

Baxa-Antonio, D., Groff, J. M. and Hedrick, R. P. 1992. Effect of water temperature on experimental *Edwardsiella ictaluri* infections in immersion-exposed channel catfish. Journal of Aquatic Animal Health 4: 148–151.

Biller-Takahashi, J. D. and Urbinati, E. C. 2014. Fish Immunology. The modification and manipulation of the innate immune system: Brazilian studies. Annals of the Brazilian Academy of Sciences 86: 1483–1495.

Billett, H. H. 1990. Hemoglobin and hematocrit. *In*: Walker, H. K., Hall, W. D., and Hurst, J. W. (eds.). Clinical methods: The history, physical, and laboratory examinations (3rd ed). Boston: Butterworths. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK340/ (25 April 2019).

Bly, J. E. and Clem, L. W. 1991. Temperature-mediated processes in teleost immunity: in vitro immunosuppression induced by in vivo low temperature in channel catfish. Veterinary Immunology and Immunopathology 28: 365–377.

Bly, J. E. and Clem, L. W. 1992. Temperature and teleost immune functions. Fish and Shellfish Immunology 2: 159–171.

Boyd, C. E. 1990. Water quality in ponds for aquaculture. Alabama: Alabama Agricultural Experiment Station, Auburn University.

Boyd, C. E. 1998. Water quality for pond aquaculture. Research and Development Series. No. 43. Alabama: Alabama Agricultural Experiment Station, Auburn University.

Boyd, C. E. and Tucker, C. S. 1992. Water quality and pond soil analyses for aquaculture. Alabama: Alabama Agricultural Experiment Station, Auburn University.

Blaxhall, P. C. and Daisley, K. W. 1973. Routine hematological methods for use with fish blood. Journal of Fish Biology 5: 771–781.

Chabot-Richards, D. S. and George, T. I. 2014. Leukocytosis. International Journal of Laboratory Hematology 36: 279–288.

Chawpaknam, C., Vorasayan, P. and Pong-in, S. 1993a. Fry nursing of two-spot glass catfish *Ompok bimaculatus* (Bloch). Technical Paper No. 6. Chonburi, Inland Fisheries Development Center.

Chawpaknam, C., Vorasayan, P. and Ranunon, N. 1993b. Embryonic development of egg larvae of two-spot glass catfish *Ompok bimaculatus* (Bloch). Technical Paper No. 37. Chonburi, Inland Fisheries Development Center.

Chawpaknam, C. and Bowonsupakijkul, S. 2003. Dietary energy requirement for *Ompok bimaculatus* (Bloch). Proceedings of 41th Kasetsart University Annual Conference: Fisheries.

Chawpaknam, C., Termvidchakorn, A. and Chaichanakasikram, S. 2004. The development of two spots glass catfish, *Ompok bimaculatus* (Bloch, 1797). Annual Conference of Department of Fisheries.

Chen, G. R., Sun, L. T., Lee, Y. H. and Chang, C. F. 1996. Characteristics of blood in common carp, *Cyprinus carpio*, exposed to low temperatures. Journal of Applied Aquaculture 5: 21–31.

Chen, W. H., Sun, L. T., Tsai, C. L., Song, Y. L. and Chang, C. F. 2002. Cold-stress induced the modulation of catecholamines, cortisol, immunoglobulin M, and

leukocyte phagocytosis in tilapia. General and Comparative Endocrinology 126: 90–100.

Cheng, A. C., Cheng, S. A., Chen, Y. Y. and Chen, J. C. 2009. Effects of temperature change on the innate cellular and humoral immune responses of orange-spotted grouper *Epinephelus coioides* and its susceptibility to *Vibrio alginolyticus*. Fish and Shellfish Immunology 26: 768–772.

Chung, S. and Secombes, C. J. 1988. Analysis of events occurring within teleost macrophages during the respiratory burst. Comparative Biochemistry and Physiology Part B Biochemistry and Molecular Biology 88: 539–544.

Cochrane, K., Young, C. D., Soto, D. and Bahri, T. (eds.) 2009. Climate change implications for fisheries and aquaculture: overview of current scientific knowledge. Fisheries and Aquaculture Technical Paper No. 530. Rome: FAO.

