VARIATION IN WATER TEMPERATURE AND ITS EFFECTS ON FISH BIOLOGY AND AQUATIC HABITAT

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เรื่อง : ความผันแปรของอุณหภูมิน้ำและผลของอุณหภูมิต่อชีววิทยาและแหล่งที่อยู่อาศัยของสัตว์น้ำ

ผู้วิจัย : ปิยะเทพ อาวะกุล

ชื่อปริญญา : ปรัชญาดุษฎีบัณฑิต

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คำสำคัญ : แม่น้ำเจ้าพระยา, อุณหภูมิน้ำ, ปลาสร้อยขาว, กำลังผลิตเบื้องต้นของแหล่งน้ำ, การเปลี่ยนแปลงของสภาพภูมิอากาศ

การศึกษาในครั้งนี้ได้มีการติดตามผลกระทบของอุณหภูมิน้ำต่อชีววิทยาและแหล่งที่อยู่อาศัย ซึ่งได้ดำเนินการศึกษาในบทที่ 2 ถึง บทที่ 4 โดยบทที่ 2 และบทที่ 3 มีวัตถุประสงค์เพื่อศึกษาผลกระทบของอุณหภูมิน้ำต่อแหล่งที่อยู่อาศัย คือ แม่น้ำเจ้าพระยา เขื่อนอุบลรัตน์ เขื่อนห้วยหลวง และเขื่อนสิรินธร ขณะที่บทที่ 4 มีวัตถุประสงค์เพื่อศึกษาผลกระทบของอุณหภูมิน้ำต่อชีววิทยาของปลาสร้อยขาว (*Henicorhychus siamensis*) โดยการศึกษาความผันแปรของคุณภาพน้ำในแต่ละช่วงเวลาและพื้นที่ของแม่น้ำเจ้าพระยาระหว่างปี พ.ศ. 2542 และ 2551 โดยใช้ค่าคุณภาพน้ำเฉลี่ยในแต่ละปีประกอบด้วยคุณภาพน้ำ 5 พารามิเตอร์ คือ ปริมาณออกซิเจนที่ละลายในน้ำ ความต้องการออกซิเจนทางชีวเคมี แบคทีเรียกลุ่มโคลิฟอร์มทั้งหมด แบคทีเรียกลุ่มฟีคัลโคลิฟอร์ม และแอมโมเนียรวม กับอุณหภูมิน้ำ จาก 32 สถานีเก็บตัวอย่าง ตลอดลำน้ำเจ้าพระยา ถูกนำมาใช้ในการคำนวณโดยวิธีแผนที่การจัดกลุ่มเอง (SOM) และการวิเคราะห์โดยวิธีสมนัย (CoA) ส่วนศึกษาการผันแปรของฤดูกาลของกำลังผลิตเบื้องต้นของแหล่งน้ำ อุณหภูมิน้ำ พีเอช ปริมาณออกซิเจนที่ละลายในน้ำ ฟอสฟอรัสรวม ไนโตรเจนรวม อนินทรีย์คาร์บอนที่ละลายน้ำได้ และคลอโรฟิลล์ เอ ถูกดำเนินการใน 3 เขื่อนหลักที่อยู่ในภาคตะวันออกเฉียงเหนือ คือ เขื่อนอุบลรัตน์ เขื่อนห้วยหลวง และเขื่อนสิรินธร ระหว่างเดือนพฤษภาคม 2555 ถึง เดือนกุมภาพันธ์ 2556 โดยตัวอย่างน้ำจะถูกเก็บบริเวณที่ลึกที่สุดซึ่งจะอยู่บริเวณหน้าสันเขื่อน น้ำตัวอย่างจะถูกเก็บตามระดับความลึกที่ระดับความเข้มแสงที่แพลงก์ตอนสามารถสังเคราะห์แสงได้ที่ร้อยละ 100, 50, 20, 10 และ 5 ตามลำดับ โดยน้ำตัวอย่างถูกวิเคราะห์ความผันแปรของฤดูกาลทั้งในแต่ละเดือน และในแต่ละเขื่อน ในขณะที่บทสุดท้ายศึกษาผลของอุณหภูมิ ซึ่งเป็นปัจจัยทางสิ่งแวดล้อมที่สำคัญต่อสัตว์น้ำโดยเฉพาะอย่างยิ่งในลูกปลาวัยอ่อนที่อุณหภูมิน้ำที่แตกต่างกัน (26, 28, 30, 32 และ 34 °C) ต่อการพัฒนาของคัพภะ และความสำเร็จในการฟักไข่ของลูกปลาสร้อยขาวกับการศึกษาผลของอุณหภูมิน้ำที่มีการเปลี่ยนแปลงอย่างฉับพลันต่อลูกปลาแรกฟัก โดยผลการศึกษาในแต่ละส่วนพบว่าคุณภาพน้ำของแม่น้ำเจ้าพระยาสามารถแบ่งตามลักษณะที่คล้ายคลึงกันในแต่ละช่วงเวลา และพื้นที่ ศึกษาได้ 4 กลุ่ม ขณะที่ความผันแปรของคุณภาพน้ำในเชิงพื้นที่ไม่ค่อยมีความแตกต่างกันอย่างชัดเจน โดยคุณภาพน้ำที่แย่ที่สุดพบบริเวณปากแม่น้ำ

เจ้าพระยาซึ่งเป็นพื้นที่ ที่มีกิจกรรมต่างๆ ของมนุษย์อย่างหนาแน่น แสดงให้เห็นว่ากลุ่มที่มีคุณภาพน้ำที่แย่ที่สุดมีความสอดคล้องกับพื้นที่มากที่สุด ขณะที่กลุ่มอื่นๆ คุณภาพน้ำมีความซ้อนทับกันในเชิงพื้นที่ ส่วนอุณหภูมิน้ำในแต่ละกลุ่มไม่มีความแตกต่างกันทางสถิติ แต่ควรระมัดระวัง และตระหนักถึงผลกระทบจากการเปลี่ยนแปลงสภาพภูมิอากาศที่น่าจะส่งผลกระทบต่อคุณภาพน้ำ ส่วนการผันแปรของฤดูกาลมีบทบาทต่อการเปลี่ยนแปลงของกำลังผลิตเบื้องต้นของแหล่งน้ำ และคุณภาพน้ำตัวอื่นๆ ส่วนการแบ่งชั้นของอุณหภูมิน้ำสามารถพบได้ในทุกเขื่อน โดยเฉพาะเขื่อนสิรินธรพบการเกิดเทอร์โมไคลน์ (Thermocline) ในเดือนกุมภาพันธ์ ขณะที่กำลังผลิตเบื้องต้นของแหล่งน้ำในแต่ละเขื่อนและในแต่ละระดับความเข้มแสงในแต่ละเดือนพบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ขณะที่เปรียบเทียบกำลังผลิตเบื้องต้นของแหล่งน้ำของทั้ง 3 เขื่อน พบว่าเขื่อนห้วยหลวงมีกำลังผลิตเบื้องต้นของแหล่งน้ำสูงที่สุด ซึ่งผลที่ได้สามารถใช้เป็นข้อมูลในการจัดการ และป้องกันระบบนิเวศน์ของแหล่งน้ำในแต่ละเขื่อนในขณะที่การพัฒนาของคัพภะถูกแบ่งออกเป็น 2 ระยะ คือ ช่วงแรกเริ่มจากระยะไซโกต (Zygote) ถึงระยะแกสทรูลา(Gastrula) และช่วงที่สองคือ ระยะเซกเมนเตชัน (Segmentation) ถึงระยะฟัก (Hatching) โดยลูกปลาสร้อยขาวไม่สามารถฟักออกจากไข่ที่อุณหภูมิน้ำ 26 และ 34 °C ซึ่งอุณหภูมิน้ำที่เหลือพบว่าระยะเวลาของการพัฒนาคัพภะในช่วงแรกมีความใกล้เคียงกัน แต่เริ่มมีความแตกต่างกันในช่วงการพัฒนาระยะที่สอง ระยะเวลาในการฟักที่อุณหภูมิน้ำ 28, 30 และ 32 °C คือ 652, 485 และ 457 นาที ตามลำดับ ร้อยละของความสำเร็จในการฟักที่อุณหภูมิน้ำ 28, 30 และ 32 °C คือ 73.76 ± 2.37, 73.90 ± 1.44 และ 61.42 ± 11.19 ตามลำดับ สำหรับผลกระทบของการเปลี่ยนแปลงของอุณหภูมิอย่างฉับพลันพบว่าจำนวนลูกปลาที่ตายไม่มีความแตกต่างกันทางสถิติระหว่างอุณหภูมิน้ำที่ 30 และ 28 °C ($P = 0.30$) ในขณะที่อุณหภูมิน้ำที่ 30 และ 32 °C มีจำนวนลูกปลาที่ตายแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P<0.01$)

ABSTRACT

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KEYWORDS : CHAO PHRAYA RIVER, WATER TEMPERATURE, SIAMESE MUD CRAP, PRIMARY PRODUCTION, CLIMATE CHANGE

This study investigated the effects of water temperature on fish biology and aquatic habitat. The main objective of Chapter 2 and Chapter 3 were to study the effects of water temperature of aquatic habitats in the Chao Phraya River and three reservoirs in North-East Thailand, Ubol Ratana, Huay Luang, and Sirindhorn. The main object of Chapter 4 was to study the effects of water temperature on fish biology. *Henicorhychus siamensis* was selected as the representative fish in this study. Spatio-temporal variations in the water quality of the Chao Phraya River were examined on an average yearly basis from 1999 to 2008 at 32 surface water stations from the river's origin to its delta. Five water quality parameters, dissolved oxygen, biochemical oxygen demand, total coliform bacteria, fecal coliform bacteria, and ammonia, were used in the analysis. Analysis was performed by the use of Self Organizing Maps and correspondence analysis. Meanwhile, seasonal variations of primary production, water temperature, pH, dissolved oxygen, total phosphorus, total nitrogen, dissolved inorganic carbon, and chlorophyll *a* were conducted in the three reservoirs from May 2012 to February 2013. The sampling stations were located in the deepest areas and water samples were collected at different levels of the water column dependent on the percentage of photo-synthetically active radiation at 100%, 50%, 20%, 10% and 5% respectively. The samples in the water column were analyzed and compared with the seasonal variations for both periods and reservoirs. The last chapter studied the effects of temperature, an important environmental factor for aquatic animals, especially fishes, particularly during the early stages of life. The study of embryo development and hatching success was done at different water temperatures (26, 28, 30, 32 and 34°C) while acute temperature change was investigated with newly-hatched larvae. The results of spatio-temporal variations in the water quality of the Chao Phraya River indicated four distinct spatially approached clusters according to the similarity of the water quality parameters, while temporal variations at most of the surface water stations were not obviously observed. The worst water quality condition was at the stations near the river delta and highly related to anthropogenic stresses. While results from the correspondence analysis showed that, except for the cluster of the worst water quality, the stations of the remaining three clusters overlapped. There was no statistical difference in water temperatures among clusters but the expected effects from climate change should be a precautionary focus since they will eventually affect the water quality. The seasonal variations of primary production influenced primary production and another variable. Thermal stratifications were observed in all reservoirs, especially Sirindhorn that thermoclines were obvious in February. Meanwhile, primary productions were significantly different among the PAR levels in all four periods of the study. Comparison of the three reservoirs showed that Huay Luang had the highest primary production. These finding's demonstrated the usefulness of aquatic habitat management and conservation. Meanwhile, the egg development was divided into two phases, firstly, from zygote to gastrula periods and secondly, segmentation to hatching periods. The larvae did not successfully hatch at the incubation temperatures of 26 and 34°C. The development times of the three remaining temperatures were relatively close in the first phase, in contrast to the second phase which was more varied. The hatching times at 28, 30 and 32°C were about 652, 485 and 457 minutes respectively. The percentages of hatching success of the three respective temperatures were 73.76±2.37%, 73.90±1.44% and 61.42±11.19% respectively. In regard to the effects of acute temperature changes, numbers of dead larvae were not significantly different between 30°C and 28°C ($P = 0.30$). There was a significant difference between 30°C and 32°C ($P<0.01$).

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CHAPTER 1
Introduction

In the twenty-first century, the global temperature has rapidly increased over the past century due to the greenhouse gases since the industrial revolution. The average temperature was increased about 0.75°C between 1880 – 2000. Meanwhile, the rate of increase of temperature in the past 25 years has been over 0.18°C per decade (Figure 1.1, Riebeek, 2012: website; Grover, 2014: 3). This phenomenon is caused by the greenhouse gases, e.g. ozone (O_3), carbon dioxide (CO_2), methane (CH_4) including artificial chemicals such as chloro-fluoro-carbons (CFCs) sulfur hexafluoride (SF_6), hydro-fluoro-carbons (HFCs) and per-fluoro-carbons (PFCs), absorb and trap the outgoing thermal radiation from the Earth, which eventually effect the Earth's temperature has warmed, i.e. global warming (Houghton, 2009: 20-22).

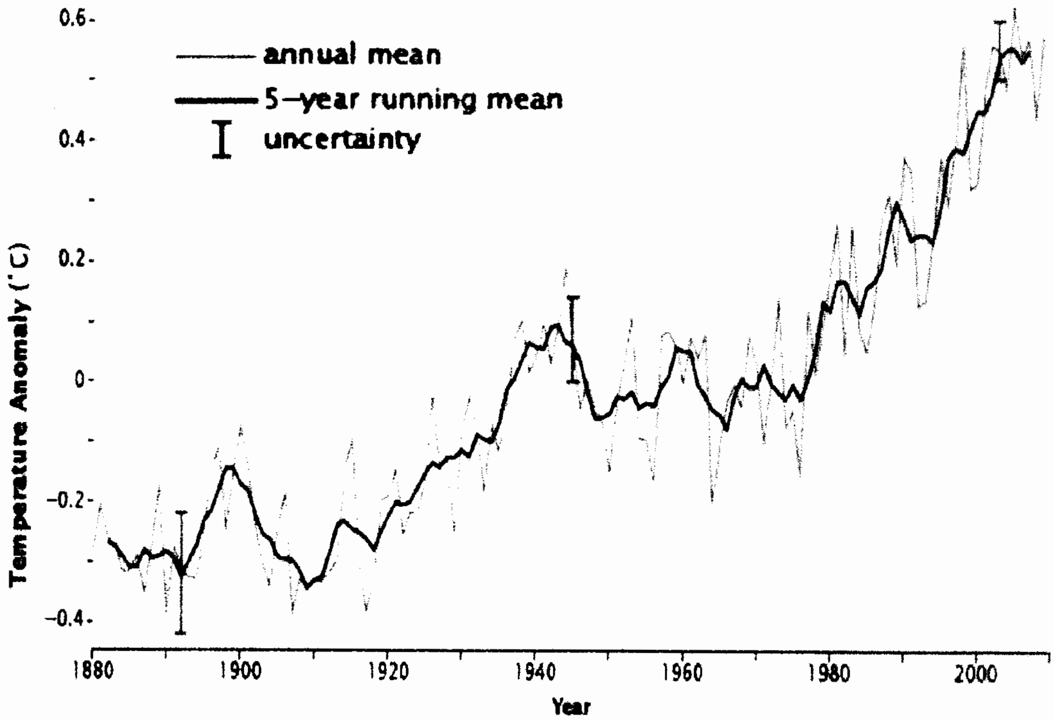


Figure 1.1 The increasing of air temperature in 1880 - 2000.

Source: applied from Riebeek (2012: website)

The global warming is important cause to climates change, which refers to change of climate elements (such as temperature, precipitation or winds) in the long-term statistics (Houghton, 2009: 10-13; Sharestha et al., 2014: 1-2) (Figure 1.2). Understanding the ecological impacts of climate change is a crucial challenge and there is lack of general rules regarding the impacts of global warming on ecosystem or biota (Daufresne et al., 2009: 12788-12789). The climate change has strong affected to hydrological systems (i.e., precipitation, evaporation and moisture) and water resources, meanwhile warmer temperature impacts on increasing evaporation. Moreover, warmer atmosphere is able to keep more moisture that can change to precipitation, as well as increasing the severe flood (Shrestha et al., 2014: 121-122). In addition, dry regions are slightly rising in temperature that will lead to significant loss of moisture as exacerbating drought and desertification that will be effect on water availability and bad water quality in water-scarce regions (Grove, 2014: 12-14).

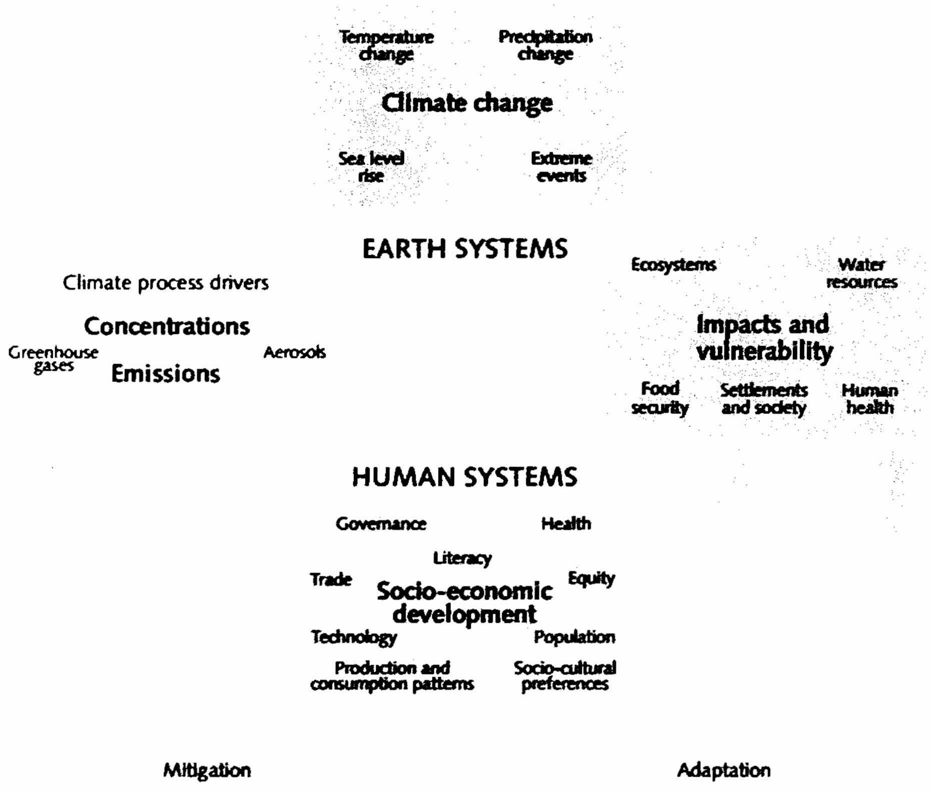


Figure 1.2 Impact of climate change

Source: Houghton (2009: 15)

1.1 The impact of climate change to river water quality

Water is a renewable resources but it can remain renewable if rate of used or harvested not over limit of renew or regenerate itself (Grove, 2014: 5). Water belong components of the climate system via atmosphere, hydrosphere, land surface and biosphere (Drake, 2000: 39). In Asia which climate change is expected to impact both seasonality and amount of river flows, while the maximum monthly flow of the Mekong that increased flooding risks during the wet season and a greater possibility of water shortages in the dry season (Shrestha et al., 2014: 116). The warm up temperature is causing of increase the global sea level that impacted on both human security and ecosystems especially in coastal regions. (Dessler, 2012: 143-144). The Intergovernmental Panel on Climate Change, IPCC (2012: 6-7) and Shrestha (2014, 121) reported frequency of heavy precipitation or proportion of total rainfall has increased in the twenty-first century over many areas, especially increase in the high latitudes and tropical regions, while flood and droughts cause significant damages every year and are responsible for a large part of water-related disasters.

Climate change is affect on extreme weather events in each season such as heavy rain fall, high evaporation and heat waves that can cause periods of intense drought or flood (Verweij and et al., 2010: 1-63). Global trends of drought associate with trends of precipitation and temperature. Global and regional projections of hydrological drought indicate a higher likelihood of hydrological drought by the end of this century in North and South America, central and southern Africa, the Middle East, southern Asia from Indochina to southern China, and central and Western Australia (Shrestha et al., 2014: 127). Overall, the negative impacts of climate change on systems and activities reliant on surface water are generally expected to be significant. Climate change is problems with water quality, while it directly affect on water temperature and indirectly affects on physical and chemical processes and it is cause of water stratification that will block of water movement in water column (Verweij and et al., 2010: 1-63). Warm up temperature in surface water will lead to algal blooms and increase microbial content that caused of increase of carbon dioxide (CO_2) from water uptake and pH can rise due to extension of phytoplankton, consequently higher alkalinity in water system (Verweij and et al., 2010: 1-63; Shrestha et al., 2014: 127-128). In addition high water temperature leads to decreased concentrations of dissolved oxygen (DO) and water velocities (Mimikou and et al., 2000: 95-109; Verweij and et al., 2010: 1-63). High precipitation will lead to high river levels and river flows that are increasing a probability of flood. The heaviest downpours will promote increase in suspended solids (turbidity), pesticides, heavy metals, nutrient load and organics because heavy runoff can lead to pollutants from terrestrial, especially urban and agriculture areas that may negative effect on water quality (Verweij and et al., 2010: 1-63; Dessler, 2012: 140-143; Shrestha et al., 2014: 127-128). Meanwhile drought will effect on decreasing of water availability and lead to bad water quality in eater-scare regions, the summer season will increase a deficit of water by evaporation as change in the volume of precipitation and changing the uncertain form of precipitation will change the timing of runoff which has important implications for water availability (Dessler, 2012: 140-143; Grover, 2012: 8). Delpha et al. (2009: 1227) performed an exhaustive literature review to project the potential impacts of climate change on water quality parameters, as shown in Table 1.1. Mimikou and et al. (2000: 95-109) reported climate change will be a significant decrease of the mean monthly runoff and highest decrease in summer season, while the rainfall scenarios will suggest a decrease in mean annual precipitation that may effect on decrease runoff values. In addition, climate change may effects on increasing of Biochemical oxygen demand (BOD) and ammonium ion (NH_4^+) values and decreasing of DO because of loss of stream dilution capacity and low water velocities. Whitehead et al. (2008: 1-115) assessed the potential impacts of climate change on water quality (orthophosphate, sediment, nitrate and ammonia) in five river at United Kingdom (Lambourn, Lugg, Tamar, Tame and Tweed river) which each river was difference of geographic. The climate change may affect on extreme weather and timing of season. Water quality in five rivers involved with water flow and water runoff from terrestrial, especially transition period that causing on sediment and nutrient load in water surface. For example at Lambourn river, orthophosphate was increasing concentrations during summer and autumn because of lower flow to reduced dilution, while Tame river similar pattern of orthophosphat in Lambourn but higher increases in winter due to diffuse urban runoff. Under climate change reduced flows in summer may affect on BOD, phosphorus and nitrate levels would increase.

Finally, water quality impacts are different that depending on geographic location and water body location within a catchment.

Table 1.1 Impacts of climate change on water quality parameters

Source	**Water quality parameters**		**Climate change factors affecting water quality**	**Water body**
Physiochemical	Basic parameters	pH	Drought, temperature increase, rainfall	Rivers, lakes
		DO		Rivers, lakes
		Temperature		Rivers
	Dissolve Organic Carbon (DOC)		Temperature and rainfall increase	Streams and lakes
	Nutrients		Temperature and rainfall increase, drought, heavy rainfall	Rivers, lakes, streams, ground water
Micropollutants	Inorganic	Metals	Temperature and rainfall increase, drought, heavy rainfall	Rivers, high alpine lakes, streams
	Organics	Pesticides	Temperature and rainfall increase, drying and rewetting cycles	Surface water and ground water
		Pharmaceutical	Temperature increase, rainfall	Streams, ground water

Table 1.1 Impacts of climate change on water quality parameters (Continue)

Source	Water quality parameters		Climate change factors affecting water quality	Water body
Biology	Pathogens		Temperature and rainfall increase	Surface waters
	Cyanobacteria		Temperature and rainfall increase	Lakes
	Cyanotoxins		Temperature increase	Lakes
	Green algae, diatoms, fish, others		Temperature increase	soils

Source: Delpha et al. (2009: 1227)

1.2 The impact of climate change to reservoir water quality and primary production (Reservoir trend enormously in Asia)

Reservoirs are an important issues in several Asian countries that expanding water resource which is very diverse both in terms of size and fisheries potential, in addition reservoirs are becoming important form animal protein and employment opportunities especially poorer sectors of community which increasing human population growth (De Silva, 2000: 5-7). Nusser (2014: 2) showed data from the inventory of the World Commission on Dams (WCD) in 2000 that reservoirs were built more than 45,000 large dams, but 5,000 of them built since 1950 (Figure 1.3). The post-World War II that reservoir was most increasing in Latin America (40-fold) and in Africa and Asia (100-fold), with China and India being among the most prolific dam-building countries in the world (De Silva, 2000: 8). In addition the total area for inland freshwater is approximately 100 million ha, the planning of reservoirs do not consider in fishery aspects that main proposed of reservoirs development are constructed for hydroelectric power and flood protection (Nusser, 2014: 8). Hence, reservoir bed was prepared to facilitate harvesting and associated management aspects put in place. Meanwhile, climate change has influence on water evaporation and shifts in precipitation pattern through will have significant influence on the stream flow and watershed hydrology. The alteration of rainfall pattern will certainly influence in the seasonal reservoir in flows and shift in the reservoir operations (Shrestha et al., 2014: 114-116; Shrestha, 2014: 9-10). Thorne and Fenner (2011: 74-87) developed a simplified climate change impact assessment tool (SCIAT) to explain climate change on the reservoir water quality that were found a significant shift in the seasonal, especially with rainy season that could be impact on change in stream flow pattern. The effect of climate change will decrease in water quality and an increased frequency of water stratification, plankton bloom and DOC in reservoir.

The primary production is the rate of synthesis of organic matter (carbohydrate molecules) from carbon source (inorganic matter) by phytoplankton that is using chlorophyll *a* and light, which process is namely photosynthesis (Rajesh and et al., 2001: 358; Ahmed and et al., 2005: 96; Lampert and Sommer, 2007: 58). Light has been known to limit primary production, while light limitation in water column occurs in optical density of the mixed layer (Torremorell and et al., 2009: 438). Dissolved inorganic carbon (DIC) is used by phytoplankton that is changing to organic carbon in photosynthesis (Schneider and et al., 2008: 597). The DIC concentration in seawater is high about 2 mM, meanwhile DIC concentration in freshwater depend on alkaline that range from 1-10 mM (Hein, 1997: 545). The elements are necessary for phytoplankton growth; moreover algal growth is depended on temperature and light (Moss, 2010: 273). The spatial and temporal variability are well known from direct on primary production and organic carbon, furthermore change ocean circulation and nutrient cycling from climate change may impact on primary production differently (Schneider and et al, 2008: 597). Moreover, availability of primary production in reservoirs is influenced by excessive load of nutrient from agriculture, waste water from urban and human activity (Saadoun et al., 2008: 67; Kaymak and et al, 2015: 132). (Chapter 3)

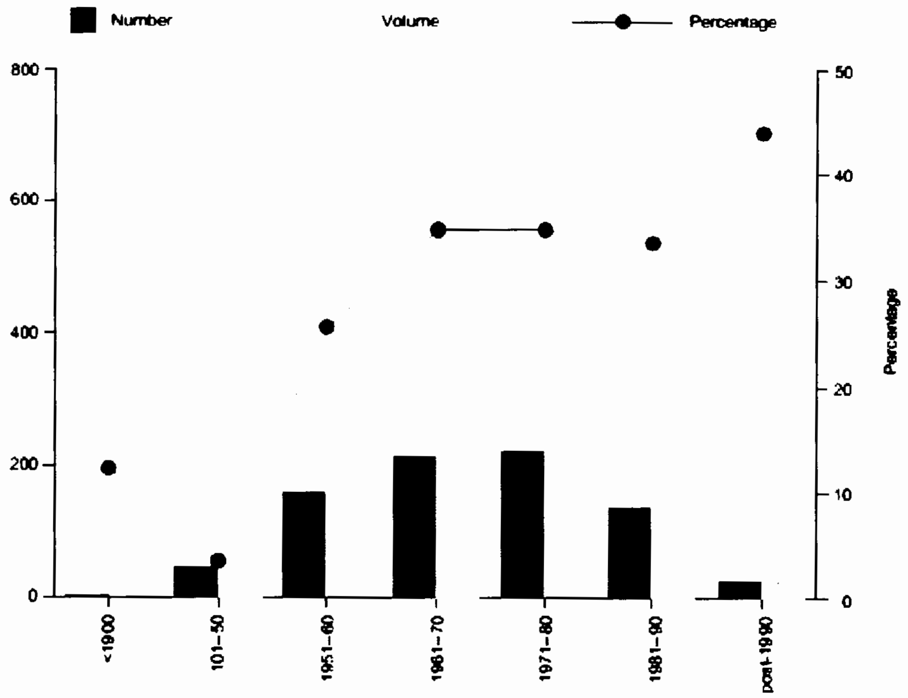


Figure 1.3 The trends in reservoir construction in Asia in comparison to that in the world

Source: De Silva (2000: 8)

1.3 General information of the studied sites and species

1.3.1 The Chao Phraya River Basin

The Chao Phraya Basin, the largest river basin in central Thailand, covers an area of approximately 160,000 km^2 representing 30 percent of the country's total area and is home to 40% of the country's population(Office of Natural Water Resources Committee of Thailand, 2003: 390-392). The Chao Phraya Basin is divided into 2 parts (Figure 1.4). The upper part, is mountainous alternating with lowland areas along the river, while the Lower Chao Phraya Basin is a vast floodplain area and covers 55,290 km^2, i.e. 35 % the total basin area (Office of Natural Water Resources Committee of Thailand, 2003: 392). The Chao Phraya River *per se* begins at the junction of four major rivers in the Upper basin, in Nakhon Sawan Province. (Office of Natural Water Resources Committee of Thailand, 2033: 391; Suvarnaraksha, 2011: 4-5). The river receives the discharge of wastewater from many sources, especially those from household, industrial and agricultural activities, and makes the water polluted. The major sources of pollutants in the upper portion were from agricultural waste and form communities and industries in the lower part of the river course (Simachaya and et al, 2000: 1-12).

The climate change may be effect on irregular pattern of runoff, especially at higher latitude that has been experiencing an increase while another part (west Africa and southern Europe) have had a decrease (Shrestha et al., 2014: 115). The global warming influences on higher air and water temperatures and changes in the timing, intensity and duration of precipitation affect the hydrological characteristics of the river, especially in terms of flow and flood, which eventually affect the water temperature and water quality of the river (Ozaki and et al., 2003: 2837-2838). Moreover, changes in stream water quality, in terms of eutrophication and nutrient transport, are very dependent on changes in stream flow and a long drought period has a noticeable effect on water quality, e.g. temperature, DO, BOD, and chloride concentration (Senhorst and Zwolsman, 2005: 9-53; Prathumratana et al., 2008: 860-866).The aims of this section is to investigate the water quality as well as water temperature along the Chao Phraya River in terms of spatio-temporal approach by using multivariate techniques (Chapter 2).

1.3.2 Sirindhorn Reservoir

Sirinthorn reservoir is the fifth biggest reservoir in terms of surface area (29,200 ha) and main proposed for hydroelectric power, which is placed in Northeast of Thailand at 15° 12′10″ N and 105° 25′ 56″ E (Figure 1.5).

The storage area of reservoir covers three district (PhiboonMungsaharn, Boontarik and Sirindhorn) in UbonRatchathani Province. The Sirindhorn Dam was closed across the Lam Dom Noi River that is a tributary of Mekong River Basin (Jutagate, 2001: 5). The geographical characteristics and water quality parameters are showed in Table 1.2 and Table 1.3, respectively.

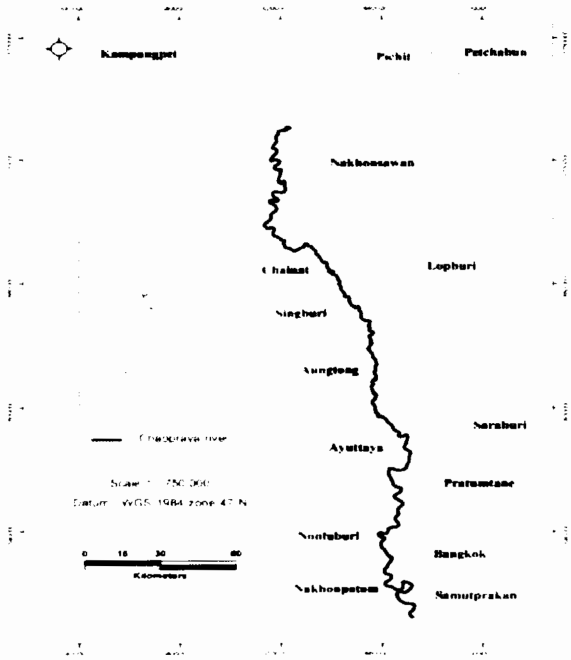


Figure 1.4 The Chao Phraya River Basin

Table 1.2 Sirindhorn geographical parameters.

Geographical characteristics	Information
Year of impoundment	1970
Type of dam	Rock fill and clay core
Dam height	42 m
Dam length	940 m
Dam width	7.5 m
Dam discharge volume (million cum./yr.)	1,500
Water surface area	288 km^2
Watershed	2,007 km^2
Maximum elevation (above MSL.)	142.2 m
Minimum elevation (above MSL.)	137.2 m
Storage capacity	1,468.8 x 10^6 m^3
Mean depth	5.1 m
Maximum depth	32 m

Source: Jutagate (2001: 12)

1.3.3 Ubol ratana Reservoir

Ubol ratana reservoir is the largest hydroelectric reservoir in Thailand. It is located in Northeast region at KhonKaen Province (Figure 1.6). The Ubol ratana reservoir was closed across the Pong and Choen Rivers at the intersection between Phu Phan and Phu Phan Kam mountains since 1965. It is a multipurpose reservoir such as hydroelectric power, irrigation, flood protection, agriculture, fisheries and recreation. The reservoir divided into three parts; upstream watersheds, reservoir area and downstream irrigation area based on different water qualities. The reservoir received water form three main tributaries (the Nam Pong, the Nam Choen and the Lam Phaniang River) and two minor tributaries are the Huay Bong and the Huay Som River (Virapat, 1993: 9-13). The geographical characteristics and water quality parameters are showed in Table 1.4 and Table 1.5, respectively.

