การหาดรรชนีทางชีวภาพในหอยแมลงภู่เพื่อวัดการปนเปื้อนของสารปรอท บริเวณแท่นผลิตปิโตรเลียมในอ่าวไทย

นายชาตรี ฤทธิ์ทอง

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IDENTIFICATION OF BIOMARKERS IN GREEN MUSSEL *Perna viridis* FOR MERCURY CONTAMINATION AT PETROLEUM PROCESSING PLATFORMS IN THE GULF OF THAILAND

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ชาตรี ฤทธิ์ทอง: การหาดรรชนีทางชีวภาพในหอยแมลงภู่เพื่อวัดการปนเปื้อนของสารปรอทบริเวณแท่นผลิตปิโตรเลียมในอ่าวไทย (IDENTIFICATION OF BIOMARKERS IN GREEN MUSSEL *Perna viridis* FOR MERCURY CONTAMINATION AT PETROLEUM PROCESSING PLATFORMS IN THE GULF OF THAILAND) อาจารย์ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ดร. เปี่ยมศักดิ์ เมนะเศวต, อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. ณรงค์ศักดิ์ พ่วงลาภ และ รศ. ดร. สมเกียรติ ปิยะธีรธิติวรกุล, 252 หน้า.

การปนเปื้อนของสารปรอท (Hg) จากกระบวนการผลิตเชื้อเพลิงปิโตรเลียมส่งผลกระทบต่อความปลอดภัยของสัตว์ที่อาศัยอยู่รอบๆ พื้นที่ผลิต นอกจากวิธีการตรวจสอบการปนเปื้อนของปรอทในระบบนิเวศที่ใช้อยู่ทั่วไปแล้ว การนำวิธีการตรวจสอบที่เน้นผลของสารปรอทในระดับต่ำ (sub lethal) ช่วยเสริมกับการติดตามฤทธิ์ของปรอทซึ่งวิธีปกติอาจไม่สามารถตรวจสอบได้นั้นจึงมีความสำคัญอย่างมากต่อการพัฒนาโปรแกรมการติดตามผลของปรอท ในการศึกษานี้ได้นำวิธีทางด้านชีวโมเลกุล มาใช้ในการตรวจสอบและติดตามการเปลี่ยนแปลงของสารปรอทที่เกี่ยวข้องกับการตอบสนองในระดับโมเลกุลในหอยแมลงภู่ *Perna viridis* ในพื้นที่รอบแท่นผลิตเชื้อเพลิงปิโตรเลียมในอ่าวไทย โดยยีนที่มีการตอบสนองและสัมพันธ์กับสารปรอท ได้แก่ยีนเมทัลโลไธโอนีน (MT) และ ซับยูนิตของยีนเมทัลโลไธโอนีนจำนวน 6 ฟอร์ม ยีนฮีทช็อกโปรตีน (HSP) และ Cytochrome P450 family 4 โดยการศึกษาครั้งนี้ได้มีการออกแบบไพรเมอร์จำเพาะ และนำไปวิเคราะห์ด้วยวิธี semi - quantitative RT - PCR เพื่อวัดระดับของ mRNA ในยีนเป้าหมายในหอยแมลงภู่ที่นำมาทดสอบ

การดำเนินการทดลองเริ่มจากเลี้ยงหอยแมลงภู่ในห้องปฏิบัติการ และเติมสารปรอทในถังเลี้ยงที่ความเข้มข้นต่ำ 0.1, 0.2, 0.5, และ 1.0 ไมโครกรัมต่อลิตร เป็นเวลา 8 สัปดาห์ ผลการวิเคราะห์พบว่าความเข้มข้นของสารปรอทในน้ำและในเนื้อเยื่อหอยแมลงภู่เพิ่มขึ้น สัมพันธ์กับปริมาณสารปรอทที่มีการเติมลงในถังเลี้ยง และพบว่าระดับของสารปรอทที่วัดได้ในเนื้อเยื่อหอยแมลงภู่มีความเข้มข้นสูงกว่าปริมาณสารปรอทที่ตรวจพบได้ในน้ำเป็นพันเท่า ภายในช่วงเวลาทดสอบ 8 สัปดาห์และได้มีการทดสอบระดับการแสดงออกของยีนเป้าหมายในตัวอย่างเดียวกัน

ผลการศึกษาพบว่าซับยูนิต pvMT07 ของยีนเมทัลโลไธโอนีน มีการตอบสนองไปในทิศทางเดียวกัน กับระดับความเข้มข้นของสารปรอทที่เพิ่มขึ้นในเนื้อเยื่อ ($p<0.05$) โดยพบความแตกต่างของการแสดงของของยีนในหอยแมลงภู่ในถังเลี้ยงที่ระดับความเข้มข้นของสารปรอท 0.2 ไมโครกรัมต่อลิตร และไม่พบความแตกต่างของการแสดงออกของยีนอื่นในหอยแมลงภู่ในถังเลี้ยงที่ระดับความเข้มข้นของสารปรอทในระดับเดียวกัน จากนั้นได้มีการตรวจสอบยืนยันผลการศึกษาในสิ่งแวดล้อมจริง โดยนำหอยแมลงภู่จากบริเวณที่ไม่มีการปนเปื้อนสารปรอท ไปแขวนเลี้ยงไว้ในพื้นที่รอบแท่นผลิตปิโตรเลียม เป็นเวลา 3 เดือน ผลที่ได้พบว่าการแสดงออกของยีน pvMT07 มีความสัมพันธ์กับระดับปริมาณสารปรอทที่เพิ่มขึ้นเช่นเดียวกัน ผลการศึกษาทั้งในห้องปฏิบัติการและในสิ่งแวดล้อมจริงยืนยันผลการศึกษาว่าการแสดงออกของยีน pvMT07 สามารถใช้เป็นดรรชนีทางชีวภาพ สำหรับการวัดการปนเปื้อนของสารปรอทในระดับต่ำในสิ่งแวดล้อมได้

การทดสอบความเป็นพิษในระดับยีน ของสารปรอท ด้วยเทคนิค (single cell gel electrophoresis หรือ comet assay) ในหลอดทดลองโดยทดสอบกับเม็ดเลือดและอสุจิของหอยแมลงภู่ ที่ได้สัมผัสกับสารปรอทในความเข้มข้นแตกต่างกัน (0.001 - 10.0 ไมโครกรัม ต่อลิตร) ที่เวลา 10, 30, และ 60 นาที ผลการวิเคราะห์ Tail length และ Tail moment พบว่าความเสียหายของ DNA เพิ่มขึ้นในระดับที่สอดคล้องกับการเพิ่มขึ้นของสารปรอท และพบความเสียหายของ DNAในระดับต่ำสุดที่ความเข้มข้นของสารปรอท 0.001 ไมโครกรัม ต่อลิตรที่เวลา 10 นาที โดยอสุจิของหอย มีแนวโน้มที่จะมีความไวต่อการเปลี่ยนแปลงของสารปรอทมากกว่าเม็ดเลือด

เทคนิคในการตรวจสอบปริมาณสารปรอท และความรู้ที่ได้จากการศึกษาครั้งนี้จะให้ข้อมูลที่มีประโยชน์สำหรับการพัฒนาการตรวจสอบสารปรอทในอนาคตและการตรวจเฝ้าระวังการปนเปื้อนสารปรอทในระบบนิเวศน์ทางทะเลได้

สาขาวิชา .....การจัดการสิ่งแวดล้อม.....ลายมือชื่อนิสิต.....ชาตรี ฤทธิ์ทอง.....

ปีการศึกษา.....2552.....ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม.....

ลายมือชื่อ อ. ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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CHATREE RITTHONG: IDENTIFICATION OF BIOMARKERS IN GREEN MUSSEL *Perna viridis* FOR MERCURY CONTAMINATION AT PETROLEUM PROCESSING PLATFORMS IN THE GULF OF THAILAND. THESIS ADVISOR: PROFESSOR PIAMSAK MENASVETA, Ph.D., THESIS CO-ADVISOR: NARONGSAK PUANGLARP, Ph.D., ASSOCIATE PROFESSOR SOMKIAT PIYATIRATITIVORAKUL., Ph.D., 252 pp.

Contamination of mercury (Hg) from the production processes of natural gas production in the off-shore area is a major concern on the welfare of animals inhabited in the surrounding areas. In addition to the chemical methods commonly used for monitoring Hg contamination in the sea, sensitive techniques emphasized on determining the sublethal effects of Hg on living organisms is crucial for the monitoring program. In this study, bioassay for monitoring the change of Hg contamination in relation to molecular response of Green mussel, *Perna viridis*, in the surrounding areas of petroleum production platforms in the Gulf of Thailand was studied. Hg responsive genes including MT and 6 of its variants, HSP, and CYP4 genes were obtained and used for designing specific primers which were then used in semi-quantitative RT-PCR for determining mRNA levels of the target genes in tested mussels.

The experiment was initially conducted by exposing mussels to Hg as $HgCl_2$ at 0.1, 0.2, 0.5, and 1.0 µg/L under laboratory condition for 8 weeks. During the experiment, Hg concentrations in water and mussel tissues were analyzed. The result showed increasing level of Hg in both water and tissue coincide with the increase of applied doses. The Hg level in tissue was found to be thousand folds higher than that in water within 8 weeks of experiment. Expression levels of target genes were determined in the same samples. The result revealed that pvMT07, one of MT variants, was correlatively responded to the increasing level of Hg. Significant difference was detected at the concentration as low as 0.2 µg/L of Hg ($P<0.05$). The expression level of the other genes showed no significant difference within the range of applied concentrations. Field validation of the obtained bioassay was carried out by transplanting mussels to the surrounding areas of petroleum production platforms for 3 months. The result showed the correlation of pvMT07 expression with Hg level. These results confirmed that the expression of pvMT07 can be used as biomarker of Hg exposure at low level.

Genotoxicity of Hg was investigated using single cell gel electrophoresis technique. Haemocytes and sperms of mussel were exposed *in vitro* with various doses of Hg as $HgCl_2$ (0.001 to 10.0 µg/L) at 10, 30, and 60 min. The result of tail length and the tail moment of the assay showed that DNA damage was increased in corresponding to the increasing level of Hg. DNA damage was detectable after exposing to Hg level as low as 0.001 µg/L for 10 min. Sperm appeared to be more sensitive to Hg exposure than haemocyte.

Hg monitoring techniques and knowledge obtained from this study will provide valuable information for the development of future Hg monitoring program in the marine ecosystem.

Field of Study: Environmental Management...Student's Signature .....
Academic Year:.....2009..... Advisor's Signature .....
Co-Advisor Signature.....
Co-Advisor Signature.....

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CHAPTER I

INTRODUCTION

1.1 Statement of Problems

Heavy metals discharged to the environment are of great concern all over the world. Heavy metals can accumulate in marine organisms and are toxic when present at high concentrations. Among them, mercury (Hg) is the most serious global pollutant. Many of its derivations are highly toxic and readily released into the environment because of their high volatility and mobility. On a molar basis, Hg is far more toxic to marine organisms than any heavy metals. Low level of Hg still has chronic effects to living organisms (Langston, 1990; Boening, 2000).

Traditional monitoring of Hg in the marine environment involves determining and comparing the metal concentrations in water, sediment and biota but each method presents its own problems and limitations. The low concentration of Hg in ambient water makes analysis difficult as contamination problems become significant and pre-concentration is required. The typical large temporal variations in the metal concentrations in water often warrant frequent sampling and analyses, which are not cost-effective. The majority of the studies associated with Hg have been designed from a toxicological approach, including the measurement of the concentrations of Hg in various tissues and organs. Obtaining an increased knowledge of biological indicators of Hg exposure would prove to be the key to understand and determine the toxic mechanisms of this metal at the cellular level (Narbonne, 2000; Lynn et al., 2001; Wiener et al., 2002).

Various numbers of mollusks including mussels are proposed as the most suitable marine organisms for monitoring the contamination levels of heavy metals in coastal water areas because of their high accumulation of many heavy metals, relatively long life span, large size of individuals enabling the analysis of individual specimens, tolerance of large temperature and salinity ranges, as well as their wide geographical distribution. *Mytilus edulis* are common mussel species widely used as a surveillance organism (Andersen, 1996). Moreover, *M. edulis* are able to synthesis the metal-binding protein, metallothionein, for metal detoxification. Green mussel, *Perna*

viridis, a close member of *M. edulis*, is a native mussel of Thailand and this mussel is widely distributed inside and around the Gulf of Thailand and it is one of suitable model species commonly used for determining heavy metal contamination. Therefore, the research designed to assess the cellular and molecular responses of living organisms such as green mussels following exposure to Hg would provide valuable information for developing techniques to determine the adverse effects of Hg to marine environment (Amiard, 2000; Lawson and Mason, 2002; NIMPIS, 2002; Nicholson and Szefer, 2003).

1.2 Objectives

This study aims to investigate the expression of Hg specific metallothionein (MT) gene and some candidate genes that are responsive to Hg exposure at low levels in both laboratory and field trails. Specific objectives of this study are:

1. To determine the expression level of Hg specific MT isoforms in *P.viridis,*
2. To clone and characterize stress responsive genes which might contain Hg specific properties in *P.viridis*, and
3. To obtain some candidate genes for the potential use as biomarkers for determining Hg contamination in water and use for monitoring petroleum activity in the gulf of Thailand.

1.3 Scope of Study

1. Determination of Hg concentration in mussel, *P.viridis*, tissue at study site (petroleum areas) and reference site (Mussel farm, Trad province) in the Gulf of Thailand.
2. Cloning and characterization of at least 2 Hg responsive genes (CYP and HSPs) in *P.viridis*
3. Determination of the expression level of Hg specific Metallothionein isoforms in *P.viridis*.
4. Evaluation of the correlation between Hg level and expression level of Hg specific genes (MT, CYP and HSPs) in *P.viridis*

5. Determination of genotoxicity of Hg on *P.viridis*

1.4 Hypotheses

Hg responsive metallothionein, cytochrome P450, and heat shock protein genes in this study can respond to the low levels of Hg contamination and can be used as biomarkers for determining Hg contamination in the Gulf of Thailand

CHAPTER II

LITERATURE REVIEW

2.1 Mercury

Mercury (Hg) is a heavy metal used in thermometers, barometers, vapor lamps, and batteries, and in the preparation of chemical pesticides. Hg is a silvery-white poisonous metallic element, liquid at room temperature. Its atomic number is 80 and atomic weight is 200.59. Melting and boiling points are -38.87 and 356.58°C, respectively. Specific gravity is 13.546 (at 20°C). Hg may occur in 3 valency states in seawater and marine sediments: zero (elemental mercury), +1 (mercurous compounds), and +2 (mercuric compound). The +2 valency state is the most common in well-oxygenated water and sediments. The major species of inorganic Hg in seawater are the chloride, $HgCl_2$ and other chloro complexes. Inorganic Hg is more abundant than organic Hg in sea-water and sediments; organic Hg usually is more abundant than inorganic Hg in air and tissues of marine organisms (Lynn et al., 2001; Neff, 2002).

Hg can accumulate in living organisms and surrounding environment in many forms and cycles in the environment as a result of natural and human (anthropogenic) activities. The amount of Hg mobilized and released into the biosphere has increased since the beginning of the industrial age. In aquatic environments, Hg cycle pathways are very complex. The various forms of Hg can be converted from one to the next. The most important form is the conversion to methyl mercury (MeHg) which is highly toxic, soluble, and can get into food chain. Ultimately, Hg ends up in the sediments, fish and wildlife, or evades back to the atmosphere by volatilization. Dietary uptake is the dominant pathway for MeHg accumulation in aquatic organisms. The bioavailability of MeHg is controlled by digestive processes rather than by constrained transfer across the gills, skin or intestinal epithelium (Neff, 2002). Fish have been estimated to assimilate from 65 to 80% of the MeHg present in the food they eat. Hg is distributed throughout the tissues and organs of the fish, but a large portion of the MeHg eventually relocates to skeletal muscle where it becomes bound

to the muscle protein of a contaminated fish. There is no way to clean or cook the fish to remove or even reduce the amount of Hg presented (Wiener, et al. 2002).

Petroleum Production Process is one of human activities that release Hg to the ocean. Thailand's first gas production started in 1981 from Erawan Gas Field which located in the Gulf of Thailand and operated by Unocal Thailand Ltd. At present, total of 37 fields are produced, 21 fields in the Gulf and 16 are onshore. In the petroleum production process, the discharged water contains high concentration of Hg and oil which must be treated before releasing to the environment or re-injected to the well head. Presently, the produced water discharged directly to the sea is reduced. However, in long-term effect, Hg that is already released from previous production process and newly discharges can still be accumulated in the sediment surrounding the production areas and can affect the environment and human. Therefore, monitoring of Hg contamination and its effect is necessary (Veil, et al. 2004; DMF, 2007).

2.2 Toxicity of mercury and molecular responses for the mercury exposure

A large number of documents clearly provide evidences that Hg is cytotoxic. Its biochemical damage at the cellular level includes DNA damage and inhibition of DNA and RNA synthesis (Khera, 1990) Hg also causes alterations in protein structure, alterations in calcium transport, along with the inhibition of glucose transport and enzyme function (O'Halloran, 1993; Goyer, 1995). It also interferes with essential nutrients by the replacement of essential minerals such as zinc at sites in enzymes. This is a part of the toxic effect of Hg that disables the enzymatic process. An inhibition of cellular enzymatic processes by binding with the hydroxyl radical (SH) in amino acids appears to be a major part of allergic/immune reactive conditions (Bagenstose et al., 1999). The effects of Hg binding with proteins also include the blockage of sulfur oxidation processes (McFadden, 1996), enzymatic processes of vitamins B6 and B12 (Srikantarah, and Radjakushnan, 1970), effects on cytochrome-c energy processes (Veltman, 1986), along with Hg's adverse effect on the cellular mineral levels of calcium, magnesium, zinc, and lithium (Danielson, 1984). Hg probably affects the inherent protein structure, which may interfere with functions relating to protein production. Hg has a strong affinity for sulfhydryl, amine phosphoryl, and carboxyl groups, and inactivates a wide range of enzyme systems,

as well as causing injury to cell membranes. Hg may also interfere with some functions of selenium, and can be an immunosuppressant.

2.2.1 Acute toxicity

The organic forms of Hg are generally more toxic to aquatic organisms than the inorganic forms. Aquatic plants are affected by Hg in the water at concentrations approaching 1 mg/L for inorganic Hg but at much lower concentrations of organic Hg (Boening, 2000). Aquatic invertebrates vary greatly in their susceptibility to Hg. Generally, larval stages are more sensitive than adults. The 96-h LC50s vary between 33 and 400 μg/L for freshwater fish and are higher for sea-water fish (WHO, 1989). Toxicity is affected by temperature, salinity, dissolved oxygen, and water hardness. A wide variety of physiological and biochemical abnormalities has been reported after fish have been exposed to sub lethal concentrations of Hg, although the environmental significance of these effects is difficult to assess (WHO, 1989). Reproduction is also affected adversely by Hg.

Hg as the reactive, free inorganic ion, and as various organomercury compounds in solution is one of the most toxic metals to marine organisms. Acutely lethal concentrations of inorganic Hg in solution to marine invertebrates are in the range of 1.0 to 10,000 μg/L (WHO, 1989). Marine and fresh water fish are somewhat more tolerant, with acutely lethal concentration in the range of 4 to 23,000 μg/L. Birds are considered quite sensitive, with acutely lethal concentrations in the diet ranging from 1 to about 5,000 mg/kg body weight (Boening, 2000).

Marine phytoplankton produced metal-chelating ligands that are capable of complexing with reactive metals, particularly Hg, in seawater, rendering them less bioavailable and toxic (Langston, 1990; Bryan and Langston, 1992). Nevertheless marine phytoplankton are among the most sensitive marine organisms to inorganic Hg. Concentrations in the range of 0.8 to 1.0 μg/L are capable of inhibiting photosynthesis and reducing growth rate of some species of marine micro algae. The species composition of natural assemblages of phytoplankton may be altered by chronic exposure to 1 to 5 μg/L dissolved inorganic Hg. Growth of sporlings of the brown macroalga, *Laminaria saccharina*, was inhibited by dissolved Hg concentration as low as 0.5 μg/L (Langston, 1990). Exposure to inorganic Hg at

concentration as low as 0.1 μg/L inhibited the enzyme glutathione-S-transferase in different foliar tissues of the seagrass, *Posidonia oceanica* (Ranvier et al., 2000). Enzyme inhibition is greater in grasses from a Hg-contaminated environment than from a relatively uncontaminated environment in the Mediterranean Sea. Marine invertebrate larvae also are sensitive to inorganic Hg. Shell growth in larval mussels, *Mytilus edulis*, was reduced by dissolved Hg concentration as low as 0.3 μg/L (Langston, 1990). Zebra fish embryos were sensitive to Hg at relatively low concentrations as measured by hatching and survival time. It was shown that 32 μg/L of $HgCl_2$ completely inhibited hatching when administered to embryos at blastula stage (Dave and Xiu, 1991)

MeHg at concentrations of 15 to 30 μg/L caused a variety of teratogenic effects, including skeletal defects, cardiovascular abnormalities, and craniofacial defects in developing embryo to marine and freshwater minnows (Sharp and Neff, 1982; Gorge, 1990; Samson and Shenker, 2000). The 4 to 8 cell stages of minnow embryos were the most sensitive to effects of MeHg. Minnows *Fundulus heteroclitus* fed MeHg-contaminated food bioaccumulate Hg in their body tissues and eggs (Matta et al., 2001). Females containing 1.1 to 1.2 μg/g wet weights MeHg produced eggs containing 0.01 to 0.63 μg/g of MeHg that decreased fertilization success and the surviving offspring altered sex ratio and reduced reproductive success (Matta et al., 2001).

2.2.2 Neurotoxicity

Hg has also been found to cause additional neurological immune system effects through immune/autoimmune reactions (Kubicka-Murranyi**,** 1996)

Generally, neurotoxicity of Hg studies in vertebrate and human. Hg is toxic to the renal, reproductive and nervous system in rat that treats with $HgCl_2$ 0.5 and 2.0 mgHg/kg. In the spontaneous cortical and hippocampal activity, altered distribution of the frequency bands was seen after 5 weeks after treatment but not at the end of the post-treatment period. Hippocampal population spikes in the treated animals were depressed and showed less potentiation, which effect was still present 19 weeks after finishing the treatment. The duration of the sensory cortical evoked potentials was shorter than in the controls (Vezer et al., 2005).

Kamakshi (2003) studied the effects of Hg on the nervous system of the mouse. It was found that 10 μM of $HgCl_2$ caused neuronal cell death on 14 day old mouse embryos. In human, Hg caused a variety of neurological and behavioral effects including central hearing loss, vestibular dysfunction, autism, mental detioration, speech difficulty, impaired vision, weakness of the extremities and ataxia, and in some cases has proven to be fetal (Chang, 1997).

Hg may enter the body as organic salt, inorganic salt or as elemental Hg. There are clinical and experimental evidences that each of these forms produces a variety of neurologic deficits, with MeHg being the most dangerous and common source of Hg toxicity. In the brain, MeHg is converted to inorganic Hg most likely by in situ demethylation. Humans and monkeys exposed chronically to MeHg have shown a high percentage of inorganic Hg in the brain. Submicromolar concentration of $HgCl_2$, an inorganic Hg compound, is shown to inhibit a variety of metabolic events in the brain by potentiating secondary neurotoxic events. Organic and inorganic Hg was reported to disrupt ion channel functions and, in turn, affect processes such as synaptic transmission and growth cone elongation. (Sirosis and Atchison, 1996). Shafer et al., (2002) observed that prolonged exposure to MeHg in low concentration reduced both Na^+ and Ca^+ ion currents in the membrane channels of cultured cells.

2.2.3 Genotoxicity

Inorganic Hg compounds were also found to induce the generation of reactive oxygen species and glutathione depletion in cultured mammalian cells. Although different Hg compounds tended to produce qualitatively comparable genetic effects, which suggests the involvement of a common toxic entity, MeHg derivatives and other ionizable organomercury compounds were more active in short-term tests than either non-ionizable Hg compounds (e.g., dimethylmercury) or inorganic Hg salts (e.g., $HgCl_2$). The results of cytogenetic monitoring in peripheral blood lymphocytes of individuals exposed to elemental Hg or Hg compounds from accidental, occupational or alimentary sources were either negative or borderline or uncertain as to the actual role played by Hg in some positive findings. Both genotoxic and non-genotoxic mechanisms may contribute to the renal carcinogenicity of Hg, which so far

has been convincingly demonstrated only in male rodents treated with $HgCl_2$ (Flora et al., 1994).

The in vivo exposure of embryos of killfish *(Fundulus heteroclitus)* to MeHg at 1 and 7 days post-fertilization enhanced the frequency of micronuclei and chromosomal abnormalities (chromosome bridges, laggard chromosomes) in embryo cells. The number of mitoses was decreased in groups of embryos exhibiting teratogenic effects (Perry et al., 1988). Exposure of larvae and embryos of the urodele amphibian newt *(Pleurodeles waltl)* to MeHg chloride, at doses similar to $HgCl_2$, induced micronuclei in red blood cells, and c-mitosis and chromosomal aberrations in embryo cells (Zoll et al., 1988).

2.3 Biomarkers

2.3.1 Definition and classification

Biomarkers are defined as quantitative measures of changes in the biological system that respond to either (or both) exposure to, and/or dose of xenobiotic substance that lead to biological effects. The term biomarker is often used restrictedly to cellular, biochemical, molecular, or physiological changes that are measured in cells, body fluids, tissues, or organ with an organism and are indicative of xenobiotic exposure and/or effect (WHO, 1993).

Biomarkers are categorized into 3 types: biomarker of exposure, effect and susceptibility. Biomarker of exposure measures an exogenous substance of its metabolite and its interaction with a biological molecule. Because a number of factors determine whether a chemical exposure reaches its biological target for a toxic response, the most accurate measurement of dose is the biologically effective dose at the target tissue, which can be more reliably measured by biomarkers of exposure than estimated by measurements of administered or ambient chemical exposure. Biomarkers of effect are measurable biochemical, physiological, behavior, or other alterations within an organism. Biomarker of effect is primarily concerned with adverse effects, although the level of evidence varies for the relationship between a given measured effect and specific, pathological responses, which occur often after a long period and after chronic exposures. There is an overlap between biomarker of exposure and biomarker of effect because the same biomarker can be used for both

measurements. Some of the same biomarkers are also used to measure interindividual differences in response and thus further serve as biomarkers of susceptibility (Barrett et al., 1997; Amiard et al., 2000; Narbonne, 2000).

The advantages of biomarker of exposure are their early response and their specificity of reaction. The latter may also be regarded as disadvantageous since the complex contamination situations are not reflected. Thus biomarkers of exposure are useful for the monitoring of hot spots of pollution or clearly defined point source inputs as well as for the characterizations of chronic unknown chemical input. Biomarkers of effect reflect pathological endpoints and are determined at each level of the biological organization. In contrast to the biomarkers of exposure, these effects mostly cannot be attributed to the impact of single contaminants and therefore serve as integrative markers of complex toxicities. The advantages are the high ecological relevance of biomarkers at high levels of organization (individual, population and community level) and the general picture of the status of environmental deterioration that can be obtained by applying this kind of biomarkers. The disadvantage is that in most of the cases the quality of contamination remains speculative. Therefore, only a combination of both kinds of biomarkers provides sufficient information for the assessment of responses reflecting the quality as well as the quantity of environmental deterioration (Broeg, et al., 2005).

2.3.2 Application of biomarkers

Biomarkers have been used routinely in recent years to assess the health of wildlife in relation to their exposure to contaminants (Peakall, and Burger, 2003). Biomarkers have been applied to various fields. For example, changes in the immune system, which can affect susceptibility to disease, may provide sensitive, early warning signals of the toxic effects of metals (Weeks, 1992; Jewett, and Lawrence, 2007). The response to acute stress, as evaluated by measurements of corticosteroids which are regulators of processes relating to energy metabolism, gland function, and a few more important processes, is another biomarker that may be affected by some trace metals (Hontela et al., 1996).

A wide range of biomarkers had been developed and suggested for use in monitoring programs. Biomarker has been used both *in vivo* and *in vitro* for the

evaluation of xenobiotic effects. One of the advantages of biomarkers is that it can indicate biological effects, while chemistry-based surveillance system cannot. It can determine the changes before a real damage has taken place. There is evidence that many biomarker responses are not directly associated with real harmful effects in the target organism (Halander, 2003). In recent years, there has been considerable interest in the use of biomarkers for the early-warning systems. This involves knowledge of their biological function and it is necessary to identify possible interferences that can influence these responses in order to standardize the analytical procedure.

2.4 Potential biomarkers for mercury exposure

There are quite a number of biomarkers commonly used in various kinds of organisms. For example, Ethoxyresorufin o-diethylase (EROD) is known to be specific to polyaromatic hydrocarbon (PAH), Polychlorobiphenyl (PCB) and dioxin contamination (Amiard et al., 2000).

2.4.1 Metallothionein

Metallothionein (MT) is the main biomarker applied for determining heavy metal contamination. MTs are low molecular weight proteins. Their physiological roles are the regulation of essential metals, such as Cu and Zn, sequestration of heavy metals, and free radical scavenging.

On the contrary, cysteinyl residues are present in large amount (about 30% in mammal MTs) (Werner, 2008). A remarkable feature of MTs is their inducibility. It is in fact well known that different factors, and in particular heavy metal cations, can stimulate the synthesis of mRNA encoding for MTs (Viarengo et al., 2001). Owing to this property and due to the high affinity of MTs for heavy metals, these proteins play an important role in regulating the physiological concentration of essential metals, such as Zn and Cu, and in detoxifying noxious metal cations penetrating into the cells (Viarengo et al., 2001; Werner et al., 2008). MT is induced by exposure to many xenobiotics including Hg. Induced MT modulates intracellular Hg concentration or gene expression and protects cells from Hg. MT has many other functions, for example, protection against carcinogenesis.

These functions might also be based on changes in intracellular Hg level or modulation of gene expression. A rapid increase in intracellular Hg concentration is important for cell signaling in mast cells. MT might be involved in a lot of cell signaling systems (Kimura and Itoh, 2008).

2.4.2 Cytochrome P450

Cytochrome P450s or CYPs are enzymes that respond to halogenated hydrocarbon exposure. This usually accompanies and often precedes toxicity in all animals. CYP is a large and ubiquitous group of heme proteins found in fish, mammals, birds, plants, and microorganisms that catalyze the oxidative biotransformation of diverse lipophilic xenobiotic and endogenous compounds. Because CYP enzymes play a critical role in the metabolism bioaccumulation, and potential toxicity of halogenated and nonhalogenated hydrocarbons found in the food chain, levels of individual CYP enzymes are important determinants of susceptibility to environmental contaminant exposure. CYP enzyme induction in fish populations has been suggested as a sensitive biochemical marker of contaminant exposure and by inference of marine ecosystem health (Miller et al., 2003). Induction of the CYP1A subfamily of enzymes can be determined by measurement of associated enzymes activities such as ethoxyreosufin O-diethylase (EROD) and benzo[a]pyrene hydroxylase or by measuring CYP1A protein using immunochemical methods and determining transcriptional levels using quantitative PCR (Miller et al., 2003; Campbell et al., 1996). Induction is an adaptive response that protects cells from toxic xenobiotics by increasing the detoxification activity. Environmental pollutant and many other xenobiotics including heavy metals enhance the metabolism of themselves and of the other co-ingested/inhaled compounds, resulting in a reduction of an increase of toxicity as a result of an increase formation of reactive metabolites (Shaw, et al., 2004). For example in leaping mullet (*Liza saliens*) exposed to 50 μM Hg^{2+}, cytochrome P450 reductase activity was inhibited completely (100%), while at the same concentrations, Cd^{+2}, Cr^{+3}, and Ni^{+2} caused 66%, 65% and 37% inhibition, respectively (Bozcaarmutlu and Arinc, 2007). Bozcaarmutlu and Arinc (2007) and Korashy and El-Kadi (2005) found that CYP1A1 mRNA levels were increased when exposed to 5 μM Hg^{2+}, 25 μM Pb^{2+} and 10 μM Cu^{2+} in Murine hepatoma Hepa 1c1c7 cells.

2.4.3 Heat Shock Protein

Heat shock proteins (HSPs) are groups of intracellular proteins that have an unusually high degree of identity at the amino acid level, among diverse organisms. As this family of proteins is induced by stressors other than heat, they are also commonly referred to as stress proteins in the literature. The term stress proteins also may refer to several other groups of proteins that respond to stressors. Stress proteins are among the most abundant intracellular proteins. Prokaryotic and eukaryotic cells react to exposition unfavorable conditions of the outer environment by increased synthesis of the stress proteins. The structure and functions of these proteins are evolutionary highly conserved and they are present in different variations in the cell of all living organisms (Iwama et al., 1998). An expression of HSPs is induced by many environmental stresses including exposure to trace metals or organic pollutants, changes in temperature or osmolarity, hypoxia, anoxia, exposure to ultraviolet radiation and reactive oxygen forms. Although during stress, proteins are expressed as the constitutive proteins, and they play the significant role even in the cells which are not exposed to the stress factor (Harboe and Quayle, 1991; Iwama et al., 1998; Feder and Hofmann, 1999; Kopećek et al., 2001). In Mediterranean mussel (*Mytilus galloprovincialis*) exposed to 0.75 μM CH_3Hg^+ for 6 days, CH_3Hg^+ inhibited MgHSP70 and induced MgHSC70 expression (Franzellitti and Fabbri, 2005).

2.5 Use of biomarkers in field studies

The use of biomarkers for the determination of xenobiotic contamination has been increasingly adopted as part of the environmental monitoring programs.

Parsont (2003) studied on MT expression in Hg-exposed mussel *P. viridis*. The RT-PCR data showed that there was a significant higher levels of MT mRNA in treated mussels within 2 to 4 weeks of Hg exposure ($p<0.05$). At concentration of 1 ppb, $HgCl_2$ induced the MT mRNA levels significantly comparing to control.

Timmermans, et al., (2005) studied on MT expression in springtail (*Orchesella cincta*, insecta) at cadmium contaminated area and reference site in Netherlands. The MT gene in cadmium tolerance of *O. cincta* was studied by means

of quantitative RT-PCR. The constitutive and Cd-induced MT mRNA expression of the laboratory cultures was measured. Results show that the mean constitutive MT mRNA expression of populations from polluted sites was significantly higher than of populations from reference sites.

Zorita, et al., (2007) studied two families of MT Isoforms (MT10 and MT20) in mussels (*Mytilus galloprovincialis*). Mussels were exposed to 200 ppb Cd and 40 ppb Cu for 2 and 9 days to characterize the tissue and isoform specificity of metal-induced MT expression. MT expression was detected in non-ciliated duct cells, stomach and gill epithelial cells, haemocytes, adipogranular cells, spermatic follicles and oocytes. RT–PCR resulted in cloning of a novel *M. galloprovincialis* isoform homologous to recently cloned *Mytilus edulis*. In gills, Cd only affected MT10 gene expression after 2 days of exposure while increases in MT protein levels occurred at day 9. In the digestive gland, a marked increase of both isoforms, but especially of MT20, was accompanied by increased levels of MT proteins and basophilic cell volume density (VvBAS) after 2 and 9 days and of intralysosomal metal accumulation in digestive cells after 9 days. Conversely, although metal was accumulated in digestive cells, lysosomes and the VvBAS increased in Cu-exposed mussels, Cu exposure did not produce an increase of MT gene expression or MT protein levels. These data suggested that MTs were expressed in a tissue-, cell- and isoform-specific way in response to different metals.

Choi, et al., (2008) studied HSP90 and MT gene in Pacific Oyster (*Crassostrea gigas*). The expression of HSP90 increased significantly with exposure to 0.01 ppm Cd for 11 days or 0.05 or 0.1 ppm Cd for 7 days. The expression of MT increased significantly with exposure to 0.01, 0.05, or 0.1 ppm Cd for 11 days. Glutamate oxaloacetate and glutamate pyruvate levels increased significantly with exposure to 0.05 or 0.1 ppm Cd for 7 days. These results indicated that HSP90 and MT played important roles in the physiological changes related to metabolism and cell protection that occur in Pacific oysters exposed to Cd.

Brulle, et al., (2007) studied on MT gene in earthworms exposed to 80 mg/kg of Cd in soil. A significant increase of the quantity of mRNA expressed after 14 h (6 fold; $p=0.012$) was observed compared to the quantity obtained in control animals. mRNA levels were 20, 24, 28, 63 folds higher after 1 day ($p=0.0003$), 2 days

(p=0.001), 6 days (p=0.026) and 14 days (p=0.035) of exposure respectively. The induction pattern was quite similar in earthworms exposed to 800 mg/kg of Cd in soil, but gene induction started earlier. Significant inductions (3 fold, 28 fold, 35 fold, 85 fold, 76 fold, 80 fold) were registered after 6 h, 14 h, 1 day, 2 days, 6 days and 14 days of exposure respectively.

Baker, et al., (2003) studied CYP isoforms in aspects of human cadmium toxicity. The possible link between non-workplace cadmium (Cd) exposure, cytochrome P450 expression and hypertension was investigated. Results of the investigation into the relationships between liver and kidney Cd burdens and the abundance of the CYP isoform 4A11 were shown. Data showed associations between non-workplace Cd exposure and changes in the abundance of hepatic and renal cortical CYP4A11. In liver, the levels of immunochemically detectable CYP4A11 were positively correlated with tissue Cd content while, in contrast, CYP4A11 abundance was inversely correlated with kidney Cd burden. These differences were most likely related to the different Cd burden of the tissues. These observations suggested the potential for involvement of Cd as a mediator of CYP4A11 expression in kidney cortex and indicated that elevations in kidney Cd content might be involved in hypertension via alteration of the expression of this particular isoform.

Poupardin, et al., (2008) studied on Cytochrome P450 monooxygenases activities in mosquito (*Aedes aegypti*) larvae, exposed to permethrin, fluoranthene and copper. Quantitative RT-PCR on different biological replicates was used to validate the expression pattern of the genes isolated from microarray experiments. The specific inductions of CYP6M6 by fluoranthene and CYP6M11 by copper were confirmed (2.2- and 3.4-fold, respectively). Interestingly, the induction of CYP9M9, CYP9M8, CYP6Z8, CYP6AL1, CYP6N12 and CCEjhe1F by one xenobiotic was confirmed but multiple inductions of these genes by other xenobiotics were also observed.

Faverney, et al., (2000) studied on heavy metals and oxidative stress in fish liver cells by measuring *CYP1A* expression in trout (*Oncorhynchus mykiss*) hepatocytes. 3-methylcholanthrene (3-MC) induced the CYP1A-related EROD activity. This induction was inhibited by concomitant exposure to Cd (II), Cu (II), Pb (II) or Zn (II). *CYP1A* mRNA levels were also reduced. Simultaneous treatment with

3-MC, a heavy metal and TEMPO suppressed both the inhibition of EROD activity and the decrease of *CYP1A* mRNA expression.

Franzellitti and Fabbri, (2005) studied on 2 genes (MgHSP70 and MgHSC70) in Mediterranean mussel (*Mytilus galloprovincialis*) exposed to heavy metals (Hg^{2+} and Cr^{6+}). Hg^{2+} (150 µg/L for different time periods) significantly induced MgHSP70 expression that reached maximum levels after 24 h, decreasing thereafter. MgHSC70 expression was inhibited after 1 day and induced after a 6-day exposure to Hg^{2+}. A 1-week exposure to Cr^{6+} (1, 10, and 50 ng/L) induced and inhibited MgHSC70 and MgHSP70 transcript levels, respectively.

Köhler and Eckwert, (1996) studied HSP70 in laboratory toxicity tests, woodlice (*Oniscus asellus*, Isopoda) exposed to a variety of different combinations of the metals. Cadmium, lead, and zinc exhibited a broad range in intensity of the induction of the 70 kDa stress protein (hsp70, stress-70).

Rios-Arana et al., (1995) studied on HSP60 in the rotifer *Plationus patulus*, exposed to various concentrations of As, Cr, Cu, Ni, Pb, and Zn. Following exposure, total protein was quantified and stress protein 60 (HSP60) was identified using Western blotting. *P. patulus* induced HSP60 as a response to single exposures to low and high heavy metal (As 10 and 50 µg/l), (Cr 10 and 50 µg /l), (Cu 10 and 50 µg/l), (Ni 10 and 50 µg /l), (Pb 10 and 100 µg /l) and (Zn 20 and 50 µg /l). HSP60 expression was increased (2 folds) in rotifers exposed to these single elements at both low and high concentrations as compared to unexposed rotifers. Arsenic exposure resulted in a 2 fold decrease in HSP induction.

2.6 Techniques used for examination of mercury exposure and toxicity

2.6.1 Toxicity test for identification of acute toxicity

Toxicity test is needed in water pollution evaluation due to chemical and physical test alone are not sufficient to assess potential effects on aquatic organisms. To examine acute toxicity, which is a relatively short-term lethal or other effect,

usually defined as occurring within 4 d for fish and macro invertebrate, toxicity test has to be done.

Toxicity test can be divided following the method of adding test solutions into 3 types:

1. Static test is the test in which solution and test organisms are placed in test chambers and kept for the duration of the test.

2. Renewal test is the test in which organisms are exposed to solutions of the same composition that are renewed periodically during the test period. This is accomplished by transferring test organisms or replacing test solution.

3. Flow-through test is the test in which solution is placed continuously in test chambers throughout the test duration.

To conduct short-term test, the technique can be static, renewal, or flow-through test. Exposure period for these tests are 48 h or 96 h. Static or renewal tests are less expensive to perform than flow-through tests. However, the flow-through test is suit for high –BOD or COD system and for the test of unstable of volatile substances (APHA, AWWA, WEF, 1992)

2.6.2 Single cell gel electrophoresis for examination of genotoxicity

To measure DNA strand breaks, the single cell gel electrophoresis or comet assay is a rapid, sensitive and in expensive method. This method has advantage over other DNA damage methods, such as sister chromatid exchange, alkali elution, and micronucleus assay, because of its high sensitivity and the DNA strand breaks are determined in individual cells.

The technique is performed by dispersing and immobilizing cultured cell or isolated cells in an agarose gel coated on appropriate support media, such as microscope slides. The fixed cells are lysed with alkaline lysis solution to disperse cell component and leave the immobilized DNA in the agarose. The DNA is denatured in an alkaline solution. Strand breaks in the denatured cellular DNA result in supercoil relaxation. The more breaks leads to the greater the degree of relaxation. The application of an electric field across the slides creates a motive force by the

charged DNA may migrate through the surrounding agarose away from the immobilized nuclear DNA. The DNA in the fixed slides in stained with a fluorescent DNA-specific dye. DNA binding dyes which can be used for comet assay included ethidium bromide, propidium iodide, etc. Stained slides are examined using a fluorescent microscope. Optimal magnification will depend on the quality of DNA in the cells being assessed for DNA damage. The migration of DNA away from the nucleus can be measured by eyes using an ocular micrometer or image analysis software to determine various parameter of the comet, i.e. tail length, percentage of DNA in tail, tail moment (Lee and steinert, 2003) (figure 2.1).

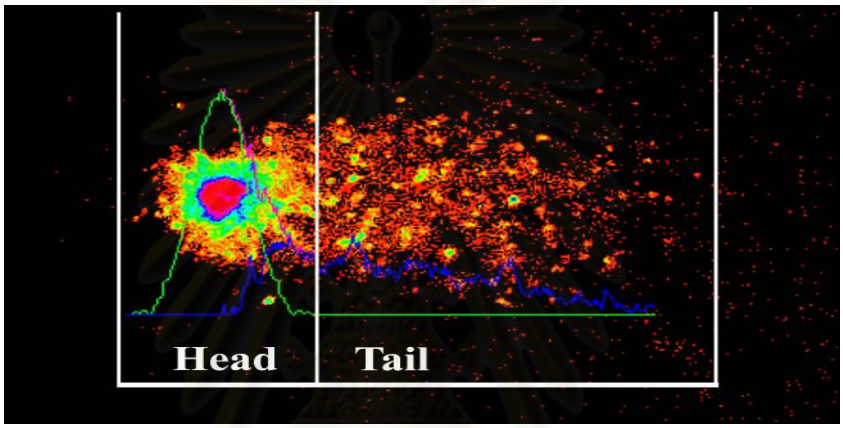


Figure 2.1 Diagram of typical comet showing distribution of DNA in tail and head.

2.6.3 RT-PCR (Reverse Transcription PCR) and Semi-quantitative RT-PCR for determination of gene expression

Reverse transcription polymerase chain reaction (RT-PCR) is a technique for amplifying a defined piece of a ribonucleic acid (RNA) molecule. The RNA strand is firstly reverse transcribed into its complementary DNA (cDNA), followed by amplification of the obtained cDNA using polymerase chain reaction. RT-PCR differs from the conventional PCR by cDNA is used as template rather than genomic DNA. The method has been used to determination of gene expression in mRNA population (Kawasaki et al., 1990)

Semi-quantitative RT-PCR is a quantitative technique used to quantitate the relative amount of mRNA as cDNA from the starting samples. Target cDNA is separately or co-amplified with the internal control gene, such as β - actin, elongation factor 1 alpha, using the same template. Use of internal control gene is under the

criterion that they are transcribed constantly and independently from the environmental stimuli.

2.7 Green Mussel

Green Mussel, *Perna viridis* is a marine mussel in Phylum: Mollusca, Class: Bivalvia, Subclass: Pteriomorphia, Order: Mytiloida, Family: Mytilidae with separate sexes and external fertilization. The life span of *P. viridis* is typically 1-2 years. Growth rates are influenced by environmental factors such as temperature, food availability and water movement.

P. viridis is a large mussel, 80-100 mm in length, occasionally reaching 165 mm. The shell tapers to a sharp, down turned beak and has a smooth surface covered with a periostracum (skin) that can be vivid green to dark brownish-green near the outer edge and olive-green near the attachment point. The ventral margin of the shell is straight or weakly concave. The interior of the shell valves is shiny and pale bluish green. The ridge which supports the ligament connecting the two shell valves is finely pitted. The beak has interlocking teeth: one in the right valve and two in the left. The wavy posterior end of the pallial line and the large kidney-shaped adductor muscle are diagnostic features of this species.