Collazos, M. E., Ortega, E. and Barriga, C. 1994. Effect of temperature on the immune system of a cyprinid fish (*Tinca tinca*, L): Blood phagocyte function at low temperature. Fish and Shellfish Immunology 4: 231–238.

Colt, J. 1984. Computation of dissolved gas concentrations in water as functions of temperature, salinity, and pressure. Bethesda, Md: American Fisheries Society, Special Publication No. 14.

Demers, N. E. and Bayne, C. J. 1997. The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. Development Comparative Immunology 21: 363–373.

De Pedro, N., Guijarro, A. I., López-Patiňo, M. A., Martínez-Álvarez, R. and Delgado, M. J. 2005. Daily and seasonal variations in haematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. Aquaculture Research 36: 1185–1196.

De Silva, S. S. and Soto, D. 2009. Climate change and aquaculture: potential impacts, adaptation and mitigation. *In* Cochrane, K., De Young, C., Soto, D. and Bahri, T. (eds.). Climate change implications for fisheries and aquaculture: overview of current scientific knowledge, 151–212 p. Fisheries and Aquaculture Technical Paper No. 530. Rome: FAO.

DOF (Department of Fisheries). nd. *Ompok bimaculatus*. Retrieved from http://www.fisheries.go.th/if-chonburi/web2/images/ompok.doc (06 February 2018).

Durborow, R. M., Crosby, D. M. and Brunson, M, W. 1997. Ammonia in fish ponds. Southern Regional Aquaculture Center. Publication No. 463.

Engelsma, M. Y., Hougee, S., Nap, D., Hofenk, M., Rombout, J. H. W. M., van Muiswinkel, W. B. and Lidy Verburg-van Kemenade, B. M. 2003. Multiple acute temperature stress affects leucocyte populations and antibody responses in common carp, *Cyprinus carpio* L. Fish and Shellfish Immunology 15: 397–410.

Esmail, M. Y., Astrofsky, K. M., Lawrence, C. and Serluca, F. C. 2015. The biology and management of the zebrafish. *In*: Fox, J. G., Anderson, L. C., Otto, G. M., Pritchett, K. R. and Whary, M. T. (eds.). Laboratory animal medicine (3rd ed). 1015–1062 p. San Diego: Academic Press.

Ficke, A. A., Myrick, C. A. and Hansen, L. J. 2005. Potential impacts of global climate change on freshwater fishes. Gland: WWF (World Wildlife Fund).

Ficke, A. A., Myrick, C. A. and Hansen, L. J. 2007. Potential impacts of global climate change on freshwater fisheries. Reviews in Fish Biology and Fisheries 17: 581–613.

Fondriest Environmental, Inc. 2014. “Water Temperature”. Retrieved from http://www.fondriest.com/environmentalmeasurements/parameters/water-quality/water-temperature (03 October 2017).

Francesco, F., Satheeshkumar, P., Senthil Kumar, D., Caterina, F. and Giuseppe, P. 2012. A comparative study of hematological and blood chemistry of Indian and Italian grey mullet (*Mugil cephalus* Linneaus 1758). Herbert Open Access Journals Biology 1: 1–5.

Francis-Floyd, R., Beleau, M. H., Waterstrat, P. R. and Bowser, P. R. 1987. Effect of water temperature on the clinical outcome of infection with *Edwardsiella ictaluri* in channel catfish. Journal of the American Veterinary Medical Association 191: 1413–1416.

Goddard, S. 1996. Feed management in intensive aquaculture. London: Chapman and Hall.

Gomonteir, B., Tanomtong, A., Supiwong, W., Sikhruadong, S., Neeratanaphan, L. and Sanoamuang, L. 2012. Standardized karyotype and idiogram of two-spot glass catfish, *Ompok bimaculatus* (Siluriformes, Siluridae) in Thailand by conventional and Ag-NOR staining techniques. The Japan Mendel Society 77: 459–464.

Green, B. S. and Fisher, R. 2004. Temperature influences swimming speed, growth and larval duration in coral reef fish larvae. Journal of Experimental Marine Biology and Ecology 299: 115–132.

Greenberg, L. A., Lester, D. and Haggard, H. W. 1943. The reaction of hemoglobin with nitrite. The Journal of Biological Chemistry 151:665–673.