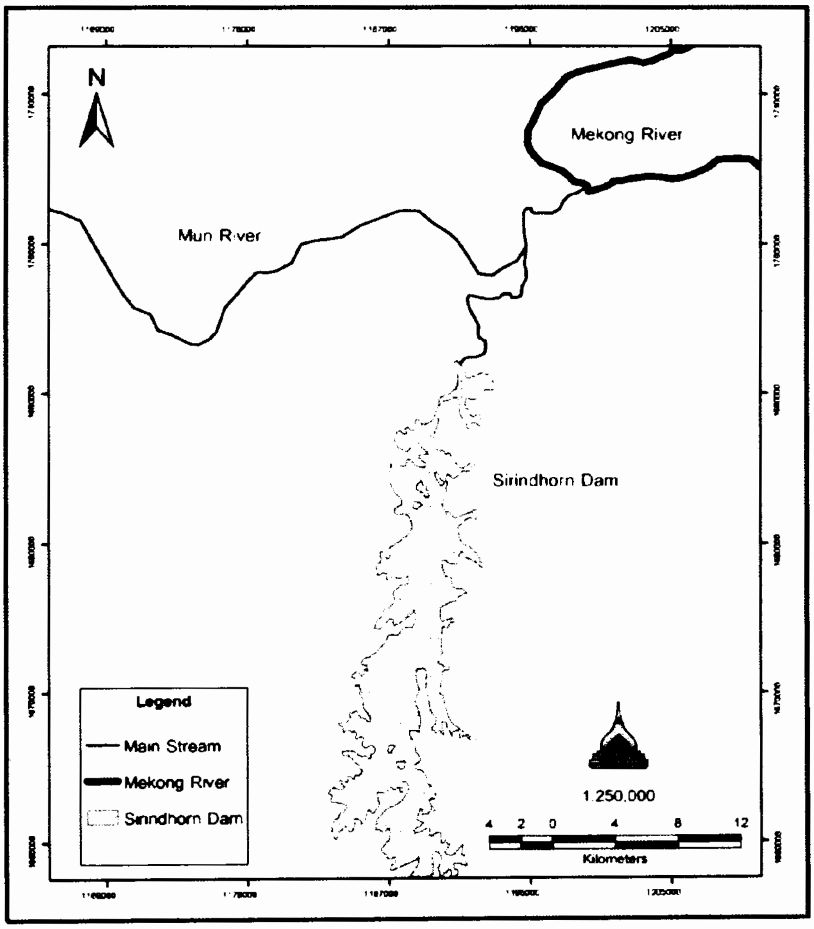


Figure 1.5 Sirindhorn reservoir

Table 1.3 Sirindhorn water quality parameters.

Water quality parameters	Informations
Surface temperature	24.1 - 29 °C
Transparency	19 - 23.3 cm
pH	6.7 - 8.1
DO (Dissolved oxygen)	5.3 - 6.8 mg/L
Alkalinity	7.5 - 15 mg/L
Hardess	4.1 - 11.5 mg/L
Orthophosphate	0.01 - 0.07 mg/L
Nitrate	0.27 - 0.98 mg/L
Total ammonia	0.01 - 0.08 mg/L
Free carbon dioxide	8.1 - 15 mg/L
Primary productivity	0.54 - 1.26 g carbon/m^2/day
Conductivity	0.02 - 0.03 mS

Source: Dumrongtripob and Janesirisak (1996: 9-10)

Table 1.4 Ubolratana and HuayLuang geographical parameters.

Geographical characteristics	Ubolratana	HuayLuang
Year of impoundment	1965	1970
Latitude	16° 30' to 16° 55'	17° 18' to 17° 23'
Longitude	102° 20' to 102° 40'	102° 33' to 102° 38'
Maximum elevation (above MSL.)	186.6 m.	201.5 m
Storage capacity	2,559 x 10^6 m^3	113.25 x 10^6 m^3
Water surface area	410 km^2	31.1 km^2
Mean depth	16 m.	4.7 m
Maximum depth	19.52 m.	11.5 m

Source: Virapat (1993: 12)

1.3.4 Huay Luang Reservoir

Huay Luang reservoir is located in Northeast region of Thailand at UdonThani Province (Figure 1.7). It was dammed across the Huay Luang and Huay Kratib Streams in 1970. The reservoir is a multipurpose reservoir such as irrigation, flood protection, agriculture, fisheries and recreation (Virapat, 1993: 13-14). The geographical characteristics and water quality parameters are showed in Table 1.4 and Table 1.5, respectively.

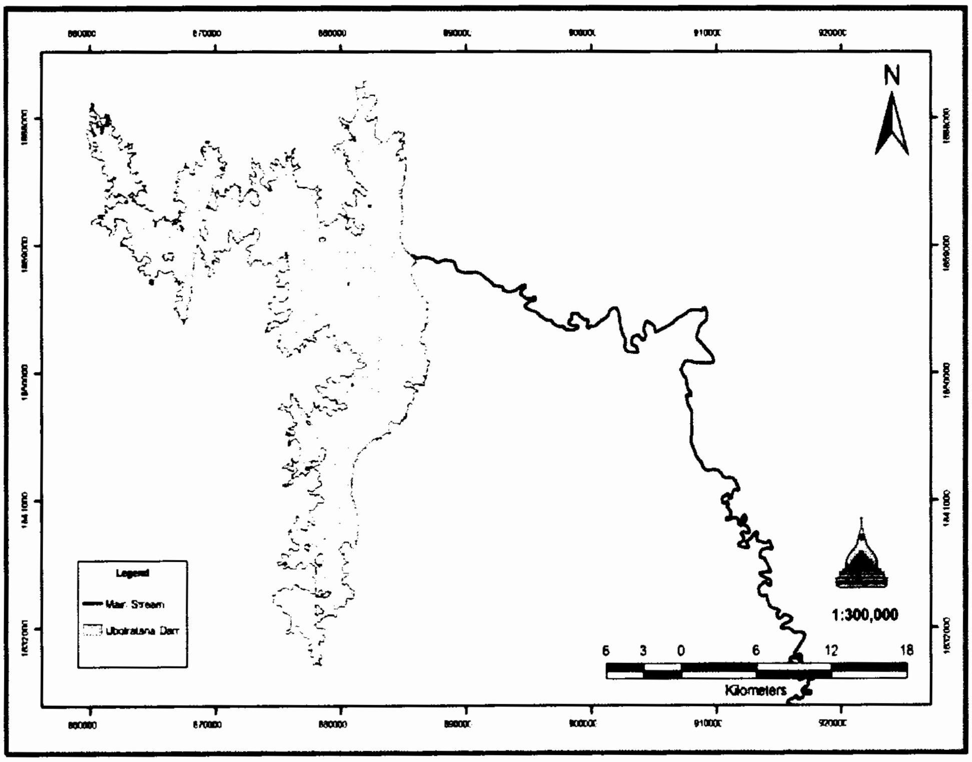


Figure 1.6 Ubol ratana reservoir

Table 1.5 Ubol ratana and Huay Luang water quality parameters.

Water quality parameters	**Ubol ratana**	**Huay Luang**
Physicochemical variables		
Transparency	20 - 248 cm	50 - 347 cm
water temperature	20.9 - 32.5 °C	23 - 31.1 °C
water level (above MSL.)	177.14 - 182.15 m	198.25 - 200.9 m
pH	6.39 - 10.24	5.31 - 9.32
Total alkalinity	35 - 167.5 mg $CaCo_3$/L	20.5 - 80 mg $CaCo_3$/L
Conductivity	125 - 220 µmho/cm	87 - 162 µmho/cm
Total carbon dioxide	0 - 23.5 ppm	0 - 9 ppm
DO (Dissolved oxygen)	3.6 - 10.9 ppm	0.4 - 12.2 ppm
Biological variables		
Chlorophyll a	2.38 - 476 µg/L	1.98 - 809.2 µg/L
Primary productivity	1,134.15 - 3,454.88 mgC/m^2/day	1,387.11 - 4,264.38 mgC/m^2/day
Meteorological variables		
Air temperature	19.9 - 33.65 °C	18.4 - 32.2 °C
Monthly rainfall	0 - 307.6 mm	0 - 363.4 mm
daily radiation	10.31 - 28.37 T MJ.M^{-2}	10.76 - 194.85 T MJ.M^{-2}

Source: Virapat (1993: 307-314)

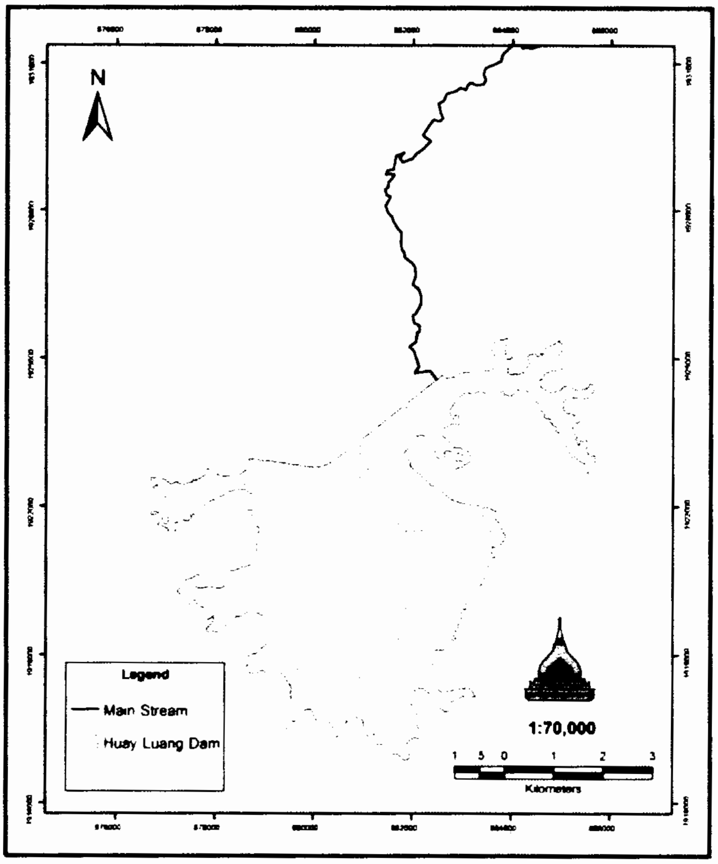


Figure 1.7 Huay Luang reservoir

1.3.5 The impact of climate change to freshwater fish (Siamese mud carp; *Henicorhynchus siamensis*)

Global climate change is predicted to result increases in water temperature, potentially affecting physiological mechanisms in species in these region (Almroth and et al., 2015: 130). Although it is too complicated to predict the impact of the climate change to fishes, the primary effect will be through change in water temperature (Brian and et al., 2011: 80). The physiological mechanisms of fishes are directly or indirectly temperature dependent, thus climate changes will affect fish by altering physiological functions such as growth, metabolism, food consumption, reproduction success and their ability to maintain homeostasis in the face of a variable external environment (Roessig and et al., 2004: 257-260). Meanwhile, the acute increase in temperature is resulted in transient oxidative stress and change in antioxidant enzyme activities (Almroth and et al., 2015: 130-137). Moreover, temperature is the main important environmental factor that affecting and governing the development of eggs, incubation time of embryos, hatching rate, survival and growth of fish larvae (Hakim and Gamal, 2009: 80-90; Le and et al., 2011: 241-245; Ahn and et al., 2012: 100-105).

The Siamese mud carp *Henicorhynchus siamensis* is a riverine species in mainland Southeast Asia (Figure 1.8). The species is a small-size cyprinid, i.e. about 20 – 25 cm TL. It is the most abundant and most economically important fish in the LMB, in particular dominated in the commercial set-net fisheries in Tonle Sap Lake, Cambodia, and the Khone Falls area in southern Laos (Rainboth, 1996: 112;Hai Yen and et al., 2009: 169-174; Fukushima and et al., 2014: 1-3). *H. siamensis* can also behaviourally adapt to the lentic environmental condition such as lakes and reservoirs and contributes the significant portion in fish catches (Suvarnaraksha and et al., 2011: 995). *H. siamensis* is a synchronous, spawning in June to September and highest in August (Suvarnaraksha and et al., 2011: 995-1000). Eggs and larvae are growing in floodplains and leave to rivers when the flood waters begin to recede at the starting of dry season (Rainboth, 1996: 112; Fukushima and et al., 2014: 1-3).Therefore, the suitable environmental condition, during the wet season, is among the important options to guarantee the survival and recruitment to the fisheries of this species. Meanwhile, there is little knowledge on its distribution and life history traits in Asia in comparison to the temperate zone as well as impacts by climate changes (Ficke et al., 2005: 581-583). This study aims of this section to provide a physiological, explaining the relationship between water temperature and embryonic development in order to observe the effect of water temperature on embryonic development, hatching time and hatching rate (Chapter 4).

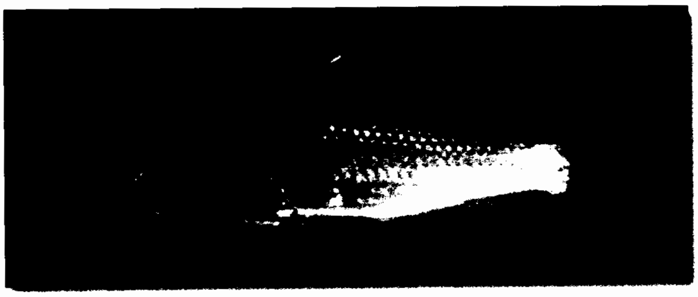


Figure 1.8 ***Henicorhynchus siamensis***

1.4 The Objectives of this Thesis

The climate change can imply that primary effect will be through change in water temperature. The effects are critical importance on both direct and indirect aquatic animal and habitat, while the natural functions in aquatic ecosystems have important intrinsic values to human society. The main objective of the study is to investigate the effect of temperature on fish biology and aquatic habitat. Meanwhile, sub objectives are 1. to examine the spatio-temporal changes in water temperature of the Chao Phraya River and investigate its relationship to the water quality parameters (Chapter 2), 2. to compare the primary productivity (and nutrient) both within and among lakes (Chapter 3) and 3. to study the effect of temperature on embryo development (Chapter 4).

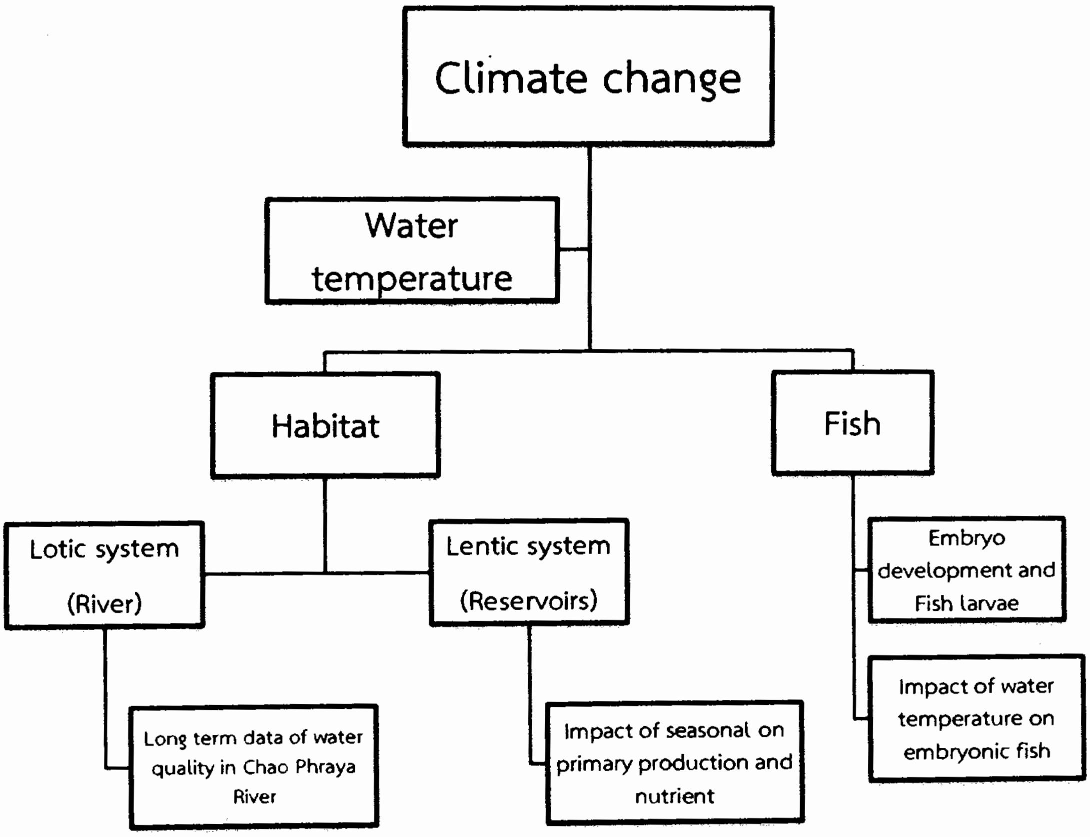


Figure 1.9 Frame work of this study

Temperature is a measure of the internal energy of an object that does not depend on size or type of object, is one important factor of climate element that has obviously effect on organism especially aquatic animals and habitats (rivers or reservoirs) (Dessler, 2012: 35; Shrestha, 2014: 25-26). Although it is too complicated to predict the impact of the climate change to fishes, the primary effect will be through change in water temperature (Brian and et al., 2011: 80-81). This study focus on effect of climate change that represented by water temperature on freshwater aquatic habitat (lotic and lentic system) and fish (especially embryo development and fish larvae) (Figure 1.9).

CHAPTER 2
SPATIO-TEMPORAL VARIATIONS IN WATER QUALITY OF THE CHAO PHRAYA RIVER, THAILAND, BETWEEN 1991 AND 2008

2.1 Introduction

The livelihoods of riparian people depend to some extent on the goods and services of water resources and water quality is among the key factors affecting the environmental health of the river system (Mekong River Commission (MRC, 2008: 1-3). Meanwhile, anthropogenic stresses as well as changes in environment during recent decades are the key issues that severely degrade the water quality in river systems elsewhere (Tudesque and et al., 2008: 732-742; Altansukh and Davaa, 2011: 398-399). However, recovery of the river system from eutrophication and poor water quality conditions is feasible through rigid control on the pollution sources (MRC, 2008: 1-3; Tudesque and et al., 2008: 732-742). Establishment of a monitoring program on water quality is, therefore, highlighted for the purpose of determining the state of pollution in any particular site in the rivers (MRC, 2008: 1-3; Tudesque and et al., 2008: 732-742; Rabee et al., 2011: 259-261). The general trends of either decreasing or increasing water quality at any monitored site indicate which areas are stepped to a good, moderate or vulnerable condition (Tudesque and et al., 2008: 732-742; Simeonov and et al., 2010: 353; Altansukh and Davaa, 2011: 398-399).

Besides the anthropogenic stressors, the condition of water quality in the river is related to hydrological properties (Ozaki and et al., 2003: 2837-2838; Rabee et al., 2011: 259-261). The global warming influences on higher air and water temperatures and changes in the timing, intensity and duration of precipitation affect the hydrological characteristics of the river, especially in terms of flow and flood, which eventually affect the water temperature and water quality of the river (Ozaki and et al., 2003: 2837-2838), for example the higher temperature reduces dissolved oxygen and extends thermal stratification, which increases the potential for anoxia. Increases in frequency and intensity of rainfall during the rainy season will produce more turbid conditions, meanwhile drought during the dry season would allow saline water to intrude into the river (Nail and Jay, 2011: 259-261). Moreover, changes in stream water quality, in terms of eutrophication and nutrient transport, are very dependent on changes in stream flow (Prathumratana et al., 2008: 860-8626) and a long drought period has a noticeable effect on water quality, e.g. temperature, DO, BOD, NH^{+}_{4} and chloride concentration (Senhorst and Zwolsman, 2005: 53-59; Prathumratana et al., 2008: 860-862).

The Chao Phraya Basin, the largest river basin in central Thailand, covers an area of approximately 160,000 km^2 representing 30 percent of the country's total area and is home to 40% of the country's population (Office of Natural Water Resource Committee of Thailand, 2003: 390-391). The Chao Phraya Basin is divided into 2 parts (Figure 2.1). The upper par, is mountainous alternating with lowland areas along the river, while the Lower Chao Phraya Basin is a vast floodplain area and covers 55,290 km^2, i.e. 35 % the total basin area (Office of Natural Water Resource Committee of Thailand, 2003: 390-391). The Chao Phraya River *per se* begins at the junction of four major rivers in the Upper basin, in Nakhon Sawan Province. The total length of river is about 380 km and drains into the Gulf of Thailand. It supplies water and supports many activities such as municipal uses, agriculture, fisheries, light and heavy industries, recreation and navigation (Office of Natural Water Resource Committee of Thailand, 2003: 390-391; Suvarnaraksha, 2011: 4-6). The river, therefore, receives the discharge of wastewater from many sources, especially those from household, industrial and agricultural activities, and makes the water polluted. The major sources of pollutants in the upper portion were from agricultural waste and form communities and industries in the lower part of the river course (Simachaya and et al., 2000: 2-9). Due to the importance of the river and the significant number of stressors along it, this paper aims to investigate the water quality as well as water temperature along the Chao Phraya River in terms of spatio-temporal approach by using multivariate techniques.

2.2 Materials and methods

2.2.1 Source of the data

The data is from the Pollution Control Department of Thailand (PCD, available at www.pcd.go.th). There are 32 surface water stations, i.e. CH1-CH32 (Figure 2.1) along the Chao Phraya river course. We used the time series data of water quality between 1999 and 2008, i.e. 10 years. The water quality parameters used in the analysis were DO, BOD, TCB, FCB, NH_3-N and water temperature. The reason why these parameters were selected is because they are input parameters for water quality index (WQI), developed by PCD, which attempts to provide a simple and understandable tool to evaluate the quality of any given water bodies (Bordalo et al., 2001: 3635-3637).

2.2.2 Data analyses

To perform statistical analysis, the annual average of each selected variable was used. The dataset was performed as rows of the observation (i.e., surface water station × year, for example, CH7_03 is the observation from station 7 in year 2003) and columns of water quality parameters (i.e., 6 variables). There were 327 observations in total for the analysis, after data cleaning by selecting the observations that had the data for all parameters. The state-of-the art multivariate analysis, Self Organizing Maps (SOM), was employed to evaluate the spatio-temporal variations of the data matrix. A SOM is an unsupervised algorithm of an artificial neural network model (ANN), proposed by Prof. T. Kohonen of Helsinki University during 1980s (Kohonen, 1982: 58-69). The basic idea of SOM is to display a high-dimensional signal manifold onto a much lower dimensional network in an orderly fashion (Simeonov and et al., 2010: 353-361).

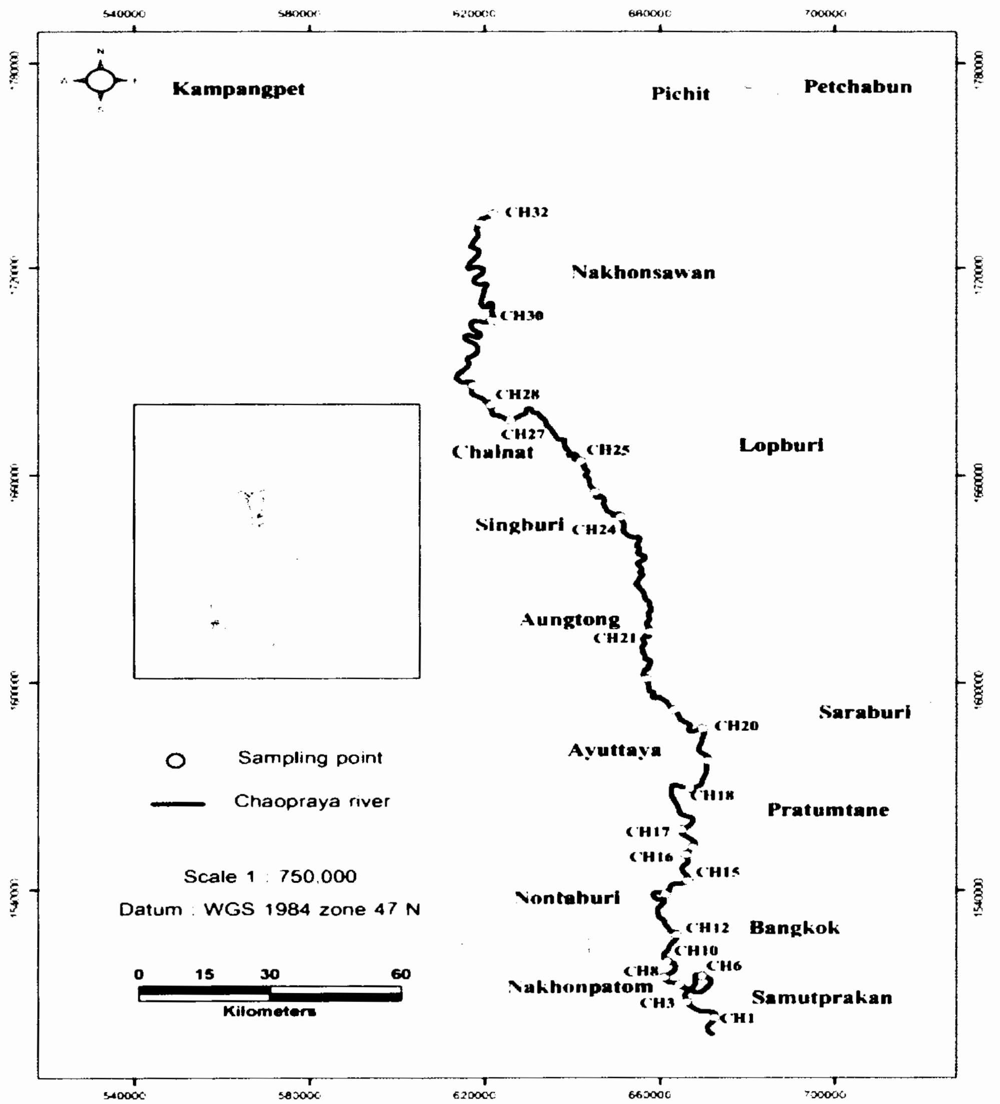


Figure 2.1 Location and map of the Chao Phraya River with the surface water stations

Since 2000, SOM has been widely applied for solving problems is capability of clustering and classification in the studies of water resources and aquatic ecology (Lek and Guegan, 2000: 1-258; Kalteh et al., 2008: 835-845). The SOM consists of two layers *viz.* the input and output layers, which are connected with the weight vectors, and the output layer is displayed as a hexagonal lattice (Lek and Guegan, 2000: 1-258; Kalteh et al., 2008: 835-845; Simeonov and et al., 2010: 353-361). The principle of SOM analysis is to classify the sample vectors (SVs), described by a set of descriptors on the map according to the similarities between the descriptors (i.e. water quality parameters). During the learning process, two SVs that are similar (from the descriptor point of view) are classified in the same or neighboring cells, whereas two different SVs are classified in separated cells that could be distant from each other and *vice versa* (Lek and Guegan, 2000: 1-258; Tudesque and et al., 2008: 732-742). The sequential algorithm used and the protocol for SOMs are widely described (Kohonen, 1982: 58-69; Lek and Guegan, 2000: 1-258; Kalteh et al., 2008: 835-845). In this study, the input layer comprised 6 neuron connected to 327 observations (327 SVs). The output layer comprised 90 neurons organized in an array with 10 rows and 9 columns. This number of neurons was defined according to the formula $C = 5 \times \sqrt{n}$ proposed by the laboratory of Computer and Information Science (CIS), Helsinki University, where C is the number of cells and n is the number of sample (i.e. observation) vectors (Lek and Guegan, 2000: 1-258; Tudesque and et al., 2008: 732-742). This SOM map size was chosen because of its minimal topographic and quantization error as well as clear classification (Kohonen, 1982: 58-69; Lek and Guegan, 2000: 1-258; Kalteh et al., 2008: 835-845). Meanwhile, the hierarchical cluster analysis (Ward linkage, Euclidean distance), was applied to help in the decision of making the clusters (Lek and Guegan, 2000: 1-258; Tudesque and et al., 2008: 732-742).

The analysis of similarity (ANOSIM) was used to test for significant difference among clusters by using occurrence probability, which is approximately estimated from the connection intensity of the SOM during the learning process. Statistical difference of each individual parameter was tested by means of analysis of variance (ANOVA) and Duncan's post-test for multiple comparisons. The correspondence analysis (CoA), the best method to examine the interrelationship between environmental variables and sampling units, was used to determine that interrelationship between the clusters and water quality parameters (Li et al., 2009: 110-121). The significance of the results was tested by a Monte-Carlo method with 1000 random permutations. The SOM was simulated and the cluster analysis was performed by MATLAB®, by using SOM-toolbox, which is developed by CIS (Tudesque and et al., 2008: 732-742). Other statistical analyses were performed by Program R (R development team. 2014: website).

2.3 Results

The observations were classified on the SOM-map according to similarity of water quality parameters presented in each observation and thus, similar observations were mapped close together and the dissimilar were mapped apart. It can be seen that the distribution of the observations on the map were scattered but visibly clustered according to the the hierarchical cluster analysis with Ward linkage method (Figure 2.2a), The outcomes of SOM were partitioned into four clusters (Figure 2.2b). From the clustering, it was obviously seen that clusters B and C were closed, meanwhile cluster A and D were clearly separated. The ANOSIM testing showed significant differences between clusters, i.e. similarity within clusters are more similar than between clusters ($R = 0.41$, $P < 0.001$, based on 1,000 permutations).

It is found that the four clusters were clearly separated from each other as spatially approached, i.e. sectioned along the longitudinal gradient (Table 2.1). Ranges and average (± sd) of each water quality parameter in each cluster are presented in Table 2.2 and it is observed that, except for temperature, other water quality parameters in each cluster are statistically different. Cluster A belonged to the lower portion of the River connected to the river delta and contained almost all observations of stations CH1 to CH10 of the whole period of the dataset. The cluster was characterized by the significantly lowest DO and highest in BOD and NH_3-N ($P < 0.05$) compared to the other clusters. Observations belonging to Cluster B, were spatially ubiquitous and showed a complex pattern along the whole river-course and also varied temporally. This cluster was highlighted by significantly high values of TCB and FCB ($P < 0.05$). Cluster C included most of the observations of the stations located in the upstream parts of the lower portion of Chao Phraya River, i.e. stations in CH12 – CH18. The water quality condition in this cluster was slightly better than cluster A, as judged by the levels of DO, BOD and NH_3-N but still worse than the other remaining 2 clusters. However, the TCB and FCB levels in this cluster were lower than cluster B. Cluster D was mostly the observations of the surface water stations from station 16 and further north up to the source of the Chao Phraya River, i.e. station CH32. This cluster can be regarded as having the best water quality condition, when compared to the other clusters, with the highest level of DO and the lowest levels of BOD, TCB, FCB and NH_3-N. In summary, it can be said that the observations in cluster A showed the worst in water quality condition and then the improving conditions were shown in the clusters B, C and D, respectively.

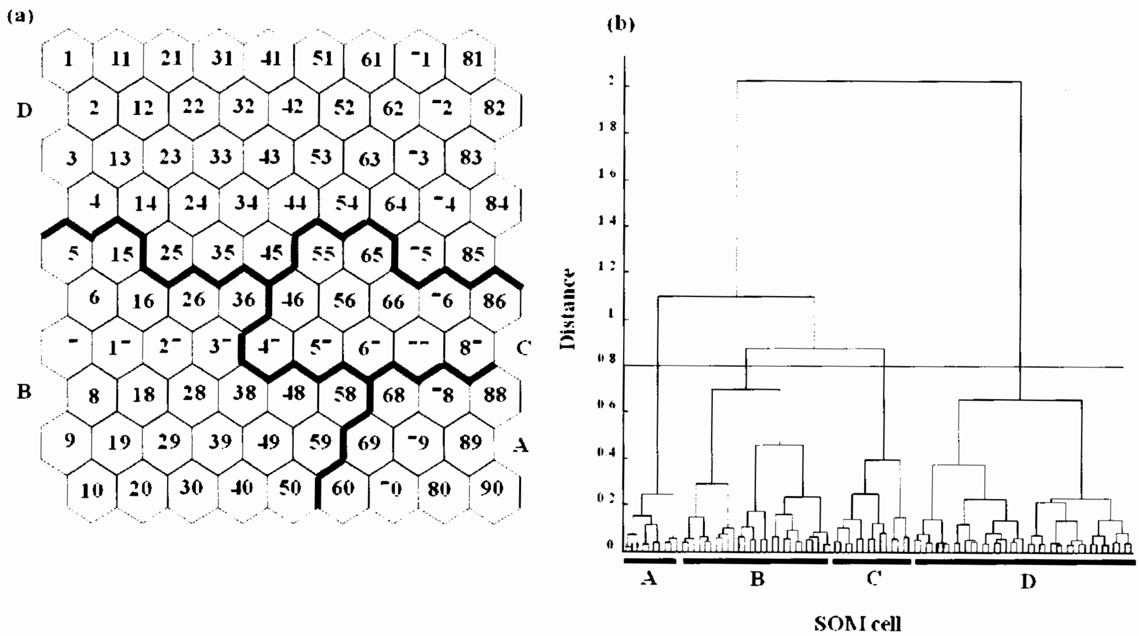


Figure 2.2 Results of SOM analysis. (a) Classification of observations on SOM using water quality data (see also Table 1) and (b) Dendrogram of the SOM output, showing groups of similarity of cells on SOM.

It is found that the four clusters were clearly separated from each other as spatially approached, i.e. sectioned along the longitudinal gradient (Table 1). Ranges and average (± sd) of each water quality parameter in each cluster are presented in Table 2.2 and it is observed that, except for temperature, other water quality parameters in each cluster are statistically different. Cluster A belonged to the lower portion of the River connected to the river delta and contained almost all observations of stations CH1 to CH10 of the whole period of the dataset. The cluster was characterized by the significantly lowest DO and highest in BOD and NH_3-N ($P < 0.05$) compared to the other clusters. Observations belonging to Cluster B, were spatially ubiquitous and showed a complex pattern along the whole river-course and also varied temporally. This cluster was highlighted by significantly high values of TCB and FCB ($P < 0.05$). Cluster C included most of the observations of the stations located in the upstream parts of the lower portion of Chao Phraya River, i.e. stations in CH12 – CH18. The water quality condition in this cluster was slightly better than cluster A, as judged by the levels of DO, BOD and NH_3-N but still worse than the other remaining 2 clusters. However, the TCB and FCB levels in this cluster were lower than cluster B. Cluster D was mostly the observations of the surface water stations from station 16 and further north up to the source of the Chao Phraya River, i.e. station CH32. This cluster can be regarded as having the best water quality condition, when compared to the other clusters, with the highest level of DO and the lowest levels of BOD, TCB, FCB and NH_3-N. In summary, it can be said that the observations in cluster A showed the worst in water quality condition and then the improving conditions were shown in the clusters B, C and D, respectively.