2.7.1 Growth and Reproduction

Sexes in this species are separated and fertilization is external. Spawning generally occurs twice a year between early spring and late autumn. However in the Philippines and Thailand, spawning occurs year round. Fertilized eggs develop into larvae and remain in the water column for two weeks before settling as juveniles. Sexual maturity typically occurs at 15-30 mm shell length (corresponding to 2-3 months of age). First year growth rates vary between locations and range from 49.7 mm/yr in Hong Kong to 120 mm/yr in India. (Rajagopal et al., 1998)

2.7.2 Feeding

This species is an efficient filter feeder, feeding on small zooplankton, phytoplankton and other suspended fine organic materials.

2.7.3 Habitat

P. viridis forms dense populations (up to 35,000 individuals per square meter) on a variety of structures including vessels, wharves, mariculture equipment, buoys and other hard substrata. It is susceptible to overgrowth from other fouling organisms that make it difficult to detect despite its vivid green appearance. It is primarily found in estuarine habitats with salinities ranging from 18-33 ppt and temperatures from 11-32 ºC, *P. viridis* has a broad salinity and temperature tolerance (in experimental testing it survived salinities of 1-80 ppt and temperatures of 7-37.5 ºC).

Many species of mussels can be used as marine biomonitoring, for example, Zebra mussel, oyster, blue mussel and green mussel, because of the mussel can accumulate the heavy metal including Hg. The tissue of *Mytilus galloprovincialis* accumulate twice as much MeHg during exposure for 35 d to the two forms of Hg in both the food and water (Fowler et al., 1978). Small mussels accumulate slightly more of both types of Hg than large mussels. (Pelletier and Larocque, 1987)

Perna viridis is commonly used as biomonitoring organisms because it is an efficient filter feeder (feeding on small zooplankton, phytoplankton and other suspended fine organic material). It has strong capacity for bioconcentration of xenobiotics. It is a common sessile animal and widely distributed in the Gulf of Thailand, It has relative long life span, large size of individuals can analyzed of individual specimens (Figure 2.2) (Amiard et al., 2000; NIMPIS, 2002).

Figure 2.2 Green mussel, *Perna Viridis*

CHAPTER III

MATERIALS AND METHODS

3.1 Test organisms

Green mussels, *P. viridis*, length 8–10 cm were collected from unpolluted-Hg area of Trad Bay at Trad Province, Thailand. This area is less polluted because of the absence of any industrial activities. Mussels were transported to the laboratory (Center of Excellence for Marine Biotechnology (CEMB), Faculty of Science, Chulalongkorn University Bangkok, Thailand) in the polystyrene foam boxes. Fouling organisms were removed from the external part of the shells. In the laboratory, mussels were acclimated for 4 weeks in seawater at the 30‰ salinity, pH 8.0 and temperature ranged from 23 to 25 ºC. Mussels were fed with unicellular algal species (*Chaetoceros* sp.) (Figure 3.1 C and D) and Brine shrimp (*Artemia* sp.) 1 time daily during acclimation and exposure periods. To avoid the accumulation of nitrite and nitrate, water was exchanged 10% daily and aeration was provided by continuous air-bubbling system.

3.2 Mercuric Chloride

Mercuric Chloride used in the experiment was obtained from local supplier while laboratory grade Hg was purchased from Ajax Finechem, Inc. (Australia). The stock solutions were prepared by diluting mercuric chloride in distilled water to give stock concentration.

3.3 Mercury analysis in mussel tissue

Total Hg concentration was determined in the mussel samples. Approximately 0.1–0.2 g of lyophilized and homogenized tissue was weighed and placed into Teflon digestion vessel. After addition of 5 ml of conc. HNO_3 (ultra pure) and 0.040 g of V_2O_5, the vessel was closed and the mixture was left to react at room temperature for 1 h. Digestion was finished by heating in microwave for 5-10 min After the digestion, samples were left in room temperature until it cooled down, then 3 ml of $K_2Cr_2O_7$ was added and diluted to 40 ml. by Mili-Q water. Total Hg concentrations in tissue and

water sample were determined by CVAAS and CVAFS the method was modified from the method of U.S. EPA (1997), Odžak (2000) and Gašpić (2006).

3.4 Experimental set up

3.4.1 Laboratory test

The experiment was conducted in five 800 L tanks. Each tank contained approximately 200 mussels put in mussel bag (15-20 mussel per 1 bag) (Figure 3.1A) with aerated seawater and bioreactor (Figure 3.1 E and F). Mercuric chloride ($HgCl_2$) was applied to each tank making up the concentration of 0.0, 0.1, 0.2, 0.5 and 1.0 µg/l, respectively. Water quality was monitored every 2 days for ammonia, nitrite, and nitrate. The water was exchanged for 10 % every day and $HgCl_2$ was applied with the new water in the ratio to maintain the same calculate concentration in every tank. Mussels were maintained in this condition for 2 months.

Sampling was performed for the period of 8 weeks. At each sampling time, 10 specimens were sampled. Gills and digestive tracts of the mussels were dissected and snap frozen in liquid nitrogen and then stored in -80 ˚C freezer. Shell length and weight of each individual specimen was measured. The collected samples were subjected to RNA extraction and remain tissue samples were kept in sealed plastic bags at -20 ˚C freezer for Hg analysis.

Figure 3.1 Green Mussel tanks maintained in Laboratory. mussel bag (A), worker (B), plankton culture tank (C and D), mussel tank show bioreactor (E), enlarge bioreactor (F)

3.4.2 Field study

Green mussels collected from the same area for laboratory test were transplanted and maintained in petroleum production platform at sites specific in figure 3.2 for 90 days. Mussels were put in net bag, each contained 15-20 specimens and tied to the rope at 5, 20 (upper thermocline), and 40 m below the sea level (below thermocline) (Yanagi, 2001). The end of the rope was weighed with heavy concrete to maintain position (Figure 3.3). Sampling was performed monthly in the period of 3 months. At each sampling time, the content of one net bag with 10 specimens was sampled. Shell length of each individual specimen was measured. Gills and digestive

tracts were dissected and mixed with RNA *later*® solution. Specimens were kept in 4 ˚C cooler when transported from experiment site to laboratory and then stored in -80 ˚C freezer (Figure 3.5). Remain tissue samples for Hg analyses were stored in sealed plastic bags at -20 ˚C freezer for total RNA extraction, cDNA preparation, and analysis of expression level of candidate genes using the same method with lab test.

Plankton was collected every time of mussel sampling using 20 micron mesh size, 50 cm diameter and 150 cm long. Plankton was sampled vertically in a water column of 40 m. depth from the sea water surface and preserved in 3% neutral formaldehyde, stored at room temperature and transported from experiment site to laboratory (Figure 3.4)

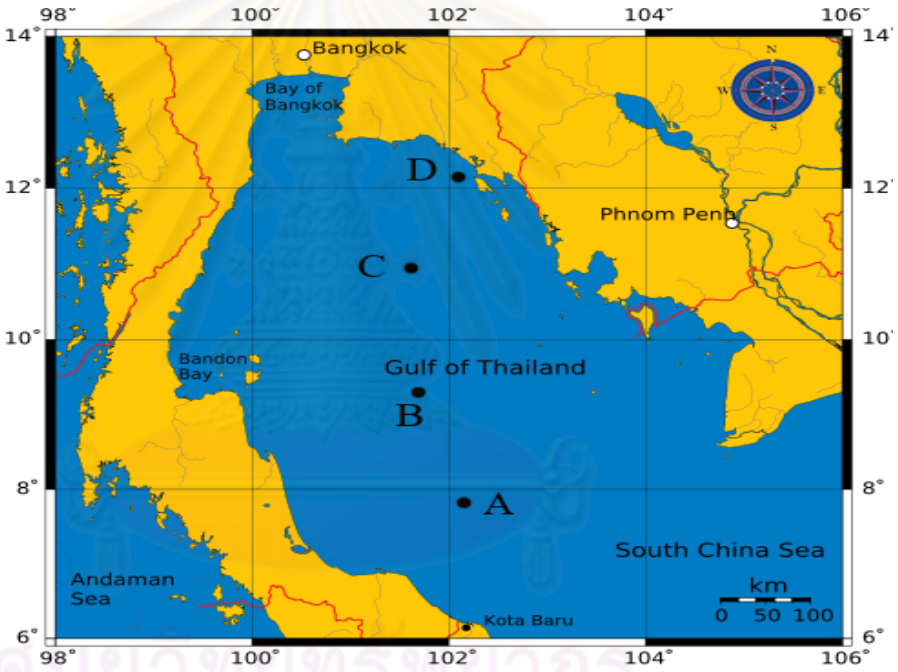


Figure 3.2 Experimental sites in the Gulf of Thailand. Station A (N7 54.765, E102 733.154), B (N9 15.633, E101 76.635), and C (N11 09.216, E101 51.367) are the locations of petroleum production platforms where the field study was carried out. Station D (N12 23.265, E101 15.0879) is the reference site near Trad Province. The map is modified from http://commons.wikimedia.org/wiki/File:Gulf_of_Thailand.svg

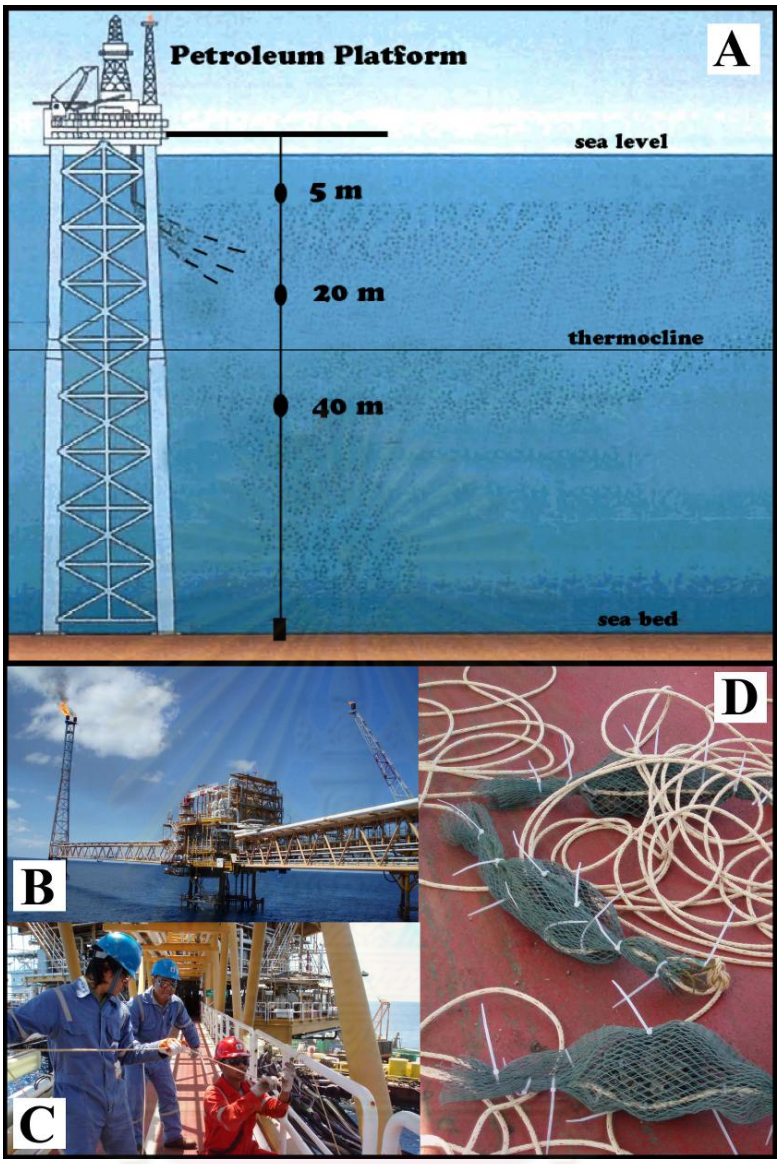


Figure 3.3 Transplantation of mussels at experimental sites. Diagram of the transplanted mussels is shown in A. Pictures of petroleum production platform, mussel transplanting process, and mussel bags attached to the string are shown in B, C, and D, respectively.

Figure 3.4 Plankton sampling at experimental sites. Planktons were collected using 20 micron mesh (A, B, and C). Collected planktons were preserved in plastic bottle containing 3% neutral formaldehyde (D and E).

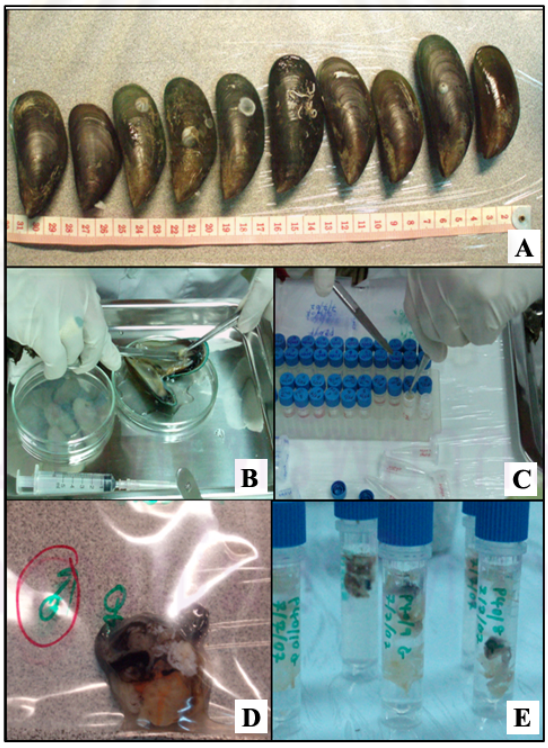


Figure 3.5 Mussel specimens and sample preparation. Mussels were collected and shell length was measured (A), tissues were dissected (B), gills and digestive tracts were preserved in RNA later (C, E), and the remaining tissues were in plastic bag and kept in -20ºC (D).

3.5 Basic techniques for molecular study

3.5.1 Nucleic acid extraction

3.5.1.1 Genomic DNA extraction

Genomic DNA was extracted from a piece of target tissue using a phenol-chloroform-proteinase K method. A piece of frozen or fresh tissue was placed in microcentrifuge tube containing 500 µl of the extraction buffer (100 mM Tris-HCl, 100 mM EDTA, 200 mM NaCl, pH 8). The tissue was briefly homogenized and added with SDS (10%) and RNase A (10 mg/ml) solutions to a final concentration of 1 % (w/v) and 100 µg/ml, respectively. The mixture was incubated at 37 ºC for 1 h. Proteinase K solution was added (300 µg/ml) and further incubated at 55 ºC for 3-4 h. An equal volume of buffer-equilibrated phenol: chloroform: isoamylalcohol (25:24:1) was added and gently mixed for 10 min. The solution was centrifuged at 10,000 rpm for 10 min at room temperature. The aqueous phase was transferred into a new microcentrifuge tube. The solvent exchange process was repeated once with phenol: chloroform: isoamylalcohol (25:24:1) and once with chloroform: isoamylalcohol (24:1). The aqueous phase was transferred into a new microcentrifuge tube. One-tenth volume of 3 M sodium acetate, pH 5.2 was added to the aqueous solution. Two volume of chilled absolute ethanol was added and gently mixed to precipitate genomic DNA. DNA pellet was recovered by centrifugation at 12,000 rpm for 10 min at room temperature, washed twice with 1 ml of 70% ethanol, air-dried, and re-suspended in 50-80 µl of TE buffer (10 mM Tris-HCl, pH 8.0 and 0.1 mM EDTA). The DNA solution was incubated at 37 ºC for 1-2 h and kept at 4 ºC until use.

3.5.1.2 RNA extraction

Tissue samples were dissected and immediately homogenized in liquid nitrogen. Ground samples were mixed with TRI REAGENT (Molecular Research Center, Inc, USA) (50-100 mg. or tissue per 1 ml of Tri reagent) and maintained for 5 min at room temperature to permit the complete dissociation of nucleoprotein complex. The mixture was then centrifuged at 12,000 g for 10 min the aqueous phase (upper phase) was transferred to a fresh tube and extracted with 0.2 ml of Chloroform per 1 ml of TRI REAGENT. The mixture was left a room temperature for 2-15 min

then centrifuged at 12,000 g for 15 min at 4 ˚C. The colorless upper aqueous phase containing RNA was transferred to a fresh tube. RNA was then precipitated by the addition of isopropanol (0.5 ml of isopropanol per 1 ml of Tri reagent originally used). The mixture was kept a room temperature for 5-10 min before centrifugation at 12,000 g for 8 min at 4 ˚C. The supernatant was discarded and RNA pellet was washed with 75% ethanol followed by centrifugation at 7,000 g for 15 min at 4 ˚C. The pellet containing total RNA was air-dried for 3-5 min, dissolved in DEPC-treated distilled water and kept at -80 ^{o}C until further used.

3.5.2 Determination of Nucleic acid

3.5.2.1 Spectrophotometry

DNA and RNA can be quantified by measuring the absorbance at the wavelength of 260 nm (A_{260}). One A_{260} unit for double strand DNA, single strand RNA, and oligonucleotide equals to 50, 40, and 33 µg/ml, respectively. The concentration of nucleic acid was calculated using the following equation:

Nucleic acid concentration (µg/ml) = A_{260} x absorbability coefficient x Dilution factor

The quality of nucleic acid was estimated by the ratio of A_{260}/A_{280}. The isolated DNA that was free from RNA and protein, the acceptable A_{260}/A_{280} ratio must be higher than 1.7.

3.5.2.2 Agarose gel electrophoresis

DNA and RNA were determined by agarose gel electrophoresis using 1.2 to 2.0 % agarose gel. Generally, agarose gel was prepared by adding agarose powder into 1x TBE buffer (89 mM Tris-Hcl, 8.9 mM boric acid, and 2.0 mM EDTA), melt in microwave oven until completely dissolved, and then poured into the gel mould with an appropriate comb. The gel was left to solidify for at least 30 min at room temperature. The comb was gently removed and the gel was transferred into the electrophoretic chamber, TBE (1x) was added to cover the gel. Five µl of PCR products was thoroughly mixed with one-tenth volume of 10x loading dye (0.25% bromophenol blue and 25% ficoll) and care fully applied into the gel slot. Two hundred µg of 100 bp DNA ladder was used as standard DNA marker.

Electrophoresis was carried out at constant voltage of 100 volts until tracking dye reach about 1 cm from the lower edge of the gel. After electrophoresis, the gel was stained with ethidium bromide (0.5 µg/ml) for 3 min and destained to remove unbound ethidium bromide by submerging in water for 10 min. The DNA fragments were visualized under the UV light using UV transiluminator. The visible bands of DNA on the stained gel were photographed using camera Pentax K1000 (Asahi Opt. Co, Ltd).

3.5.3 First strand cDNA synthesis using Reverse Transcription Polymerase Chain Reaction

Total RNA isolation from tissue of mussel was subjected to single stranded cDNA synthesis by the reverse transcriptions of mRNA to cDNA using oligodT_{15} primer. The reverse transcription reaction was performed in the final volume of 20 µl, at 42^{o}C, for 90 min using Improm II ^{TM} reverse transcription kit. The condition includes 1 U of Improm II ^{TM}, 2 µl of 1 x Improm II ^{TM} reactive buffer, 2.5 mM $MgCl_2$, 0.5 mM dNTP mix, 0.5 µg Oligo dT, and 2.0 U of recombinant RNasin® Ribonocluose Inhibitor. The obtained first strand cDNA template was kept at -20 ^{o}C until use.

3.5.3.1 PCR

The target cDNA is amplified from single stranded cDNA template by PCR using degenerated primers designed from conserved sequences of genes. The reaction mixture of PCR contains 1X PCR buffer (10 mMTris-Hcl pH 8.8, 50mM KCl, 0.1%TritonX-100), 0.4 mM dNTPs, 1.5 mM $MgCl_2$, 1U of Taq DNA polymerase, 100 ng of cDNA template and 0.5 µM of forward and reverse primers. The reaction mixture was carried out in thermal cycler.

3.5.4 RACE-PCR

3.5.4.1 Primer design

Gene specific primers (GSPs), including Metallothionein, Cytochrome P450, and Heat Shock Protein are designed from the obtained nucleotide sequence resulting from cloning and sequencing analysis.

3.5.4.2 First strand cDNA synthesis

Total RNA extracted from gill and digestive tract using TRI REAGENT® (Molecular Research Center, Inc) was subjected to mRNA purification using illustra^{TM} QuickPrep Micro mRNA Purification Kit (GE Healthcare UK Limited).The purified mRNA was further reversed transcribed to RACE-Ready cDNA using a SMART^{TM}RACE cDNA Amplification Kit (Clontech Laboratory, Inc). The reverse transcription is performed by mixing of 1.0 µg mRNA, 1µl of 5' CDS primer A and 1 µl of SMART II A oligonucliotide for 5'RACE-PCR and 1.0 µg of mRNA, 1µl of 3' CDS primer A for 3'RACE-PCR. The solution was gently mixed and briefly centrifuged. The reaction was incubated at 70 ^{o}C for 2 min and immediately placed on ice of 2 min. The reaction tube was briefly centrifuged and added with 2 µl of 5X first strand buffer, 1 µl of (20mM) DDT, 1 µl of dNTP Mix (10mM each) and 1 µl of MMLV Reverse Transcriptase. The reaction was then incubated at 42 ^{o}C for 1.5 h. The first strand reaction products were diluted with 125 µl of Tricine-EDTA buffer and heat at 72 ^{o}C for 7 min.

3.5.4.3 Rapid Amplification of cDNA Ends (RACE)

Master Mix of 5' and 3' RACE PCR reaction were prepared in a volume of 41.50 µl for each reaction. The mixture contains 34.5 µl of PCR-Grade water, 5 µl of 10X Advantage 2 PCR buffer, 1 µl of dNTP mix (10 mM each) and 1 µl of 50X Advantage 2 polymerase mix. PCR reaction was performed for 25 cycles of 94 ºC for 30 sec, 68 ºC for 30 sec, and 72 ºC for 3 min. The 5' and 3' RACE-PCR products were analyzed using 1.2% agarose gel electrophoresis.

3.5.5 SSCP (Single-Strand Conformation Polymorphism)

SSCP was the electrophoretic separation of single-strand nucleic acids base on subtle differences in sequence (often a single base pair) which results in a different secondary structure and a measurable difference in mobility through a gel. The method used in this experiment was a method described by Hein et al., (2003) using Polyacrylamide Electrophoresis, PROTEAN® II xi Cell (Bio-Rad, USA). Polyacrylamide gel (1.5% gel, 2.66% cross-link) (16x20x0.4cm) was prepared. The gel was allowed to polymerize for 4 h and pre-run in the gel box in a 4 ^{o}C cold room

for at least 5 min. PCR product (8 µl) from each sample was mixed with 32 µl of loading dye and heated at 95 ^{o}C for 5 min and immediately transferred to the ice box. Sample was loaded onto polyacrylamide gel and running until the dye reaches the bottom of the gel. After the gel was removed with on side attached to the glass, placed into the fix-stop solution for 20 min, and washed with distilled water, the gel was stained with 0.1% silver nitrate for 30 min., washed again with distilled water for 10 sec., and placed into the developing solution. Once the band of DNA starts to appear, the gel was transferred into freshly prepare developing solution and shaken until all DNA bands are visualized. Following gel staining, separating band of ssDNA was analyzed. Each ssDNA band was cut out of the gel and washed 3 times with ultrapure water. Each band was added with 20 µl of ultrapure water and incubated at 37 ^{o}C for 24 h to allow DNA to diffuse to from gel to the water. This cDNA was used as template for amplification of each variation of genes. PCR product was loaded onto 1% agarose gel and the band of interest is cut out and DNA was eluted.

3.5.6 Cloning and Sequencing

Target DNA obtained from PCR was purified using QIAquick gel extraction kit (QIAGEN). Method was conducted following manufacture protocol. Purified DNA was ligated to pGEM-T easy vector by performing in a final volume of 10 µl ligation reaction that contains 3 µl of target PCR product, 25 ng of pGEM-T easy vector, 5 µl of 2x rapid ligation buffer (60 mM Tris-HCl pH 7.8, 20 mM DTT, 2 mM ATP and 10% PEG 8000) and 3 Weiss unit of T4 DNA ligase. The ligation solution was gently mixed by pipetting and incubated at 4 ^{o}C overnight. Ligation product was then transformed into *E.Coli* strain JM109 cells by mixing the resulting ligation solution into 200 µl of the competent cells, placing in a 42 ^{o}C water bath for 45 sec, and immediately placing on ice for 5 min. The solution was then added into a tube containing 1 ml of SOC medium. The solution was incubated at 37 ^{o}C with vigorous shaking for 1.5 h. The solution was transferred to a microcentrifuge tube and centrifuged at 8,000 rpm for 1 min at room temperature. The supernatant was discarded and the cell pellet was resuspended with 100 µl of SOC medium. The cell solution was spread on LB agar containing 50 µg/ml of ampicillin, 25 µg/ml of IPIG, and 20 µg/ml of X-gal. The plate was incubated at 37 ^{o}C overnight. The recombinant clones containing inserted DNA were observed as white colony whereas the clones

without inserted DNA are blue colony. The recombinant plasmid was screened for the size of inserted DNA using colony PCR. The PCR was performed in a volume of 25 µl containing 1x buffer, 1.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, dTTP, 0.2 µM of pUC1 (5'CCGGCTCGTATGTTGTGTGGA-3') and pUC2 (GTGCTGCAAGGCGATTAAGTTGG-3') primers and 0.5 U of DyNAzyme^{TM}II DNA Polymerase (Finnzymes). Individual of recombinant colony was picked using micropipette tip and mixed in the amplification reaction. The PCR profiles was predenatured at 94 ^{o}C for 3 min, followed by 35 cycles of 94 ^{o}C for 30 sec, 55 ^{o}C for 60 sec, and 72 ^{o}C for 90 sec, and a final extension at 72 ^{o}C for 7 min, The resulting PCR product was analyzed using agarose gel electrophoresis. Alternatively, the recombinant clones showing expected size of target DNA insert can be detected by restriction enzyme digestion. The method was conducted by isolating plasmid from cultured cells using QIAprep® Miniprep Kit (QIAGEN GmbH, D-40724 Hilden). The insert size of recombinant plasmid was examined by digestion with *ECo*RI. The digestion was performed in a volume of 15 µl reaction containing 1x restriction buffer (90mM Tris-HCl, pH 7.5, 10mM NaCl, and 50 mM $MgCl_2$), 1 µg of recombinant plasmid and 2-3 units of *ECo*RI and incubated at 37 ^{o}C for 3-4 h. The resulting digestion was analyzed using agarose gel electrophoresis. Recombinant plasmid containing target DNA fragment was subjected to sequence analysis. The cloned DNA fragment was sequenced by automated DNA sequencer using M13 forward and/or M13 reverse primers as the sequencing primer by MACROGEN (Korea). The obtained nucleotide sequences were subjected to BLAS search (NCBI) to identify homologous nucleotide sequence.

3.6 Cloning and characterization of mercury inducible genes in *P.viridis*

Target genes including metallothionein, cytochrome P450, and heat shock proteins that reported in various animals to respond significantly to heavy metal exposure or recognized as sensitive biomarkers for heavy metal were subjected to cloning and characterization. cDNA partial sequences of the target genes were determined by performing degenerate and specific PCR and full length sequences were obtained by RACE-PCR.

3.6.1 Primer design

To amplify fragments of the target genes in *P.viridis*, degenerated and specific primers for PCR amplification were designed. Primers specific to each target gene are shown in Table 3.1. For Metallothionein gene, degenerated primers were designed from conserved regions specific to each isoform of MT.

Table 3.1 Sequence, length and the melting temperature of primers designed for MT genes and some of its isoforms from *P.viridis*.

Gene	Sequence	Length (bp)	Tm ^{o}C
Metallothionein	Sense 5′ATgCCCAgCCCTTgTAATTg 3′	20	57.8
	Anti sense 5′TTATTTgCACgAACAACTgg 3′	20	53.7
β-actin	Sense 5′TTgggACgATATggAgAAgAT 3′	21	56.2
	Anti sense 5′ACgACCAgAggCgTACAgAg 3′	20	57.5
pvMT01	Sense 5′CTTgTAATTgCATTgAAACAA 3′	21	51.9
	Anti sense 5′CATgCACACTCTCCTggC 3′	18	54.3
pvMT02	Sense 5′ggTgCAgCggAgAAgA 3′	16	51.7
	Anti sense 5′CTggAgTCACATTTACAggTg 3′	21	52.7
pvMT03	Sense 5′gTgggAgTggATgCAgC 3′	17	53.8
	Anti sense 5′CCACACgCACACgCAT 3′	16	54.2
pvMT07	Sense 5′gTgggAgTgggTgCAgA 3′	17	54.0
	Anti sense 5′TACAggTCTTTggTCCCg 3′	18	53.4
pvMT08	Sense 5′TgggAgTgggTgCAgA 3′	16	52.2
	Anti sense 5′TACAggTCTTTggTCCCA 3′	18	50.7
pvMT11	Sense 5′ATgCCCAgCCCTTgTAAT 3′	18	55.1
	Anti sense 5′TgCACACTCTCCTggCTg 3′	18	54.9
CYP4	Sense 5′ATCCgAgCggAAgTCgATACTT 3′	22	62.1
	Anti sense 5′gTggTTCATgCCAgACAgTTgg 3′	22	62.5
HSP71	Sense 5′ACTCgCAAAgACAggCAACAA 3′	21	60.8
	Anti sense 5′CTCAgACgACgAACAgCTCTCTT 3′	23	60.8

3.6.2 PCR amplification

PCR was conducted to amplify fragments of target gene using total RNA extracted from gill and digestive tract of *P.viridis* . PCR reaction was performed in a final volume of 25 µl, which composed of 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.2 µM Forward Primer, 0.2 µM Reverse Primer, 1U of DyNAzyme^{TM} II DNA Polymerase (Finnzymes), The typical PCR profiles was predenaturing at 94 ^{o}C for 5 min, followed by 35 cycles of 94 ^{o}C for 30 sec, 50-65 ^{o}C for 45 sec (depending on the melting temperature of the primer), and 72 ^{o}C for 45 sec, and a final extension at

72 ^{o}C for 5 min. Amounts of first strand cDNA template and annealing temperature for each gene are shown in Table 3.2

Table 3.2 Details on template and annealing temperature used for gene amplification

Gene	First Strand cDNA (ng)	Anealing Temperature (^{o}C)	PCR Product (bp)
Metallothionein	500	50	220
β-actin	500	50	208
pvMT01	500	50	151
pvMT02	500	50	154
pvMT03	500	50	118
pvMT07	500	50	147
pvMT08	500	50	146
pvMT11	500	50	159
CYP4	250	55	355
HSP71	250	55	337

3.7 Expression analysis of the gene in mussel exposed to mercury

Expression levels of target genes, including various subunits of metallothionein, cytochrome P450, heat shock protein 70 in mussels exposed to Hg (lab test) and mussels transplanted at petroleum platforms (field test) were analyzed using semi-quantitative RT-PCR analysis. β-actin was used as internal control gene.

3.7.1 Exposure of mussel to mercury

Acclimated mussels were exposed to mussel in 5 exposure series: 0, 0.1, 0.2, 0.5 and 1 µg/l. Exposed mussel (N=10) were collected from each treatment after 1, 2, 3, 4, 5, 6, 7 and 8 weeks of exposure and their gills and digestive tracts were subjected to the total RNA extraction and first strand cDNA synthesis as described in 3.5.1.2 and 3.5.3.

3.7.2 Semi-quantitative analysis

3.7.2.1 Primer design

Primer designed specifically for each target gene and used for semi-quantitative RT-PCR are shown in Table 3.1

3.7.2.2 Optimization of PCR condition

Prior to the quantitative analysis, the appropriate PCR condition including temperature, template concentration, number of cycles, and $MgCl_2$ concentration for each of target genes and reference gene were verified based on the criteria that the PCR product must be on the log phase of amplification.

PCR was performed in a PCR thermal cycler (Hybraid Limited, England). The PCR reaction was based on the standard condition consisted of 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.2 μM Forward Primer, 0.2 μM Reverse Primer, 1U of DyNAzyme^{TM} II DNA Polymerase (Finnzymes), The typical PCR Profiles was predenaturing at 94 ^{o}C for 5 min, followed by 35 cycles of 94 ^{o}C for 30 sec, 50-65 ^{o}C for 45 sec (depending on the melting temperature of the primer), and 72 ^{o}C for 45 sec, and a final extension at 72 ^{o}C for 5 min. The condition was optimized as follow.

First, the annealing temperature for each target gene was adjusted within several degrees to obtain the best intensity and specificity of the target band. Then, PCR reactions with various concentrations of DNA templates (between 50-1000 ng) and amplified in different numbers of PCR cycles (20, 25, 30 and 35 cycles) were carried out. The condition that amplified the PCR product in the exponential range and did not reach a plateau level was chosen. Also, the applications of $MgCl_2$ and primer concentration, ranged from 1.5, 2, 3, and 5 mM for $MgCl_2$ and 0.05, 0.1, 0.15 and 0.2 μM for primers, and were determined, the concentrations that gave the highest yield and specificity were chosen.

3.7.2.3 Semi-quantitative RT-PCR

Semi-quantitative RT-PCR for each target gene was conducted using the optimize condition as shown in Table 3.4. The PCR product was analyzed using

agarose gel electrophoresis. The intensity of target band was examined using the Quantity I Program (BioRad). The expression ratio of target gene and β-actin gene was analyzed using statistical package in SPSS Version 15 for Window. Significant different among group of treatments was examined using Duncan's test and $P<0.05$.

Table 3.3 Optimal condition for Semi-quantitative RT-PCR of gene in gill and digestive tract of Hg exposed mussel.

Gene	Template (ng/µl)	$MgCl_2$ (µM)	Primer (µM)	Anealing Temp. (^oC)	PCR cycle Number	PCR product (bp)
Metallothionein	500	50	25	50	28	220
β-actin	500	50	25	50	28	208
pvMT01	500	50	25	50	28	151
pvMT02	500	50	25	50	28	154
pvMT03	500	50	25	50	28	118
pvMT07	500	50	25	50	28	147
pvMT08	500	50	25	50	28	146
pvMT11	500	50	25	50	28	159
CYP4	250	50	25	55	28	355
HSP71	250	50	25	55	28	337

3.8 Determination of the correlation between Hg levels and expression levels of candidate genes.

Correlation of Hg levels and expression level of candidate genes will be evaluated by correlations and one way analysis of variance (ANOVA) followed by Duncan's new multiple range tests. Significant comparisons will be considered when the P value is < 0.05.

3.9 Single cell gel electrophoresis analysis or comet assay, (Genotoxicity test)

In vitro testing was conducted to examine the genotoxic effects of Hg on mussel haemocytes and sperms using single cell gel electrophoresis assay. The method was performed according to Pereira et al., (2010) with modification.

3.9.1 Haemocyte

Haemolymph was withdrawn from coelomic fluid of mussel using 24 G needle and 1 ml syringe containing 10% sodium citrate as anticoagulant (1:1 dilution). Haemolymph was centrifuged at 600 g for 2 minutes to precipitate haemocyte and then resuspended in 100 µl of 10% sodium citrate.

Haemolymp obtained from healthy mussel were separated into 5 treatments with 3 replications. The test was conducted by adding 10 µl of 10% Sodium citrate to haemolymp and diluted to the solution that contained approximately 10^4 haemocytes. The solution was then mixed with mercuric chloride stock solution which was diluted to make the final concentration of 0, 0.001, 0.01, 0.1, 1.0 and 10.0 µg/l, respectively.

The exposed haemocytes were then mixed with melted 1% low melting-point agarose, subsequenctly layered on 1 % agarose-precoated microscope slide, covered with a cover slip, and allowed to solidify at 4 ^{o}C. The coverslip was removed and a second layer of low melting-point agarose was placed on the top of the solidified layer. The gel was then covered with the cover slip and stored at 4 ^{o}C for solidification. After the removal of cover slip, the slide was subjected to a lysis step by placing the slide into chilled lysis solution (2.5 M NaCl, 10 mM Tris-HCl, 0.1 M Na_2EDTA, 1X Triton X, 10% Dimethylsulfoxide, pH 10) at 4 ^{o}C for 1 h. At the end of lysis step, the slide was placed in an electrophorisis chamber containing alkaline electrophoresis buffer (0.3 M NaOH, 1 mM Na_2EDTA, 0.2% Dimithylsulfoxide)for 30 min to allow DNA to unwind. The electrophoresis was conducted for 10 min at 26 V and 300 mA. After completion of the electrophorisis , the DNA was neutralized by placing the slide into neautralization buffer (400 mM Tris-HCl pH 7.5), stained with ethidium bromide, and dried for immersing in absoluted ethanol. For DNA damage visualization and analysis, micoscopic analysis was conducted using Olympus BX 50 Microscope. Randomly chosen nuclei image ($N \geq 50$) from each slide were taken using Pentax 20D and categorized into ghost cells or cells damaged by cytotoxicity and comet cells or cells whose DNA was damaged by genotoxicity, the comet cells were then analyzed using Comet Score software (CometScore^{TM}, TriTek Corporation, USA). DNA tail length was measured from the exposed haemocytes. DNA tail moment was calculated (tail length x % DNA in tail), and used as parameters for determining the degree of DNA damage.

Viability of haemocytes and the values of tail length and tail moment were analyzed using the statistical package in SPSS Version 15 for Window. The difference of parameter among group of treatment for each experiment was tested for normality and variance homogeneity using Shapiro-Wilk and Levene's test. For the genotoxicity test, the parameter was averaged tail length and tail moment. Significant different among group of treatments was examined using Duncan's test at $P<0.05$.

3.9.2 Sperm

Sperms was withdraw from sperm sac of mussel using 1 ml pipettes tip and diluted to 10^6 cell/ml. in 10% sodium citrate as anticoagulant approximately 500 µl.

Prior to the assay, *in vitro* cell was examined on the normal and Hg exposed haemocytes and sperms after 10, 30 and 60 min of exposure using PBS buffer. The mixture was left at room temperature.

The method for comet assay study in sperms similar with study in haemocytes cells.

3.10 Statistical Analysis

Variables of each experiment were typically analyzed using the statistical package in SPSS Version 15 for Window. The difference of each variable among group of treatment was tested for normality and variance homogeneity using Shapiro-Wilk and Levene's test. Significant different among group of treatments was examined using Duncan's test at $P<0.05$.

CHAPTER IV

RESULTS

4.1 Cloning and characterization

4.1. Degenerate PCR amplification

Degenerate primers designed from conserved regions of HSP70, HSP90, and CytochromeP450A1 of Pacific oyster, *Crassostrea gigas* (GenBank accession no. CB617404), Abalone, *Haliotis asinina*, (GenBank accession no.EF621884), and *Crassostrea gigas* (GenBank accession no. AJ305315), respectively (Table 4.1), were used for DNA amplification. As shown in figure 4.1, DNA fragments at the sizes of 400, 180, and 250 bp, were obtained from the PCR of HSP70, HSP90, and CYP1A, respectively. Sequence analysis these PCR products were shown in figure 4.2-4.4. BLAST result (Table 4.2) indicated that these 3 fragments were no match to the sequences of HSP70, HSP90, and CYP1A.

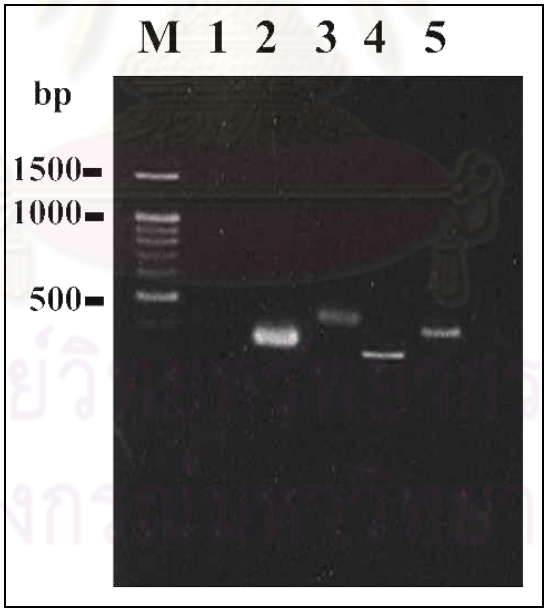


Figure 4.1 PCR product of HSP70 (lane 3), HSP90 (lane4), CYP1A (lane5) and β-actin gene (land 2) as positive control. Lane M is 100 bp DNA ladder.

Table 4.1 Degenerate primers designed for amplifying target genes in mussel.

Gene	Primer sequence	Length (bp)	Size (bp)
HSP70	Forward 5′ ggAATAgATCTTggAACCACATA 3′	23	
	Reward 5′ TTgCCAAgATATgCTTCTgCAgT 3′	23	382
HSP90	Forward 5′ ggTgAATgTTACCAAggAAgg 3′	21	
	Reward 5′ gTTACgATACAgCAAggAgATg 3′	22	126
CYP1A	Forward 5′ gTgCATCAAAgAATTTTggATAC 3′	23	
	Reward 5′ TgCAATAATTTTTgAAgCCCCgg 3′	23	248

GGAATAGATCTTGGAACCACATAATTAGAGCTAGTAAGCACTGTTTAAGTACACTAAAACAT
TTTCTCCCCTTTCCACAAAGTCATAAGCATTATGTGCTAGTCAATAGCAGGAAAAATTAATT
ACTTTAAATTGAAAAAAAAGAAAAACTATAAACTTATATCACAGAGATGTTTCCCCTGTCAC
ATATGGATGTTGTTATTCGAGACTTGATTGGTGGTAACAGTGTTACTAATTTCATATGTAAT
GGATAAAATATGATATCAATTCAACTTTTTGACACTACCTAAAAGGATAGATTCCTCGGGTA
TAGTTTGTTTGCATATTGACCTGTGTTAAAGTGATAATCTCAAACCATG**ACTGCAGAAGCAT**
ATCTTGGCAA

Figure 4.2 Nucleotide sequence of 400 bp fragment. Primers used in PCR are indicated in bold and underlined.

GTGAATGTTACAAGGAAGGAGGCTGAGAGTTGTGATTGCCTTCAGGGATTCCAGCTTACACA
TTCCTTGGGAGGTGGAACTGGATCTGGTATGGGAACCCTGCT**CATCTCCTTGCTGTATCGTA**
AC

Figure 4.3 Nucleotide sequence of 180 bp fragment. Primers used in PCR are indicated in bold and underlined.

GTGCATCAAAGAATTTTGGATACTCTGGAAACATTATCGAAAGAGGATTGCGAAGAAAAAGG
AATCTATCATACGCTTAAAGAAGCGACGGAGGAAAAAGACGAAAATGGGGAACCGTGTATAA
ATGAGGATAACATATATGGAATACTTTTTAATCTTGCTGGAGCTGGATATTTAACAACACGG
GGAACTCTATTATCCGTAATTCAGATCCTTGCAAAAAGA**CCGGGGCTTCAAAAATTATTGCA**

Figure 4.4 Nucleotide sequence of 250 bp fragment. Primers used in PCR are indicated in bold and underlined.

.

Table 4.2 BLAST results of PCR products amplified from first strand cDNA template of mussel tissue using degenerate primers designed from HSP70, HSP90, and CYP1A genes of closest species.

Gene	Detected size (bp)	Actual size (bp)	Putative gene	Species	Expected value	Figure
HSP70	400	382	Unknown	-	-	4.1
HSP90	180	126	Beta-tubulin	*Rhizoctonia solani*	0.58	4.1
CYP1A	250	248	Unknown	-	-	4.1

4.1.2 Specific PCR amplification

Specific primers (table 4.3) designed from HSP71 and CytochromeP450 family4 (CYP4) of green mussel, *P.viridis*, (GenBank accession no. DQ988328 and EU429566, respectively) were amplified and subjected to sequence analysis. The results of PCR and sequencing (figure 4.6 and 4.7) confirmed the identity of HSP71 and CYP4 genes of *P.viridis*.

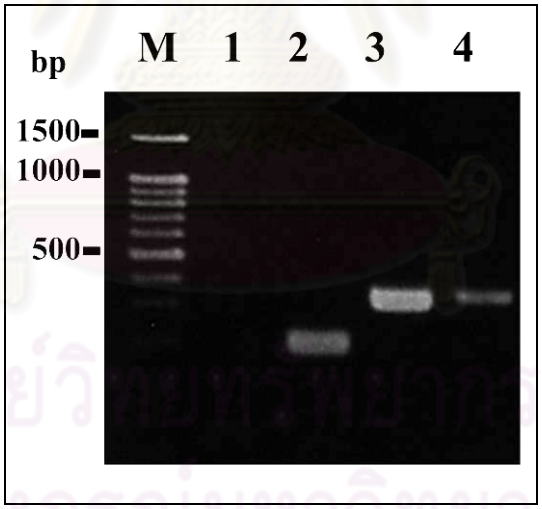


Figure 4.5 PCR product of HSP71 (lane 3), CYP4 (lane4) β-actin gene (land 2) as positive control, and negative control (lane1). Lane M is 100 bp DNA ladder.

Table 4.3 Specific primers designed from HSP71 and CYP4 genes of *P.viridis.*

Gene	Primer sequence	Length (bp)	Size (bp)
HSP71	Forward 5′ ACTCgCAAAgACAggCAACAA 3′ Reward 5′ CTCAgACgACgAACAgCTCTCTT 3′	21 23	337
CYP4	Forward 5′ ATCCgAgCggAAgTCgATACTT 3′ Reward 5′ gTggTTCATgCCAgACAgTTgg 3′	22 22	355

ACTCGCAAAGACAGGCAACAAAAGATGCTGGAACCATCTCTGGAATGAATGTGCTGCGTATCATCAATG
AACCTACAGCTGCAGCTATTGCCTATGGTTTAGACAAAAAGGCTACAGGTGAAAGAAATGTTCTCATTT
TTGATCTTGGAGGAGGAACTTTTGATGTATCCATTCTGACAATTGAAGATGGTATCTTTGAAGTCAAGT
CCACCTCTGGAGACACTCACTTAGGTGGGGAAGATTTTGACAACAGAATGGTGAATCACTTCATCCAAG
AATTCAAACGCAAACACAAGAAAGACATTAGTGAAAAC**AAGAGAGCTGTTCGTCGTCTGAG**

Figure 4.6 Nucleotide sequence of HSP71. Primers used in PCR are indicated in bold and underlined.