Hallare, A. V., Schirling, M., Luckenbach, T., Köhler, H. R. and Triebskorn, R. 2005. Combined effects of temperature and cadmium on developmental parameters and biomarker responses in zebrafish (*Danio rerio*) embryos. Journal of Thermal Biology 30: 7–17.

Hargreaves, J. A. and Tucker, C. S. 2004. Managing ammonia in fish ponds. Southern Regional Aquaculture Center. Publication No. 4603.

Harrahy, L. N. M. 2000. The effects of elevated temperature and stress on immune function in juvenile chinook salmon (*Oncorhynchus tshawytscha*) (Master's thesis, Oregon State University, Oregon, USA). Retrieved from https://ir.library.oregonstate.edu/downloads/vq27zs11k (23 February 2019).

Hassan, E. S., Mahmoud, M. M., Kawato, Y., Nagai, T., Kawaguchi, O., Iida, Y., Yuasa, K. and Nakai, T. 2012. Subclinical *Edwardsiella ictaluri* infection of wild ayu *Plecoglossus altivelis*. Fish Pathology 47: 64–73.

Hawke, J. P. 2015. Enteric septicemia of catfish. Southern Regional Aquaculture Center. Publication No. 477.

Hawke, J. P., Kent, M., Rogge, M., Baumgartner, W., Wiles, J., Shelley, J., Savolainen, C. L., Wagner, R., Murray, K. and Peterson, T. S. 2013. Edwardsiellosis caused by *Edwardsiella ictaluri* in laboratory populations of zebrafish *Danio rerio*. Journal of Aquatic Animal Health 25: 171–183.

Haylor, S. G. and Mollah, F. M. 1995. Controlled hatchery production of African catfish, *Clarias gariepinus*: The influence of temperature on early development. Aquatic Living Resources 8: 431–438.

Health, A. G. 1995. Water pollution and fish physiology (2nd ed). Boca Raton: Lewis Publishers.

Hernández, J. M., León-Santana, M. and León, C. J. 2007. The role of the water temperature in the optimal management of marine aquaculture. European Journal of Operational Research 181: 872–886.

Hersey-Benner, C. 2013. Protein, total. *In*: Mayer, J. and Donnelly, T. M. (eds.). Clinical veterinary advisor: Birds and exotic pets. 642–643 p. USA: Saunders.

Hoang, M. and Dvorsky, C. 2014. Temperature effects on the solubility of gases. Retrieved from https://nchem.libretexts.org/Core/Physical_and_Theoretical_hemistry/Equilibria/Solubilty/Temperature_Effects_on_the_Solub-ility_of_Gases (04 October 2017).

Houston, A. H., Dobric, N. and Kahurananga, R. 1996. The nature of hematological response in fish. Fish Physiology and Biochemistry 15: 339–347.

Jobling, M. 1997. Temperature and growth: Modulation of growth rate via temperature change. *In*: Wood, C. M. and McDonald, D. G. (eds.). Global warming: implications for freshwater and marine fish, 223–254 p. Cambridge: Cambridge University Press.

Johnson, S. L. and Hinck, L. W. 1985. Effects of temperature and crowding on the pathogenicity of *Edwardsiella ictaluri* in channel catfish (*Ictalurus punctatus*). Journal of the Arkansas Academy of Science 39: 55–58.

Kausar, R. and Salim, M. 2006. Effect of water temperature on the growth performance and feed conversion ratio of *Labeo rohita*. Pakistan Veterinary Journal 26: 105–108.

Kroupova, H., Machova, J. and Svobodova, Z. 2005. Nitrite influence on fish: A review. Veterinarni Medicina 11: 461–471.

Langston, A. L., Hoare, R., Stefansson, M., Fitzgerald, R., Wergeland, H. and Mulcahy, M. 2002. The effect of temperature on non-specific defence parameters of three strains of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). Fish and Shellfish Immunology 12: 61–76.

Larsen, H. M. and Snieszko, S. F. 1961. Modification of the microhaematocrit technique with trout blood. Transactions of the American Fisheries Society 90: 139–142.

Lopes, J. M., Silva, L. V. F. and Baldisserotto, B. 2001. Survival and growth of silver catfish larvae exposed to different water pH. Aquaculture International 9: 73–80.