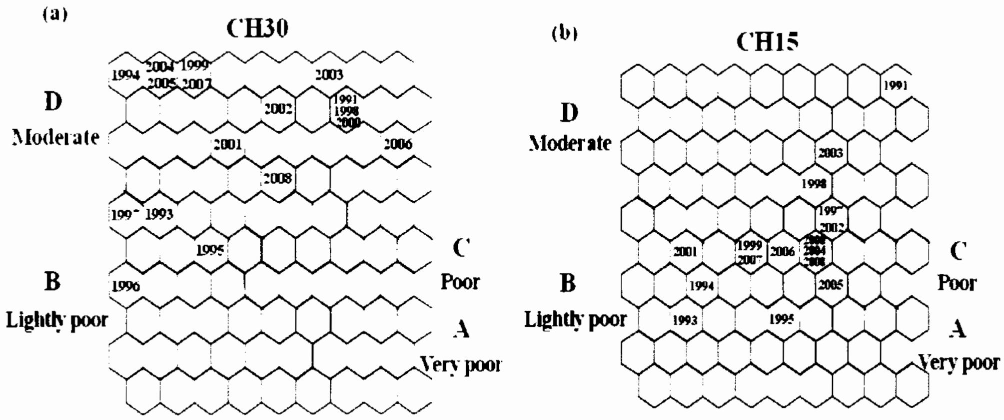


Figure 2.3 Temporal representation of the example stations shift in the SOM map between 1991 and 2008 (a) CH30, from lightly poor to moderate and (b) CH15 from moderate to poor.

In terms of temporal approach, some particular stations showed a trend of better conditions, such as station CH30 (Figure 2.3a), or *vice versa*, such as station CH15 (Figure 2.3b). However, most of the surface water stations were stuck to one particular cluster, i.e. no trend to better or worse in water quality of the station, during the whole monitoring period (Table 2.1). The Monte Carlo test revealed significant relations among the water quality parameters (Monte-Carlo testing, 1,000 permutations, $P < 0.001$), when applying CoA. The ordination by CoA showed that only the observations belonging to cluster A were obviously separated, while the observations of the remaining three clusters were overlapped to some degree (Figure 2.4). This implies that the water quality of the "very poor condition" surface stations was highly separated from the others. The "short-arm" length of temperature compared to the other water quality parameters, implies low fluctuations in annual average water temperature during the study period. Cluster A showed the most fluctuation in terms of average temperature, however the differences were less than ± 2 °C in each cluster during the whole study period (Figure 2.5).

2.4 Discussion

Different rates of anthropogenic stress along the river course result in different water quality conditions in any particular site and period. The results of this study reveal a clear spatial variation in water quality along the Chao Phraya River. The analyses were conducted by means of multivariate procedures, which have been proved to be more suitable for the complicated nature of pollution-induced ecological disturbances (Lek and Guegan, 2000: 1-258; Aguilera and et al., 2001: 4053-4062; Tudesque and et al., 2008: 732-742; Li et al., 2009: 110-121) and they have been successfully applied in environmental quality assessment and management (Lek and Guegan, 2000: 1-258; Aguilera and et al., 2001: 4053-4062; Kalteh et al., 2008: 835-845). Moreover, applying the univariate approach (e.g. WQI) for water quality monitoring always have low interpreting value for decision makers (Aguilera et al., 2001: 4053-4062; Simeonov and et al., 2010: 353-361), because they want to know which water quality parameter should be of the most concern in any particular site. Because of the dense population in the lowland of the Central plain (Li et al., 2009: 110-121), poor water quality condition in the lower portion of the river is common and found not only in Chao Phraya River (i.e. cluster A) but also the other rivers in the region, i.e. Bangpakong (Bordalo et al., 2001: 3635-3642), Mae Klong (Thongdonphum et al., 2011: 178-188) and Tha Chin (Meksumpun and Meksumpun, 2008: 2303-2311), in which all selected parameters, except temperature, were below the national standard of surface water quality of Thailand at the lowest level, i.e. fairly clean used (Simachaya and et al., 2000: 1-59). Municipal wastes and untreated industrial effluents are the main sources in decreasing the DO level and increasing BOD level. Moreover, contaminants from the industries (e.g. chemical agents and heavy metal) also caused a reduction in DO and are increase in BOD in the lower Chao Phrya River (Pholprasert, 1982: 775-784; Kunacheva and et al., 2011: 684-692). Meanhile, high NH3-N and derivatives are commonly loaded from surrounding agricultural land uses (Meksumpun and Meksumpun, 2008: 2303-2311; Thongdonphum et al., 2011: 178-188). FCB and TCB are commonly used indicators of sanitary quality of the water, especially wastes from human and warm-blooded animals (Campos and Cachola, 2007: 31-41). Apart from cluster A, FCB and TCB in cluster B were very far beyond the criteria for “fairly clean used” water quality standard in Thailand i.e. 4,000 and 20,000 MPN/100ml, respectively (Simachaya and et al, 2000: 1-59). The extensive scattering of the observations from cluster B imply the tremendous increase of coliform bacteria along the Chao Phraya river course compared to the data during 1980s, where the high values of FCB and TCB were limited to the lower portion (Mimamara and Sale, 1994: 501-516).

Reduction in DO along the river course (i.e., from clusters D to A) is also related to the flow condition in the river and seasonal effects (Voutasa and et al., 2001: 13-32; Qadir et al., 2008: 43-59). The high influx of freshwater during the monsoon season may have led to marked dilution of the stream resulting in a significant increase in DO levels while the reverse occurred in the dry season (Qadir et al., 2008: 43-59). The natural river flow system in the Chao Phraya is likely to be changed in the near future according to the plan for flood protection in the central plain of the country after the big flood in 2011 (Auynirundronkool and et al., 2012: 245-255). Good condition of water quality in cluster D, in which all parameters were in the range of the “very clean used” criteria related to the fact that the land uses of the upper portion are still sparsely urbanized and also due to dilution from the mass of water from the four upper tributaries (Simachaya and et al., 2000: 1-59).

Table 2.1 Observations (Station x Year), belonged to each cluster, after SOM analysis

Clusters	Station (year)
A	CH1 (1991, 1993, 1994, 1995, 1999, 2002, 2005, 2008), CH2 (1997) CH3 (1991, 1993, 1995, 1997, 1998, 1999, 2000, 2002, 2003, 2005, 2008) CH4 (1997), CH6 (1995, 1997, 1998, 1999, 2000, 2002, 2003, 2004, 2005, 2006, 2007, 2008) CH8 (1991, 1993, 1995, 1997, 1998, 1999, 2005, 2008), CH9 (1996) CH10 (1991, 1995, 1997, 1998, 1999, 2005), CH12 (2005)
B	CH1 (1996, 2001, 2003, 2004, 2006, 2007), CH2 (1994, 1996), CH3 (1994, 1996, 2001, 2004, 2006, 2007) CH4 (1994, 1996), CH5 (1994), CH6 (1994, 1996, 2001), CH7 (1994) CH8 (1994, 1996, 2000, 2001, 2002, 2003, 2004, 2006, 2007), CH9 (1994) CH10 (1993, 1994, 1996, 2000, 2001, 2002, 2003, 2004, 2006, 2007, 2008), CH11 (1994, 1997) CH12 (1994, 1995, 1996, 1997, 1999, 2002, 2004, 2006, 2007, 2008), CH13 (1994, 1995, 1996, 1997) CH15 (1993, 1994, 1999, 2001, 2007), CH16 (1994), CH17 (1994, 1995), CH18 (1994, 1995, 1996, 1997) CH19 (1995, 1996, 1997), CH20 (1993, 1995, 1996, 1997), CH21 (1994, 1995, 1996, 1997, 2001) CH24 (1996, 1997, 2004), CH25 (1996, 1997), CH28 (1993, 1996, 1997), CH29 (1997) CH30 (1993, 1995, 1996, 1997), CH31 (1996), CH32 (1995, 1996, 1997, 2000)
C	CH1 (1997, 1998, 2000), CH2 (1995), CH12 (1991, 1998, 2000, 2003) CH15 (1995, 1997, 2000, 2002, 2004, 2005, 2006, 2008), CH16 (1995, 2000, 2006), CH17 (1997, 2008), CH18 (2000, 2002), CH20 (2007), CH21 (2008)
D	CH12 (2001), CH15 (1991, 1998, 2003) CH16 (1991, 1996, 1997, 1998, 1999, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008) CH17 (1991, 1993, 1996, 1998, 1999, 2000 ,2001, 2003, 2004, 2005, 2006, 2007) CH18 (1991, 1993, 1998, 1999, 2001, 2003, 2004, 2005, 2006, 2007, 2008) CH19 (1994), CH20 (1991, 1994, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2008) CH21 (1991, 1993, 1998, 1999, 2000, 2002, 2003, 2004, 2005, 2006, 2007, 2008), CH22 (1995, 1997) CH23 (1997), CH24 (1991, 1993, 1995, 1998, 1999, 2000, 2001, 2002, 2003, 2005, 2006, 2007, 2008) CH25 (1993, 1995, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008), CH26 (1995, 1997) CH27 (1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008) CH28 (1991, 1995, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008), CH29 (1994, 1995) CH30 (1991, 1994, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008) CH31 (1994, 2006, 2007, 2008) CH32 (1993, 1994, 1998, 1999, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008)

Table 2.2 Mean value (± SD) of the six selected water quality parameters in each cluster

Cluster	Temperature (°C)	DO (mg/l)	BOD (mg/l)	TCB (MPN/100ml)	FCB (MPN/100ml)	NH_3-N (mg/l)
A	29.7±0.6^{a}	1.2±0.6^{a}	4.3±1.5^{a}	317,734±749,693^{b}	59,502±70,292^{a}	2.3±2.9^{a}
B	29.3±0.9^{a}	3.6±1.9^{b}	2.1±0.9^{b}	701,740±1642,924^{a}	165,751±409,245^{b}	0.3±0.4^{c}
C	30.1±0.9^{a}	2.9±1.2^{b}	1.9±0.9^{b}	35,789±37,540^{c}	8,761±10,173^{c}	1.4±3.7^{b}
D	29.6±0.8^{a}	5.5±1.1^{c}	1.2±0.5^{c}	21,757±33,296^{d}	3,841±5,906^{d}	0.1±0.8^{d}

Note: Superscripts in each column indicate significant differences between clusters (Duncan's post- test, $P < 0.05$)

Although the low fluctuation in average water temperature was observed, this parameter must be approached cautiously and closely monitored. Climate change is also expected to have some effects with increasing temperatures and changed rainfall patterns. The average maximum daily temperature in the central plain of the country in 2030 is estimated at 40 °C, with the baseline about 35 °C (Boonprakrop and Hattirat, 2006: 1-24). Meanwhile the precipitation rates are expected to be higher but also more intense falling in a shorter time (Boonprakrop and Hattirat, 2006: 1-24). An increase in air temperature resulted in an increase in biological oxygen demand, coliform bacteria and suspended solids, and a decrease in dissolved oxygen, which eventually has a deteriorating effect on water quality in the river (Ozaki and et al., 2003: 2837-2853; Al-Jebouri and Edham, 2012: 32-38) then eventually aquatic biota and human.

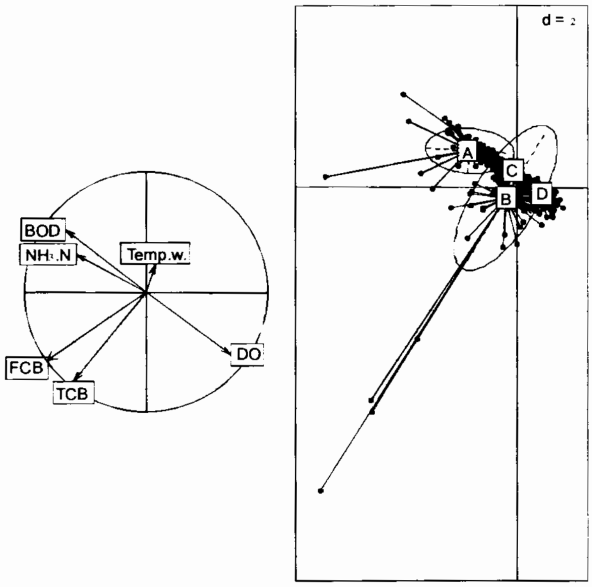


Figure 2.4 Result from CoA showed the gradients of 6 selected water quality parameters to the observations of each cluster.

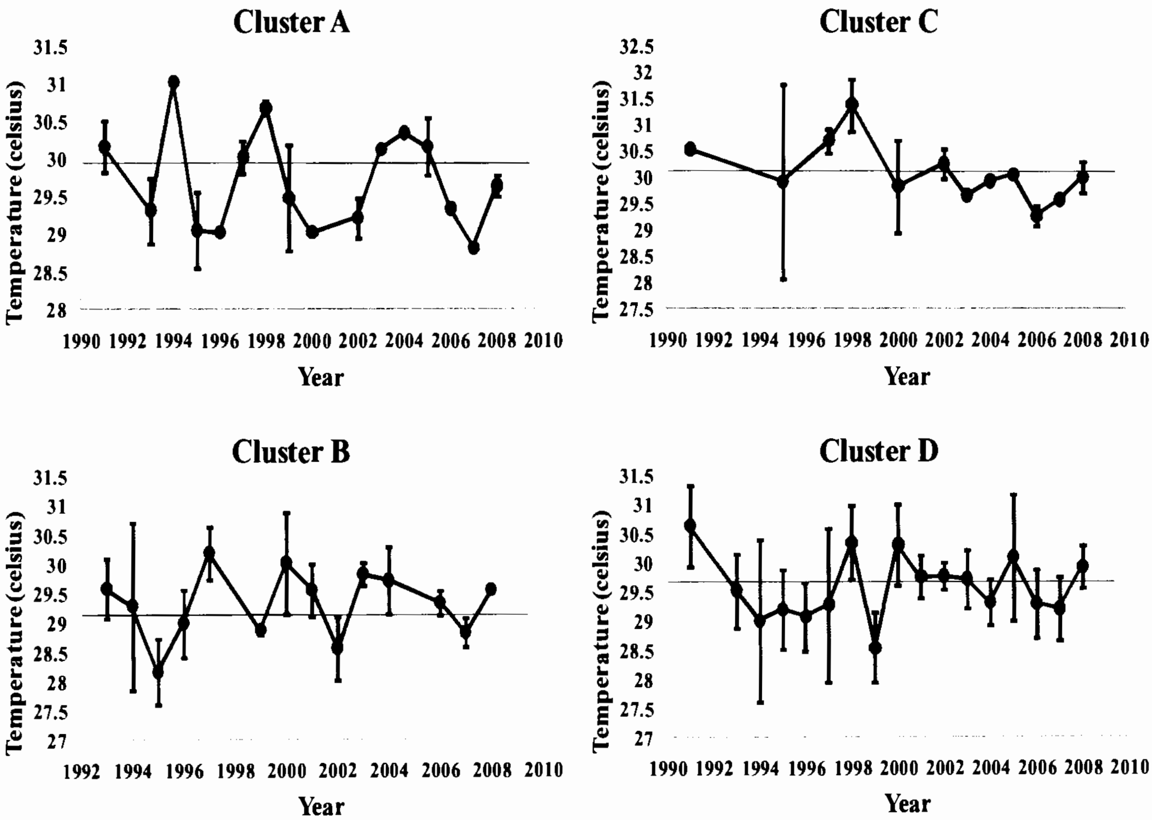


Figure 2.5 Fluctuations of the annual average water temperature in each cluster between 1991 and 2008.

2.5 Conclusion

Distinct spatial differences in water quality along the Chao Phraya River were clearly identified and highly related to anthropogenic stresses. However, there was no clear temporal trend for most of surface water stations, either positive or negative. Nonetheless, as the purpose to provide a big picture, the results were still insufficient to provide the detail in terms of inter-annual variation in water quality of the river. Thus, further studies on the inter-annual variation of water quality of the Chao Phraya River should be focused in the next step.

CHAPTER 3
THE SEASONAL VARIATION OF PRIMARY PRODUCTION AND WATER QUALITY PARAMETERS IN THREE RESERVOIRS, THAILAND

3.1 Introduction

The primary production is the rate of synthesis of organic matter (carbohydrate molecules) from carbon source (inorganic matter) by phytoplankton that is using chlorophyll *a* and light, which process is namely photosynthesis (Rajesh and et al., 2001: 358; Ahmed and et al., 2005: 96; Lampert and Sommer, 2007: 58). The light, that includes the photosynthetically active radiation (PAR; 400 – 700 nm), is important energy source for aquatic ecosystems, especially freshwater habitat (Lampert and Sommer, 2007: 19; Torremorell and et al., 2009: 437). The rate of photosynthesis could be possible limit by two resources as light (energy source) and inorganic matter (Lampert and Sommer, 2007: 60). Light has been known to limit primary production, while light limitation in water column occurs in optical density of the mixed layer (Torremorell and et al., 2009: 438). In addition, Photosynthesis and phytoplankton depend on several environmental factor such as temperature, nutrient (nitrogen and phosphorus) and inorganic carbon (Zoppini and et al., 1995: 494; Hein, 1997: 545; Gurung et al., 2006: 141). Dissolved inorganic carbon (DIC) is comprised of carbon dioxide (CO_2), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) that each abundance form depends on pH in water (Lampert and Sommer, 2007: 17, 64; Emerson and Hedges, 2008: 101-102). DIC is used by phytoplankton that is changing to organic carbon in photosynthesis (Schneider and et al., 2008: 597). Inorganic carbon limitation is possible because air-saturated water contains only about 13μM free CO_2 at 20 °C and the diffusion coefficient for CO_2 is 10^4 fold in water lower than air that those cause may effects on limited photosynthesis by the rate of inorganic carbon supply (Hein, 1997: 545-546).

The DIC concentration in seawater is high about 2 mM, meanwhile DIC concentration in freshwater depend on alkaline that range from 1-10 mM (Hein, 1997: 545). The 20 elements are necessary for phytoplankton growth but only three elements, such as C (carbon), P (phosphorus) and N (nitrogen), are likely to limit growth rates, moreover algal growth is depended on temperature and light. (Moss, 2010: 273). Both phytoplankton and nutrient dynamics are closely linked to algal growth when uptake nutrient is removed dissolved nutrient from water (Saadoun et al., 2008: 68). Phosphorus is found in the waste products of animal and is released during the decomposition of organic matter, meanwhile almost algae are uptake soluble inorganic P form (Knud-hansen, 1998: 17-18). Nitrogen has three primary forms, such as ammonium ion (NH_4^+), nitrate (NO_3^-) and nitrogen gas (N_2) that was utilized by algal growth (Knud-hansen, 1998: 22).

Moreover, spatial and temporal variability are well known from direct on primary production and organic carbon, furthermore change ocean circulation and nutrient cycling from climate change may impact on primary production differently (Schneider and et al, 2008: 597). Cota and et al. (1996: 247-248) and Gurung (2006: 141) reported seasonal variation has been strong affected on phytoplankton primary production, abundance and vertical distribution of oxygen, nitrate and phosphate in upper mixed layer and the upper halocline. Reservoir water quality and productivity are influenced and controlled by inputs of quantity and quality of external nutrient and sediment loading because can reduce of primary production by decreasing light intensity (Knoll et al., 2003: 608-609; Saadoun et al., 2008: 67). Moreover, availability of primary production in reservoirs is influenced by excessive load of nutrient from agriculture, waste water from urban and human activity (Saadoun et al., 2008: 67; Kaymak and et al., 2015: 132).

Ubol Ratana (UB), Huay Luang (HU) and Sirindhorn (SI) reservoirs are multipurpose reservoir which are highly utilized for irrigation, agriculture, recreation and electricity for countries people in Northeast of Thailand (Virapat, 1993: 9; Jutagate, 2001: 5). In the other hand, wastewater, nutrient and sediment are loaded to three reservoirs by human and natural activity that those activity will be effect on water quality and primary production. Meanwhile, the study of seasonal variation of three reservoirs in vertical primary production and nutrition has a litter bit

(Virapat, 1993: 248). This aim of study is to examine the seasonal variations of vertical primary production and water quality parameters in three reservoirs.

3.2 Materials and Methods

3.2.1 Study areas

The water samples were collected at deepest areas which were located at front of dam in three reservoirs (Figure 1.10, 1.11 and 1.12). Three reservoirs are composed of Ubol Ratana, Huay Luang and Sirindhorn reservoirs that are placed in Northearth of Thailand. All reservoirs have different geographical characteristics that are compared by size and depth viz, Ubol Ratana reservoir is large and shallow, Huay Luang reservoir is small and shallow and Sirindhorn reservoir is large and depth, respectively.

3.2.2 Water samples collection and measurement

The water samples were collected quarterly during May 2012 to February 2013 that all samples were send and analyzed at National Institute for Environmental Studies (NIES), Japan. The water temperature (WT), depth and dissolved oxygen (DO) in water column were measured only three months that were represented of Thailand season with HYDROLAB MS5. Thailand season, which may be divided three seasons, is summer or pre-monsoon season (mid-February to mid-May), rainy or southwest monsoon season (mid-May to mid-October) and winter or northeast monsoon season (mid-October to mid-November) (Meteorological Department, 2014: 2). Alkalinity was measured only water surface by titration of 100 ml water sample with 0.02 N H_2SO_4 until pH nearest 4.2 which was stopped process and record.

The photosynthetically active radiation (PAR) was measured in water column, using LI-250A light meter. The record was every kept at 0 (surface), 0.25, 0.5 to 3 m then afterward every 2 m until photosynthetically active radiation equal to zero. Correlation between dept and photosynthetically active radiation was calculated by using semi-log paper. Calculation corresponded with depth and 100%, 50%, 20%, 10% and 5% of photosynthetically active radiation.

Water samples were collected at five depths that using a 2 L of plastic van Dorn sampler. Water samples all depths were strictly protected from light. In each depth, 50 ml water sample was collected in plastic bottle and were stored frozen until analytical for total phosphorus (TP) and total nitrogen (TN) by Auto analyzer Type: AACSII (BRAN LUEBBE).

Chlorophyll *a* (Chl *a*) was filtered by 300 ml water sample. A Glass fibre filter was folded half and was wrapped with aluminium foil. It was kept in freezer. Chlorophyll *a* was analysed by spectrophotometric, according to APHA (1999: 2449-2474).

Dissolved inorganic carbon (DIC) was conducted by 60 ml glass bottle that was filled water sample until full without gas bobble. It immediately injected 0.6 ml of 35 % formalin. Dissolved inorganic carbon was analysed by NDIR analyzer (Non-dispersive Infrared).

The primary production (PP) was measured using ^{13}C fixation with simulated *in situ* incubations (Hama and et al, 1983: 31-36). Two set of 500 ml plastic bottles (control (C) and sample (S) bottles) from each five depths were covered black plastic bag to protect light. Both control and sample bottles were filled water sample until full. Sample bottles were added $NaH^{13}CO_3$ (concentration depended on alkalinity, appendix I) and were kept in the dark until incubation. Control bottles were injected 4.75 ml of 35% formalin and were stored in normal temperature. Sample bottles were incubated in water for 2-3 hours (10.00 – 12.00 am.) in Figure 3.1. After incubation both control and sample bottles were injected 4.75 ml of 35% formalin. Water samples (250 ml) from control and sample bottles were filtered. Each bottle was replicated 2 times. Glass fibre filters were dried and were stored in plastic case. $^{13}C/^{12}C$ atomic ratio of particulate matter was analyzed by using mass spectrometer (Flash 2000/DEL TA V) advantage, Thermo Scientific.

Table 3.1 Sampling site of three reservoirs.

Station	Longitude	Latitude
UB	102°36′34′′E	16°46′19′′N
HU	102°34′35′′E	17°21′14′′N
SI	105°25′45′′E	15°11′54′′N

3.2.3 Statistical analysis

Scatter plot were analysed between two water quality parameters (water temperature and dissolved oxygen) and vertical depth in each reservoirs and season (rain in August, winter in November and summer in February).

Principal component analysis (PCA) is multivariate statistical method which is designed to reduce the number of variables. The matrix data was arranged for input that was comprised 60 sampling site (three reservoirs x four periods x five PAR) and nine water quality variables. The PCA was employed to examine trend of relationship of sampling site with primary production, dissolved oxygen, pH, water temperature, photosynthetically active radiation, total phosphorus, total nitrogen, dissolved inorganic carbon and chlorophyll *a*.

Comparative primary production, dissolved oxygen, pH, water temperature, total phosphorus, total nitrogen, dissolved inorganic carbon and chlorophyll *a* with three reservoirs (UB, HU and SI reservoir) in each period. The reservoir was designed in block and this data was not replication. The statistic test was conducted by Friedman test. If significant difference was used Mann-Whitney test for different between groups.

Comparative primary production, dissolved oxygen, pH, water temperature, total phosphorus, total nitrogen, dissolved inorganic carbon and chlorophyll *a* with both percents of photosynthetically active radiation (100%, 50%, 20%, 10% and 5%) and four periods (May 2012, August 2012, November 2012 and February 2013) within each reservoir were calculated by Kruskal Walis test, when $P < 0.05$ is significant difference. All statistics was conducted by R program (R development team, 2014: website).

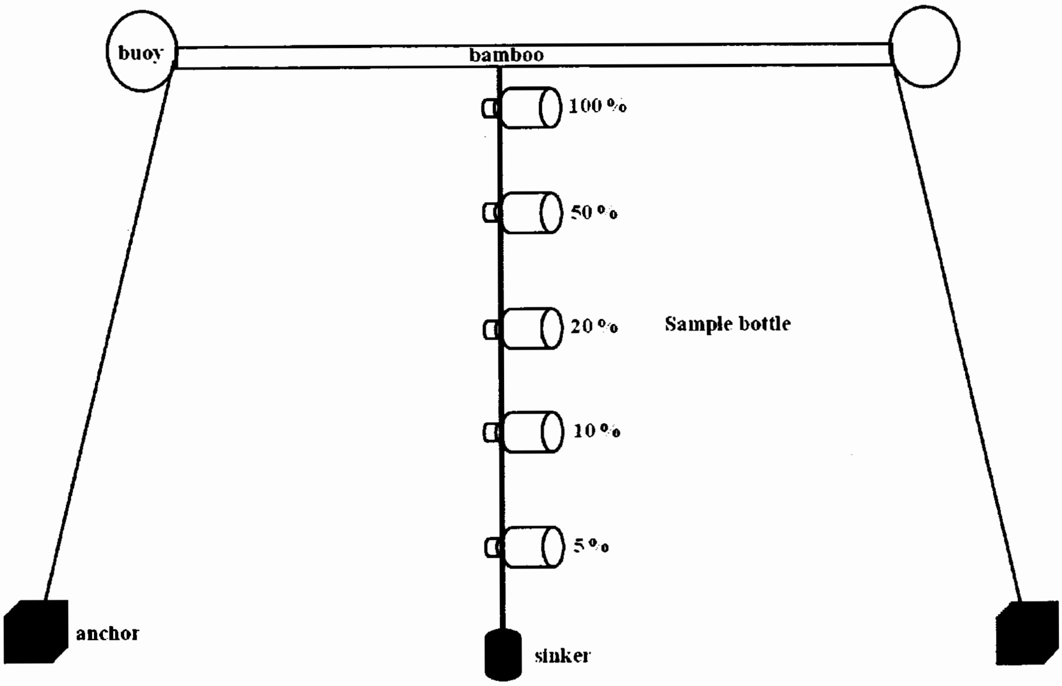


Figure 3.1 Incubation of sample bottles

3.3 Results

3.3.1 Water temperature and Depth

The vertical water temperature profile in UB, HU and SI is illustrated in Figure 3.2. SI reservoir was the deepest (17.79 m) in summer afterward UB (10.13 m) and HU (6.77 m) reservoirs in winter. The maximum depth of UB reservoir in rain (August), winter (November) and summer (February) were 8.13, 10.13 and 8.92 m, respectively. UB reservoir was found the highest water surface temperature in winter (28.53 °C) and lowest water surface temperature in summer (27.67 °C). The thermal stratification was found in winter and clearness in summer. Thermal stratification was developed at around 1 m depth in both seasons. The vertical water temperature in rainy season was about 27.90 $\pm$ 0.11 °C. The water depths at HU reservoir in rain, winter and summer season were 5.97, 6.77 and 5.54 m, respectively. HU reservoir was found the highest water surface temperature in rainy season (32.20 °C) and lowest water surface temperature in summer (30.07 °C). The thermal stratification was obviously found in summer. Thermal stratification developed at around 2.80 m depth. The vertical water temperatures in rain and winter were against depth. The maximum depths of SI reservoir in rain, winter and summer season were 14.42, 15.88 and 17.79 m, respectively. The highest and lowest water surface temperatures in SI reservoir were not difference that water temperature between 29.28 °C and 29.27 °C. The thermocline was clearly found in summer and was developed at around 6.45 m depth. The water temperature in rainy season was 29.00 $\pm$ 0.15 °C through water column but winter season were against depth.

3.3.2 Dissolved oxygen concentration (DO)

The vertical DO concentration profile in UB, HU and SI is showed in Figure 3.2. The UB reservoir was highly DO concentration (8.31 $\pm$ 0.18 mg/L) through water column in rainy season but winter was 3.53 $\pm$ 0.11 mg/L of DO concentration through water column. DO concentration of summer was increased from 8.91 mg/L at surface to 9.19 mg/L at 1.82 m depth after that was decreased to 4.44 mg/L in bottom. Summer and rainy season of HU reservoir had two layers of DO concentration in vertical depth. Rain and summer were the highest DO concentration at surface to 1.05 m depth (9.5 – 10.06 mg/L) in rain and at surface to 1.43 m depth in summer (8.97 – 9.89 mg/L) after that DO concentration was gradually declined to 1.7 and 4.69 mg/L in bottom, respectively. DO concentration in winter was against depth but was sharply decreased to 0.08 mg/L at bottom after 3.78 m depth. SI reservoir had two layers of DO concentration in rain and summer. Both seasons were found the highest DO concentration at surface until 12.18 m (7.75 $\pm$ 0.13 mg/L) in rain and at surface to 6.91 m (7.87 $\pm$ 0.07 mg/L) in summer afterward were rapidly declined to 4.21 and 1.77 mg/L in bottom. DO concentration in winter was continually decreased from 6.29 at surface to 0.48 mg/L in bottom.

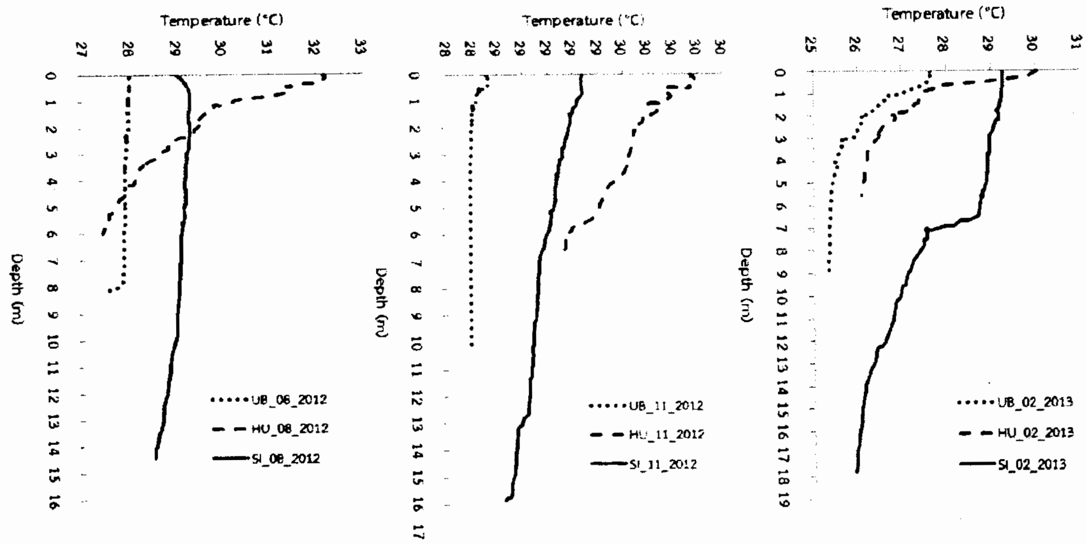


Figure 3.2 The vertical water temperature in three reservoirs

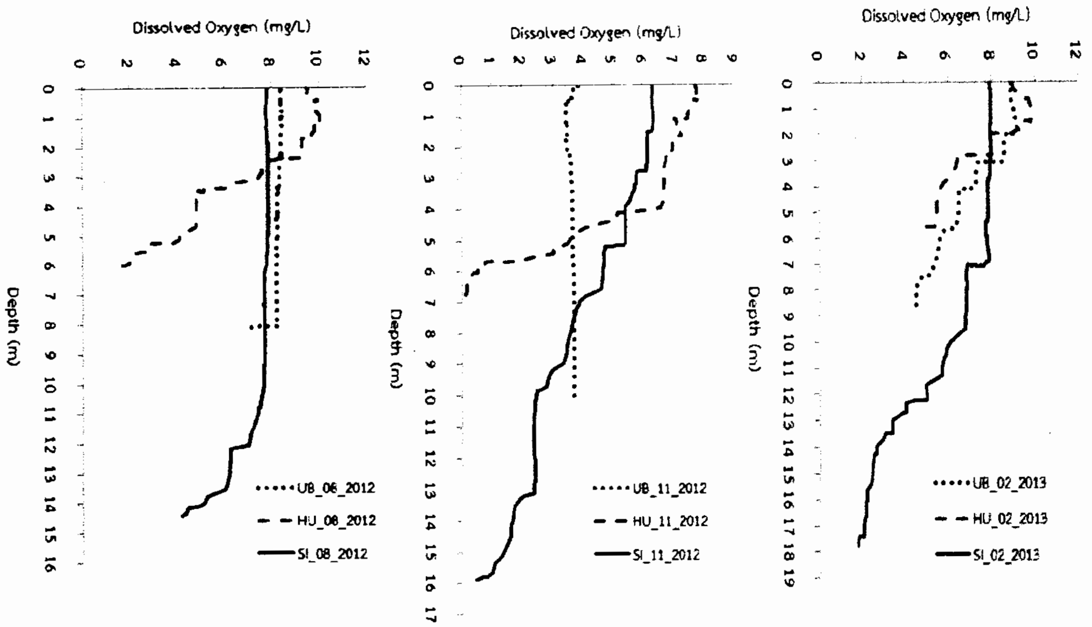


Figure 3.3 The vertical dissolved oxygen in three reservoirs

3.3.3 Relationships of sampling site and water quality parameters

The PCA was explained a combined of two axis (Figure 3.3). The eigen value of total variation was 60.13% of the full data. The eigen value of principle component axis 1 (PC1) and principle component axis 2 (PC2) were represented 33.10% and 20.90% of variance, respectively. The length of vector indicates the importance of variable on the PCA plots. The PC1 was described by primary production, total phosphorus, total nitrogen, chlorophyll *a* and dissolved inorganic carbon. The water temperature, pH, dissolved oxygen and photosynthetically active radiation were loaded in PC2. Primary production was high relationship with total phosphorus and total nitrogen. The composition of water quality parameters were related to sampling site, especially HU reservoir.