ATCCGAGCGGAAGTCGATACTTTCTTGTTTGCAGGTCATGATACTACGACCAGTGCAATGAC
TTGGATTCTGTATGAGCTTGCAAAACAACCGGATTACATGATGCAATGTCAGGAAGAAATTG
ATATTGTCTTAAAGGATAGCCATGGCGTTGTAAAGTGGGATAGTTTGGAAAAATTAGAATTT
TTGACTCAGTGCATTAAGGAAGGTATGAGACTTCATTCACCTGTTCCGATCATCGGTAGACA
GGCGTCAAGACCCTATACCATAGATGGTGTAACATTCCCAGCGAAAACTTACTTTTCTGTGC
AAGTGTATGCCTTACACCACAAC**CCAACTGTCTGGCATGAACCAC**

Figure 4.7 Nucleotide sequence of CYP4. Primers used in PCR are indicated in bold and underlined.

Table 4.4 BLAST results of PCR products amplified from first strand cDNA template of mussel tissue using specific primers designed from HSP71 and CYP4 genes of *P.viridis*. (Appendix C)

Gene	Detected size (bp)	Size (bp)	Putative gene	Species	Expected value	Figure
HSP71	337	337	Heat shock protein 71	*P. viridis*	$2x10^{-56}$	4.5
CYP4	355	355	Cytochrome P450 family4	*P. viridis*	$6x10^{-65}$	4.5

4.2. Laboratory study

4.2.1. Mercury concentration (Total mercury) in mussel tissues

Average Hg levels in mussel tissues (whole soft tissue) measured in tank 1 (control), 2, 3, 4, and 5 were 0.0104±0.0090, 0.0579±0.0241, 0.0885±0.0500, 0.1378±0.0505, 0.1644±0.0500, 0.1383±0.0803 µg/g, respectively (Table 4.5). In the first week, Hg concentrations in un-exposed (control) and other Hg expose mussels remained the same level except in tank 3 (200 ng/L) which were significantly higher (Fig.4.8A). At the end of the experiment (week 8), the differences between treatments were statistically significant (Fig.4.8B). Hg levels increased in corresponding to the increasing levels of Hg that applied to the mussels. The average level of Hg in the mussel before treatment was 0.0104±0.0091 µg/g while the level from the highest Hg treatment for 8 weeks was 0.1383±0.0803 µg /g. The Hg level was approximately 10 times higher than that of initial mussel.

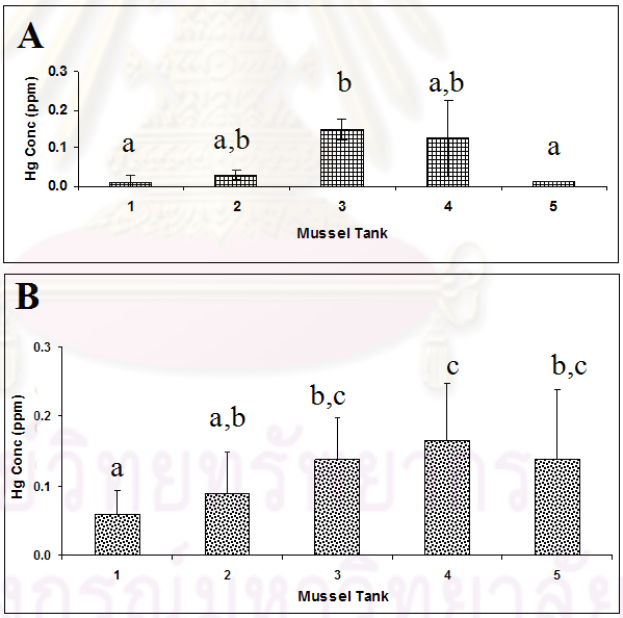


Figure 4.8 Hg concentrations in mussel tissue. The results of Hg level in mussel tissue at week 1(A) and average 8 weeks (B)

Table 4.5 Mercury concentration (mean ± SD) in tissue of experiment mussels exposed to different concentration of $HgCl_2$ (µg/g)

Time of Exposure (week)	Mussel tanks				
	Tank 1 (Control)	**Tank 2** (0.1 µg/L)	**Tank 3** (0.2 µg/L)	**Tank 4** (0.5 µg/L)	**Tank 5** (1.0 µg/L)
0	0.0104±0.0090	0.0104±0.0090	0.0104±0.0090	0.0104±0.0903	0.0104±0.0090
1	0.0110±0.0191	0.0300±0.0138	0.1490±0.0262	0.1266±0.0998	0.0140±0.0010
2	0.0926±0.0371	0.0651±0.0265	0.1582±0.0196	0.1176±0.0295	0.2076±0.1000
3	0.0601±0.0291	0.1142±0.0694	0.0539±0.0159	0.1506±0.0554	0.1048±0.0368
4	0.0559±0.0144	0.0793±0.0252	0.1057±0.0476	0.1237±0.0954	0.0747±0.0378
5	0.0650±0.0383	0.0898±0.0452	0.1464±0.0725	0.1966±0.0692	0.1499±0.0269
6	0.0601±0.0396	0.1930±0.0663	0.2081±0.0587	0.2550±0.1094	0.2493±0.1023
7	0.0608±0.0337	0.0454±0.0243	NA	0.1810±0.0836	0.0167±0.1312
8	NA	1.0914±0.0316	NA	NA	NA
Average	0.0579±0.0241	0.0885±0.0500	0.1378±0.0505	0.1644±0.0500	0.1383±0.0803

Remark: NA = data is not available due to mortality of mussel.

4.2.2 Mercury concentration in experiment water

After 8 weeks of mercuric chloride treatment, the average level of Hg detected in water of tank 1 (control) to tank 5 (0, 100, 200, 500, and 1000 ng/L, respectively) were 2.7514±1.6959, 4.9286±2.5461, 10.8600±7.2910, 18.0443±12.1593 and 40.1314±37.1110 ng/L, respectively (Table 4.6 and Figure 4.9). This revealed that Hg levels remained at 0.049, 0.054, 0.036, and 0.040 %, respectively, of the applied concentrations.

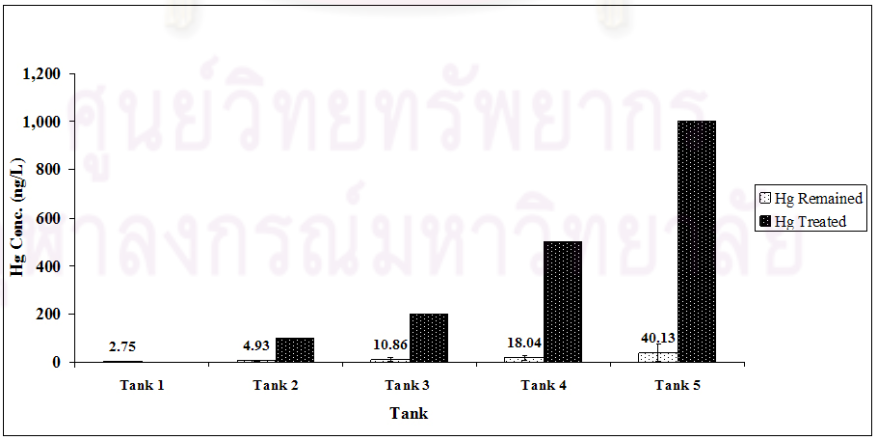


Figure 4.9 Hg concentrations in the mussel rearing water in tank 1 (0), tank 2 (0.1 µg/L), tank 3 (0.2 µg/L), tank 4 (0.5 µg/L), tank 5 (1.0 µg/L)

Table 4.6 Mercury concentrations in water of the experiment mussel tanks (ng/l)

Time of Exposure (week)	Mussel tanks				
	Tank 1 (Control)	**Tank 2** (0.1 µg/L)	**Tank 3** (0.2 µg/L)	**Tank 4** (0.5 µg/L)	**Tank 5** (1.0 µg/L)
0	2.60	8.48	23.14	27.93	109.5
1	0.72	4.65	4.82	6.64	15.60
2	1.53	2.37	3.88	6.60	18.82
3	2.57	3.06	6.34	9.26	15.31
4	2.03	2.23	7.54	17.38	22.67
5	5.85	6.00	12.44	19.26	24.03
6	3.96	7.71	17.86	39.24	74.99
7	NA	NA	NA	NA	NA
8	NA	NA	NA	NA	NA
Average	2.75	4.93	10.86	18.04	40.13

Remark: NA = data is not available due to lost of sample in analysis process

4.2.3 Water quality

Water qualities (amount of nitrogenous waste) were determined every 2 days. As shown in figure 4.10-4.11, the levels of ammonia, nitrite, and nitrate were between 0.8365±0.6971, 0.0243±0.0031, and 11.8290±007898 mg/L, respectively. Ammonia and nitrite were initially increased but reduced to the safety level within 1 and 2 weeks, respectively. Nitrate level was also increased constantly but reduced gradually after water exchange. These results indicated that the amounts of nitrogenous waste in all experiment tanks did not reach the toxic level.

Figure 4.10 Experiment tanks for rearing of green mussels (A) and details of bioreactor used for nitrogenous waste treatment (B, C, and D).

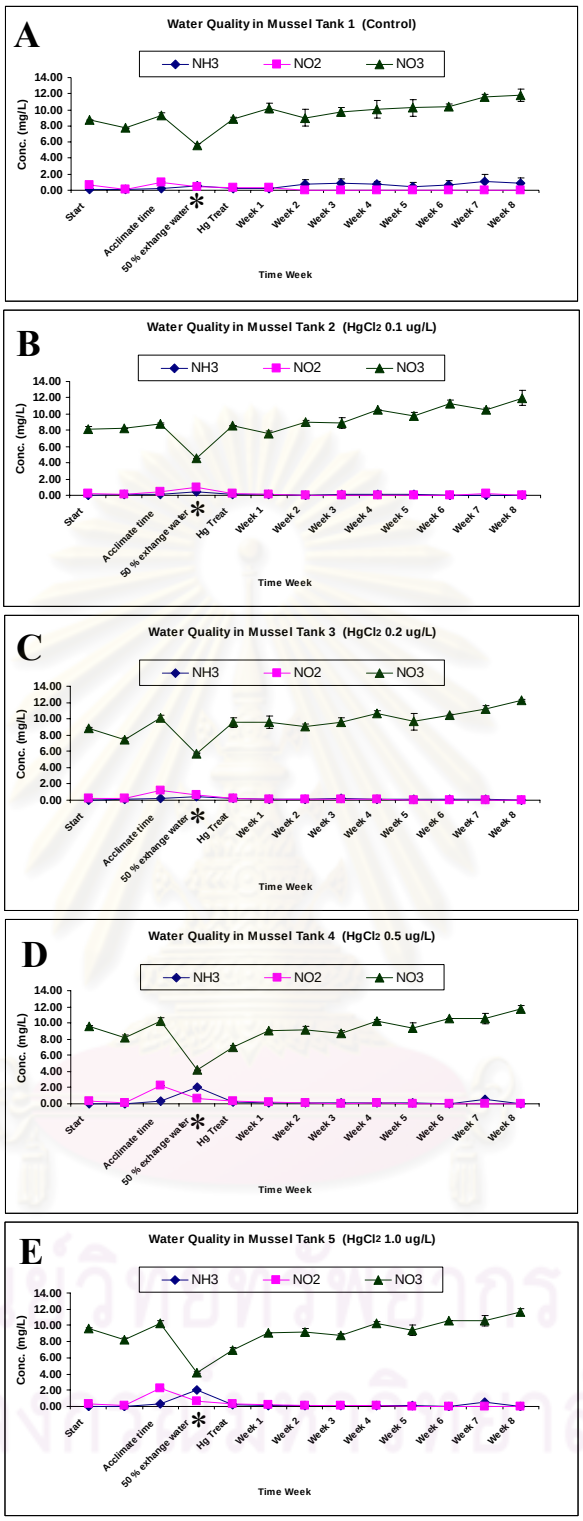


Figure 4.11 Water qualities of the experiment tanks. A= tank1 (control), B = tank 2 (0.1 µg/L), C =tank 3 (0.2 µg/L), D = tank 4 (0.5 µg/L), E = tank 5 (1.0 µg/L)

Remark**:** * Water was exchanged 50 %, 1 week before start treat Hg in the mussel tank.

4.3 Field study

4.3.1 Mercury concentration in mussel tissue

Hg concentrations in mussel tissues (whole soft tissue) measured from the mussel transplanted to petroleum platforms were shown in Table 4.7. Hg levels ranged between less than 0.0100 to 0.1725 μg/g. The concentration of Hg in mussels maintained at 5 m. from station A and B were in the same level as station D (reference site) while that of station C 30D (Fig 4.12) was significantly higher than that of the other stations ($P<0.05$).

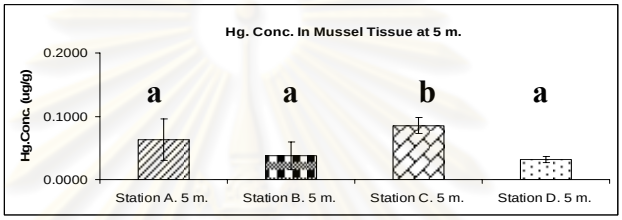


Figure 4.12 Hg concentration in mussel tissue from Station A, B, C and D at 5 m. depth. 30D.

When the levels of Hg in mussels from each station were compared according to time, the results showed that after 30 days, the level of Hg in tissue tended to increase. (Fig.4.13B, C, and E) At station A, the level of Hg in mussels at 20 m depth (Fig. 4.13E) at day 30 was significantly lower than that of day 60 and 90 ($P<0.05$).

When the levels of Hg in mussels from each station were compared according to depth, the result showed that there was significant difference between the level of Hg from the mussels exposed at 5 m, 20 m and 40 m depth at station B and C. At day 60, the level of Hg at 5m was significantly higher than that of 20 m and 40 m, respectively ($P<0.05$) (Fig. 4.14E and H)**.**

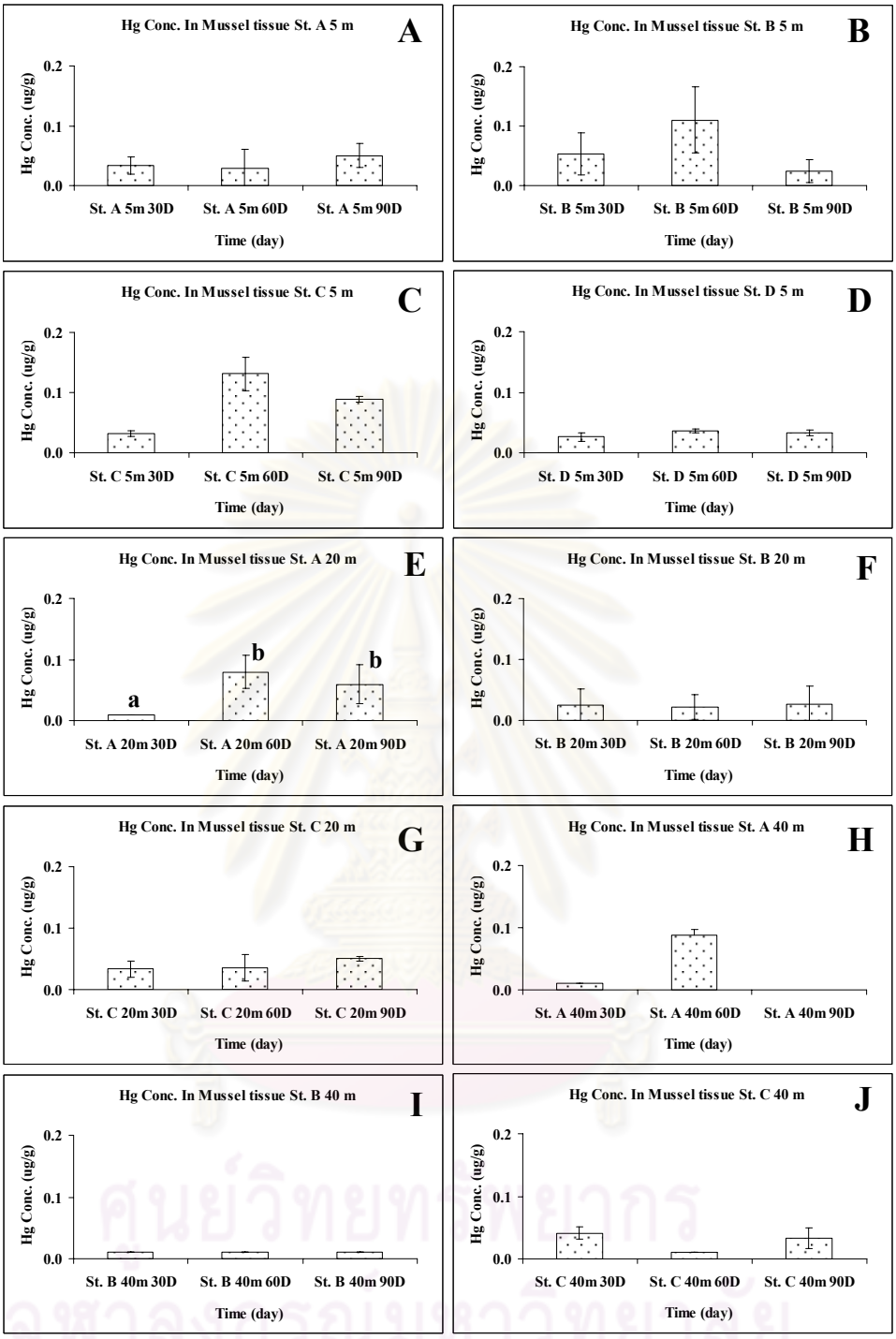


Figure 4.13 Hg concentration in mussel tissue according to time of exposure. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C respectively. H to J indicates the result from 40 m. depth at station A to C respectively.

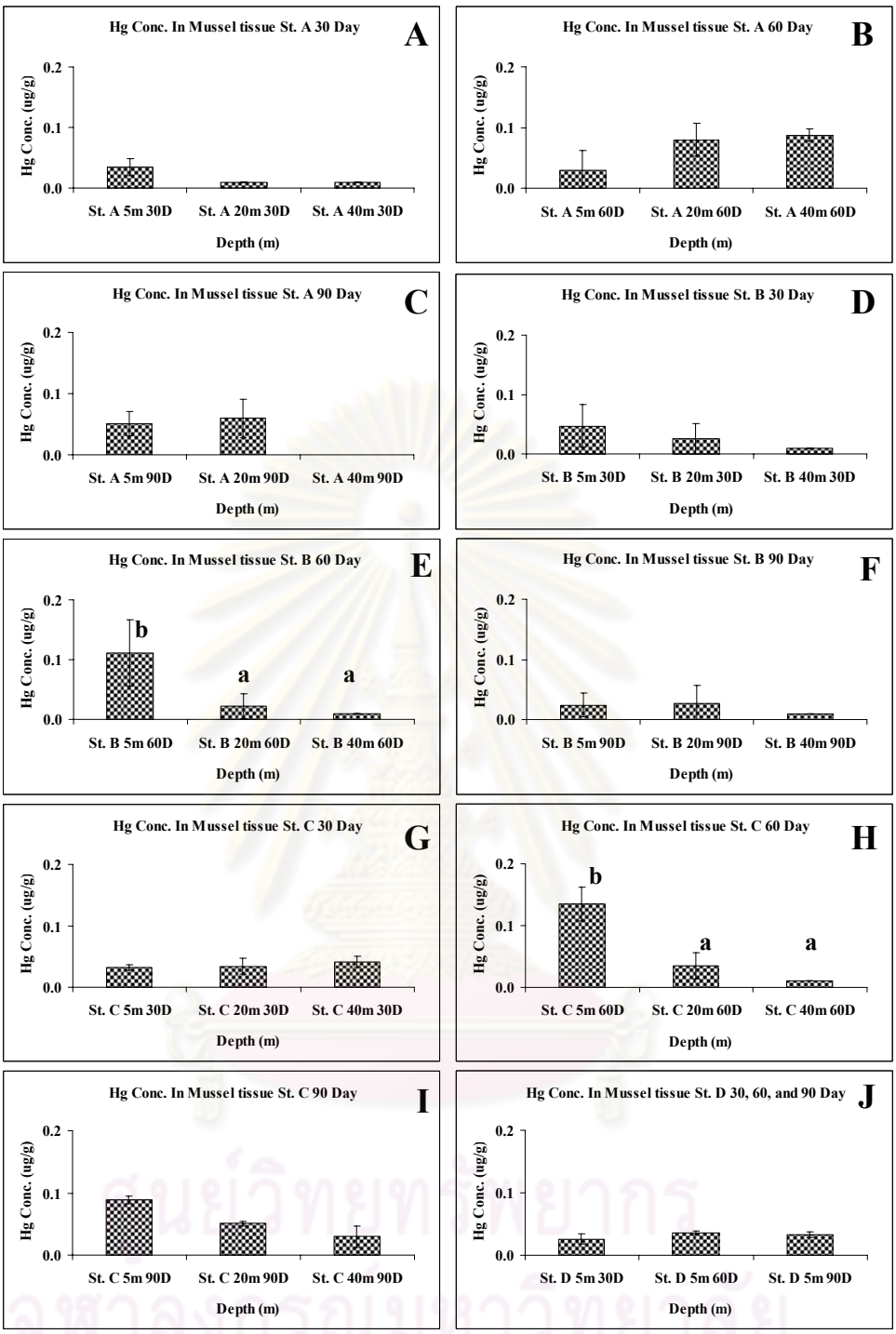


Figure 4.14 Hg concentration in mussel tissue according to depth of exposure. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C respectively. H to J indicates the result from 40 m. depth at station A to C respectively.

Table 4.7 Mercury concentrations (Mean ± SD) in mussel (μg/g) tissue from petroleum production platform station A, B, C, and D (reference station)

Station/Depth (m)	Days of Exposed		
	30 Day	**60 Day**	**90 Day**
St.A 5 m	0.0341±0.0143	0.0288±0.0326	0.0502±0.0198
St.A 20 m	0.0100±0.0000	0.0797±0.0275	0.0596±0.0316
St.A 40 m	0.0100±0.0000	0.0876±0.0097	NA
St.B 5 m	0.0535±0.0262	0.1104±0.0555	0.0243±0.0192
St.B 20 m	0.0250±0.0260	0.0219±0.0206	0.0269±0.0292
St.B 40 m	0.0100±0.0000	0.0100±0.0000	0.0100±0.0000
St.C 5 m	0.0319±0.0051	0.1313±0.0213	0.0888±0.0054
St.C 20 m	0.0337±0.0130	0.0349±0.0216	0.0505±0.0040
St.C 40 m	0.0412±0.0094	0.0100±0.0000	0.0293±0.0166
St.D 5 m	0.0261±0.0075	0.0360±0.0027	0.0328±0.0048

Remark: NA = data was not available due to mortality of mussel.
Result in station D (reference site) showed only in 5 m. depth due to shallow water at mussel farms.

4.3.2 Plankton composition

The average composition of planktons detected from different sites was shown in fig 4.15. No significant difference were found for the composition of plankton (*t*-test, ns) within the offshore stations (Station A, B, and C) while the near shore station which was reference site (Station D) was significantly higher than that of Station A, B and C ($p < 0.005$). The dominant groups of planktons in station A, B, and C was blue green algae while the dominant group of station D was diatoms.

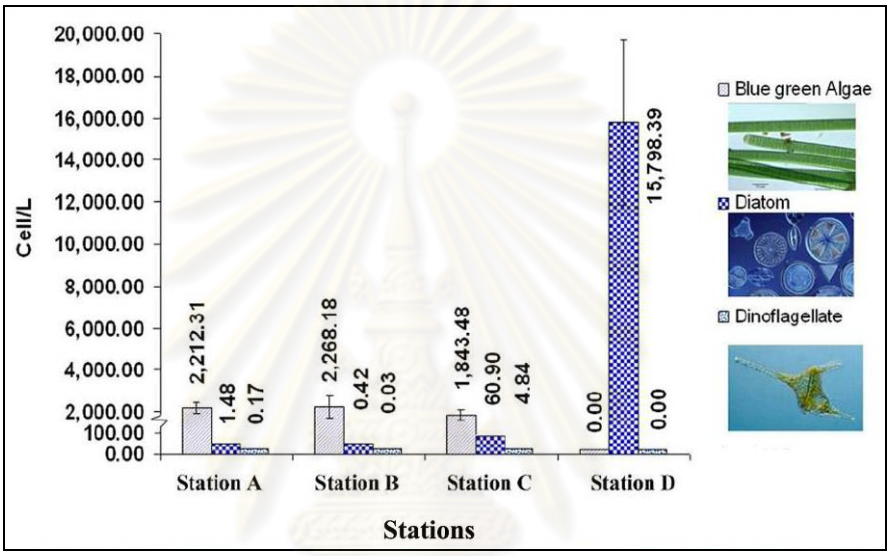


Figure 4.15 Plankton compositions from Petroleum Production Platforms (Station A, B and C) and reference site (Station D) in September 2007.

4.3.3 Survival rate of mussel

Transplanted mussels were collected (3 bags) from each station at 5, 20, and 40 m. and referent site. Survival rates of the mussels were determined and shown in Fig 4.16A-D.

Comparing the mussels maintained in different water depth, no significant difference between survival rates was found (*t*-test, ns) except at 90D station A and B survival rates of mussels at 20 m tend to higher than that of 5 and 40 m depth . When compared between stations, that of the mussels from Station D, which was a reference station, were significantly higher than that of the other station (*t*-test, s) ($p < 0.005$). During 3 months of experiment, the survival rates of mussel tend to decrease when increasing time of exposure.

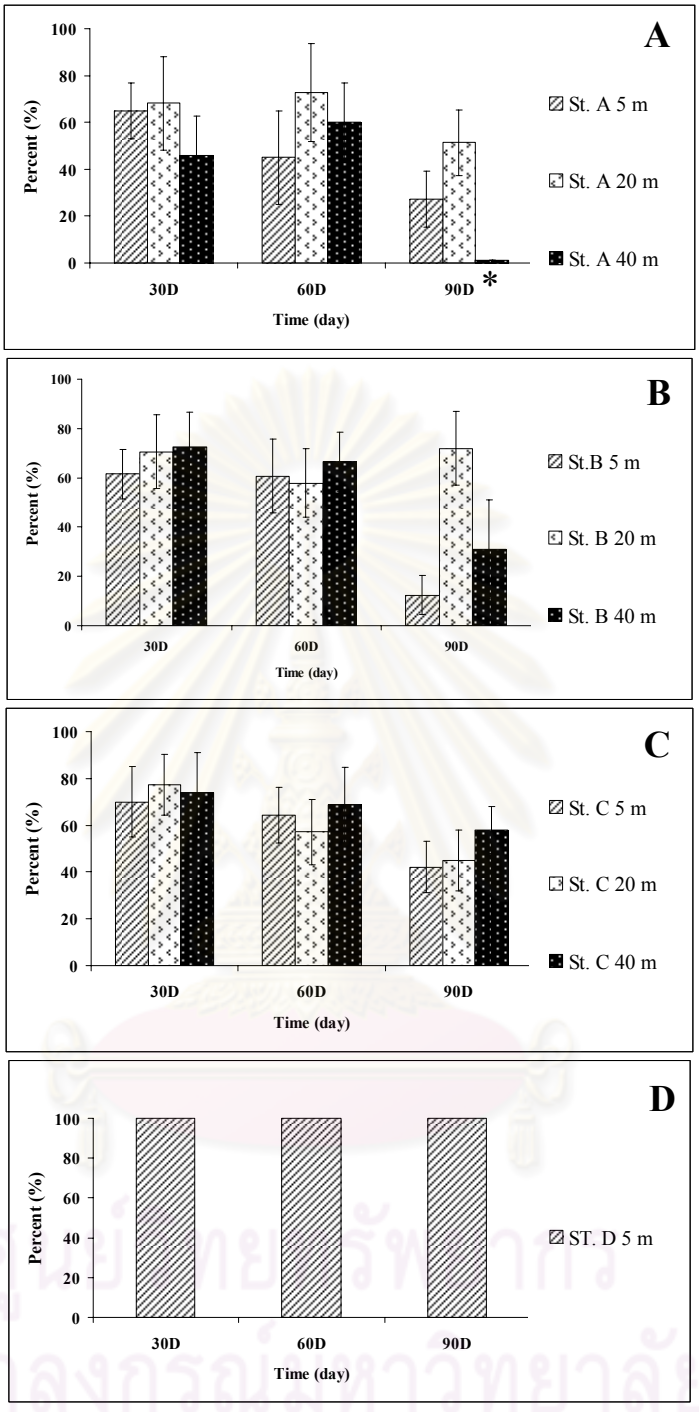


Figure 4.16 Survival rates of Mussel, *Perna viridis*, from Petroleum Production Platform. A, B, and C represent data from station A, B, and C, respectively while D indicates station D (reference site).

Remark: * Lost of sample (mussel string shear) at station A 40 m 90D

4.3.4 Growth rate of transplanted mussels

The mussel growth rate was determined by measuring the average shell length of 10 mussels from each treatment. Mussels from Station D. (TRAD reference site) appeared to have larger sizes than the mussels from other stations. The results were shown in Fig 4.17. By comparing between different depths and stations, no significant difference was found for shell length of mussel (*t*-test, ns) between stations.

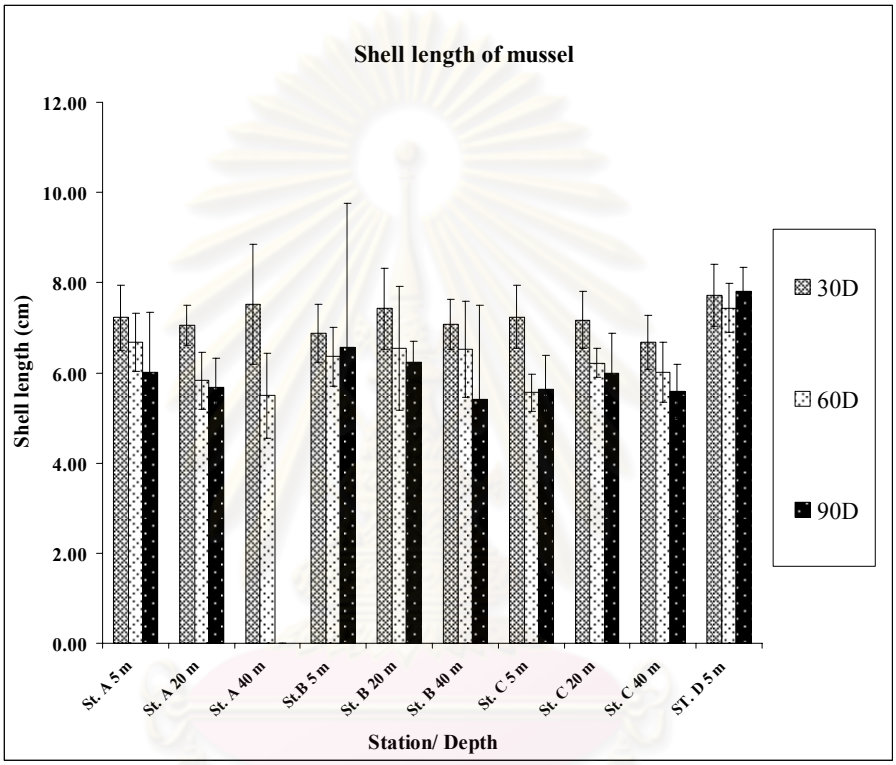


Figure 4.17 Shell lengths of transplanted mussel, *Perna viridis*, from Petroleum Production Platform

4.4 Determination of MT, HSP71, and CYP4 gene expression in Hg exposed mussels

4.4.1 Optimization of PCR condition

Prior to the quantitative analysis, the appropriate PCR condition including temperature, template concentration, number of cycles, and $MgCl_2$ concentration for each of target genes and reference gene were verified based on the criteria that the PCR product must be on the log phase of amplification. The condition was optimized as follow.

First, the annealing temperature for each target gene was adjusted within several degrees to obtain the best intensity and specificity of the target band. Then, PCR reactions with selected annealing temperature were conducted with various concentration of $MgCl_2$ (0.5, 1.0, and 1.5 mM) and the concentration that provide the best and specific target band was chosen. The optimal primer concentration was examined with the concentration ranging from 0.10, 0.20 0.25, and 0.30 μM using PCR with optimal $MgCl_2$ concentration and the concentration that gave highest yield and specificity was chosen. Finally, optimal $MgCl_2$ and primer concentration was used to identify the suitable PCR cycle number with various concentration of DNA template (between 100 to 1,000 ng). The cycle number and amount of template that amplified the PCR product in the experimental range and did not reach a plateau level was chosen.

4.4.1.1 Metallothionein gene and its variants

Six subunits of MT gene (pv-MT01, pv-MT02, pv-MT03, pv-MT07, pv-MT08, and pvMT11) previously identified were subjected to expression analysis. Six pairs of primers specific to each subunit were designed and used for PCR amplification in comparison to total MT primers previously obtained for amplifying total MT gene. Figure 4.18 showed the PCR products of each subunit which were amplified from the same stock of first strand cDNA of mussel gills using specific primers of each subunit.

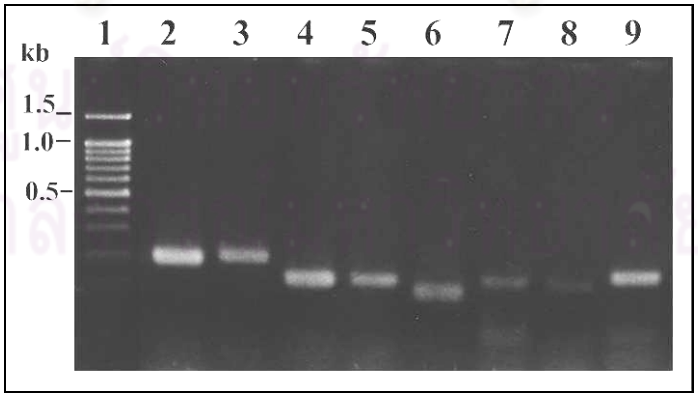


Figure 4.18 PCR products of MT variants of *P.viridis* using the first strand cDNA extracted from gill (lane 1 to 9 represent 100 bp ladder, ß-Actin (200 bp), MT (220 bp), pvMT01 (151 bp), pvMT02 (154 bp), pvMT03 (118 bp), pvMT07 (147 bp), pvMT08 (146 bp), pvMT11 (159 bp), respectively.

The optimal PCR condition of total MT gene and 6 of its variants were shown in Fig. 4.19-4.25 and summary of optimal condition shown in table 4.8

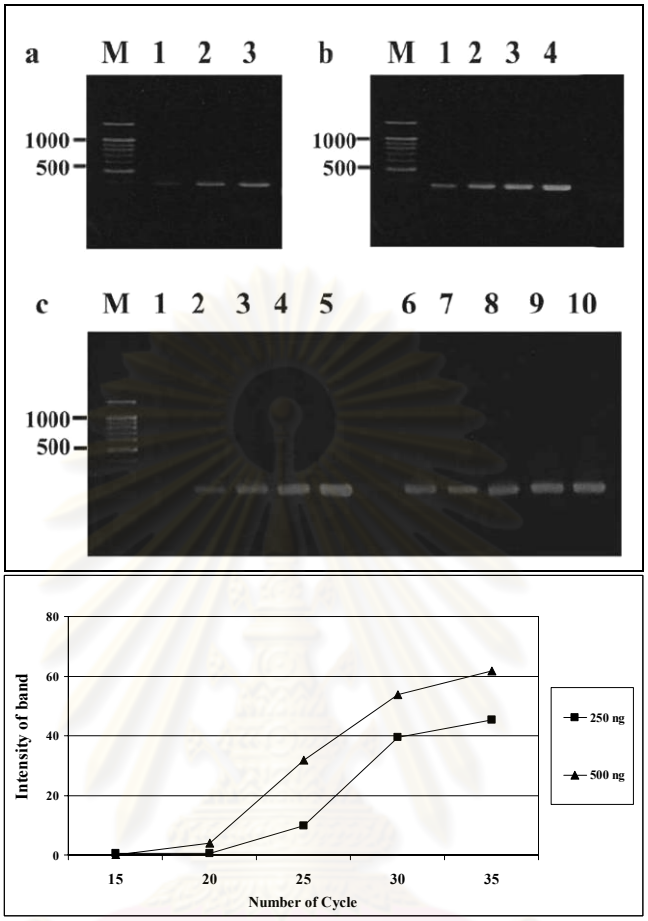


Figure 4.19 Optimization of PCR condition for quantifying the expression level of total MT gene. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM.(Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 µM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

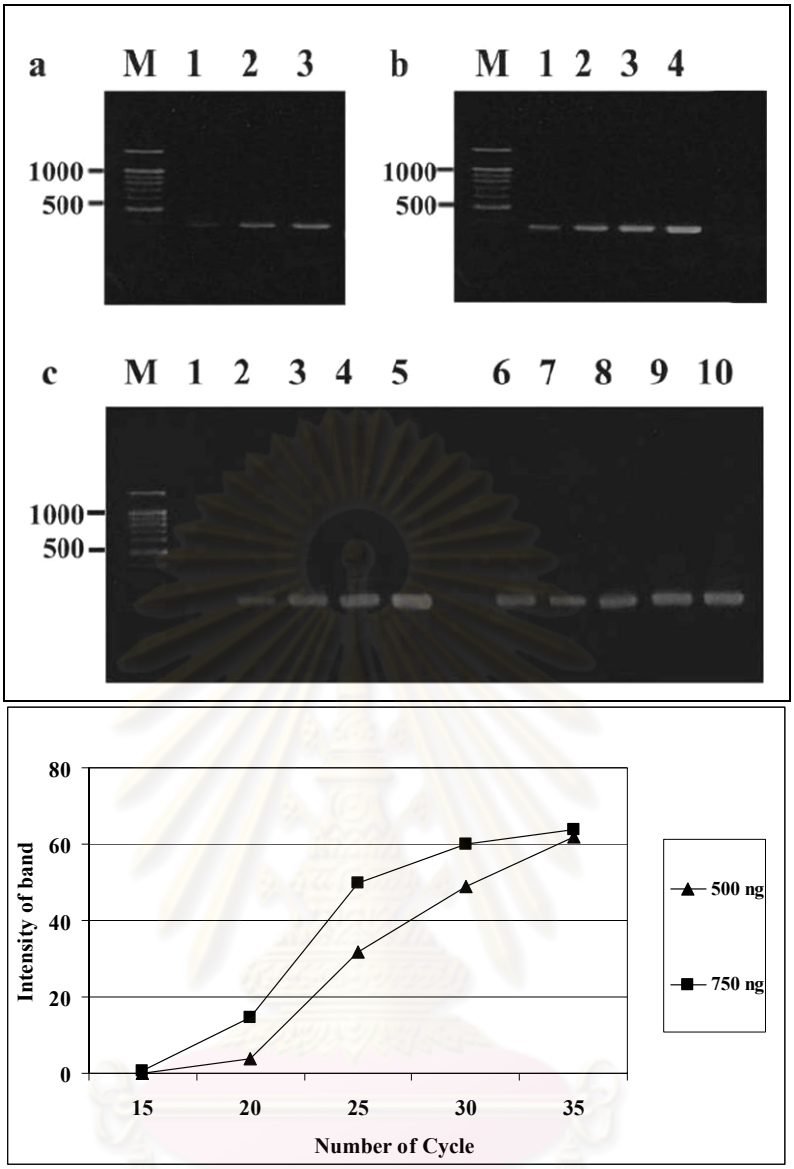


Figure 4.20 Optimization of PCR condition for quantifying the expression level of pvMT01. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM. (Lane a1-a3). Primer was examined from the concentration of 0.10. 0.10, 0.25 and 0.30 µM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

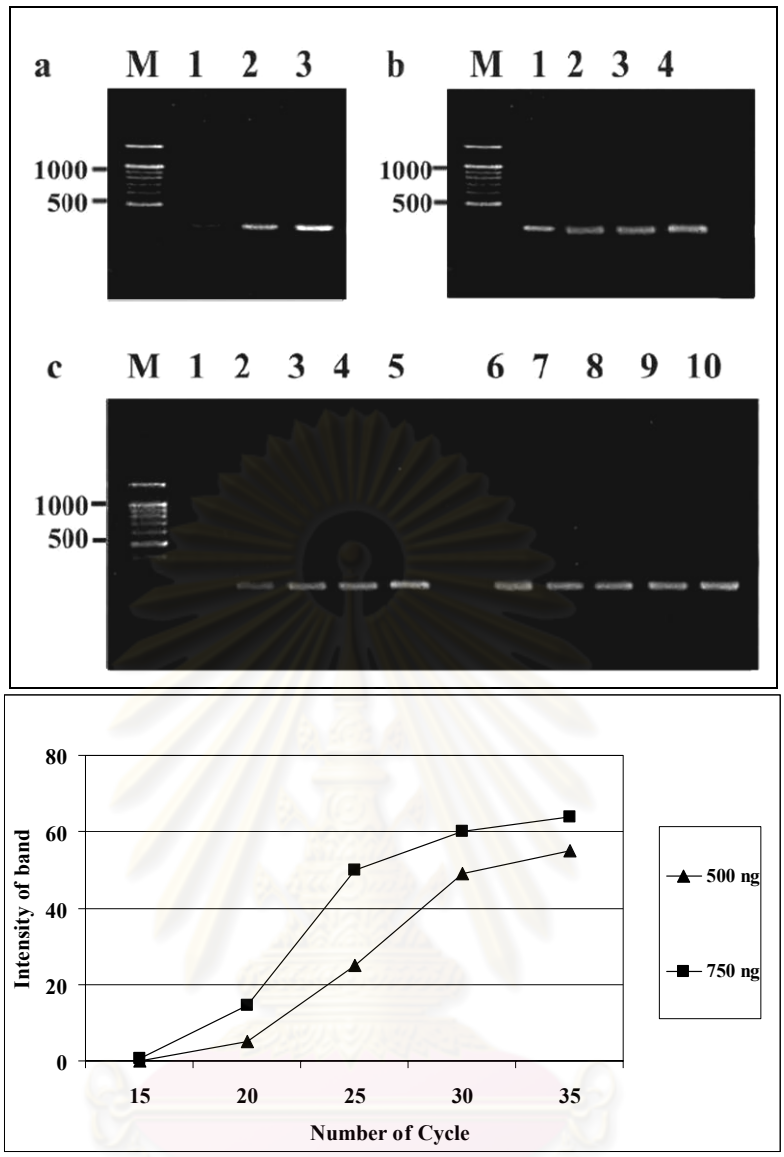


Figure 4.21 Optimization of PCR condition for quantifying the expression level of pvMT02. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM.(Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 μM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

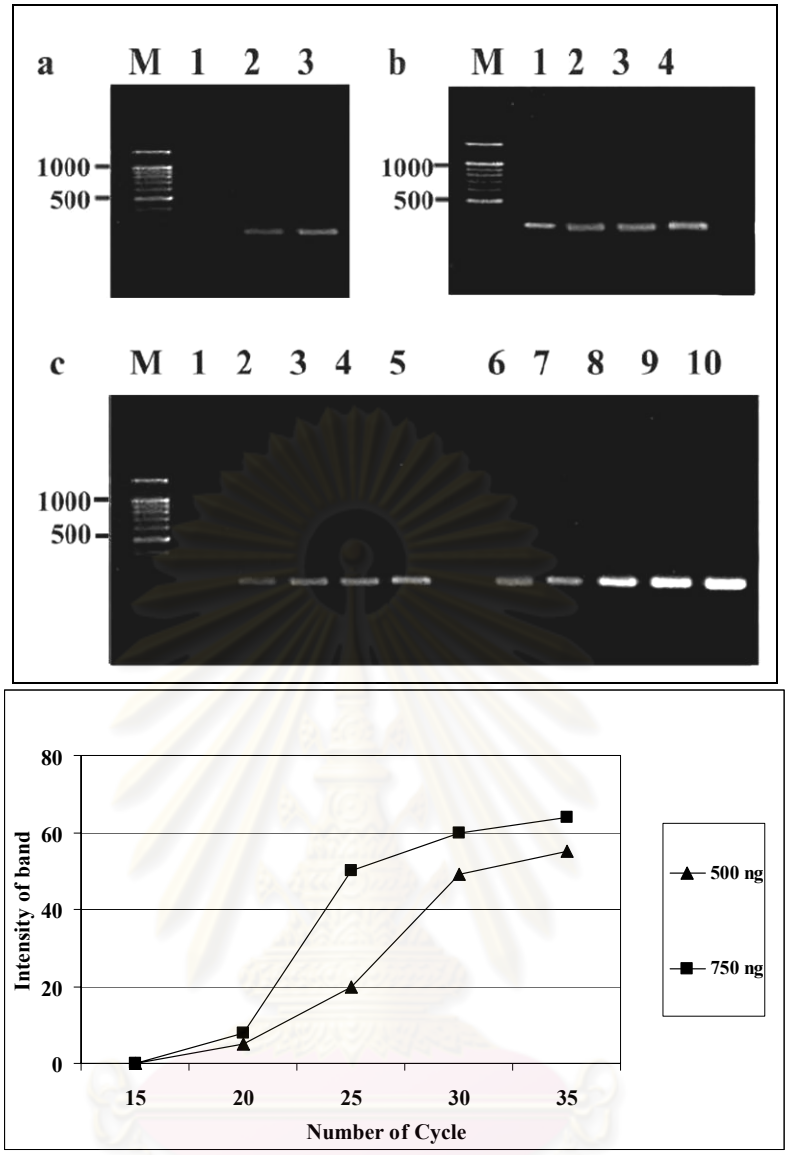


Figure 4.22 Optimization of PCR condition for quantifying the expression level of pvMT03. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM. (Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 μM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