Magnadóttir, B. 2006. Innate immunity of fish (overview). Fish and Shellfish Immunology 20: 137–151.

Martinez, D., Vargas-Lagos, C., Oyarzún, R. Loncoman, C. A., Pontigo, J. P., Yáñez, A. J. and Vargas-Chacoff, L. 2018. Temperature modulates the immunological response of the sub-antarctic notothenioid fish *Eleginops maclovinus* injected with *Piscirickettsia salmonis*. Fish and Shellfish Immunology 82: 492–503.

Martins, M. L., Xu, D. H., Shoemaker, C. A. and Klesius, P. H. 2011. Temperature effects on immune response and hematological parameters of channel catfish *Ictalurus punctatus* vaccinated with live theronts of *Ichthyophthirius multifiliis*. Fish and Shellfish Immunology 31: 774–780.

Meisner, H. M. and Hickman, JR. C. P. 1962. Effect of temperature and photoperiod on the serum proteins of the rainbow trout, *Salmo gairdneri*. Canadian Journal of Zoology 40: 127–130.

Meyer, F. 1991. Aquaculture disease and health management. Journal of Animal Science 69: 4201–4208.

Modrá, H., Svobodová, Z. and Kolářová, J. 1998. Comparison of differential leukocyte counts in fish of economic and indicator importance. Acta Veterinaria Brno 67: 215-226.

Morvan, C. L., Troutaud, D. and Deschaux, P. 1998. Differential effects of temperature on specific and nonspecific immune defences in fish. The Journal of Experimental Biology 201: 165–168.

Muñoz, N. J., Farrell, A. P., Heath, J. W. and Neff, B. D. 2018. Hematocrit is associated with thermal tolerance and modulated by developmental temperature in juvenile chinook salmon. Physiological and Biochemical Zoology 91: 757–762.

Nagai, T. and Nakai, T. 2014. Water temperature effect on *Edwardsiella ictaluri* infection of ayu *Plecoglossus altivel*. Fish Pathology 49: 61–63.

Ndong, D., Chen, Y. Y., Lin, Y. H., Vaseeharan, B. and Chen, J. C. 2007. The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to

Streptococcus iniae under stress in low and high temperatures. Fish and Shellfish Immunology 22: 686–694.

Ndubuisi, U. C., Chimezie, A. J., Chinedu, U. C., Chikwem, I. C. and Alexander, U. 2015. Effect of pH on the growth performance and survival rate of *Clarias gariepinus* fry. International Journal of Research in Biosciences 4: 14–20.

Ng, H. H. and Hadiaty, R. K. 2009. *Ompok breviricus*, a new catfish (Teleostei: Siluridae) from Sumatra. Zootaxa 2232: 50–60.

Ng, H. H., Tenzin, K. and Pal, M. 2010. *Ompok bimaculatus*. The IUCN Red List of Threatened Species 2010: e.T166616A6248140. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.20104.RLTS.T166616A6248140.en (04 February 2018).

NOAA National Centers for Environmental Information. 2019. State of the climate: global climate report for May 2019. Retrieved from https://www.ncdc.noaa.gov/sotc/global/201905 (July 3, 2019).

Noga, E. J. 2010. Fish disease: Diagnosis and treatment (2nd ed). New York: John Wiley and Sons, Inc.

Pascoli, F., Lanzano, G. S., Negrato, E., Poltronieri, C., Trocino, A. and Radaelli, G. 2011. Seasonal effects on hematological and innate immune parameters in sea bass *Dicentrarchus labrax*. Fish and Shellfish Immunology 31: 1081–1087.

Pearson Education, Inc. (n.d.). In measuring temperature and metabolic rate. Retrieved from http://www.phschool.com/science/biology_place/labbench/lab10/temprate. html (03 October 2017).

Pethiyagoda, R. 1991. Freshwater fishes of Sri Lanka. Colombo: The Wildlife Heritage Trust of Sri Lanka.

Phuc, N. T. H. 2015. Effects of temperature and salinity on growth performance in cultured tra catfish (*Pangasianodon hypophthalmus*) in Vietnam (PhD thesis, Queensland University of Technology, Brisbane, Australia). Retrieved from https://eprints.qut.edu.au/86693/4/Phuc_Nguyen_Trong_Hong_Thesis.Pdf (21 December 2018).