Table 3.2 The loading scores of the first two principal components for nine water quality parameters.

Parameters	PC1	PC2
Primary production (µgC/L/h)	-0.4653	0.1954
Total phosphorus (mg/L)	-0.4913	0.1839
Total nitrogen (mg/L)	-0.4098	0.1412
Chlorophyll *a* (µg/L)	-0.4461	-0.1915
Dissolved inorganic carbon (mg/L)	-0.2559	-0.2133
Water temperature (°C)	-0.1234	0.5779
pH	-0.2236	-0.5396
Dissolved oxygen (mg/L)	-0.1981	-0.3504
Photosynthetically active radiation (µmol/m^2/s)	-0.0700	0.2788

3.3.4 The comparative of primary production and water quality parameters in water column at four periods in each reservoir.

3.3.4.1 Ubol Ratana reservoir

Comparative primary production, dissolved oxygen, pH, water temperature, total phosphorus, total nitrogen, dissolved inorganic carbon and chlorophyll *a* with both percent of photosynthetically active radiation and four periods were showed in boxplot (Figure 3.5). The primary production was the highest value in November (35.40 $\pm$ 13.31 µgC/L/h) and significant difference with May (11.16 $\pm$ 7.02 µgC/L/h) that was lowest primary production. Both August (20.20 $\pm$ 10.73 µgC/L/h) and February (19.96 $\pm$ 6.67 µgC/L/h) were not significant difference from the highest and lowest primary production. The primary production in vertical light intensity that found first two of PAR was significant difference from 5% of PAR. The highest primary production was found in 50% of PAR. Meanwhile, both 20 and 10% of PAR were not significant difference from 100, 50 and 5% of PAR. Water temperature and pH of all periods were significantly different. The maximum and minimum of water temperature were found in May (31.40 $\pm$ 0.31 °C) and February (26.34 $\pm$ 0.88 °C) while, maximum and minimum of pH were exhibited in August (8.43 $\pm$ 0.02) and November (7.24 $\pm$ 0.09), respectively. In vertical light intensity, all percent of PAR of water temperature and pH were not significantly different. Meanwhile, The highest water temperature was found in water surface (100% of PAR) after that water temperature was continually declining. The pH at first four of PAR was ranged 7.91 – 7.99 but pH at 5% of PAR was lowest (7.83 $\pm$ 0.54).

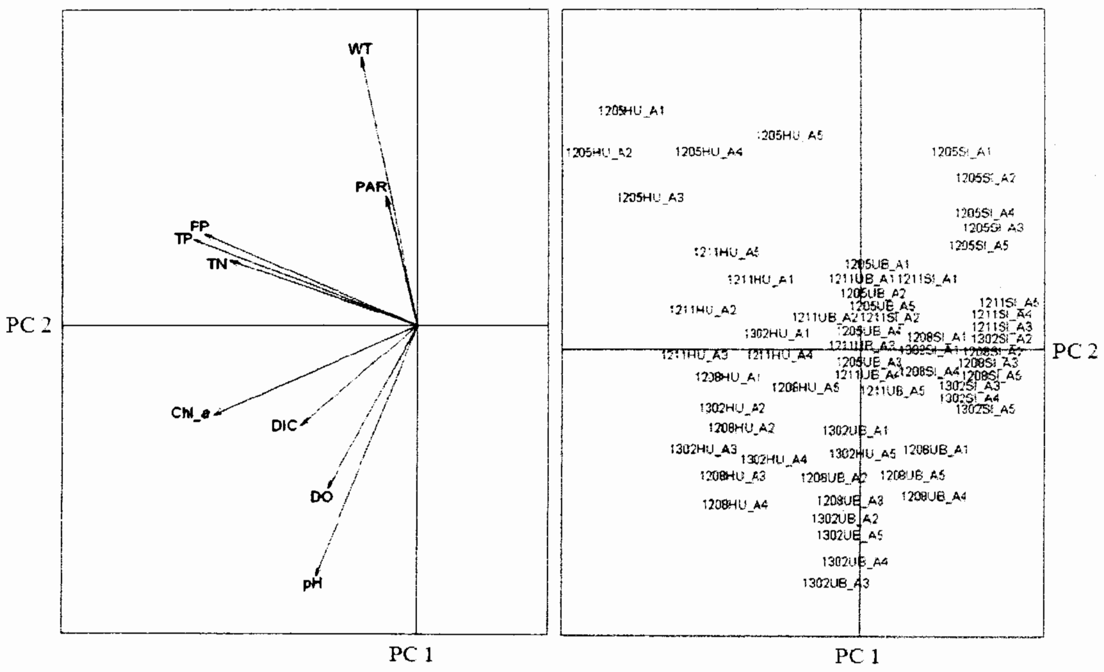


Figure 3.4 Principal component analysis of nine water quality parameters and 60 sampling sites.

The maximum of dissolved oxygen was established in both August (8.33 $\pm$ 0.09 mg/L) and February (8.23 $\pm$ 1.01 mg/L) that was significant difference from May (4.28 $\pm$ 0.13 mg/L) and November (3.58 $\pm$ 0.14 mg/L). The dissolved oxygen in each percent of PAR was not significantly different.

The highest dissolved oxygen was found in 100% of PAR (water surface) and was reducing against depth. The total phosphorus was not significant difference in all periods that maximum of total phosphorus was found in May (0.014 $\pm$ 0.005 mg/L) and November (0.014 $\pm$ 0.001 mg/L). Total phosphorus in each percent of PAR was not significant difference and ranged between 0.010 – 0.013 mg/L. The total nitrogen was significant difference in two groups. First group comprised May (0.29 $\pm$ 0.04 mg/L) and August (0.24 $\pm$ 0.01 mg/L) were minimum group. Second group was November (0.34 $\pm$ 0.01 mg/L) and February (0.39 $\pm$ 0.09 mg/L) were maximum group. Dissolved inorganic carbon was significant difference in three groups. Maximum value was found in November (15.01 + 0.22 mg/L). Minimum value was found in August (12.63 $\pm$ 0.24 mg/L). Chlorophyll *a* was significant difference in period. The highest value was found in November (9.00 $\pm$ 0.70 µg/L) and lowest was found in May (4.80 $\pm$ 0.44 µg/L). The PAR was not significant difference in total nitrogen, Dissolved inorganic carbon and chlorophyll *a*. The best period of UB reservoir was November, especially PAR at 100% to 50%, because high primary production was corresponding with total phosphorus, total nitrogen, dissolved inorganic carbon and chlorophyll *a*.

3.3.4.2 Huay Luang reservoir

The comparative between water quality parameters with HU reservoir was exhibited in boxplot (Figure 3.6). Primary production had the highest value in May (129.86 $\pm$ 73.26 µgC/L/h) and lowest in February (41.54 $\pm$ 20.39 µgC/L/h). Conversely, all periods were not significantly different. Percent of PAR was significant difference that was separated two groups. The first group was composed of 100, 50 and 20% of PAR that 50% of PAR was the highest primary production. Second group was 10 and 5% of PAR that lower primary production than first group. Water temperature was significant difference in May (33.30 $\pm$ 0.81 °C). Water temperature had the highest value in water surface (100% of PAR) afterward continually decreasing. The maximum pH was found in August (8.39 $\pm$ 0.56) and February (8.30 $\pm$ 0.11) and minimum pH was found in May (7.10 $\pm$ 0.68) and November (7.50 $\pm$ 0.26). Both maximum and minimum pH were significantly different. PAR was not significant difference that maximum pH was found in 50 and 20% of PAR. Dissolved oxygen both periods and PAR were not significantly different. The maximum and minimum water temperature were 8.72 $\pm$ 2.15 and 6.54 $\pm$ 1.67 mg/L in August and May, respectively. Dissolved oxygen was declining from 100 to 5% of PAR that maximum of dissolved oxygen was found in 50% of PAR (8.89 $\pm$ 1.02 mg/L). Total phosphorus and total nitrogen were significant difference in each period which was found the highest value in May (0.054 $\pm$ 0.003 and 0.73 $\pm$ 0.04 mg/L) and lowest value in August (0.026 $\pm$ 0.004 and 0.34 $\pm$ 0.01 mg/L). Both total phosphorus and total nitrogen were not significant difference in PAR. Dissolved inorganic carbon was significant difference in all periods. The maximum value was 9.43 $\pm$ 0.03 and 8.86 $\pm$ 0.09 mg/L in November and May. Chlorophyll *a* was highly significant difference in August (17.00 $\pm$ 2.12 µg/L). PAR of dissolved inorganic carbon and chlorophyll *a* were not significantly different. High productivity of HU reservoir was found in May, especially at surface to 20% of PAR, because this time was highly primary production, total phosphorus, total nitrogen and dissolved inorganic carbon.

3.3.4.3 Siridhorn reservoir

The statistical test was conducted for water quality parameter in SI reservoir was illustrated in Figure 3.7. Primary production was not significant difference in four periods. The high value was established in August (5.20 $\pm$ 4.49 µgC/L/h) and November (3.40 $\pm$ 2.30 µgC/L/h). Primary production in PAR was significantly different. The high value was found in 100 to 20% of PAR, particularly in 100 and 50%. Water temperature was highly significant in May (31.88 $\pm$ 0.14 mg/L). Although PAR was not significantly different, water quality value was not less than 29.00 mg/L through water column. The maximum and minimum pH were found in February (7.42 $\pm$ 0.13) and May (6.87 $\pm$ 0.10) that were significantly different. pH in PAR was not significantly different. Dissolved oxygen was significant difference that the highest value was found in August (7.80 $\pm$ 0.10 mg/L) and February (7.72 $\pm$ 0.35 mg/L). Dissolved oxygen was not significant difference in PAR and maximum value was found in surface (100% of PAR). Meanwhile, Dissolved oxygen was declining with depth. Total phosphorus and total nitrogen were not significant difference both all period and PAR. The maximum value of total phosphorus and total nitrogen were found in August (0.004 $\pm$ 0.001 mg/L) and November (0.22 $\pm$ 0.06 mg/L), respectively. While, both total phosphorus and total nitrogen were high value in 100 to 20% of PAR. Dissolved inorganic carbon and chlorophyll *a* were high value in August and November. But, PAR of both dissolved inorganic carbon and chlorophyll *a* were not significantly different. The best of productive in SI reservoir was found in August and November because both months was supported by primary production, total phosphorus, total nitrogen, dissolved inorganic carbon and chlorophyll *a*.

3.3.5 The comparative of primary production and water quality parameters in water column at three reservoirs in each period.

3.3.5.1 May 2012

The comparison of nine water quality parameters with three reservoirs was exhibited in Figure 3.8. UB, HU and SI reservoirs were significantly different. The highest of primary production and water temperature were found in HU. The pH was significant difference in SI, while maximum value was found in UB and HU. The maximum value of dissolved oxygen was found in HU and SI reservoirs that both reservoirs were significant difference with UB reservoirs. The total phosphorus, total nitrogen and chlorophyll *a* had the highest value in HU reservoir that was significant difference with SI and UB reservoirs. Meanwhile, dissolved inorganic carbon was significant difference in all reservoirs that maximum value was found in UB reservoir.

3.3.5.2 August 2012

The primary production, water temperature, pH, total phosphorus, total nitrogen and chlorophyll *a* had the highest in HU reservoir that was significant difference from SI and UB reservoirs. While dissolved oxygen in three reservoirs was not significantly different, the maximum value was still in HU reservoir. In the other hand, the highest dissolved inorganic carbon was found in UB reservoir that was significant difference from HU and SI reservoirs. The result of statistical analysis was illustrated in boxplot (Figure 3.9).

3.3.5.3 November 2012

The maximum value of primary production, water temperature, dissolved oxygen, total phosphorus, total nitrogen and chlorophyll *a* were found in HU reservoir that was significant different with SI and UB reservoirs. Although the pH of three reservoirs was not significantly different, the highest value was still in HU reservoir. Dissolved inorganic carbon had the highest value in UB reservoir that was significant difference with HU and SI reservoirs. All result was showed in boxplot (Figure 3.10).

3.3.5.4 February 2013

The primary production of HU reservoir was significant difference with SI reservoir, while the maximum value was found in HU reservoir. The maximum of water temperature was found in SI reservoir that was significant difference with UB reservoir. The pH and total nitrogen were high value in HU and UB reservoirs that were significant difference with SI reservoir. Dissolved oxygen of three reservoirs was not significantly different, while the maximum value was found in HU reservoir. Chlorophyll *a* and total phosphorus were found the highest value in HU reservoir that was significant difference with SI and UB reservoirs. The maximum value of dissolved inorganic carbon was found in BU reservoir, while all reservoir was significantly different All result was showed in boxplot (Figure 3.11).

The high value of water quality parameters, especially primary production total phosphorus and total nitrogen, in each time periods were correspond to HU reservoir. These evidences would imply that HU reservoir was high production than UB and SI reservoirs.

3.4 Discussion

UB, SI and HU reservoirs are obviously found thermal stratification in summer (February), meanwhile SI reservoir is clearly found thermocline in summer. SI reservoir was fund the water deepest in summer season which solar radiation can not through in bottom. Jindarojana and et al. (1978: 125) and Virapat (1993: 309-310) reported the thermal stratification in UB reservoir is occurred during February and March. The thermal stratification depends on solar radiation, wind and air temperature that can effect on circulation patterns of lake (Knud-hansen, 1998: 11; Tundisi and Matsumura-Tundisi 2011: 81). DO concentration in water column is occurred two layers in summer at all reservoirs. The maximum upper layer depth is not excess than 7 m, except SI reservoir in rainy season, which PRA can through. The oxygen in water can be supported by exchange from atmosphere and release from photosynthesis. Which related with organic matter is synthesized and release oxygen in upper layer, while lower layer which organic matter is decomposed and consumption oxygen (Lampert and Sommer, 2007: 24; Gurung et al., 2006: 143-146). The PAC showed high relationship between primary production and both nutrients (TN and TP), especially TP. Downing and McCauley (1992: 936) reported the form of the relationship of TN to TP in the pelagic zone of variety of the world's lakes due to the lake is rich phosphorus (P) also often rich in nitrogen (N) that is positive correlation between the TN and TP concentrations of lake. Thus, the form of the relationship between TN and TP in lake may reveal how the processes that control N:P vary with lake trophic status. The primary sources of new nutrient to lake is terrestrial runoff and atmospheric input that yield nutrients to lake is predominantly in fixed organic form, both solution and particles, and can vary widely in the concentration and ratio of total phosphorus and total nitrogen as well as in the proportion of organic and inorganic form of these nutrient (Downing and McCauley, 1992: 940; Guildford and Hecky, 2000: 1214). In addition, total phosphorus and total nitrogen are import nutrient source for algal, particularly algal biomass and algal growth rate are controlled by phosphorus than nitrogen (Downing and McCauley (1992: 938; Knud-hansen, 1998: 17-22; Guildford and Hecky, 2000: 1221-1222). UB reservoir was found the highest primary production in November at 50-20% of PAR, while SI reservoir had the highest primary productivity in August at 50–20% of PAR. Both UB and SI were supported by total phosphorus, total nitrogen, dissolved inorganic carbon and chlorophyll *a*. Meanwhile, August to November is heavy rainfall in Thailand (Meteorological Department, 2014: 4). High precipitation will lead to high river levels and high river flows that is increasing a probability of flood. The heaviest downpours will promote increase in suspended solids (turbidity), pesticides, heavy metals, nutrient load and organics. (Verweij and et al., 2010: 1-63; Dessler, 2012: 140-143; Shrestha et al., 2014: 127-128). HU reservoir had the highest primary production value in May at 50% of PAR. In addition, Virapat (1993: 313) reported maximum primary production in UB and HU were usually found in summer season. When compared the primary production in each reservoir, HU reservoir was high production than UB and SI reservoirs. Although, The geographical HU reservoir is shallow and small size when compared another reservoirs, meanwhile proportion of littoral zone and pelagic zone is high (Virapat, 1993: 9-14). While difference in primary production rates may be a result of differences in hydrological condition, the increasing of water level fluctuation has been implicated in limiting the literal ecosystem which can contribute significantly to pelagic production, especially in small to medium-size lake (Rysgaard et al., 1999: 14; Matzinger and et al., 2007: 2630). Vertical primary production in UB and HU reservoirs was highly occurred at between 50–20 % of PAR that was support by pH, dissolve oxygen and chlorophyll *a*. The depth at 50-20% of PAR of both reservoir are 0.5-1.1 in UB and 0.25–0.9 m in HU that those depth are called trophogemic zone which organic matter is synthesized and oxygen produced (Lampert and Sommer, 2007: 24), while at water surface (100% of PAR) is the highest water temperature and PAR which may be against primary production. In contrast, Torremorell and et al. (2009: 443) described the behaviour of primary production at high light intensities by photosynthetic parameters which showed marked seasonal trends that correlated with the levels of incident solar radiation, and *vice versa*. Meanwhile, maximum primary production in water column at SI reservoir was found between 100-50% of PAR that corresponds with pH, dissolved oxygen, total phosphorus, dissolved inorganic carbon and chlorophyll *a*. The depth at 50% of PAR was 1.7 m. In three reservoirs, high primary production was accepted in a middle depth than the surface. However, a decrease of the primary production on the surface was not able to clarify the strong light inhibition or the high temperature inhibition.

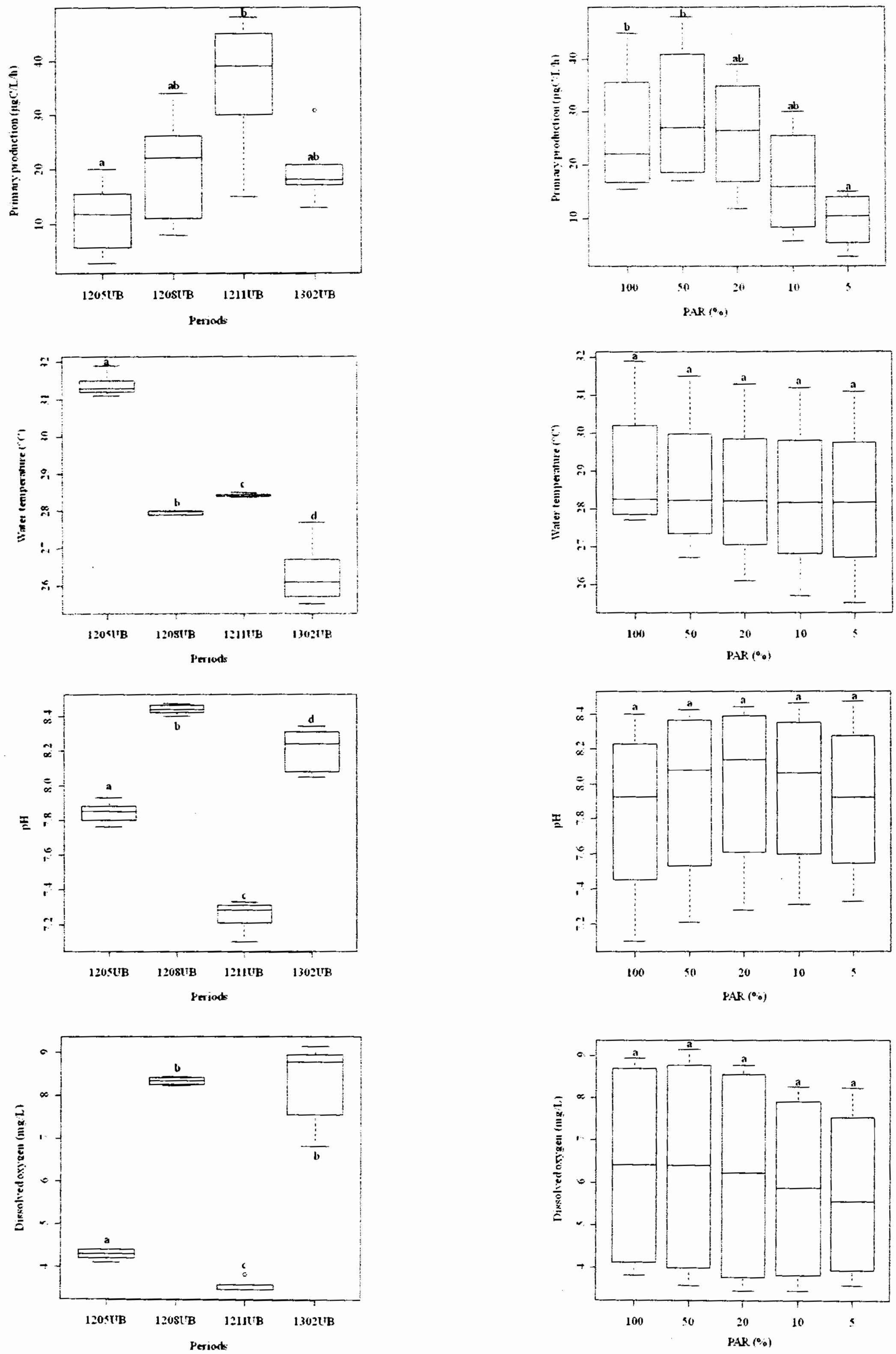


Figure 3.5 Primary production and water quality parameters in water column at Ubol Ratana reservoir (different letters indicate significant difference at P < 0.05).

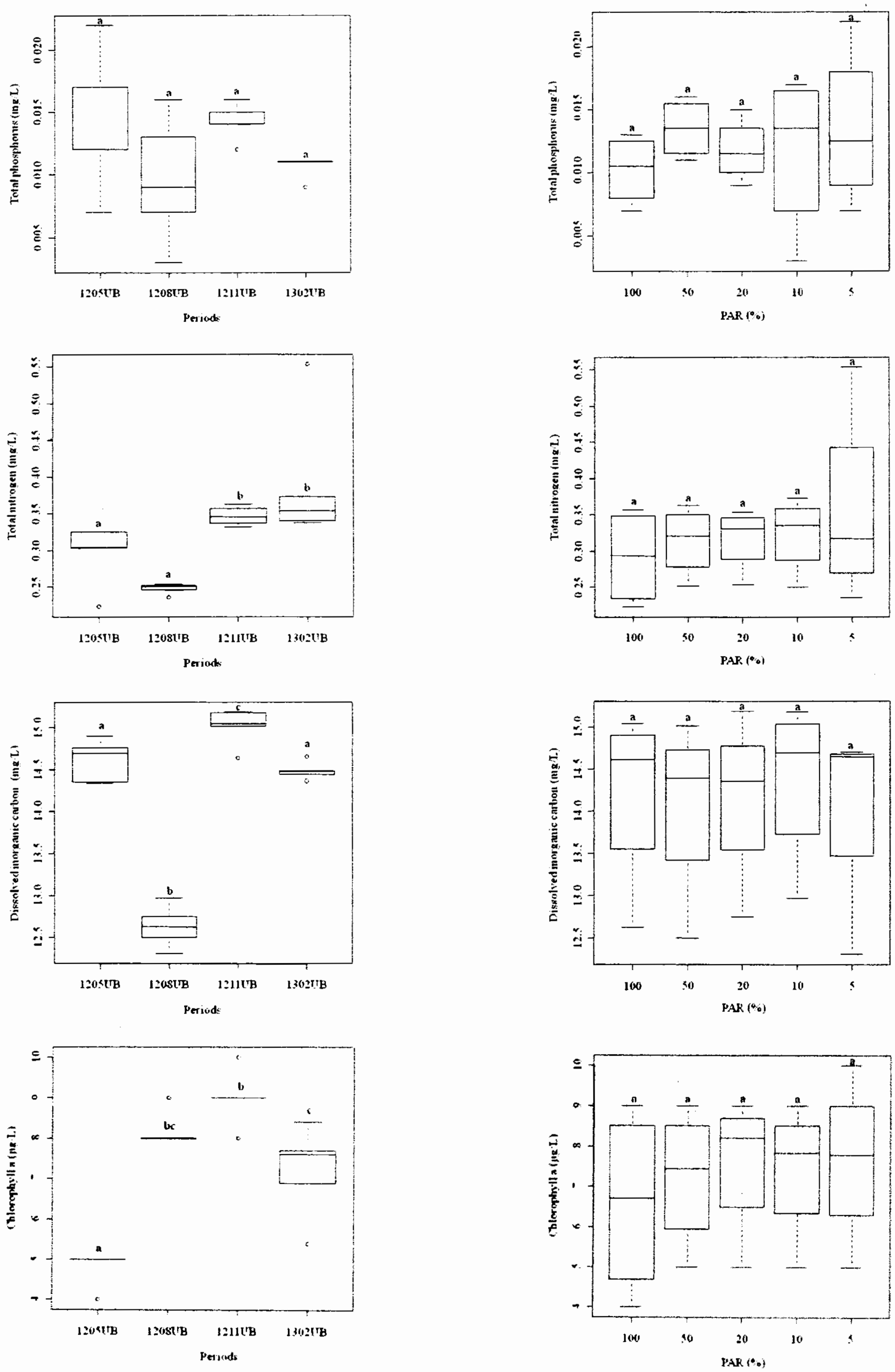


Figure 3.5 Primary production and water quality parameters in water column at Ubol Ratana reservoir (different letters indicate significant difference at P < 0.05). (Continued)

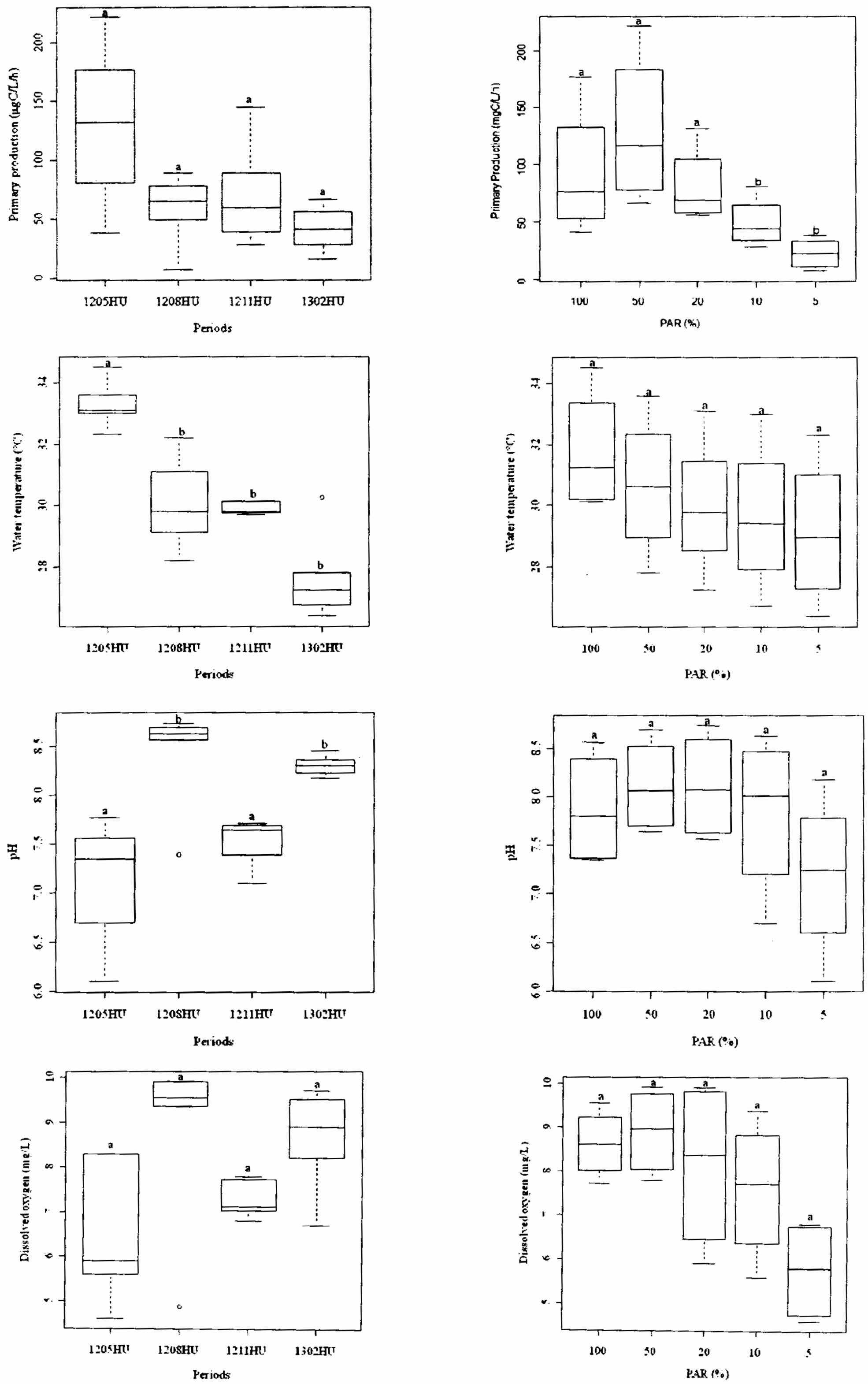


Figure 3.6 Primary production and water quality parameters in water column at Huay Luang reservoir (different letters indicate significant difference at P < 0.05).

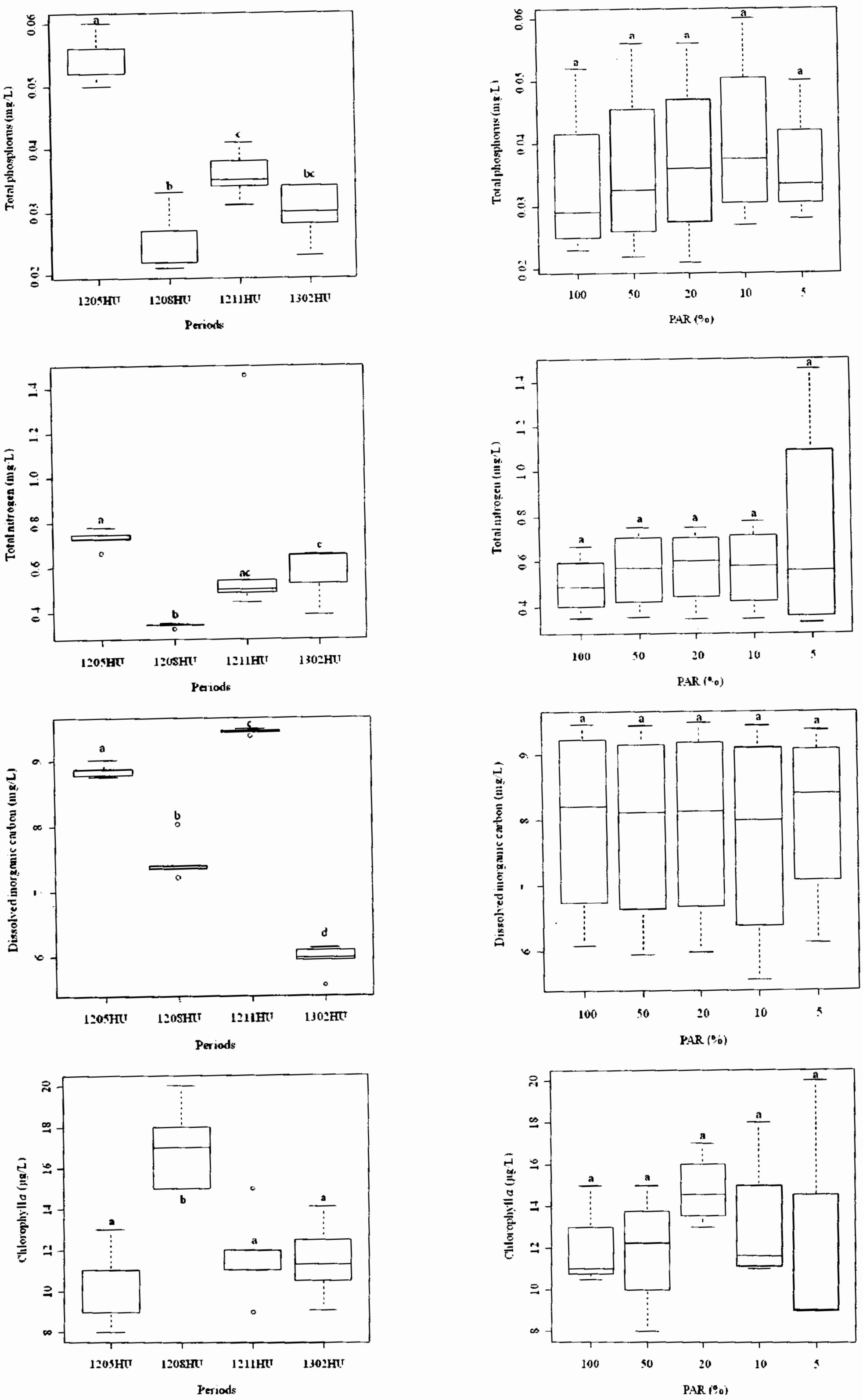


Figure 3.6 Primary production and water quality parameters in water column at Huay Luang reservoir (different letters indicate significant difference at P < 0.05). (Continued)

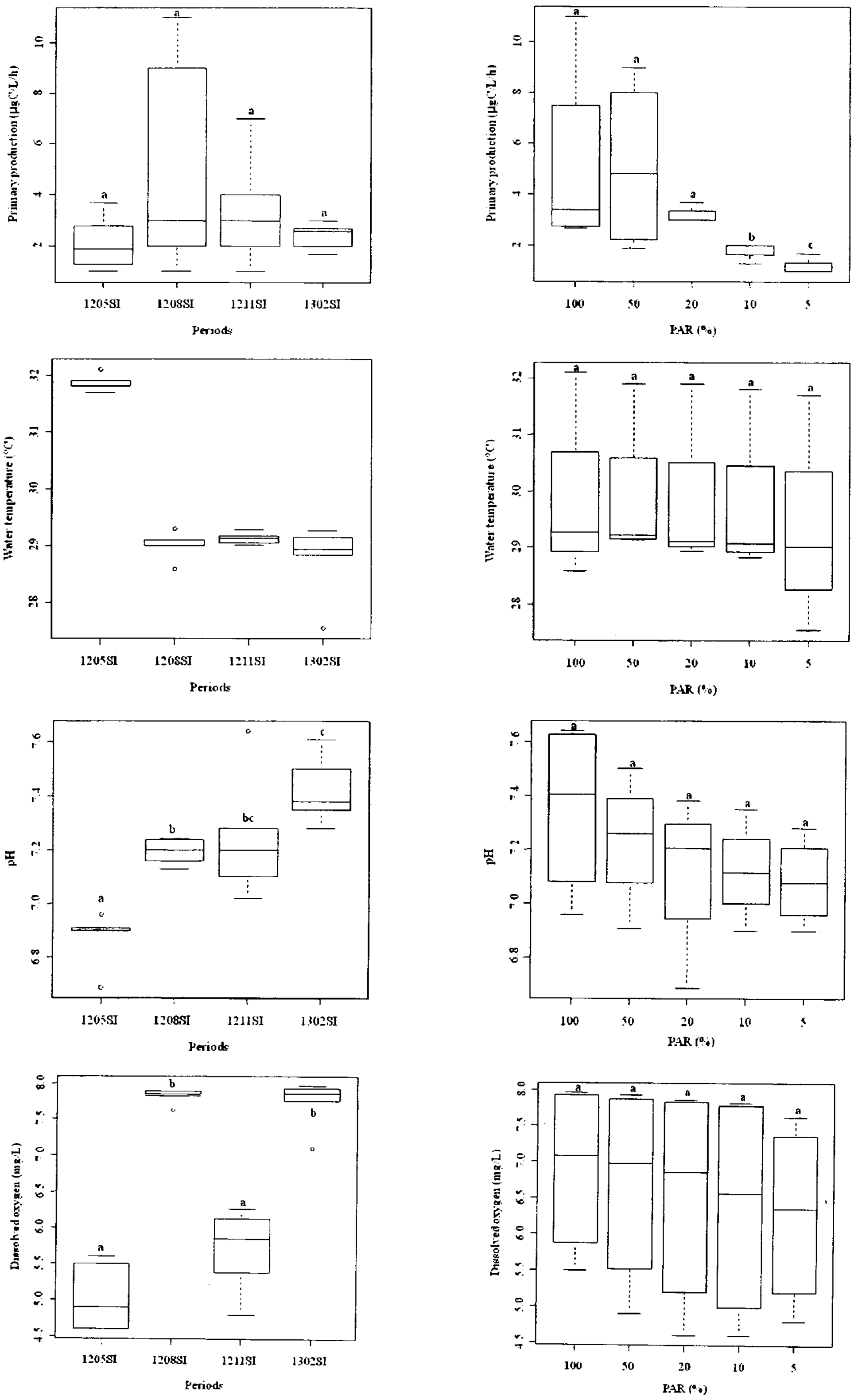


Figure 3.7 Primary production and water quality parameters in water column at Sirindhorn reservoir (different letters indicate significant difference at $P < 0.05$).