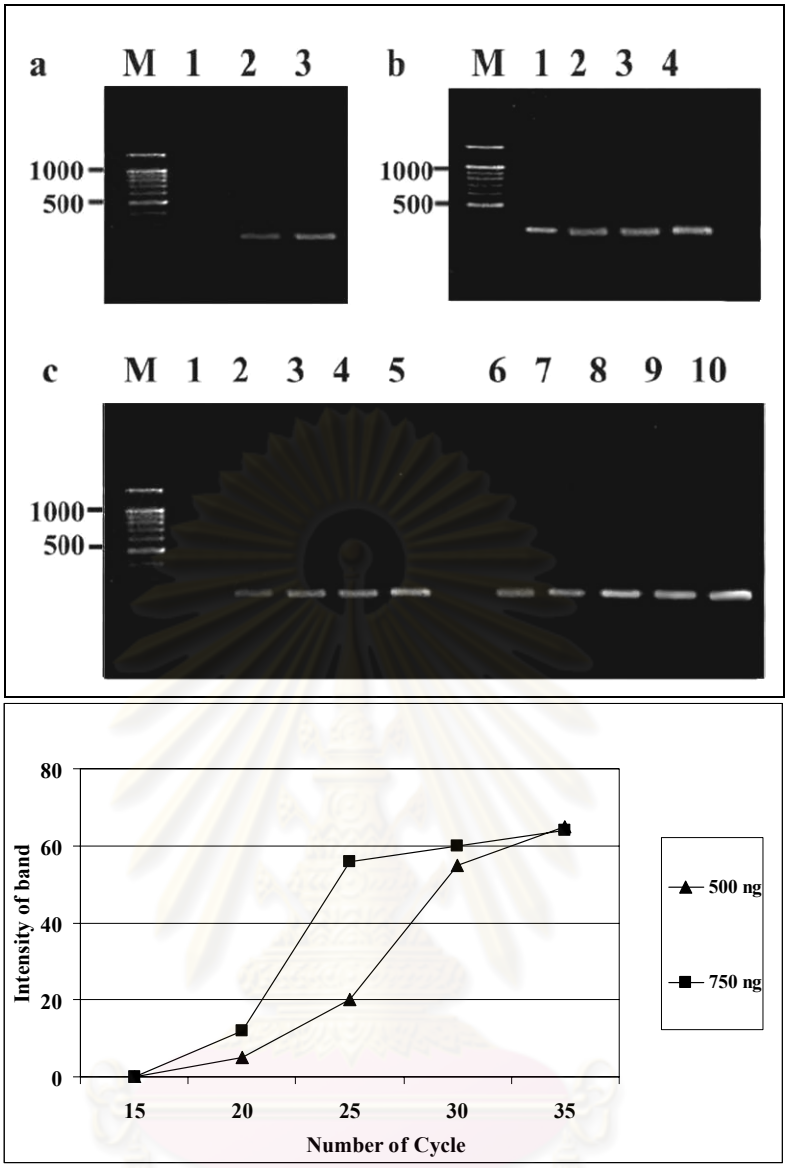


Figure 4.23 Optimization of PCR condition for quantifying the expression level of pvMT07. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM. (Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 μM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

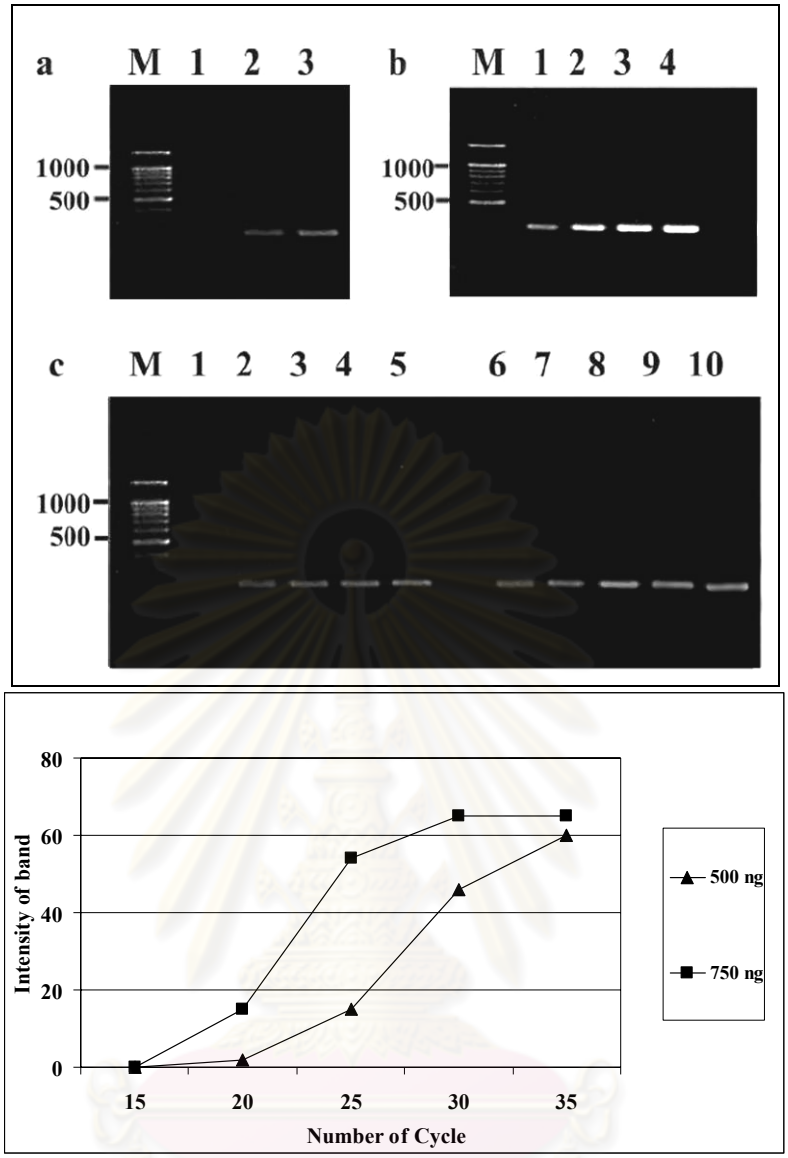


Figure 4.24 Optimization of PCR condition for quantifying the expression level of pvMT08. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM. (Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 μM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

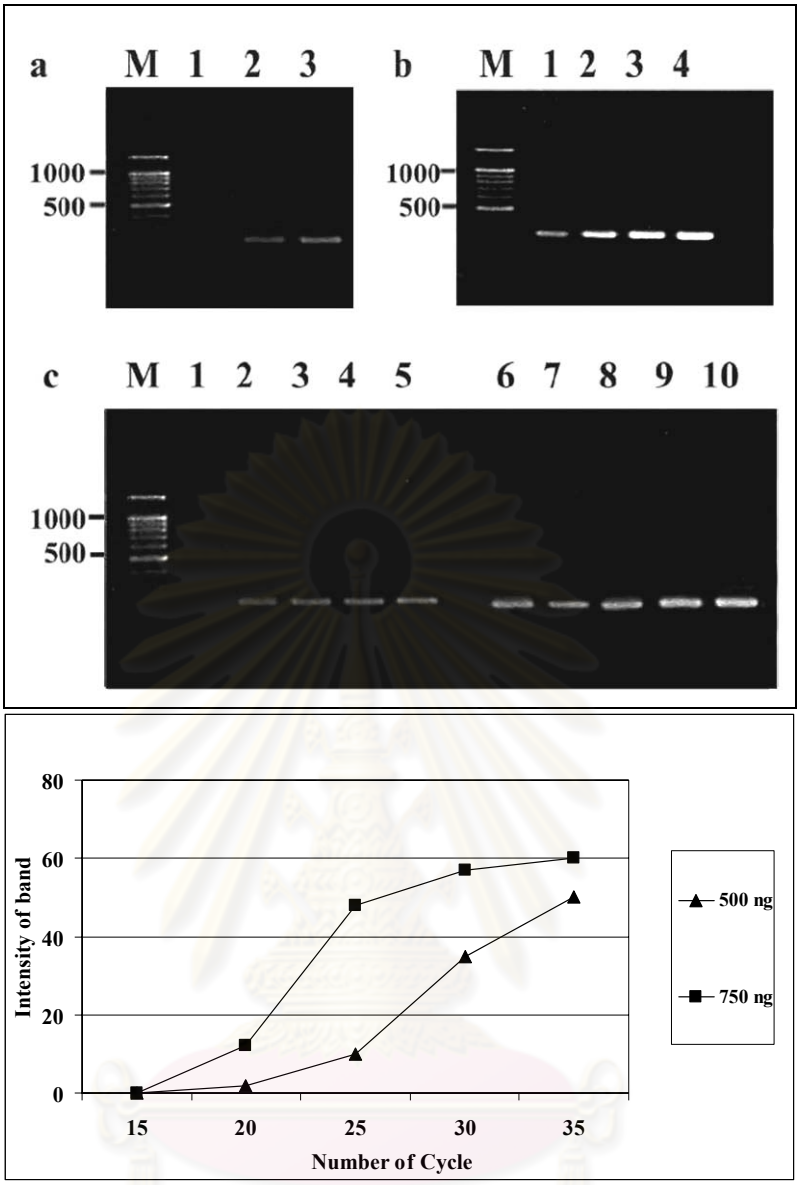


Figure 4.25 Optimization of PCR condition for quantifying the expression level of pvMT11. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM. (Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 μM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

4.4.1.2 HSP71 gene

The optimal PCR condition of total HSP71 gene was shown in fig. 4.26

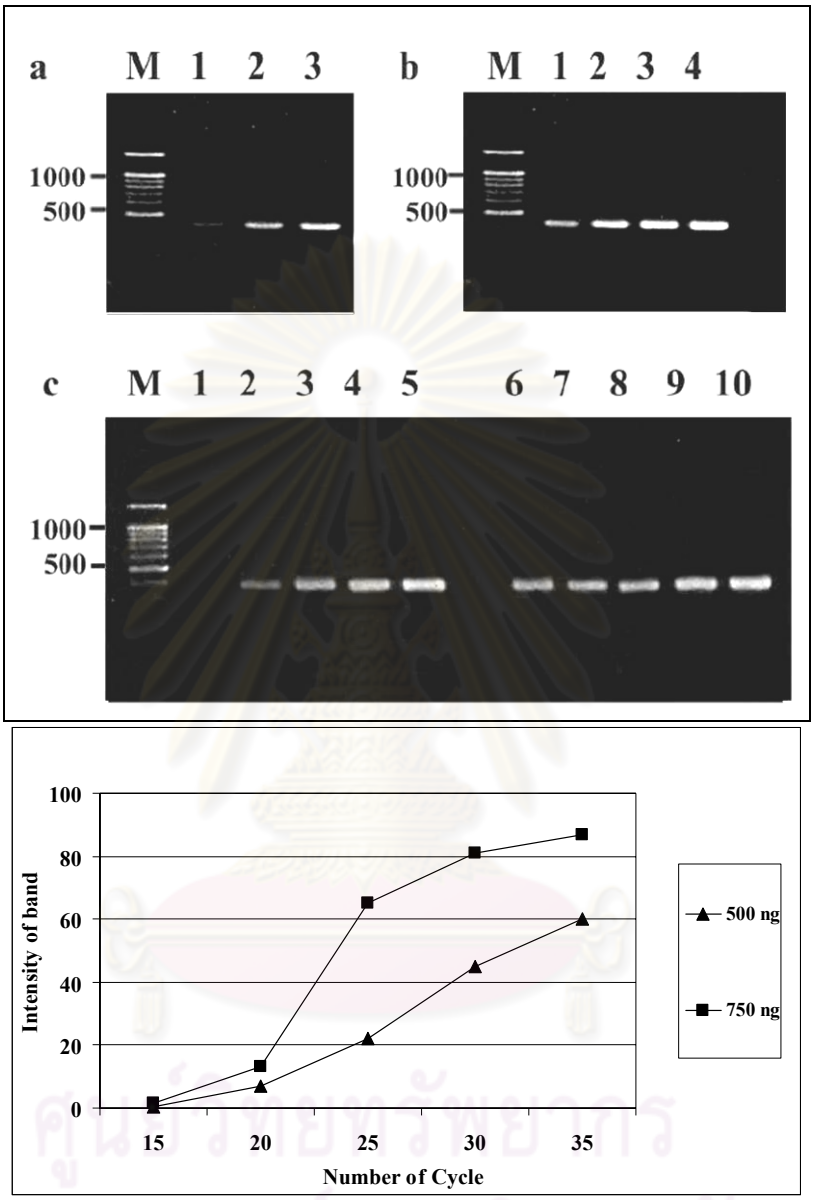


Figure 4.26 Optimization of PCR condition for quantifying the expression level of HSP71 gene. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM. (Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 μM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

4.4.1.3 CYP4 gene

The optimal PCR condition of total CYP4 gene was shown in fig. 4.27

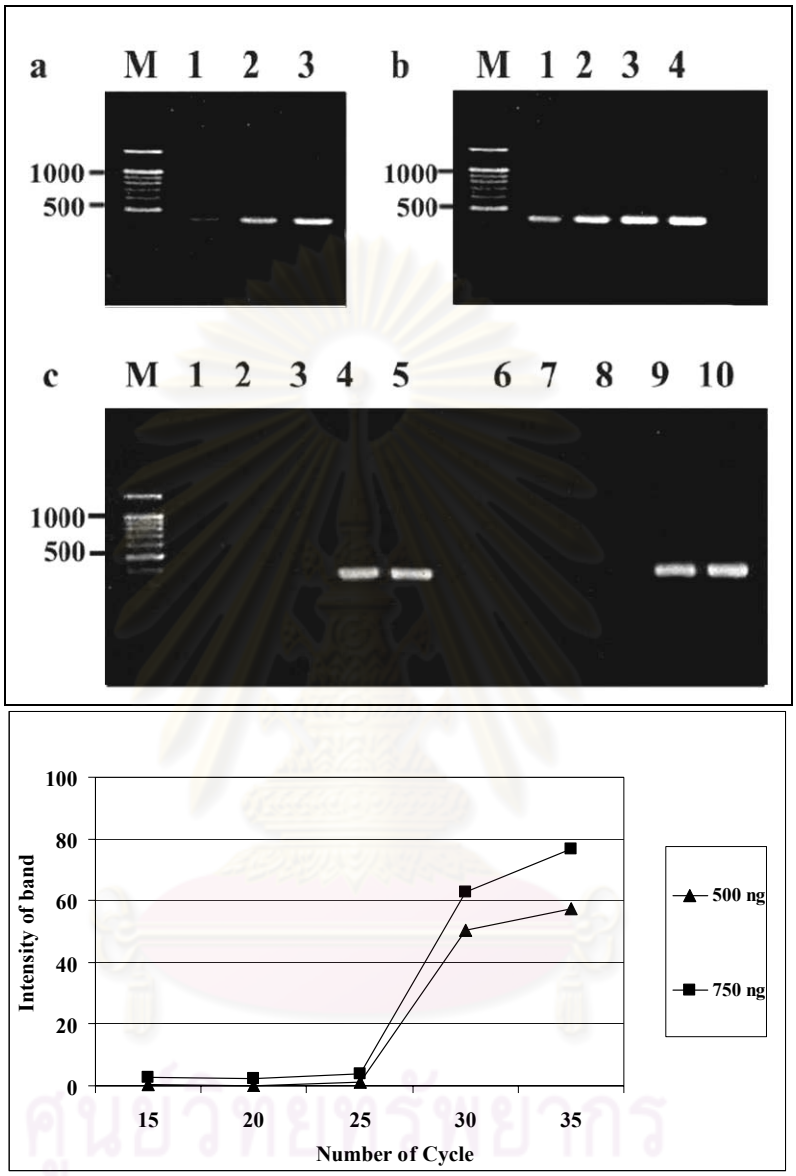


Figure 4.27 Optimization of PCR condition for quantifying the expression level of CYP4 gene. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM. (Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 μM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles for 250 ng (Lane c1-c5)and 500 ng (Lane c6-c10) of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

4.4.1.4 β-actin gene

The optimal PCR condition of total β-actin gene was shown in fig 4.28

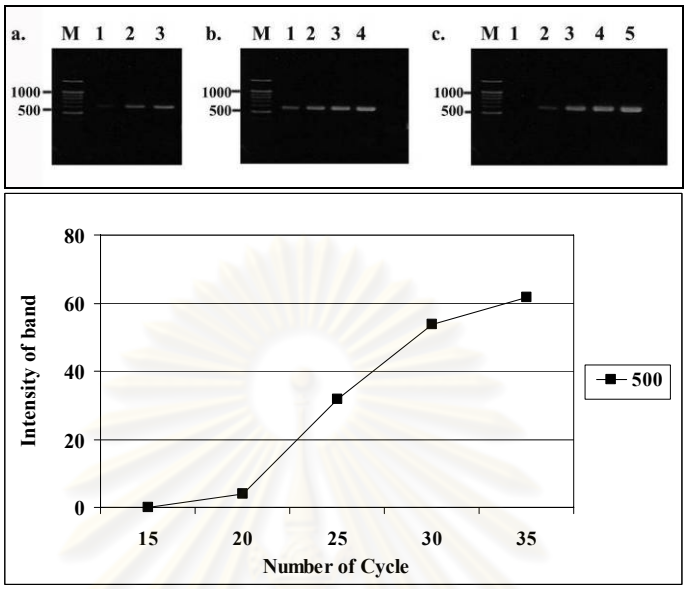


Figure 4.28 Optimization of PCR condition for quantifying the expression level of β-actin gene. $MgCl_2$ concentration was examined from the varied concentration of 0.5, 1.0, and 1.5 mM. (Lane a1-a3). Primer was examined from the concentration of 0.10. 0.20, 0.25 and 0.30 µM (Lane b1-b4). Number of cycle was examined from the varied number of 15, 20, 25, 30, and 35 cycles (Lane c1-c5) for 500 ng of gill first strand cDNA template. Lane M is DNA ladder. The intensity of amplified product was plotted against the number of amplification cycle (d.)

Table 4.8 Summary of optimal condition for semi-quantitative RT-PCR of gene in gill of mercury exposed mussel.

Gene	Template (ng/µl)	$MgCl_2$ (mM)	Primer (µM)	Anealing Temp. (^oC)	PCR cycle Number	PCR product (bp)
1.Metallothionein	500	0.50	0.25	50	28	220
2. pvMT01	500	0.50	0.25	50	28	151
3. pvMT02	500	0.50	0.25	50	28	154
4. pvMT03	500	0.50	0.25	50	28	118
5. pvMT07	500	0.50	0.25	50	28	147
6. pvMT08	500	0.50	0.25	50	28	146
7. pvMT11	500	0.50	0.25	50	28	159
8. HSP71	250	0.50	0.25	55	28	337
9. CYP4	250	0.50	0.25	55	28	355

4.4.2 Semi-quantitative RT-PCR of MTs, HSP71, and CYP4 genes

4.4.2.1 Laboratory study

4.4.2.1.1 Expression level of total MT gene in Hg exposed mussels

Total MT gene expression levels were determined from the mussels exposed to 0, 0.1, 0.2, 0.5, and 1.0 ppb of mercuric chloride for 8 weeks. The results (table 4.9 and figure 4.29 A-H) revealed no significant difference between mussels from control and other treatments ($P>0.05$). Within week 7, complete mortality of mussel was obtained from 0.2 ppb treatment. Complete mortalities were increasingly obtained within week 8 (control, 0.1, 0.5, and 1.0 ppb treatments).

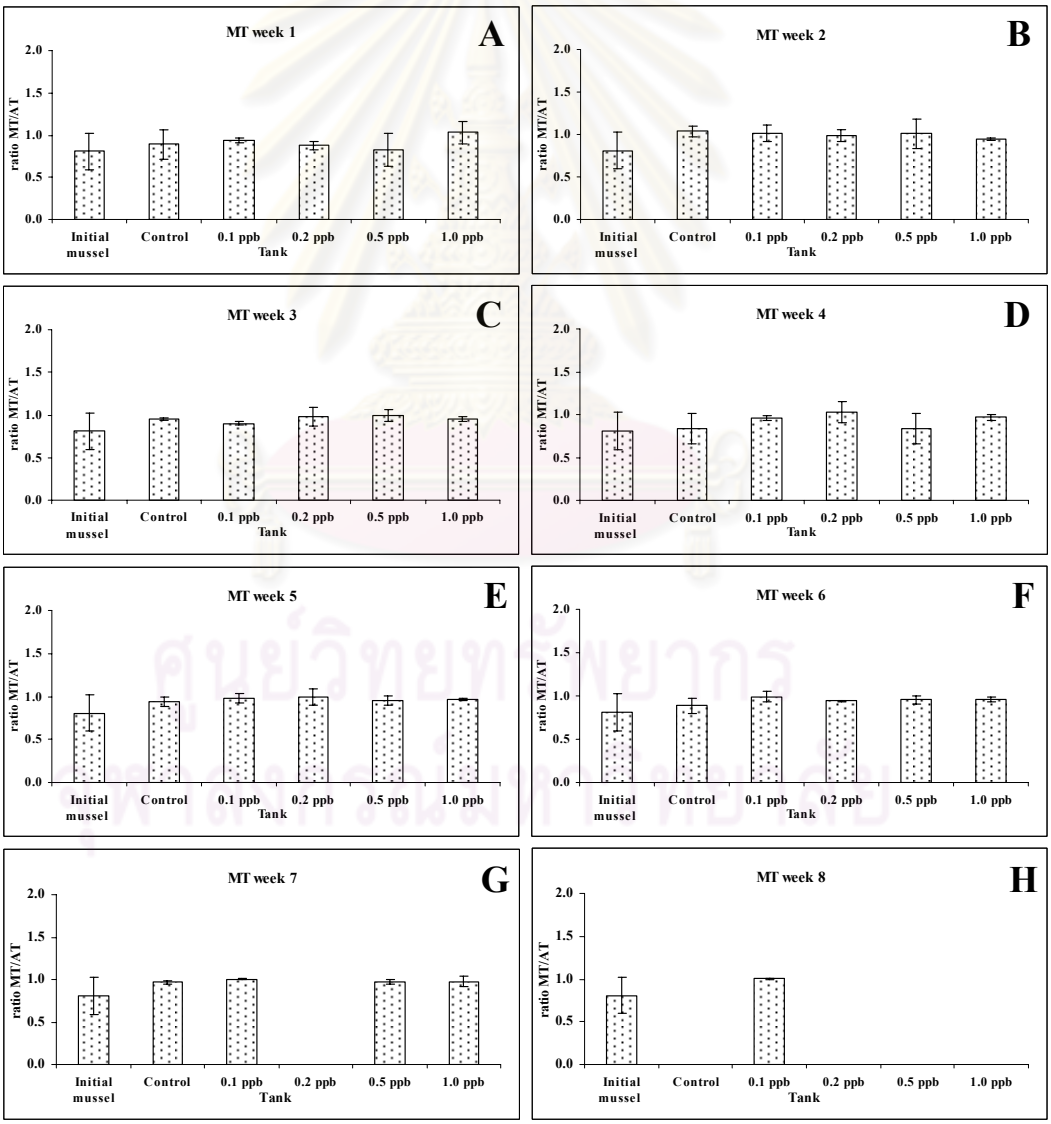


Figure 4.29 Relative expression level of MT gene in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

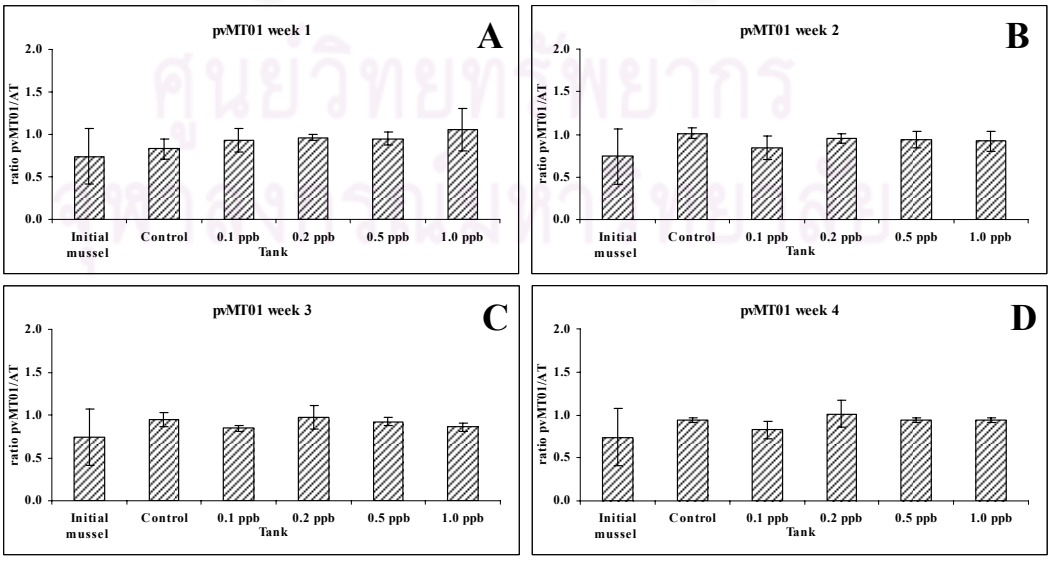
Table 4.9 Relative expression levels of MT gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.81±0.22	0.81±0.22	0.81±0.22	0.81±0.22	0.81±0.22
1	0.89±0.18	0.94±0.02	0.88±0.05	0.82±0.20	1.03±0.13
2	1.04±0.06	1.02±0.10	0.99±0.07	1.01±0.17	0.95±0.01
3	0.95±0.01	0.90±0.02	0.98±0.11	0.99±0.07	0.95±0.02
4	0.84±0.18	0.96±0.03	1.03±0.12	0.84±0.18	0.97±0.04
5	0.94±0.05	0.97±0.06	0.99±0.09	0.96±0.05	0.97±0.01
6	0.89±0.09	0.99±0.06	0.94±0.01	0.95±0.04	0.96±0.03
7	0.97±0.02	1.00±0.01	NA	0.97±0.03	0.97±0.06
8	NA	1.00±0.00	NA	NA	NA

Remark: NA = data not available due to mortality of mussel.

4.4.2.1.2 Expression level of pvMT01 in Hg exposed mussels

The results (table 4.10 and figure 4.30A-H) revealed no significant difference between mussels from control and other treatments (P>0.05). Also, no significant difference of the expression level of pvMT01 was detected in all mussels during the experiment.



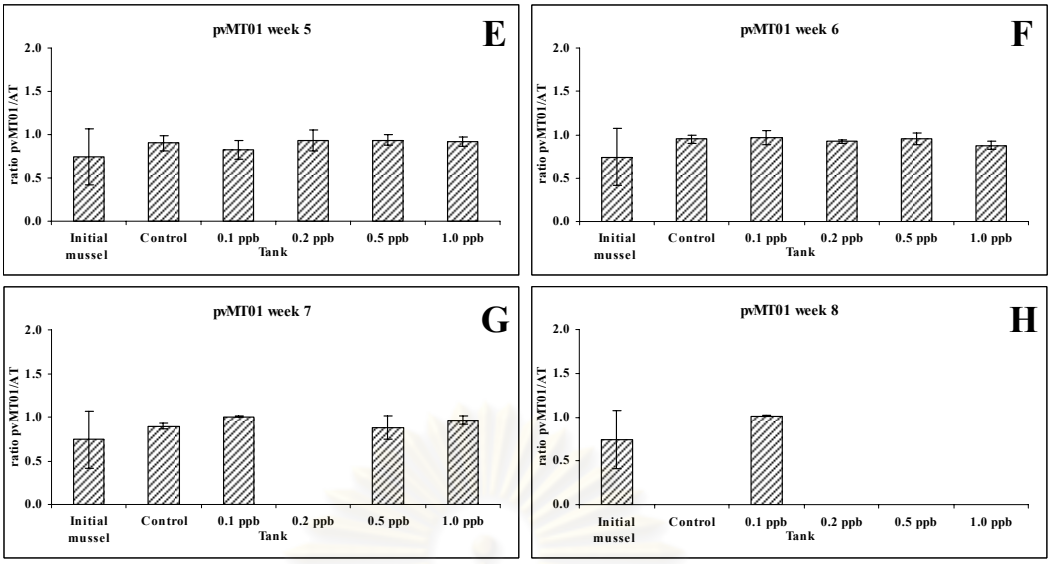


Figure 4.30 Relative expression level of pvMT01 in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

Table 4.10 Relative expression level of pvMT01 gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.74±0.33	0.74±0.33	0.74±0.33	0.74±0.33	0.74±0.33
1	0.83±0.12	0.93±0.14	0.96±0.04	0.95±0.08	1.05±0.25
2	1.01±0.06	0.85±0.14	0.95±0.06	0.94±0.10	0.92±0.11
3	0.94±0.08	0.85±0.04	0.97±0.13	0.92±0.05	0.86±0.05
4	0.94±0.03	0.83±0.10	1.01±0.16	0.94±0.03	0.94±0.03
5	0.90±0.09	0.82±0.10	0.93±0.12	0.94±0.05	0.92±0.06
6	0.95±0.05	0.96±0.08	0.92±0.02	0.96±0.06	0.88±0.04
7	0.90±0.03	1.01±0.00	NA	0.88±0.13	0.97±0.05
8	NA	1.01±0.01	NA	NA	NA

Remark: NA = data was not available due to mortality of mussel.

4.4.2.1.3 Expression level of pvMT02 in Hg exposed mussels

The results (table 4.11 and figure 4.31A-H) revealed no significant difference between mussels from control and other treatments ($P>0.05$). Also, no significant difference of the expression level of pvMT02 was detected in all mussels during the experiment.

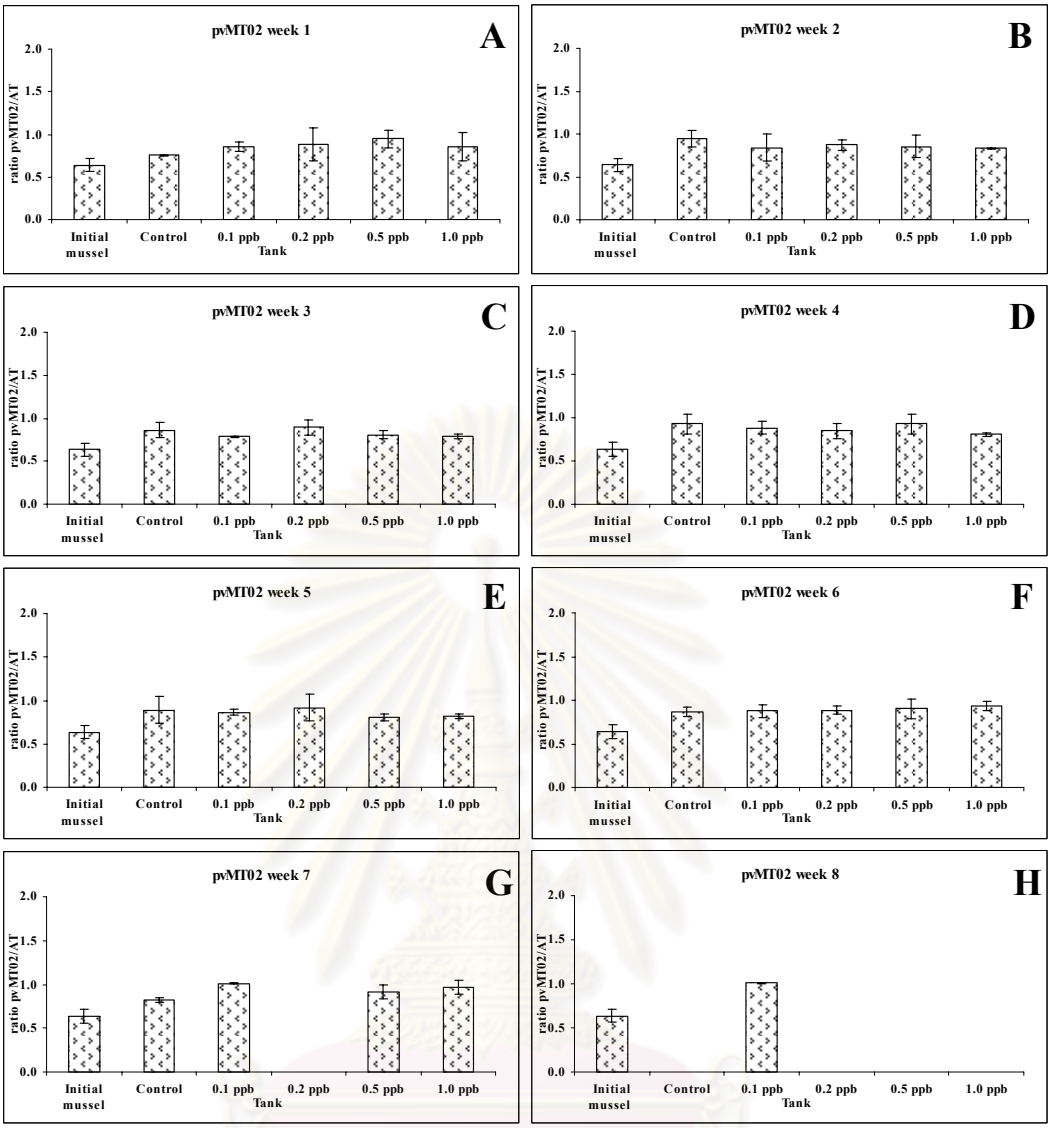


Figure 4.31 Relative expression level of pvMT02 in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

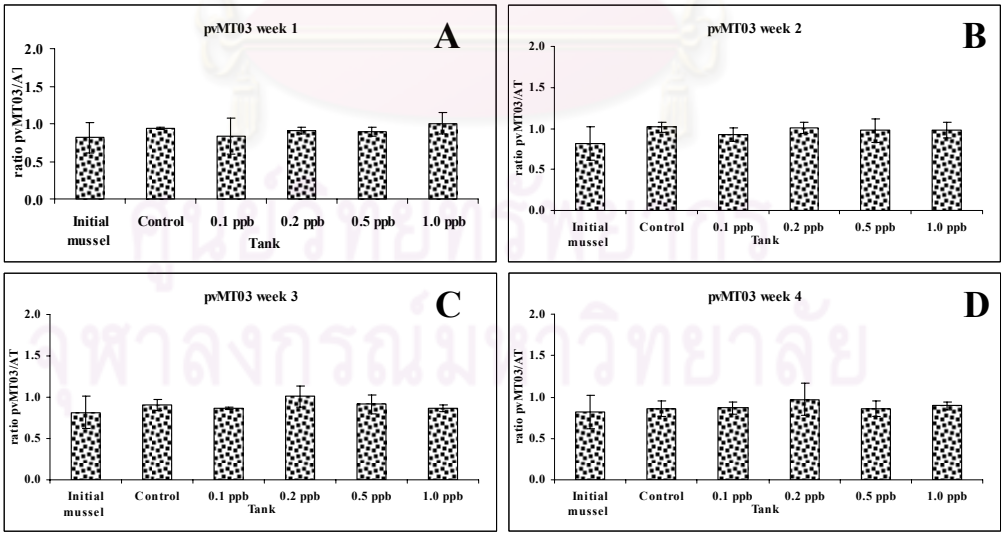
Table 4.11 Relative expression level of pvMT02 gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.64±0.08	0.64±0.08	0.64±0.08	0.64±0.08	0.64±0.08
1	0.75±0.01	0.86±0.06	0.88±0.20	0.94±0.10	0.85±0.16
2	0.95±0.09	0.84±0.16	0.87±0.06	0.85±0.13	0.83±0.01
3	0.86±0.09	0.78±0.01	0.89±0.09	0.81±0.05	0.79±0.03
4	0.93±0.11	0.88±0.07	0.85±0.09	0.93±0.11	0.80±0.02
5	0.89±0.15	0.86±0.04	0.92±0.15	0.80±0.04	0.82±0.03
6	0.87±0.05	0.88±0.07	0.89±0.05	0.90±0.11	0.94±0.05
7	0.82±0.02	1.01±0.01	NA	0.91±0.08	0.97±0.08
8	NA	1.01±0.00	NA	NA	NA

Remark: NA = data was not available due to mortality of mussel.

4.4.2.1.4 Expression level of pvMT03 in Hg exposed mussels

The results (table 4.12 and figure 4.32A-H) revealed no significant difference between mussels from control and other treatments (P>0.05). Also, no significant difference of the expression level of pvMT03 was detected in all mussels during the experiment.



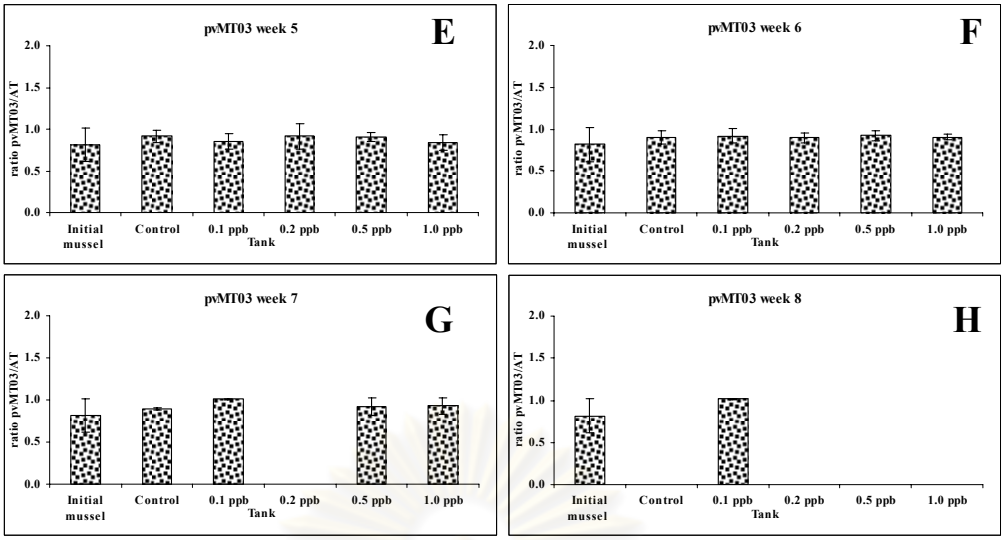


Figure 4.32 Relative expression level of pvMT03 in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

Table 4.12 Relative expression level of pvMT03 gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.82±0.20	0.82±0.20	0.82±0.20	0.82±0.20	0.82±0.20
1	0.94±0.01	0.83±0.24	0.91±0.04	0.90±0.06	1.01±0.14
2	1.02±0.06	0.92±0.08	1.01±0.07	0.97±0.14	0.98±0.10
3	0.90±0.07	0.86±0.01	1.01±0.13	0.91±0.11	0.86±0.04
4	0.86±0.09	0.87±0.08	0.97±0.19	0.86±0.09	0.90±0.05
5	0.92±0.07	0.85±0.10	0.92±0.15	0.91±0.06	0.84±0.09
6	0.90±0.08	0.92±0.09	0.90±0.06	0.92±0.06	0.90±0.03
7	0.89±0.02	1.01±0.01	NA	0.92±0.10	0.93±0.10
8	NA	1.01±0.02	NA	NA	NA

Remark: NA = data was not available due to mortality of mussel.

4.4.2.1.5 pvMT07 expression in mussels tissue

Significant differences between the expression levels of pvMT07 in mussels from different treatments were detected (table 4.13 and figure 4.33A-H). It was increasing in correlation with the increasing level of Hg applied to the mussels. Expression level of pvMT07 from mussels exposed to 0.2 ppb of mercuric chloride was significantly higher than that of control mussels within the first week ($p<0.05$) (Fig. 4.34A). At the end of the experiment (8 weeks), the average pvMT07 expression

level from all Hg treatments (tank 2-5) appeared to be significantly higher than that of control mussels (tank 1) ($p<0.05$) (Fig. 4.34B).

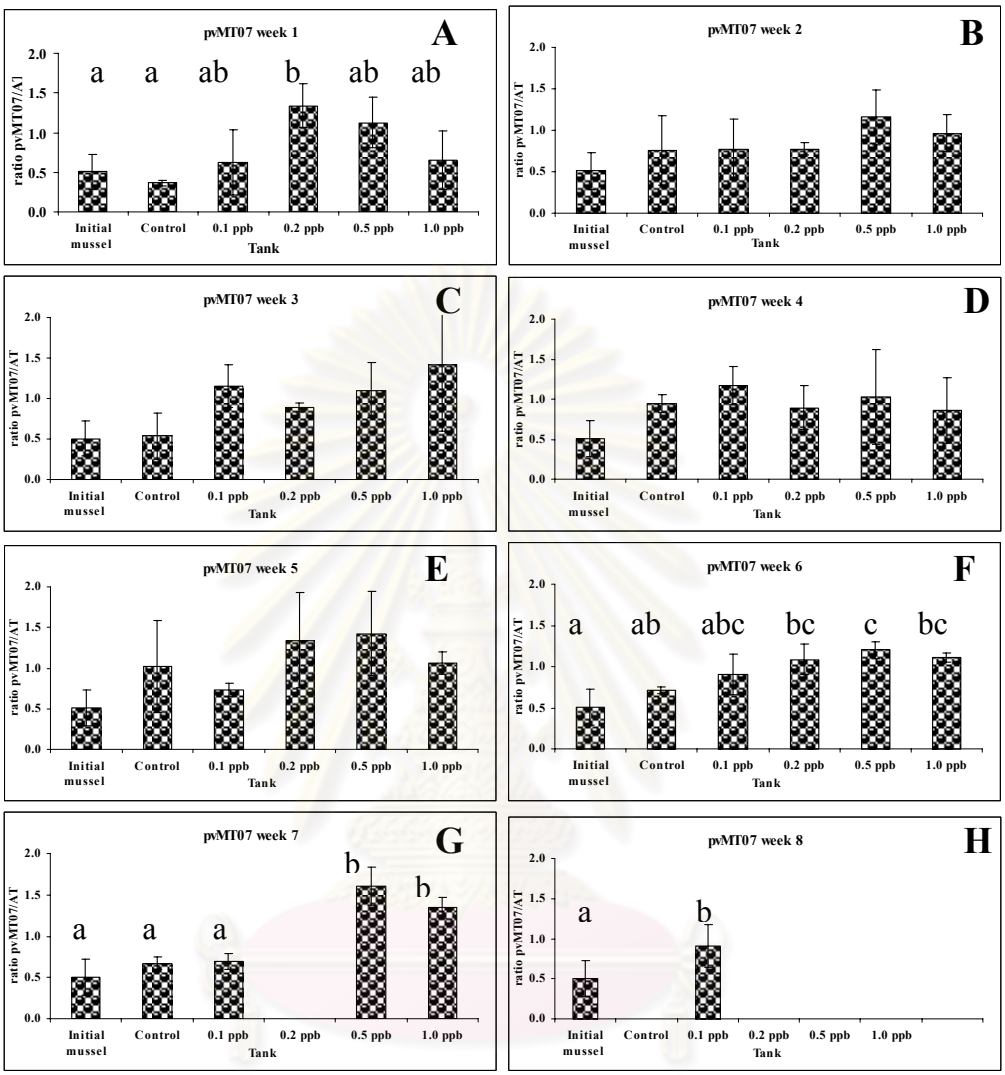


Figure 4.33 Relative expression level of pvMT07 in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

Remark: The same superscripts indicated that the relative expression level was not significantly different ($P\geq0.05$) amount group of treatment within the same period of exposure.

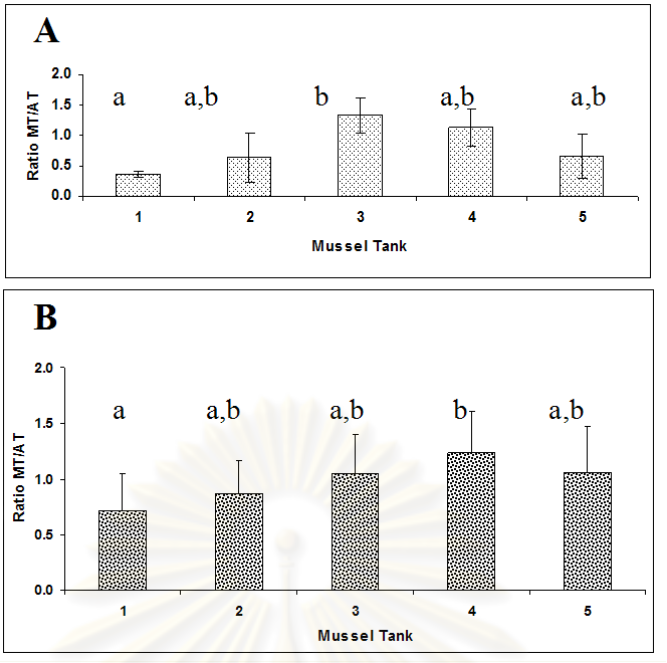


Figure 4.34 Average expression level of pvMT07 at week 1 (A) and average 8 week (B).

Remark: The same superscripts indicated that the relative expression level was not significantly different ($P \geq 0.05$) amount group of treatment within the same period of exposure.

Table 4.13 Relative expression level of pvMT07 gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.51 ± 0.22^{a}	0.51 ± 0.22^{a}	0.51 ± 0.22^{a}	0.51 ± 0.22^{a}	0.51 ± 0.22^{a}
1	0.37 ± 0.04^{a}	$0.63\pm0.41^{a,b}$	1.33 ± 0.28^{b}	$1.13\pm0.32^{a,b}$	$0.65\pm0.36^{a,b}$
2	$0.76\pm0.42^{a,b}$	$0.78\pm0.36^{a,b}$	$0.77\pm0.08^{a,b}$	$1.16\pm0.33^{a,b}$	$0.96\pm0.23^{a,b}$
3	0.54 ± 0.29^{a}	$1.15\pm0.26^{a,b}$	$0.89\pm0.06^{a,b}$	$1.10\pm0.34^{a,b}$	$1.42\pm0.82^{a,b}$
4	$0.94\pm0.11^{a,b}$	$1.16\pm0.24^{a,b}$	$0.89\pm0.27^{a,b}$	$1.03\pm0.59^{a,b}$	$0.86\pm0.41^{a,b}$
5	$1.02\pm0.56^{a,b}$	$0.73\pm0.08^{a,b}$	$1.34\pm0.58^{a,b}$	$1.42\pm0.52^{a,b}$	$1.06\pm0.13^{a,b}$
6	$0.72\pm0.04^{a,b}$	$0.91\pm0.25^{a,b}$	$1.09\pm0.19^{a,b}$	$1.20\pm0.10^{a,b}$	$1.11\pm0.05^{a,b}$
7	$0.67\pm0.08^{a,b}$	$0.69\pm0.10^{a,b}$	NA	1.61 ± 0.23^{b}	$1.35\pm0.13^{a,b}$
8	NA	0.91±0.27	NA	NA	NA

Remark: NA = data was not available due to mortality of mussel. The same superscripts indicated that the relative expression level was not significantly different ($P \geq 0.05$) amount group of treatment within the same period of exposure.

