การตอบสนองทางสรีรวิทยาของกุ้งกุลาดำ *Penaeus monodon* วัยรุ่นต่อผลร่วมของความเค็มและปริมาณน้ำมันดิบส่วนที่ละลายน้ำ

นางสาว วรวรรณ ศุกระฤกษ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ลิขสิทธิ์บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

PHYSIOLOGICAL RESPONSES OF POSTLARVA BLACK TIGER PRAWN *Penaeus monodon* TO COMBINED EFFECTS OF SALINITY AND WATER SOLUBLE FRACTION OF DUBAI CRUDE OIL

Miss Warawan Sukrarurk

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พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสีเขียวนี้เพียงแผ่นเดียว

วรวรรณ ศุภระฤกษ์ การตอบสนองทางสรีรวิทยาของกุ้งกุลาดำ *Penaeus monodon* วัยรุ่นต่อผลร่วมระหว่างความเค็มและปริมาณน้ำมันดิบส่วนที่ละลายน้ำ (The Physiological Responses of Postlarva Black Tiger Prawn *Penaeus monodon* to Combined Effect of Salinity and Water Soluble Fraction of Dubai Crude Oil)
อ.ที่ปรึกษา: รศ.ดร. ลมเกียรติ ปิยะธีรธิติวรกุล. 71 หน้า.ISBN 974-333-140-9.

การศึกษาผลของความเค็มและระดับของความเข้มข้นของน้ำมันดิบส่วนที่ละลายน้ำ (WSF) ของน้ำมันดิบดูไบต่อกุ้งกุลาดำ*Penaeus monodon* วัยรุ่นโดยออกแบบการทดลอง 2 ขั้นตอนได้แก่การทดลองหาค่าความเป็นพิษเฉียบพลัน เพื่อหาระดับความเป็นพิษที่ทำให้กุ้งตายทันที (LC_{50}) ภายในระยะเวลา 24, 48, 72 และ 96 ช.ม. โดยทดลองระดับเปอร์เซ็นต์ความเข้มข้นของ WSF ที่ใช้มี 4 ระดับคือ 100%WSF, 75%WSF, 50%WSF และ 25%WSF ส่วนระดับความเค็มที่ใช้มี 4 ระดับ 14, 21, 28 และ 35 ppt.อีกขั้นตอนหนึ่งคือการทดลองหาความเป็นพิษเรื้อรัง ทำการทดลองแบบ factorial design เพื่อศึกษาการตอบสนองทางสรีรวิทยาของกุ้งกุลาดำ *Penaeus monodon* ที่มีต่อความเค็ม 4 ระดับ (14, 21, 28และ 35 ppt.) และระดับความเข้มข้นของ (WSF) 4 ระดับ (0,20,40และ 60ug/l) ทุกชุดการทดลองทำ 3 ซ้ำ เลี้ยงกุ้งในสภาวะที่กำหนด 30 วัน ก่อนการทดลองทำการปรับสภาพกุ้งให้เคยชินกับภาวะของการทดลองเป็นเวลา 1 สัปดาห์ ผลการทดลองทำการทดลองหาความเป็นพิษเฉียบพลัน พบว่า ค่า 96-hr LC_{50}ของWSFของน้ำมันดิบดูไบที่มีต่อกุ้งกุลาดำที่ความเค็ม14, 21, 28 และ35ppt.เท่ากับ 81.548, 13.555, 40.651 และ 80.054 ug/l ตามลำดับ ส่วนผลการทดลองจากการวัดค่าการตอบสนองทางสรีรวิทยา อัตราการใช้ออกซิเจน อัตราการขับถ่าย และอัตราการกินอาหาร พบว่าความเค็มและระดับความเข้มข้นของ WSF มีปฏิสัมพันธ์กัน ($p<0.005$) อัตราการใช้ออกซิเจนสูงสุดที่ความเค็ม 14 ppt.มีค่าเท่ากับ 0.008±0.005 mg O_2/gdw/hr ส่วนอัตราการใช้ออกซิเจนต่ำสุดที่ความเค็ม 35 ppt.เท่ากับ 0.007±0.006 mg O_2/gdw/hr ค่าเฉลี่ยของอัตราการขับแอมโมเนียสูงสุดที่ความเค็ม 14 ppt.เท่ากับ 1.5666±1.222 mg NH_3/l/gdw/hr ส่วนค่าต่ำสุดที่ความเค็ม 28 ppt. เท่ากับ 0.932±0.671 mgNH_3/l/gdw/hr และค่าเฉลี่ยของอัตราการกินอาหารของกุ้งกุลาดำสูงสุดที่ความเค็ม 14 ppt.เท่ากับ 0.809± 0.248 mg *Artemia*/gdw/hr ค่าเฉลี่ยต่ำสุดที่ 35 ppt.เท่ากับ 0.354±0.108 mg *Artemia*/gdw/hr ค่าเฉลี่ยของอัตราการใช้ออกซิเจนที่ความเข้มข้นต่าง ๆ มีค่าใกล้เคียงกันอยู่ในช่วง0.700±0.005 mg O_2/l/gdw/hr ค่าเฉลี่ยของอัตราการขับแอมโมเนียสูงสุดที่ความเข้มข้น 40 ug/l เท่ากับ 1.360±0.989 mg NH_3/l/gdw/hr และต่ำสุดที่ 0 ug/l เท่ากับ 0.825±0.541 mg NH_3/l/gdw/hr ค่าเฉลี่ยของอัตราการกินอาหารสูงสุดที่ 20ug/l เท่ากับ 0.543±0.285 mg *Artemia*/gdw/hr และต่ำสุดที่ 0 ug/l มีค่าเท่ากับ 0.422±0.137 mg *Artemia*/gdw/hr จากผลการทดลองพบว่า ค่าพลังงานที่ใช้ในการหายใจและการขับถ่ายมากกว่าค่าพลังงานที่ได้มาจากอาหาร เพราะฉะนั้น อัตราการเจริญเติบโตจึงมีค่าเป็นลบ แสดงให้เห็นว่าในสภาวะที่มีการปนเปื้อนของน้ำมันดิบดูไบกุ้งมีการใช้พลังงานในการซ่อมแซมส่วนที่สึกหรอของร่างกายมากกว่าในการการเจริญเติบโต

ภาควิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อนิสิต .....
สาขาวิชา วิทยาศาสตร์ทางทะเล ลายมือชื่ออาจารย์ที่ปรึกษา .....
ปีการศึกษา 2542 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสีเขียวนี้เพียงแผ่นเดียว

##3971562723 : MAJOR MARINE SCIENCE
KEY WORD: *Penaeus monodon* / PHYSIOLOGICAL RESPONSE / WATER SOLUBLE FRACTION OIL / SALINITY.
WARAWAN SUKRARURK: PHYSIOLOGICAL RESPONSE OF POSTLARVA BLACK TIGER PRAWN *Penaeus monodon* TO COMBINED EFECT OF SALINITY AND WATER SOLUBLE FRACTION OF DUBAI CRUDE OIL.THESIS ADVISOR: ASSOC.PROF.SOMKIAT PIYATIRATITIVORAKUL, Ph.D 71 pp. : ISBN 974-333-140-9

A study of effects of salinity and concentrations of water soluble fraction (WSF) of Dubai crude oil on black tiger prawn *Penaeus monodon* postlarvae was divided into 2 processes; acute toxicity testing and sublethal toxicity testing. The purpose of acute toxicity testing is to determine LC_{50} of WSF to *P.monodon* after exposure of 24,48,72 and 96 hrs. The level of WSF concentrations (100%,75%,50% and 25% of WSF) and salinity (14,21,28 and 35 ppt.) were used for LC_{50} determination. Sublethal toxicity testing was done at 4 levels of salinity (14,21,28 and 35 ppt.) and 4 levels of WSF concentrations (0,20,40 and 60 ug/l) by a completely randomized design involved factorials. *Penaeus monodon* were acclimated for a week before testing. In acute toxicity testing, 96-hr LC_{50} of WSF of Dubai crude oil on *P.monodon* at salinity 14,21,28 and 35 ppt was 81.55, 13.56, 40.65 and 80.05 ug/l, respectively. In the sublethal testing, there were interaction between salinity and concentrations of WSF on oxygen consumption rate, ammonia excretion rate and feeding rate. The highest oxygen consumption rate was 0.008 ± 0.005 mg O_2/gdw/hr found in postlarval shrimp at salinity 14 ppt. and the lowest oxygen consumption rate 0.007 ± 0.006 mg O_2/gdw/hr was found at salinity 35 ppt. Ammonia excretion rate was the highest in salinity 14 ppt., 1.5666 ± 1.222 mg NH_3 /gdw/hr and the lowest ammonia excretion rate was 0.932 ± 0.671 mg NH_3/gdw/hr at salinity 28 ppt. Feeding rate was highest at salinity 14 ppt and lowest at salinity 35 ppt (0.809 ± 0.248 mg dry weight *Artemia*/gdw/hr and 0.354 ± 0.108 mg dry weight *Artemia*/gdw/hr, respectively). For different concentrations of WSF, the feeding rate of *P.monodon* was found the highest value in WSF concentration 20 ug hydrocarbon/l (0.543 ± 0.285 mg dry weight *Artemia*/gdw/hr) and the lowest in 0 ug hydrocarbon/l (0.422 ± 0.137 mg dry weight *Artemia*/gdw/hr), respectively. The result indicated that total energy of prawns received from food was mainly deplenished in respiratory and excretory rather than growth in contamination environment, so that scope for growth (SFG) was lower than zero.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

ภาควิชา วิทยาศาสตร์ทางทะเล ลายมือชื่อนิสิต .....

สาขาวิชา วิทยาศาสตร์ทางทะเล ลายมือชื่ออาจารย์ที่ปรึกษา .....

ปีการศึกษา 2542 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

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CHAPTER I

INTRODUCTION

Petroleum contamination of the marine environment can introduced from varieties of sources., for example, offshore operation, coastal oil refineries, industrial waste and accidental oil spill and shipping mishaps. All of these cases are the possible sources of petroleum contamintaion in the sea. Fauna and flora are affected if they lived in contamination region. Accidental oil spill is the one source that less opportunity to happen but if it occur, many marine organisms are affected. The example case is in 1978 when Amoco Cadiz, 216,000 tons of crude oil were released into the waters of the coast of Brittany (France).

Estuaries and other coastal waters represent areas of prime interest for a multitude of human activities such as navigation, fisheries and sewage disposal. However, it also represent the most productive zone. Many of man's activities in coastal areas and area nearby involve the use of petroleum that also other sources of hydrocarbon contamination in the sea and would harm to animal and flora nearby.

In Thailand, a several accidental oil spill happened and affected mangroves and marine lifes. For example in March 6,1996, an accident of "Visahakit 5"(ESSO standard Thailand ltd.) released 480,000 barrels of Diesel oil to the sea. Other was example is the accident of "Deltasigma" in 1974 released oil at ChoaPhraya river mouth. , "Shintaku" in 1978 , "Vigous Victory" in 1981 and "Navakhun 4" in 1992 accidently oil spill in Thai waters and harmed to many marine organsims (Sarin, 1994) .

The knowledge we obtained for oil pollution today is derived from work carried out in different kinds of coastal waters and in laboratories. Some scientists prefer to study oil pollution and it's effect in real field but it cause more problems on an uncontrolable conditions. In laboratory. Two levels of toxic test; acute and chronic

toxicity test were used to determine effect of toxic substance to organisms that can be possibled used for field comparison.

Presentlty, there is only few information of oil pollution effect on tropical marine organisms especially on some economic Important species. Black tiger prawn *Penaeus monodon* is a suitable presenter of organism that its life stages postlarvae and juvenile live in coastal areas where oil contamination can be occurred . Black tiger shrimp is an economic important shrimp for tropical aquaculture in coastal zone so information of oil contamination on this species may be helpful to farmers. They may use the data to manage and get rid of oil contamination in their farm system.

The objectives of this thesis are:

1. To evaluate toxic level of water soluble fraction (WSF) of Dubai crude oil on physiological effects of black tiger prawn.
2. To calculate the response activities that changed by the combination effects between salinity and concentration of WSF.

LITERATURE REVIEW

Selection of species

Selection of species for toxicity testing depends upon several criteria, such as availability of the species, ability of species to live under laboratory conditions, convenient size and available biological data on biology of the species.(Table I).

A survey of the published literature in toxicity testing indicates that a high percentage of the test organisms are from estuaries and shallow inshore waters(Donald J.,1984). They are convenient to collect in large numbers and their biology was known. The organisms must be of a convenient size. Crustecean were the most sensitivity to toxicants and they have highly adaptable ability in laboratory (Table 1)(Donald J.,1984)

Testing shrimp that selected is Black tiger prawn *Penaeus monodon Fabricius* It's in infraorder Penaeidae. It is a biggest shrimp in genus Penaeus. Their habitats are in Thailand, Taiwan, Malaysia, Indonesia and the Philippines coastal waters. They live throughout tropical zone including the coast and mangrove estuaries. The life cycle of *Penaeus monodon* can be divided into different stages; 6 nauplius stages, 3 zoeal stages and postlarval 5 stages. Postlarva and early juvenile stages was selected becaused they live in area that oil contamination easier to occur.

Petroleum and its characteristic.

Crude oil (Crude oil or petroleum) is a complex substances that compose of carbon and hydrogen more than 97%, which from molecules ranging in size and complexity from methane with one carbon atom and four hydrogen atoms to compound containing more than 50 carbons atoms arranged in straight or branch chains and rings. The other constituents are sulphur and nitrogen. There are 4 types of hydrocarbons classified on properties of the compound. There are aliphatic hydrocarbon, alicyclic hydrocarbon, olefinnic hydrocarbon ad aromatic hydrocarbon.

Table 1 Criteria for selection marine species to use in toxicity testing (Donald J,1984)

Species	A	B	C	D	E	F	G	H.
Poiychaetes								
-catapitella capitata	+	+	+	-	-	+	4	+
-ctenodrillus serratus	2	+	+	-	-	+	4	1
-Neanthes virens	+	+	+	-	3	-	4	+
Pelecypods								
-C.virginica	+	+	+	+	-	-	5	+
-Mytilud edulis	+	+	+	+	-	-	5	+
Crustecean								
-callinectes sapidus	+	+	+	+	-	-	6	+
Homarus americanus	+	+	+	-	+	-	6	+
Palaemonetes pugio	+	+	+	-	-	+	6	+
Fish								
-Mugil cephalus	+	+	+	-	-	-	4	+
-Clevelandia ios	+	+	+	-	-	-	4	+

Notes: A=Ease of collection, B =Convenient size, C= Adaptable laboratory
D=Ecological important, E Econimically valueable, F=Laboratory culture
G=Sensitivity to toxicants, and H= Bioaccumulation
+ / - = Yes/No
2-6= poor to highest sensitivity

หอสมุดกลาง สถาบันวิทยบริการ
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Figure 1 Development stages of Black tiger prawn; *Penaeus monodon* (Motoh,1981)

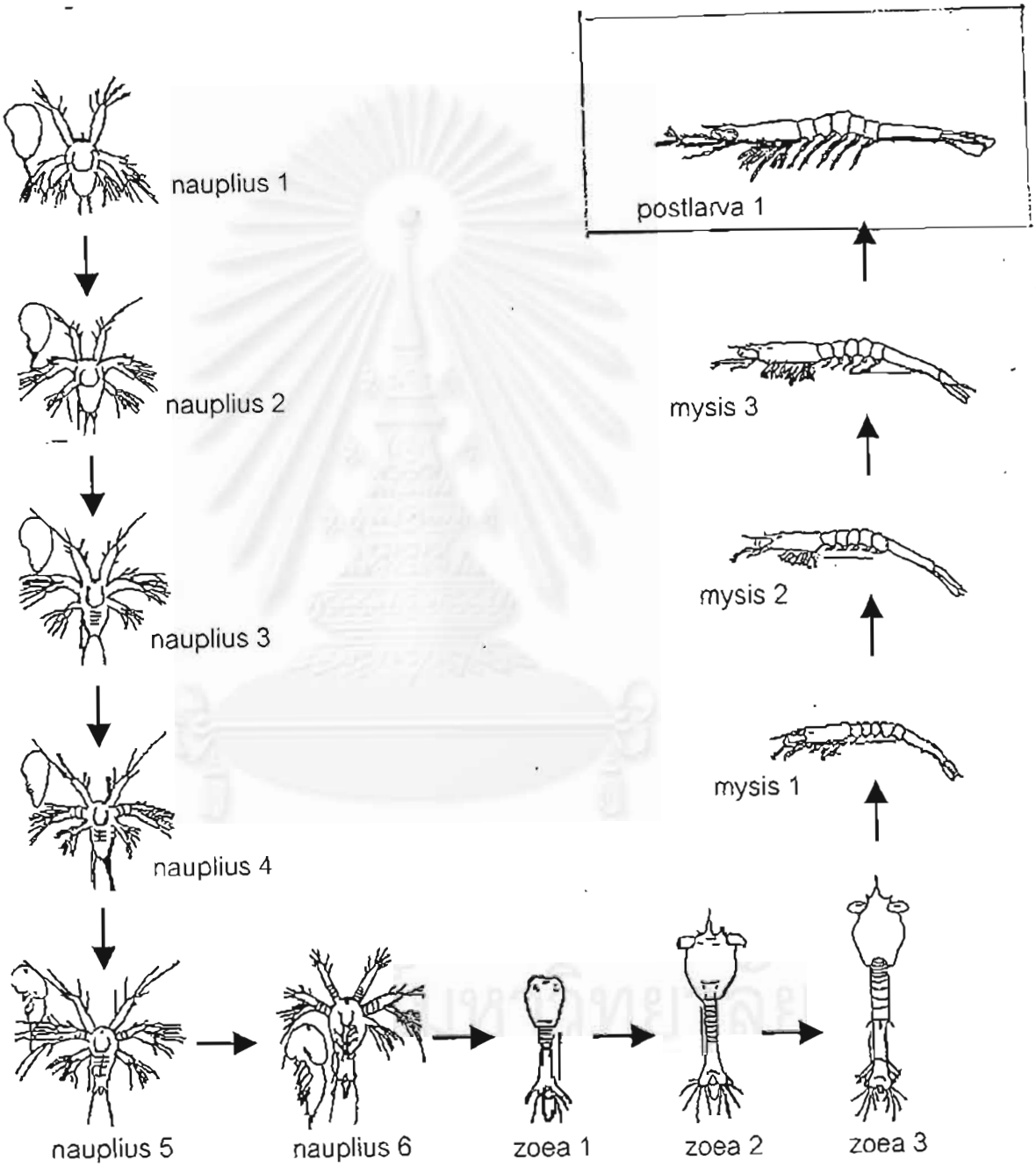
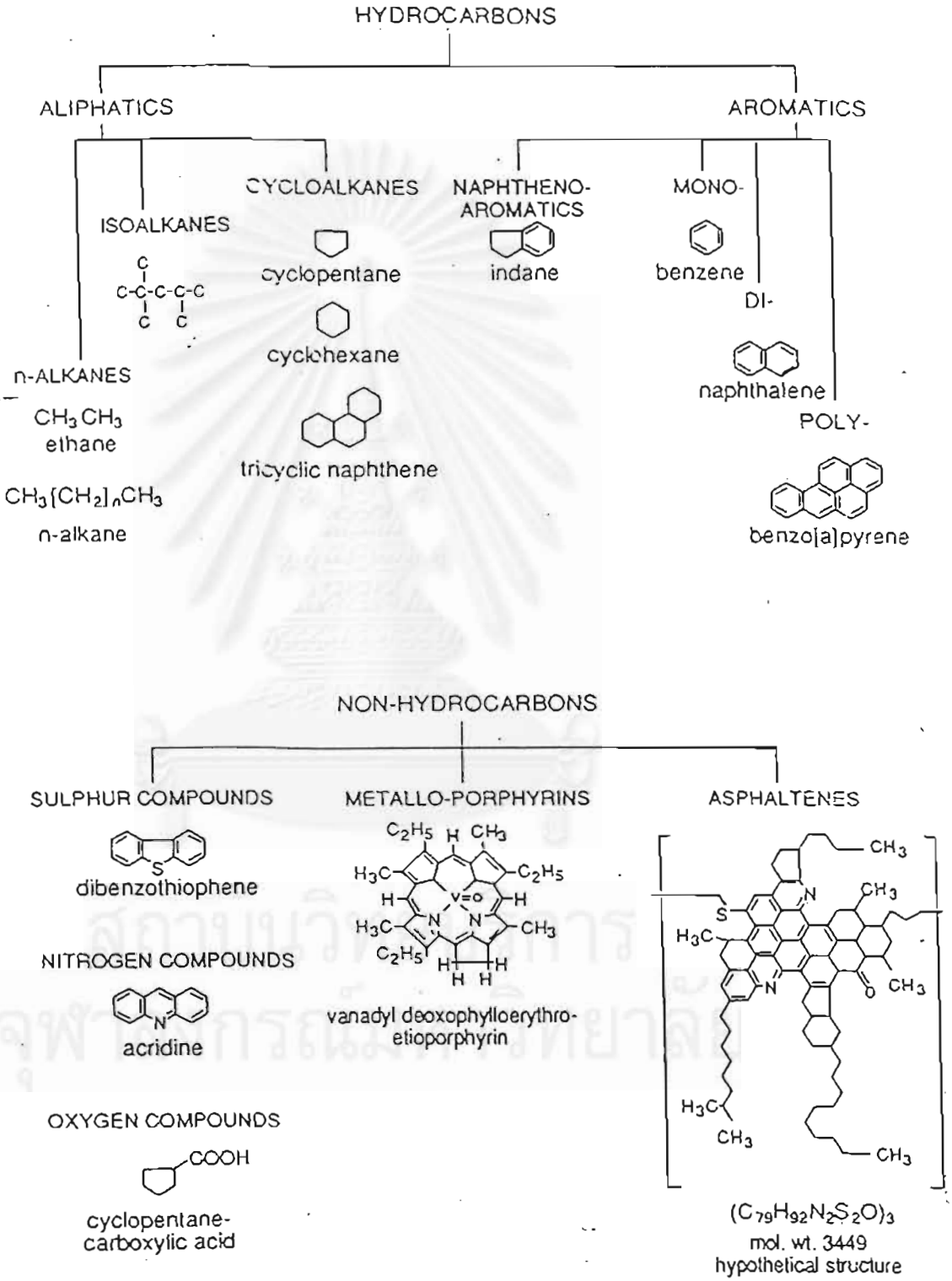