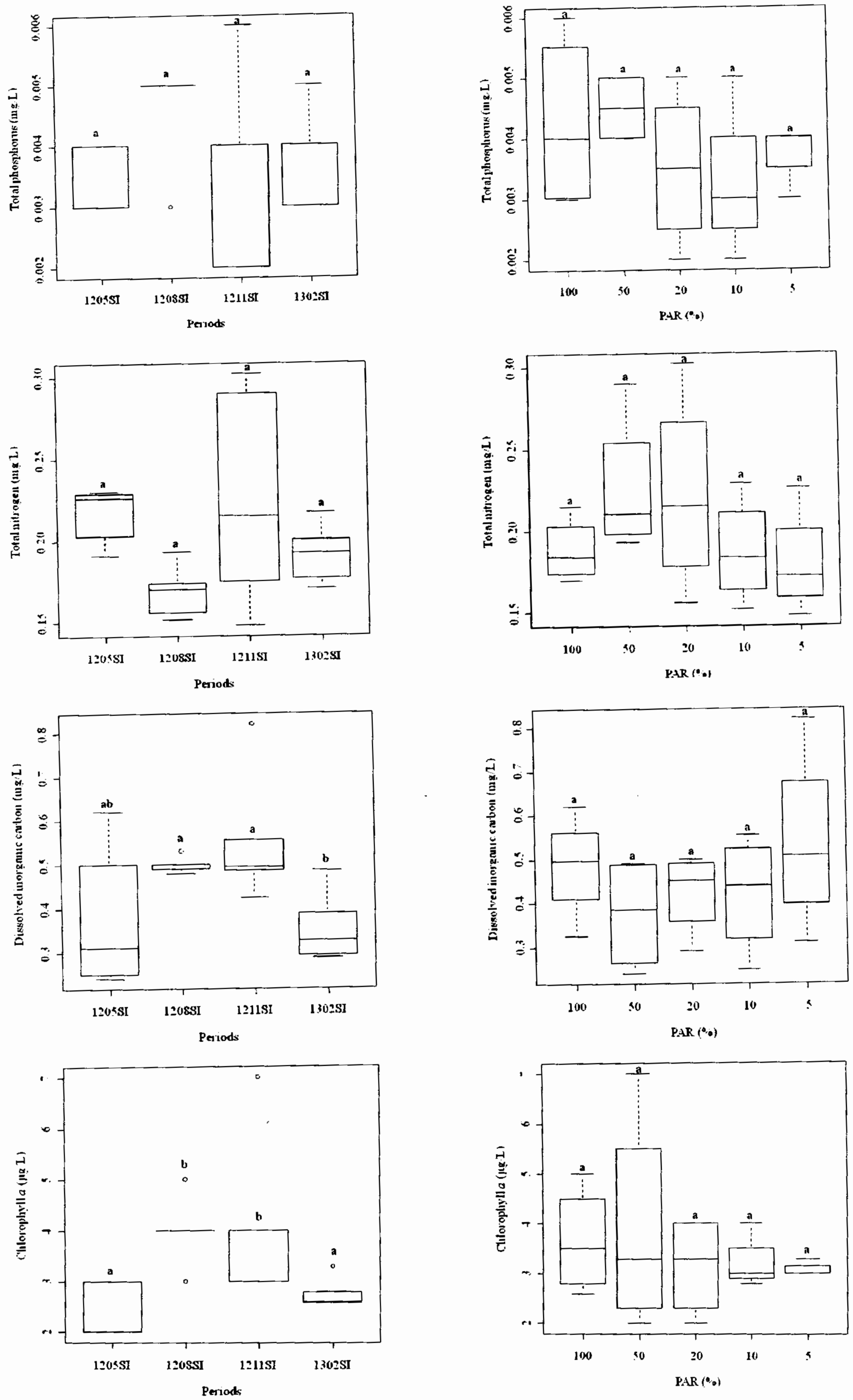


Figure 3.7 Primary production and water quality parameters in water column at Sirindhorn reservoir (different letters indicate significant difference at P < 0.05). (Continued)

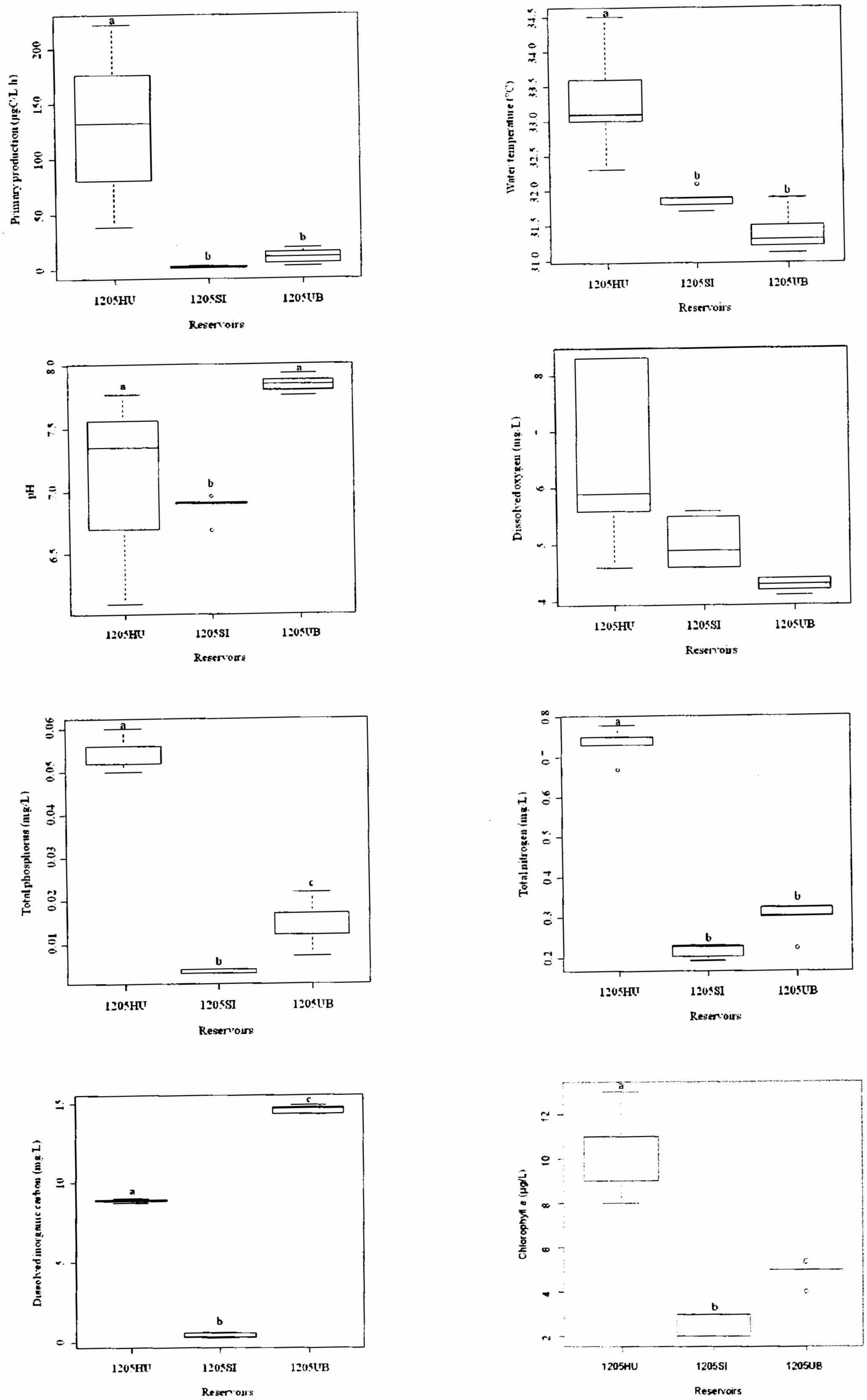


Figure 3.8 Primary production and water quality parameters in water column in May 2012 (different letters indicate significant difference at P < 0.05).

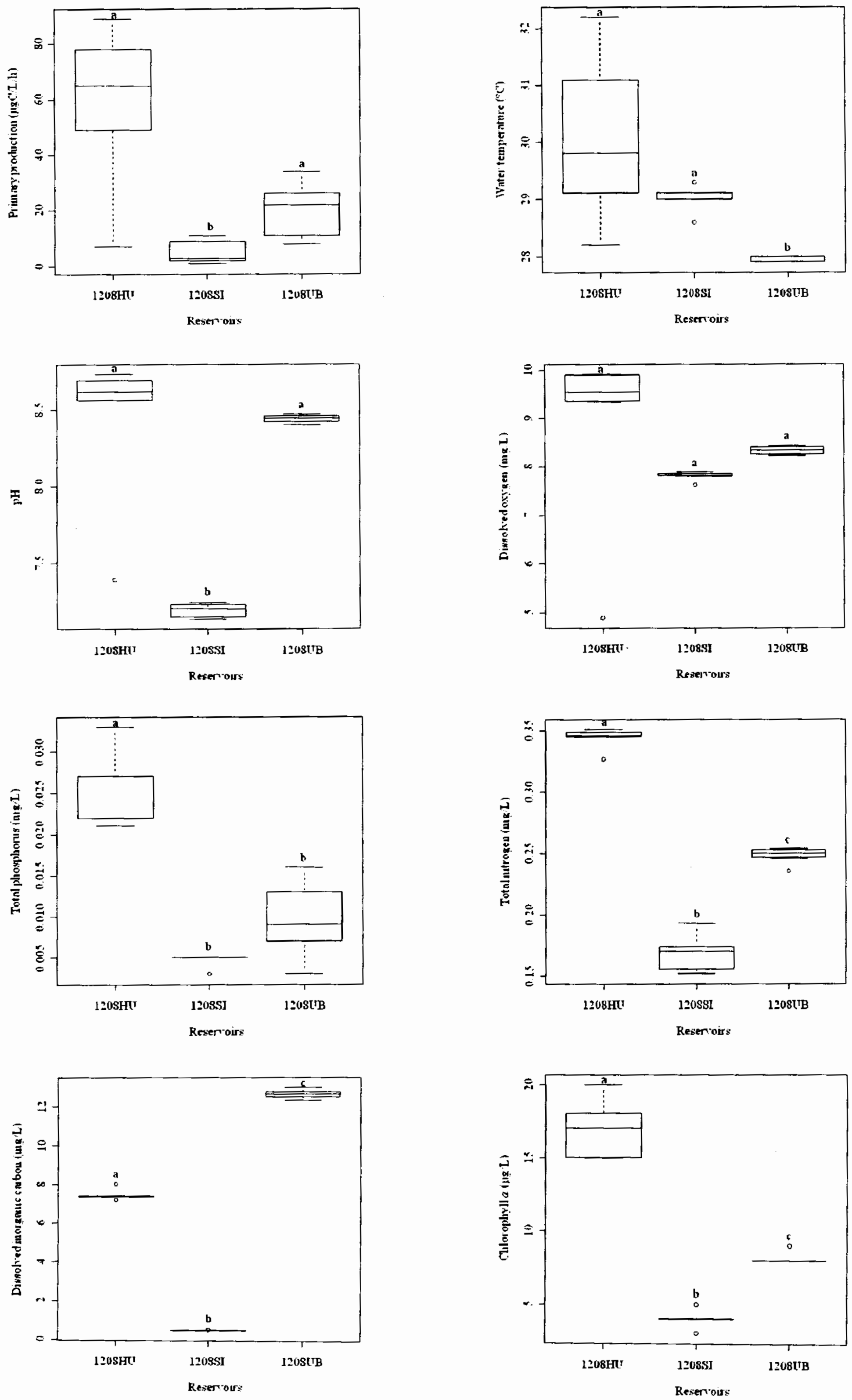


Figure 3.9 Primary production and water quality parameters in water column in August 2012 (different letters indicate significant difference at P < 0.05).

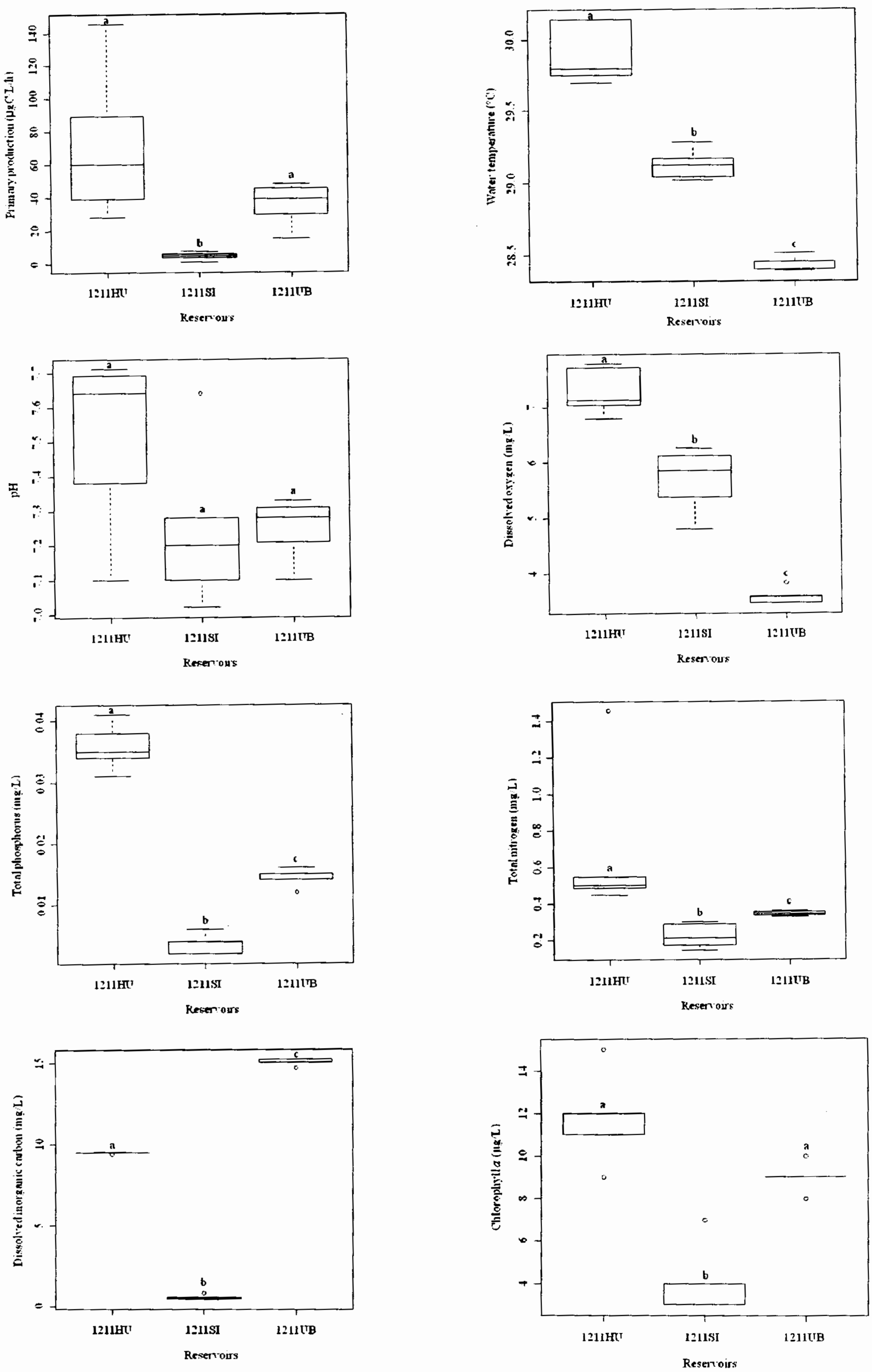


Figure 3.10 Primary production and water quality parameters in water column in November 2012 (different letters indicate significant difference at P < 0.05).

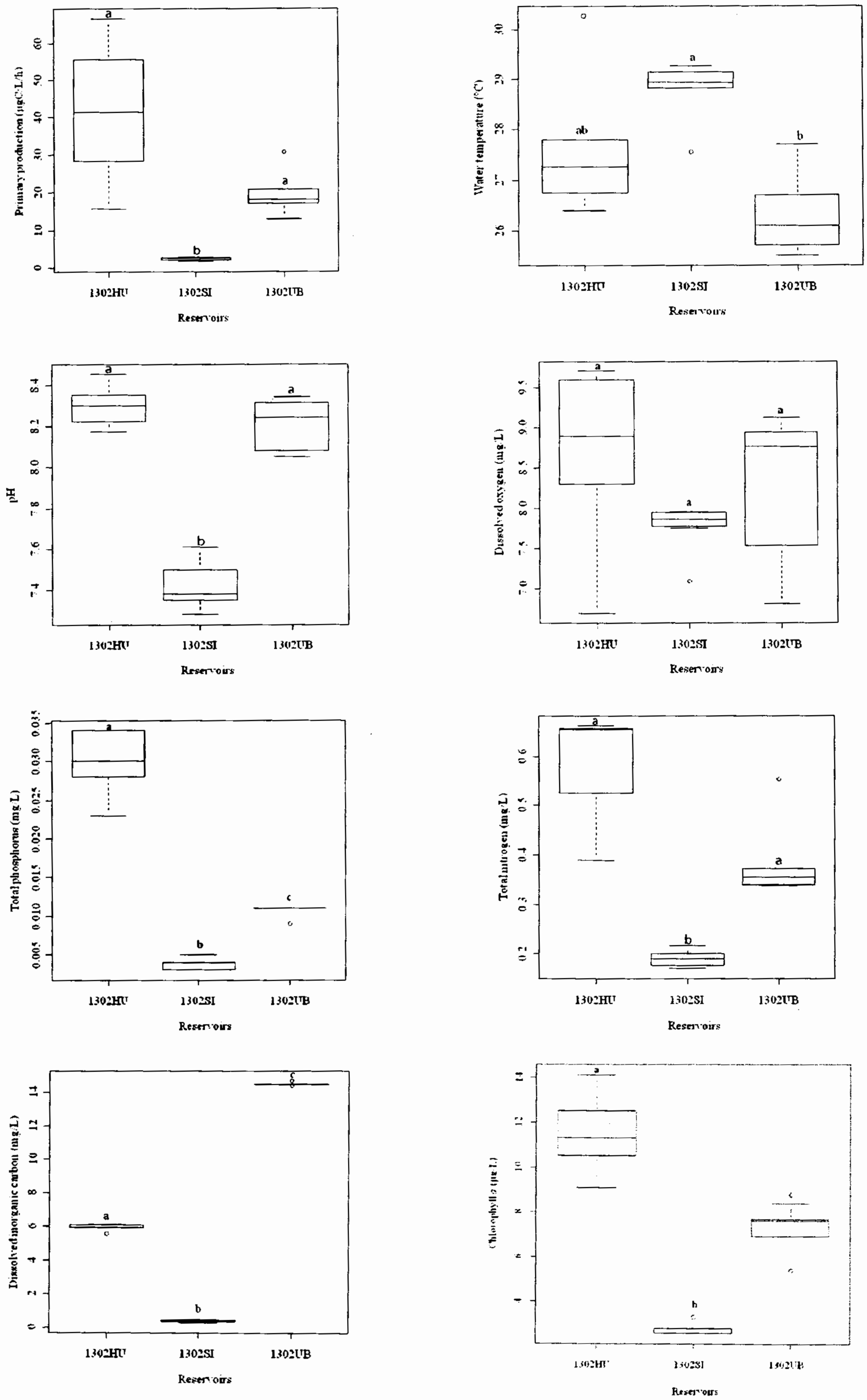


Figure 3.11 Primary production and water quality parameters in water column in February 2013 (different letters indicate significant difference at P < 0.05).

3.5 Conclusion

Although, our study showed that predominant of temperature effected on vertical primary production in three reservoirs by seasonal variation but may seem less likely to suffer negative impacts from increases in global warming. The results also provide useful information for habitat management for protection of the local aquatic ecosystem.

CHAPTER 4

EFFECTS OF WATER TEMPERATURE ON EMBRYONIC DEVELOPMENT, HATCHING SUCCESS AND SURVIVAL OF LARVAE OF SIAMESE MUD CRAP *Henicorhynchus siamensis* (Sauvage 1881)

4.1 Introduction

Although it is too complicated to predict the impact of climate change to fishes, the primary effect of climate change will be through changes in water temperature (Brian and et al., 2011: 80-81). The physiological mechanisms of fishes are directly or indirectly temperature dependent, thus climate changes will affect fish by altering physiological functions, such as growth, metabolism, food consumption, reproduction success and their ability to maintain homeostasis in the face of a variable external environment (Roessig and et al., 2004: 257-260). Moreover, water temperature is the most important environmental factor that affects and governs the development of eggs, incubation time of embryos, hatching rate, survival and growth of fish larvae (Hakim and Gamal, 2009: 80-90; Le and et al., 2011: 241-245; Ahn and et al., 2012: 100-105).

The countries in the Lower Mekong Basin (LMB) are expected to be impacted by climate change with significant changes in rainfall and temperature (Mekong River Commission (MRC), 2009: 2-4). In Thailand, for example, it is estimated that the rainfall will intensity in shorter periods, and temperature will be increase about 2 – 3 °C and be highly variable from season to season (Greenpeace, 2006: 11-15). Air temperatures have risen by 0.5 to 1.5 °C in the past 50 years and continue to rise across the LMB and are likely to shift outside the present comfort zone, which the most suitable condition for species and ecosystem in the basin particularly in the wet season (International Centre for Environmental Management (ICEM), 2013: 7-12).

Continual temperature increases will inevitably impact the fish diversity and fisheries in the LMB, in which about 2 million tones of fishes and other aquatic animals are harvested each year, and eventually to the food security in the LMB (ICEM, 2010: 1-80). There is another report by Lauri and et al. (2012: 4603-4619), which shows the difference in maximum and minimum daily average water temperature in the Mekong River could be more than 1 °C in 2032 onward. This is the cumulative impact of climate change and reservoir operation, i.e. released water from dam.

The Siamese mud carp *Henicorhynchus siamensis* a riverine species in mainland Southeast Asia. The species is a small-size cyprinid, i.e. about 20 – 25 cm TL (total length). It is the most abundant and most economically important fish in the LMB. It is the dominate species in the commercial set-net fisheries in Tonle Sap Lake, Cambodia, and the Khone Falls area in southern Laos (Rainboth 1996: 112; Hai Yen and et al., 2009: 169-174; Fukushima and et al., 2014: 1-3). Not only dose this fish provide protein, but also vitamins and minerals to the people in the LMB (Roos and et al., 2007: 1106-1109). *H. siamensis* also adapts to lentic environmental conditions, such as lakes and reservoirs, and contributes a significant portion in fish catches (Suvarnaraksha and et al., 2011: 995). Therefore, it is one of the most important candidates for a fish stock enhancement program to increase fish production in inland waters (Jutagate, 2009: 104-105).

H. siamensis is a synchronous, i.e. single spawned species that clearly showed a single peak of gonadosomatic index in June to September and highest in August (Suvarnaraksha and et al., 2011: 995-1000). During the wet season, this species migrates into floodplains for spawning (Fukushima and et al., 2014: 1-3). Eggs and larvae grow in the floodplains and the larvae migrate back to rivers when the flood waters begin to recede at the starting of dry season (Rainboth, 1996: 112; Fukushima and et al., 2014: 1-3). Therefore, suitable environmental conditions, during the wet season, are very important to guarantee the survival and recruitment to the fisheries of this species.

Little is known on fish distribution and life history traits in Asia in comparison to fish in temperate zones as well as possible impacts of climate changes on fish (Ficke et al., 2007: 581-583). Besides, it is widely accepted that the impact of climate change is unavoidable and must be serious concern to the developing world, as many of Asian countries, where fish is among the major sources for protein food security (Food and Agriculture Organization of the United Nations (FAO), 2007: 1-16). Regarding on the role of *H. siamensis* as highly valuable fisheries resources throughout the LMB, this study aims to explain the relationship between water temperature and embryonic development as well as the effect of acute changes in water temperature to mortality of the newly hatched *H. siamensis*. The findings will lead to the better understanding on impact of temperature to this species and further support to improve climate risk management.

4.2 Materials and Methods

4.2.1 Broodstock

Broodstock of *H. siamensis* were obtained from Ubon Ratchathani Inland Fisheries Research and Development Center Ubon Ratchathani Province, Thailand, during April to May 2013. The parental fish were reared in a fiber tank (60 liters) for a week before breeding. The average water temperature of both tanks was kept at 30 ± 1 °C. The broodstock was divided into three lots (1 female:2 males per lot) and each lot was kept in separate tank. The average water temperature in each tank was 30 ± 1 °C. The Suprefact was mixed with 10 mg of Motilium and then injected into the females (80- 150 g) and males (50- 100 g) at doses of 10 and 5 $\mu g{\cdot}kg^{-1}$, respectively. The parental fish were spawned after 8 hours of injection.

4.2.2 Experiment and data analysis

Five water temperature treatments, i.e. at 26, 28, 30, 32 and 34 °C, were prepared in aquaria (24 x 40 x 28 cm^3) with a water volume of 25 liters. One thousand eggs were rapidly transferred from the brood stock tanks into each experimental aquarium. The incubated eggs of *H. siamensis* is were examined at 5 temperature levels from 26 °C to 34 °C of the interval by 2 °C in order to observe the effect of water temperature on embryonic development, hatching time and hatching success. The study was conducted in the air conditioning room, where the room and air temperatures were at about 20 and 24 °C, respectively. Then, there were 3 replications for each treatment and the water temperatures were kept constant, at the set temperatures, by using EHEIM JAGER TSRH 300 W. This experiment was conducted for 12 hours, in which all aquaria were aerated and the pH was maintained around 7.

The developmental periods of embryo from fertilization to hatching were divided into seven periods *viz.*, zygote, cleavage, blastula, gastrula, segmentation, pharyngula and hatching (Kimmel and et al., 1995: 253-310). The developmental periods were observed and recorded every 5 minutes in early periods (zygote, cleavage, blastula and gastrula) and then every 30 minutes until hatching. The study on duration for embryonic development was conducted by sub-sampling the water about 0.1 liter from each aquarium and then 20 eggs were taken to determine the periods of development and considered as complete when more than 60% of the sub-samples were reached that period at each time interval. Hatching success (%), i.e. number of hatch by total number eggs, was then calculated. The analysis of variance (ANOVA) was applied to examine the effects of water temperature on hatching success. The Tukey's HSD test at 95% confidence interval was applied when ANOVA revealed significance.

The effect of the acute change in temperature was examined by the experimental design of "Before-After Control-Impact (BACI)".The setting of the experiment was similar to the first trail, i.e. effect of temperature to the embryonic development periods. The temperature were set at 3 levels viz., 30 °C as a control and the impacts were set at 28 °C and 32 °C, which respectively represented temperature at -2 and +2 °C of the control, since the estimated fluctuation daily water temperature in the Mekong could be beyond 1 °C in 2032 onward (Lauri and et al., 2012: 4603-4619). Each temperature was replicated 3 times, in which 100 newly hatched *H. siamensis* were in each replicate, i.e. aquaria (24 x 40 x 28 cm^3). The analysis was done by Randomized Intervention Analysis (RIA), on the difference in cumulative number of dead larvae between the control and each impact. The "before" was the period that the larvae were in control temperature for 20 minutes and then they were transferred, i.e. starting of the intervention of 100 larvae to the designated aquaria. The record was kept at minutes 5, 15, 30 and 60 then afterward every 60 minutes. The test was run by 999 iterations to random permutations of the impact-control data to generate the P-value. The statistical analyses were carried out with R software (R development team, 2014: website).

4.3 Results

The durations for development at each period and hatching varied, depending on water temperature at their incubation (Table 4.1). Development time from zygote to gastrula period was relatively closed at all water temperatures. However, the development of blastula and gastrula periods at 26 °C took longer than the other treatments. The development period from segmentation to hatching, showed the clear fluctuation. It is also worthy to note that the lower the temperature, the longer the time from segmentation period to hatching (Figure. 4.1). Development was stopped at the pharyngula period at 26 °C. Eggs did not hatch at 26 and 34 °C. For the remaining 3 levels, the time consuming from the zygote until hatching, were obviously longer at 28 °C than 30 and 32 °C (Table 4.1). The hatching success was highest at 30 °C (73.90% ±1.44%) but not significant different at 28 °C (73.76% ±2.37%, Figure 4.2). Meanwhile, at high temperature, 32 °C, hatching success decreased (61.42%±11.19%) and was significantly different to the remaining two temperature levels. Dead larvae were observed after the intervention both in the control- and impact- manipulations. The average numbers of dead larvae at control and 28 °C were relatively low, i.e. less than 10 individuals. However, the acute change in water temperature from 30 to 32 °C yielded high numbers of dead larvae, which was up to 30 larvae after 9 hours. The RIA results indicated the non-significant difference in average number of dead larvae between the control and 28 °C (P-value = 0.30; Figure 4.3) but the difference between the control and 32 °C were significantly different (P-value < 0.01; Figure 4.4).

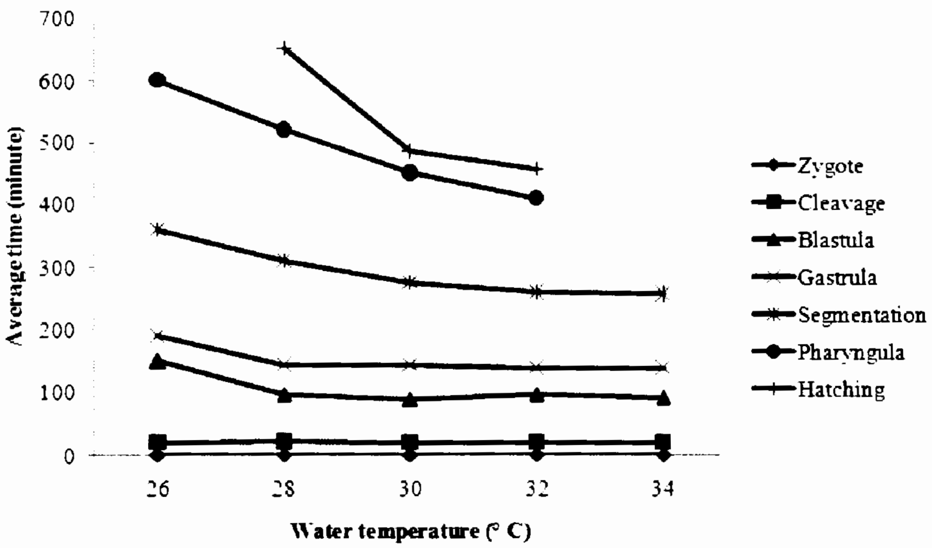


Figure 4.1 The durations of *H. siamensis* embryonic development at each studied temperature.