4.4.2.1.6 Expression level of pvMT08 in Hg exposed mussels

The results (table 4.14 and figure 4.35A-H) revealed no significant difference between mussels from control and other treatments (P>0.05). Also, no significant difference of the expression level of pvMT08 was detected in all mussels during the experiment.

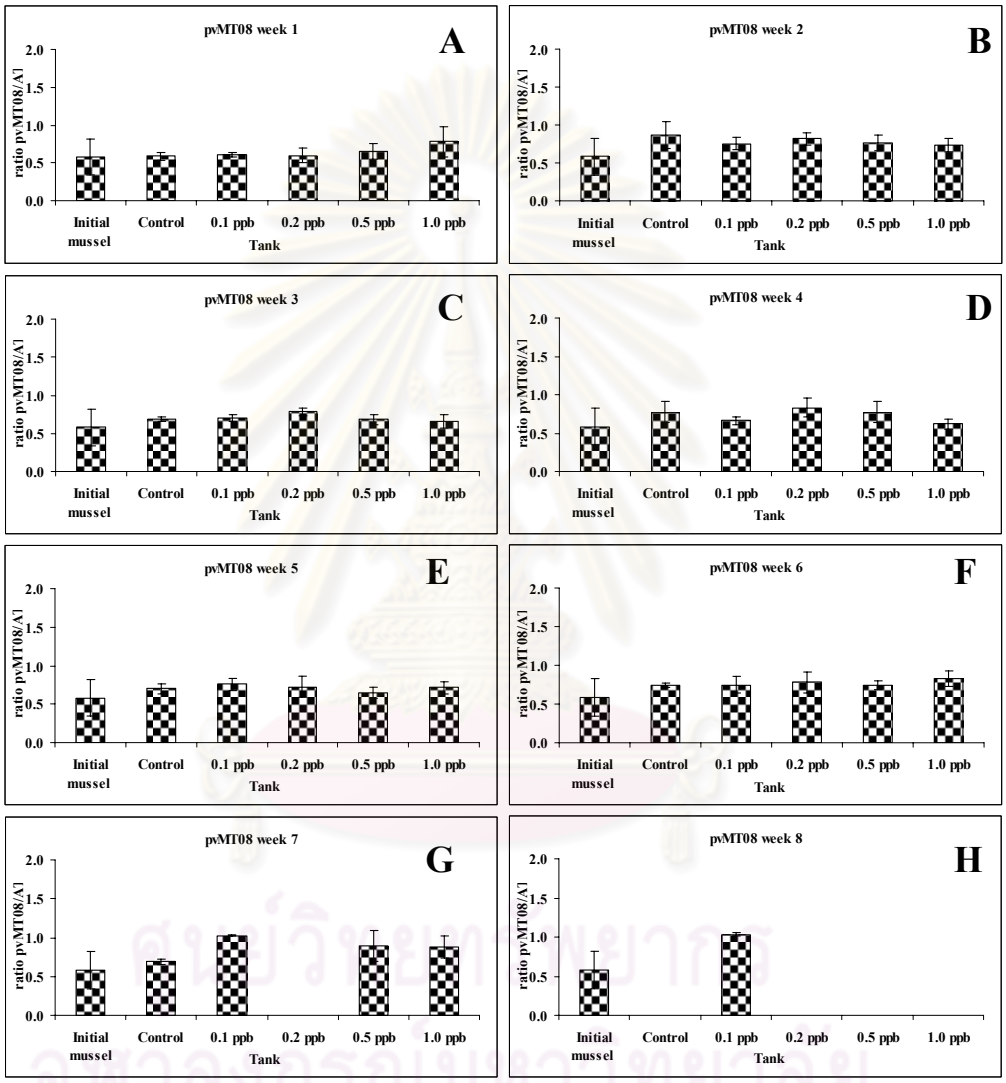


Figure 4.35 Relative expression level of pvMT08 in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

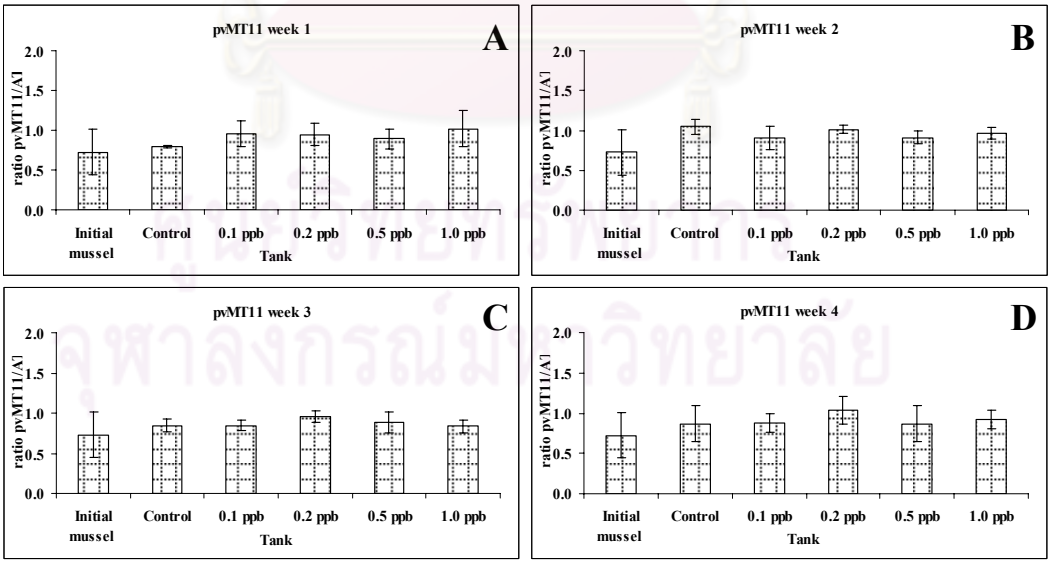
Table 4.14 Relative expression level of pvMT08 gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.58±0.24	0.58±0.24	0.58±0.24	0.58±0.24	0.58±0.24
1	0.59±0.06	0.61±0.03	0.65±0.11	0.78±0.20	1.03±0.13
2	0.87±0.18	0.76±0.08	0.76±0.11	0.73±0.09	0.95±0.01
3	0.68±0.03	0.70±0.04	0.68±0.07	0.66±0.09	0.95±0.02
4	0.77±0.14	0.66±0.05	0.77±0.14	0.62±0.07	0.97±0.04
5	0.70±0.07	0.76±0.07	0.64±0.07	0.71±0.08	0.97±0.01
6	0.74±0.03	0.75±0.11	0.74±0.06	0.83±0.10	0.96±0.03
7	0.69±0.04	1.03±0.01	NA	0.89±0.14	0.97±0.06
8	NA	1.03±0.02	NA	NA	NA

Remark: NA = data was not available due to mortality of mussel.

4.4.2.1.7 Expression level of pvMT11 in Hg exposed mussels

The results (table 4.15 and figure 4.36A-H) revealed no significant difference between mussels from control and other treatments (P>0.05). Also, no significant difference of the expression level of pvMT11 was detected in all mussels during the experiment.



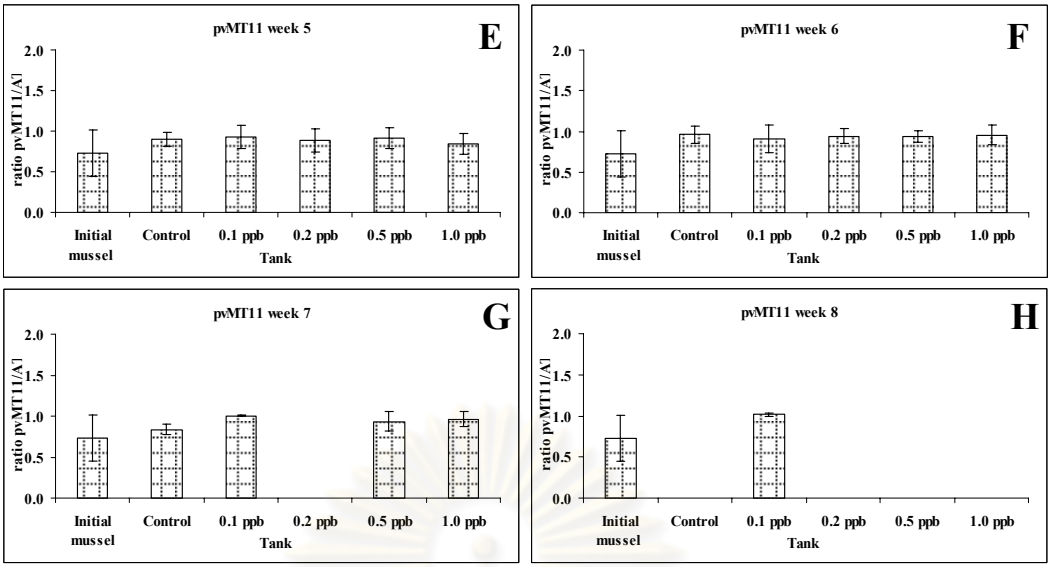


Figure 4.36 Relative expression level of pvMT11 in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

Table 4.15 Relative expression level of pvMT11 gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.73±0.28	0.73±0.28	0.73±0.28	0.73±0.28	0.73±0.28
1	0.79±0.02	0.95±0.17	0.95±0.14	0.89±0.12	1.02±0.23
2	1.05±0.10	0.90±0.15	1.01±0.06	0.91±0.08	0.96±0.07
3	0.85±0.07	0.84±0.06	0.96±0.07	0.88±0.13	0.84±0.08
4	0.87±0.22	0.87±0.12	1.03±0.17	0.87±0.22	0.93±0.12
5	0.90±0.09	0.93±0.14	0.89±0.14	0.92±0.13	0.84±0.12
6	0.96±0.11	0.91±0.16	0.94±0.09	0.94±0.07	0.96±0.12
7	0.84±0.06	1.01±0.00	NA	0.93±0.12	0.96±0.09
8	NA	1.02±0.02	NA	NA	NA

Remark: NA = data was not available due to mortality of mussel.

4.4.2.1.8 Expression level of HSP71 gene in Hg exposed mussels

The results of HSP71 gene expression (table 4.16 and figure 4.37A-H) revealed no significant difference between mussels from control and other treatments (P>0.05). No significant difference of the expression level was also detected in all mussels during the experiment.

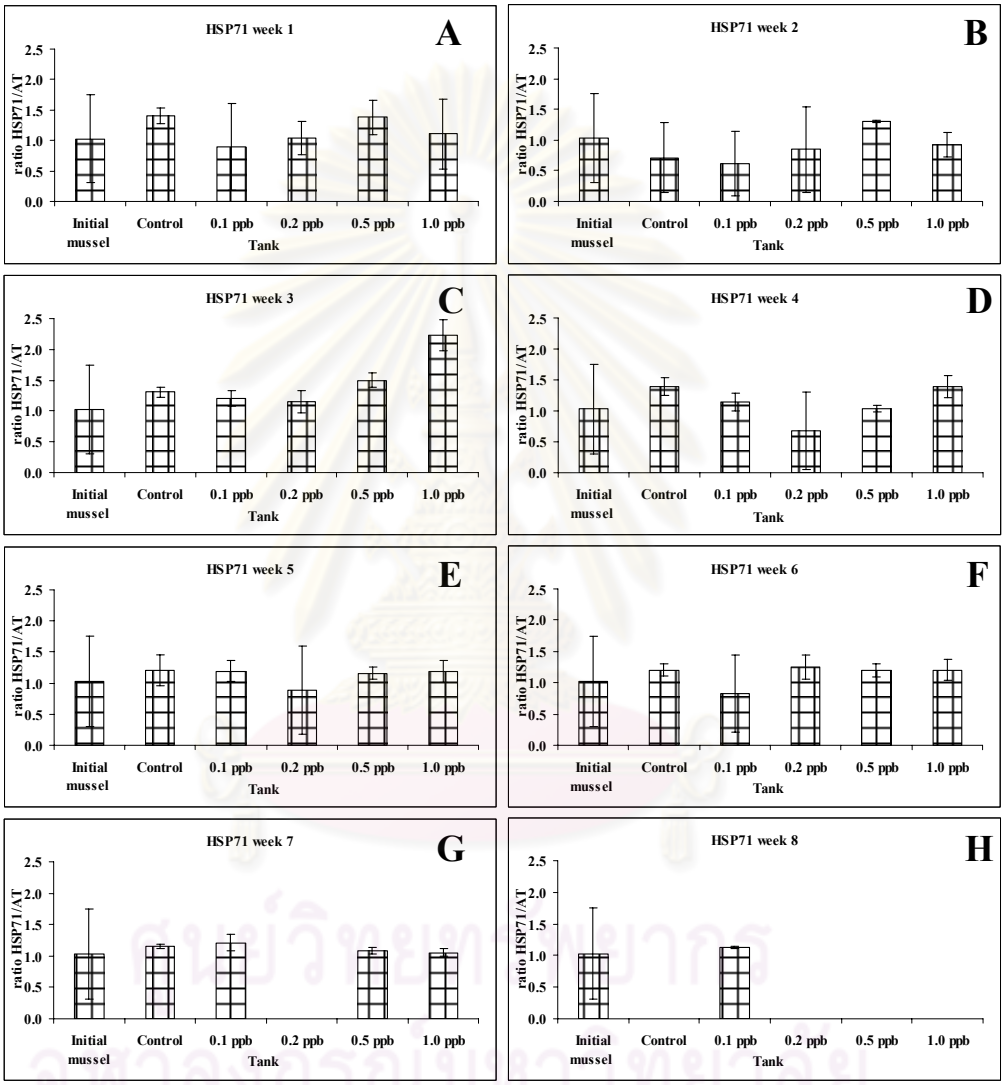


Figure 4.37 Relative expression level of HSP71 gene in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

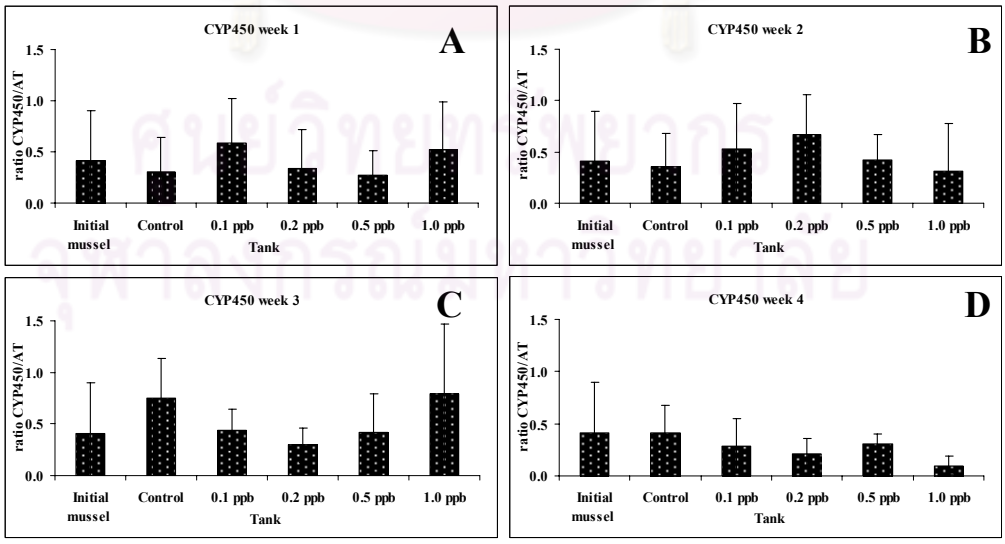
Table 4.16 Relative expression level of HSP71 gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.81±0.22	0.81±0.22	0.81±0.22	0.81±0.22	0.81±0.22
1	0.89±0.18	0.94±0.02	0.88±0.05	0.82±0.20	1.03±0.13
2	1.04±0.06	1.02±0.10	0.99±0.07	1.01±0.17	0.95±0.01
3	0.95±0.01	0.90±0.02	0.98±0.11	0.99±0.07	0.95±0.02
4	0.84±0.18	0.96±0.03	1.03±0.12	0.84±0.18	0.97±0.04
5	0.94±0.05	0.97±0.06	0.99±0.09	0.96±0.05	0.97±0.01
6	0.89±0.09	0.99±0.06	0.94±0.01	0.95±0.04	0.96±0.03
7	0.97±0.02	1.00±0.01	NA	0.97±0.03	0.97±0.06
8	NA	1.00±0.00	NA	NA	NA

Remark: NA = data was not available due to mortality of mussel.

4.4.2.1.9 Expression level of CYP4 gene in Hg exposed mussels

The results of CYP4 gene expression (table 4.17 and figure 4.38A-H) revealed no significant difference between mussels from control and other treatments (P>0.05). No significant difference of the expression level was also detected in all mussels during the experiment.



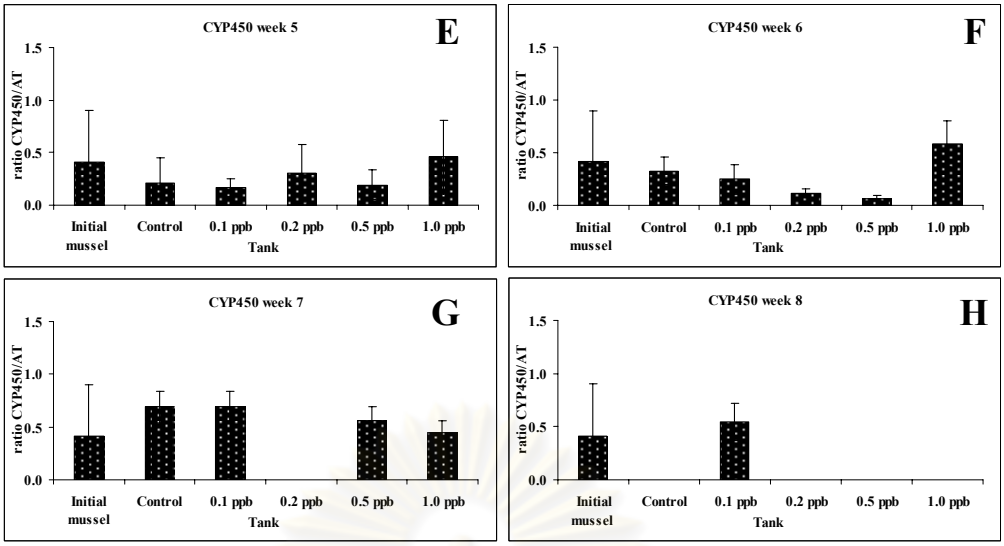


Figure 4.38 Relative expression level of CYP4 gene in experiment mussels. A to H indicates the result from week 1 to 8, respectively.

Table 4.17 Relative expression level of CYP4 gene of mussels exposed to different concentrations of $HgCl_2$

Time of Exposure (week)	$HgCl_2$ Concentration (µg/L)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
0	0.81±0.22	0.81±0.22	0.81±0.22	0.81±0.22	0.81±0.22
1	0.89±0.18	0.94±0.02	0.88±0.05	0.82±0.20	1.03±0.13
2	1.04±0.06	1.02±0.10	0.99±0.07	1.01±0.17	0.95±0.01
3	0.95±0.01	0.90±0.02	0.98±0.11	0.99±0.07	0.95±0.02
4	0.84±0.18	0.96±0.03	1.03±0.12	0.84±0.18	0.97±0.04
5	0.94±0.05	0.97±0.06	0.99±0.09	0.96±0.05	0.97±0.01
6	0.89±0.09	0.99±0.06	0.94±0.01	0.95±0.04	0.96±0.03
7	0.97±0.02	1.00±0.01	NA	0.97±0.03	0.97±0.06
8	NA	1.00±0.00	NA	NA	NA

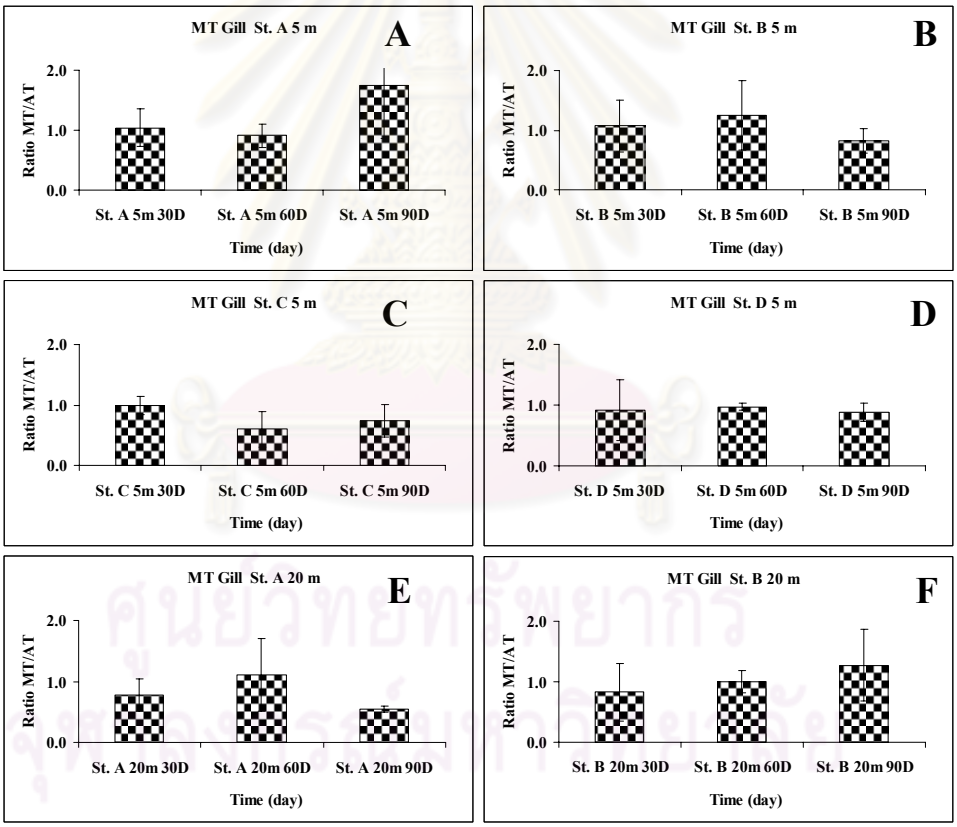
Remark: NA = data was not available due to mortality of mussel.

4.4.2.2 Field study

Expression levels of target genes including MT gene and its variants, HSP71, and CYP4 genes in mussels transplanted at petroleum production platforms were determined.

4.4.2.2.1 Expression level of total MT gene in transplanted mussels

Expression levels of total MT gene were determined in gills and digestive tracts of mussels transplanted at 3 offshore stations (station A, B, and C) in comparison with reference station (station D). The results of total MT expression levels in mussels at different depths during 3 months of experiment were shown in figure 4.39a, 4.39b and table 4.18a and 4.18b.



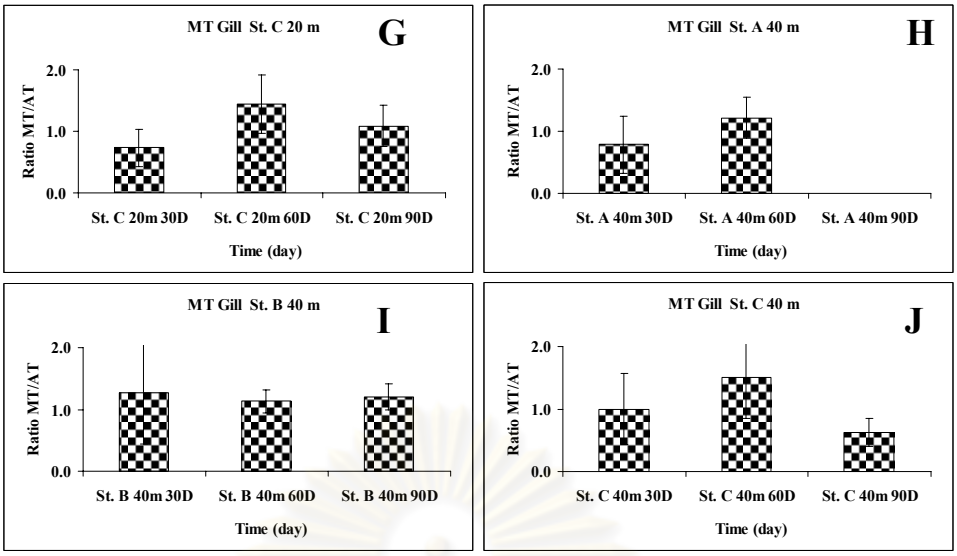
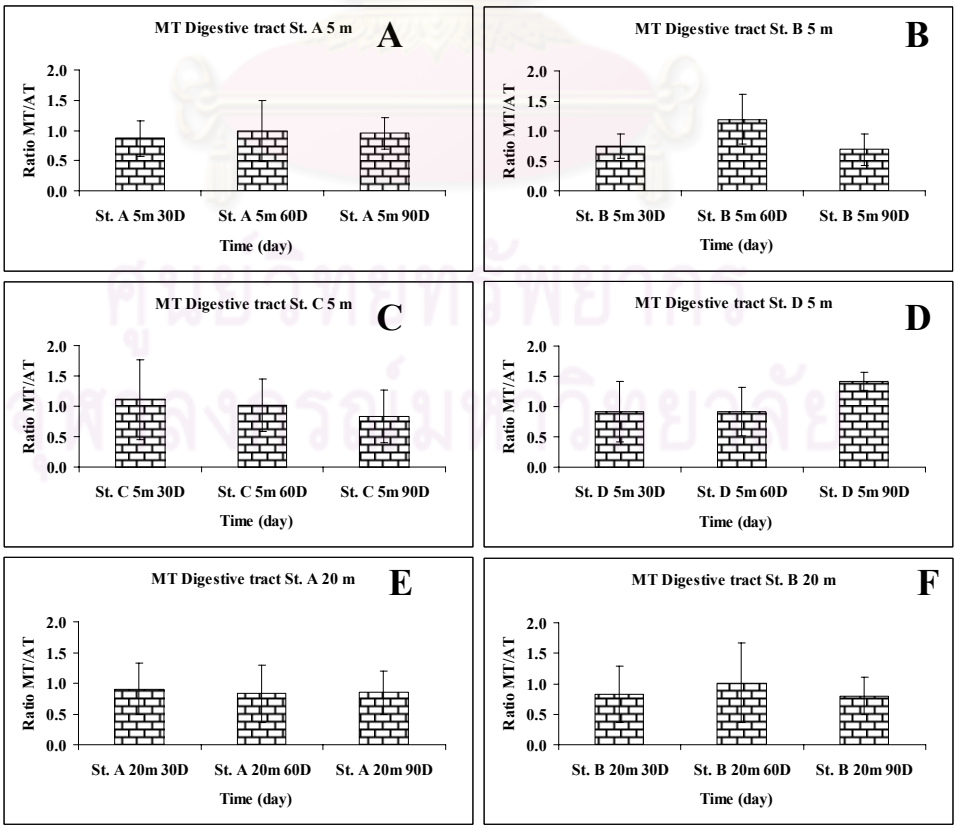


Figure 4.39a Relative expression level of total MT gene in gill of mussels transplanted at 4 studied sites. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.



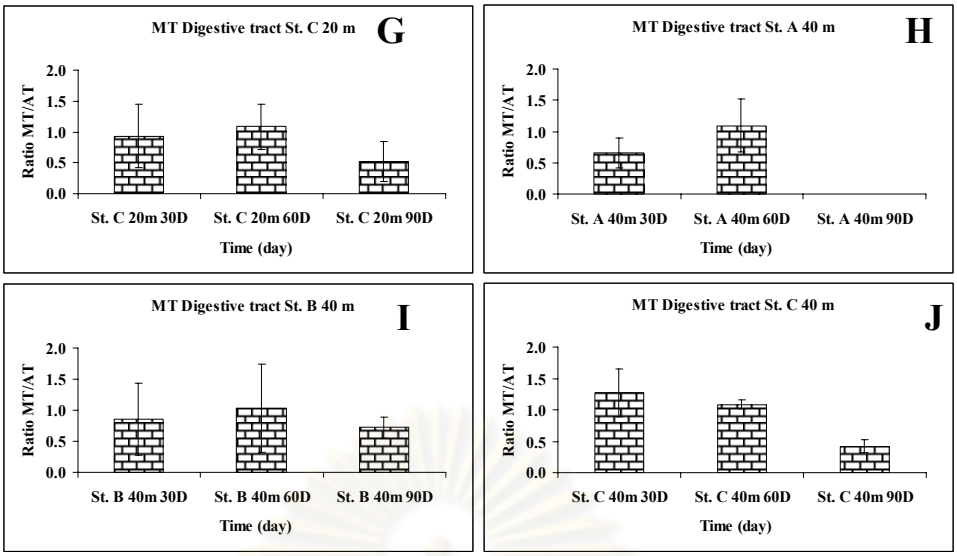


Figure 4.39b Relative expression level of total MT gene in digestive tract of mussels transplanted at 4 studied sites. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.18a Relative expression level of total MT gene in gill of mussels transplanted at 4 studied sites (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	60 Day	90 Day
ST.A 5 m	1.04±0.31	0.91±0.19	1.75±0.89
ST.A 20 m	0.77±0.26	1.10±0.60	0.55±0.06
ST.A 40 m	0.79±0.46	1.22±0.33	NA
ST.B 5 m	1.07±0.44	1.25±0.59	0.81±0.21
ST.B 20 m	0.83±0.47	0.99±0.18	1.27±0.60
ST.B 40 m	1.28±0.83	1.13±0.18	1.20±0.21
ST.C 5 m	0.99±0.15	0.60±0.07	0.29±0.03
ST.C 20 m	0.73±0.30	1.44±0.48	1.09±0.33
ST.C 40 m	1.00±0.57	1.50±0.66	0.62±0.23
ST.D 5 m	0.91±0.50	0.97±0.07	0.89±0.15

Remark: NA = data was not available due to mortality of mussel.

Table 4.18b Relative expression level of total MT gene in digestive tract of mussels transplanted at 4 studied sites (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	0.87±0.29	0.99±0.51	0.95±0.26
ST.A 20 m	0.91±0.41	0.83±0.47	0.85±0.35
ST.A 40 m	0.65±0.24	1.10±0.43	NA
ST.B 5 m	0.75±0.20	1.19±0.41	0.69±0.27
ST.B 20 m	0.83±0.47	1.01±0.66	0.80±0.31
ST.B 40 m	0.85±0.58	1.03±0.71	0.72±0.17
ST.C 5 m	1.11±0.66	1.01±0.43	0.83±0.43
ST.C 20 m	0.93±0.51	1.08±0.36	1.51±1.23
ST.C 40 m	1.27±0.39	1.09±0.07	0.41±0.10
ST.D 5 m	0.91±0.50	0.92±0.40	1.41±0.15

Remark: NA = data was not available due to mortality of mussel.

The total MT expression levels appeared to be various among different depths, time, and stations. Also, the average MT expression levels of mussels from some stations (station A and B) seemed to be higher than that of station D (reference site) and that of the gill appeared to be expressed higher than that of reference site. However, there were no significant differences between the results from these stations due to the diverse results between samples. This can be indicated that there is no influence factors on the expression level of total MT gene from mussels at reference site (station D) and petroleum production platforms (station A, B, and C). Similar result was obtained from the study of digestive tract (Fig. 4.40A and B)

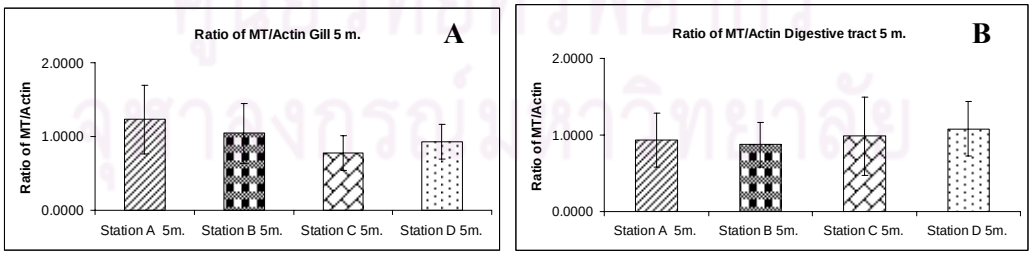


Figure 4.40 Ratio of MT expression in mussels at 5 m. depth (A = ratio of MT in gill, B = ratio of MT in Digestive tract)

When compared the levels of total MT gene from each study site according to time, the results showed that after 30 days, the level of total MT gene tended to increase in tissue. (Fig. 4.41) However, the difference was not statistically significant.

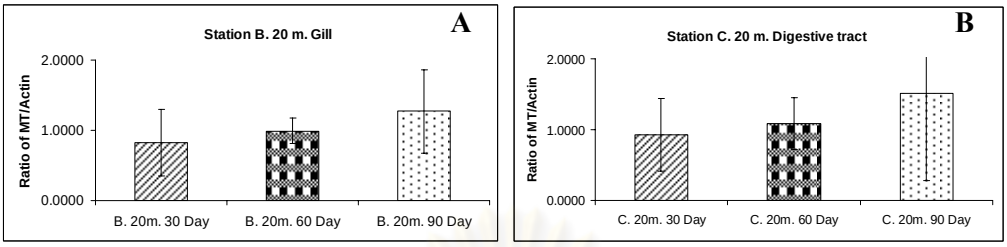


Figure 4.41 Ratio of MT expression in mussel during times of experiment (A = MT gill at station B at 20m, B = MT digestive tract at station C at 20 m)

4.4.2.2.2 Expression level of pvMT01 in transplanted mussels

The pvMT01 expression levels appeared to be various among different depths, time, and stations. (Table 4.19) Also, the average pvMT01 expression levels of mussels from some stations (station A 60D) (Fig.4.42A) seemed to be higher than that of station D 30D and 60D (reference site) (Fig.4.42D) However, there were no significant differences between the results from these stations.

When compared the levels of pvMT01 gene from each study site according to time, the results showed that after 30 days, the level of pvMT01 gene tended to increase in tissue. (Fig. 4.42A, B, C, D, E, G, and F) However, the difference was not statistically significant.

When compared the level of pvMT01 gene between depths, the results showed the level of pvMT01 gene tended to decrease in tissue (station B 5, 20, and 40m.). (Fig. 4.42B, F, and I) However, the difference was not statistically significant.

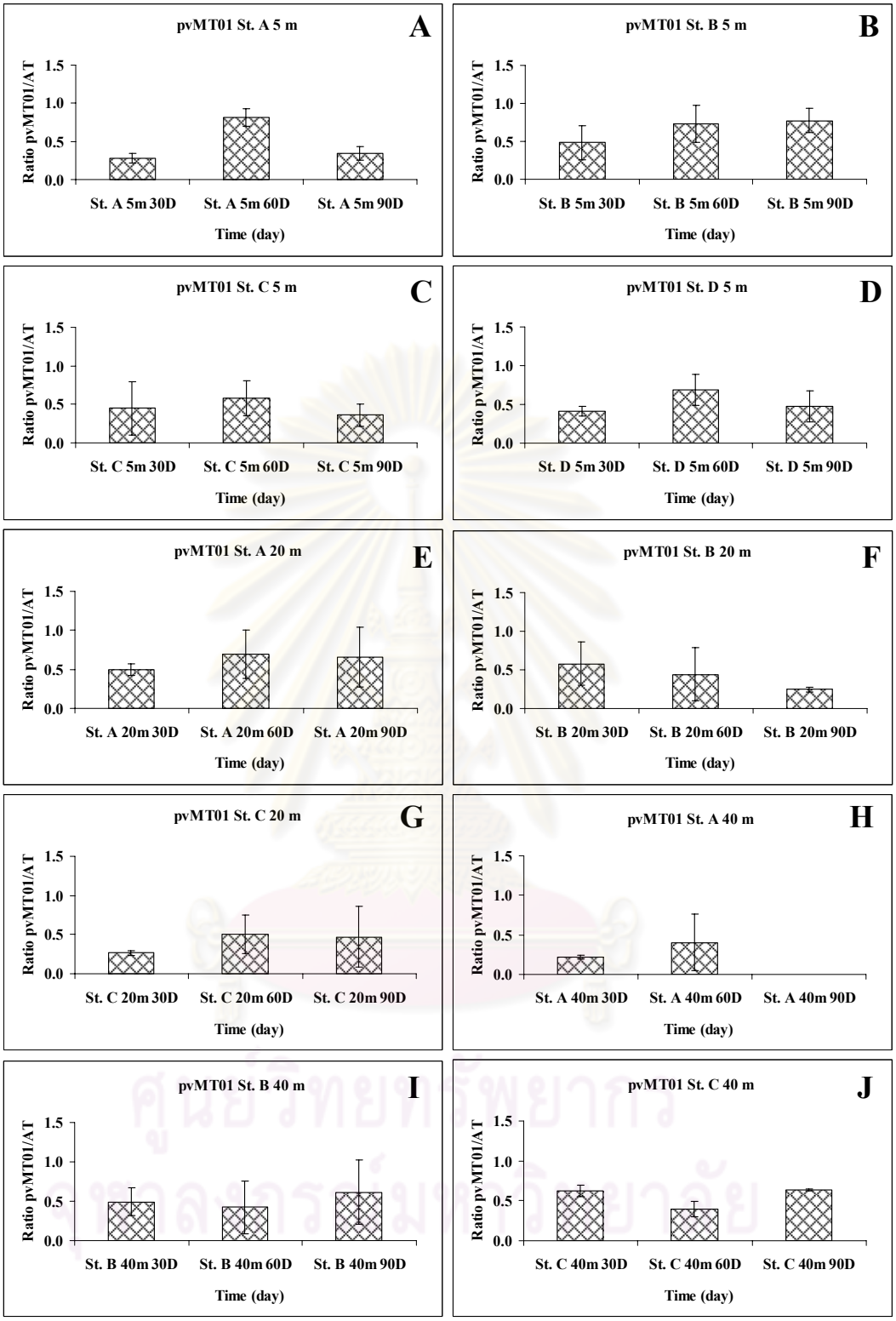


Figure 4.42 Relative expression level of pvMT01 in gill of transplanted mussels. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.19 Relative expression level of pvMT01 in gill of transplanted mussels (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	0.27±0.06	0.81±0.12	0.34±0.09
ST.A 20 m	0.50±0.07	0.69±0.31	0.65±0.39
ST.A 40 m	0.22±0.03	0.40±0.36	NA
ST.B 5 m	0.48±0.22	0.73±0.24	0.78±0.16
ST.B 20 m	0.58±0.28	0.44±0.34	0.24±0.03
ST.B 40 m	0.49±0.18	0.42±0.34	0.62±0.41
ST.C 5 m	0.45±0.35	0.58±0.23	0.36±0.14
ST.C 20 m	0.27±0.03	0.51±0.25	0.47±0.39
ST.C 40 m	0.63±0.39	0.40±0.04	0.64±0.27
ST.D 5 m	0.42±0.06	0.69±0.20	0.48±0.20

Remark: NA = data was not available due to mortality of mussel.

4.4.2.2.3 Expression level of pvMT02 in transplanted mussels

The pvMT02 expression levels appeared to be various among different depths, time, and stations (Table 4.20). Also, the average pvMT02 expression levels of mussels from some stations (station B and C at 5M 30, and 60D) (Fig.4.43B and C) seemed to be higher than that of station D (reference site) (Fig.4.43D) However, there were no significant differences between the results from these stations.

When compared the levels of pvMT02 gene from each study site according to time, the results showed that after 30 days, the level of pvMT02 gene tended to increase in tissue. (Fig. 4.43E) However, the difference was not statistically significant.

When compared the level of pvMT02 gene between depths, the results showed the level of pvMT02 gene tended to decrease in tissue (station C 5, 20, and 40m.). (Fig. 4.43C, G, and J) However, the difference was not statistically significant.

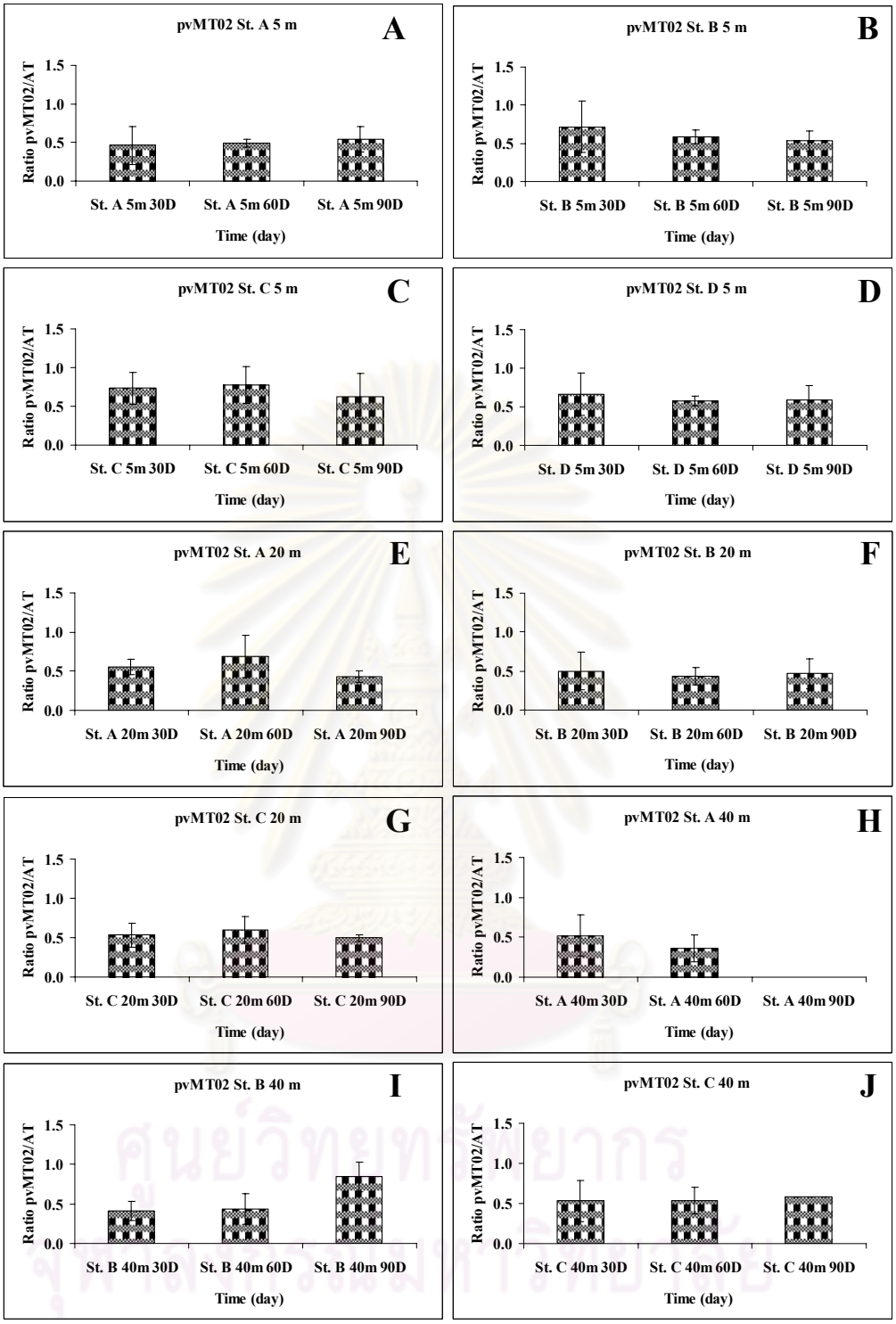


Figure 4.43 Relative expression level of pvMT02 in gill of transplanted mussels. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.20 Relative expression level of pvMT02 in gill of transplanted mussels (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	0.46±0.25	0.49±0.05	0.54±0.16
ST.A 20 m	0.55±0.09	0.69±0.27	0.43±0.07
ST.A 40 m	0.52±0.26	0.36±0.17	NA
ST.B 5 m	0.72±0.34	0.59±0.09	0.53±0.14
ST.B 20 m	0.50±0.24	0.44±0.11	0.47±0.19
ST.B 40 m	0.41±0.12	0.43±0.19	0.85±0.19
ST.C 5 m	0.74±0.21	0.77±0.24	0.63±0.29
ST.C 20 m	0.53±0.15	0.60±0.17	0.50±0.04
ST.C 40 m	0.53±0.24	0.53±0.19	0.58±0.21
ST.D 5 m	0.66±0.27	0.57±0.06	0.59±0.19

Remark: NA = data was not available due to mortality of mussel.

4.4.2.2.4 Expression level of pvMT03 in transplanted mussels

The pvMT03 expression levels appeared to be various among different depths, time, and stations. (Table 4.21) Also, the average pvMT03 expression levels of mussels from some stations (station A, B, and C) (Fig.4.44A, B, and C) seemed to be higher than that of station D (reference site) (Fig.4.44D) However, there were no significant differences between the results from these stations.

When compared the levels of pvMT03 gene from each study site according to time, the results showed that after 30 days, the level of pvMT03 gene tended to increase in tissue. (Fig. 4.44A, C, and E) However, the difference was not statistically significant.

When compared the level of pvMT03 gene between depths, the results showed the level of pvMT03 gene tended to decrease in tissue (station C 5, 20, and 40m.). (Fig. 4.44C, G, and J) However, the difference was not statistically significant.