Figure 2 Structure and types of petroleum hydrocarbon (Miller and Connell,1982 cited by Well et.al,1993)



Source of hydrocarbon compounds in marine environment.

There are 2 types of hydrocarbon in marine environment; a natural source and an antropogenic source of hydrocarbon. The latter is the hydrocarbon that entered urbanized estuaries, coastal metropolitan areas and rivers flowing into the sea, finally. The natural sources of hydrocarbon are biogenic and geochemical process hydrocarbon. The geochemical process is original from any process of oil. They are the natural disperse of oil and the natural seep process. The antropogenic hydrocarbon is from the oil spill and petroleum discharge that flow into estuaries from coastal metropolitan areas and rivers. There are a large amounts of petroleum enters estuaries and such areas by transportation, any processes in industries, marine transportation cause oil spill by accident or direct discharge from vessels. Moreover, they are a lot of offshore production and offshore operation in coastal zone area., too. The coastal refineries and coastal municipal and industrial wastes are the major causes of oil spill. Another cause is incomplete combustion process by any vehicle and any machines in factories. Loading estimates give a sense of the magnitude of the problem and depend upon amount of urbanized area, the size of watershed, the flushing and circulation of estuaries. The toxicity of petroleum hydrocarbon is related to the types of oil present and its physical forms.

Once petroleum entering the marine environment, it is subject to form complex process that disperse and degrade the oil. Oil will move and the relative important of major process such as evaporation, mixing due to turbulance and microbial decomposition. Such process can degrade oil and decrease its concentration. There processes are follow: evaporation, emulsifying process, chemical decompostition and microbial decomposition.

Respond level of marine organisms to petroleum hydrocarbon

The response of organisms on contaminated hydrocarbon can be divided into 4 levels; biochemical cellular , organismal, population and community. Each level has types of response and effect to organisms so shown in table x.

Table 2 Respond level of marine organisms to petroleum hydrocarbon (Donald J.,1984)

Level	Types of response	Effects at next level
I. Biochemical-cellular	Impairment of metabolic pathways Detoxification	-Disruption in energetics
II. Organisms	Metabolic changes Behavior changed Increased incidence of disease Reduction in growth and reproduction	-Reduction in performance of populations -Regulation and adaptation of population
III. Population	Changed in population dynamics	-Effects on coexisting organisms and community -No change at community level
IV. Community	Adaptations of populations to stress Changes in species composition Reduced energy flow Ecosystem adaptation	-Detorioration of community -Reduced secondary production -No change in community stability

Impact of hydrocarbon substances on marine environment

Constituents of petroleum vary by the origin of oil and the toxicity of oil depends upon aromatic hydrocarbon and alkane in petroleum. Moreover, the toxicity is depend on the period and stability of oil in marine environment and the evaporation capacity of oil, too.

The level and effect of petroleum on marine organsims can be divided into:

1. The highly toxic level when petroleum cover on any living organisms. The organsim cannot move and breath. The organism will die immediately.

2. The toxic of petroleum that disturbed the metabolisms process of living organisms. The different concentration level of hydrocarbon in living tissue may cause the mulfunctional metabolism and activities.

Toxicity testing

Aquatic toxicology has been defined as a study of effects of chemicals and/or other foreign agents on aquatic organisms with special emphasis on adverse of harmful effects Toxicity tests are used to evaluate the concentrations of the chemical and the duration of exposure required to produce the criterion effect. Effects of chemical may be in such a minor significant to aquatic organisms which is able to carry on its functions in a normal or additional stress conditions.(changes in temperature of salinity.) More effects of chemical substances to living organisms may also result from the interaction of small amounts of some chemicals and large amounts of the other chemicals without any additional stress.

Theorethically, aquatic toxicity tests are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms. Since, these effects are not necessarily harmful, a principal function of the test is to identify chemicals that can have antagonistic effects on aquatic organisms. Moreover, the tests also provide a data base that can be used to access risk strategies in which chemical agent, the organism, and the exposure conditions are defined.

The aquatic toxicity test is typically called a bioassay. An appropriate definition of bioassay is a test to evaluate the relative potency of a chemical by comparing its in vitro effect on a living organism with in a standard preparation. A bioassay is performed to determine the strength of the chemical from the degree of response elicited from the

test organisms, not to estimated the in vivo concentrations of the chemical which is toxic to those organisms.

A toxicity test is then performed to measure the degree of response produced by a specific level of chemical stimulus (Rand and Petrocelli, 1985). A variety of toxicity test methods has been developed by the American Public Health Association(APHA), U.S. Environmental Protection Agency(U.S EPA), American Society for testing Materials (ASTM), and Organization for Economic Cooperation and Development(OECD) to evaluate the hazard and potential toxicity of various materials to aquatic organisms.

The study of the role and effect of toxicants to living organisms.

The study of effect of toxicants on role and functions and process of all activities in marine organisms is divided into 2 levels. There are acute toxicity test and chronic toxicity test. Acute toxicity test is designed to determine and evaluate the acute toxic level or highest concentration of petroleum that made 50% or shrimp suddenly died in arranged time., such as 24,48,72 and 96 hrs. After that, Such value was calculated as LC_{50}.Chronic toxicity test is an experiment that evaluate the level of petroleum that gather in marine organism and bothered any metabolism process. This value is lower than acute level. A study is a sublethal level, on the effects of toxicants that bother any physiological parameters and metabolisms also uses bioassay method .The bioassay method is experiment in toxicology laboratories carefully controlled conditions, selected species are exposed to petroleum at a given concentration.

University of Texas which attempted to define the relative to toxicity of several crude oil and refined oil on sensitivity of a variety of marine animals are found the effects of petroleum that can be summarized as follow:

1. The toxicity of crude from different oil fields and fuel from different refineries vary.
2. The sensitivity of different species to a given oil also varies

3. The form in which the oil is presented to the test organisms is a major determinant of its toxicity. The toxicity of various refined oil and crude oil changes drastically depending upon whether the whole oil or only the water soluble fraction in assayed.

The suitable measurement to measure the response activity is to measure survival rate and we will see the relationship between lethal concentration level and % survival(Brown,1973 and Finney,1971). The result of experiment is show in tolerance limit value of TL. The resistance concentration of exposed animal may survived 50% called TL_{50} or TLm and change this value to LC_{50}. In many reports, Scientists show their result in LC_{50} value at 24,48,72 and 96 hours. In the past US standard method used 48 hr- LC_{50}. value. In present 96 hr- LC_{50}. is well- defined to be used for toxic compasrison .(Sprage,1973). The toxic ranges substances are:

Partially nontoxic	>10,000 mg/l
Slightly toxic	1000-10,000 mg/l
Moderately toxic	100-1000 mg/l
Toxic	1-100 mg/l
Very toxic	< 1 mg/l

The indicator that indicates stress stage of marine organisms that live in contamination environment.

The experiment that concerns with the measurement of the level of contamination will care about the suitability of criteria that is chose to access. Bayne, (1985) listed criteria for accessing physiological response to be used for environmental stress measurement. The criteria are follows:

1. A quantitative relationship should exist between the response and contaminant dose
2. The response should have ecological significant and be shown to be related to an adverse effect on the growth, reproduction or survival of individual.
3. The sensitivity to the contaminant should exist to provide a large scope for response throughout the range of exposure.

4. The response should be measured in laboratory and easily to use a suitable and moderate expensive costs equipment.

In any contamination environments, such as oil spill. A lot of marine organisms are subject in and thus environment is called stress. Marine organisms show an unusal physiological response. The physiological parameter that's changed were respiration rate, excretion rate, feeding rate and absorption efficiency. Finally, all of this parameter were choose to calculate scope for growth(SFG). SFG is an integrated physiological parameter reflecting the energetic balance between process of energy consumption (feeding and absorption) and energy expenditure(metabolism and excretion). The SFG are widely used to evaluated aquatic environmental quality and to test the toxic effects of environmental contaminants. The measurement of SFG are measured in energy form in any metabolism activities that influence growth rate. Each components of parameter is converted into energy equivalents and alternation into growth and production can be described by balance energy equation of Winberg(1960)

$$C = P + R + U + F$$

$$P = A - (R + U)$$

P is an estimated from difference between the energy gains and energy losses

A is the product of energy consumed and the absorption efficiency of energy from food

U is energy loss in excrete

R is respiration energy expenditure.

The SFG is defined as an index of the energy available for growth and Reproduction (Bayne et.al,1985). It's not only provides an integration of the physiological parameters but also an understanding of the impact of environmental stress.

The advantage of using SFG in environmental biomonitoring are

1. SFG provides a sensitive and quantitative of stress and therefore responsive to environmental pollutants.

2. SFG is an early warning indicator of cellular and metabolic responses at the individual level

3. SFG represents cost effectively in both laboratory and field situations.

The bioenergetics approach

Energy is a primary requirement of living organism in order to survive and perpetuate life. It is used for a number of measurable life process, including maintenance, activities, growth and reproduction.

Klekowski and Duncan(1975) defined bioenergetic as the study of energy transformations in living organisms. There are several approaches to bioenergetics which deal with the same events but at different level of biochemical organization. The first is the molecullar-biochemical level, dealing with cells and subcellular structures. At present, this level is the most fundamental since the site of energy transformation is inside the cell. The second level of approach is called the physiological level since it deals with the utilization of energy In the whole organism and the interest is in organism not divorce from their normal habitat of their normal conditions of life. The third level of approach is the ecological level, in which the interest is in energy transformation under simulated field conditions. The basic unit studied in ecological bioenergetic may vary: it may focus on the species throughout it's life cycle, the population, the biocoenosis, the community and ecosystem, single trophic level or a single food chain.

Bioenergetic has focused on estimation of a complete energy budget, especially as influenced by environmental fluctuation, pollution, and diet. In addition, the energy budget has been estimated either in an individual, in different life history stages, in a population of a given species or by ecological modelling(Vernberg,1987).

The impact of crude oil on marine organisms.

Oil spill is the main cause that affect any organisms in the sea. There constituents changed and would harm to marine organisms. The impacts of petroleum hydrocarbon to marine organisms depend on the resistance of such toxicants, the receiving level and the tolerant ability of marine. The impact of oil may influence to metabolism, growth and survivial. Survival rate and fertilization rate may be affected. The impacts of petroleum hydrocarbon to organisms are seperated into 4 levels. The first one is the impact of petroleum hydrocarbon to cell. These cause the unusual of any biochemical process in cell. Secondly, the impact of petroleum hydrocarbon on individuals, any normal behavior and any metabolisms are changed. It's easier to catch any pathogens. Growth rate will decrease and finally die. Thirdly, that impact is influence to population level , these cause the changed in the equilibrium of any ecosystems and organisms will adapt to such stress level. Finally, that impact is influence to community level. A lot of species in that community are changed. The sensitive of organisms to petroleum hydrocarbon is depends on life cycle, species type and forms of petroleum hydrocarbons. In chronic exposure conditions, toxicant will effect the equilibrium of habitats that organisms live. Organisms must adapt and resist in the tolerance conditions or stress conditions. These cause the reduction of fertilization and it's makes the population in constant level. (Capuzzo J.M.,1985)

Related scientific literatures that concerns to impact of oil to marine organisms are reviewed and follow:

There were several papers that support the bioenergetic study approach on marine organisms. Most of studies concern about the impact of environmental contamination on marine organisms. Anderson(1976) reported that the tolerance limit of pollutants in any marine organisms depends on seasons because in winter, animals are gathered lipids more than others seasons so the level of toxicants that solute in lipids are gathering and more. For example in oyster, the level of toxicants are different in alter seasons.

Capuzzo and Lancaster(1982) set the experiment to study the impact of crude oil to the american lobster *Homarus americanus* by compare the respiration rate ,ammonia excretion rate and O:N ratio. They found that Both of the control group and treatment group are different. In control group, the respiration rate at 24 ht is 2.3 UgO2/hr/mg dry wt. and constant after 72 hrs. In oil treatment group, respiration rate at 24 hr was 3.5 ugO2/hr/mg dry wt. These higher than 24 hr. About the excretion rate, In control group at 24 hr. The rate is 0.15 ug-NH4/mg/dry wt. But this rate in test group is 0.18 ug-NH4/mg/dry wt. The excretion rate at 72 hr. is higher than in test group but the control group it's constant. The excretion rate at 72 hr. is 0.23 ug-NH_3/mg dry wt.

The physiological rates of marine organisms were changed because of pollutants contaminated in their environments. The main physiological rates that were changed are oxygen consumption rate, excretion rate, feeding rate and so they were observed as the main changed in this experiment too. Metabolism were changed when compared with the control group that have no pollutants. Edward(1978) study the metabolism of shrimp *cragon cragon* that mix with water soluble fraction of oil. He found that the growth rate and respiration rate are decreased when the concentration is higher.

The study of the changes of energy in any metabolism of organisms in contamination conditions are called bioenergetic study by measurement any activities and SFG indicator.(Warren,1971 ; Bayne et.al,1979; Cole,1979)

Malins and Hodgins(1981) studied the toxicity of WSF of Cook Inlet crude oil and No.2 fuel oil to 39 types of animal in subarctic alsaska by using bioassay techique , they found that the organisms in sensitive group that sensitive to WSF of this oil have TLm value in range 1-3 mg/l. The intermediate resistance organisms will have 16-hr TLm in range 3-8 mg/l. The toxicity of WSF of No.2 fuel oil is higher than the toxicity of WSF of Cook Inlet crude oil.

John and Miller(1982) studied the bioenergetics of crab *cancer irroratus* in the variety of copper ad cadmium solution. LC50 at 96 hr were measured. The control experiment of copper solution is 100 ppb. And cadmium solution is 250 ppb. Another experiment ,the concentration of copper and cadmium are various from 3.3 ppb to 220 ppb. . The respiration rate of crab was different between control and treatment.

Lindane (1978) studied the impact of WSF of crude oil and distilled oil that pass distillation process. He found that crude oil is effect the growth of larva by delay the growth and development of larva such as fish larva of Baltic herring *Clupea harengus.*

The impact of oil and water soluble fraction of crude oil on shrimp were studied. Respiration rate, excretion rate, feeding rate and growth rate were measured. Any indicator that refered the contamination level were measured and calcualte., too.

Stickle , Kapper , Shirley and Rice (1987) set the experiment to studied impact of Cook Inlet crude oil on *Pandalus borealis.* Respiration rate, growth rate were measured and follow the behavior and calculate SFG. They found that LC_{50} was decreased after 2 days. This LC_{50} is decreased from 1200 ppb. to 500 ppb. After 2 days, Total energy intake is decreased after 28 days from 100 J-g- wet w/gt/day. When the aromatic hydrocarbon is 50 ppb. And continuely decreased to 40 J-g- wet w/gt/day. O:N ratio at 28 days is 13.37 higher than O:N ratio at 56 days that is 10.77 and higher than O:N ratio at 84 days(10.08). They conclude that the level of concentration and contamination were influence to the resistance and metabolism process of shrimp *Pandalus borealis.*

Wang and Stickle(1987) ,measured the tolerance limit and metabolism activities and growth of crab *Callinectes sapidus.* that exposed in WSF of South Lousiana crude oil. The LC50 at 7 days is 4501 ppb. and decreased to 3927 ppb. at 21 days. Energy intake varied with the concentration of aromatic hydrocarbon and it's higher than all energy that received in control group. The SFG at 21 days exposure to the concentration

of WSF at 820,1476 and 2504 ppb. were 67,50 and 30 % of SFG in control ,respectively. The SFG value in control is 125 ppb. The SFG value was still decreased. When the aromatic hydrocarbon concentration is 3000 ppb result in the SFG is 50 ppb. They concluded that the SFG were varied with the concentration of petroleum hydrocarbon in the same trend.