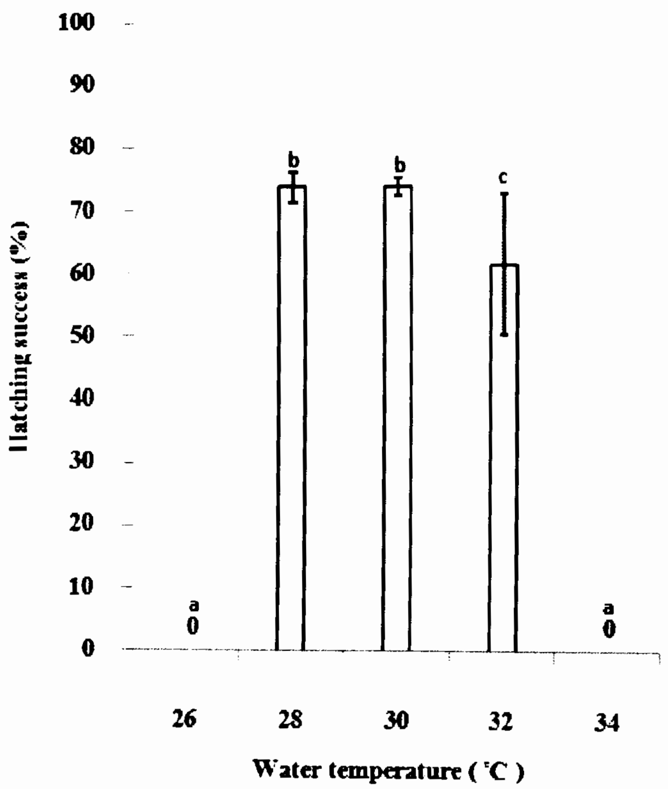
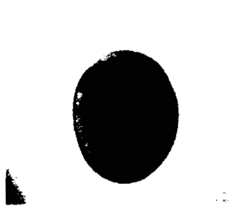
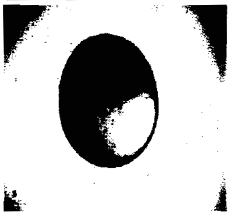
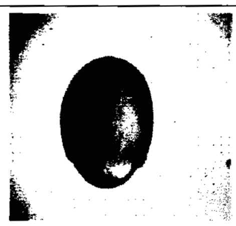
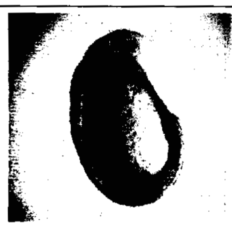


Figure 4.2 Hatching success of *H. siamensis* at each studied temperature. (The different letters, above each bar, indicate statistical different at α = 0.05).

4.4 Discussion

Changes in water temperature produced a strong effect on embryo development and hatching of *H. siamensis*. Temperatures, which are beyond the tolerance range, results in unsuccessful development and hatching failure (Kucharczyk and et al., 1997: 214-224; Kupren et al., 2011 70-80; Ahn and et al., 2012: 100-105). In this study, water temperatures at 24 and 34 °C are considered as the respective "minimum limiting"- and "maximum limiting"- temperatures (Cruz et al., 2002: 91-82) for the egg development of *H. siamensis*. In general, the lower temperature causes unsuccessful egg development and the high temperature causes mortality of the embryo (Kupren et al., 2011: 70-80; Ahn and et al., 2012: 100-105). The maximum limiting temperature is the main concern because water temperatures in Thai inland waterways can reach 34 °C during the rainy season (Choo-In and et al., 2013: 1794-1797), i.e. the spawning season for *H. siamensis*. Also increasing temperatures are expected on both daily maximum temperature and daily minimum temperature in the LMB, where the daily maximum temperature could be beyond 35 °C (Helsinki University of Technology (TKK) and Southeast Asia START Regional Center, 2009: 1-75). The minimum limiting temperature may also be of concern, when cold water from dams is released (Lugg and Copeland, 2014: 71-79), if this phenomenon overlaps into the spawning season.

Table 4.1 Average time (minutes) of embryonic development at five water temperature treatments

Period	Temperature (°C)	Average time*(minutes)	Reference photographs	Description
Zygote	26	0.0		The chorion swells and cytoplasm streams toward animal pole to form the blastodisc.
	28	0.0		
	30	0.0		
	32	0.0		
	34	0.0		
Cleavage	26	19.8		Blastodisc is vertically cleavaged from 1 to 64 blastomeres.
	28	21.0		
	30	19.8		
	32	19.8		
	34	19.8		
Blastula	26	150.0		Blastoderm covers about 30 % of egg and blastocoel is presented.
	28	96.6		
	30	90.0		
	32	96.6		
	34	91.8		
Gastrula	26	189.6		Germ ring is visible and covers 90 % of egg.
	28	144.6		
	30	144.6		
	32	139.8		
	34	139.6		
Segmentation	26	360.6		Fisrt somite expands to 26 somites and eyes vesicle, optic veasicle and tail bud are visible.
	28	309.6		
	30	274.8		
	32	259.8		
	34	256.8		
Pharyngula	26	600.0		Body is straight and well developed of somitesto the end of tail. Binary symmetry and pigment are visible.
	28	519.6		
	30	450.0		
	32	408.6		
	34	No hatching		
Hatching	26	No hatching		Basical organs are well developed, especially pectoral fin, jaws and gills and then hatching.
	28	652.2		
	30	484.8		
	32	457.2		
	34	No hatching		

Although there were non-significant differences in embryonic development time at each stage, there was a trend towards a decrease of development time when the incubation temperature increased for *H. siamensis*. This implied that a lower temperature retards the rate of embryonic development, and a higher water temperature accelerates the larval development, which conforms to the negative second order polynomial trend-line for the temperate- and most of marine fishes (Chambers and Tripple, 1997: 599; Kawahara and et al., 1997: 239-250; Yang and Chen, 2005: 173-179; Kupren et al., 2011: 70-80). The reason for faster hatching of embryo at high water temperature is because of the increase of mobility in warmer water and early excretion of the hatching enzyme (Kupren et al., 2011: 70-80).

The optimum temperature, which is suitable for fish embryo development and success in hatching as well as their survival, is usually correlated with spawning temperature (Kucharczyk and et al., 1997: 214-224; Ahn and et al., 2012: 100-105). The temperature experienced by the parents may influence the optimal temperature of egg incubation (Bermudes and Ritar, 1999: 245-255). In this experiment, the treatment at 30 °C, i.e. similar to the spawning aquaria, yielded the highest hatching success. Meanwhile, the water temperatures in the treatments that prominently deviated from average spawning aquaria at±4 °C, i.e. 24 and 34 °C, damaged *H. siamensis* eggs resulting in hatching failure. Notwithstanding this fact for *H. siamensis*, Landsman et al. (2011: 1200-1212) reported that deviated temperature of ± 2 °C during the egg stage for Atlantic cod can generate huge variations in recruitment, i.e. can be up to 1000-fold. This probably results from elevated metabolic rate, causing accelerated consumption of energy store or from thermal degradation of proteins that impart a direct effect on cell function such as substrate binding activity and stress protein synthesis (Landsman et al., 2011: 1200-1212).

Acute increase of water temperature causes significant decrease in survival of newly-hatched larvae rather than acute decrease of water temperature (Landsman et al., 2011: 1200-1212; Lahnsteiner et al., 2012: 977-986). In general, deviations in metabolic rate could be observed in fishes during the acute change in temperature, in which cold-acclimated individuals typically show a large increase in metabolic rate when exposed to warm temperatures, while warm-acclimated fish often show a considerable decrease when acutely exposed to colder temperatures (Killen and et al., 2007: 431-438), in which daily development and mortality rates trend to increase with acute increasing temperature (Pepin, 1991:503-518).

4.5 Conclusion

Although it is believed that tropical fishes have evolved to survive in very warm water and may seem less likely to suffer negative impacts from increases in global temperature (Ficke et al., 2007: 581-613), our results showed that the prominent deviation in water temperature affect embryonic developments and hatching of *H. siamensis*. The results also provide useful information for habitat management for protection of the local aquatic ecology and as well to aquaculture system. Further studies are needed to understand such impact is on the abnormality of the larvae and their growth performances.

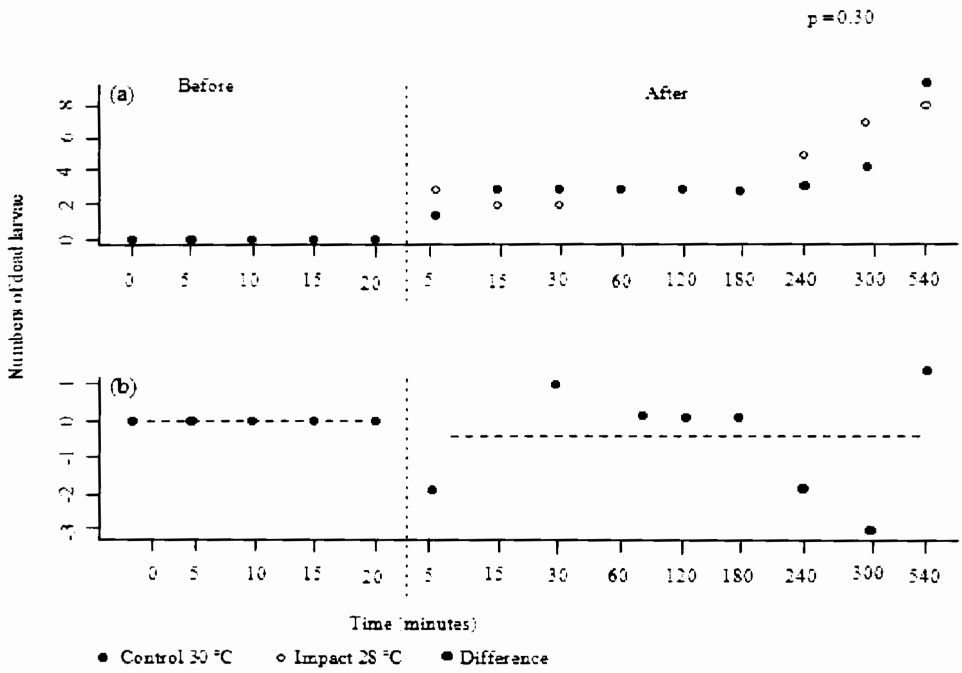


Figure 4.3 Numbers of death *H. siamensis* larvae of the control (30 °C) and impact (28 °C). Graph (a) depicts the raw data used for RIA and graph (b) shows the differences between control and impact.

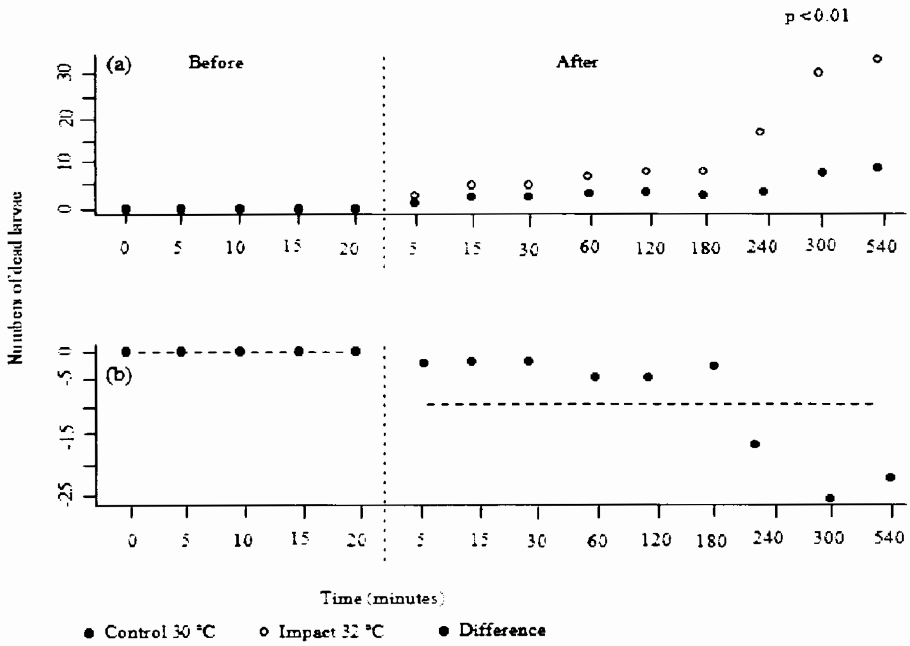


Figure 4.4 Numbers of death *H. siamensis* larvae of the control (30 °C) and impact (32 °C). Graph (a) depicts the raw data used for RIA and graph (b) shows the differences between control and impact.

CHAPTER 5
GENERAL CONCLUSION

Climate change have been effected in the past decades, especially the 21st century that air temperature has increasing from 1.8 to 4.0 °C, while an increase in global temperature is cause of precipitation which will significantly affect the hydrological regimes of many river systems.(Shrestha and et al., 2013: 1). In Southeast Asia which will be effect from climate change that are indicated by increasing trend in mean of surface air temperature during the past several decades about 0.1-0.3 °C, rainfall trending down, sea levels up and extreme weather events such as heavy precipitation, heat waves. Those have led to massive flood, landslide and droughts (Beilfuss and Triet, 2014: 7-8). Meanwhile, Keskinen and et al. (2010: 103) reported that climate change can directly affect the hydrologic cycle and through the quantity and quality of water resource, as its will direct impact on water availability for flora and fauna.

The Mekong River Basin is affected by climate change such as increasing of mean temperature and annual rainfall as well as the season will shift and change pattern that may also lead to the implication with water quality in term of suspended solids and flush out the pollution, especially in longer dry season (Shrestha and et al., 2013: 1-2). The Low Mekong River Basin is tropical climate that high heat and humidity as minimum average monthly temperature not less than 20 °C (Mekong River Commission (MRC), 2010: 14). Change in temperature and precipitation patters that including the volume, timing and intensity of rainfall as those events may affect on timing, magnitude, duration of river flows, the frequency of droughts and floods and rate of evaporation. Flood frequency is affected by changes precipitation in year-to-year and by change in short term rainfall, while frequency of drought is affected primary by changes in the seasonal distribution of precipitation (Beilfuss and Triet, 2014: 17-18).

In this study, Chapter 2: The water qualities in Chao Phraya River were divided four clusters that mostly poor water quality was closely community, especially lower portion of Chao Phraya River. The distinct spatial differences in water quality along the Chao Phraya River were clearly identified and highly related to anthropogenic stresses. Meanwhile, there was no clear temporal trend for most of surface water stations, either positive or negative and results were still insufficient to provide the detail in terms of inter-annual variation in water quality of the river.

Chapter 3: seasonal variation may be affected on primary production in reservoirs. Meanwhile, many study primary production in reservoir are indicate main effect on primary production from nutrient load that come from terrestrial and fluctuation of water level in reservoirs. In future study should be collected the nutrient load from terrestrial with soil around reservoir and variation of the pattern of rainfall on primary production and river water quality.

Chapter 4: the water temperature have strong affected on egg and newly hatched larvae of *H. siamensis*. The optimum water temperature is usually correlated with spawning temperature. Meanwhile, water temperature fluctuation from optimum water temperature must be effect on egg development and newly hatched larvae that can imply to abnormal pattern season may be effect on aquatic fish.

This study may show the trend of water temperature on fish biology and aquatic habitat, especially egg development, newly hatched larvae. Meanwhile, aquatic habitat may be affected by seasonal variation in term of runoff. In addition, the human activity will accelerate the accumulative impact of extreme climate that should be taking under consideration.

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APPENDIX

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Spatio-Temporal Variations in Water Quality of the Chao Phraya River, Thailand, between 1991 and 2008

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ABSTRACT

Spatio-temporal variations in the water quality of the Chao Phraya River, Thailand, were examined, on average-yearly basis, between 1999 and 2008, from 32 surface water stations from the river origin to the delta. Five water quality parameters *viz.*, DO, BOD, TCB, FCB, NH3-N and water temperature were used in the analysis. Analysis was performed by using the Self Organizing Maps. Four distinct spatially approached clusters were classified, according to the similarity of water quality parameters, while temporal variations of most of the surface water stations were not obviously observed. The worst water quality condition was at the stations near the river delta and highly related to anthropogenic stresses. Result from the correspondence analysis showed that, except for the cluster of the worst water quality, the stations of the remaining three clusters were overlapped. There was no statistical difference in water temperature among clusters but the expected effects from climate change should be a precautionary focus since the will eventually affect the water quality.

Keywords: Surface Water; Self Organizing Maps; Anthropogenic Stresses; Temperature

1. Introduction

The livelihoods of riparian people depend to some extent on the goods and services of water resources and water quality is among the key factors affecting the environmental health of the river system [1]. Meanwhile, anthropogenic stresses as well as changes in environment during recent decades are the key issues that severely degrade the water quality in river systems elsewhere [2,3]. However, recovery of the river system from eutrophication and poor water quality conditions is feasible through rigid control on the pollution sources [1,2]. Establishment of a monitoring program on water quality is, therefore, highlighted for the purpose of determining the state of pollution in any particular site in the rivers [1,2,4]. The general trends of either decreasing or increasing water quality at any monitored site indicate which areas are stepped to a good, moderate or vulnerable condition [2, 3,5].

Besides the anthropogenic stressors, the condition of water quality in the river is related to hydrological properties [4,6]. The global warming influences on higher air and water temperatures and changes in the timing, intensity and duration of precipitation affect the hydrological characteristics of the river, especially in terms of flow and flood, which eventually affect the water temperature and water quality of the river [6], for example the higher temperature reduces dissolved oxygen and extends thermal stratification, which increases the potential for anoxia. Increases in frequency and intensity of rainfall during the rainy season will produce more turbid conditions, meanwhile drought during the dry season would allow saline water to intrude into the river [7]. Moreover, changes in stream water quality, in terms of eutrophication and nutrient transport, are very dependent on changes in stream flow [8] and a long drought period has a noticeable effect on water quality, e.g. temperature, DO, BOD, NH_4^+, and chloride concentration [8,9].

The Chao Phraya Basin, the largest river basin in central Thailand, covers an area of approximately 160,000 km^2 representing 30 percent of the country's total area and is home to 40% of the country's population [10]. The Chao Phraya Basin is divided into 2 parts (**Figure 1**). The upper par, is mountainous alternating with lowland areas along the river, while the Lower Chao Phraya Basin is a vast floodplain area and covers 55,290 km^2, *i.e.* 35% the total basin area [10]. The Chao Phraya River *per se* begins at the junction of four major rivers in the Upper basin, in Nakhon Sawan Province. The total length of river is about 380 km and drains into the Gulf of Thailand. It supplies water and supports many activities such

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as municipal uses, agriculture, fisheries, light and heavy industries, recreation and navigation [10,11]. The river, therefore, receives the discharge of wastewater from many sources, especially those from household, industrial and agricultural activities, and makes the water polluted. The major sources of pollutants in the upper portion were from agricultural waste and form communities and industries in the lower part of the river course [12]. Due to the importance of the river and the significant number of stressors along it, this paper aims to investigate the water quality as well as water temperature along the Chao Phraya River in terms of spatio-temporal approach by using multivariate techniques.

2. Materials and Methods

2.1. Source of the Data

The data is from the Pollution Control Department of Thailand (PCD, available at www.pcd.go.th). There are 32 surface water stations, *i.e.* CH1-CH32 (**Figure 1**) along the Chao Phraya river course. We used the time series data of water quality between 1999 and 2008, *i.e.* 10 years. The water quality parameters used in the analysis were DO, BOD, TCB, FCB, NH_3-N and water temperature. The reason why these parameters were selected is because they are input parameters for water quality index (WQI), developed by PCD, which attempts to provide a simple and understandable tool to evaluate the quality of any given water bodies [13].

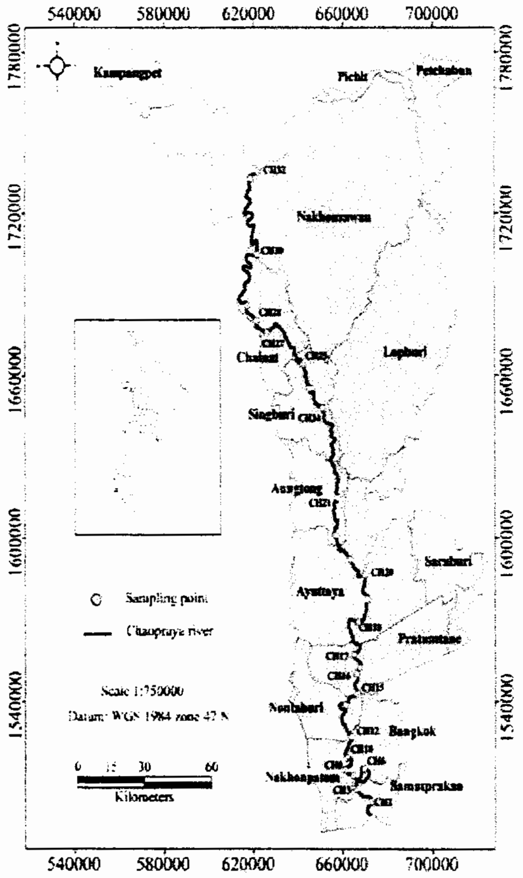


Figure 1. Location and map of the Chao Phraya River with the surface water stations.

2.2. Data Analyses

To perform statistical analysis, the annual average of each selected variable was used. The dataset was performed as rows of the observation (*i.e.*, surface water station × year, for example, CH7_03 is the observation from station 7 in year 2003) and columns of water quality parameters (*i.e.*, 6 variables). There were 327 observations in total for the analysis, after data cleaning by selecting the observations that had the data for all parameters. The state-of-the art multivariate analysis, Self Organizing Maps (SOM), was employed to evaluate the spatio-temporal variations of the data matrix. A SOM is an unsupervised algorithm of an artificial neural network model (ANN), proposed by Prof. T. Kohonen of Helsinki University during 1980s [14]. The basic idea of SOM is to display a high-dimensional signal manifold onto a much lower dimensional network in an orderly fashion [5]. Since 2000, SOM has been widely applied for solving problems is capability of clustering and classification in the studies of water resources and aquatic ecology [15, 16].

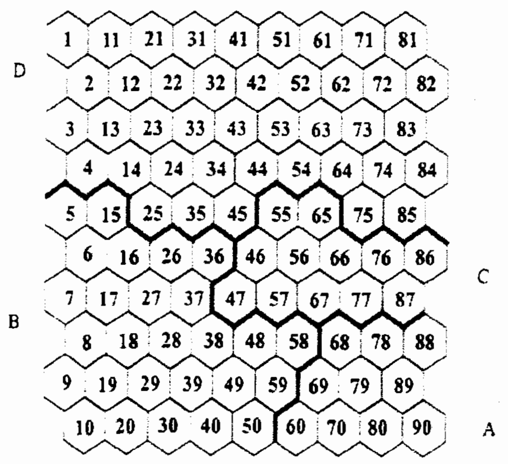
The SOM consists of two layers *viz.* the input and output layers, which are connected with the weight vectors, and the output layer is displayed as a hexagonal lattice [5, 15,16]. The principle of SOM analysis is to classify the sample vectors (SVs), described by a set of descriptors on the map according to the similarities between the descriptors (*i.e.* water quality parameters). During the learning process, two SVs that are similar (from the descriptor point of view) are classified in the same or neighboring cells, whereas two different SVs are classified in separated cells that could be distant from each other and *vice versa* [2,16]. The sequential algorithm used and the protocol for SOMs are widely described [14-16]. In this study, the input layer comprised 6 neuron connected to 327 observations (327 SVs). The output layer comprised 90 neurons organized in an array with 10 rows and 9 columns. This number of neurons was defined according to the formula $C = 5 \times \sqrt{n}$ proposed by the laboratory of Computer and Information Science (CIS), Helsinki University, where C is the number of cells and n is the number of sample (*i.e.* observation) vectors [2,16]. This SOM map size was chosen because of its minimal topographic and quantization error as well as clear classification [14-16]. Meanwhile, the hierarchical cluster analysis (Ward linkage, Euclidean distance), was applied to help in the decision of making the clusters [2,16].

The analysis of similarity (ANOSIM) was used to test for significant difference among clusters by using occurrence probability, which is approximately estimated from the connection intensity of the SOM during the learning process. Statistical difference of each individual parameter was tested by means of analysis of variance (ANOVA) and Duncan's post-test for multiple comparisons. The correspondence analysis (CoA), the best method to examine the interrelationship between environmental variables and sampling units, was used to determine that interrelationship between the clusters and water quality parameters [17]. The significance of the results was tested by a Monte-Carlo method with 1000 random permutations. The SOM was simulated and the cluster analysis was performed by MATLAB®, by using SOM-toolbox, which is developed by CIS [2]. Other statistical analyses were performed by Program R [18].

3. Results

The observations were classified on the SOM-map according to similarity of water quality parameters presented in each observation and thus, similar observations were mapped close together and the dissimilar were mapped apart. It can be seen that the distribution of the observations on the map were scattered but visibly clustered according to the the hierarchical cluster analysis with Ward linkage method (**Figure 2(a)**). The outcomes of SOM were partitioned into four clusters (**Figure 2(b)**). From the clustering, it was obviously seen that clusters B and C were closed, meanwhile cluster A and D were clearly separated. The ANOSIM testing showed significant differences between clusters, *i.e.* similarity within clusters are more similar than between clusters ($R = 0.41$, $P < 0.001$, based on 1000 permutations).

It is found that the four clusters were clearly separated from each other as spatially approached, *i.e.* sectioned along the longitudinal gradient (**Table 1**). Ranges and average (±sd) of each water quality parameter in each cluster are presented in **Table 2** and it is observed that, except for temperature, other water quality parameters in each cluster are statistically different. Cluster A belonged to the lower portion of the River connected to the river delta and contained almost all observations of stations CH1 to CH10 of the whole period of the dataset. The cluster was characterized by the significantly lowest DO and highest in BOD and NH_3-N ($P < 0.05$) compared to the other clusters. Observations belonging to Cluster B, were spatially ubiquitous and showed a complex pattern along the whole river-course and also varied temporally. This cluster was highlighted by significantly high values of TCB and FCB ($P < 0.05$). Cluster C included most of the observations of the stations located in the upstream parts of the lower portion of Chao Phraya River, *i.e.* stations in CH12-CH18. The water quality condition in this cluster was slightly better than cluster A, as judged by the levels of DO, BOD and NH_3-N but still worse than the other remaining 2 clusters. However, the TCB and FCB levels in this cluster were lower than cluster B. Cluster D was mostly the observations of the surface water stations from station 16 and further north up to the source of the Chao Phraya River, *i.e.* station CH32. This cluster can be regarded as having the best water quality condition, when compared to the other clusters, with the highest level of DO and the lowest levels of BOD, TCB, FCB and NH_3-N. In summary, it can be said that the observations in cluster A showed the worst in water quality condition and then the improving conditions were shown in the clusters B, C and D, respectively.



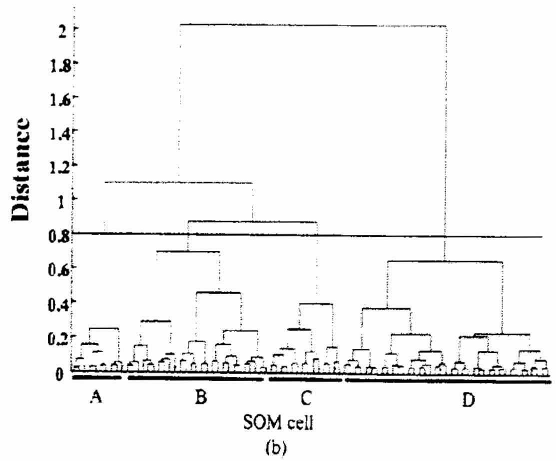


Figure 2. Results of SOM analysis. (a) Classification of observations on SOM using water quality data (see also Table 1) and (b) Dendrogram of the SOM output, showing groups of similarity of cells on SOM.

Table 1. Observations (station × year), belonged to each cluster, after SOM analysis.

Cluster	Station (year)
	CH1 (1991, 1993, 1994, 1995, 1999, 2002, 2005, 2008), CH2 (1997)
	CH3 (1991, 1993, 1995, 1997, 1998, 1999, 2000, 2002, 2003, 2005, 2008)
A	CH4 (1997), CH6 (1995, 1997, 1998, 1999, 2000, 2002, 2003, 2004, 2005, 2006, 2007, 2008)
	CH8 (1991, 1993, 1995, 1997, 1998, 1999, 2005, 2008), CH9 (1996)
	CH10 (1991, 1995, 1997, 1998, 1999, 2005), CH12 (2005)
	CH1 (1996, 2001, 2003, 2004, 2006, 2007), CH2 (1994, 1996), CH3 (1994, 1996, 2001, 2004, 2006, 2007)
	CH4 (1994, 1996), CH5 (1994), CH6 (1994, 1996, 2001), CH7 (1994)
	CH8 (1994, 1996, 2000, 2001, 2002, 2003, 2004, 2006, 2007), CH9 (1994)
	CH10 (1993, 1994, 1996, 2000, 2001, 2002, 2003, 2004, 2006, 2007, 2008), CH11 (1994, 1997)
B	CH12 (1994, 1995, 1996, 1997, 1999, 2002, 2004, 2006, 2007, 2008), CH13 (1994, 1995, 1996, 1997)
	CH15 (1993, 1994, 1999, 2001, 2007), CH16 (1994), CH17 (1994, 1995), CH18 (1994, 1995, 1996, 1997)
	CH19 (1995, 1996, 1997), CH20 (1993, 1995, 1996, 1997), CH21 (1994, 1995, 1996, 1997, 2001)
	CH24 (1996, 1997, 2004), CH25 (1996, 1997), CH28 (1993, 1996, 1997), CH29 (1997)
	CH30 (1993, 1995, 1996, 1997), CH31 (1996), CH32 (1995, 1996, 1997, 2000)
	CH1 (1997, 1998, 2000), CH2 (1995), CH12 (1991, 1998, 2000, 2003)
C	CH15 (1995, 1997, 2000, 2002, 2004, 2005, 2006, 2008), CH16 (1995, 2000, 2006), CH17 (1997, 2008)
	CH18 (2000, 2002), CH20 (2007), CH21 (2008)
	CH12 (2001), CH15 (1991, 1998, 2003)
	CH16 (1991, 1996, 1997, 1998, 1999, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008)
	CH17 (1991, 1993, 1996, 1998, 1999, 2000 ,2001, 2003, 2004, 2005, 2006, 2007)
	CH18 (1991, 1993, 1998, 1999, 2001, 2003, 2004, 2005, 2006, 2007, 2008)
	CH19 (1994), CH20 (1991, .994, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2008)
D	CH21 (1991, 1993, 1998, 1999, 2000, 2002, 2003, 2004, 2005, 2006, 2007, 2008), CH22 (1995, 1997)
	CH23 (1997), CH24 (1991, 1993, 1995, 1998, 1999, 2000, 2001, 2002, 2003, 2005, 2006, 2007, 2008)
	CH25 (1993, 1995, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008), CH26 (1995, 1997)
	CH27 (1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008)
	CH28 (1991, 1995, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008), CH29 (1994, 1995)
	CH30 (1991, 1994, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008)
	CH31 (1994, 2006, 2007, 2008) CH32 (1993, 1994, 1998, 1999, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008)

Table 2. Mean value (±SD) of the six selected water quality parameters in each cluster.

Cluster	Temperature (C)	DO (mg/l)	BOD (mg/l)	TCB (MPN/100ml)	FCB (MPN/100ml)	NH_3-N (mg/l)
A	29.7 ± 0.6^a	1.2 ± 0.6^a	4.3 ± 1.5^a	$317,734 \pm 749,693^c$	$59,502 \pm 70,292^a$	2.3 ± 2.9^a
B	29.3 ± 0.9^a	3.6 ± 1.9^b	2.1 ± 0.9^b	$701,740 \pm 1642,924^c$	$165,751 \pm 409,245^b$	0.3 ± 0.4^c
C	30.1 ± 0.9^a	2.9 ± 1.2^b	1.9 ± 0.9^b	$35,789 \pm 37,540^c$	$8761 \pm 10,173^c$	1.4 ± 3.7^b
D	29.6 ± 0.8^a	5.5 ± 1.1^c	1.2 ± 0.5^c	$21,757 \pm 33,296^d$	3841 ± 5906^d	0.1 ± 0.8^d

Note: Superscripts in each column indicate significant differences between clusters (Duncan's post-test, $P < 0.05$).

In terms of temporal approach, some particular stations showed a trend of better conditions, such as station CH30 (**Figure 3(a)**), or *vice versa*, such as station CH15 (**Figure 3(b)**). However, most of the surface water stations were stuck to one particular cluster, *i.e.* no trend to better or worse in water quality of the station, during the whole monitoring period (**Table 1**). The Monte Carlo test revealed significant relations among the water quality parameters (Monte-Carlo testing, 1000 permutations, $P < 0.001$), when applying CoA. The ordination by CoA showed that only the observations belonging to cluster A were obviously separated, while the observations of the remaining three clusters were overlapped to some degree (**Figure 4**). This implies that the water quality of the "very poor condition" surface stations was highly separated from the others. The "short-arm" length of temperature compared to the other water quality parameters, implies low fluctuations in annual average water temperature during the study period. Cluster A showed the most fluctuation in terms of average temperature, however the differences were less than ±2˚C in each cluster during the whole study period (**Figure 5**).

4. Discussion

Different rates of anthropogenic stress along the river course result in different water quality conditions in any particular site and period. The results of this study reveal a clear spatial variation in water quality along the Chao Phraya River. The analyses were conducted by means of multivariate procedures, which have been proved to be more suitable for the complicated nature of pollution-induced ecological disturbances [2,16,17,19] and they have been successfully applied in environmental quality assessment and management [15,16,19]. Moreover, applying the univariate approach (e.g. WQI) for water quality monitoring always have low interpreting value for decision makers [5,19], because they want to know which water quality parameter should be of the most concern in any particular site.