Phuc, N. T. H., Mather, P. B. and Hurwood, D. A. 2017. Effects of sub-lethal salinity and temperature levels and their interaction on growth performance and

hematological and hormonal levels in tra catfish (*Pangasianodon hypophthalmus*). Aquaculture International 25: 1057–1071.

Poff, L. N., Brinson, M. M. and Day, J. W. 2002. Aquatic ecosystems and global climate change: potential impacts on inland freshwater and coastal wetland ecosystems in the United States. Pew Center on Global Climate Change. Arlington, Virginia.

Price, E. E., Stauffer, J. R. and Swift, M. C. 1985. Effect of temperature on growth of juvenile *Oreochromis mossambicus* and *Sarotherodon melanotheron*. Environmental Biology of Fishes 13: 149–152.

Rainboth, W. J. 1996. Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. Rome: FAO.

Ravichandra, J. A. 2012. Influence of acute temperature stress on hemoglobin content in snakeheaded fish, *Channa punctatus* Godavari River, Nanded. International Journal of Biomedical and Advance Research 3: 823–827.

Roberts, R. J. 2012. Fish pathology (4th ed). New York: John Wiley and Sons, Inc.

Rohlenová1, K., Morand, S., Hyršl, P., Tolarová, S., Flajšhans, M. and Šimková1, A. 2011. Are fish immune systems really affected by parasites? an immunoecological study of common carp (*Cyprinus carpio*). Parasites and Vectors 4: 120.

Sapkale, P. H., Singh, R. K. and Desai, A. S. 2011. Optimal water temperature and pH for development of eggs and growth of spawn of common carp (*Cyprinus carpio*). Journal of Applied Animal Research 39: 339–345.

Schäperclaus, W. 1992. Fish diseases (volume 1). Rotterdam: Balkema, A.A.

Secombes, C. J. 1996. The nonspecific immune system: Cellular defenses. *In*: Iwama. G, and Nakanishi, T. (eds.). The fish immune system: Organism, pathogen and environment. 63-103 p. San Diego: Academic Press.

Singh, S. P., Sharma, J. G., Ahmad, T. and Chakrabarti, R. 2013. Effect of water temperature on the physiological responses of Asian catfish *Clarias batrachus* (Linnaeus 1758). Asian Fisheries Science 26: 26–38.

Small, C. B. and Bates, D. T. 2001. Effect of low temperature incubation of channel catfish *Ictalurus punctatus* eggs on development, survival, and growth. Journal of the World Aquaculture Society 32: 189–194.

Snyder, G. 2011. Water temperature effects on fish and aquatic life. Retrieved from http://sciencefairwater.com/physical-waterqualityparameters/ water temperature/water-temperature-effects-on-fish-and-aquatic-life/ (29 September 2017).

Stasiak, A. S. and Baumann, C. P. 1996. Neutrophil activity as a potential bioindicator for contaminant analysis. Fish and Shellfish Immunology 6: 537–539.

Stickney, R. R. 1979. Principles of warmwater aquaculture. New York: John Wiley and Sons, Inc.

Stickney, R. R. 1994. Principles of aquaculture. New York: John Wiley and Sons, Inc.

Stone, N. M. and Thomforde, H. K. 2004. Understanding your fish pond water analysis report. Cooperative Extension Program, University of Arkansas at Pine Bluff.

Strange, R. J. 2004. Recirculation aquaculture: water quality: ammonia. Retrieved from http://web.utk.edu/~rstrange/wfs556/html-content/05-ammonia.html (25 March 2019).

Stuart, L. M and Ezekowitz, R. A. B. 2005. Phagocytosis: Elegant complexity. Immunity 22: 539–550.

Suja, B., Phillips, H., Lochmann, R. and Chen, R. 2009. Effect of temperature on growth, feed utilization, and immune status of channel catfish in recirculating system. North American Journal of Aquaculture 71: 64–72.

Supamattaya, K. 1995. Laboratory manual for fish disease and parasites. Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University.

Supamattaya, K., Ruangsri, J., Kiriratnikom, S. and Songsrichan, N. 2000. The immune system in black tiger shrimp, *Penaeus monodon,* Fabricius. Songklanakarin Journal of Science and Technology 22 (suppl.): 597–603.