Victor and Jennifier (1987) studied the bioenergetic of mussel *Venus verrunosa* that exposed to crude oil for 145 days. They found that the feeding rate was varied with time . When time was increased, feeding rate increased, too. The feeding rate at 63 days, 105 days and 145 days were 65%, 72% and 80%, respectively. The SFG that measured at 27 days that compared between control and treatment group were 2 and 2.9 J/h/g, respectively. In treatment group, SFG is decreased when compared with control.

In Thailand there were a lot of literatures that studied about petroleum contamination and their effects but there were few that studied and demonstrated the toxicity of petroleum hydrocarbon and toxictiy of WSF to marine organisms. Bioenergetics studied were few. The literatures review about them are follow:

Siravajanakul V.(1999) studied the accumulate of petroleum hydrocarbon in mussel and oyster tissue at Ang-Sila ,Srichang Sriracha ,Chonburi province by extracted and analyzed then using Gas chromatography method. The result found a contamination of petroleum contamination in the tissue of both organisms . The amount of contamination were in range 34.97 to 74.08 ug/g dry weight. It is safety for consumer.

Phetongkum (1979) sets an experiment to calculate the acute toxicity level of crude oil, diesel oil and benzene oil to shrimp *Penaues marguensis*. He found that the acute toxicity level that killed 50% of test shrimp at 96 hr or 96 hr- LC50 were different at alter temperature .There were 0.54, 0.78, and 0.74 in 23, 28 and 33 C., respectively.

Popongvivat (1994) measured the O:N ratio in green mussel *Perna viridis* by calculate respiration rate, excretion rate and feeding rate. This rates have been compared between control group and treatment group in different salinity. The excretion rate increased with salinity . O:N ratio in control at salinity 20 ppt is 72.7. This is the max value. But in treatment, the concentration in 25 %WSF, the O:N ratio was in range 45.4 to 46.2.

CHAPTER II

MATERIAL AND METHODS

Testing animal

Black tiger prawn *Penaeus monodon* Fabricius postlarvae with an average length of 5.50 mm were used in this experiment. They were in between moderately postlarval stage and early juvenile stage. The testing shrimp were taken from shrimp farms in Chonburi and Chachengsao province. After acclimating for 2 days in the designed salinty . The shrimp were used to further experiments.

The acute toxicity testing used the shrimp from Chonburi culture farm and the sublethal toxicitty testing used the shrimp from Chachengsoa culture farm.

Seawater

The sea water that used were prepared from high salinity seawater preserved tank at department of Marine Science, Chulalongkorn University. Salinity 14, 21, 28 and 35 ppt. were used in the experiments.

Oil chemical

Oil used in the experiment was Dubai crude oil from Bang – Jak Oil terminal, Bangkok

Laboratory Procedures

1. Preparation of water soluble fraction of crude oil (WSF)

Dubai crude oil was mixed with any level of seawaters of different salinity; the crude oil mixed with 14 ppt. seawater, 21 ppt seawater , 28 ppt seawater and 35 ppt. seawater. Water soluble fraction (WSF) of Dubai oil were prepared by mixed 1 part of Dubai crude oil with 9 part of seawater in each salinity and stirred them for 24 hours by magnetic stirrer. After 24 hr, magnetic stirrer were turn off and letting it settling for 10 minutes then seperated layer was observed. The lower part of solution was pipetted to 250 ml flask to

be the 100%WSF (Stickle,1987). After that the 100% WSF was diluted in each salinity to 75%, 50%, and 25% by added seawater that was the same salinity of its source. All diluted WSF were analyzed to determined concentrations of petroleum hydrocarbon content.

2. Volume reducing by Rotary evaporator

After added 50 ml of 100% WSF with 50 ml of hexane and mixed together by shaking for 5 minutes and settling for 5 minutes . The lower part was rinsed to 250cm^3 beaker and add 5 mg ash of sodium thiosulphate and shaked well. Rotary evaporator was used to reduced volume. The suitable temperature was in range 50$\pm$3 degree celcious. The reducing time was about 40 minutes to 1 hour. The solution was reduced until nearly 5 ml then the sample was analyzed for petroleum hydrocarbon concentration using fluorescent spectrophotometer LS 50 B.

3. Acute toxicity testing

The experiment procedures are follow:

3.1 Set the level of salinity and concentration

The volume of testing bottle was 750 cm^3.The salinity of seawater used in the experiment was 14, 21, 28 and 35 ppt. The level of petroleum hydrocarbon concentration used in acute toxicity test on postlarvae of *P.monodon* were 100% WSF, 75% WSF, 50% WSF and 25% WSF. The experiment was run for 24, 48, 72 and 96 hrs. Every 6 hours, shrimp in each experiment unit was observed to determined any dead shrimp. The suitable volume of seawater in the bottle and suitable amount of WSF were calculated by $M_1V_1=M_2V_2$ formula. A control was used to determine degradation of WSF at different period of the experiment.

3.2 Finding LC_{50}(median lethal concentration)

Same size of black tiger prawn was selected to exposing in the 750 cm^3 bottle. Six prawns were exposed in different salinity and different concentration of WSF for 96 hrs. After exposing shrimp for 24, 48, 72 and 96 hr, died shrimp were counted and

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recorded. *P.monodon* postlarvae were acclimated for 2 days before testing and they were fed with artemia and shrimps 's commercial feed. Each treatment was done in tripicate. The recorded data were in percent mortality and used for LC_{50} determination.

3.3 Physiological response measurment

The concentration of WSF of oil that lower than 96-hr LC_{50} were selected for used in sublethal toxicity testing . The concentration of WSF at 0, 20, 40, 60 mg/l hydrocarbon in WSF were selected of the test.. The experiment was done in 750 cm^3 testing bottle. Each of treatment unit contained 6 *P.monodon* postlarvae. The rearing period was 30 days in a CRD involved factorial consisted of 4 levels of WSF(0, 20, 40 and 60 ug hydrocarbon /l) and 4 levels of salinities (14, 21 ,28 and 35 ppt.). Every 2 days the same condition in each treatment unit was renew and any dead shrimp were removed. Each treatment unit was provided with small cur buble to ensure enough of dissolved oxygen. Feeding of the shrimp was done 3 times a day. After acclimating the shrimp for 7 days, measurement of physiological respopnse in each experimental combination of salinity and WSF was done by the following techiques.

14 ppt

6 shrimps SW14ppt. Conc.0	6 shrimps SW14 ppt. Conc.20	6 shrimps SW 14ppt. Conc.40	6 shrimps SW14 ppt. Conc.60

There are 3 replicates.

21 ppt.

6 shrimps SW 21ppt. Conc.0	6 shrimps SW21 ppt. Conc.20	6 shrimps SW 21ppt. Conc.40	6 shrimps SW 21ppt. Conc.60

There are 3 replicates.

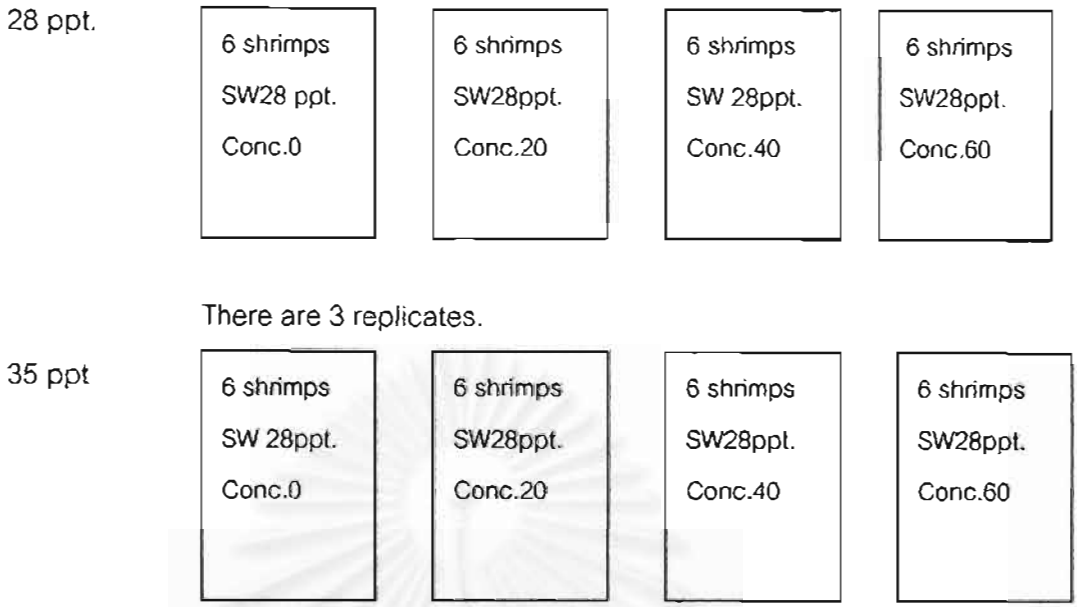

Figure 3 The experiment chart show the experiment of sublethal toxicity testing. Salinity 4 level:14,21,28and 35 ppt. Concentration in Water soluble fraction of oil were 0,20,40,60 ug/l

The physiological response measurement are follow:

1. Respiration rate An oxygen consumption rate of tesing P.monodon in one hour were measured by using BOD bottle techique. Six shrimp were transferred into filtered seawater with the same salinity ans WSF in a BOD bottle in room temperature. After one hour, the water was fixed and then dissolved oxygen in each bottle was determined using Strickland and Parsons (1972) and The shrimp in each BOD bottle was collected to determined their dry weight and then respiration rate of the shrimp was calculated as:

$$\text{Respiration rate (mg O2/gdw/hr)} = O_2t_2\text{-}O_1t_1 / t_2\text{-}t_1$$

Where,

O_2 = the amount of oxygen (mg)

T_1 = the beginning of time and

T_2 = the end of time

2. Excretion rate This rate was measured by the amount of ammonia in NH_3-N form that excreted by testing shrimp in the chamber after 1 hr. Techique of the experiment was similar to the our explained for respiration rate. Seawater in each treatment were took to measure the amount of ammonia by Strickland and Parsons (1972) ammonia measurement method. Spectrophotometer was used to measured ammonia by compare with standard curve and calculated by excretion rate comparing to shrimp dry weight..

3. Feeding rate Feeding rate was determined by using the proportion of food that testing shrimp ate during 1 hr. The amount of *Artemia* at the beginning of time to the amount of *Artemia* at the end of wxperiment was used to dalculated feed uptake of the shrimp. The test was done in 1 hr and then fixed in formalin, the different number of Artemia then was used for feeding rate calculation:

Feeding rate (Cr) =V(logeC1 - logeC2) / t

Where,

V= volume of water in chamber

C1 and C2= the concentration of *Artemia* at beginning and end of time interval t

t= time in the experiment (1 hr)

4. Absorption efficiency(Ab) The efficiency of food absorption in testing shrimp was calculated using Widdow (1984). The method was sone as follow:

1. Filter paper GF/C was weighted after oven drying at 65 degree celcious for 2 hours

2. This filter papers were used to filter faeces of tesing shrimp each chamber of treatment by using Vacuum Pump and took this filter papers into the oven to drying again at 60 degree celcious then took them to burn in Muffler Furnance machine for 2 hours per one treatment
3. The filtered paper GF/C were weighted again and calculate the absorption efficiency by using Widdow(1984)

$$\text{Absorption efficiency} = (F - E)/ (1 - E)F$$

F = ash free dry weight of food / dry weight of food and

E = she free dry weight of faeces / dry weight of faeces

5. Scope for growth

Scope for growth (SFG) is an indicator to estimate and assessment of growth efficiency and reproductive efficiency of test animal was calculated using energy balance equation . SFG is in energy form by changing and apply any physiological rate parameters into energy budget.

$$SFG = P = A-(R+U)$$

A is feeding rate * absorption efficiency

R is respiration rate

U is ammonia excretion rate

Each parameters above was measured every day at the end at day. But after that measured these parameters on the 11^{th},14^{th},17^{th},21^{th},24^{th},28^{th} and 30^{th} of the experiment in each treatment combination.

Statistical Analysis

All statistical analyzes were performed using the Statistical Analysis System(SAS

Institute. Data on the telerance *of P.monodon* to WSF of Dubai crude oil were analyzed and used probit analysis and are presented as the LC_{50} ± 95% confiducial limits (Silverstone,1975). All physiological rate functions were standardized to weight specific rates. Scope for growth was calculated by integration the various energy budget components of individuals rather than means of treatment group. Analysis of variance was used to analyzed variation among treatments and Duncan's Multiple Range test was used to identify significant difference between treatments.

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CHAPTER III

RESULTS

The results of the experiment were described in 3 parts as follows:

1. Concentration of Water soluble fraction (WSF) of Dubai oil that measured by UV-fluorescent Spectrophotometer LS-50B

The standard curve was plotted between hydrocarbon in standard chrysene and fluorescent intensity shown in figure 4. This relationship was used to determined concentrations of petroleum hydrocarbon of percent WSF in various salinity. The result was shown in table 3.

The higher percent of WSF, the higher petroleum hydrocarbon concentration was found in each salinity. The highest petroleum hydrocarbon was in 100% WSF at salinity 21 ppt and the lowest LC_{50} was in 25%WSF at salinity 21 ppt, too.

2. LC_{50} of WSF of Dubai crude oil on *P.monodon* postlarvae

LC_{50} data came from the percent mortality and plotted data between probit scale and log of WSF concentration so LC_{50} values are discovered. All percent mortality were shown in table 4.

The LC_{50} of WSF of Dubai oil to *Penaeus monodon* were shown in table 5. The 24-hr LC_{50} is the highest LC_{50} value and usually higher than 48-hr LC_{50}, 72-hr LC_{50} and 96-hr LC_{50}.

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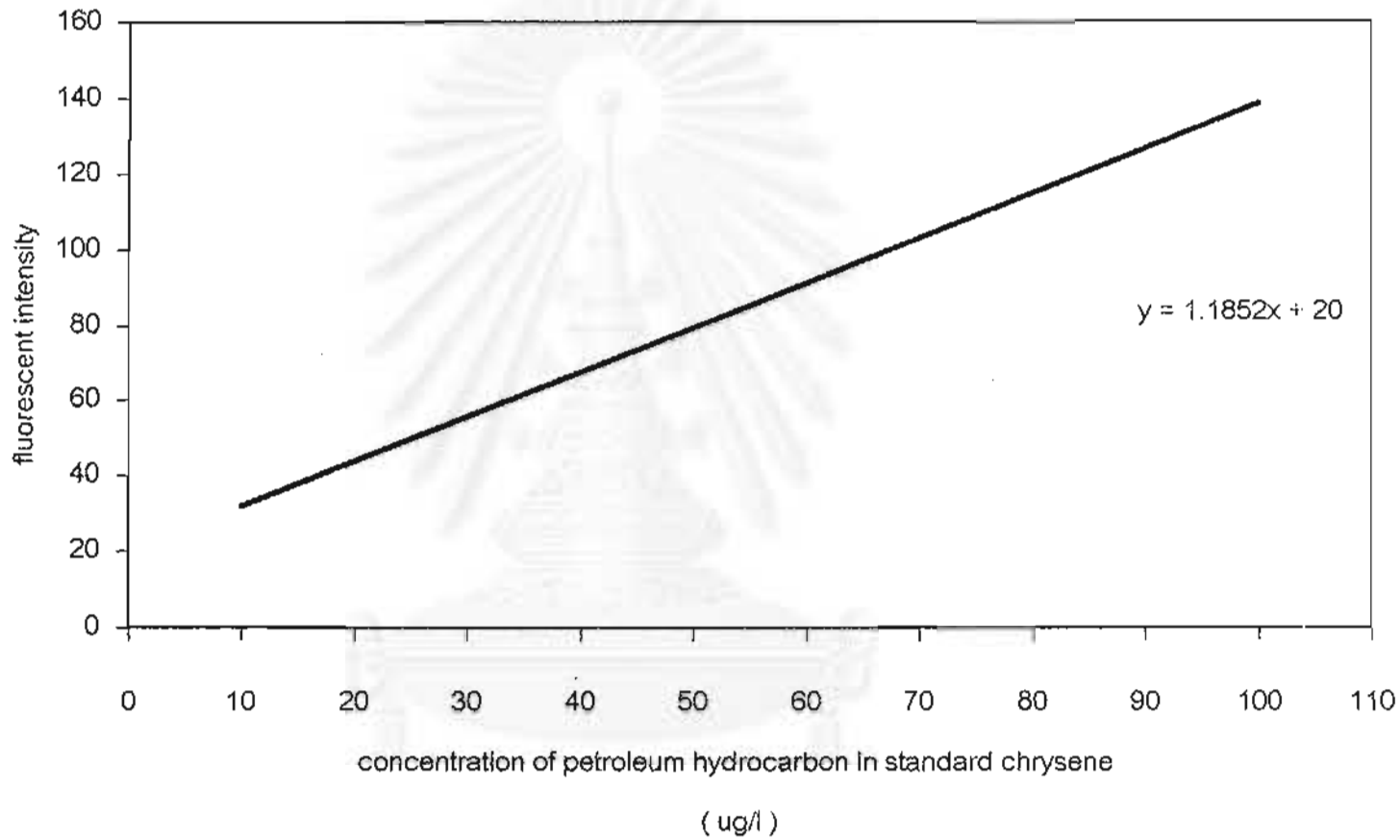

Figure 4 Relationship between concentration of hydrocarbon in standard chrysene andfluorescent Intensity that measured by fluoorescent Spectrophotometer LS50B(excitation wavelength=360 nm. and emission wavelength=310 nm)

Table 3 The concentration of petroleum hydrocarbon(ug/l) in water soluble fraction(WSF) of Dubai crude oil that measured by Fluorescent Spectrophotometer LS 50B Perkin Elmer

Salinity	%WSF and dilution	concentration of petroleum hydrocarbon in WSF(ug/l)
14	100	111.586
	75	107.379
	50	94.666
	25	80.828
21	100	169.947
	75	163.686
	50	117.358
	25	13.097
28	100	134.109
	75	74.703
	50	72.504
	25	44.249
35	100	101.895
	75	91.041
	50	88.334
	25	82.233

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Table 4 The percent mortality of *Penaeus monodon* postlarva exposed to different salinity and concentrations of water soluble fraction(WSF) of Dubai oil during 24,48,72 and 96 hrs.

Salinity (ppt.)	Concentration of WSF (%)	% mortality at 24 hr.	% mortality at 48 hr.	% mortality at 72 hr.	% mortality at 96 hr.
14	100	50.00	66.67	100.00	100.00
	75	33.33	50.00	100.00	100.00
	50	33.33	50.00	83.33	83.33
	25	16.67	16.67	50.00	50.00
21	100	50.00	83.33	100.00	100.00
	75	33.33	66.67	66.67	83.33
	50	16.67	66.67	83.33	83.33
	25	16.67	33.33	33.33	50.00
28	100	50.00	83.33	100.00	100.00
	75	33.33	50.00	100.00	100.00
	50	33.33	66.67	100.00	100.00
	25	16.67	33.33	66.67	66.67
35	100	33.33	83.33	83.33	100.00
	75	16.67	83.33	66.67	83.33
	50	16.67	50.00	50.00	66.67
	25	0.00	16.67	33.33	66.67

At 24 hr of salinity 14 ppt., the percent mortality of testing shrimp; *P.monodon* in 100% WSF was higher than 75%,50% and 25% WSF. This trend was the same as salinity 21 ppt.,28 ppt and 35 ppt. The percent mortality of shrimp in 100% WSF is usually higher than 75%,50% and 25% in every salinity. The highest value is 100%.It's means there are completely died in such treatment. At 72 hr, *P.monodon* exposed in salinity 14,21 and 28 ppt are completely died(100%). But at salinity 35 ppt, they are completely died after 96 hr.