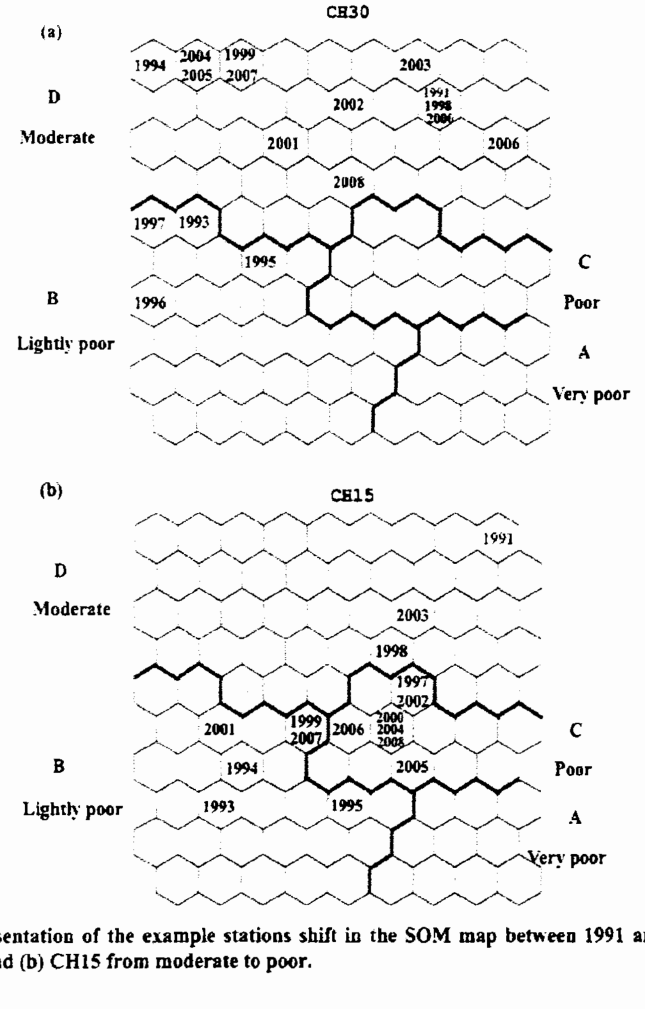


Figure 3. Temporal representation of the example stations shift in the SOM map between 1991 and 2008 (a) CH30, from lightly poor to moderate and (b) CH15 from moderate to poor.

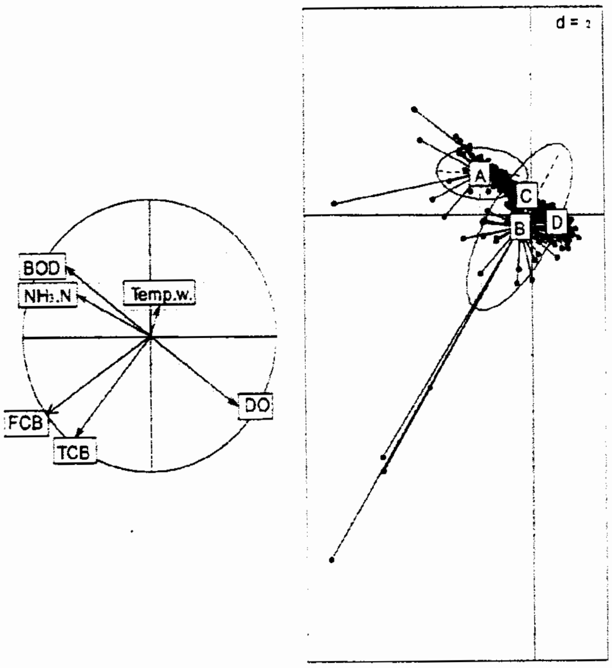


Figure 4. Result from CoA showed the gradients of 6 selected water quality parameters to the observations of each cluster.

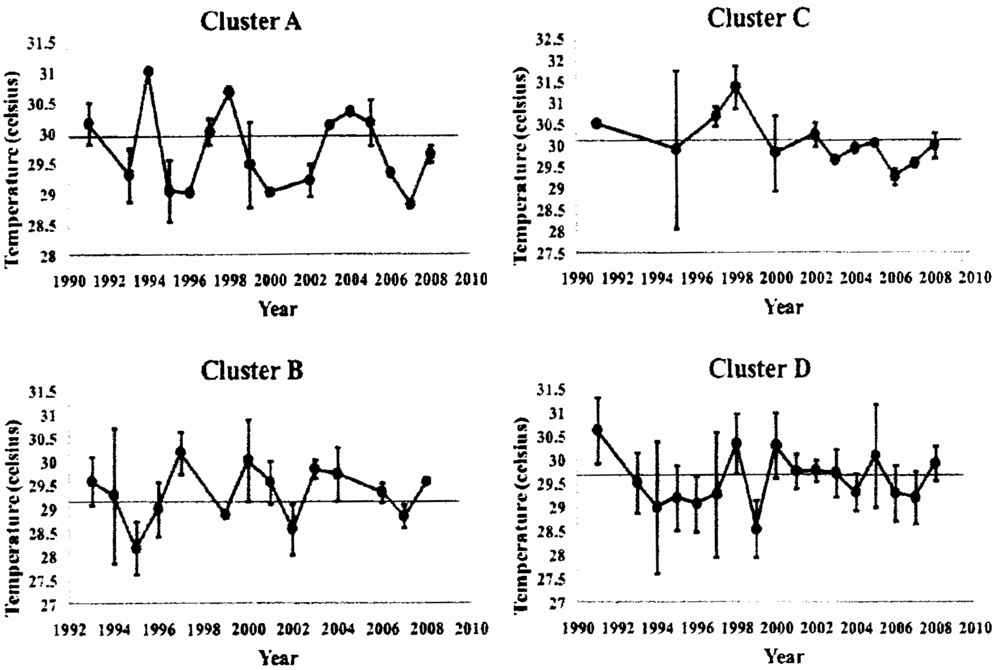


Figure 5. Fluctuations of the annual average water temperature in each cluster between 1991 and 2008.

Because of the dense population in the lowland of the Central plain [12], poor water quality condition in the lower portion of the river is common and found not only in Chao Phraya River (*i.e.* cluster A) but also the other rivers in the region, *i.e.* Bangpakong [13], Mae Klong [20] and Tha Chin [21], in which all selected parameters, except temperature, were below the national standard of surface water quality of Thailand at the lowest level, *i.e.* fairly clean used [22]. Municipal wastes and untreated industrial effluents are the main sources in decreasing the

DO level and increasing BOD level. Moreover, contaminants from the industries (e.g. chemical agents and heavy metal) also caused a reduction in DO and are increase in BOD in the lower Chao Phrya River [23,24]. Meanwhile, high NH3-N and derivatives are commonly loaded from surrounding agricultural land uses [20,21].

FCB and TCB are commonly used indicators of sanitary quality of the water, especially wastes from human and warm-blooded animals [25]. Apart from cluster A, FCB and TCB in cluster B were very far beyond the criteria for "fairly clean used" water quality standard in Thailand *i.e.* 4000 and 20,000 MPN/100 ml, respectively [22]. The extensive scattering of the observations from cluster B imply the tremendous increase of coliform bacteria along the Chao Phraya river course compared to the data during 1980s, where the high values of FCB and TCB were limited to the lower portion [26].

Reduction in DO along the river course (*i.e.*, from clusters D to A) is also related to the flow condition in the river and seasonal effects [27,28]. The high influx of freshwater during the monsoon season may have led to marked dilution of the stream resulting in a significant increase in DO levels while the reverse occurred in the dry season [28]. The natural river flow system in the Chao Phraya is likely to be changed in the near future according to the plan for flood protection in the central plain of the country after the big flood in 2011 [29]. Good condition of water quality in cluster D, in which all parameters were in the range of the "very clean used" criteria [22] related to the fact that the land uses of the upper portion are still sparsely urbanized [12,22] and also due to dilution from the mass of water from the four upper tributaries [12].

Although the low fluctuation in average water temperature was observed, this parameter must be approached cautiously and closely monitored. Climate change is also expected to have some effects with increasing temperatures and changed rainfall patterns. The average maximum daily temperature in the central plain of the country in 2030 is estimated at 40°C, with the baseline about 35°C [30]. Meanwhile the precipitation rates are expected to be higher but also more intense falling in a shorter time [30]. An increase in air temperature resulted in an increase in biological oxygen demand, coliform bacteria and suspended solids, and a decrease in dissolved oxygen, which eventually has a deteriorating effect on water quality in the river [31,32] then eventually aquatic biota and human.

5. Conclusion

Distinct spatial differences in water quality along the Chao Phraya River were clearly identified and highly related to anthropogenic stresses. However, there was no clear temporal trend for most of surface water stations, either positive or negative. Nonetheless, as the purpose to provide a big picture, the results were still insufficient to provide the detail in terms of inter-annual variation in water quality of the river. Thus, further studies on the inter-annual variation of water quality of the Chao Phraya River should be focused in the next step.

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Effects of Water Temperature on Embryonic Development, Hatching Success and Survival of Larvae of Siamese Mud Carp *Henicorhynchus siamensis* (Sauvage 1881)

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Abstract

Temperature is an important environmental factor for aquatic animals especially fishes, in particular during the early life stages. The objectives of this study are to investigate (a) the effect of different water temperature (26, 28, 30, 32 and 34 °C) on embryo development and newly-hatched larvae of Siamese mud carp *Henicorhynchus siamensis* (Sauvage 1881) and (b) the effect of acute temperature change to the newly hatched *H. siamensis* larvae. The development was divided into two phases *viz.*, firstly, from zygote to gastrula periods and, secondly, segmentation to hatching periods. The *H. siamensis* larvae did not successfully hatch at the incubation temperatures of 26 and 34 °C. The development times of the three remaining temperatures were relatively closed at the first phase, in contrast to the second phase, which were quite varied. The hatching times at 28, 30 and 32 °C were about 652, 485 and 457 min, respectively. The percentages of hatching success of the three respective temperatures were 73.76±2.37%, 73.90±1.44% and 61.42±11.19%, respectively. For the effect of acute temperature changes, numbers of dead larvae were not significantly different between 30 and 28 °C (P-value = 0.30), but there was a significant difference between 30 and 32 °C (P-value < 0.01).

Introduction

Although it is too complicated to predict the impact of climate change to fishes, the primary effect of climate change will be through changes in water temperature (Brian et al. 2011). The physiological mechanisms of fishes are directly or indirectly temperature dependent, thus climate change will affect fish by altering physiological functions, such as growth, metabolism, food consumption, reproduction success, and their ability to maintain homeostasis in the face of a variable external environment (Roessig et al. 2004).

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Moreover, water temperature is the most important environmental factor that affects and governs the development of eggs, incubation time of embryos, hatching success, survival and growth of fish larvae (Hakim and Gamal 2009; Le et al. 2011; Ahn et al. 2012).

The countries in the Lower Mekong Basin (LMB) are expected to be impacted by climate change with significant changes in rainfall and temperature (MRC 2009). In Thailand, for example, it is estimated that the rainfall will be intense in a shorter period, and temperature will increase about 2 – 3 °C and be highly varied from season to season (Greenpeace 2006). Air temperatures have risen by 0.5 to 1.5 °C in the past 50 years and continue to rise across the LMB and are likely to shift outside the present comfort zone, which is suitable for the LMB fishes for living (ICEM 2013). Continual temperature increase will inevitably impact the fish diversity and fisheries in the LMB, in which about 2 million tonnes of fishes and other aquatic animals are harvested each year, and which will eventually contribute to the food security in the LMB (ICEM 2010). There is another report by Lauri et al. (2012), which showed the difference between maximum- and minimum- daily average water temperature in the Mekong River could be more than 1 °C in 2032 onward. This is the cumulative impact of climate change and reservoir operation, i.e. the released water from dam.

The Siamese mud carp *Henicorhynchus siamensis* (Sauvage 1881) is a riverine species in mainland Southeast Asia. The species is a small-size cyprinid, i.e. about 20-25 cm TL (total length). It is the most abundant and most economically important fish in the LMB. It is the dominant species in the commercial set-net fisheries in Tonle Sap Lake, Cambodia, and the Khone Falls area in southern Laos (Rainboth 1996; Hai Yen et al. 2009; Fukushima et al. 2014). Not only does this fish provide protein, but also vitamins and minerals to the people in the LMB (Roos et al. 2007). *Henicorhynchus siamensis* also adapts to lentic environmental conditions, such as lakes and reservoirs, and contributes a significant portion in fish catches (Suvarnaraksha et al. 2010). Therefore, it is one of the most important candidates for a fish stock enhancement programme to increase fish production in inland waters (Jutagate 2009).

Henicorhynchus siamensis is a synchronous, i.e. single spawned species that clearly shows a single peak of gonadosomatic index in June to September and the highest in August (Suvarnaraksha et al. 2010). During the wet season, this species migrates into floodplains for spawning (Fukushima et al. 2014). Eggs and larvae grow in the floodplains and the larvae migrate back to rivers when the floodwaters begin to recede at the starting of dry season (Rainboth 1996; Fukushima et al. 2014). Therefore, suitable environmental conditions, during the wet season, are very important to guarantee the survival and recruitment to the fisheries of this species.

Little is known on fish distribution and their life history traits in Asia as well as possible impacts of climate change on fish, in comparison to fish in temperate zones (Ficke et al. 2007). Besides, it is widely accepted that the impact of climate change is unavoidable and is a serious concern to the developing world, including many Asian countries, where fish is among the major sources for protein food security (FAO 2007).

Henicorhynchus siamensis is a highly valuable fisheries resource throughout the LMB, and this study aims to explain the relationship between water temperature and embryonic development as well as the effect of acute changes in water temperature to mortality of the newly hatched *H. siamensis*. The findings will lead to a better understanding of the impact of temperature to this species which will help to improve climate risk management.

Materials and Methods

Broodstock

Broodstock of *H. siamensis* were from Ubon Ratchathani Inland Fisheries Research and Development Center in Ubon Ratchathani Province, Thailand. The parental fish were reared in a fibre-glass tank (60 L) for a week before breeding. The average water temperature was kept at 30±1 °C. The broodstock was divided into three lots (1 female: 2 males per lot) and each lot was kept in separate tanks. The average water temperature in each tank was 30±1 °C. The Suprefact was mixed with 10 mg of Motilium and then injected into the females (80- 150 g) and males (50- 100 g) at doses of 10 and 5 $\mu g \cdot kg^{-1}$, respectively. The parental fish spawned 8 h after injection.

Experiment and data analysis

Five water temperature treatments, i.e. at 26, 28, 30, 32 and 34 °C, were prepared in aquaria (24 x 40 x 28 cm) with a water volume of 25 L. One thousand eggs were rapidly transferred from the broodstock tanks into each experimental aquarium. The incubated eggs of *H. siamensis* were examined at 5 temperature levels from 26 to 34 °C at intervals of 2 °C in order to observe the effect of water temperature on embryonic development, hatching time and hatching success. The study was conducted in an air conditioning room, where the room- and air- temperatures were at about 20 and 24 °C, respectively. There were three replications for each treatment and the water temperatures were kept constant, at the set temperatures, by using the aquarium thermostat heater (Brand: EHEIM JAGER; model: TSRH 300 W). This experiment was conducted for 12 h, in which all the aquaria were aerated and the pH was maintained at 7.

The developmental periods of embryo from fertilisation to hatching were divided into seven periods *viz.*, zygote, cleavage, blastula, gastrula, segmentation, pharyngula and hatching (Kimmel et al. 1995). The developmental periods were observed and recorded every 5 min in early periods (zygote, cleavage, blastula and gastrula) and then every 30 min until hatching. The study on the duration for embryonic development was conducted by sub-sampling about 0.1 L of water from each aquarium and then 20 eggs were taken to determine the periods of development which was considered complete when more than 60% of the sub-samples reached that period at each time interval. Hatching success (%), i.e. number of hatch in total number of eggs, was then calculated.

The analysis of variance (ANOVA) was applied to examine the effects of water temperature on hatching success. The Tukey's HSD test at 95% confidence interval was applied, when ANOVA revealed significance.

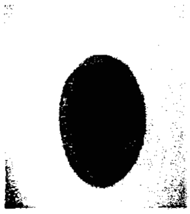
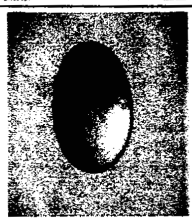
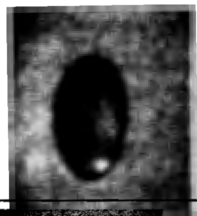
The effect of the acute change in temperature was examined by the experimental design "Before-After Control-Impact (BACI)". The setting of the experiment was similar to the first trial, i.e. effect of temperature on the embryonic development periods. The temperatures were set at 3 levels *viz.*, 30 °C as a control and the impacts were set at 28 °C and 32 °C, which respectively represented temperature at -2 and +2 °C of the control, since the estimated daily fluctuation of the water temperature in the Mekong could be beyond 1 °C in 2032 onward (Lauri et al.2012). Each temperature was replicated three times, in which 100 newly hatched *H. siamensis* were used in each replicate, in the aquaria (24 x 40 x 28 cm). The analysis was done by Randomized Intervention Analysis (RIA), on the difference in cumulative number of dead larvae between the control and each impact. The "before" was the period that the larvae were in control temperature for 20 min and then they were transferred, i.e. starting of the intervention of 100 larvae to the designated aquaria. Records were kept at 5, 15, 30, and 60 min then afterward every 60 minutes. The test was run by 999 iterations to random permutations of the impact-control data to generate the P-value. The statistical analyses were carried out with R software (R Development Core Team 2013).

Results

The durations for development at each period and hatching varied, depending on water temperature at their incubation (Table 1). Development time from zygote to gastrula period was relatively similar at all water temperatures. However, the development of blastula and gastrula periods at 26 °C took longer than the other treatments. The development period from segmentation to hatching, showed clear fluctuation. It is also worthy to note that the lower the temperature, the longer the time from segmentation period to hatching (Fig. 1). Development was stopped at the pharyngula period at 26 °C. Eggs did not hatch at 26 and 34 °C. For the remaining 3 levels, the time from the zygote stage until hatching, were obviously longer at 28 °C than 30 and 32 °C (Table 1).

The hatching success was highest at 30 °C (73.90±1.44%) but not significantly different at 28 °C (73.76±2.37%; Fig.2). Meanwhile, at the high temperature of 32 °C, hatching success decreased (61.42±11.19%) and was significantly different from the remaining two temperature levels. Dead larvae were observed after the intervention both in the control- and impact- manipulations. The average number of dead larvae at the control and 28 °C were relatively low, i.e. less than 10 individuals. However, the acute change in water temperature from 30 to 32 °C yielded high numbers of dead larvae, which was up to 30 larvae after 9 h. The RIA results indicated that there was no significant difference in average number of dead larvae between the control and 28 °C (P-value = 0.30; Fig. 3) but the difference between the control and 32 °C were significantly different (P-value < 0.01; Fig. 4).

Table 1. Average time (minutes) of embryonic development at five water temperature treatments.

Period	Temperature (°C)	Average time*(minutes)	Reference photographs	Description
Zygote	26	0.0		The chorion swells and cytoplasm streams toward animal pole to form the blastodisc
	28	0.0		
	30	0.0		
	32	0.0		
	34	0.0		
Cleavage	26	19.8		Blastodisc is vertically cleavaged from 1 to 64 blastomeres.
	28	21.0		
	30	19.8		
	32	19.8		
	34	19.8		
Blastula	26	150.0		Blastoderm covers about 30 % of egg and blastocoel is presented.
	28	96.6		
	30	90.0		
	32	96.6		
	34	91.8		
Gastrula	26	189.6		Germ ring is visible and covers 90 % of egg.
	28	144.6		
	30	144.6		
	32	139.8		
	34	139.6		
Segmentation	26	360.6		Fisrt somite expands to 26 somites and eyes vesicle, optic veasicle and tail bud are visible.
	28	309.6		
	30	274.8		
	32	259.8		
	34	256.8		
Pharyngula	26	600.0		Body is straight and well developed of somitesto the end of tail. Binary symmetry and pigment are visible.
	28	519.6		
	30	450.0		
	32	408.6		
	34	No hatching		
Hatching	26	No hatching		Basical organs are well developed, especially pectoral fin, jaws and gills and then hatching.
	28	652.2		
	30	484.8		
	32	457.2		
	34	No hatching		

* The average time (minutes) for each development period is considered to be completed when more than 60% of eggs had reached the period.

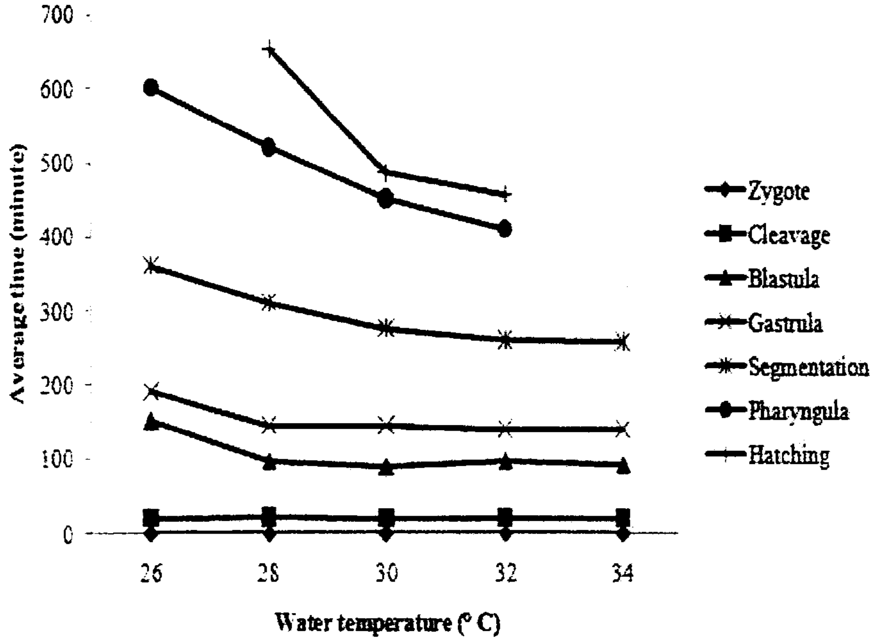


Fig. 1.The durations of *Henicorhynchus siamensis* embryonic development at each studied temperature.

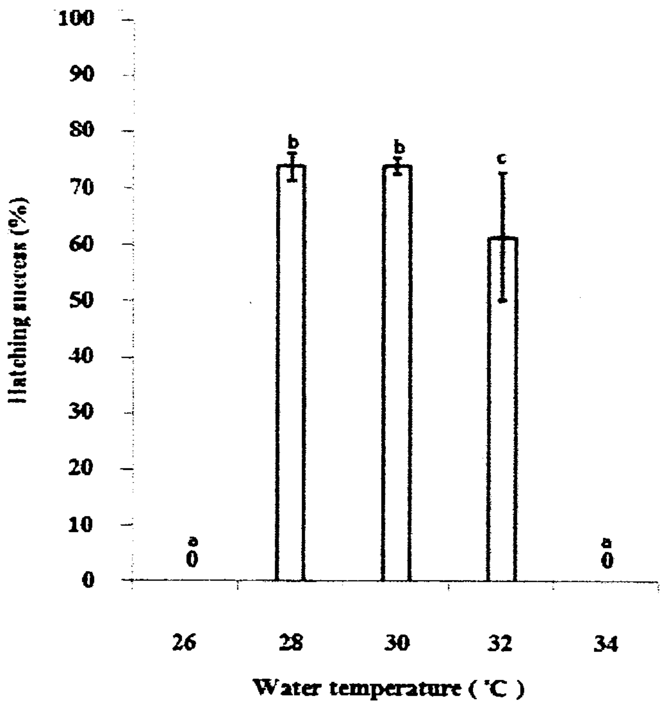


Fig. 2. Hatching success of *Henicorhynchus siamensis* at each studied temperature (The different letters, above each bar, indicate statistical difference at $\alpha = 0.05$.)

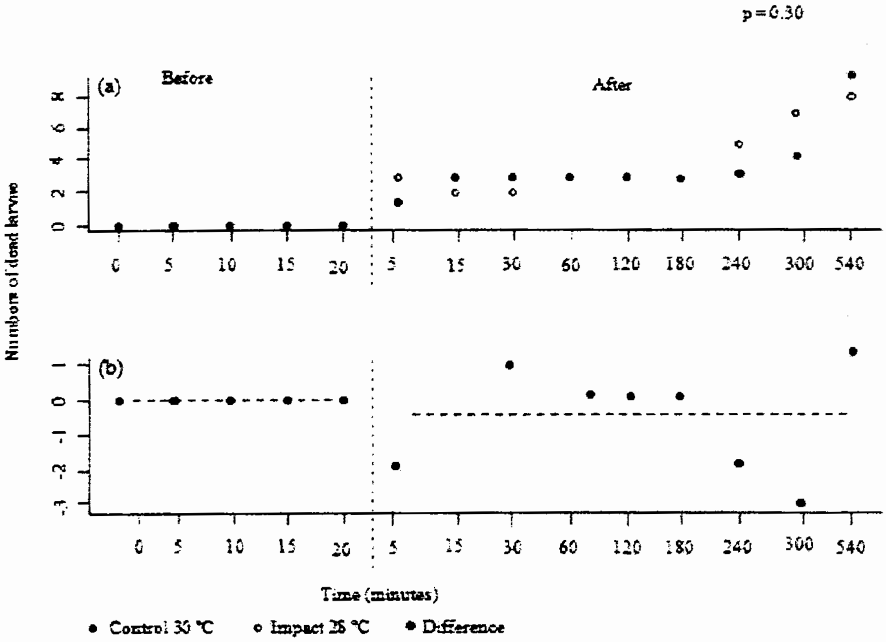


Fig. 3. Number of dead *Henicorhynchus siamensis* larvae of the control (30 °C) and impact (28 °C) treatments. Graph (a) depicts the raw data used for RIA and graph (b) shows the differences between control and impact.

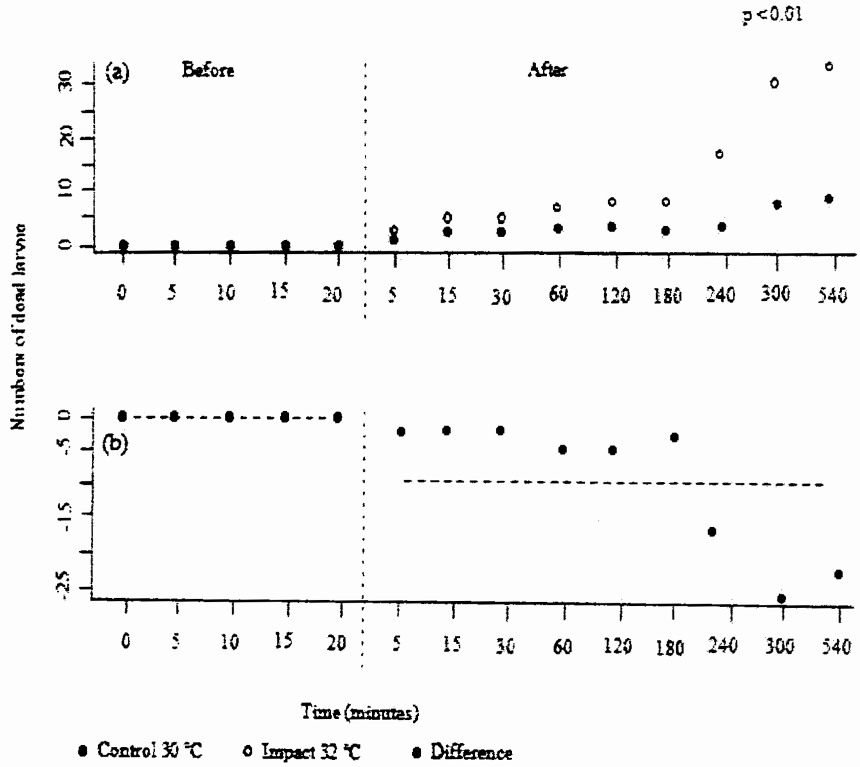


Fig. 4. Number of dead *Henicorhynchus siamensis* larvae of the control (30 °C) and impact (32 °C) treatments. Graph (a) depicts the raw data used for RIA and graph (b) shows the differences between control and impact.

Discussion

Changes in water temperature produced a strong effect on embryo development and hatching of *H. siamensis*. Temperatures, which are beyond the tolerance range, results in unsuccessful development and hatching failure (Kucharczyk et al. 1997; Kupren et al. 2011; Ahn et al. 2012). In this study, water temperatures at 24 and 34 °C are considered as the respective "minimum limiting"- and "maximum limiting"- temperatures (Cruz et al. 2002) for the egg development of *H. siamensis*. In general, the lower temperature causes unsuccessful egg development and the high temperature causes mortality of the embryo (Kupren et al. 2011; Ahn et al. 2012). The maximum limiting temperature is the main concern because water temperatures in Thai inland waterways can reach 34 °C during the rainy season (Choo-In et al. 2013), i.e. the spawning season for *H. siamensis*. Also increasing temperatures are expected on both daily maximum temperature and daily minimum temperature in the LMB, where the daily maximum temperature could be beyond 35 °C (TKK & SEA START RC 2009). The minimum limiting temperature may also be of concern, when cold water from dams is released (Lugg and Copeland 2014), and if this phenomenon overlaps into the spawning season.

Although there were non-significant differences in embryonic development time at each stage, there was a trend towards a decrease of development time when the incubation temperature increased for *H. siamensis*. This implies that a lower temperature retards the rate of embryonic development, and a higher water temperature accelerates the larval development, which conforms to the negative second order polynomial trend-line for the temperate- and most of marine fishes (Kawahara et al. 1996; Chambers and Tripple 1997; Yang and Chen 2005; Kupren et al. 2011). The reason for faster hatching of embryo at high water temperature is because of the increase of mobility in warmer water and early excretion of the hatching enzyme (Kupren et al. 2011).

The optimum temperature, which is suitable for fish embryo development and success in hatching as well as their survival, is usually correlated with spawning temperature (Kucharczyk et al. 1997; Ahn et al. 2012). The temperature experienced by the parents may influence the optimal temperature of egg incubation (Bermudes and Ritar 1999). In this experiment, the treatment at 30 °C, i.e. similar to the temperature in the spawning aquaria, yielded the highest hatching success. Meanwhile, the water temperatures in the treatments that prominently deviated from the average temperature in the spawning aquaria at ± 4 °C, i.e. 26 and 34 °C, damaged *H. siamensis* eggs and resulting in hatching failure. Notwithstanding this fact for *H. siamensis*, Landsman et al. (2011) reported that deviated temperature of ± 2 °C during the egg stage for Atlantic cod can generate huge variations in recruitment, i.e. can be up to 1000-folds. This probably results from elevated metabolic rate, causing accelerated consumption of energy store or from thermal degradation of proteins that impart a direct effect on cell function such as substrate binding activity and stress protein synthesis (Landsman et al. 2011).

Acute increase of water temperature causes significant decrease in survival of newly-hatched larvae rather than acute decrease of water temperature (Landsman et al. 2011; Lahnsteiner et al. 2012). In general, deviations in metabolic rate could be observed in fishes during acute changes in temperature. The cold-acclimated individuals typically show a large increase in metabolic rate when exposed to warm temperatures, while warm-acclimated fish often show a considerable decrease when acutely exposed to colder temperatures (Killen et al. 2007). Hence, the daily development and mortality rates trend to increase with acute increasing temperature (Pepin 1991).

Conclusion

Although it is believed that tropical fishes have evolved to survive in very warm water and may seem less likely to suffer from increases in global temperature (Ficke et al. 2007), our results showed that the prominent deviation in water temperature affect embryonic developments and hatching of *H. siamensis*. The results also provide useful information for habitat management for protection of the local aquatic ecology as well as to aquaculture system. Further studies are needed to understand such impacts on the abnormality of the larvae and their growth performances.

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The authors wish to thank the Fisheries and Aquaculture section of Sirindhorn Reservoir for providing the facilities of this research and the Ubon Ratchathani Inland Fisheries Research and Development Center for fish samples.We also thank Professor Dr. Michael D. Hare, Faculty of Agriculture, Ubon Ratchathani University, for English editing of the manuscript.

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Environment Research & Technology Development Fund

D-1202

(Dam development and environmental conservation in the international river, the Mekong - the assessment of ecological services in reservoirs)

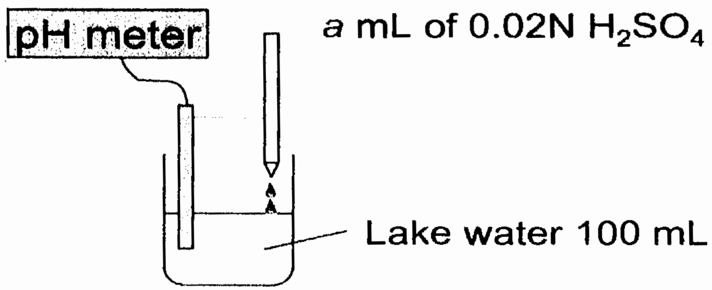
17 December, 2012

National Institute for Environmental Studies, Japan

Table of Contents

Section 1. Measuring alkalinity for ^{13}C inoculation at Stations

- Titrate lake surface water (100 mL) with sulfuric acid (0.02N) to determine H_2SO_4 volume (*a* mL) to achieve a pH endpoint of 4.2.



<u>Previous estimates of *a*</u>

Sirindhorn < 1 mL

Lam Poa ≈ 3 mL

Ubolratana ≈ 8 mL

Huay Luang ≈ 6 mL

Tonle Sap ≈ 4 mL

Nam Ngum ≈ 4 mL

- Calculate the volume of $NaH^{13}CO_3$ to inoculate sample water with for primary productivity measurement (see Section 3) as follows:

If a > 1.8 mL, add 0.204 a mL of $NaH^{13}CO_3$ solution (5 mg mL^{-1}) to S bottles.

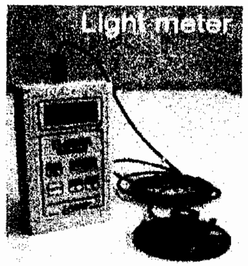
If a < 1.8 mL, add 1.020 a mL of $NaH^{13}CO_3$ solution (1 mg mL^{-1}).