Svobodová, Z., Lloyd, R., Máchová, J. and Vykusová, B. 1993.Water quality and fish health. EIFAC Technical Paper. No. 54. Rome: FAO.

Taylor, P. W. 1977. Serum protein and hemoglobin characteristics of various catfish (*Ictalurus* sp.) under normal and diseased conditions (PhD thesis, Auburn University, Auburn, Alabama).

Thuvander, A., Norrgren, L. and Fossum, C. 1987. Phagocytic cells in blood from rainbow trout (*Salmo gairdneri* Richardson) characterized by flow cytometry and electronmicroscopy. Journal of Fish Biology 31: 197–208.

Tort, L., Rotllant, J., Liarte, C., Acerete, L., Hernández, A., Ceulemans, S., Coutteau, P. and Padros, F. 2004. Effects of temperature decrease on feeding rates, immune indicators and histopathological changes of gilthead sea bream *Sparus aurata* fed with an experimental diet. Aquaculture 229: 55–65.

Tyagi, A., Ali, S. and Barat, A. 2013. Impact of exposure of cold temperature on hematological parameters and plasma electrolytes of cold water fish *Shizothorax richardsonii*. Journal of Information, Knowledge and Research in Biomedical Engineering 2: 38–41.

Uribe, C., Folch, H., Enriquez, R. and Moran, G. 2011. Innate and adaptive immunity in teleost fish: A review. Veterinarni Medicina 56: 486–503.

Verma, A. K., Pal, A. K., Manush , S. M., Das, T., Dalvi , R.S. Chandrachoodan, P. P., Ravi, P. M. and Apte, S.K. 2007. Persistent sub-lethal chlorine exposure augments temperature induced immunosuppression in *Cyprinus carpio* advanced fingerlings. Fish and Shellfish Immunology 22: 547–555.

Wedemeyer, G. A. 1996. Physiology of fish in intensive culture systems. London: Chapman and Hall.

Witeska, M. 2015. Anemia in teleost fishes. Bulletin-European Association of Fish Pathologists 35: 148–160.

Worldfish Center. 2007. The threat to fisheries and aquaculture from climate change. Penang, Malaysia.

Yazdi, D. and Shakouri, D. 2010. The effects of climate change on aquaculture. International Journal of Environmental Science and Development 5: 378–382.

Zhou, T., Yuan, Z., Tan, S., Jin, Y., Yang, Y., Shi, H., Wang, W., Niu, D., Gao, L., Jiang, W., Gao, D. and Liu, Z. 2018. A review of molecular responses of catfish to bacterial diseases and abiotic stresses. Frontiers in Physiology 9: 1–16.

Appendix

Chemical and growth media for the culturing of bacteria preparations

1. Chemical preparation

1.1 Phosphate buffer saline (PBS)

Add 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_4), and 0.24 g of potassium hydrogen phosphate (KH_2PO_4) to 800 ml of distilled water. Stir to dissolve, adjust the pH, and dilute to 1000 ml with distilled water. Store in a bottle and sterilize in an autoclave for 15 minutes at 121°C with 15 psi of pressure. Keep the bottle at room temperature.

1.2 Yokoyama's fluid

Solution A:

Add 4 g of sodium chloride (NaCl) to distilled water which has already sterilized before using. Stir to dissolve and then add 1.25 mg of dextrose (D-glucose), 0.25 g of sodium bicarbonate ($NaHCO_3$), and 0.2 g of KCl into the previous solution and dilute to 200 ml. After that, add 50 ml of formaldehyde (concentration: 40%), stir to dissolve properly and keep the solution at 4°C.

Solution B:

Dissolve 0.075 g of methyl violet in sterile distilled water and add 0.075 g of pyronin B, then stir to dissolve and dilute to 250 ml. Keep the solution at 4°C.

Dissolve solution A and solution B in 1:1 ratio and filter with the filter paper (Whatman filter paper Grade 1, diameter: 25 mm) before using. Then working solution is ready to use.