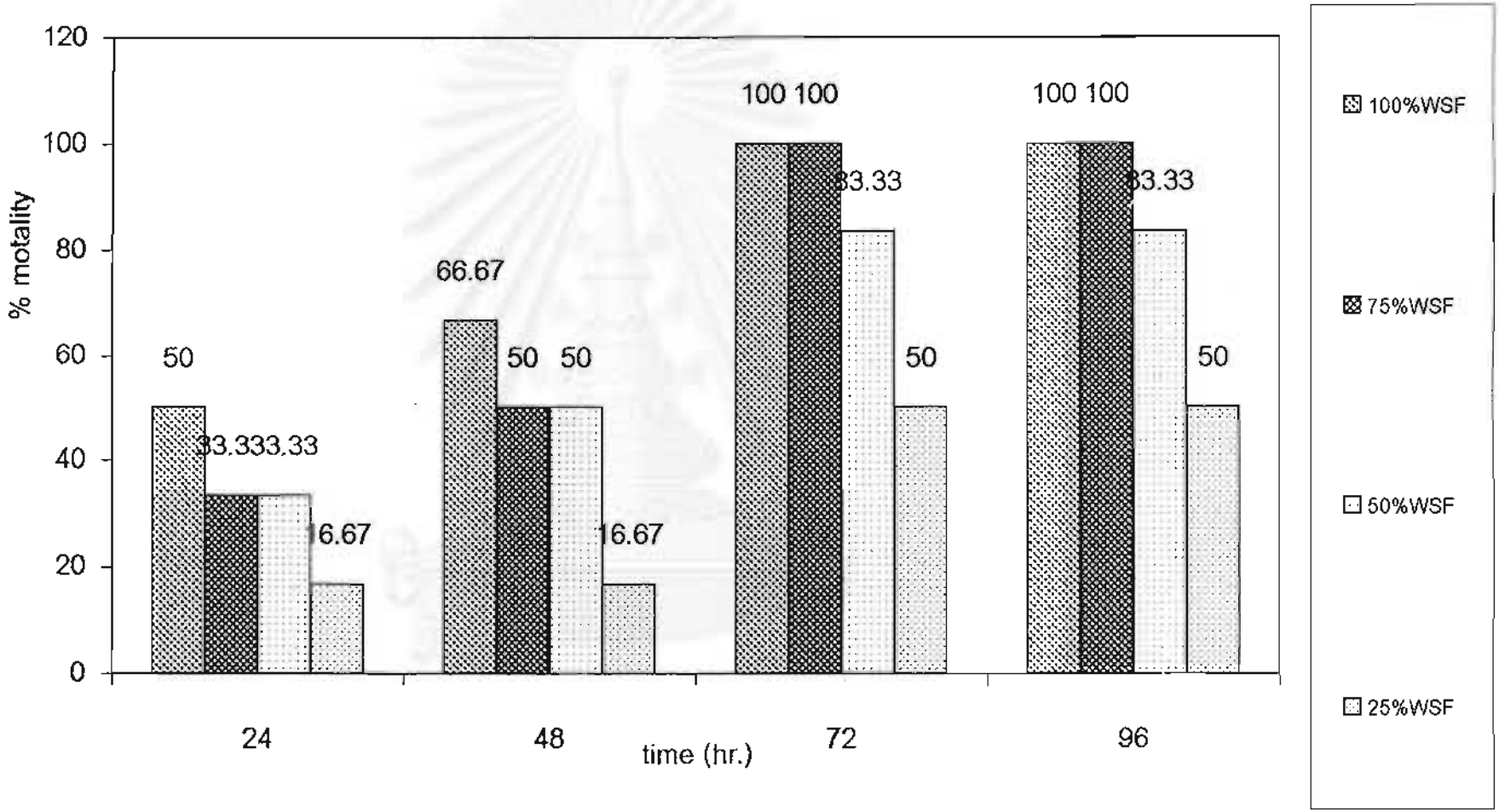

Figure 5 Mortality of *Penaeus monodon* postlarva exposed to different concentrations of Water soluble fraction(WSF) of Dubai oil(ug/l) during 24,48,72 and 96 hrs. at salinity 14 ppt

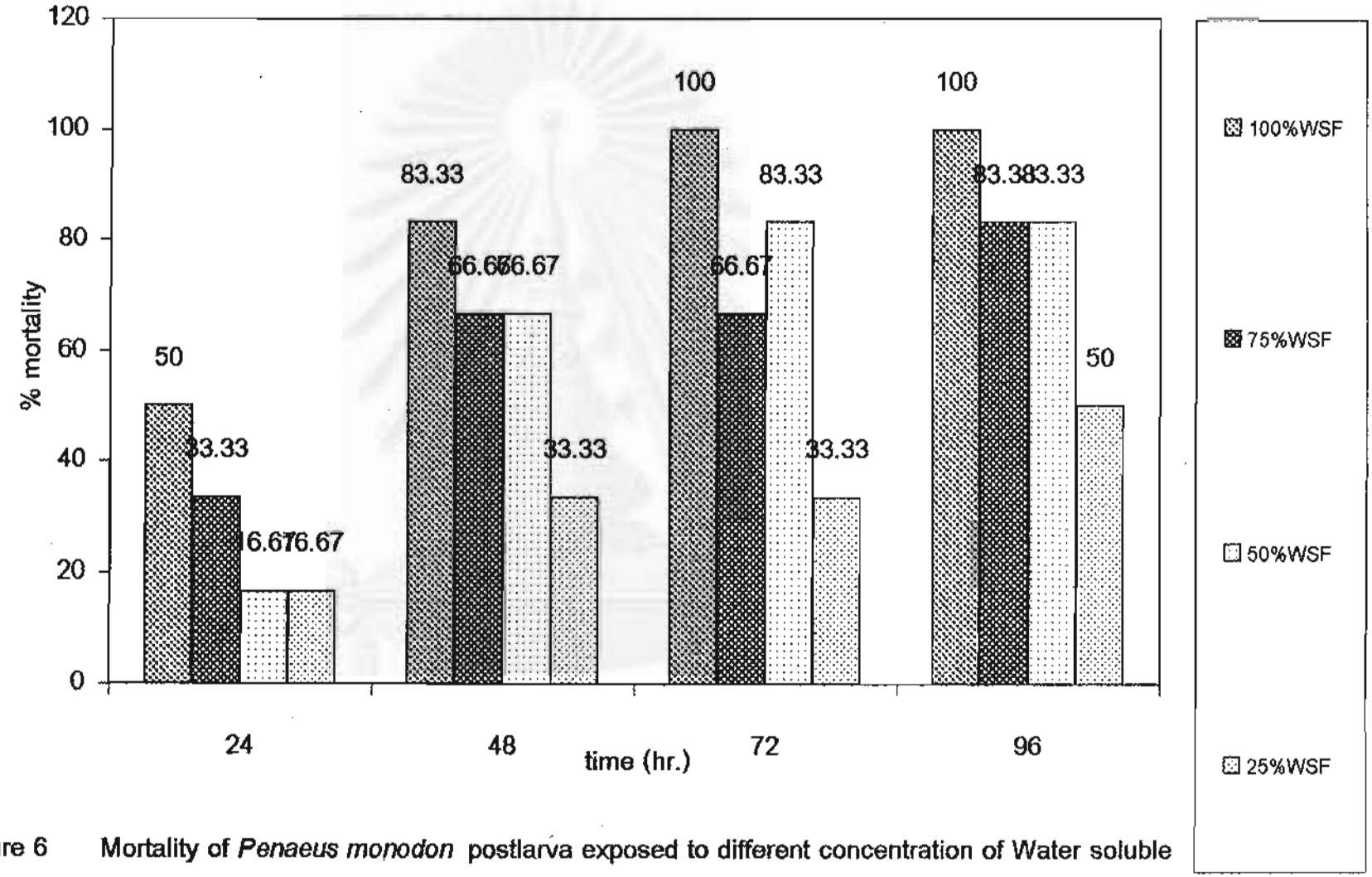

Figure 6 Mortality of *Penaeus monodon* postlarva exposed to different concentration of Water soluble fraction of Dubai oil during 24,48,72 and 96hrs. at salinity 21 ppt.

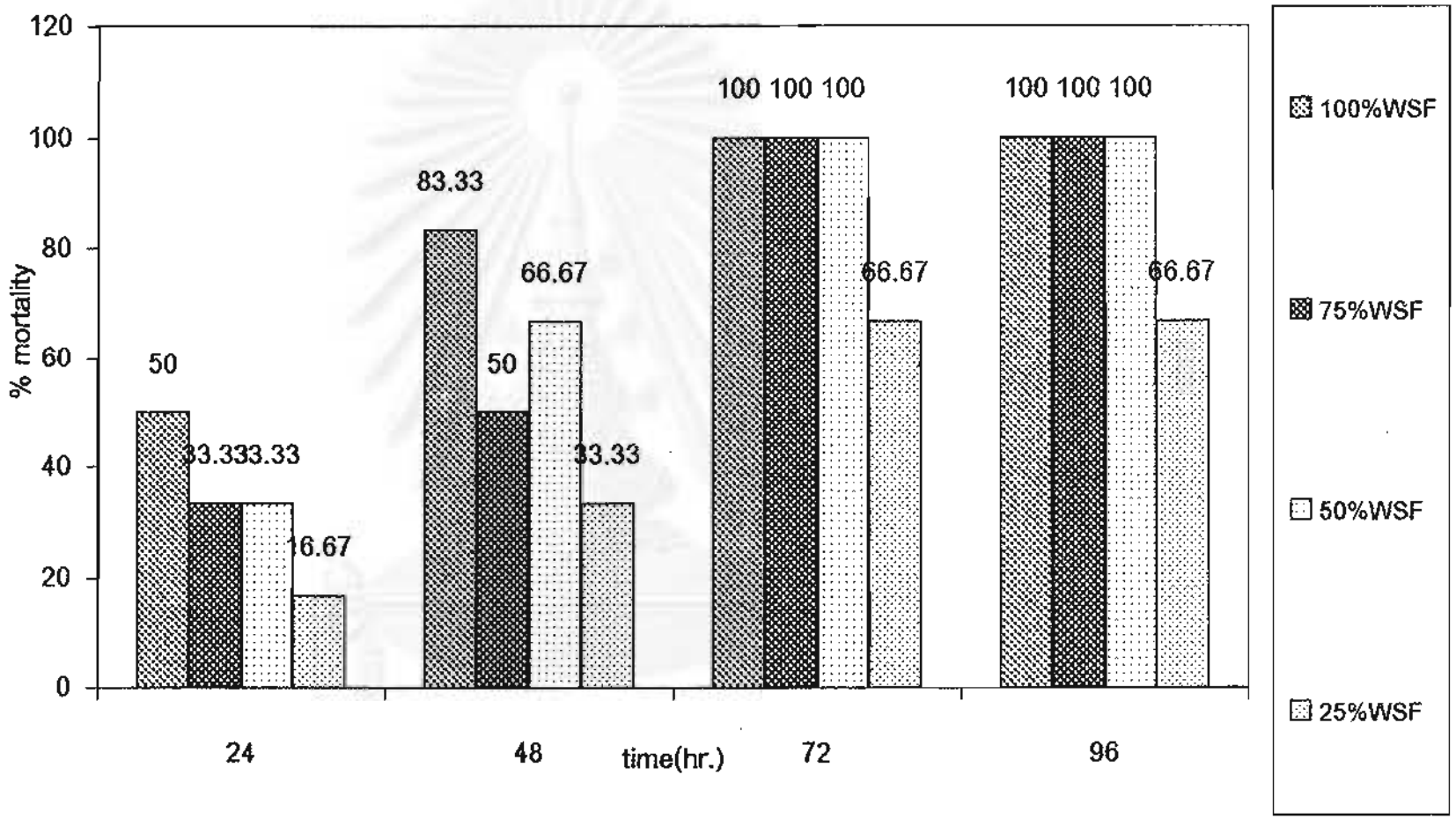

Figure 7 Mortality of *Penaeus monodo* n exposed in different concentrations of Water soluble fraction of Dubai oil during 24,48,72 and 96 hrs. at salinity 28 ppt.

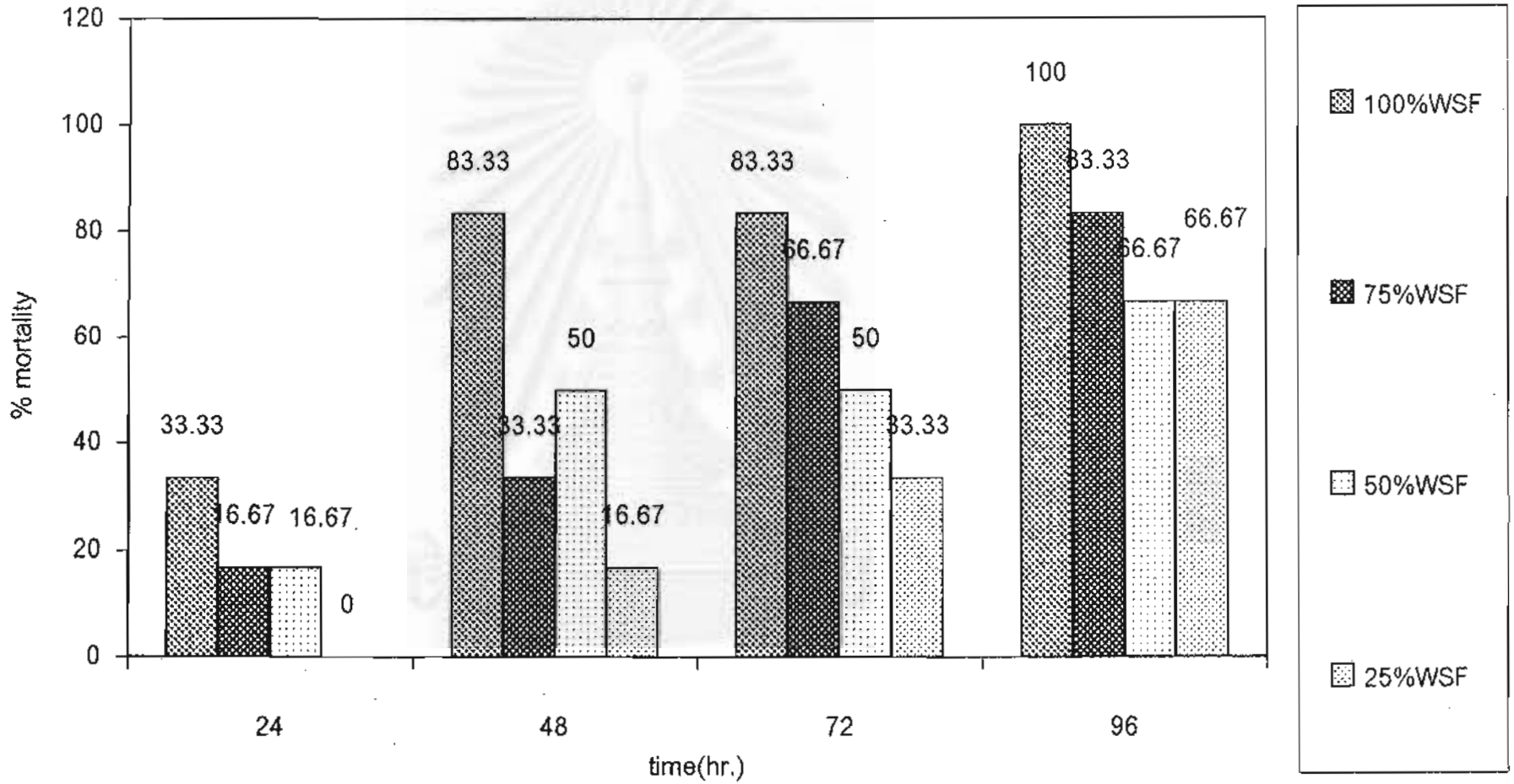

Figure 8 Mortality of *Penaeus monodon* postlarva exposed in different concentration of Water soluble frac tion of Dubal oil during 24,48,72 and 96 hrs.at salinity 35 ppt.

Table 5 The LC_{50} value of Water soluble fraction(WSF) of Dubai crude oil to *Penaeus monodon* postlarva that exposed during 24,48,72 and 96 hrs.(LC_{50} is the lethal concentration of pollutant that killed 50% of shrimp)

Salinity ppt.	LC_{50} at 24 hr. (ug/l)	LC_{50} at 48 hr. (ug/l)	LC_{50} at 72 hr. (ug/l)	LC_{50} at 96 hr. (ug/l)
14	117.409	101.257	81.548	81.548
21	712.713	36.387	27.425	13.555
28	106.064	62.176	40.651	40.651
35	107.382	91.764	87.341	80.054

The most highest LC_{50} (712.71 ug hydrocarbon/l) was in 24 hr of salinity 21 ppt. These was 712.713. The lowest LC_{50} was 96 hr-LC_{50} at salinity 21 ppt. The 96 hr-LC_{50} was 13.555.