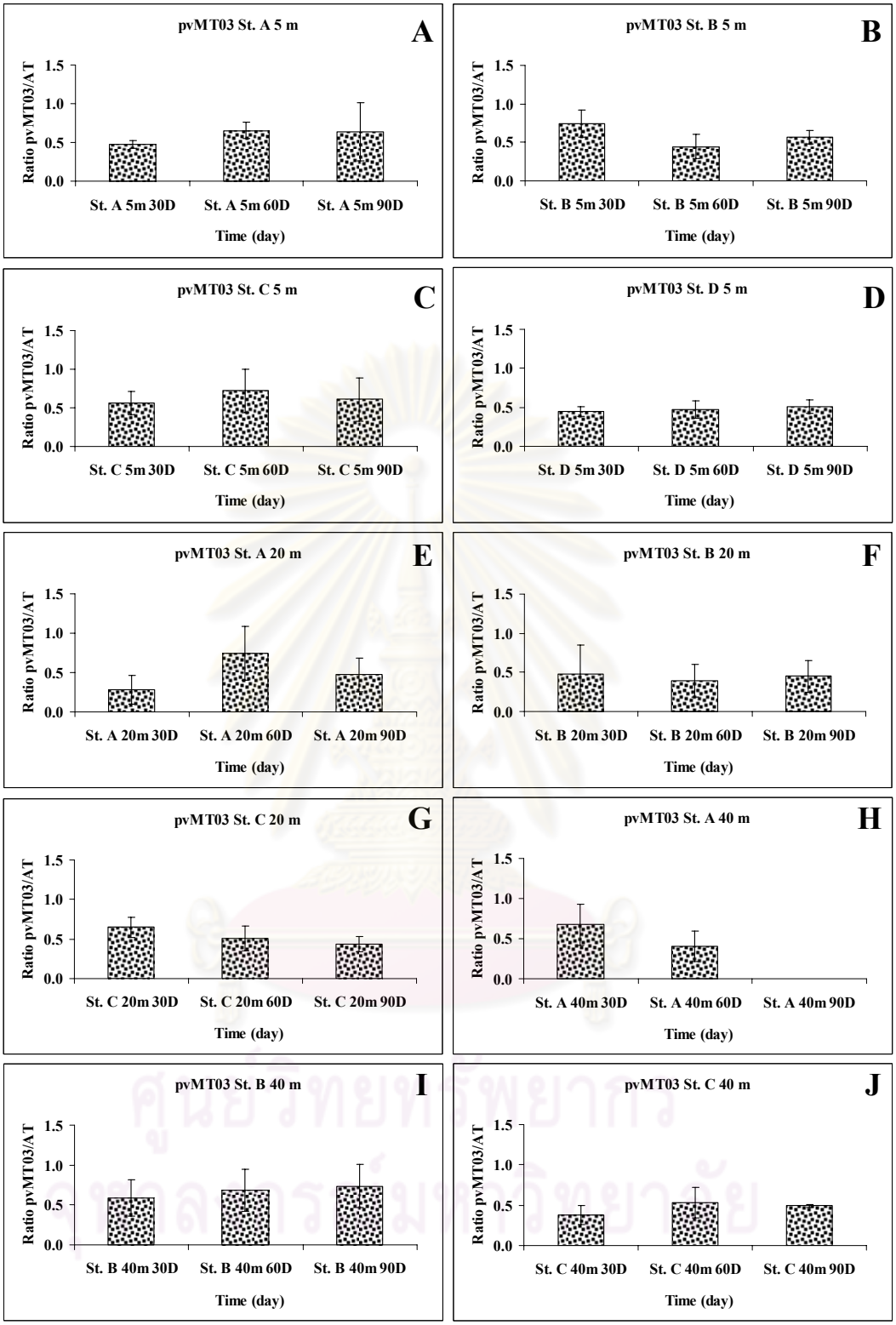


Figure 4.44 Relative expression level of pvMT03 in gill of transplanted mussels. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.21 Relative expression level of pvMT03 in gill of transplanted mussels (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	0.47±0.05	0.65±0.11	0.64±0.37
ST.A 20 m	0.28±0.18	0.74±0.34	0.47±0.21
ST.A 40 m	0.68±0.26	0.40±0.19	NA
ST.B 5 m	0.74±0.17	0.45±0.16	0.57±0.09
ST.B 20 m	0.48±0.37	0.39±0.20	0.45±0.20
ST.B 40 m	0.59±0.23	0.68±0.26	0.74±0.27
ST.C 5 m	0.56±0.14	0.72±0.28	0.61±0.28
ST.C 20 m	0.65±0.13	0.51±0.16	0.44±0.09
ST.C 40 m	0.38±0.12	0.53±0.19	0.49±0.02
ST.D 5 m	0.45±0.06	0.47±0.11	0.51±0.08

Remark: NA = data was not available due to mortality of mussel.

4.4.2.2.5 Expression level of pvMT07 in transplanted mussels

The pvMT07 expression levels appeared to be various among different depths, time, and stations. (Table 4.22) Also, the average pvMT07 expression levels of mussels from some stations (station A, B, and C) (Fig.4.45A, B, and C) seemed to be higher than that of station D (reference site) (Fig.4.45D) However, there were no significant differences between the results from these stations.

When compared the levels of pvMT07 gene from each study site according to time, the results showed that after 30 days, the level of pvMT07 gene tended to increase in tissue. (Fig. 4.45A, B, E, H, and J) However, the difference was not statistically significant.

When compared the level of pvMT07 gene between depths, the results showed the level of pvMT07 gene tended to decrease in tissue (station C 5, 20, and 40m.). (Fig. 4.45C, G, and J) However, the difference was not statistically significant.

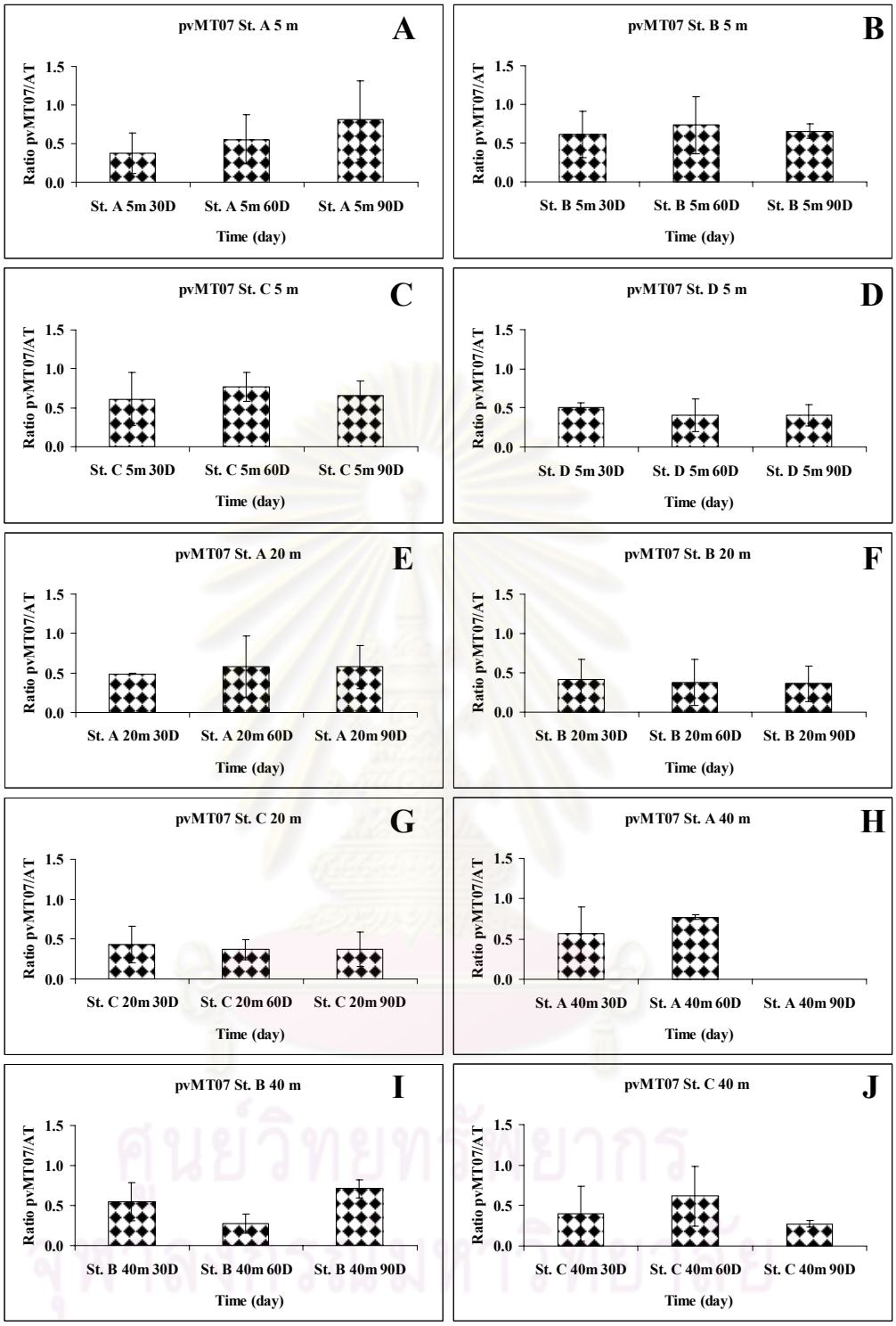


Figure 4.45 Relative expression level of pvMT07 in gill of transplanted mussels. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.22 Relative expression level of pvMT07 in gill of transplanted mussels (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	0.37±0.26	0.55±0.32	0.81±0.50
ST.A 20 m	0.49±0.01	0.58±0.39	0.58±0.27
ST.A 40 m	0.57±0.03	0.77±0.03	NA
ST.B 5 m	0.61±0.31	0.74±0.37	0.66±0.09
ST.B 20 m	0.41±0.26	0.38±0.29	0.36±0.23
ST.B 40 m	0.54±0.24	0.28±0.12	0.71±0.11
ST.C 5 m	0.61±0.34	0.76±0.18	0.65±0.20
ST.C 20 m	0.44±0.23	0.37±0.12	0.37±0.22
ST.C 40 m	0.40±0.34	0.62±0.37	0.27±0.04
ST.D 5 m	0.51±0.06	0.41±0.21	0.41±0.14

Remark: NA = data was not available due to mortality of mussel.

4.4.2.2.6 Expression level of pvMT08 in transplanted mussels

The pvMT08 expression levels appeared to be various among different depths, time, and stations. (Table 4.23) Also, the average pvMT08 expression levels of mussels from some stations (station A, B, and C) (Fig.4.46A, B, and C) seemed to be higher than that of station D (reference site) (Fig.4.46D) However, there were no significant differences between the results from these stations.

When compared the levels of pvMT08 gene from each study site according to time, the results showed that after 30 days, the level of pvMT08 gene tended to increase in tissue. (Fig. 4.46A, C, E, and J) However, the difference was not statistically significant.

When compared the level of pvMT08 gene between depths, the results showed the level of pvMT08 gene tended to decrease in tissue (station C 5, 20, and 40m.). (Fig. 4.46C, G, and J) However, the difference was not statistically significant.

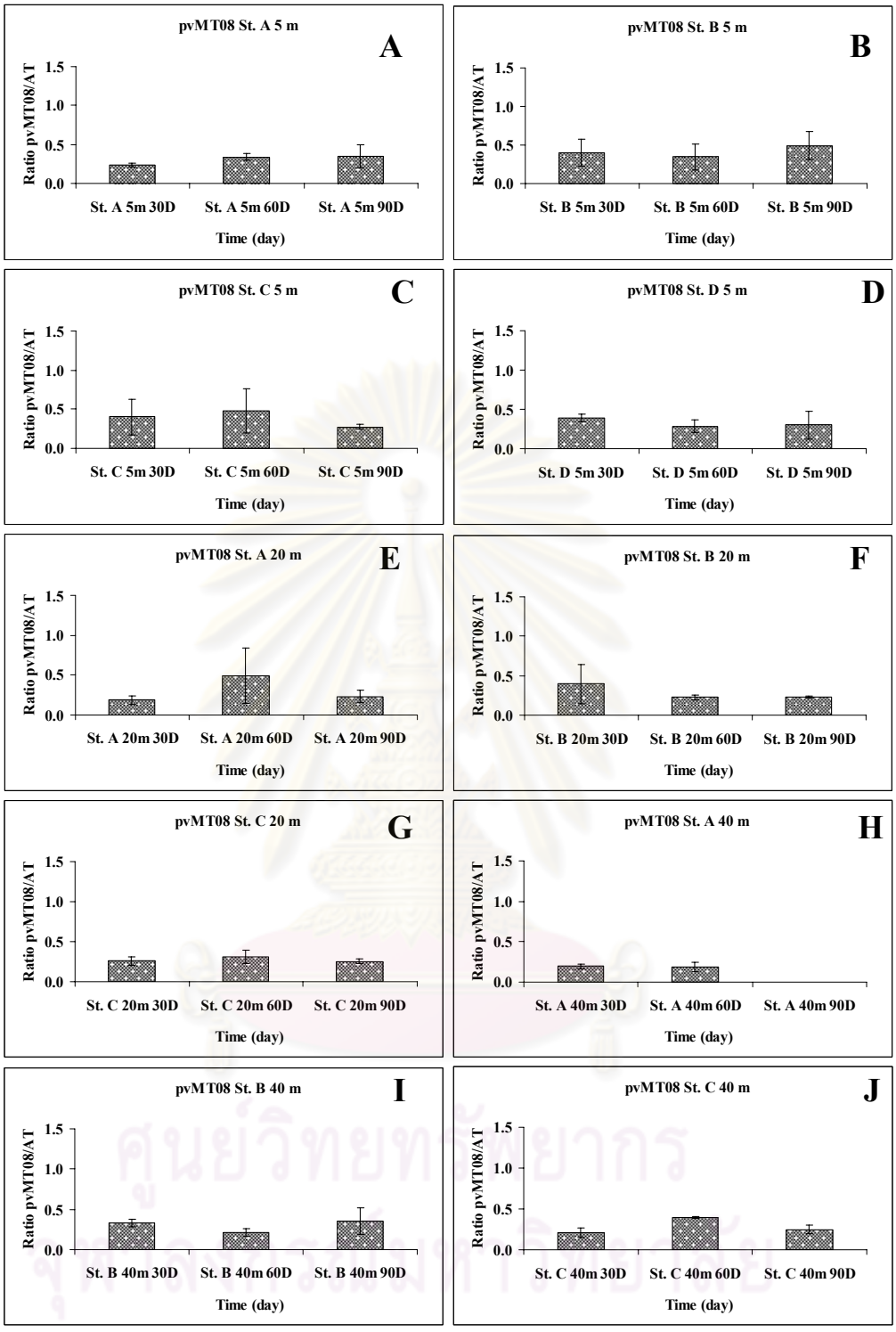


Figure 4.46 Relative expression level of pvMT08 in gill of transplanted mussels. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.23 Relative expression level of pvMT08 in gill of transplanted mussels (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	0.23±0.03	0.34±0.05	0.35±0.15
ST.A 20 m	0.19±0.06	0.50±0.35	0.23±0.07
ST.A 40 m	0.19±0.03	0.19±0.06	NA
ST.B 5 m	0.40±0.18	0.34±0.17	0.49±0.18
ST.B 20 m	0.40±0.25	0.22±0.03	0.23±0.01
ST.B 40 m	0.33±0.05	0.21±0.05	0.35±0.16
ST.C 5 m	0.40±0.23	0.48±0.28	0.27±0.03
ST.C 20 m	0.26±0.06	0.31±0.09	0.25±0.03
ST.C 40 m	0.21±0.06	0.39±0.01	0.25±0.05
ST.D 5 m	0.39±0.05	0.28±0.08	0.30±0.18

Remark: NA = data was not available due to mortality of mussel.

4.4.2.2.7 Expression level of pvMT11 in transplanted mussels

The pvMT11 expression levels appeared to be various among different depths, time, and stations. (Table 4.24) Also, the average pvMT11 expression levels of mussels from some stations (station A, B, and C) (Fig.4.47A, B, and C) seemed to be higher than that of station D (reference site) (Fig.4.47D) However, there were no significant differences between the results from these stations.

When compared the levels of pvMT11 gene from each study site according to time, the results showed that after 30 days, the level of pvMT11 gene tended to increase in tissue. (Fig. 4.47C, E, G, and J) However, the difference was not statistically significant.

When compared the level of pvMT11 gene between depths, the results showed the level of pvMT11 gene tended to decrease in tissue (station C 5, 20, and 40m.). (Fig. 4.47C, G, and J) However, the difference was not statistically significant.

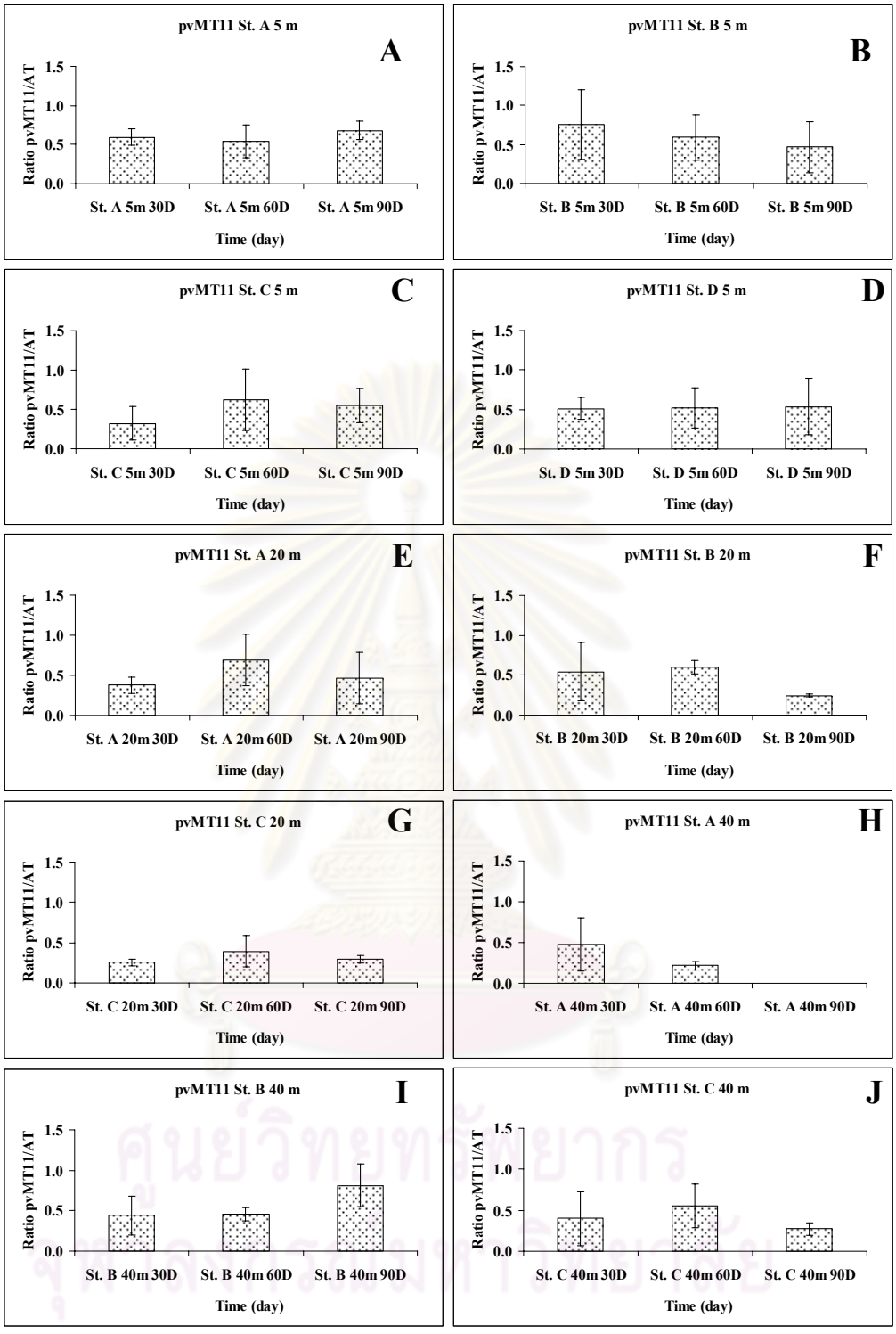


Figure 4.47 Relative expression level of pvMT11 in gill of transplanted mussels. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.24 Relative expression level of pvMT11 in gill of transplanted mussels (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	0.59±0.10	0.54±0.21	0.68±0.11
ST.A 20 m	0.38±0.10	0.69±0.32	0.46±0.32
ST.A 40 m	0.47±0.32	0.22±0.05	NA
ST.B 5 m	0.75±0.45	0.59±0.29	0.47±0.33
ST.B 20 m	0.55±0.37	0.60±0.08	0.24±0.02
ST.B 40 m	0.44±0.24	0.46±0.08	0.81±0.27
ST.C 5 m	0.32±0.22	0.63±0.39	0.55±0.22
ST.C 20 m	0.25±0.04	0.40±0.19	0.30±0.04
ST.C 40 m	0.40±0.33	0.56±0.27	0.27±0.07
ST.D 5 m	0.51±0.14	0.52±0.25	0.54±0.36

Remark: NA = data was not available due to mortality of mussel.

4.4.2.2.8 Expression level of HSP71 gene in transplanted mussels

The HSP71 expression levels appeared to be various among different depths, time, and stations. (Table 4.25) Also, the average HSP71 expression levels of mussels from some stations (station A, B, and C) (Fig.4.48A, B, and C) seemed to be higher than that of station D (reference site) (Fig.4.48D) However, there were no significant differences between the results from these stations.

When compared the levels of HSP71 gene from each study site according to time, the results showed that after 30 days, the level of total HSP71 gene tended to increase in tissue. (Fig. 4.48A, C, and F) However, the difference was not statistically significant.

When compared the level of HSP71 gene between depths, the results showed the level of total HSP71 gene no significant difference were found between depths.

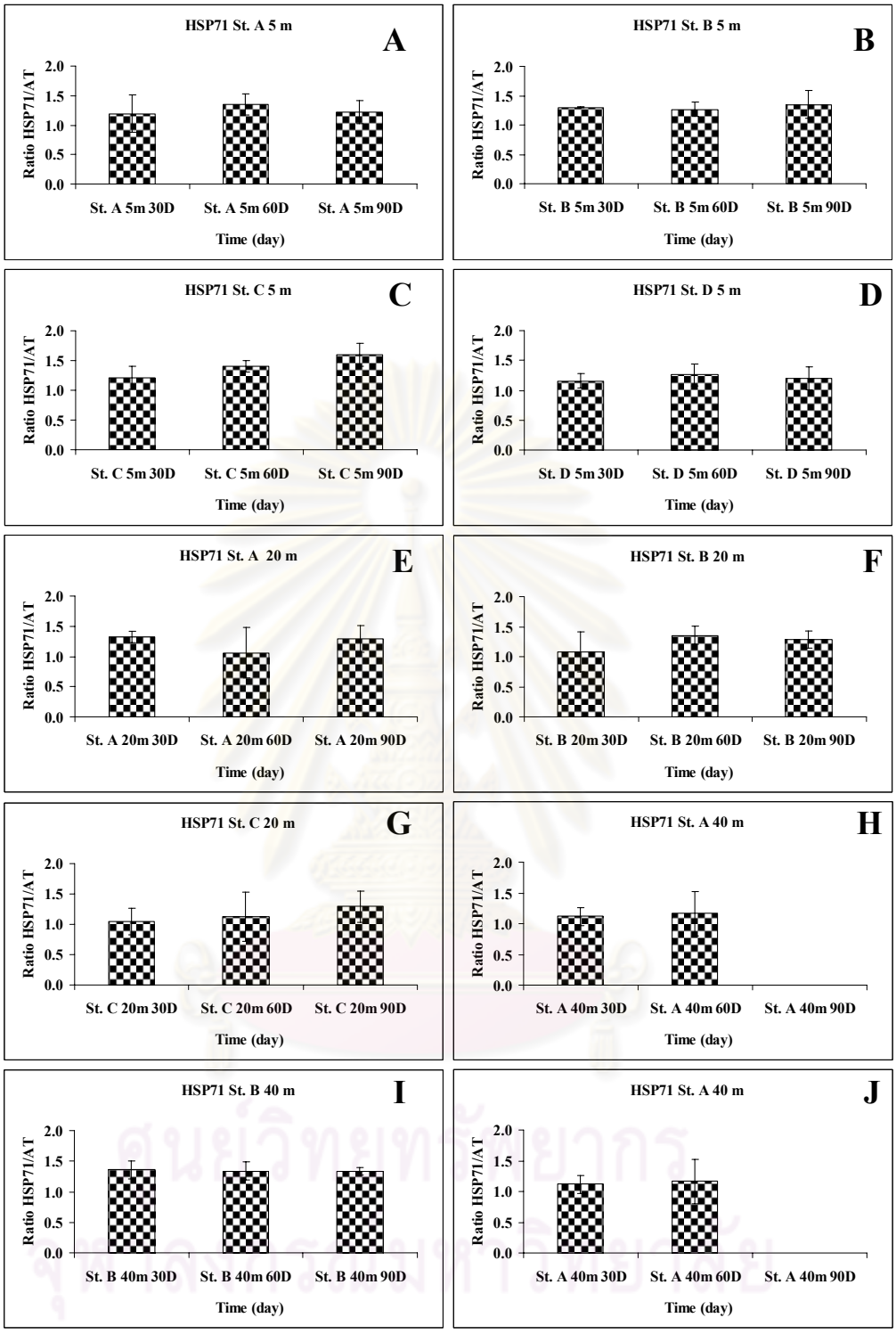


Figure 4.48 Relative expression level of HSP71 gene in gill of transplanted mussels. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.25 Relative expression level of HSP71 gene in gill of transplanted mussels (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	1.19±0.31	1.35±0.17	1.21±0.19
ST.A 20 m	1.32±0.10	1.06±0.41	1.29±0.22
ST.A 40 m	1.12±0.15	1.16±0.37	NA
ST.B 5 m	1.30±0.01	1.27±0.13	1.35±0.24
ST.B 20 m	1.08±0.33	1.35±0.16	1.28±0.14
ST.B 40 m	1.36±0.15	1.34±0.14	1.34±0.06
ST.C 5 m	1.21±0.19	1.41±0.10	1.60±0.19
ST.C 20 m	1.05±0.22	1.12±0.41	1.29±0.41
ST.C 40 m	1.32±0.13	1.52±0.09	1.26±0.03
ST.D 5 m	1.16±0.13	1.26±0.18	1.20±0.20

Remark: NA = data was not available due to mortality of mussel.

4.4.2.2.9 Expression level of CYP4 gene in transplanted mussels

The CYP4 expression levels appeared to be various among different depths, time, and stations. (Table 4.26 and Fig.4.49) Also, the average CYP4 expression levels of mussels between stations no significant differences were found.

When compared the levels of CYP4 gene from each study site according to time, the results showed no significant difference were found between levels of total CYP4.

When compared the level of CYP4 gene between depths, the results showed the level of total CYP4 gene no significant difference were found.

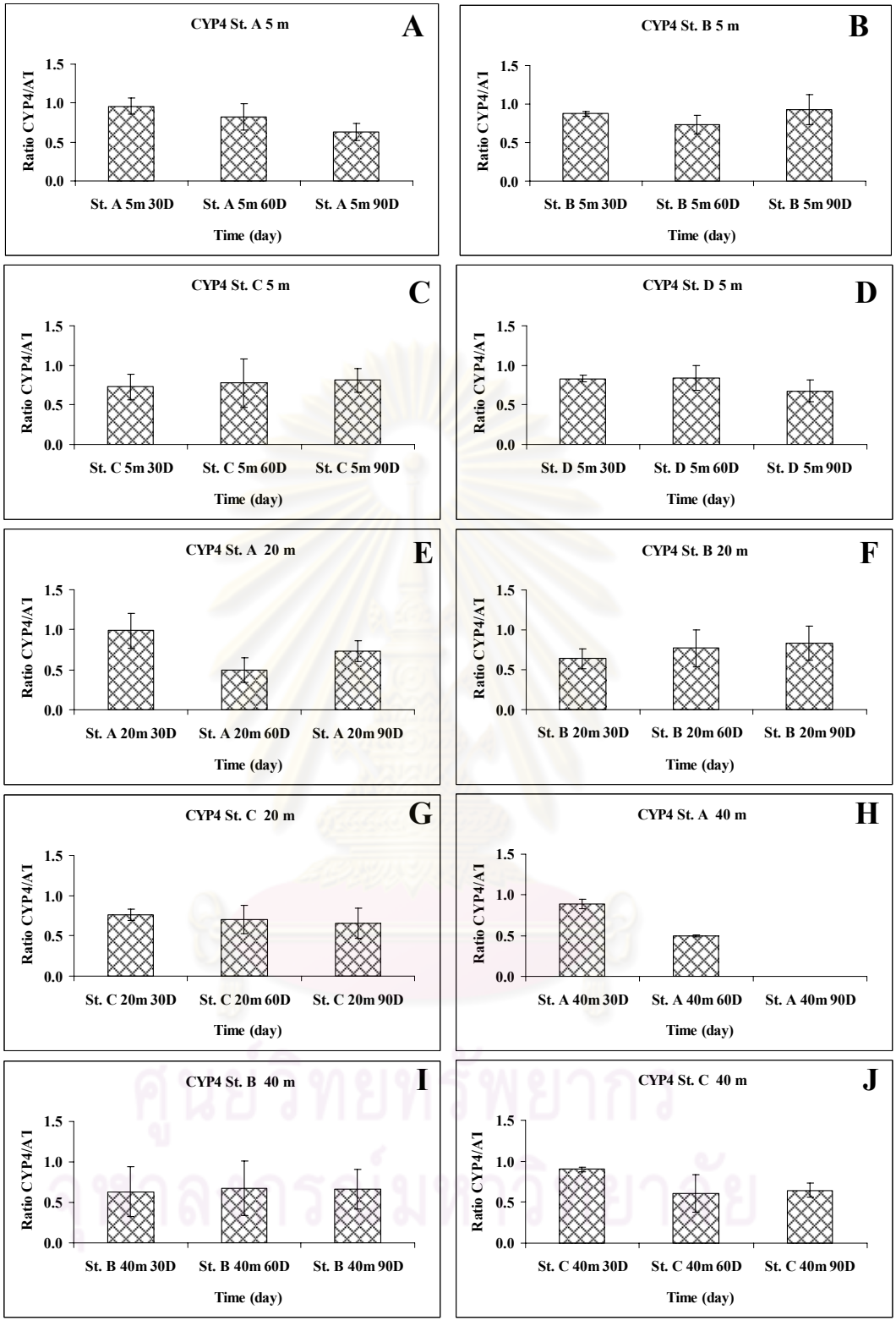


Figure 4.49 Relative expression level of CYP4 gene in gill of transplanted mussels. A to D indicates the result from 5 m. depth at station A to D, respectively. E to G indicates the result from 20 m. depth at station A to C, respectively. H to J indicates the result from 40 m. depth at station A to C, respectively.

Table 4.26 Relative expression level of CYP4 gene in gill of transplanted mussels (n=3)

Stations/ Depth (m)	Time of Exposure (Day)		
	30 Day	**60 Day**	**90 Day**
ST.A 5 m	0.96±0.10	0.83±0.17	0.63±0.11
ST.A 20 m	0.99±0.22	0.50±0.16	0.73±0.13
ST.A 40 m	0.89±0.06	0.50±0.01	NA
ST.B 5 m	0.87±0.03	0.73±0.12	0.93±0.20
ST.B 20 m	0.64±0.13	0.77±0.23	0.83±0.21
ST.B 40 m	0.63±0.31	0.67±0.34	0.67±0.24
ST.C 5 m	0.73±0.16	0.78±0.31	0.81±0.15
ST.C 20 m	0.76±0.07	0.71±0.18	0.65±0.19
ST.C 40 m	0.90±0.03	0.60±0.23	0.64±0.09
ST.D 5 m	0.83±0.04	0.84±0.16	0.68±0.14

Remark: NA = data was not available due to mortality of mussel.

4.4.3 Correlation between gene expression level and mercury concentration in mussel

4.4.3.1 Correlation between MT gene and Hg concentration in mussel

4.4.3.1.1 Total MT gene

Correlation between expression levels of total MT gene and Hg concentration were analyzed in gills of Hg treated mussels (laboratory study) and mussels transplanted at petroleum production platforms (field study). The results showed in figure 4.50 (laboratory data) and figure 4.51 (field data). The result revealed that expression level of MT gene did not correlated with the Hg level in the same mussel tissue.

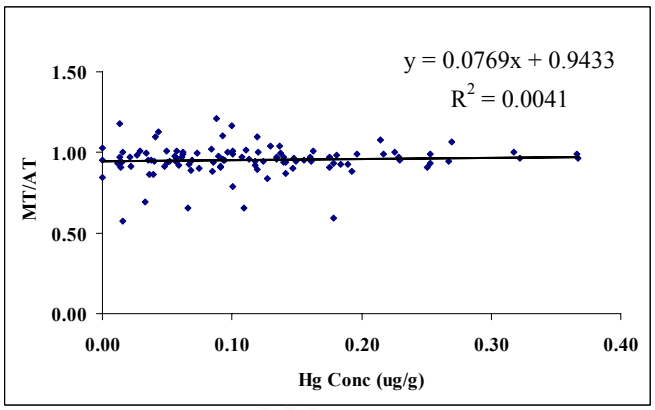


Figure 4.50 Analysis of correlation between MT gene expression and Hg concentration in mussel tissue (Laboratory study)

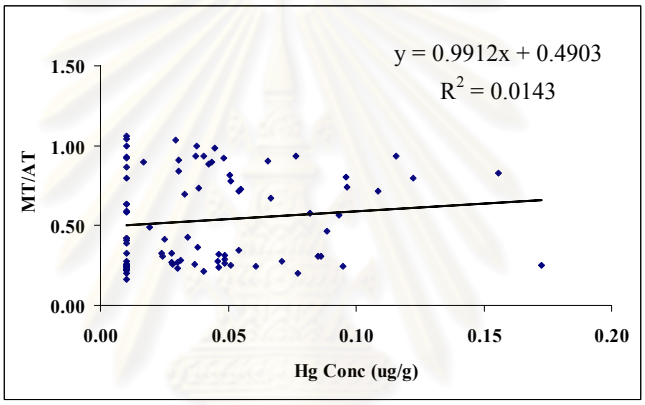


Figure 4.51 Analysis of correlation between MT gene expression and Hg concentration in mussel tissue. (Field study)

4.4.3.1.2 pvMT01

The expression level of pvMT01 gene was not in agreement with the increasing level of Hg concentration in mussel tissue. Therefore, there was no correlation between pvMT01 and Hg concentration in both laboratory and field studies. The results were shown in Fig. 4.52 (Laboratory data) and Fig 4.53 (Field data).

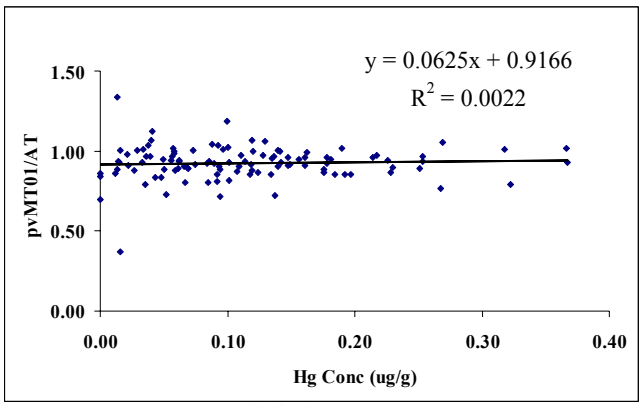


Figure 4.52 Analysis of correlation between pvMT01 gene expression and Hg concentration in mussel tissue (Laboratory study).

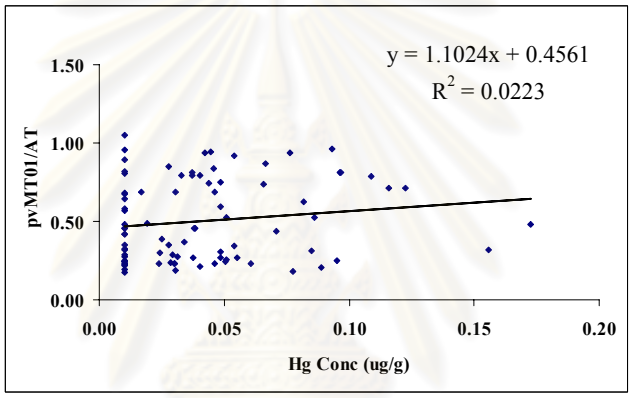


Figure 4.53 Analysis of correlation between pvMT01 gene expression and Hg concentration in mussel tissue (Field study).

4.4.3.1.3 pvMT02 gene

The expression level of pvMT02 gene was not in agreement with the increasing level of Hg concentration in mussel tissue. Therefore, there was no correlation between pvMT02 and Hg concentration in both laboratory and field studies. The results were shown in Fig. 4.54 (Laboratory data) and Fig 4.55 (Field data.)

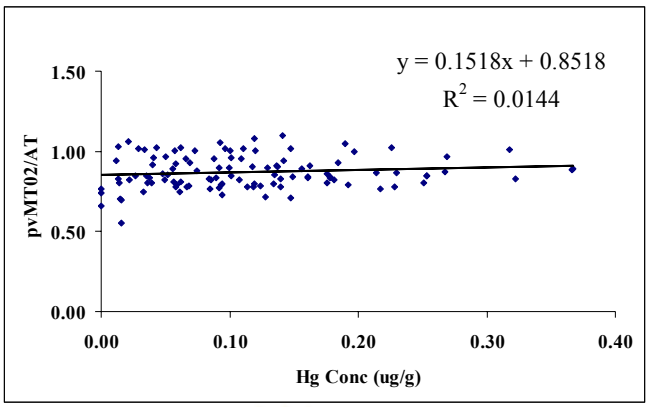


Figure 4.54 Analysis of correlation between pvMT02 gene expression and Hg concentration in mussel tissue (Laboratory study)

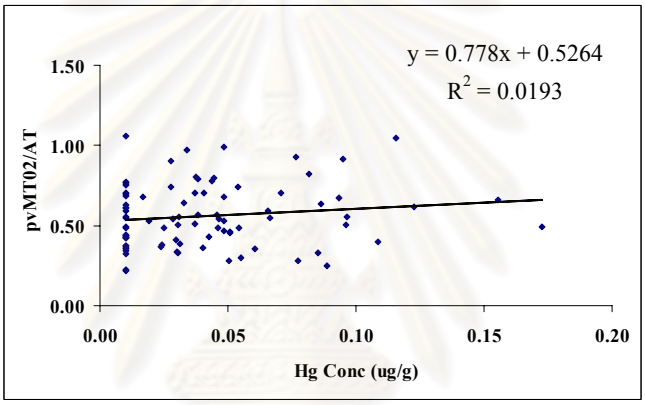


Figure 4.55 Analysis of correlation between pvMT02 gene expression and Hg concentration in mussel tissue (Field study)

4.4.3.1.4 pvMT03 gene

The expression level of pvMT03 gene was not in agreement with the increasing level of Hg concentration in mussel tissue. Therefore, there was no correlation between pvMT03 and Hg concentration in both laboratory and field studies. The results were shown in Fig. 4.56 (Laboratory data) and Fig 4.57 (Field data.)

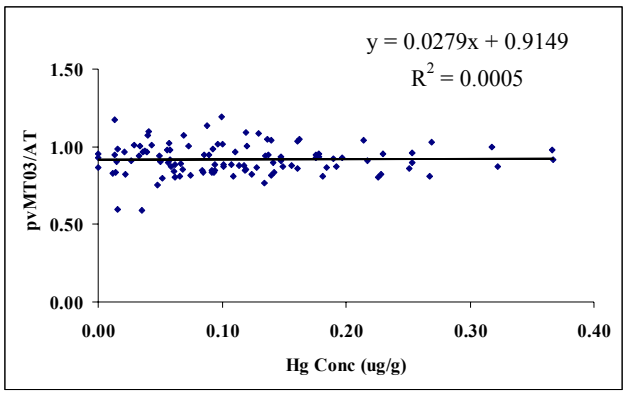


Figure 4.56 Analysis of correlation between pvMT03 gene expression and Hg concentration in mussel tissue (Laboratory study)

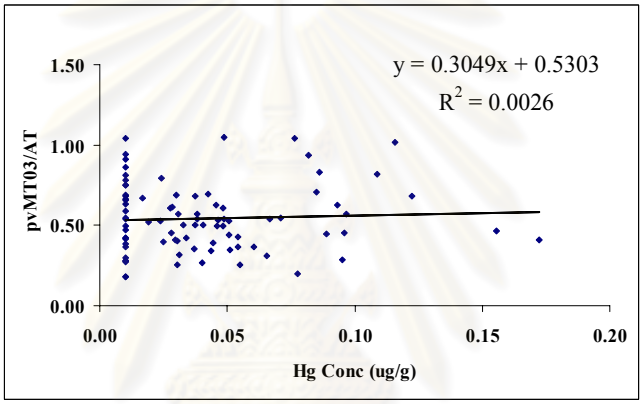


Figure 4.57 Analysis of correlation between pvMT03 gene expression and Hg concentration in mussel tissue (Field study).

4.4.3.1.5 pvMT07 gene

The expression level of pvMT07 gene appeared to be in agreement with the increasing level of Hg concentration in mussel tissue. Therefore, the correlation was statistically significant ($p<0.01$). The results were shown in Fig. 4.58 (Laboratory data) and Fig 4.59 (Field data).

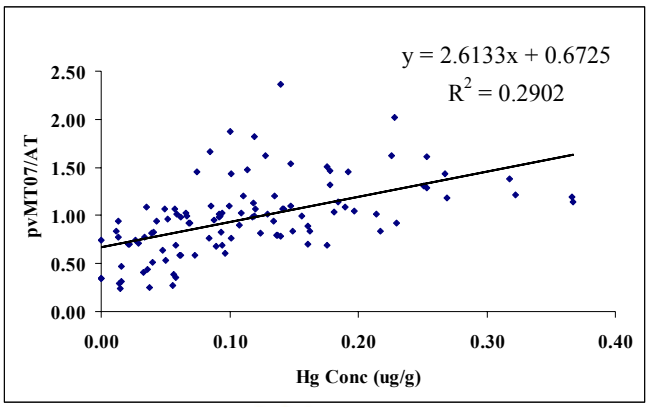


Figure 4.58 Analysis of correlation between pvMT07 gene expression and Hg concentration in mussel tissue (R = 0.539** p<0.01) (Laboratory study)

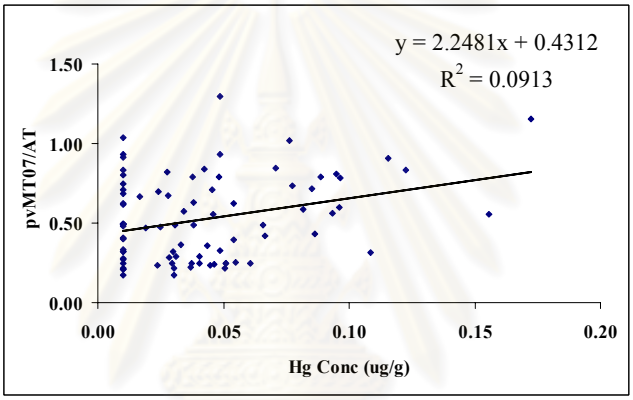


Figure 4.59 Analysis of correlation between pvMT07 gene expression and Hg concentration in mussel tissue (R = 0.302** p<0.01) (Field study)

4.4.3.1.6 pvMT08 gene

The expression level of pvMT08 gene was not in agreement with the increasing level of Hg concentration in mussel tissue. Therefore, there was no correlation between pvMT08 and Hg concentration in both laboratory and field studies. The results were shown in Fig. 4.60 (Laboratory data) and Fig 4.61 (Field data.)

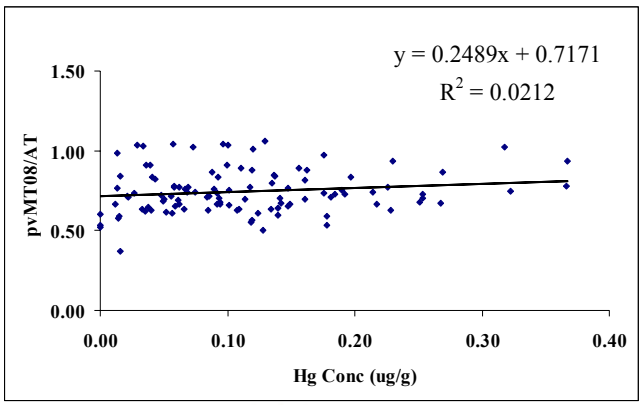


Figure 4.60 Analysis of correlation between pvMT08 gene expression and Hg concentration in mussel tissue (Laboratory study)

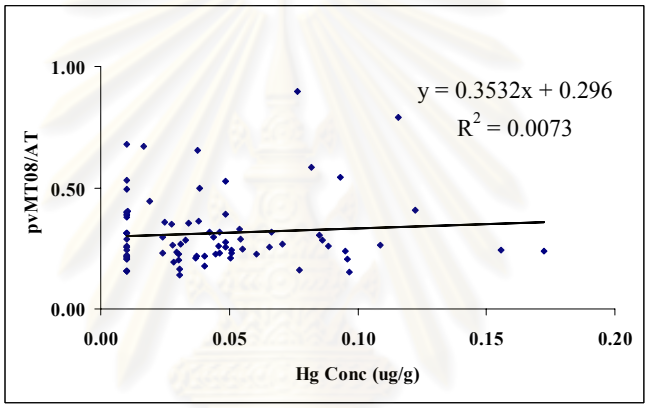


Figure 4.61 Analysis of correlation between pvMT08 gene expression and Hg concentration in mussel tissue (Field study).

4.4.3.1.7 pvMT11 gene

The expression level of pvMT11 gene was not in agreement with the increasing level of Hg concentration in mussel tissue. Therefore, there was no correlation between pvMT11 and Hg concentration in both laboratory and field studies. The results were shown in Fig. 4.62 (Laboratory data) and Fig 4.63 (Field data.)