1.3 Drabkin's solution

Dissolve 1 g of $NaHCO_3$, 0.05 g of potassium cyanide (KCN), 0.2 g of potassium ferricyanide ($K_3Fe(CN)_6$) in sterile distilled water and dilute to 1000 ml, then store the solution in amber glass bottle at 4°C.

1.4 Alkaline copper solution

Solution A

To prepare copper sulfate ($CuSO_4 \cdot 5H_2O$) 0.5%, dissolve 0.015 g of $CuSO_4 \cdot 5H_2O$ in the deionized distilled water and dilute to 3 ml.

Solution B

To prepare potassium sodium tartrate ($KNaC_4H_4O_6$) 1%, dissolve 0.03 g of $KNaC_4H_4O_6$ in the deionized distilled water and dilute to 3 ml.

Solution C

To prepare sodium carbonate (Na_2CO_3) 1%, dissolve 2 g of sodium hydroxide (NaOH) in the deionized distilled water and add 1 g of Na_2CO_3. Stir to dissolve and dilute to 100 ml.

Dissolve solution A, solution B, and solution C in 1:1:50 ratio and then keep in the dark and cold place.

1.5 Folin's reagent solution

Dilute the stock Folin's reagent to 1:10-ratio with deionized distilled water and keep in the dark and cold place.

1.6 Anticoagulant acid citrate dextrose (ACD) solution

Dissolve 4 g of citric acid ($C_6H_8O_7 \cdot$anhydrous), 11.3 g of sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), and 11 g of dextrose (D-glucose) in sterile distilled water and dilute to 500 ml. Bring the solution to sterilize in an autoclave for 15 minutes at 121°C with 15 psi of pressure.

1.7 NaCl 0.85%

Dissolve 8.5 g of NaCl in 1000 ml of distilled water. Store in a glass bottle and sterilize in an autoclave for 15 minutes at 121°C with 15 psi of pressure.

1.8 Nitro blue tetrazolium (NBT) solution with concentration 0.2% in NaCl solution with concentration 0.85%

Dissolve 0.02 g of NBT in 10 ml of NaCl solution (0.85%). Store the solution in an amber glass bottle and keep at 4°C.

1.9 Potassium hydroxide (KOH) solution with 2N

Dissolve 11.2 g of KOH in 100 ml of distilled water and keep at room temperature.

1.10 Phosphate citrate buffer solution with pH 5.8

Solution A:

Dissolve 7.089 g of disodium phosphate in deionized distilled water and dilute to 500 ml.

Solution B:

Dissolve 10.507 g of citric acid in deionized distilled water and dilute to 500 ml.

Dissolve solution A and solution B in 3:1 ratio and adjust to pH 5.8. If the pH is lower than 5.8, add solution A to increase the pH. If the pH is higher than 5.8, add solution B to decrease the pH. Then store in a glass bottle and bring the solution to sterilize in an autoclave for 15 minutes at 121°C with 15 psi of pressure. Keep the solution in room temperature and the working solution is ready to use.

1.11 *Micrococcus lysodeikticus* solution 0.075%

Dissolve 0.0375 g of bacteria *M. lysodeikticus* (powder) in phosphate citrate buffer solution with pH 5.8 and dilute to 50 ml.

1.12 Hen egg white lysozyme solution

Dissolve 0.01 g of hen egg white lysozyme powder in phosphate citrate buffer solution with pH 5.8 and dilute to 10 ml. Then we receive the standard solution of hen egg white lysozyme with 1000 µg/ml. Keep it at -80°C until ready to use.

1.13 Fetal calf serum solution: Bring fetal calf serum from the freezer at -80°C to keep at room temperature until it becomes normal liquid before using.

1.14 Phosphate buffer saline (PBS) at pH 7.2

Dissolve 8.5 g of NaCl, 1.07 g of dihydrogen phosphate (H_2PO_4), and 0.39 g of sodium dihydrogen phosphate (H_2NaPO_4) in 900 ml of deionized distilled water, adjust to pH 7.2 and dilute to 1000 ml. Then store in a glass bottle and sterilize in an autoclave for 15 minutes at 121°C with 15 psi of pressure.

1.15 Heparin solution 1000 unit

Dilute heparin solution at the concentration 5000 units with PBS at pH 7.2 in 1:4 ratio. Keep the heparin solution 1000 units at 4°C until ready to use.

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List of Publication

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