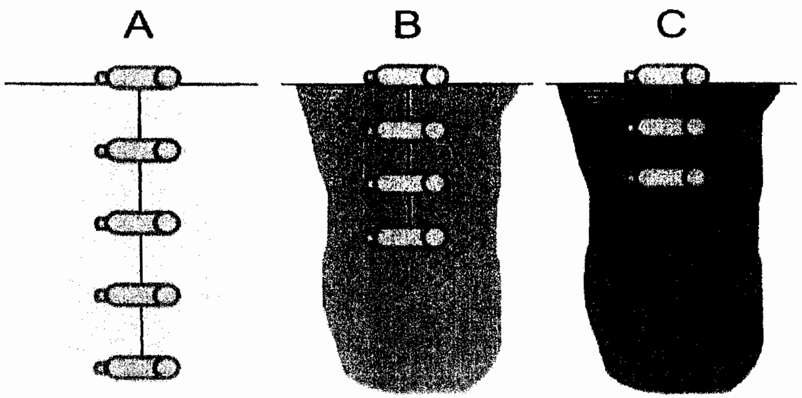
- S bottles = Sample bottles (see Section 3)
- Always calibrate pH meter before use using standard pH buffers.
- For more details, refer to Appendix I.

Section 2. Collecting water at Stations

2-1. Water depths to deploy S bottles in an incubation experiment

- Fill in the log sheet with date, weather, water depth, transparency, etc.
- Measure underwater Photosynthetically Active Radiation (PAR) using a light meter from surface to bottom at a prescribed distance interval to determine depths to deploy S bottles (do this as quickly as possible without holding PAR readings).
- Determine water depth (D_5) at which light intensity corresponds to 5% of light intensity at water surface for the lowermost S bottle.
- Divide D_5 by 4. The other S bottles are hung at water depths corresponding to 1/4, 1/2, and 3/4 of D_5 shallower as well as at the surface (total of 5 bottles are hung) (A).
- Divide D_5 by 3 (or 2) and hang 4 (or 3) bottles if D_5 is too short with increasing turbidity (B & C).

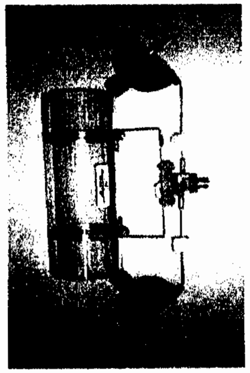




2-2. Collecting water from each depth

Reservoirs in Thailand and Laos (Nam Ngum)

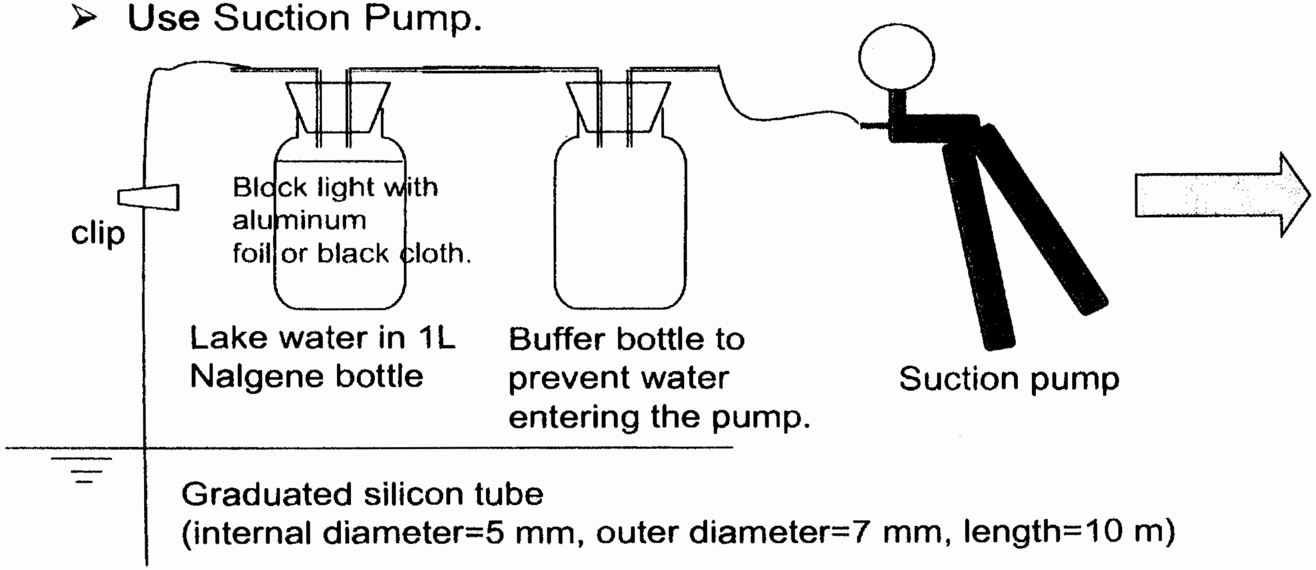
- Use Van Dorn sampler.
- Pour the collected water into a bucket before distributing the water among sample bottles.
- Repeat water sampling with Van Dorn sampler from all the target depths.

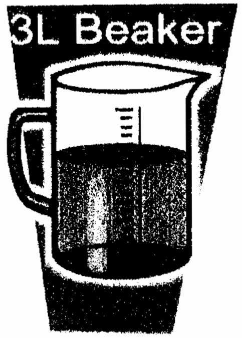


Van Dorn Sampler (capacity=1.6 L)

Tonle Sap

- Use Suction Pump.





Block light with aluminum foil or black cloth

Section 3. Distributing the water among various bottles

Sample bottles (S bottles: 1S, 2S, ...)	➢ Decant water from each target depth into each S bottle (half full). ➢ Inoculate each bottle with the prescribed volume of $NaH^{13}CO_3$ (see Section 1). ➢ Decant water again to fill the bottle up (≈600 mL). ➢ Keep the bottles in black cloths until all bottles are ready for incubation.
Control bottles (C bottles: 1C, 2C, ...)	➢ Decant *500 mL* of water from each depth into each C bottle (I-Boy) with 4.75 ml of formalin which is added in advance.
Glass bottles for Dissolved Inorganic Carbon (DIC)	➢ Fill the bottle up with lake water. ➢ Insert a needle to a rubber cap to allow air to escape. ➢ Immediately add 0.6 mL of formalin using another needle.
1-L plastic bottles	➢ Fill each bottle up with water from each target depth. ➢ Keep the bottles in a cooler box until filtration.

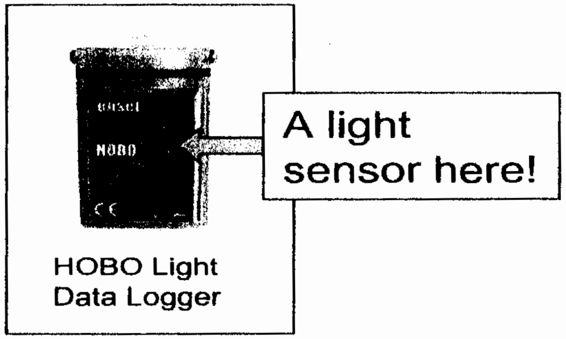
- Total amount of water required from each depth ≈ 3 L, which is equivalent to 2 times of Van Dorn sampling.
- Bottles and tools must be rinsed with sample water before use.

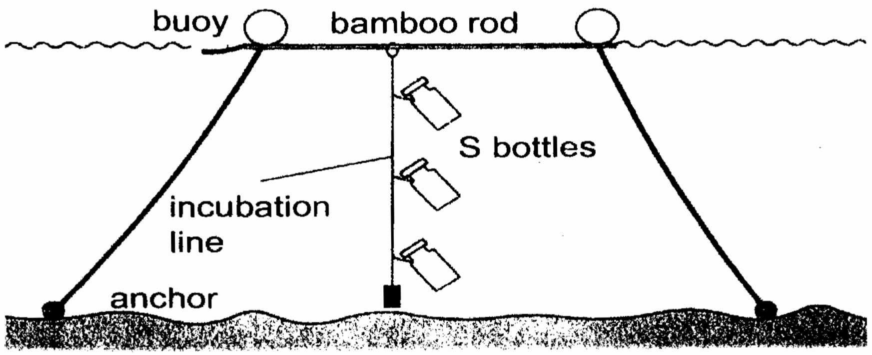
Section 4. Conducting *in situ* primary production incubation

4-1. Initiation of an incubation experiment

- Attach S bottles to an incubation line at water depths from which water samples were collected (see Section 2).
- Set up the incubation apparatus (i.e., bottles, rope, rod, buoys, etc.) and gently lower the incubation line with the S bottles into the lake.
- Deploy an illuminance logger on the roof of the boat with the sensor facing upward.

- Record the initiation time and GPS coordinates of the Station on the log sheet.
- Recording a waypoint with a GPS is recommended to return to Station easily and accurately.





Incubation apparatus for primary productivity measurement

- Be careful not to cover the light sensor during data logging.

4-2. Termination of the experiment (typically after 1-2 hours of incubation)

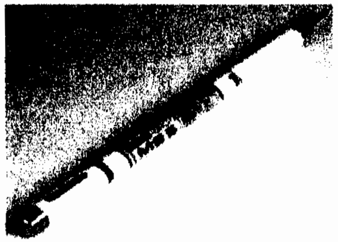
- Retrieve S bottles from a lake or a reservoir to the boat.
- Pour the water of S bottles to 500 mL I-Boy with 4.75 mL of formalin already added.
- Retrieve the illuminance logger (and store it in a box).

- *Record the termination time on the log sheet.*
- Longer incubation time is needed if cloudy.
- The illuminance logger should be outside of a box only during an incubation period.

Section 5. Collecting limnological data at Stations

Thai reservoir and Nam Ngum

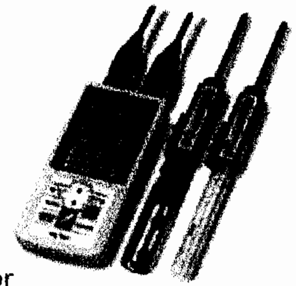
- Use HYDROLAB to record profiles of pH, DO and water temperature from surface to bottom.
  - If HYDROLAB data look aberrant, use other instrument such as TOA DKK pH-DO meter to double-check the data.



HYDROLAB

Tonle Sap

- Use TOA DKK pH-DO meter.
- Measurement is done at all depths of incubation bottles as well as the depths of every 0.5 m from surface to bottom.
- Fill in the log sheet with the data.
  - Jiggle the sensor for accurate DO measurement.
  - Note this pH-DO meter does not automatically store the data.
  - Always read temperatures from the pH sensor to maintain consistency (DO sensor also has a temperature sensor).



TOA DKK pH-DO meter

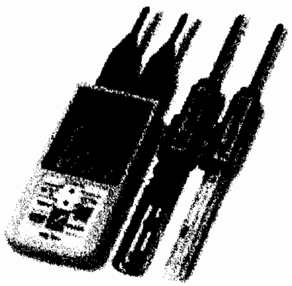
Section 6. Collecting water and limnological data at Sites

6-1. Water sampling

- Fill a bucket with surface water at each Site.
- Decant the water into *two* 1-L plastic bottles.
- Keep the bottles in a cooler box until filtration.

6-2. Collecting limnological data

- Measure depth, transparency, temperature, pH and DO at water surface and bottom at Site.
- Fill in the log sheet with the data.
- Record an approximate distance from the bank to the Site in the log sheet.

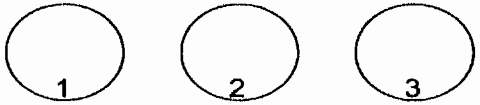


- Read temperatures from the pH sensor.
- Jiggle the sensor (TOA DKK) for accurate DO measurement.

Section 7. Filtering water samples in the laboratory

7-1. Three types of GF/F filters are used.

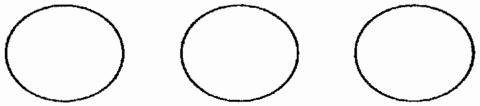
Write serial numbers *in situ* with a pencil on each filter.
If days are different, the same numbers can be used. ⇨ **Filter A**



....

Used for primary production and isotopes (^{15}N, ^{13}C) measurement

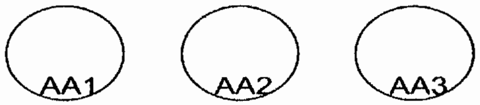
No numbers are written on the filter. ⇨ **Filter B**



....

Used for chlorophyll *a* and DNA measurement

Filters are pre-numbered as AA1, AA2, ... ⇨ **Filter C**



....

Used for POC measurement

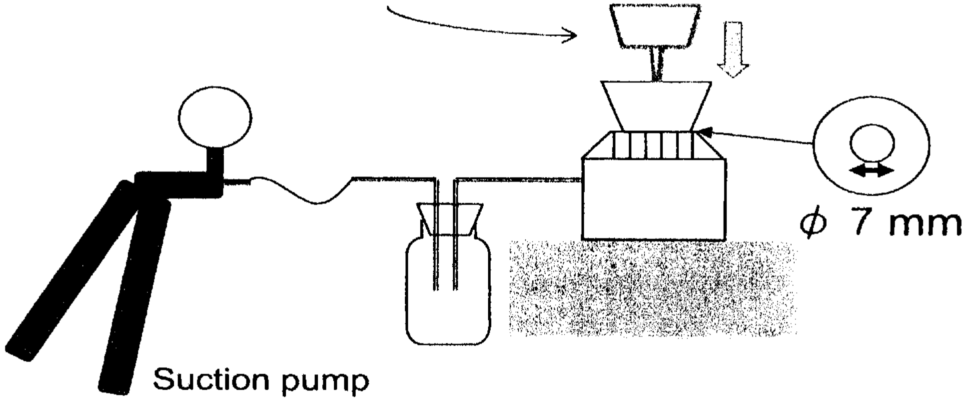
- POC needs to be accurately weighed using Filter C which is pre-combusted and constant-mass attained.

7-2. Water from incubation bottles collected at Stations

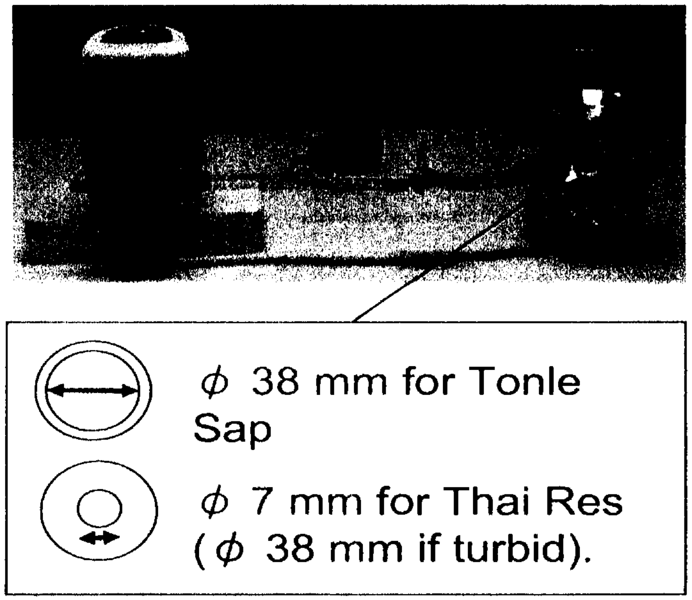
7-2-1. Filtration apparatus

Nam Ngum

Use a funnel to concentrate water in the center of GF/F filter.
(If it takes too long time for water to drain with this funnel, use a 50-ml syringe instead.)



Tonle Sap & Thai reservoirs



- The diameter of filter base is smaller (7 mm) for Thai and Lao reservoirs than for Tonle Sap (38 mm) because of too low phytoplankton concentrations in the former to obtain accurate primary productivity estimates.
- Formula for the primary productivity estimation is given in Appendix II.
- Correct set up for the electric pump is given in Appendix III.

7-2-2. Filtration procedure for incubation bottles

Filtration for primary productivity and N & C isotopes

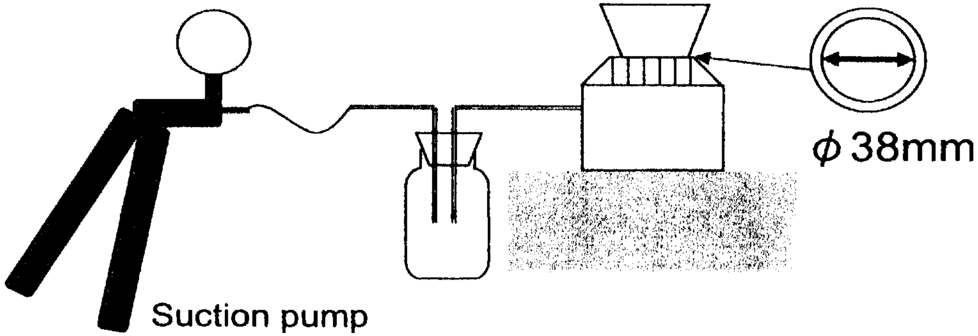
- Filter up to 250 mL of water in C and S bottles using **Filter A** (< 250 mL if turbid).
- Remove the filter and place it on wrinkled aluminum foil in the dehydrator.
- Repeat the filtration two time to make *duplicate* samples of filters.
- Dispose of filtrate.
- Clean the filter funnel and measuring cylinder with distilled water before filtering the next water sample.

- Filter all C bottles first and then S bottles.
- Minimize a filtration area of each filter except for Tonle Sap filters, so that thicker material remains on the filter to increase the sensitivity of the isotopic analysis.
- When the water sample is turbid, pour only about 50 mL to the filter funnel. If filtration takes too long a time (say, 3 min.), pour no more water (see Appendix IV).
- Shake the sample bottles well before pouring the water to prevent particulate material from depositing on the bottom.
- No need to record the volume of water filtered.

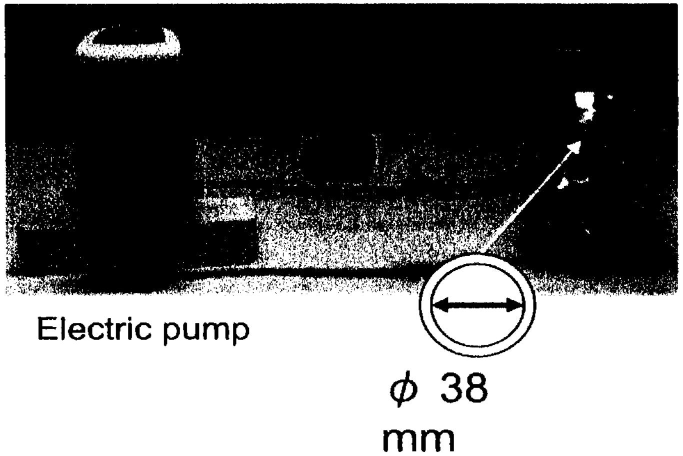
7-3. Water from 1-L plastic bottles from both Stations and Sites

7-3-1. Filtration apparatus

Nam Ngum



Tonle Sap and Thai reservoirs



- The diameter of filter base is 38 mm for both the reservoirs and Tonle Sap.

7-3-2. Filtration procedure for 1-L bottles

- Rinse the filtering apparatus and the measuring cylinder with distilled water every time before the next water sample is processed. The measuring cylinder should also be rinsed with the sample water before use.

<u>Filtration for Chlorophyll *a*</u>

- Filter 150 mL of water in the 1st 1-L bottle using **Filter B** (< 150 mL if turbid).
- If water is filtered smoothly, pour another 150 mL (300 mL total). If not, no more water.
- Fold the filter in half (organic matter inside) and wrap with aluminum foil.
- Attach a label on the sachet of the aluminum foil.
- Store five sachets into one plastic bag.
- Record the sample volume filtered and dispose of the filtrate.

<u>Filtration for POC</u>

- Filter the same or less volume of water as above from the 1st bottle using **Filter C**.
- Place the filter on aluminum foil in the dehydrator.
- Record the filter number and the sample volume in a notebook.
- Decant 50 mL* of filtrate each into the bottles of DTN and Nutrient (see 7-4).

* Filter more water if filtrate is not enough to make two 50-ml bottles.

Filtration for phytoplankton DNA

- Filter water of the same volume as chlorophyll in the 1st bottle using **Filter B**.
- Spray 70-75% EtOH on the GF/F filter and wait a few seconds until the water (and EtOH) disappear from above the filter.
- Repeat above step a few time.
- Fold the filter in half (organic matter inside) and wrap with aluminum foil.
- Attach a label to the sachet of the aluminum foil.
- Store five sachets into one plastic bag.
- Record the sample volume filtered.

- Decant 50 mL of the water in the 1st 1-L bottle into 50-mL plastic bottles for TNP (all the 1-L bottles) and for Phytoplankton (only the surface water at Stations) (see 7-4).

Filtration for ^{15}N and ^{13}C isotopes (Sites only)

- Filter 200 mL of sample water (<200 mL if turbid) in the *2nd* 1-L bottle using **Filter A**.
- If water is filtered smoothly, pour the rest of the water (up to 1 L in total).
  (no need to record the sample volume filtered)

- When the water sample is turbid, pour only about 50 mL to the filter funnel. If filtration takes too long (say, 3 min.), pour no more water. Record the amount of water filtered for the filters of chlorophyll *a*, POC and DNA. See Appendix IV.
- Wet filters (i.e., chlorophyll *a* and DNA) must be refrigerated.

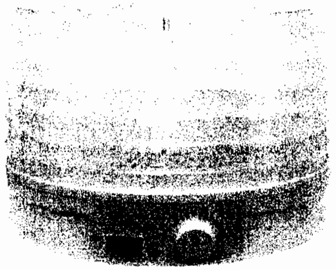
7-4. Preserving lake water and filtrate

Only from 1-L bottles collected at water surface of Stations	From all the 1-L bottles		
Phy	TNP	DTN	Nutr.
Lake water 50 mL	Lake water 50 mL	filtrate 50 mL	filtrate 50 mL + 2M HCl 0.5 mL
No processing	No processing	No processing	
Refrigeration and immediate delivery to NIES required.	Refrigeration desirable.		

- Do not forget to attach a label to each sample bottle.

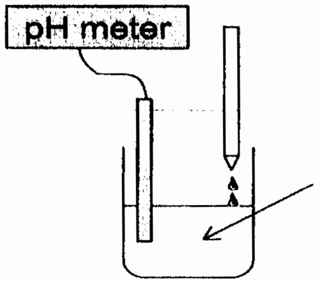
Section 8. Dehydrating filters

- Dehydrate the filters for at least 3 hours at the highest temperature setting, preferably over-night (The dehydrator must be kept outdoor or in a shower room with ventilation on).
- After the filters become completely dehydrated, store the filters into plastic cases, one by one.
- Attach a label to each plastic case.
- Store the plastic cases in a plastic bag with desiccant agent.



Dehydrator

Appendix I. The volume of $NaH^{13}CO_3$ to add to a sample bottle



- Titrate 100 mL of lake water with 0.02N H_2SO_4 to pH 4.2.
- Record the volume of titrant (*a* mL).

water volume 100 mL

Alkalinity ($meq \cdot L^{-1}$) ≈ Dissolved Inorganic Carbon (DIC) (mN)

$$\text{Alkalinity } (meq \cdot L^{-1}) = a\ (mL) \times \frac{1000\ (mL)}{\text{water volume } (mL)} \times 0.02\ (N) \times f^{*} \qquad \cdots\cdots\cdots\cdots (1)$$

*factor=1.00

$NaH^{13}CO_3$ equivalent (b mg L^{-1}) = Alkalinity x 85**

** molecular weight of $NaH^{13}CO_3$

Weight of $NaH^{13}CO_3$ required for 600-mL*** sample water (c mg) = b x 0.6 x 0.10****

*** the volume of an incubation bottle (S bottle) .... (2)

**** 10% quantity of DIC

Volume of 5 mg mL^{-1} $NaH^{13}CO_3$ solution = $c/5$ (mL)

= 0.204 a (mL) .... (3)

Or equivalently, 1 mg mL^{-1} $NaH^{13}CO_3$ solution = $c/1$ (mL) = 1.02 a (mL) ...... (4)

Appendix II. Calculation of photosynthetic production rate, P (μg C L^{-1} h^{-1})

$$P = \frac{(a_{is} - a_{ns})}{(a_{ic} - a_{is})} \times \text{POC} \times \frac{1}{t} \qquad \text{........... (5)}$$

where

a_{is} = ^{13}C atomic % of particulate matter after incubation in S bottles,

a_{ns} = ^{13}C atomic % of particulate matter in nature measured in C bottles,

a_{ic} = ^{13}C atomic % of DIC in nature and $NaH^{13}CO_3$ additive in S bottles,

POC = particulate organic carbon concentration before incubation (μg C L^{-1}) (see 7-3-2),

and

t = incubation time (h).

^{13}C atom % of DIC in natural fresh water is about 1.1%.

<u>Experimental condition</u>

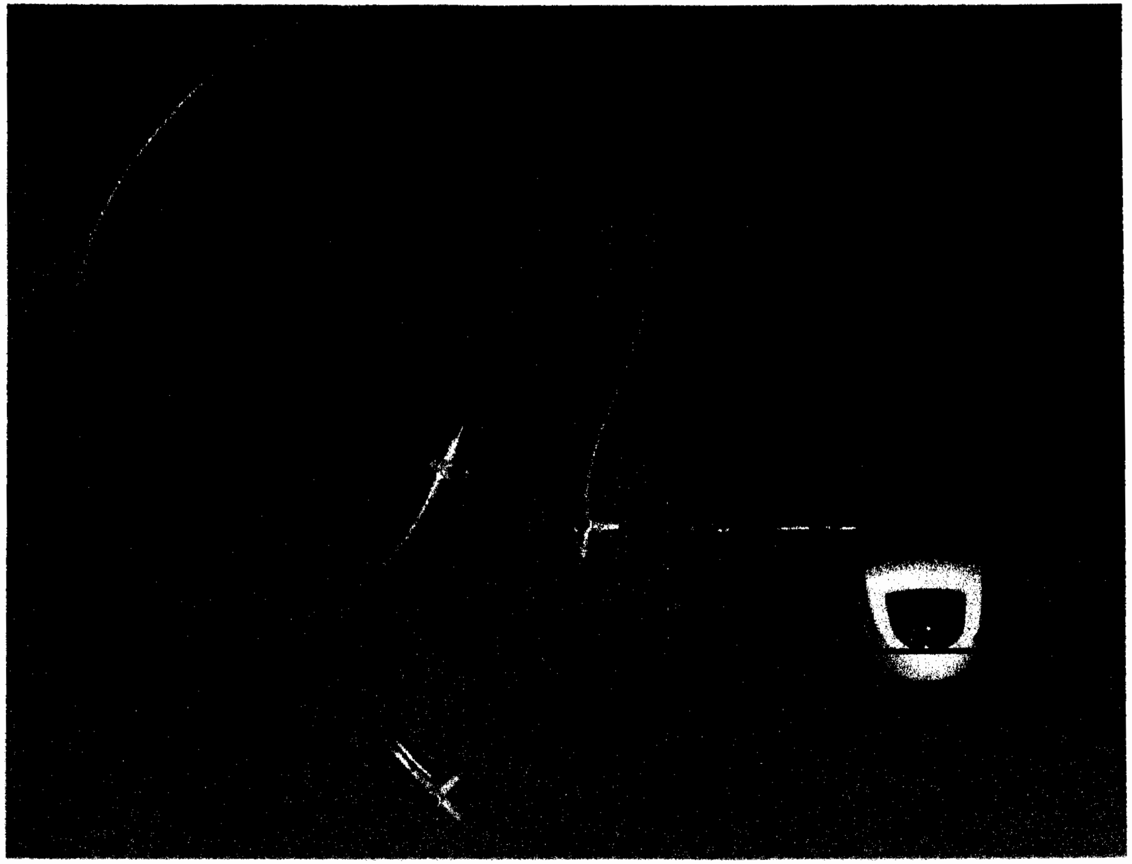
a_{ic} >> ^{13}C atomic % of DIC in nature (≈1.1%)

Typically add just enough $NaH^{13}CO_3$ for the final ^{13}C concentration in S bottles to be 10 %.

Excess amount of $NaH^{13}CO_3$ additive could influence photosynthetic activity.

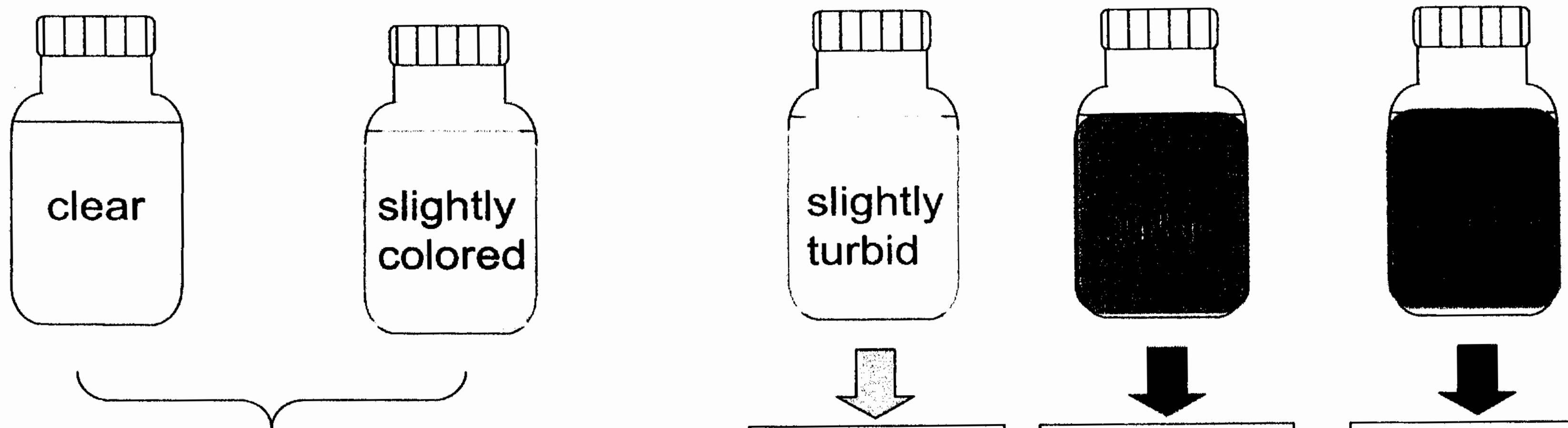
Reference:
Hata et al. 1993. ^{13}C Tracer Methodology in Microbial Ecology with Special Reference to Primary Production Processes in Aquatic Environments. In Jones, J.G. edit. Advances in Microbial Ecology. Springer.

Appendix III. Correct connection method of tubes for an electric pump



Appendix IV. Determination of the volume of water to be filtered

Be careful not to overload sample water for filtering!



Pour 150ml of water to the filter funnel. Add more water if it is filtered smoothly to a maximum of:

1) 250 ml for primary productivity bottles;
2) 300 ml for Chl *a*, POC and DNA; and
3) 1,000 ml for N & C isotopes.

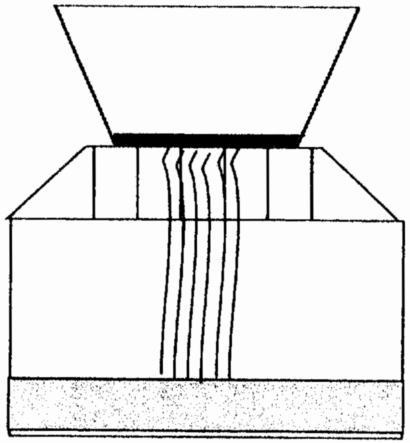
Try 100ml of water and see how it goes.

Try 50ml and see.

Try 30ml.

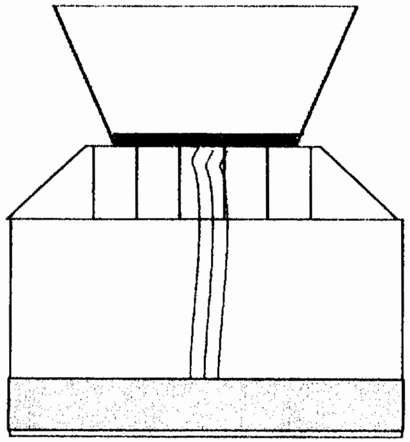
Appendix IV. Determination of the volume of water to be filtered (continued)

Furthermore, watch out for the speed of filtration as illustrated below.



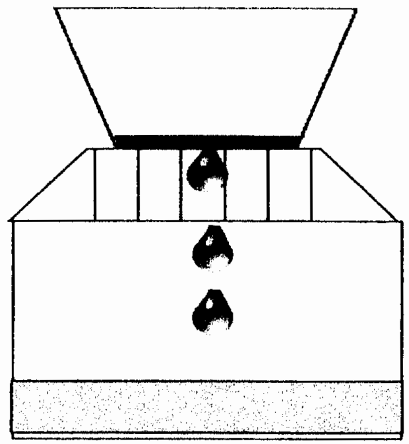
If smoothly filtered,

Add more water.



If filter gets clogged,

Add little or no water.

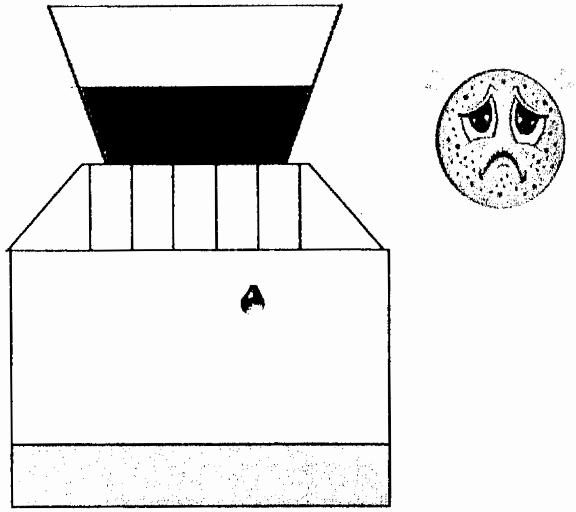


If completely clogged,

Add no water.

Appendix IV. Determination of the volume of water to be filtered (continued)

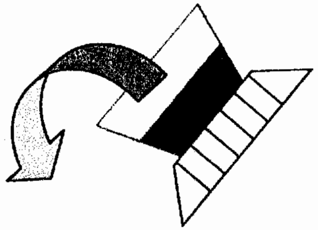
What if a GF/F filter is clogged up
while water is still left
on the upper part of a funnel?



Actions to take:

1. Stop using pumps immediately.

2. Throw away water from the funnel and the filter.



3. Repeat filtration with less sample water after washing the apparatus.

CURRICULUM VITAE

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CURRICULUM VITAE (CONTINUED)

Jutagate T, P. Phomikong, **P. Avakul** and S. Saowakoon. "Age and growth determinations of chevron snakehead Channa striata by otolith reading", In **Proceedings of 51st Kasetsart University Annual Conference: Veterinary Medicine, Fisheries**. p. 1-10. Bangkok: Kasetsart University, 2013.

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