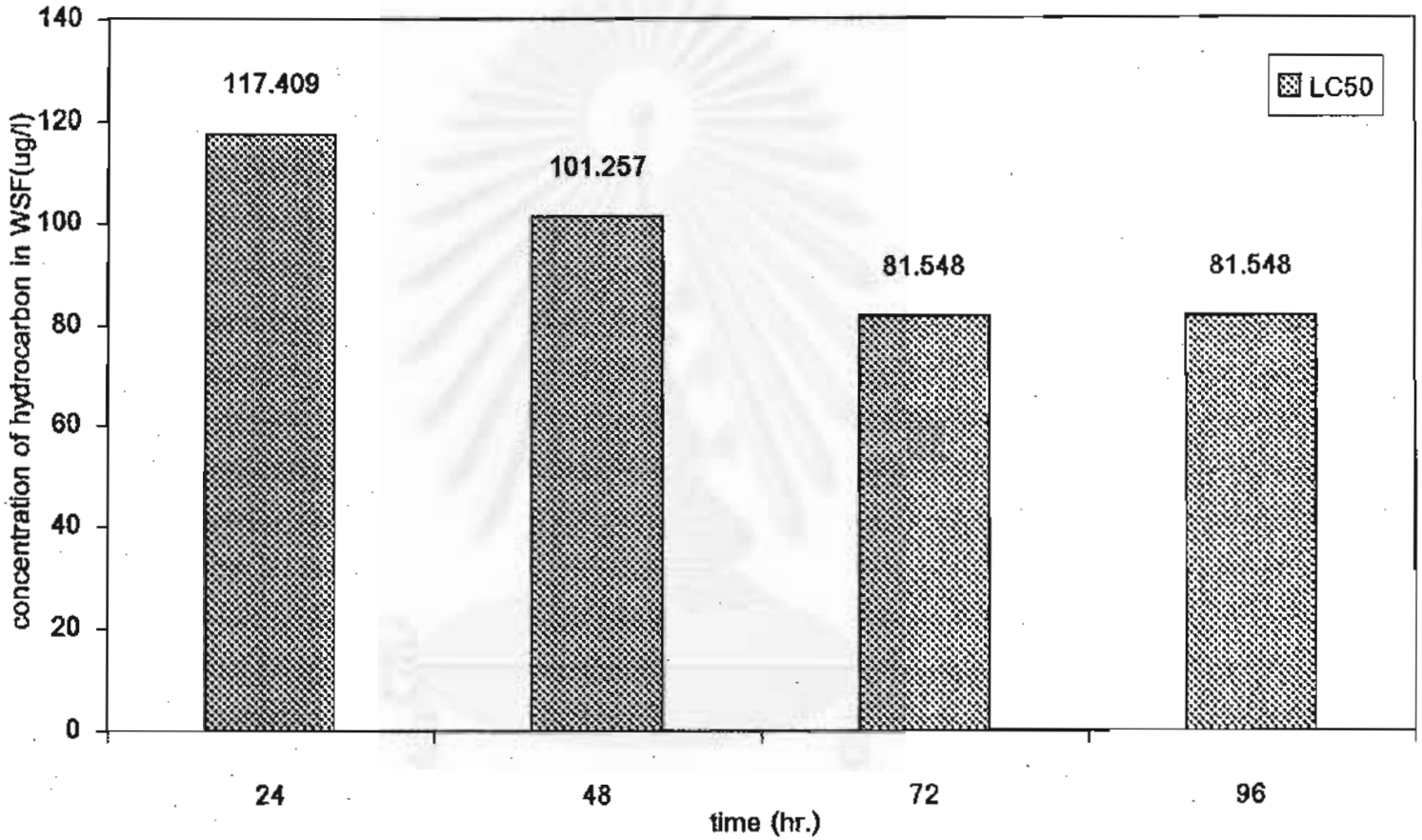

Figure 9 LC50 of Water soluble fraction of Dubai crude oil that *Penaeus monodon* exposed during 24,48,72,96 hrs.at salinity 14 ppt.

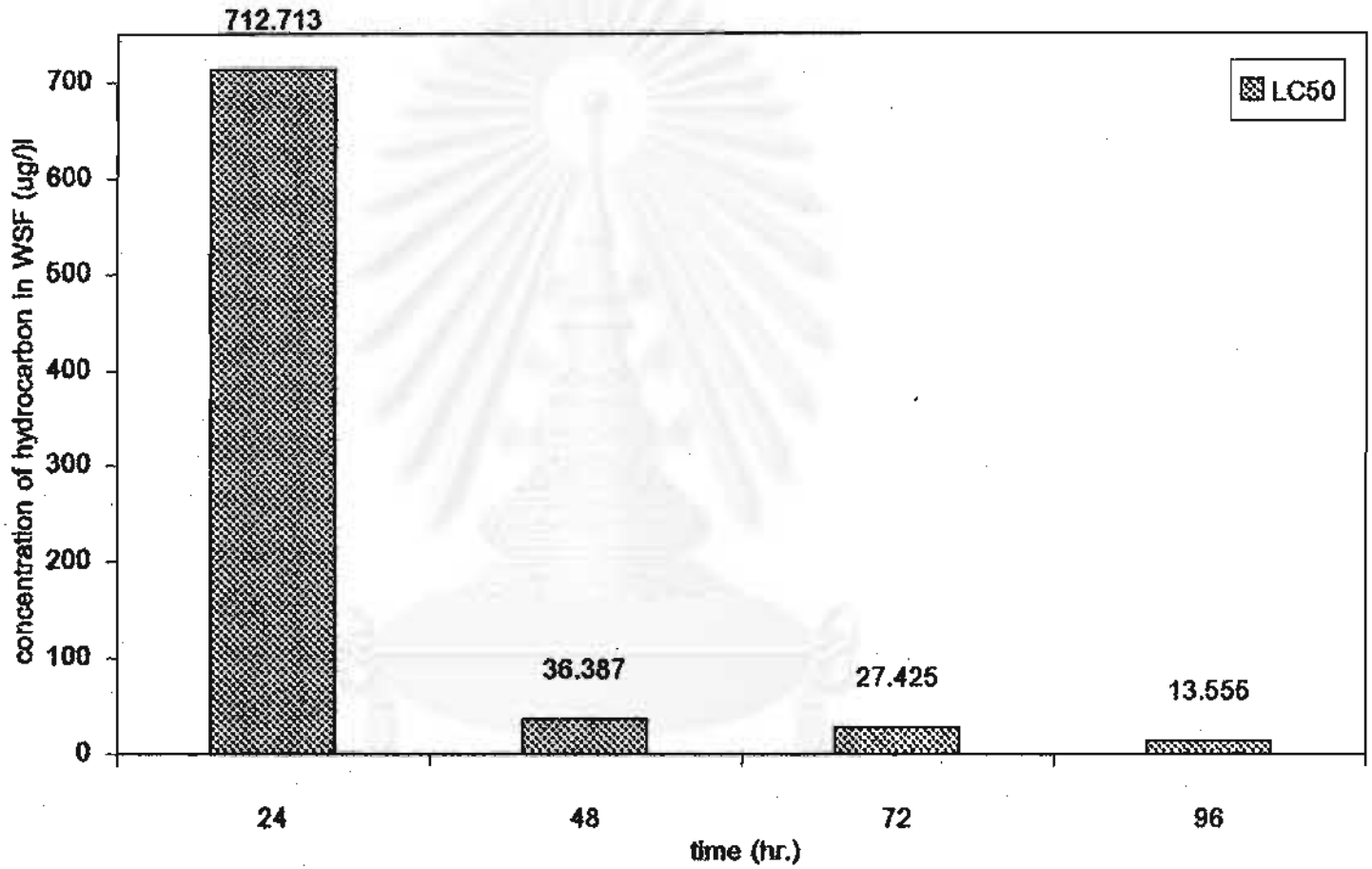

Figure 10 LC50 of Water soluble fraction of Dubai oil that *Penaeus monodon* exposed during 24,48,72 and 96 hrs.at salinity 21 ppt.

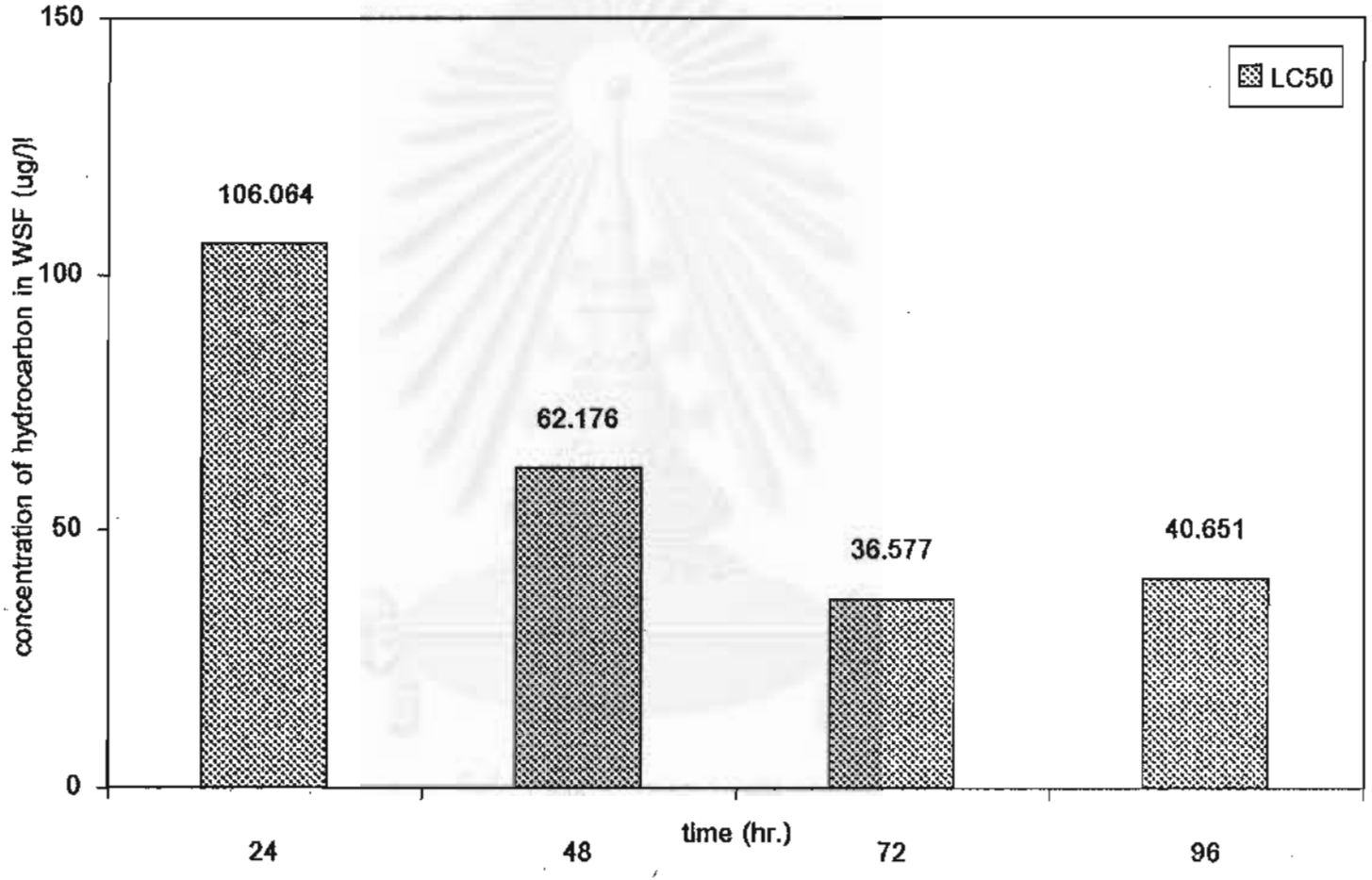

Figure 11 LC50 of Water soluble fraction of Dubai oil that *Penaeus monodon* exposed during 24,48,72 and 96 hrs. at salinity 28 ppt.

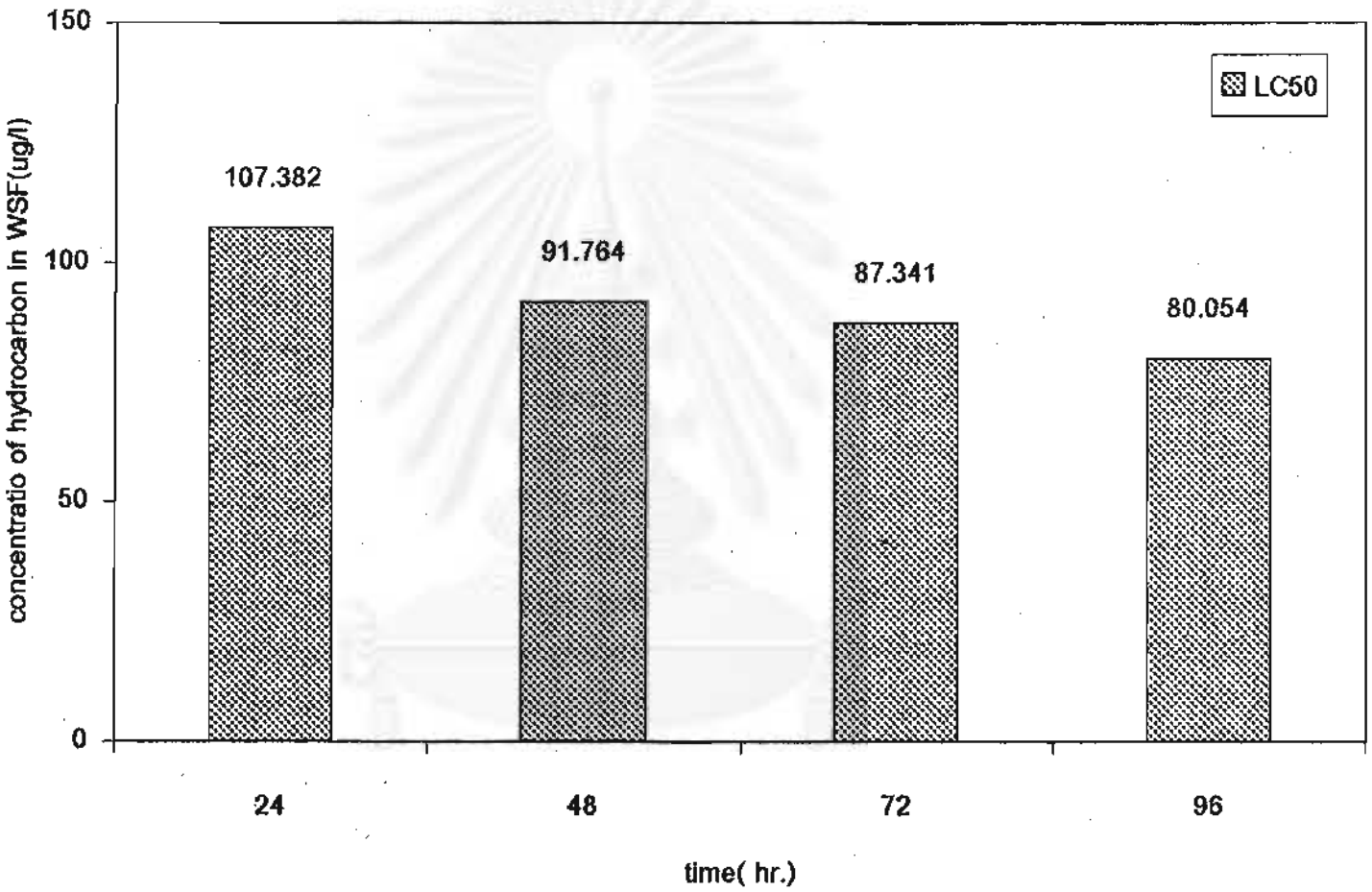

Figure 12 LC50 of Water soluble fraction of Dubai oil that *Penaeus monodon* exposed during 24,48,72 and 96 hrs. at salinity 35 ppt

3. The results of experiment that physiological response of P.monodon on sublethal effect of WSF of Dubai crude oil.

Oxygen consumption rate, ammonia excretion rate , feeding rate and absorption efficiency were determined in each level of salinity and concentrations of WSF. There were interaction between salinity and concentration of WSF. The result of the study could be described as follows.

1.1 Oxygen consumption rates

There was an effect of salinity and WSF of crude oil on oxygen consumption rate of *P. monodon* larvae was shown in table 6.The result of salinity and WSF of crude oil on oxygen consumption rate of the shrimp was the described seperately by salinity and WSF as shown in Figure 13 and figure 14

Table 6 **Oxygen consumption rate of *Penaeus monodon* exposed in different salinity and concentration of hydrocarbon in water soluble fraction of Dubai oil that show the interaction between salinity and concentration of hydrocarbon in water soluble fraction of Dubai oil**

Source	DF	Meansquare	F value	Pr >F
Sal	3	0.000385	19.23	0.0001
Conc	3	0.000031	1.56	0.1986
Sal*conc	9	0.000121	6.03	0.0001

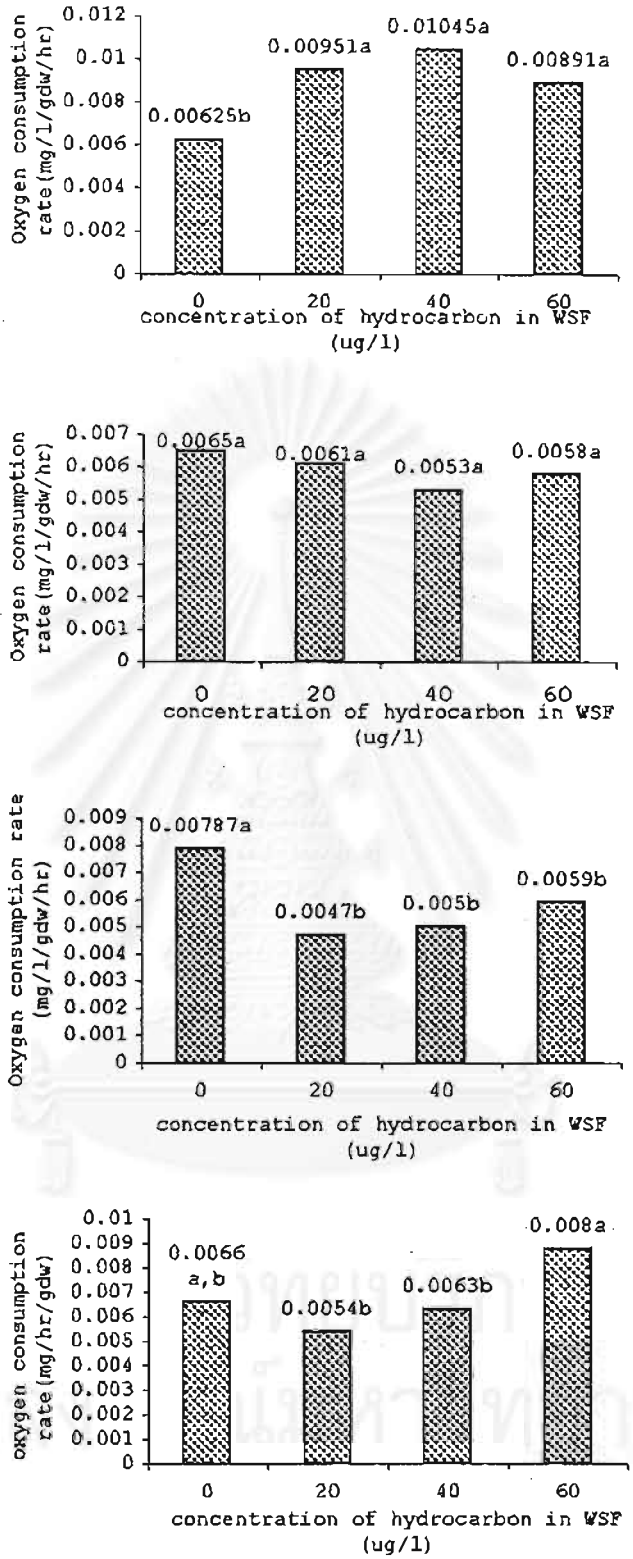

Figure 13 Oxygen consumption rate of *Penaeus monodon* exposed in different concentration of hydrocarbon in water soluble fraction(WSF) of Dubai oil (0,20,40 and 60 ug/l); A is salinity14 ppt., B is salinity 21 ppt., C is salinity 28 ppt. and D is salinity 35 ppt.