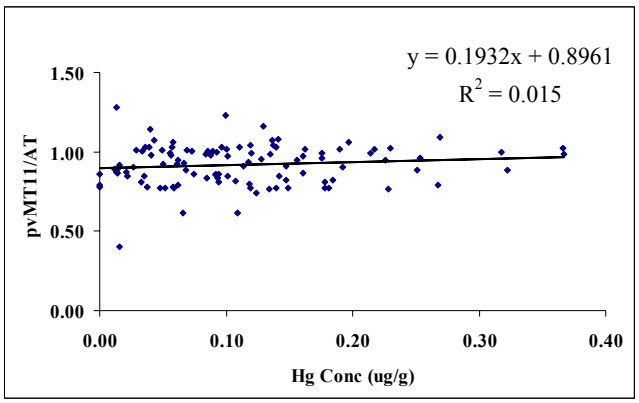


Figure 4.62 Analysis of correlation between pvMT11 gene expression and Hg concentration in mussel tissue (Laboratory study).

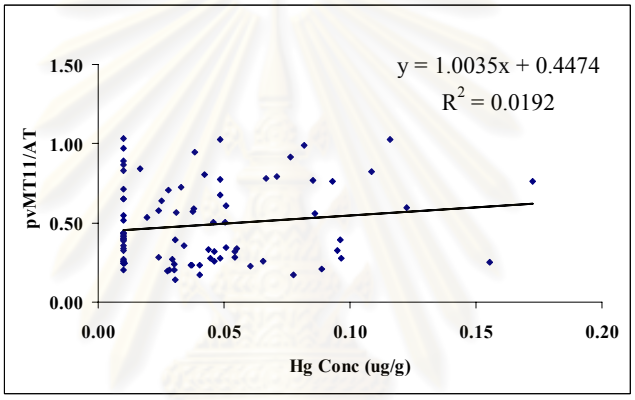
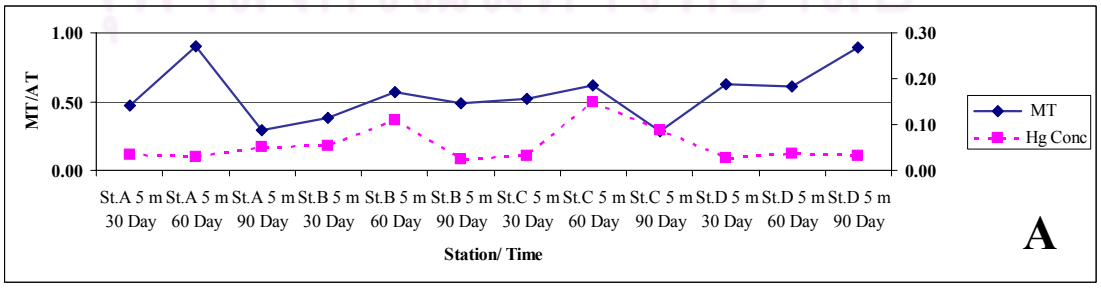


Figure 4.63 Analysis of correlation between pvMT11 gene expression and Hg concentration in mussel tissue (Field study).

As the results of correlation between Hg concentration and 6 of MT variants, it is indicated that the expression of pvMT07 was significantly correlated with Hg concentration of tested mussels better than the other subunits of MT gene ($p<0.01$) (figure 4.64E).



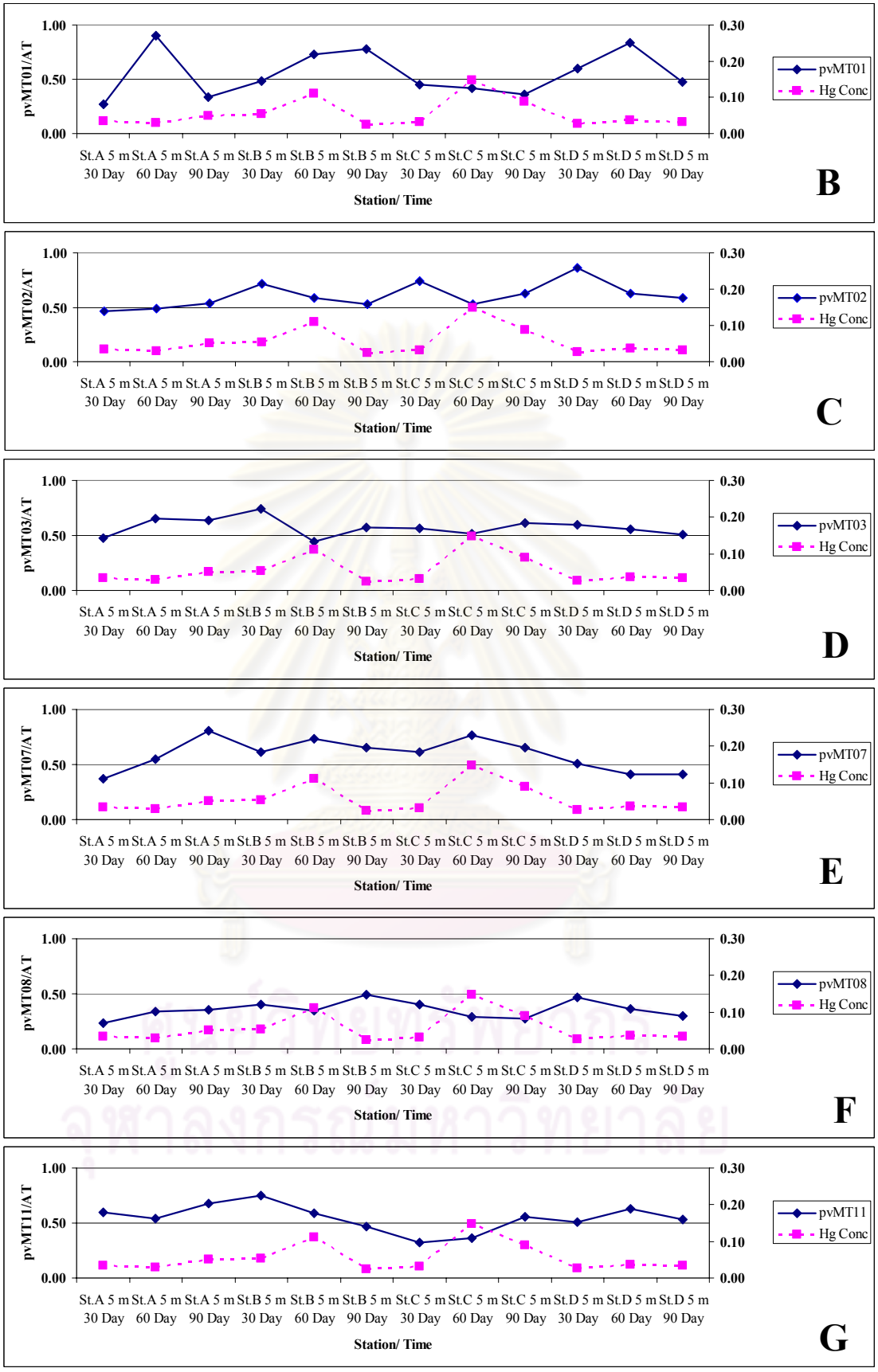


Figure 4.64 Correlation between the expression levels of 6 MT subunits including total MT in the same mussel tissue of *P.viridis* transplanted at petroleum processing

platform (Continuous line represents the expression of MT subunit while dotted line represents Hg concentration). A to G indicates correlation levels of genes total MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08, and pvMT11, respectively.

4.4.3.2 Correlation between HSP71 gene and Hg concentration in mussel tissue

The expression level of HSP71 gene was not in agreement with the increasing level of Hg concentration in mussel tissue. Therefore, there was no correlation between HSP71 and Hg concentration in both laboratory and field studies. The results were shown in Fig. 4.65 (Laboratory data) and Fig 4.66 A and B (Field data.)

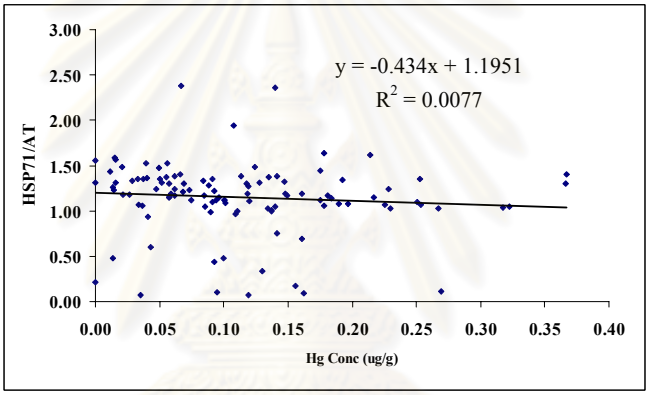
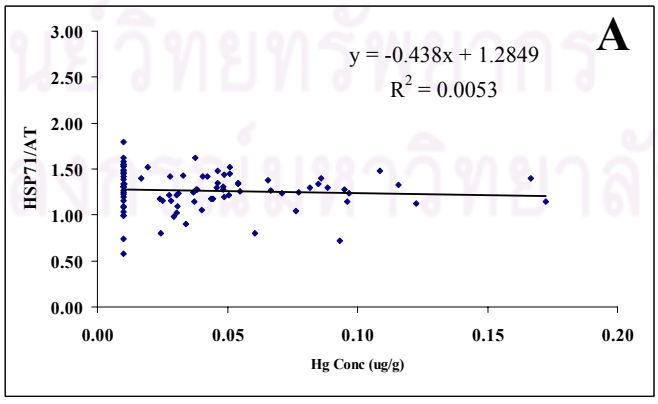


Figure 4.65 Analysis of correlation between HSP71 gene expression and Hg concentration in mussel tissue (Laboratory study)



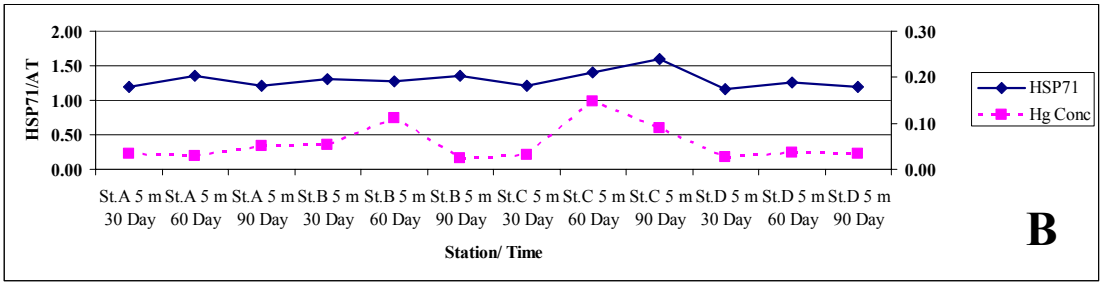


Figure 4.66 Analysis of correlation A = Correlation between HSP71 gene expression and Hg concentration in mussel tissue (Field study). B = Correlation between the expression levels of HSP71 in the same mussel tissue of *P.viridis* transplanted at petroleum processing platform (Continuous line represents the expressions of MT subunit while dotted line represents Hg concentration).

4.4.3.3 Correlation between CYP4 gene and Hg concentration in mussel tissue

The expression level of CYP4 gene was not in agreement with the increasing level of Hg concentration in mussel tissue. Therefore, there was no correlation between CYP4 and Hg concentration in both laboratory and field studies. The results were shown in Fig. 4.67 (Laboratory data) and Fig 4.68 A and B (Field data.)

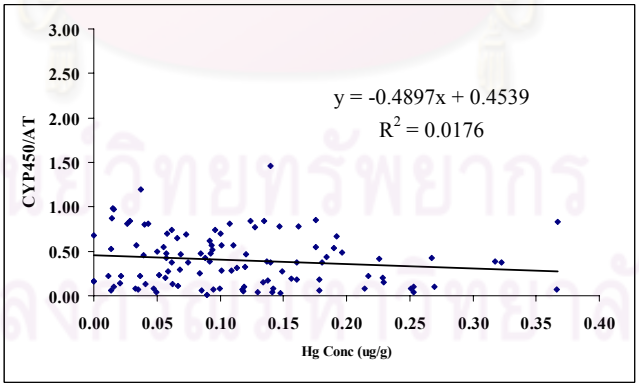


Figure 4.67 Analysis of correlation between CYP4 gene expression and Hg concentration in mussel tissue (Laboratory study)

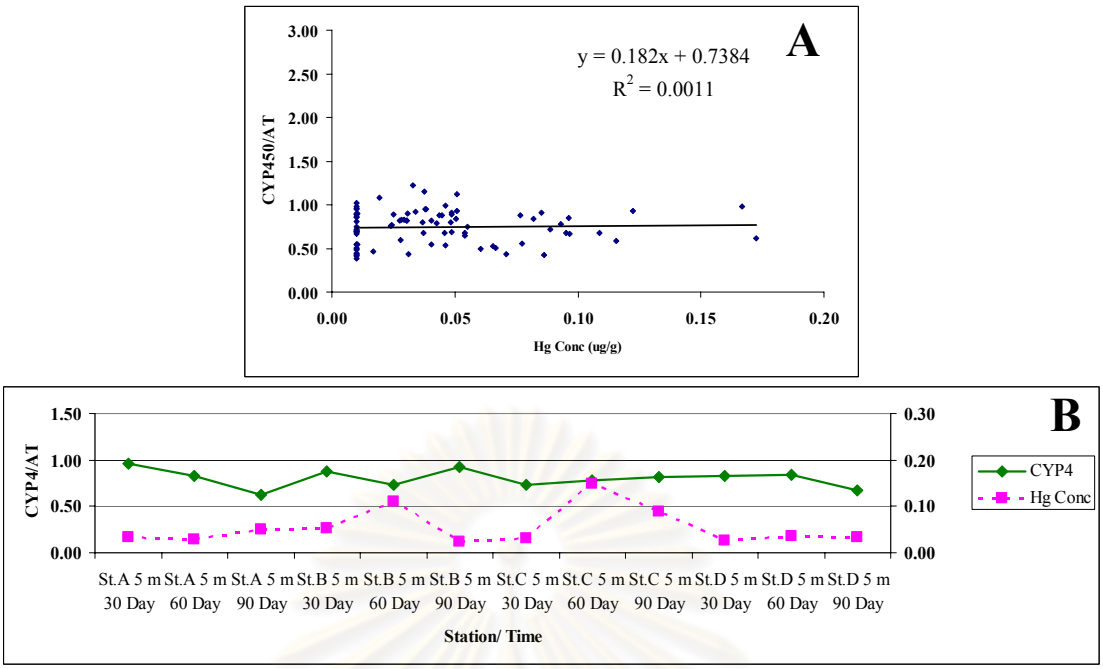


Figure 4.68 Analysis of correlation A = Correlation between CYP4 gene expression and Hg concentration in mussel tissue (Field study). B = Correlation between the expression levels of CYP4 in the same mussel tissue of *P.viridis* transplanted at petroleum processing platform (Continuous line represents the expressions of MT subunit while dotted line represents Hg concentration).

4.5 Single cell gel electrophoresis analysis (Comet assay)

DNA damage caused by exposing to Hg in vitro was measured in haemocytes (figure 4.69, 4.73) and sperm (figure 4.70, 4.74) of mussel. The degree of damage was estimated from tail length as the extent of the migration of the genetic material in the direction of the anode and the tail moment which is calculated by multiplying the tail length with % of DNA in tail. Mean tail length of comets obtained by Hg exposures are given in table 4.27 and 4.28. The trend of increase in comet tail length with increasing Hg concentration and duration is depicted in Figure 4.71A and B. At 10 min exposure, the comet tail lengths of the haemocyte treated with 0, 0.001, 0.01, 0.1, 1.0, and 10.0 µg/L of $HgCl_2$ were 53.56±24.25, 168.48±41.59, 181.91±51.88, 191.91±46.52, 212.54±36.40, and 245.80±31.91 in length (Table 4.27), which were 1.0, 3.15, 3.40, 3.58, 3.97, and 4.59 folds longer than that of control (Table 4.31, Fig.4.75a) and at 10 min exposure, the comet tail lengths of the sperms treated with 0, 0.001, 0.01, 0.1, 1.0, and 10.0 µg/L of $HgCl_2$ were 106.20±66.26, 128.35±43.75, 161.42±39.41, 225.06±48.51, 254.58±56.35, and 273.72±44.53 in length (Table

4.28), which were 1.0, 1.21, 1.52, 2.12, 2.40, and 2.58 folds longer than that of control (Table 4.31 and Fig.4.75a).

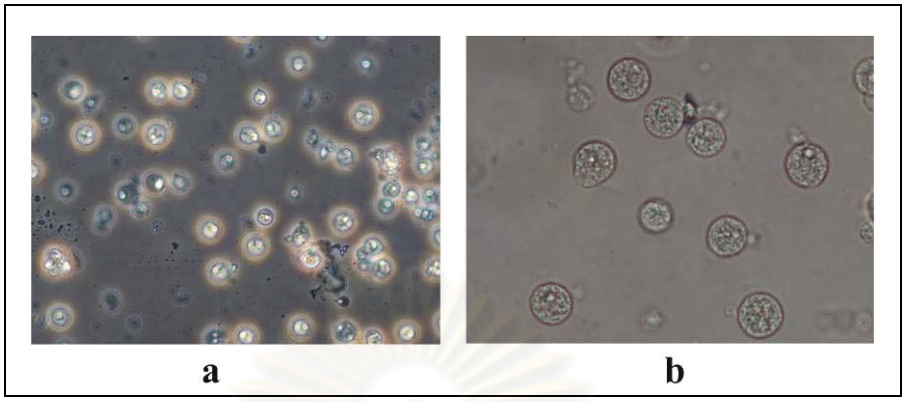


Figure 4.69 Mussel haemocytes visualized by microscope at 200X (a) and 600X (b)

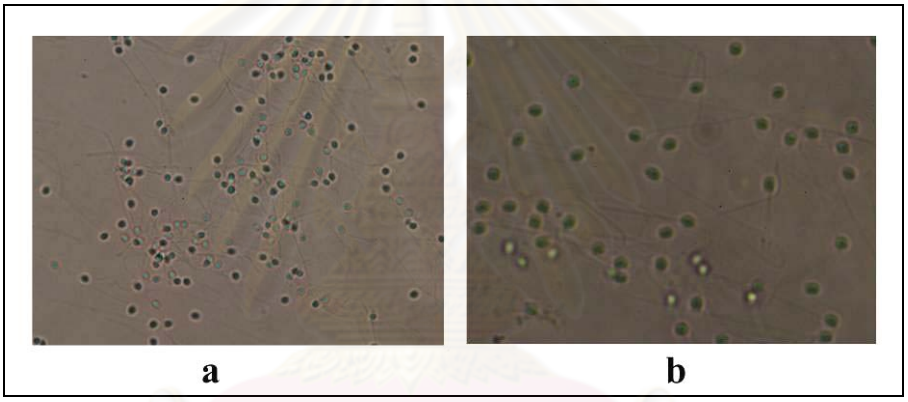


Figure 4.70 Mussel sperm visualized by microscope at 200X (a) and 400X (b)

All concentrations evoked significant DNA damage ($p<0.05$) when compared with controls. The results also showed significant differences between treatment groups.

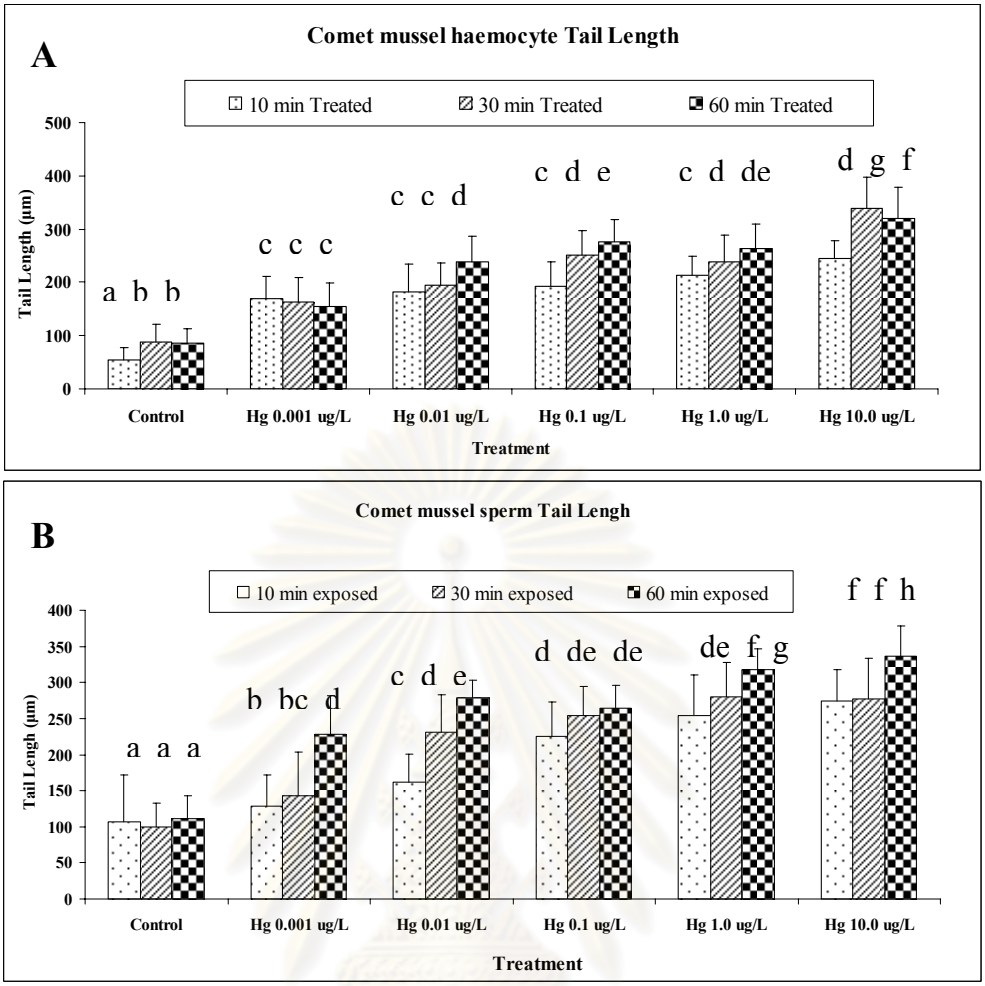


Figure 4.71 Comet Tail length. A indicates comet in haemocyte and B indicates comet in sperm

Table 4.27 DNA tail length (µm) (mean ± SD) from haemocyte after 10, 30, and 60 min of $HgCl_2$ exposure

Exposure Time (min)	$HgCl_2$ concentration (µg/L)					
	0	**0.001**	**0.01**	**0.1**	**1.0**	**10.0**
10	53.56±24.25^{a}	168.48±41.59^{c}	181.91±51.88^{c}	191.91±46.52^{c}	212.54±36.40^{c}	245.80±31.91^{d}
30	87.68±34.06^{b}	162.72±46.88^{c}	195.08±40.81^{c}	250.38±47.72^{d}	239.07±49.54^{d}	339.11±58.03^{g}
60	86.48±27.42^{b}	154.14±44.76^{c}	237.66±48.03^{d}	277.02±41.18^{e}	263.88±45.25^{de}	321.58±58.77^{f}

Remark: The same superscripts indicated that the DNA tail length was not significantly different (P≥0.05) amount group of treatment within the same period of exposure.

Table 4.28 DNA tail length (μm) (mean ± SD) from sperms after 10, 30, and 60 min of $HgCl_2$ exposure

Exposure Time (min)	$HgCl_2$ concentration (μg/L)					
	0	**0.001**	**0.01**	**0.1**	**1.0**	**10.0**
10	106.20±66.26[a]	128.35±43.75[b]	161.42±39.41[c]	225.06±48.51[d]	254.58±56.35[de]	273.72±44.53[f]
30	99.11±33.59[a]	142.69±61.41[bc]	230.37±53.20[d]	253.59±40.59[de]	280.21±47.16[f]	277.90±55.05[f]
60	111.72±31.62[a]	228.50±53.43[d]	278.75±24.08[e]	264.21±32.06[de]	317.66±29.04[g]	335.85±41.99[h]

Remark: The same superscripts indicated that the DNA tail length was not significantly different ($P \geq 0.05$) amount group of treatment within the same period of exposure.

The comet tail moment of the haemocyte treated with 0, 0.001, 0.01, 0.1, 1.0, and 10.0 μg/L of $HgCl_2$ at 10 min were 6.77±6.48, 49.96±30.27, 69.27±37.97, 71.68±34.55, 88.36±40.34, and 135.12±27.96 (Table 4.29), which were 1.0, 1.21, 1.52, 2.12, 2.40, and 2.58 folds higher than that of control (Table 4.31, Fig.4.75A) and at 10 min exposure, the comet tail moment of the sperms treated with 0, 0.001, 0.01, 0.1, 1.0, and 10.0 μg/L of $HgCl_2$ were 25.83±27.80, 53.81±32.60, 87.22±36.79, 150.80±51.50, 172.94±61.86, and 187.08±52.81 (Table 4.30), which were 1.0, 2.08, 3.38, 5.84, 6.70, and 7.24 folds higher than that of control (Table 4.32 and Fig.4.76A). The values of comet tail moment of DNA damage significant differences on the DNA tail moment of haemocyte and sperms exposed to different concentrations of $HgCl_2$ were obtained ($p<0.05$). within 10 min of exposure, there was a small difference in the extent of comet tail moment between sperms and haemocytes treated with 0.001 μg/L $HgCl_2$ and control group, statistically significant ($p<0.05$). It appeared that sperms of mussel responded to Hg quicker that haemocytes.

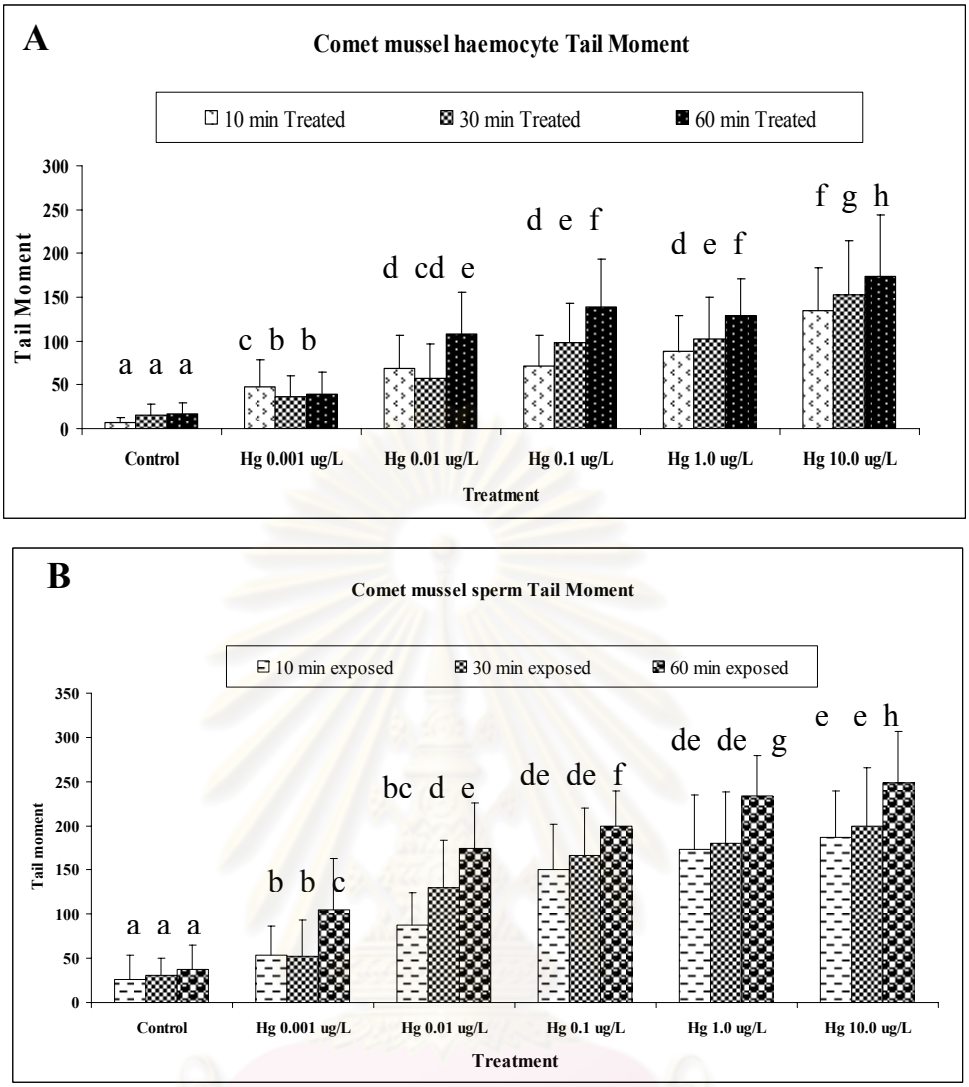


Figure 4.72 Tail moment in mussel haemocyte (A) and sperm (B)

Table 4.29 DNA tail moment (mean ± SD) from haemocyte representing DNA damage after 10, 30, and 60 min of $HgCl_2$ exposure

Exposure Time (min)	$HgCl_2$ concentration (µg/L)					
	0	**0.001**	**0.01**	**0.1**	**1.0**	**10.0**
10	6.77 ± 6.48^{a}	47.96 ± 30.27^{c}	69.27 ± 37.97^{d}	71.68 ± 34.55^{d}	88.36 ± 40.34^{d}	135.12 ± 47.96^{f}
30	15.08 ± 13.09^{a}	37.05 ± 23.34^{b}	57.74 ± 39.25^{cd}	98.83 ± 44.83^{e}	102.29 ± 48.37^{e}	152.63 ± 61.71^{g}
60	16.68 ± 12.68^{a}	38.94 ± 25.08^{b}	108.51 ± 47.58^{e}	139.12 ± 53.99^{f}	128.35 ± 42.30^{f}	174.18 ± 69.85^{h}

Remark**:** The same superscripts indicated that the DNA tail length was not significantly different ($P\geq0.05$) amount group of treatment within the same period of exposure.

Table 4.30 DNA tail moment (mean ± SD) from sperms representing DNA damage after 10, 30, and 60 min of $HgCl_2$ exposure

Exposure Time (min)	$HgCl_2$ concentration (µg/L)					
	0	**0.001**	**0.01**	**0.1**	**1.0**	**10.0**
10	25.83±27.80[a]	53.81±32.60[b]	87.22±36.79[bc]	150.80±51.50[de]	172.94±61.86[de]	187.08±52.81[e]
30	30.65±19.67[a]	52.55±40.57[b]	129.94±54.08[d]	166.39±53.62[de]	180.22±58.25[de]	199.29±65.94[e]
60	37.61±27.41[a]	104.51±58.84[c]	173.93±51.38[e]	199.41±39.61[f]	234.06±45.14[g]	248.42±57.73[h]

Remark**:** The same superscripts indicated that the DNA tail length was not significantly different (P≥0.05) amount group of treatment within the same period of exposure.

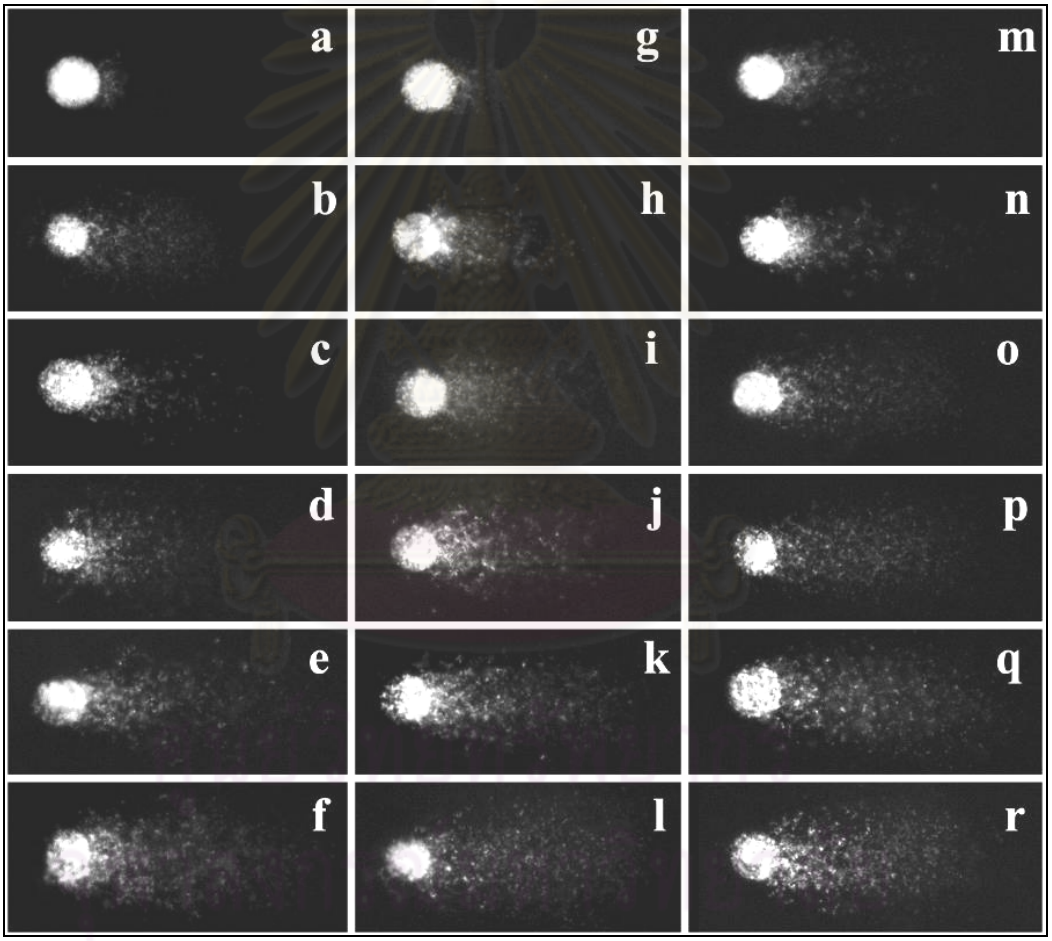


Figure 4.73 Comet result of mussel haemocytes exposed to 0, 0.001, 0.01, 0.1, 1.0, and 10.0 µg/L is show in a, b, c, d, e, and f, respectively, within 10, 30, and 60 min. (a-f = 10 min treated, g-l. = 30 min treated and m-r = 60 min treated)

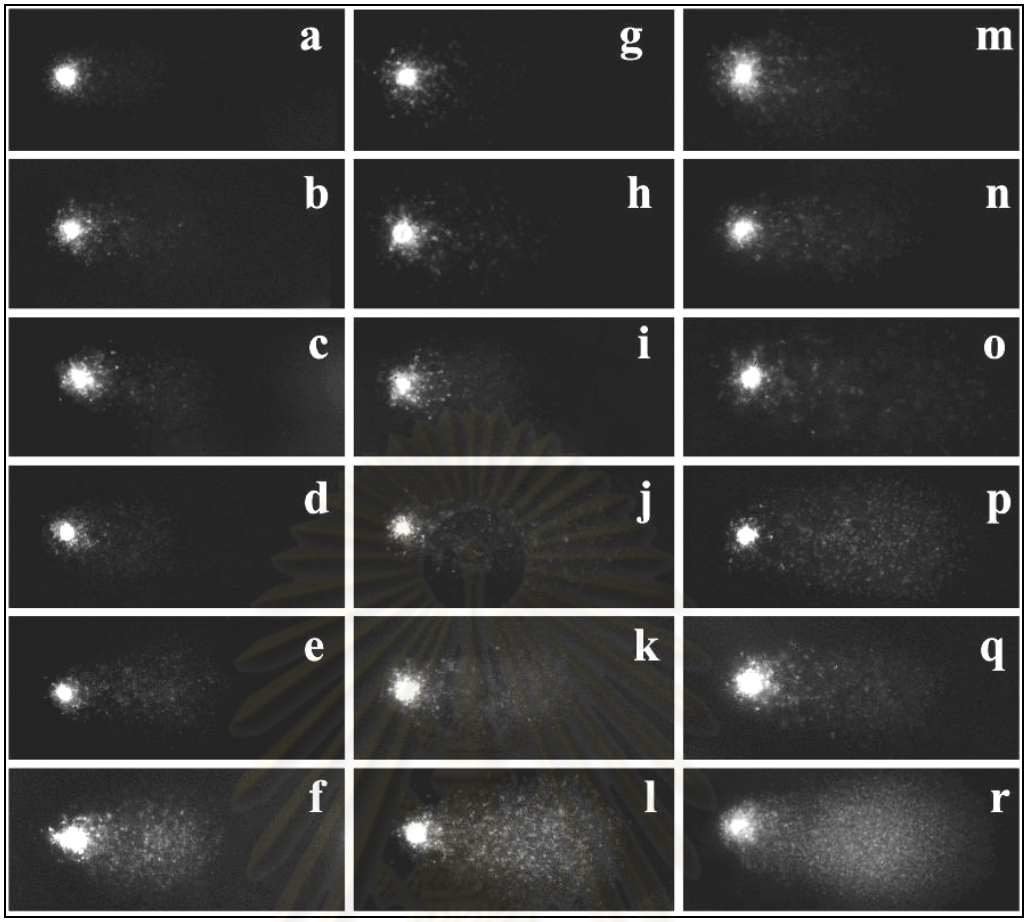


Figure 4.74 Comet result of mussel sperms exposed to 0, 0.001, 0.01, 0.1, 1.0, and 10.0 µg/L is show in a, b, c, d, e, and f, respectively, within 10, 30, and 60 min. (a-f = 10 min treated, g-l = 30 min treated and m-r = 60 min treated)

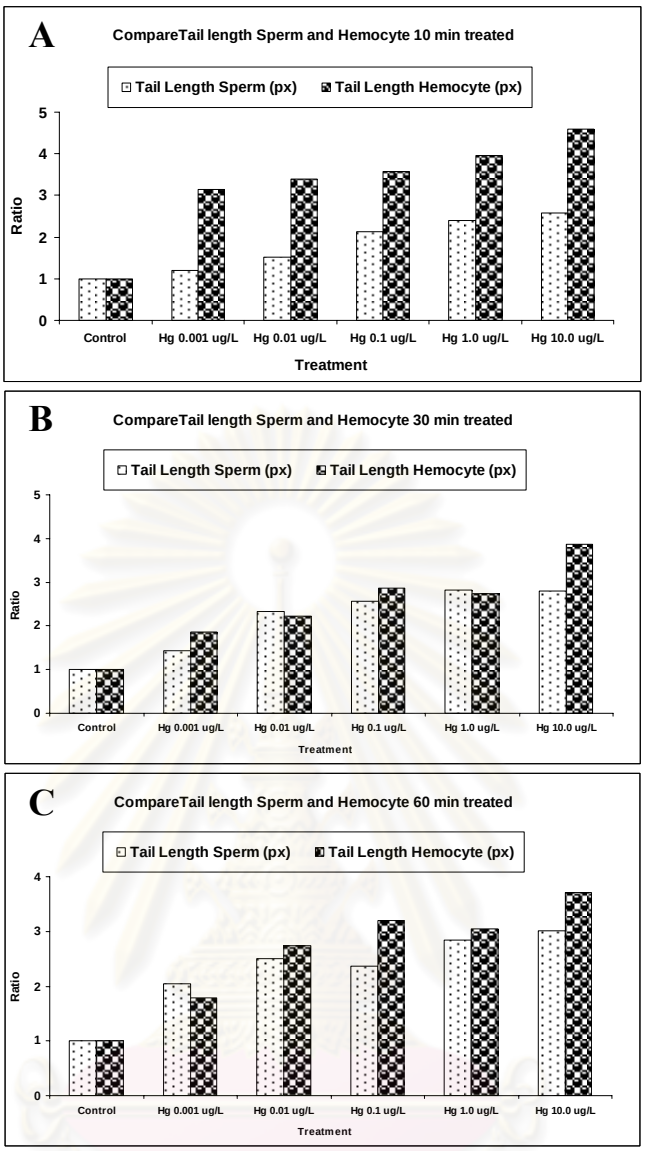


Figure 4.75 Ratio of tail length of mussel haemocyte and sperms compare with control, A=10 min treated, B = 30 min treated, C= 60 min treated.

Table 4.31 Ratio of tail length of mussel haemocyte and sperms compare with control, A=10 min treated, B = 30 min treated, C= 60 min treated.

Exposure Time (min)/target tissues	$HgCl_2$ concentration (µg/L)					
	0	**0.001**	**0.01**	**0.1**	**1.0**	**10.0**
10 sperm	1.00	1.21	1.52	2.12	2.40	2.58
10 haemocyte	1.00	3.15	3.40	3.58	3.97	4.59
30 sperm	1.00	1.44	2.32	2.56	2.83	2.80
30 haemocyte	1.00	1.86	2.23	2.86	2.73	3.87
60 sperm	1.00	2.05	2.50	2.36	2.84	3.01
60 haemocyte	1.00	1.78	2.75	3.20	3.05	3.71

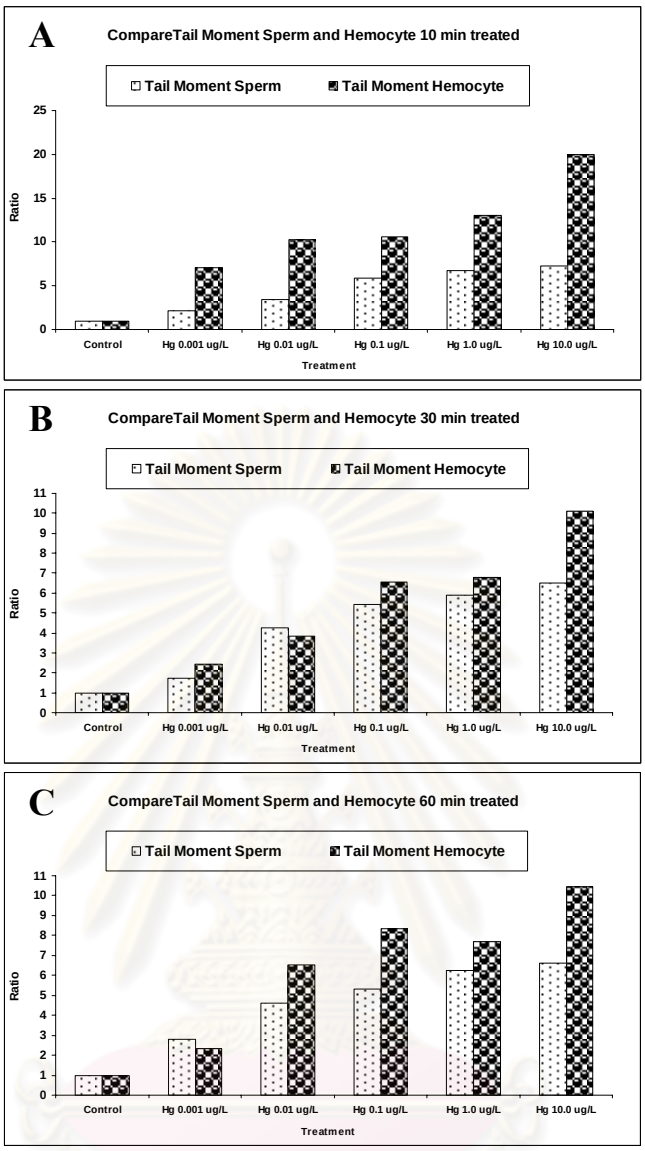


Figure 4.76 Ratio of tail moment of mussel haemocyte and sperms compare with control, A=10 min treated, B = 30 min treated, C= 60 min treated.

Table 4.32 Ratio of tail moment of mussel haemocyte and sperms compare with control, A=10 min treated, B = 30 min treated, C= 60 min treated.

Exposure Time (min)/target tissues	$HgCl_2$ concentration (µg/L)					
	0	**0.001**	**0.01**	**0.1**	**1.0**	**10.0**
10 sperm	1.0	2.08	3.38	5.84	6.70	7.24
10 haemocyte	1.0	7.08	10.23	10.59	13.05	19.96
30 sperm	1.0	1.71	4.24	5.43	5.88	6.50
30 haemocyte	1.0	2.46	3.83	6.55	6.78	10.12
60 sperm	1.0	2.78	4.62	5.30	6.22	6.61
60 haemocyte	1.0	2.33	6.51	8.34	7.70	10.44

CHAPTER V

DISCUSSION

5.1 Use of mussel as model animal for Hg bio-monitoring

Various metal-accumulating biomaterials, such as plants (AI- Shayeb et al., 1995), non-parasite organisms (lichens, mosses, algae) (Antonelli et al., 2001; Conti and Cecchetti, 2003), and animal tissues and organs (feathers, livers, kidneys, bones) (Catsiki and Strogyloudi, 1999; Dauwe et al., 2006) have been used as environmental bioindicators because they are those with low-cost, ease of sampling, and showing a good correlation with environmental quality change of ecosystems. Mollusks, especially mussels, were found promising for monitoring the change of heavy metal contamination in aquatic systems (Claisse et al., 2001; Astudillo et al., 2002; Nicholson, 2003). However, using living organisms often times can be limited because they are not always a natural component of the ecosystem and sometimes appear there spontaneously. In this study, green or green lipped mussel, *Perna viridis*, were used as bioindicator for monitoring Hg in petroleum production platform by transplanting into the sites to be monitored because this mussel is a common sessile animal and widely distributed in the Gulf of Thailand.

During 3 months of mussel transplantation at petroleum production platforms, increasing mortality was detected. However, no significant difference between survival rates of the mussels was found between different water depths, and stations. There was no significant difference between the growth rates of transplanted mussels from different petroleum platform stations. However, significant different growth rate can be detected between mussels when compared to those from reference site. This was probably due to the less abundance of food. This was confirmed by the result of plankton composition between stations where the amounts of diatoms from reference site which was the natural habitat of mussel were much higher than any of the test stations. These results indicated that mussels could be transplanted and survived in un-natural habitat such as petroleum production platforms in the middle of the Gulf of Thailand where food was much less abundant for up to 3 months without significant physical change.