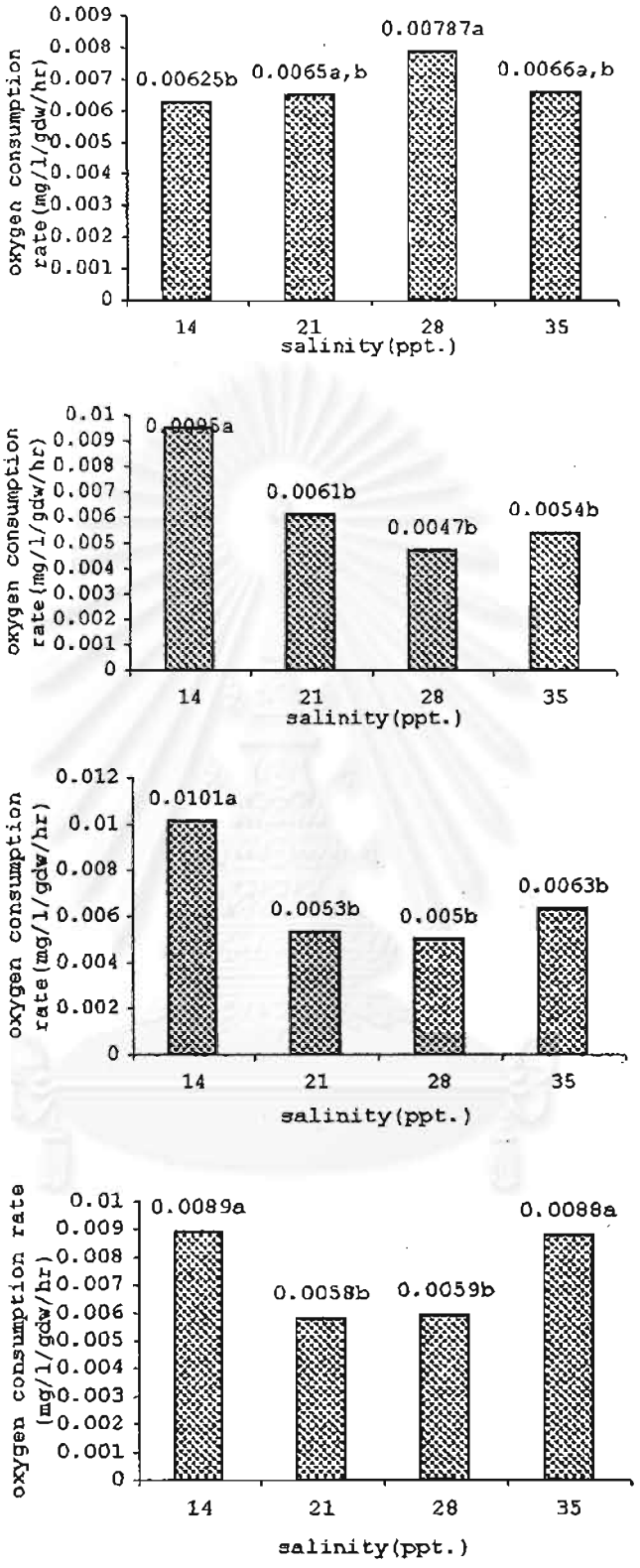

Figure 14 Oxygen consumption rate of *Penaeus monodon* exposed in different salinity of seawater 14,21,28 and 35 ppt.; A is concentration 0 ug/l of WSF, B is concentration 20 ug/l of WSF, C is concentration 40 ug/l of WSF and D is concentration 60 ug/l of WSF.

Salinity effects:

At salinity 14 ppt., oxygen consumption rate of *P.monodon* were highest and lowest at concentration 40 ug of hydrocarbon/l in WSF and 0 ug of hydrocabon/l in WSF, respectively. There was statistic different between oxygen consumption rate at 0 ug of hydrocarbon/l of WSF and the group of treatment 20,40and 60 ug of hydrocarbon/l of WSF.

At salinity 21 ppt., oxygen consumptionrate was highest at 0 ug of hydrocabon/l but no statistical difference among WSF concentrations

At salinity 28 ppt., oxygen consumption rate was highest at 0 ug of hydrocabon//l of WSF. Oxygen consumption rate in 0 ug/l of WSF was had statistic different with group of 20,40 and 60 ug of hydrocabon//l of WSF.

At salinity 35 ppt., oxygen consumption rate at concentration 60 ug of hydrocabon//l of hydrocarbon in WSF was significantly higher than concentration 20 and 40 ug/l of hydrocarbon in WSF.

The most highest oxygen consumption rate among concentrations was in salinity 14 ppt. of 40 ug of hydrocabon//l of WSF. At concentrations 20 and 60 ug of hydrocabon//l of WSF, there were obviously have statistic different between 0 ug/l of WSF and 20,40,60 ug of hydrocabon//l of WSF group.

1.2. Ammonia excretion rate

This rate is measured by spectrophotometer and compared with standard curve. The ammonia excretion rate were depends upon salinity and concentration and interaction between salinity and concentration that shown in table7 and result of them was in figure 15,16.

Table 7 Ammonia excretion rate of *Penaeus monodon* exposed in different salinity and concentration of hydrocarbon in water soluble fraction of Dubai oil that show the interaction between salinity and Concentration

Source	DF	Meansquare	F value	Pr>F
Sal	3	12.5591	23.60	0.0001
Conc	3	9.7969	18.41	0.0001
Sal*conc	9	4.8295	9.08	0.0001

Salinity effects:

At salinity 14 ppt., ammonia excretion rate of *P.monodon* exposed in 2040 and 60 ug/l of WSF were shown significantly difference with concentration 0 ug hydrocarbon /l of WSF. The highest ammonia excretion rate was 1.894 ug NH_3/hr/gdw at concentration 60 ug hydrocarbon /l of WSF at salinity 14 ppt.

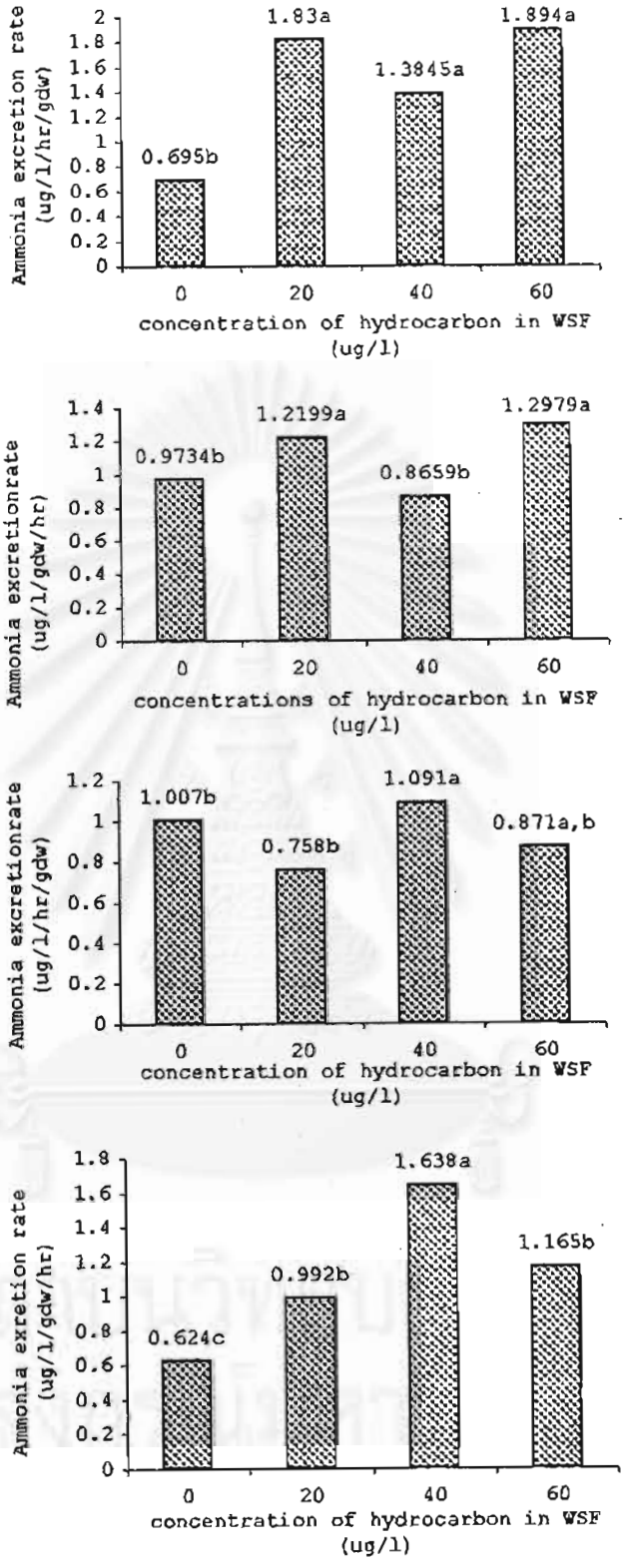

Figure 15 Ammonia excretion rate of *Penaeus monodon* exposed in different concentration of hydrocarbon in water soluble fraction(WSF) of Dubai oil (ug/l)(0,20,40 and 60 ug/l) ; A is salinity14 ppt., B is salinity 21 ppt., C is salinity 28 ppt. and D is salinity 35 ppt.

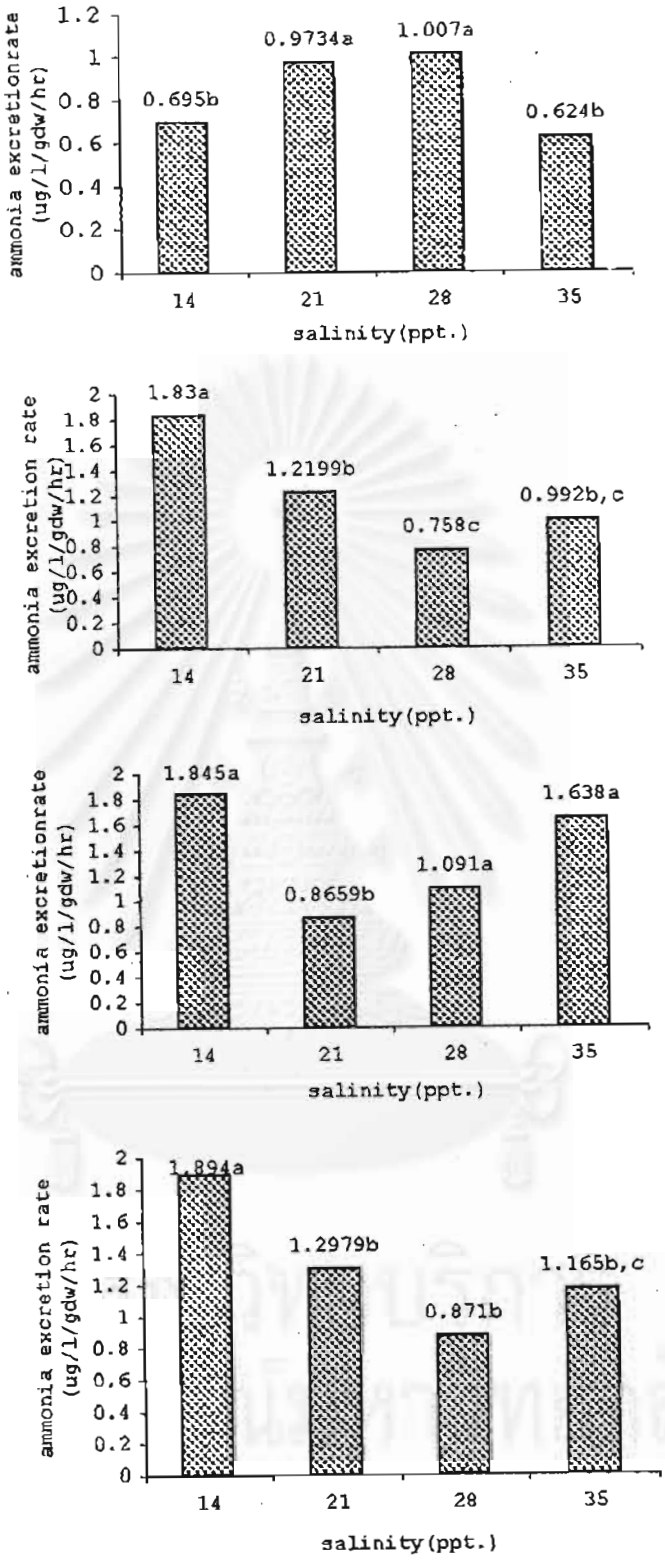

Figure 16 Ammonia excretion rate of *Penaeus monodon* exposed in different salinity of seawater 14,21,28 and 35 ppt.; A is concentration 0 ug/l of WSF, B is concentration 20 ug/l of WSF, C is concentration 40 ug/l of WSF and D is concentration 60 ug/l of WSF.

WSF concentrations effects:

At concentration 0 ug hydrocarbon/l of WSF, There was none of statistic different betweeen ammonia excretion rate in 21 and 28 ppt of exposure and 14,35 ppt. exposure but these two group were have statistical different.

At concentration 20 ug/l of WSF, ammonia excretion rate at salinity 14,21 and 28 ppt. were obviously had statistical different but at salinity 21 ppt. was the same as 35 ppt. At concentration 40 ug hydrocarbon/l of WSF, there was statistic diffferent of ammonia excretion rate betweeen 2 groups; 14,28,35 ppt. and 21 ppt.

At concentration 60 ug hydrocarbon /l of WSF, ammonia excretion rate at salinity 14 ppt. was obviously had statistical different with 21,28 and 35 ppt. group. But ammonia excretion rate at salinity 21 ppt. is not different from salinity 35 ppt.

1.3 Feeding rate of *P.monodon*

Feeding rate significantly depended upon salinity, WSF concentration and the interaction between salinity and concentration that shown in table 8.

Table 8 **The feeding rate of *Penaeus monodon* exposed in different salintiy and concentration of hydrocarbon in water soluble fraction of Dubai oil show the interaction between salinity and concentration.**

Source	DF	Meansquare	F value	Pr>F
Sal	3	9.2508	559.42	0.0001
Conc	3	0.5804	35.10	0.0001
Sal*conc	9	1.1958	72.25	0.0001

The result of feeding rate of *P.monodon* were shown in figure 17 and figure 18.

Salinity effects:

At salinity 14 ppt., feeding rate of *P.monodon* at concentration 0 ug hydrocarbon /l of WSF had statistical difference with 20,40 and 60 ug hydrocarbon /l of WSF group.

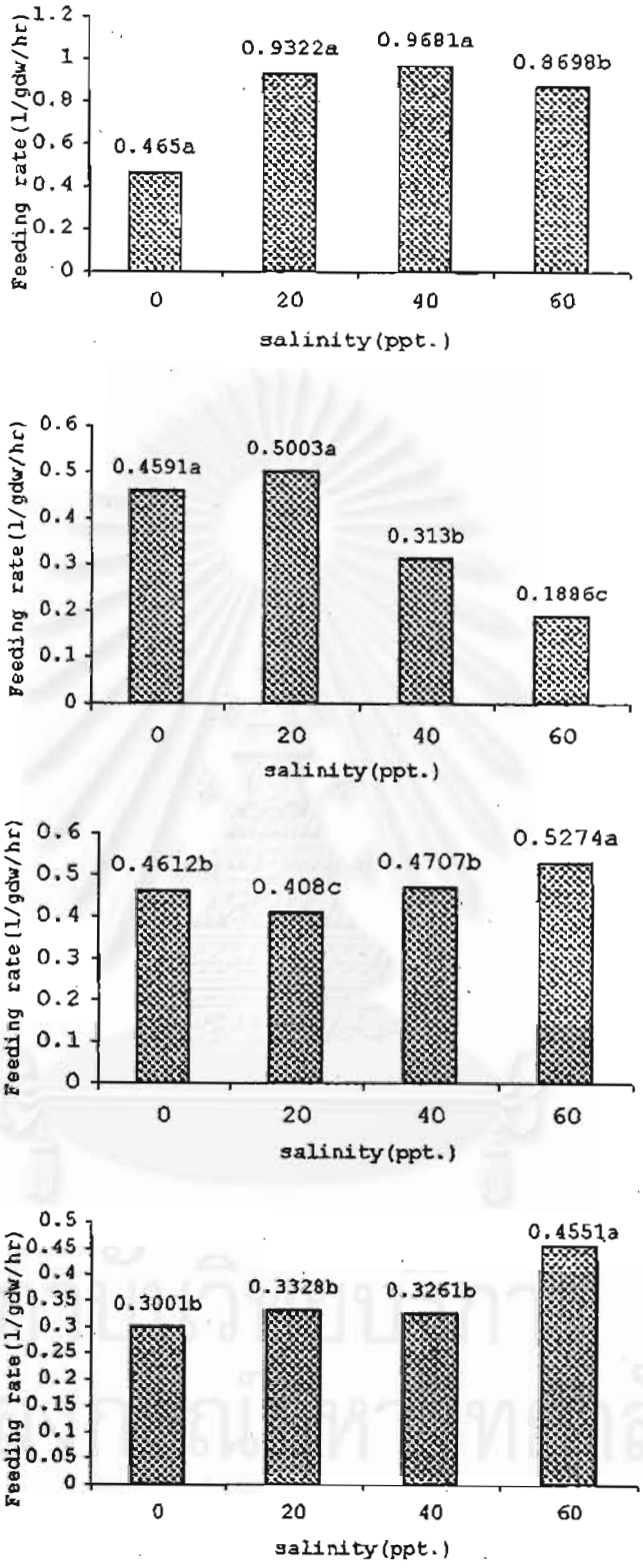

Figure 17 Feeding rate of *Penaeus monodon* exposed in different salinity of seawater 14,21,28 and 35 ppt.; A is concentration 0 ug/l of WSF, B is concentration 20 ug/l of WSF, C is concentration 40 ug/l of WSF and D is concentration 60 ug/l of WSF.

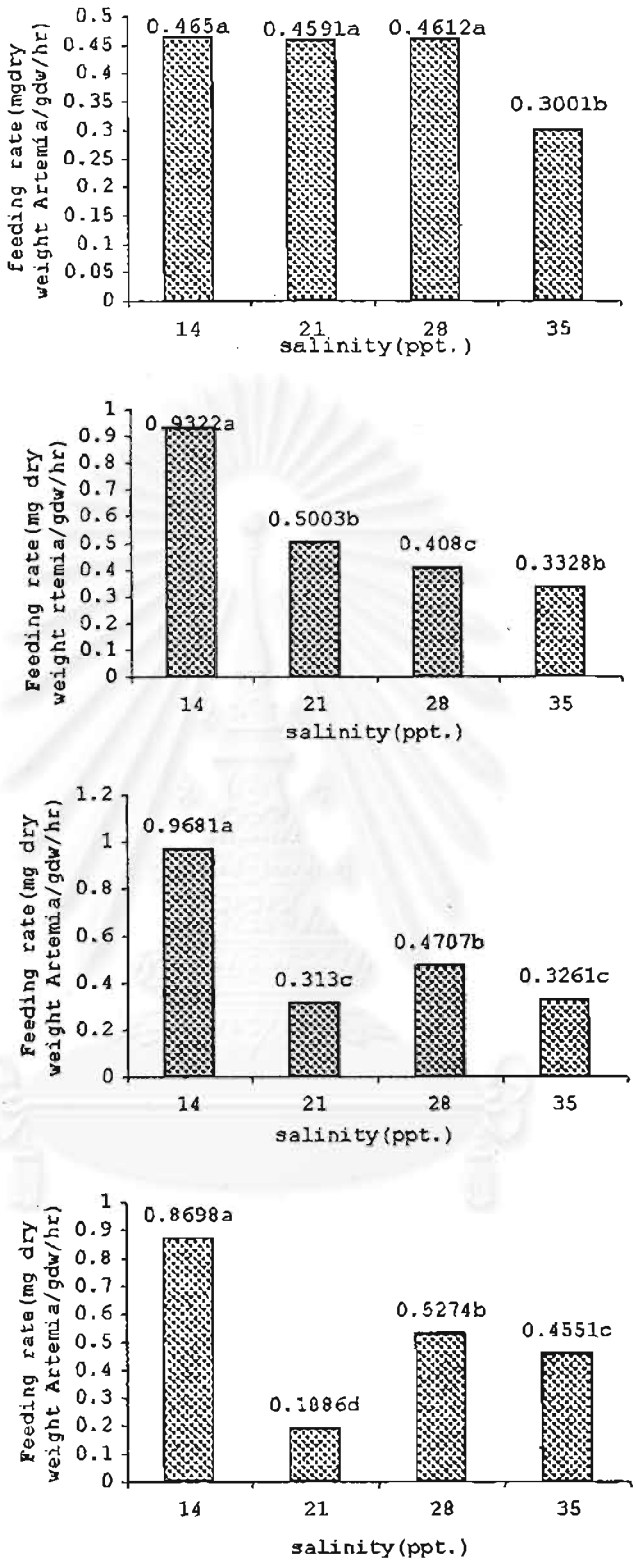

Figure 18 Feeding rate of *Penaeus monodon* exposed in different concentration of hydrocarbon in water soluble fraction(WSF) of Dubai oil(ug/l)(0,20,40 and 60 ug/l).; A is salinity14 ppt., B is salinity 21 ppt., C is salinity 28 ppt. and D is salinity 35 ppt.

At salinity 21 ppt., feeding rate of *P.monodon* at concentration 0 and 20 ug hydrocarbon /l of WSF was not had statistic different. But was different from concentration 40 and 60 ug hydrocarbon /l.

At salinity 28 ppt., feeding rate of *P.monodon* at concentration 0and 40 ug hydrocarbon /l of WSF was not had statistic different but this rates had statistical difference with the rate in concentration 20 and 60 ug hydrocarbon /l of WSF.

At salinity 35 ppt., WSF concentrations of 60 ug of hydrocarbon/l of WSF showed significantly higher feeding rate than at WSF concentration of 0, 20 and 40 ug hydrocarbon/l of WSF.

WSF concentrations effects:

At concentration 0 ug hydrocarbon /l exposure, feeding rate of *P.monodon* at salinity 14,21 and 28 ppt. was similar but had statistical different but this group was different from feeding rate at 35 ppt. At concentration 20 ug of hydrocarbon /l of WSF, salinity 14 ppt had higher feeding rate than those of at salinity 21, 28 and 35 ppt. At concentrations 40 and 60 ug hydrocarbon /l of WSF indicated the highest feeding rate at salinity 14 ppt. There were significant differences of feeding rate.

At concentration 60 ug hydrocarbon /l exposure, feeding rate of *P.monodon* at 4 level of salinity were statistical different. There were 0.8698^{e},0.1886^{d},0.5274^{b} and 0.4551^{c} at salinity 14,21,28 and 35 ppt., respectively.

1.4 Absorption efficiency(Ab)

Ab was significant affected by salinity and concentration and interaction of salinity and WSF concentration. The interaction result is shown in table 9.

Table 9 **Absorption efficiency of *Penaeus monodon* exposed in different salinity and concentration of water soluble fraction of Dubai oil show the interaction of salinity and concentrations.**

Source	DF	Meansquare	F value	Pr>F
Sal	3	1.02620	158.98	0.0001
Conc	3	0.03633	5.62	0.0008
Sal*conc	9	0.15361	23.380	0.0001

The result of absorption efficiency of *P.monodon* exposed in different salinity and cocnentrations were shown in figure 19 and figure 20.

Salinity effects:

At salinity 14 ppt , Ab of *P.monodon* at concentration 20 ug/l of WSF was had significantly different from 0, 40 and 60 ug hydrocarbon /l of WSF group. At salinity 21 ppt., Ab of *P.monodon* at concentration 0 ug hydrocarbon/l of WSF was had statistical different from 20,40,60 ug hydrocarbon /l of WSFgroup.

At salintiy 28 ppt., Ab of *P.monodon* exposed in concentration 0, 20 and 40 ug hydrocarbon /l of WSF were had statistic different but Ab at concentration 20 ug hydrocarbon /l of WSF and 60 ug/l of WSF were not different.

At salinity 35 ppt., Ab of *P.monodon* exposed in concentrations 60 ug hydrocarbon/l of WSF was significantly higher than 0, 20 and 60 ug hydrocarbon/l of WSF group.

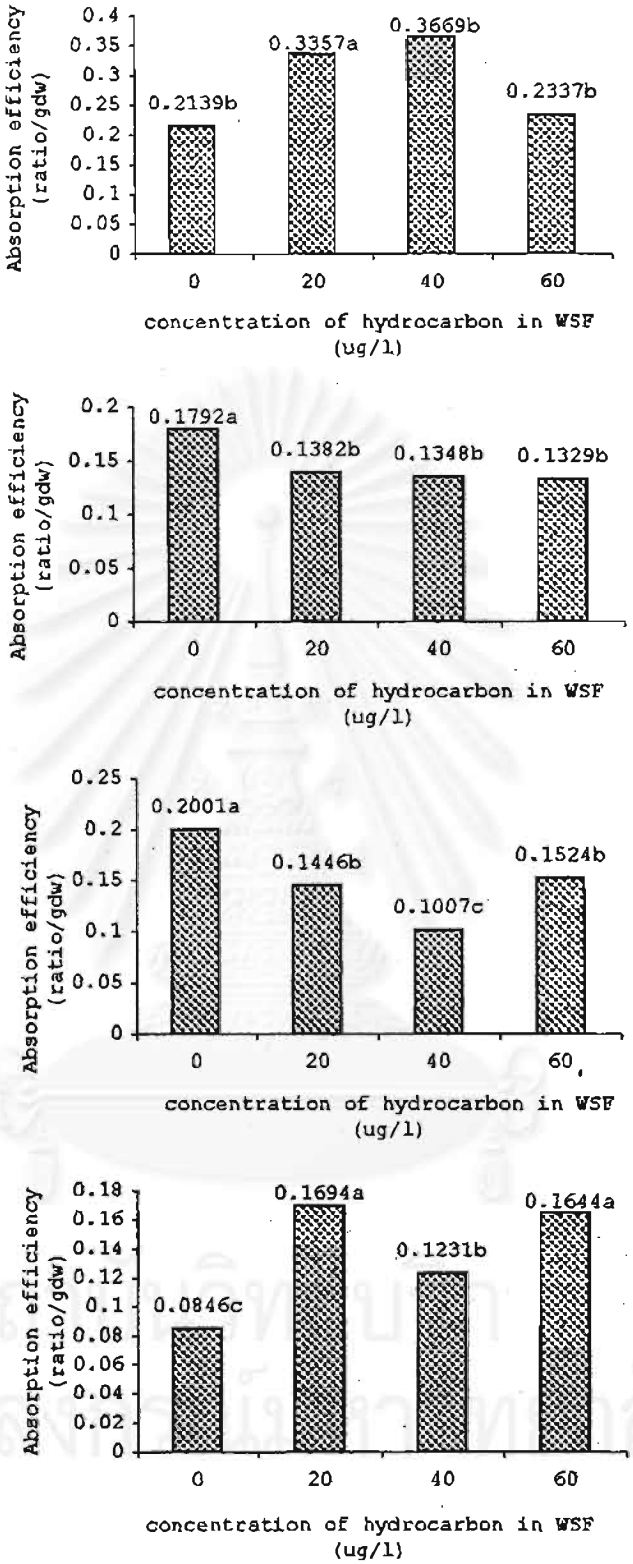

Figure 19 Absorption efficiency of *Penaeus monodon* exposed in different concentration of hydrocarbon in water soluble fraction(WSF) of Dubai oil (ug/l)(0,20,40 and 60 ug/l).; A is salinity14 ppt., B is salinity 21 ppt., C is salinity 28 ppt. and D Is salinity 35 ppt.

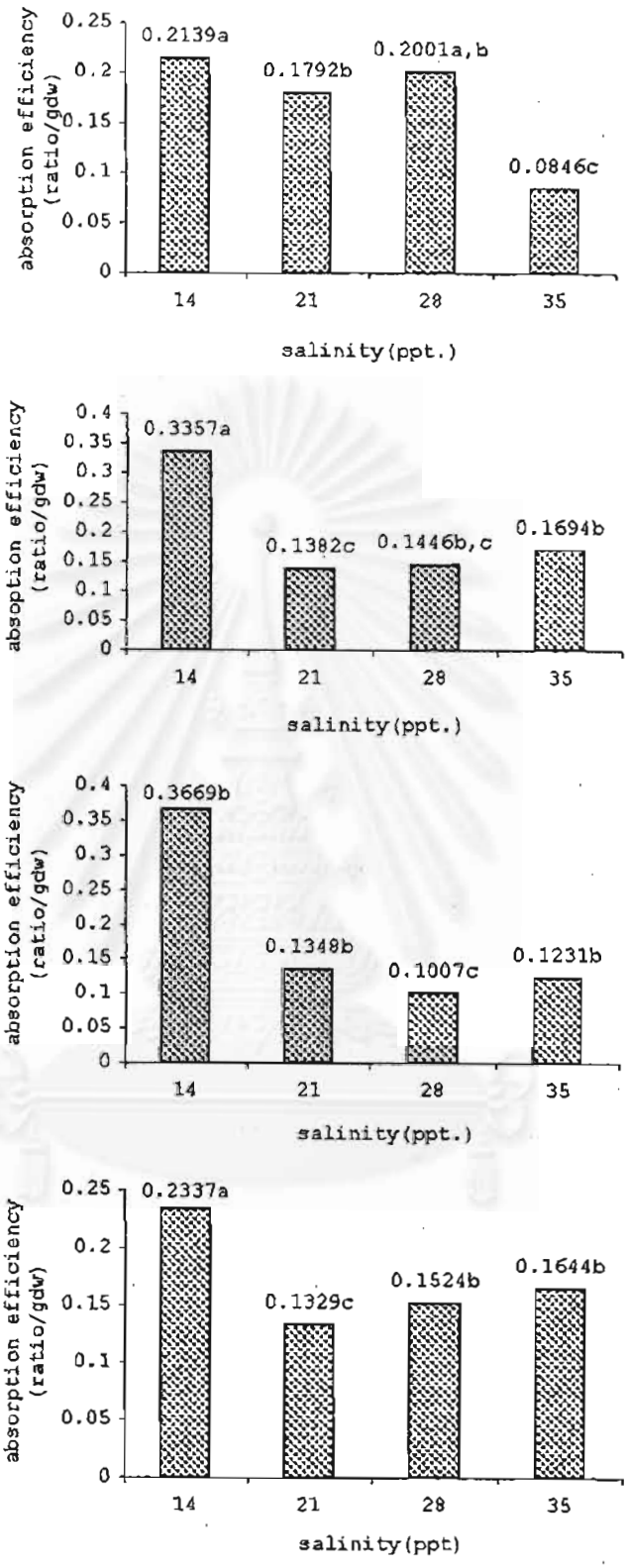

Figure 20 Absorption efficiency of *Penaeus monodon* exposed in different salinity of seawater 14,21,28 and 35 ppt.; A is concentration 0 ug/l of WSF, B is concentration 20 ug/l of WSF, C is concentration 40 ug/l of WSF and D is concentration 60 ug/l of WSF.

At concentration 0 ug/l of WSF, Ab was not had statistic different between 21,28 ppt. But it's different from 14 and 35 ppt. At concentration 40 ug hydrocarbon /l of WSF, Ab of *P.monodon* exposed in salinity 14,21 and 35 ppt. were similar but these group were different from Ab at salinity 28 ppt. At concentration 60 ug hydrocarbon /l of WSF, Ab at salinity 14 were different from salinity 21 ppt. but these group were not different from 28 and 35 ppt. group.

Consequently, Oxygen consumption rate was significantly depends upon salinity and interaction between salinity and concentration, excretion rate , feeding rate and absorption efficiency were significantly depends on salinity ,concentration and interaction with salinity and concentration. All mean±S.D of physiological responses that measured were in table 10.

Table 10 **Table that show the mean±S.D. of oxygen consumption rate, ammonia excretion rate ,clearance rate and absorption efficiency of *Penaeus monodon* exposed to WSF of Dubai crude oil in different salinity and concentrations**

Salinity	Oxygen consumption rate (mg O_2/l/gdw/hr)	Excretion rate (mg NH_3/gdw/hr)	Cr. Rate (mg*Artemia*/ gdw/hr)	Absorption Efficiency
14	0.008±0.005	1.5666±1.122	0.809±0.248	0.285±0.122
21	0.006±0.003	1.089±0.476	0.365±0.208	0.146±0.057
28	0.006±0.003	0.932±0.671	0.467±0.102	0.149±0.075
35	0.007±0.006	1.105±0.769	0.354±0.108	0.135±0.094

Next:

Conc. (mg/l)	Oxygen consumption rate (mg O_2/l/gdw/hr)	Excretion rate (mg NH_3/gdw/hr)	Cr. Rate (mg*Artemia*/ gdw/hr)	Absorption Efficiency
20	0.006 ±0.005	1.201±0.809	0.543±0.285	0.197±0.103
40	0.007±0.005	1.360±0.989	0.519±0.299	0.181±0.130
60	0.007±0.005	1.307±0.806	0.510±0.254	0.168±0.115
Con	0.007±0.004	0.825±0.541	0.422±0.137	0.169±0.082

4. Scope for growth (SFG)

SFG is estimated from difference between the energy gains and the energy losses (Energy expenditure via respiration and excretion). SFG in treatments that exposed to water soluble fraction of oil lower than control except at salinity 28 ppt. There were interaction between salinity and concentration of WSF on SFG *of P.monodon* postlarvae as a result shown in table 11.

Table 11 **Scope for growth of *Penaeus monodon* exposed in different salinity and concentration of hydrocarbon in water soluble fraction of Dubai oil show the interaction of salinity and concentration.**

Source	DF	Meansquare	F value	Pr>F
Sal	3	0.07648	16.77	0.0001
Conc	3	0.00799	1.75	0.1547
Sal*conc	9	0.02386	5.23	0.0001

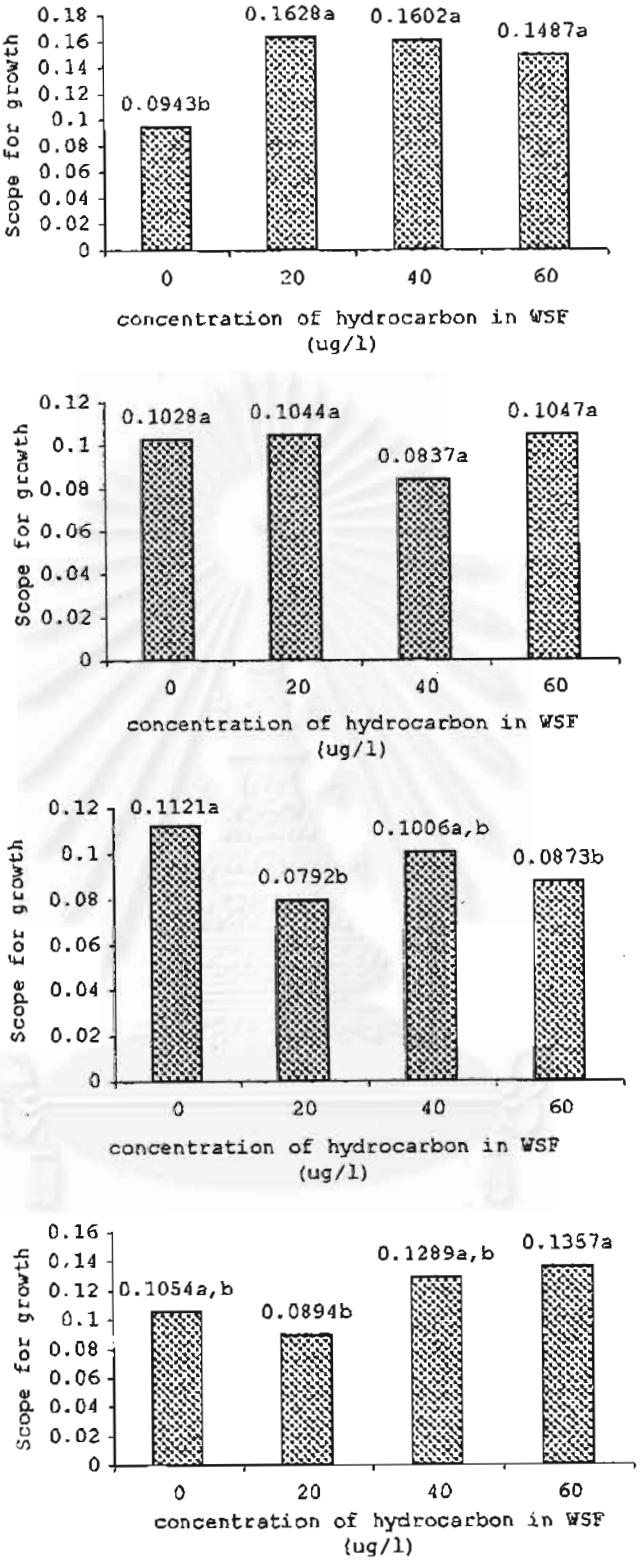

Figure 21 Scope for growth of *Penaeus monodon* exposed in different concentration of hydrocarbon in water soluble fraction(WSF) of Dubai oil (ug/l)(0,20,40 and 60 ug/l). A is salinity14 ppt., B is salinity 21 ppt., C is salinity 28 ppt. and D is salinity 35 ppt.

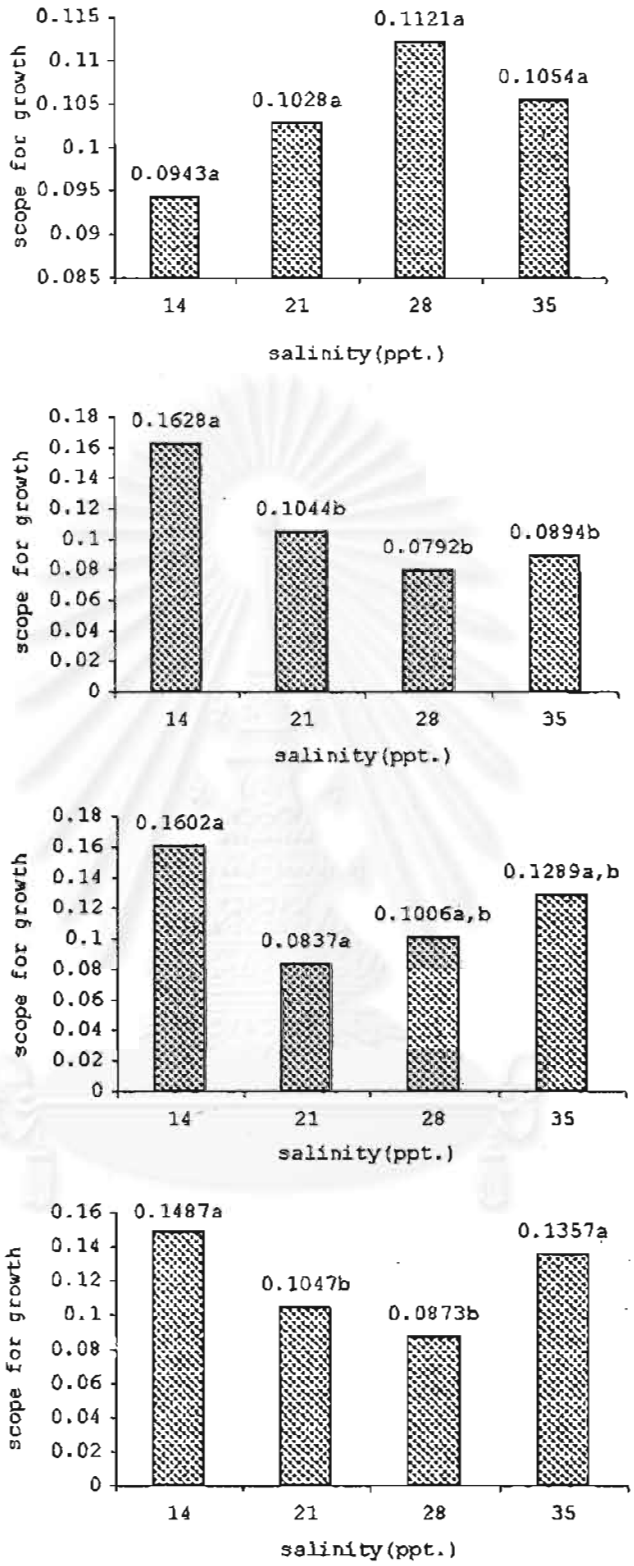

Figure 22 Scope for growth of *Penaeus monodon* exposed different salinity of seawater 14,21,28 and 35 ppt.; A is concentration 0 ug/l of WSF, B is concentration 20 ug/l of WSF, C is concentration 40 ug/l of WSF and D is concentration 60 ug/l of WSF.

The scope for growth of *P.monodon* expsoed in different salinity and cocnentrations was shown in figure 21 and figure 22.

At salinity 14 ppt., SFG of *P.monodon* at concentration 20,40 and 60 ug hydrocarbon /l of WSF was significantly higher than that of concentration 0 ug hydrocarbon /l of WSF.

At salinity 21 ppt. , SFG of *P.monodon* at all level of concentration of WSF was similar. At salinity 28 ppt., SFG of *P.monodon* at concentration 0 ug/l of WSF was significantly higher at 60 ug hydrocarbon/l of WSF. All other concentrations seemed to have no effects on SFG.

WSF concentrations effects:

At concentration 0 ug hydrocarbon/l of WSF, SFG of *P.monodon* at all salinity was not significantly different with salinity 21,28 and 35 ug/l group.

At concentration 20 ug hydrocarbon/l of WSF, SFG of *P.monodon* at salinity 14 ppt. was significantly higher than others salinities.

At concentration 40 ug hydrocarbon/l of WSF, SFG of P.monodon at salinity 14 ppt. was higher than salinity 21 and 28 ppt.

At concentration 60 ug hydrocarbon/l of WSF, SFG of *P.monodon* at salinity 14 ppt. was similar with salinity 35 ppt. but was significantly higher than that at salinity 21 and 28 ppt.

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CHAPTER IV

DISCUSSION

Penaeus monodon postlarvae were moderately tolerant to long term petroleum hydrocarbon exposure. The higher LC_{50} is observed in early exposure. At 24 hr,50% survival was observed up to 24-hr LC_{50} =117.409 ppm.. The tolerance of shrimp to the water soluble fraction (WSF) of Dubai oil concentration declined with duration of exposure. The 24-hr LC_{50} was higher than 48-hr LC_{50} ,72-hr LC_{50} and 96-hr LC_{50}. Salinity and concentration of WSF seemed to have some interactive affects on percent mortality of *P.monodon* larvae.

In sublethal conditions, the concentration that selected are 20,40,60 ug hydrocarbon /l WSF. It's lower than 96-hr LC_{50} value. All physiological parameters were measured and determined. Oxygen consumption rate, Ammonia excretion rate, Feeding rate were measured and Absorption efficiency. About respiration rate or oxygen consumption rate, There is a considerable body of published information with respiratory response of marine invertebrate to the physiological stress induced by changes in environmental salinity and dissolved oxygen (Fry,1971). Chronic and acute exposure to sublethal concentrations of petroleum hydrocarbon may constitute a physiological stress to marine organisms. The major component of the energy budget affected by petroleum hydrocarbon exposure (Wang and Stickle,1987). Chronic or acute exposure to sublethal concentrations of petroleum may also constitute a physiological stress to marine organisms. Marine organisms might be expected to show a respiratory response while exposed in sublethal concentrations of oil mixtures.(Anderson , Neef and Cox ,1984)

Respiration rate of shrimp, it's decreased when salinity was increased. This trends confirm by other investigators that concerning with the influence of environment stressors on respiration rate. For example, Edward (1978) reported that when WSF of oil increased result in the decrease of respiration rate in *Cragon cragon* and percent

mortality were observed. Oil that in WSF forms may attach at gills of shrimp and increased of disturbed the oxygen exchanges system so oxygen decreased. Furthermore, the aromatic hydrocarbon that is the most soluble forms and the most toxic forms are affected the respiratory system by disturbed generating blood cells system and decrease affinity of the cell to pass oxygen to any tissue.

Capuzzo and Lancaster (1982) reported that the disruption in energetics with reducing respiration rates as a result on an inhibition of a lipid utilization among larval lobsters has been observed with exposure to WSF concentrations. However, respiration rates were not always the same patterns. For example reductions in respiration rate among larval lobsters exposed to WSF of oil. Stage I larva were the most sensitive to WSF of oil. But others investigators; Edward,1978;Capuzzo et.al.(1984) reported that rates of oxygen consumption were reduced when exposed to sublethal concentrations of crude oil.

Anderson et.al (1984) demonstrated and reported that oxygen consumption rate of shrimp *Penaeus azetecus* exposed to WSF of #2 fuel oil and WSF of South Louisiana crude oil showed lower oxygen consumption rate particularly at the lower exposure concentration. At intermediate WSF concentrations, the shrimp generally had respiration rates near those of the controls. At all exposure concentrations of the #2 fuel oil, means respiration rate was found to be significantly different from the control rate. However, at exposure concentrations between 30 and 70 % WSF, mean respiration rates of the larger shrimp were higher than those of the controls.

Other supported literature of Wang ans Stickle (1987) was studied of respiration rate that utilizing in grass shrimp *Palaeomonetes pugio* that exposure to WSF of Kuwait crude oil, the result is the greatest respiratory depression appeared to occur following exposure to the lowest concentration of Kuwait WSF. It' would appear that exposure to Kuwait WSF caused a temporary but significant depression of oxygen consumption by the grass shrimp. They concluded that the magnitude of the respiratory response of

marine animals to exposure to sublethal concentrations of oil is species-dependent and also apparently dependent on the hydrocarbon composition and concentration of the exposure water. The respiration rate result of *P. monodon* is similar to trend of respiration rate of blue crab, *Callinectes simillis* that increased respiration at low salinity. This result may indicated that *C. similis* incurs greater costs due to osmoregulation.

Ammonia excretion rates were inversly with oxygen consumption rates. Increasing salinity were resulted on increasing ammonia excretion rate. All rates in treatments were higher than control. But this rate has unclear relationship with WSF concentrations. However, patterns of respiration rate and ammonia excretion rate were not always uniform (Edward,1978), for example, Carr and Lindane(1984) recorded that increased rates of oxygen consumption rate and ammonia excretion rate in *Gammarus salinus* exposed to North sea crude oil. Reduced oxygen consumption rate was found in *Cragon cragon* and *Homarus americanus* exposed to the combined effect of a numbers of factors, such as salinity and composition of pollutants. Another supported data is Widdows (1987) reported the ammonia excretion rate *of Mytilus edulis* .

He found that the rate of ammonia excretion by *M.edulis* during oil exposure and the period of recovery did not show a consistent pattern of reponse in *M.edulis*. Ammonia excretion rates patterns in cruteceans are highly variable and difficulty to access (Mullin et.al,1975). Part of this difficulty is due to the effects of life history stages, salinity ,temperature, sex, light, food and stravation, body size density and other physico-chemical parameters and another part is due to difficulty of excretion measurement tecniques (Vernberg and Vernberg,1987)

The primarly component of the energy budget of *P.monodon* which determined variation In scope for growth was the rate of energy consumption(feeding rate) and absorption. Variations in feeding rate has been shown to be the major component that determines the energy budget variation of several marine invertebrate species adapted

to several points along a gradient of stressors intensity.(Shirley and Stickle,1982; Stickle,1985; Stickle et.al,1984)

Other physiological rate is feeding rate. The feeding rate was not uniformly depends with salinity but increasing concentration of WSF as a result on reducing of feeding rates. John and Peachnix (1980) reported that reduced feeding rates as a result of exposure to petroleum hydrocarbons ,have been demonstrated in larvae of crustecean (*Cancer irrotatus*) and gastropod and bivalve. It's assumed that reduction in food consumption exposed to oil may reflect a narcotizing effect of oil on sensory functions (John and Miller,1972). Wang and Stickle (1987) demonsrated the feeding rate in blue crab *Calinectes sapidus* ,reducing feeding rate as a result of exposure to petroleum hydrocarbon. Widdows and Stickle,1985 were studied feeding rate in bivalve *Mytilus edulis* He found that reduced feeding rate as a result of exposure to petroleum hydrocarbon .

In addition, feeding rate was the same patterns of absorption efficiency. These two parameters are total energy intake of the shrimp. Food absorption efficiency is inversed relation to faeces loss. Feeding rates reduced when exposed to sublethal concentrationss of WSF. The supported literature is Widdow,1984 ,feeding rate was determined under WSF exposures .Feeding rate of *M.edulis* that exposed to water accomodated fraction of diesel oil was significantly reduced and showed inversly relationship with oil concentration. The same result was found in the present study whereas the oil exposure, too.

Derby and Atema(1981) and Pearson et..al 1981 have demonstrated impairment in the ability of crusteceans to detect food cues during exposure to petroleum hydrocarbons. Difficulty in locating food was observed during in WSF treatment. But it's not clear, In view of the further studies in such areas as chemosensory modality , food palatability were needed to enhance our standing of mechanism regulating energy intake in stressed organisms.

The reduction of food consumption rates in the present study was similar to result of Stickle W. and Kapper (1987) that reported food consumption rate in shrimp *Pandalus borealis* is decreased when higher concentration of WSF of Cook Inlet crude oil. It's similar with that rate in *P.monodon* . Other supported were Peter and John (1976) reported that reduction of food consumption in lobsters larvae exposed to oil may have negative contribution to slow development and increased frequency of moulting. Lower foood consumption have a result to weakening and may affected by oil interference to chemosensory and feeding behavior of adult lobsters(Atema and stein,1974).It is clear that the physiological effects on oil exposure to aquatic animals varied via ability of adaptaion life cycle and other environmntal factors such as temperature , salinity , light and pressure, pH and dissolved oxygen.

Scope for growth

Scope for growth is defined as an index of the energy available for growth and reproduction (Bayne et.al.,1985) can be provide an integration of the physiological parameters involved in the energy budget, but also an understanding of the impact of an environmental stress which can discriminate between natural and antropogenic stress. SFG is an early waming indicator of cellular and metabolic response at the individual level which allows detection of responses to environmental change prior to measurable effects on growth reproduction of survival of the individuals. A scope for growth in treatment of *P.monodon* that exposed to WSF of Dubai oil are lower than control. The highest scope for growth is 0.157 in salinity 14 ppt. Reduced growth as a result on long term exposure to petroleum hydrocarbon both exposure duration and level were important in reducing growth of *P.monodon*.

Capuzzo and Lancaster (1982) reported that in juvenile *C.sapidus* that with long term exposure to South Lousiana crude oil, reducing growth and delayed development were observed . Scope for growth or energy retained for somatic growth and reproduction was greatly reduced in crabs exposed to WSF of crude oil as a result of

reduced energy intake without a consistent reduction in maintainance costs. All values of scope for growth in this experiment are negative. It's means that test animal *P.monodon* cannot accumulate any energy to growth and they use energy to maintain and repair their body returns to normal. It's very seriously sublethal condition. Scope for growth is an early warning indicator of cellular and metabolic responses at the individual levels, which allows detection of responses to an environment change prior to measurable effects on the growth, reproduction or survival of the individual (Asd and John,1994) When shrimp exposed to WSF of oil, they tried to maintain their energy budget for normal metabolic use only, so growth rate may have a negative output.

Disruption in energetics and developments of larval crustacean as a result to petroleum hydrocarbons has been reported by several investigators. The main effect is reducing growth (Tatem,1977, Laughlin and Neff,1980). The negative value showed the unbalanced status between energy acquisition (feeding and absorbtion) and energy expenditure(metabolism and excretion). Otherwise, negative values when the animal is severly stressed and utilization body reserves. Energy reserved is not enough to growth and developments. Wang and Stickle(1987) demonstrated that scope for growth or energy retained for somatic growth and reproduction was greatly reduced in crabs exposed to crude oil WSF as a result of reduced energy intake. Other supported paper is Gunderson (1996) that studied subacute toxicity of the WSF of Kuwait crude oil on *Menidia beryllina* and found reduction in SFG in fish exposed to WSF. The trend of reducing SFG is similar with the result of Stickle et.al.(1987) who reported that SFG of pink shrimp *P.borealis* was reduced with increasing WSF concentration or a long period of WSF exposure . SFG showed a same trend of *P.borealis* only *P.monodon* allocated their energy for respiration and escretion higher and provided less energy to growth while exposed to WSF of Cook inlet crude oil for long time. But all SFG were positive throughout sublethal concentration. This different from SFG in *P.monodon*. It's means the pink shrimp can metabolized food and use energy to growth because energy expenditure were lower than energy intake by foods it contrast with *P.monodon* but they used energy in respiration and excretion more than energy compose. Enhanced SFG at

elevated concentrations of WSF was due to enhanced feeding, this suggest the occurance of hormesis, an enhancement of physiological process by low concentrations of environmental pollutants as first described by Laughlin et.al (1981) .Stebbing (1979) has indicated that hormesis is a functional component of stress etiology

The marked reduction of scope for growth was demonstrated in *Mytilus edulis* by Widdows,1987, too. SFG or growth potential of mussels chronically exposed to oil is primarily the result of a decline feeding rate and food absorption efficiency. During exposure to high oil concentrations mussels had a negative SFG and had to utilize body reserves in order to survival in stressed thus resulting in degrowth.

In many cases, the SFG responses appears to depend mainly on the feeding rate response, which seems to respond more sensitively than other components of the energy budget (Widdows and Johnson,1988; Buttler et. al ,1990) .Feeding rate reduce while enhancing respiration rate, and consequently the response is best integrated and expressed as SFG (Widdows and Donkins,1991). Donkin et.al. (1989) made a comparisons of the effects of hydrocarbon on feeding rate of *Mytilus edulis* and mortality of *Daphnia magna* and Artemia. Feeding rate appears to be sensitive parameters on the effects of low-molecular weight hydrocarbon which is ascribed to the narcotic effect of these compounds.

Alteration in biochemical composition of inefficient utilization of reserves are critical to the development of larval crustacean larvae and may result in the developmental abnormalities experienced with oil exposure. The reduction in lipid utilization of larval lobsters and shrimp in the present study with oil exposure could account for the sublethal effects on growth and development observed among the larval stages of other crustaceans species. It can not be ruled out. However, that decreased lipid utilization may be a defense mechanism against incorperating lipophillic petroleum hydrocarbons in metabolic pathways and disruption in energetic is a consequence of the reduction in energy available for growth .

These finding of bioenergetic of *Penaeus monodon exposed* to WSF of Dubai crude oil agrees with physiological and biochemical evidences from other studies. There are little evidence to described any beneficial effects under exposure and there are little information on the effects of crude oil exposure on the bioenergetic of shrimp. In addition there were few experimental data correlating changes in scope for growth with simultaneous growth or reproductive measurement. In my opinion the scope for growth is a good parameters to indicate status of environments. SFG provide a sensitive ,quantitative and integrated stress over a wide range of conditions from optimal to lethal and is therefore responsive to environmental pollutants. There are advantages of using SFG in environmental biomonitoring.

CHAPTER V

CONCLUSION

1. The 24-hr LC_{50},48-hr LC_{50},72-hr LC_{50} and 96-hr LC_{50} of exposed *Penaeus monodon* in WSF of Dubai crude oil at salinity 14 ppt. are 117.409,101.257,81.548 and 81.548 ug/l., respectively.
2. The 24-hr LC_{50},48-hr LC_{50},72-hr LC_{50} and 96-hr LC_{50} of exposed *Penaeus monodon* in WSF of Dubai crude oil at salinity 21 ppt. are 712.713,36.387,27.425 and 13.555 ug/l, Respectively.
3. The 24-hr LC_{50},48-hr LC_{50},72-hr LC_{50} and 96-hr LC_{50} of exposed *Penaeus monodon* in WSF of Dubai crude oil at salinity 28 ppt. are 106.064,62.176,40.651 and 40.651 ug/l, respectively.
4. The 24-hr LC_{50},48-hr LC_{50},72-hr LC_{50} and 96-hr LC_{50} of exposed *Penaeus monodon* in WSF of Dubai crude oil at salinity 35 ppt. are 107.382,91.746,87.341 and 80.054 ug/l, respectively.
5. Salinity and concentrations of WSF of Dubai crude oil are affected the growth rate of exposed *Penaeus monodon*. Scope for growth (SFG) in exposed conditions are lower than normal.
5. Salinity are affected oxygen consumption rate, ammonia excretion rate and feeding rate of exposed *Penaeus monodon* postlarva in 4 levels of salinity and WSF of Dubai crude oil. The highest oxygen consumption rate , ammonia excretion rate and feeding rate are at salinity 14 ppt.
6. Concentraions of WSF of Dubai crude oil are affected only ammonia excretion rate and feeding rate of *Penaeus monodon* postlarva. The highest ammonia excretion rate and feeding rate are at 40 ug Hydrocarbon/l of WSF and 20 ug hydrocarbon /l WSF , respectively.
7. Salinity and concentrations are affected scope for growth(SFG).SFG of *Penaeus monodon* postlarva in WSF exposure are lower than control.

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