5.2 Mercury concentration in water and mussel

Hg monitoring programs have been carried out globally (Amiard et al., 2000). Advances in analytical techniques over the last decade have allowed extremely accurate determinations of Hg and Hg species. As the result, determination of Hg level in various sources is still the most important part for facilitating the monitoring strategy in the activities involving Hg.

In this study, mussels were exposed to very low levels of inorganic Hg (between 0.1 to 1.0 µg/L) under controlled laboratory condition. The levels of Hg in rearing water decreased rapidly. Less than 0.1% (between 0.03 to 0.05%) of total Hg was detected after 24 h of application. This result coincides with most experiments (Sanchez et al., 1998) since inorganic Hg has been known to be changed quickly in aquatic environment and mostly transformed into organic Hg by living organisms (Kannan et al., 1998). This indicated that the amount of Hg measured from the water only represent very small amount of Hg that actually released into water.

Microorganisms in sediments produce most of organic Hg as methylated forms which are then concentrated in aquatic food chain. Predatory organisms at the top of the food chain can accumulate MeHg in their diets and present elevated concentrations. While the concentration at the bottom of the aquatic food chain may be at the low parts per trillion levels, at the top, fish tissue can present Hg concentrations in excess of 1 ppm. Bioconcentration factors can be on the order of 10 thousand to100 thousand times (Fowler et al., 1978; Phillips and Buhler, 1978; Thompson et al., 1990; Yamada et al., 2003).

Mercuric chloride was used as source of Hg in this study because it was one form of inorganic Hg which was proved to be the dominant toxic species in water. Although MeHg is the most toxic form of Hg, its concentration was found to be less in water and tends to accumulate in organisms and sediment (Sayler et al., 1975; Pentreath, 1976; Barkay et al., 1997). This is also the case in this study that the increasing level of Hg in mussel tissue coincide with the increasing level of Hg applied to the test tank and the tissue Hg levels were thousand folds higher than that of water (Table 5.1 and 5.2)

Table 5.1 Average Hg concentration in experiment water (mussel tank) during 8 weeks of experiment

Mercury Concentration (µg/l)				
Control	**Tank 2**	**Tank 3**	**Tank 4**	**Tank 5**
0.0028±0.0017	0.0050±0.0025	0.0109±0.0073	0.0180±0.0122	0.0401±0.0371

Table 5.2 Average Hg concentration in experiment mussel during 8 weeks of experiment

Mercury Concentration (µg/kg)				
Control	**Tank 2**	**Tank 3**	**Tank 4**	**Tank 5**
57.9±24.1	88.5±50.0	137.8±50.5	164.4±50.0	138.3±80.3

Concentrations of Hg in coastal water and estuaries generally are much higher than those in the open ocean. Concentration in relatively uncontaminated coastal and estuarine water may be as high as 19 ng/L dissolved Hg. in British estuaries (Law et al., 1994) (Table 5.3). Hg concentration as high as 350 ng/L were reported in the Derwent Estuary, Tasmania (Plaschke et al., 1997). In the Gulf of Thailand, the levels of Hg were between 0.31 to 4.54 ng/L (Table 5.3) (DMF, 2008) which were in the same level as those found in the Offshore Great Britain, and English channal this level of Hg is quite low in the safe level less than standard (0.1 µg/L) (PCD, 1997).

Table 5.3 Concentrations of dissolved total Hg in oceanic and coastal waters of the world based on recent determinations. Concentrations are ng/L

Location	Total Mercury (ng/L)	Reference
Darwent Estuary, Tasmania	350.0	Plaschke et al., 1997
Dogger Bank, North Sea	0.19-0.12	Fileman et al., 1991
North Sea, Offshore	0.34	Coquery & Cossa, 1995
North Sea, Nearshore	0.72	Coquery & Cossa, 1995
Offshore Great Britain	<0.2-6.7	Law et al., 1994.
English Channal	0.19-4.1	Cossa & Fileman, 1991
Straits of Dover	0.12-1.3	Cossa & Fileman, 1991
British Estuaries	0.35-19.0	Law et al., 1994.
Lapdev Sea, N. Russia	0.80-2.7	Coquery et al., 1995
Kara Sea, N. Russia	0.14-3.4	Coquery et al., 1995
North Atlantic Surface water	0.31	Mason et al., 1995
Patuxent River Estuary, MD	0.04-0.30	Bernoit et al., 1998
North Atlantic Ocean	0.10-0.50	Guentzel et al., 1996
Mediterranean Sea 1-5000 m	0.16-1.28	Cossa et al., 1997
South Florida Estuarine	3.0-7.4	Kannan et al., 1998
Gulf of Thailand Offshore	0.31-4.54	DMF, 2008

In field study, Hg concentration in the water around platform was monitored every 3 or 4 years by the Department of Mineral Fuel, Ministry of Energy. The standard value for the offshore seawater regulation is less than 0.1 (μg/l) (PCD, 1997). The average Hg concentrations of water detected at Station A, B, C, and D (reference site) were 3.51-4.54, 1.25-2.00, 0.44, and 0.31 ng/l, respectively (Table 5.4). Hg concentration at every platform was lower than the standard limit values of the offshore seawater regulation.

Table 5.4 Mercury concentrations in the Gulf of Thailand at Station A, B, C and D (reference site) (DMF, 2008)

Year	Station	Platform	Distances form Platform	Hg-Total (ng/l)
2004	Station A	Average	surface	4.54
		Average	bottom	3.51
2004	Station B	Average	surface	2.00
		Average	bottom	1.25
2003	Station C	Average	surface	0.44
		Average	bottom	NA
2003	Reference site	Average	surface	0.31
		Average	bottom	NA

Remark: NA = data is not available.

Concentration of Hg in the whole soft tissues of mussel of marine organisms from throughout the world generally falls in the range of 0.003 to 264 μg/g dry weight (Table 5.5). The average level of Hg in the mussel before treatment was 0.0104±0.0091 μg/g while the level from the highest Hg treatment for 8 weeks was 0.1383±0.0803 μg /g which were approximately 10 times higher than that of initial mussels. At petroleum platforms, Hg concentrations in mussel tissues were in the range between 0.0100 to 0.1725 μg/g. The average level was slightly lower than that of laboratory study (0.0100 to 0.3644 μg/g.). Although, the amount of Hg applied to laboratory test mussels was many folds higher than Hg levels in the field, the Hg levels obtained from laboratory appeared to be slightly higher than that of field study. However, both results were still in the same range of Hg found in mussels studied by Neff (2002) (0.004-11.7 μg /g. dry wt.) as shown in table 5.5 In previous study, the levels of Hg detected from tested mussels reached between 1.3200 to 2.2900 μg /g after exposing to 1-5 μg /L of Hg for 4 weeks (Parsont, 2003). This level was more than 10 times higher than the result of this study, indicating that Hg accumulated in

mussel tissue could be very high if the mussel were treated with very high level of Hg.

The average level of Hg in tissue of mussels transplanted to petroleum platforms for 3 months was 0.0413 µg/g. This value was much lower than that of mussel located at Map Ta Phut areas (0.175µg/g) where industrial activities were heavily operated (PCD, 2010). Minimal risk level (MRL) which has been set for Hg exposure in human recommends that people can ingest Hg between 0.002 to 0.007 mg/kg/day without appreciable risk of adverse non-cancer health effect (ATSDR, 2010). According to this MRL guideline, average people (weight at 60 kg) should not consume Hg more than 0.42 mg/day which can be roughly calculated as 44 kg of transplanted mussels and 10 kg of mussels from Map Ta Phut areas per day. This calculation reveals the degree of possible Hg risk to human consuming mussels from the lowest and highest level of Hg found in the Gulf of Thailand.

Table 5.5 Range of Hg concentrations in mussel or whole soft tissues of marine organism from throughout the world (Neff, 2002). Concentrations are µg/g dry weight

Taxon	No. Analyses	Hg Conc. µg/g	Remark
All	858	0.003-264	Neff, 2002
Macroalgae	15	0.1-46.8	Neff, 2002
Polychaetes	16	0.085-7.3	Neff, 2002
Snails	38	0.025-3.7	Neff, 2002
Mussels	60	0.004-11.7	Neff, 2002
Oysters	74	0.003-8.0	Neff, 2002
Scallops	5	0.05-0.35	Neff, 2002
Clams	32	0.005-85	Neff, 2002
Cephalopods	18	0.013-8.2	Neff, 2002
Shrimp	27	0.02-6.2	Neff, 2002
Lobsters	14	0.05-12.6	Neff, 2002
Crabs	20	0.015-2.3	Neff, 2002
Echinoderms	5	0.031-1.4	Neff, 2002
Sharks	57	0.035-52.5	Neff, 2002
Fish	379	0.01-115	Neff, 2002
Sea turtles	8	0.04-1.78	Neff, 2002
Marine Birds	24	0.15-25.0	Neff, 2002
Marine Mammals	27	0.005-264	Neff, 2002
Mussel (*P. viridis*)	87	0.04-0.69	This study (Field)
Mussel (*P. viridis*)	108	0.04-1.46	This study (Lab)

Remark: from this study conversation factor change wet weight to dry weight is multiply by 4.0

5.3 Bioaccumulation of mercury in tested mussel

The average level of Hg in the acclimated mussels before treatment was 0.0104±0.0091 μg/g while the level from the highest Hg treatment for 8 weeks was 0.1383±0.0803 μg/g. The Hg level rose approximately 10 times higher than that of initial mussels. The accumulation of Hg in tested mussels can be roughly determined by calculating the amount of Hg applied to the rearing tank and that detected from mussel tissues. The result revealed that mussel in experiment tanks accumulated 48.89%, 27.56%, 14.52%, and 4.51% of Hg in tank 2-5, respectively.

The accumulation efficiencies of Hg vary greatly between organisms, especially in marine mollusks (Neff, 2002). The assimilation efficiency of Hg from food of Mussel *Mytilus edulis* was reported to be 1-9 % for inorganic Hg and 30-87% for MeHg (Gagnon & Fisher, 1997). In this study, the maximum accumulation of Hg (48.89 %) was found in mussels exposed to the lowest level of Hg (100 ng/L) (Table 5.6). The levels of accumulation seemed to be reduced when the level of Hg applied to the mussels was increased. This un-expected result was probably due to excess level of Hg applied to the mussels. Also, nitrifying bacteria from bioreactors and algae might play some parts in absorbing Hg from the treated water. It was quite interesting to note that detectable amount of Hg was obtained from control treatment (both tissue and water) where Hg was not applied to the tank (Table 5.6). The amount of Hg detected in control mussel tissues and water were in the same level as those detected from reference site in field study, indicating that the Hg detected in control samples were the background concentration of Hg normally found in mussels from natural habitat.

Table 5.6 Summary for mass balance of Hg in mussel water tank after 8 weeks of experiment (Laboratory study)

Mussel tank	Hg conc. Exposed to mussel	Hg in water tank (%)								
		Hg added		Hg accumulated in mussel tissue		Hg remained in water		Hg loss		Remark
		μg	%	μg	%	μg	%	μg	%	
Tank 1	0	0	-	75.31	-	15.41	-	-	-	Control
Tank 2	100 ng/L	304.00	100	148.64	48.89	27.60	9.08	127.77	42.03	
Tank 3	200 ng/L	608.00	100	167.59	27.56	60.81	10.00	379.60	62.43	
Tank 4	500 ng/L	1,520.00	100	220.71	14.52	108.22	7.12	1,191.07	78.36	
Tank 5	1,000 ng/L	3,040.00	100	137.10	4.50	224.74	7.40	2,678.16	88.09	

Hg in inorganic or (such as $HgCl_2$) can accumulate through plankton because phytoplanktons can bioaccumulate inorganic Hg at the cell membranes of plant in a relative non-bioavailable forms. Also, an organic form or MeHg can accumulate through methylation of inorganic Hg by sediment bacteria (Mason et al., 1995). Lobsters, *Nephrops norvegica*, accumulated inorganic and organic Hg from both water and food. Hg, particularly inorganic forms and taken up from the water, accumulated preferentially in gills while Hg, accumulated from food, concentrated in hepatopancreas (Canli and Furness, 1995). All organic Hg (MeHg) is associated with soft tissues, whereas 15 % of the inorganic Hg is bound to the exoskeleton (study on copepod) (Lawson and Mason, 1998).

5.4 Expression analysis of Hg responding genes in mussels exposed to very low level of Hg

Hg is a trace component of all fossil fuels including natural gas, gas condensates, crude oil, and coal. The production processes of these fuels provide the main opportunity for emissions of Hg to the environment. Speciation techniques for Hg compounds in water have evolved along with the development of the very sensitive detectors. Hg and its compounds can now be measured in aqueous media at below parts per trillion (ng/L) levels. But the use of these advanced techniques is still limited due to the requirement of sophisticated equipments and special operators. Therefore, this study focuses on the feasibility and validity of using candidate genes, which were earlier reported as Hg responsive genes, as biomarkers of Hg contamination in the surrounding areas of petroleum production platforms in the Gulf of Thailand.

Bioassay was established using semi-quantitative RT-PCR for quantitative evaluation of the transcripts of the target genes which included MT and its variants, HSP71, and CYP4 of mussel. The assay was initially conducted on the mussels exposed to 0 to 1.0 μg/L of Hg in laboratory controlled condition. Consequently after an appropriate condition for each gene was obtained, field validation was carried out by measuring the expression of candidate genes from mussels transplanted to the petroleum production platforms

5.4.1 Laboratory study

Twelve forms MT of *P.viridis* were previously identified. They were divided into two main subforms, defined as mop1 and mop2 that contained 6 isoforms, similar to mop1 and mop2 of mollusk metallothioneins (Figure 5.1).

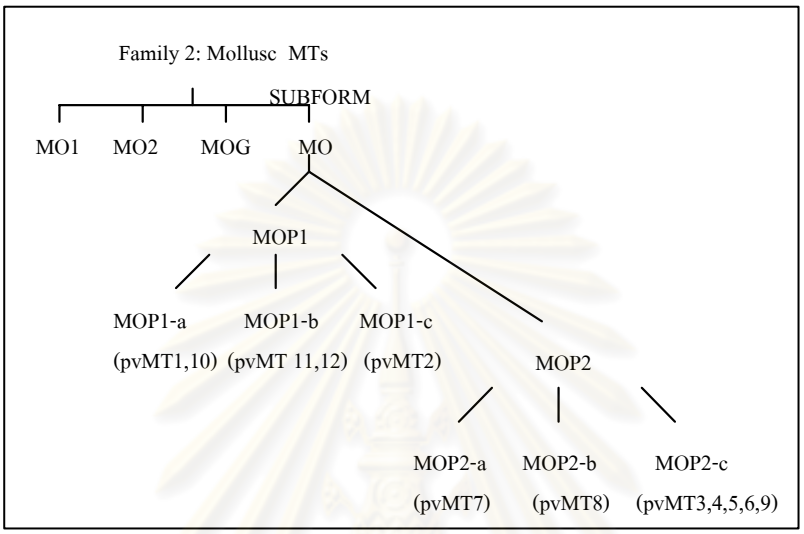


Figure 5.1 Diagram of classification of variant metallothionein gene from gill and digestive tract. The members of isoform mop1a are pvMT1 and pvMT10. The members of isoform mop1b are pvMT11 and pvMT12. The member of isoform mop1c is pvMT2. The member of isoform mop2a is pvMT7. The member of isoform mop2b is pvMT8. The members of isoform mop2c are pvMT3, pvMT4, pvMT5, pvMT6, and pvMT9 (Parsont, 2003).

Six variants pvMTs (pv-MT01, pv-MT02, pv-MT03, pv-MT07, pv-MT08, and pvMT11) out of 12 pvMTs were selected for primer design due to limited regions for possible specific primer production.

Expression levels of 6 subunits and total MT gene were analyzed on the gills of mussels exposed to various levels of Hg (0-1.0 μg/L). The result revealed that only the expression of pvMT07 responded and correlated significantly to the amount of Hg at very low levels (lower than 0.2 μg/L) within the first week of experiment while total MT, which was previously responsive to Hg at the concentrations of 1-5 μg/L (Parsont, 2003), did not show significant difference among mussels from all

treatments. The result indicated that pvMT07 is the most sensitive form among tested pvMT variants when mussels were exposed to Hg at the level lower than 1 μg/L.

Metal-specific forms of MT have been reported in a number of organisms (Roesijadi, 1992). Most mammals tissues examined by far contained two major MT isoforms, designated as MT-I and MT-II (Kojima and Kagi, 1978; Kagi, 1993). In mouse, 4 isoforms of MT (MTI, MTII, MTIII, and MTIV) were found and studied on the effect of zinc and cadmium. The result showed that only MTI isoform was specific to zinc (Andrew, 2000). So far, a few DNA sequences of molluscan MTs have been characterized. These include MTs in the mussels, *Mytilus edulis* (Lemoine et al., 2000; Soazig and Marc 2003), *Perna viridis* (Khoo and Patel, 1999) and the oyster *Crassostrea virginica* (Roesijadi, 1992; Unger and Rosejadi, 1996). To date, the highest number of isoforms found in aquatic species was in mussel, *M. edulis* MT (at least nine Cd-induced isoforms). Evidences also indicated that some forms were more specific to curtain metal than the others (Rigaa et al., 1998).

Buouwer (2002) studied 3 isoforms of MT gene in blue crab, *Callinectes sppidus*, (MTI, MTII and MTIII). His result revealed that MTI was induced by cadmium, zinc and copper; MT-II was induced by cadmium and zinc, and MTIII was induced by copper only. The data also showed that one gene could be specific to a few contaminants. Two isoform of MT, (MT10 and MT20) were found in *Mytilus edulis*. The result showed that MT20 was more specific to cadmium than MT10 (Soazig, 2003). In this study, only one isoform of MT (pvMT07) was found to be more specific to very low level of Hg (<0.1 μg/g Hg in tissue) than the others. No Hg related gene ever report on the response of Hg at this very low level.

For the expression analysis of other Hg-related genes, a few attempts to clone HSP70, HSP90, and CYPA1 genes have been conducted using degenerate primers designed from conserved regions of those genes from closest species reported in GenBank database. None of them were successfully obtained. Therefore, reported genes including HSP71 and CYP4 of mussel were obtained and used for designing specific primers for quantitative evaluation analysis. The result showed that the expressions of HSP71 and CYP4 genes were not significantly different in mussels exposed to Hg level lower than 1 μg/L.

Usually, these 2 genes are proved to be sensitive to many toxicants including Hg (Shaw, 2002; Micovic, 2009). They did not show any dose-related responses to Hg in this study was possible because of 2 reasons; one was the doses of Hg used in this study was much lower than the threshold of these genes or the forms of these genes used in this study were not specific to Hg.

Study on HSP70 and HSC70 of Mediterranean mussel, *Mytilus galloprovincialis*, exposed to Hg^{2+} at 150 µg/L revealed that expression of HSP70 was induced and reached maximum levels after 24 h of exposure while HSC70 level was inhibited after 1 day and induced after 6 days of exposure (Franzellitti, and Fabbri, 2005).

Members of the Cytochrome P450 (CYP) family are key detoxification enzymes which metabolize many chemicals such as plant metabolites and pharmaceutical contaminants (Nebert et al., 1989). The CYP isozymes superfamily consists of over 800 genes (Nelson, 2010). CYP enzymes are potentially induced in response to specific environmental xenobiotics (Gonzalez, 1998). Among them, CYP1A isoform, the most frequent used biomarkers, was reported to be induced by PAHs, PCBs, and dioxin. CYP1a1 mRNA levels in Murine hepatoma Hepa 1c1c7 cells were found to be increased when exposed to 5 µM Hg^{2+} (Korashy and El-Kadi , 2005; Bozcaarmutlu and Arinc, 2007). CYP4 isoform was known to be induced by phthalate ester plasticizers, and chlorinated aryl phenoxy herbicides (Stein et al., 1998). HSP72 has also been induced by Hg (II) in NRK-52E cells significantly after 24 h. of treatment of 40 µM $HgCl_2$ (Stacchiotti, 2009) Duffy et al., (1999) study on HSP70 and HSP60 from 31 gills of Alaska fish. The expression level of HSP70 significantly correlation with Hg level higher than 1.0 µg/g. but no statistical relationship between increased levels of HSP60 in gills and increased Hg levels. Bozcaarmutlu and Arinc (2007) study the effect of Hg on CYP in leapin mullet (*Liza saliens*) the result shown 50 mM Hg concentration inhibited the Cytochrome P450 reductase activity completely (100%).

5.4.2 Field validation

Optimized condition of semi-quantitative RT-PCR for each genes used with tested mussels in laboratory study were carried out with samples collected from field study sites. Quantitative evaluation revealed similar result obtaining from laboratory study where only the expression of pvMT07 correlated with the Hg level in tissue of mussels.

Dose-related expressions of MTs and other Hg related genes are generally induced by sublethal and lethal doses of various metals including Hg. They have been successfully used as biomarkers in various numbers of environmental monitoring programs (Rigaa et al., 1998). An increasing number of investigations on the environmental impact of heavy metal using the induction of MT gene as indicator have been applied to the real world conditions. Use of MT gene expression as biomarker for determining the environmental impact of heavy metals has increasingly been applied to the real world condition. For example, MT expressions were analyzed in springtail, *Orchesella cincta*, at cadmium contaminated area and reference site in Netherlands. The study also included MT gene that involved cadmium tolerance Results showed that the mean constitutive MT mRNA expression of populations from polluted sites was significantly higher than of populations from reference sites (Astudillo, et al., 2002).

For the environmental impact of Hg, the assessment is more complicated due to the lethal dose of Hg is much lower than most heavy metals (Zoll, 1988). Investigation on chronic effect of Hg in various species of diverse ecosystem has become even harder since the chronic dose is many folds lower than the acute concentration and in many cases, chronic levels of Hg are in proximity to the levels currently considered safe (Hontela, 1996; Vezer, 2005). In this study, pvMT07, one subunit of *P.viridis*, has been proved to be sensitive to the induction of Hg at very low level (< 1.0 μ/L). Furthermore, its feasibility for determining the exposure of Hg in mussels has been confirmed in both laboratory and field studies. The results from the investigations on other stress inducible genes such as HSP71 and CYP4 in *P.viridis* revealed no significant change in their expression levels after exposing to low levels of Hg in the field, indicating that these genes were not sensitive enough to detect the exposure of Hg at the levels and times tested in this study. Further investigation on

these 2 genes in mussels exposed to higher concentrations of Hg will clarify their sensitivity and availability as biomarker of exposure for the higher doses of Hg. Generally, these 2 genes belong to heat shock proteins and cytochrome P450 families which are recognized as stress responsive genes in various organisms (Campbell, 1996). In invertebrates, CYP4 are mainly reported and the expression level of its genes has become one of the potential biomarkers for determining chemical contaminants in marine environment (Simpson, 1997; Snyder, 1998; Chaty, 2004). CYP1A is usually found in most vertebrates and commonly used as biomarker of exposure for many substances including heavy metals (Goksoyr, 1992; Ueng, 1996 Shaw, 2002). For example, cytochrome P450 expression and hypertension of human was investigated on the possible link between non-workplace cadmium (Cd) exposure. The results indicated that the relationships between liver and kidney Cd burdens and the abundance of the CYP isoform 4A11 were shown (Baker, 2003).

5.5 Genotoxicity of Hg on haemocytes and sperms of mussels

Scoring of comets can be conducted in several ways including the percentage of DNA in the comet tail, the length of the tail, and DNA tail moment (product of the fraction of DNA in the tail and tail length). Tail moment is considered to be one of the best indices of induced DNA damage among the various parameters (De Boeck et al., 2000). For the evaluation of DNA damage in this study, 2 parameters were monitored; tail length and tail moment. Significant results with regard to comet tail length and tail moment were observed with Hg exposure over a range of concentrations from 0.001 to 10.0 μg/L at different interval times (10, 30, and 60 min) in comparison with hydrogen peroxide treatment. Similar results were obtained from both parameters. Increases were found when measuring Comet tail length, but the greatest changes were in tail moment which showed that the extent of DNA damage was proportional to the concentration of Hg. The result indicated that the lowest level of Hg was still highly toxic to both somatic and germ cells of mussels.

Sperms appeared to be more sensitive to Hg exposure than haemocytes. In mammals, effects of Hg on reproductive development are well documented. Studies of occupational exposure indicate that exposure to elemental Hg may affect human reproduction. Possible effects are increased spontaneous abortions, congenital anomalies, and reduced fertility among women (Khan, 1987). In aquatic invertebrates,

their susceptibility varies greatly to Hg. Generally, larval stages are more sensitive than adults (WHO, 1989).

Hg is known to induce genetic damage in vertebrates (Ben-Ozer et al., 2000; Ruiz et al., 2008). In hamster, ovary cells exposed to 75 μM of Hg for 60 min created DNA damage (Orazio, 1984). Hg is well studied in terms of its bioavailability, bioaccumulation, biomagnification, and cellular toxicity, especially in bivalves (G´eret et al., 2002). Nevertheless, despite its high toxicity and genotoxicity (in vertebrates), only few studies reported that Hg was shown to be genotoxic to invertebrates. One example was found in a mollusk, *Mytilus galloprovincialis* (Bolognesi et al., 2004). It was found that exposure of *M. galloprovincialis* to 32 μg/L of $HgCl_2$ for 5 days caused a significant increase in the frequency of micronuclei in both gill cells and haemocytes. In *M. edulis*, single strand breaks could be detected using the Comet assay in haemocytes exposed to $HgCl_2$ at the concentration of 20 μg/L for one day (Tran et al., 2007) (Table 5.7). In this study, DNA damage was detectable in both haemocytes and sperms of mussels after exposing in vitro to $HgCl_2$ at the concentration of 0.001 μg/L for only 10 min. This indicates that mussel cells are more sensitive to Hg than other mollusks. In higher Hg concentration leading to more DNA in comet tails, similar result was obtained from the study on haemocyte of bivalve mollusk, *Scrobicularia plana* (Petridis, 2009).

Table 5.7 Assessment of DNA damage by comet assays after *in vitro* exposure of aquatic animal cells to genotoxicants.

Animal	Tissue/cell	Chemical treated	Assessment method	Response ± (D-R;I)	Reference
Mussel (*M,edulis*)	Gills, haemocytes Digestive gland, sperm	H_2O_2 ,NDMA MX, BP, NP, Cu, NF, araC, MNNG	Empirical Score Percentage of DNA in tail Tail length	D-R; I	Steinert, 1996 Wilson, 1998 Michelmore, 1998a Micchelmore, 1998b
Mussel (*M,edulis*)	Haemocyte	Hg, Se	% DNA in tail	D-R; I	Tran, 2007
Mud welk (*N. tegula*)	Haemocyte	H_2O_2, Cu	Tail length	I	Sastre, 1997.
Ribbed mussel (*M. senhousia*)	Haemocyte	H_2O_2	Tail length	I	Sastre, 1997.
Flounder (*P. americanus*)	Blood	H_2O_2, MNNG	Tail length	D-R; I	Cotelle, 1999. Nacci, 1996.
Bivalve mollusk (*S. plana*)	Haemocyte	H_2O_2,(xeno-) estrogens	Tail length, Tail moment	D-R; I	Petridis, 2009.
Brown trout (*Salmo trtta*)	Hepatocyte	MNNG	Tail length	I	Micchelmore, 1998b
Mussel (*P. viridis*)	Haemocyte, sperm	H_2O_2, $HgCl_2$	Tail length, Tail moment	D-R; I	This study

Remark: ± Dose-response (D-R) curves and /or significant increase above control (I) MNNG = n-methyl-nitrosoguanidine, BP = benzo(a)pyrene, MX = 3-chloro-4-

(dichloromethyl)-5-hydroxy-2[5H]-furanone, NP =1-nitropyrene, NF = nitrofurantoin, araC = cytosine-β-D-arabinofuranoside, NDMA = N-nitrosodimethylamine.

In this study, the ratio of tail length of mussel haemocyte and sperms in comparison with control showed significant increase when the levels of Hg increase from 1.5 to 4.5 times higher than control. The same result was found in haemocyte of flounder treated with H_2O_2 in low dose (5μM of H_2O_2). Average tail length was approximately 2.2 times higher than control. In high dose (500μM H_2O_2), average tail length increased 11-17 times higher than control (Nacci, 1996). The effect of $HgCl_2$ at 20 μg/L was reported to cause DNA damage in *M. edulis* (Tran et al., 2007).

The DNA must be considered a target site of its toxic action. If induction of DNA lesions and active repair of these lesions are important for mutagenicity of carcinogenicity of a chemical agent, then Hg may be expected to have mutagenic activity. Additionally, the DNA lesions induced by $HgCl_2$ may result in miscoding during DNA replication. However, $HgCl_2$ has been shown to inhibit cell growth specifically in S phase (Costa, 1983) and therefore miscoding during DNA replication must occur at concentrations of $HgCl_2$ that allow this process to proceed in order to achieve a mutagenic response in a surviving cell. These mechanistic findings may help explain the low mutagenic/carcinogenic activity displayed by $HgCl_2$ in a number of experimental systems (Leonard, 1983)

The DNA damage results revealed a great deal of information about the various ways mussels respond to different contaminants and suggest a pathway of toxicity that has not been considered in previous studies. When compared to germ cell DNA damage, somatic cells appear to have a high capacity to repair damage than germ cell (Steinert, 1998). The ability to distinguish between somatic and germ cell DNA damage is one of the most informative aspects of the Comet assay. The organism capacity to cope with contaminant exposure can be compared to overall sustained damage levels. In addition, germ cell damage shows a more rapid response and in the future may represent a rapid measurement.

5.6 Applications and future prospects

Apart form the natural emission, the primary sources of Hg released into the Gulf of Thailand are the discharge of offshore petroleum operation and anthropogenic

activities along the coastline. There were an increasing number of platforms for oil and gas exploration and production. It was reported that 21 oil rigs were operating in the middle of the Gulf by the year 2002 (DMF, 2008). This led to the increasing amount of Hg released into the Gulf.

Pollution Control Department (PCD) has performed comprehensive monitoring program for determining Hg contamination in water, sediment, and marine organisms in the vulnerable areas surrounding the oil and gas processing platforms. The result in 1995 showed high Hg concentrations in seawater around the area and it was calculated in 1997 that Hg released into water was between 40-300 kg/year or loading into water from 20-740 μg/L (Pornsook et al, 2010). In 1998, Hg level in tissue samples ranged from 0.023 to 1.57 μg/g dry weight, while in 2001, the level of Hg in sea water was 0.0008 μg/L and tissue samples were between 0.001 to 0.51 μg/g (Pornsook et al, 2010). Until now, the results have shown that Hg in water and animal tissues are decreasing over time and the ranges of Hg levels measured were still lower than the standard limit (standard allowable values are 0.1 μg/L in sea water and 0.5 μg/g (wet weight) or 1.25 μg/g (dry weight) in animal tissues) (PCDa, 2010). On the other hand, the levels of Hg in sediment from different platforms varied the value at one platform to be between 0.015 and 0.02 μg/g (sediment) and the other location was between 0.02-5.01 μg/g (sediment) (Pornsook et al, 2010). In addition, Hg levels in the sediment collected from central production platform were higher than sediment from the distance radius points. The results reveal a trend of decreasing Hg levels in sediments with increasing distance from the platform. This indicates that Hg can accumulate further in sediment and if the discharge continues, perhaps finally Hg can go beyond the prescribed standard while Hg levels in water and animal tissues are still below standard (1μg/g sediment dry weight) (PCDa, 2010). It is well aware that inorganic Hg can transform quickly into organic Hg and accumulate in sediment and microorganisms. Therefore, monitoring Hg level in the water cannot adequately indicate the activity of Hg in aquatic environment.

Another major source of Hg is the anthropogenic activities in the areas of industrial ports and estates where heavy industries are operated in the vicinity to the coastline of the inner Gulf. Map Ta Phut and Laem Chabang areas, located at eastern part of Thailand, are the classic examples of industrial estates that are confronting with pollution caused by industrial activities. Map Ta Phut also has a high-capacity

industrial port to serve heavy industries with a wide range of public utilities and infrastructure services. It is presently the biggest industrial port in Thailand, located in a strategic location suitable for all types of industries. PCD has conducted pollution monitoring program using samples collected from surrounding areas and the results generally indicated that the levels of most pollutants, especially heavy metals, were lower than the standard limit (PCDb, 2010) while the data of National Cancer Institute of Thailand indicated that during 1997-2001, all types of cancer are increasingly found in Map Ta Phut areas, 3-5 folds higher than the people in the other areas (Jadsri, 2006; Sangrajang, 2008; Thai Post, 2008). A number of people having symptoms relating to respiratory tract also increased significantly (Bureau of Occupational and Environmental Diseases, Department of Disease Control Ministry of Public Health, Thailand, 2010). These contradictory evidences clearly showed that parameters routinely used for determining the effects of harmful chemicals in the areas were not sensitive enough for early detection of contaminant exposure and/or the toxic effects.

The ability of low level of pollutants and their derivatives to affect their toxic actions can complicate the assessment based solely on environmental levels. Deleterious effects on populations are often difficult to detect in organisms since most effects tend to be clear only after longer periods of time. When the effect finally becomes obvious, the destructive process may have gone beyond the point where it can be reversed by remedial actions or risk reduction. Standard method commonly used to measure the toxicity of chemicals by evaluating mortality values can only provide a measure of short-term acute toxicity and are not always useful for predicting the ecological consequences of exposure to a particular chemical where effects are observed at concentrations well below the lethal value. Hence, the need for associate methods to assess, monitor, and mitigate the impact of Hg is important. This scenario has triggered the research to establish early-warning signals, or biomarkers, reflecting the adverse biological responses towards environmental toxins.

In this study, we assessed the use of *P.viridis* MTs as biomarker of exposure by exposing healthy mussels to 2 conditions: laboratory exposures where mussels were exposed at arrange of very low concentration of Hg and field exposure where mussels were exposed to Hg contaminated water at the real-world condition from

petroleum processing facilities in the middle of the Gulf of Thailand. This study found that pvMT07 induction threshold lies near 0.2-0.5 µg/L of Hg while expression of other forms of pvMT weakly correlates with this range. It is proved that pvMT07 can be applied as a suitable biomarker for Hg monitoring at the areas such as petroleum production platforms in the Gulf of Thailand where Hg level is close to the background value. This Biomarker can provide information on the potential adverse impacts of Hg contaminants and can act as early warning signals of impending environmental damage.

There is increasing interest in assessing the impact of genotoxicants which are chemicals capable of causing damage to genetic materials released into coastal marine ecosystems. The level of cellular DNA damage has been proposed as a sensitive biomarker in environmental biomonitoring. In the present study, single cell gel electrophoresis or comet assay is used as the technique to preliminarily determine the DNA damage of haemocyte and sperm of mussels caused by *in vitro* exposure of Hg. The result provides some evidences on the potential genotoxic property of Hg. Nevertheless, the nominal concentrations used in this study may not reflect the exact concentration that mussel cells may respond to Hg since the experiment was conducted *in vitro* where cells were collected and experimented in artificial media. Further study in the *in vivo* condition is needed to verify the exact situation where the whole bodies of living animals are exposed to Hg before the assessment of DNA damage in aquatic animals collected from contaminated sites is carried out. Additionally, since variations in factors such as feeding, reproduction, sexual status and lipid content, as well as DNA repair rate can affect the pollutant uptake of organisms, it would be more instructive to relate a biological response to the body burdens of the toxicant concerned rather than the nominal concentrations. As genotoxicants are often present at contaminated marine sites, it is suggested that the comet assay could be beneficially used at many sites to determine if there are linkages between DNA damage and effects at the population and community levels.

Application of the assays on both pvMT07 and DNA damage established in the present study can be further conducted in most polluted areas such as Map Ta Phut and Laem Chabang industrial ports and estates along the coastal line of inner gulf. It is suitable for applying these assays in these contaminated areas because they are natural habitat to mussels which are used as bioindicators in the assay. Even in

some sites where mussels are not available, mussels from reference site in Trad can be easily transplanted to the target sites. However, the situation facing one specific problem and requiring one biomarker is rare. Generally, ecosystem has to confront with multiple causes of disturbances and it is virtually impossible to monitor all contaminants that are potential threat to the environment. Various number of biomarkers used in environmental studies include enzymes, receptors, biogenic amines, vitamins, hormones, DNA damage, antioxidants, immunological, reproductive cycle, skeletal abnormalities and other pathological effects and the ability to integrate data from different platforms is not a straightforward procedure. Therefore, a panel of complementary and ecologically relevant biomarkers would be necessary and extensive research associating biomarker and ecological studies in the ecosystems of different quality are needed in order to validate the use of biomarkers for modeling environmental quality.

CHAPTER VI

CONCLUSION

The production of all fossil fuels is one of the main sources for emissions of mercury to the environment. It is important that the cycle of Hg and released compounds including their effects on living organisms are monitored efficiently. In this study, bioassay for determining Hg contamination in marine environment at the surrounding areas of petroleum platform in the Gulf of Thailand was established. The study focused on the feasibility and validity of using candidate genes, which were earlier reported as Hg responsive genes, as biomarkers. In addition, the genotoxicity of Hg was investigated using comet assay. Green mussel, *P.viridis*, was used as model species for monitoring the change of Hg contamination in target areas.

The study was initially conducted by testing the response of mussels to low level of Hg in controlled condition. The result indicated that growth and survival rate of the experiment mussels from laboratory and field studies were relatively normal when compared to the mussels rearing in their natural habitat. Transplantation of mussels at petroleum production platforms showed no sign of any physical anomalies on experiment samples. This showed that transplanted mussels could be maintained in un-natural habitat such as petroleum production platforms in the middle of the Gulf of Thailand where food was much less abundant for up to 3 months without significant physical change.

Sublethal levels of inorganic Hg (between 0.1 to 1.0 µg/L) were applied to experiment mussels for 8 weeks in order to monitor Hg concentration in both water and tissues of the mussels. The result revealed that Hg level in water decreased rapidly. Less than 0.1% was detected after 24 h of application while Hg level in tissue increased significantly; indicating that the majority of Hg applied to the experiment tank was absorbed into the tested mussels.

The average level of Hg in the mussel before treatment was 0.0104 µg/g while the level from the 10 µg/L Hg (the highest dose) treatment for 8 weeks was 0.1383 µg/g. It was more than 10 times higher than in the initial mussels. This indicates that Hg can be uptaken into living organisms almost completely within 24 h and Hg can

be accumulated in the mussel tissue at the concentration more than 1,000 folds higher than the Hg level in the surrounding water.

It was calculated that almost 50% of Hg applied to the tested mussels during the experiment were accumulated in the mussel body. The accumulation rate of Hg is reduced when Hg level in the surrounding water increases. This is presumably because the uncertain level of bacteria growing in the water-treated bioreactor and the excess amount of Hg applied to the mussels or it could be the higher efficiency of mussel to eliminate Hg from their bodies when the homeostasis was interfered. Although, Hg level of transplanted mussels appeared to be slightly lower than that of laboratory tested mussels due to the experiment period was shorter and the level of Hg in the surrounding water was much lower, the difference between Hg levels of water and tissue from mussels transplanted at the field sites was still in agreement with the result obtained from the laboratory study.

In order to develop a reliable and easy-to-use method to monitor the effect of sublethal level of Hg on marine organisms, molecular response of mussels exposed to Hg at very low level were analyzed. Expression levels of MT genes and their 6 variant forms, together with other 2 Hg responsive genes, HSP71 and CYP4, were analyzed in mussels exposed to sublethal level of Hg (0 to 1.0 μg/L) in laboratory condition. Bioassay for quantitative evaluation of the transcripts of the target genes was established using semi-quantitative RT-PCR technique. The result of laboratory study indicated that pvMT07, one of MT variant form, responded to Hg at very low levels (lower than 0.2 μg/L) within the first week of experiment while the other candidate genes showed no difference in each treatment. Expression level of pvMT07 also correlated significantly with the increasing level of Hg applied to the tested mussels.

In order to study the feasibility and validity of the obtained method, the optimal condition of the assay used in laboratory study was used for analyzing the expression level of those candidate genes in mussels transplanted at petroleum platform. The result of all candidate genes provide no detectable change on their expression among sampling mussels due to the Hg level in the surrounding areas was lower than the minimal level of the assay. However, the correlation between expression levels and Hg level in mussels still confirmed that pvMT07 was dose-related to Hg even at very low level. The results from both laboratory and field studies

show that the capacity of pvMT07 and the method can be used as a tool to monitor Hg activity at very low level.

Study of Hg genotoxicity to mussel was conducted on haemocyte and sperm cells using single cell gel electrophoresis. Target cells were exposed *in vitro* to $HgCl_2$ at the concentration between 0.001 to 10.0 μg/L at different time interval (10, 30, and 60 min). By measuring the tail length and the tail moment of the assay, similar result was obtained from the assay of both haemocyte and sperm which indicated that the extent of DNA damage was proportional to the level of Hg and the DNA damage could be detected after exposing to Hg level as low as 0.001 μg/L for 10 min. Sperm which is a germ cell appeared to be more sensitive to Hg exposure than haemocyte which is a somatic cell.

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APPENDICES

APPENDIX A

1. LB Broth (per Liter)

10 g of NaCl

10 g of tryptone

5 g of yeast extract

Add deionized H_2O to a final volume of 1 liter. Adjust to pH 7.0 with 5 N NaOH and autoclave.

2. LB Agar (per Liter)

- 10 g of NaCl
- 10 g of tryptone
- 5 g of yeast extract
- 20 g of agar

Add deionized H_2O to a final volume of 1 liter. Adjust to pH 7.0 with 5 N NaOH and autoclave. After, pour into petri dishes (~25 ml/100 mm plate)

3. LB-Ampicillin Agar (per Liter)

- Prepare 1 liter of LB agar. Autoclave and cool to 55 ºC
- Add 50 ml of filter-sterilized ampicillin
- Pour into petri dishes (~25 ml/100-mm plate)

4. 1x TAE Buffer

- 40 mM Tris-acetate
- 1 mM EDTA

5. SOB Medium (Per liter) :

- Bacto-tryptone	20 g
- Yeast extract	5 g
- NaCl	0.5 g

6. Ampicillin

Stock solution. 25 mg/ml of the sodium salt of ampicillin in water. Sterilize by filtration and store in aliquots at –20 ºC

7. 5 M NaCl

Dissolve 292.2 g of NaCl in 800 ml of H_2O. Adjust volume to 1 liter. Dispense into aliquots and sterilize by autoclaving.

8. 1 M $MgCl_2$

Dissolve 203.3 g of $MgCl_2 \cdot 6H_2O$ in 800 ml of H_2O. Adjust volume to 1 liter. Dispense into aliquots and sterilize by autoclaving.

9. 3 M Sodium acetate (pH 5.2)

Dissolve 408.1 g of sodium acetate $\cdot 3H_2O$ in 800 ml of H_2O. Adjust pH to 5.2 with glacial acetic acid. Adjust volume to 1 liter. Dispense into aliquots and sterilize by autoclaving.

10. 10% Sodium dodecyl sulfate (SDS) (also called sodium lauryl sulfate)

Dissolve 100 g of electrophoresis-grade SDS in 900 ml of H_2O. Heat to 68 ºC to assist dissolution. Adjust the pH to 7.2 by adding a few drops of concentrated HCl. Adjust volume to 1 liter. Dispense into aliquots.

11. Ethidium bromide 10 mg/ml

Add 1 g of ethidium bromide to 100 ml of H_2O. Stir on a magnetic stirrer for several hours to ensure that the dye has dissolved. Wrap the container in aluminum foil or transfer to a dark bottle and store at 4 ºC.

12. TE pH 8.0

- 10 mM Tris · Cl (pH 8.0)
- 1 mM EDTA (pH 8.0)

13. Tris-Borate (TBE)

-Working solution

- 0.089 M Tris-borate
- 0.089 M boric acid
- 0.002 M EDTA

- Concentrated stock solution (5x)

Per liter:

- Tris base	54 g
- Boric acid	27.5 g
- 0.5 M EDTA (pH 8.0)	20 ml

14. Gel-Loading Buffer Type II

- 10x buffers
- 0.25% bromophenol blue
- 0.25% xylene cyanol
- 25% Ficoll (type 400) in H_2O
- Store at room temperature.

15. 10x TEN buffers

- 0.1 M Tris-Cl (pH 8.0)

- 0.01 M EDTA (pH 8.0)

- 0.1 M NaCl

16. Glycerol (10% v/v)

Dilute 1 volume of molecular-biology-grade glycerol in 9 volume of sterile pure H_2O. Sterilize the solution by passing it through a prerinsed 0.22 µM filter. Store in 200-ml aliquots at 4 °C

17. IPTG (20% w/v, 0.8 M)

IPTG is isopropylthio-B-D-galactoside. Make a 20% solution of IPTG by dissolving 2 g of IPTG in 8 ml of distilled H_2O. Adjust the volume of the solution to 10 ml with H_2O and sterilize by passing it through a 0.22 µM disposable filter. Dispense the solution into 1-ml aliquots and store them at -20 °C

18. X-gal solution (2% w/v)

X-gal is 5-bromo-4-chloro-3-indolyl-B-d-galactoside. Make a stock solution by dissolving X-gal in dimethylformamide at a concentration of 20 mg/ml solution. Use a glass or polypropylene tube. Wrap the tube containing the solution in aluminum foil to prevent damage by light and store at -20 ºC. It is not necessary to sterilize X-gal solution by filtration.

19. 10% (w/v) Ammonium persulfate

Ammonium persulfate (sigma) 1.0 g is dissolved in 10 ml of dH_2O.

20. Resolving gel buffers : 3 M Tris-HCl pH 8.8

Tris 36.3 g is dissolved in 40 ml of dH_2O, adjusted with 1 M HCl to pH 8.8 and adjusted to 100 ml final volume with dH_2O.

21. Phosphate Buffer Saline (PBS)

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g

Dissolve in 800 ml of dH_2O, adjust pH to 6.8 and adjust to 1000 ml final volume with dH_2O

22. 0.1 % DEPC- dH_2O

Diethyl pyrocarbonate 97 %	1 g

Add dH_2O to 1000 ml and incubate overnight at 37 °C then autoclave.

APPENDIX B

PCR product of MTs, HSP71 and CYP4 gene (Laboratory study)

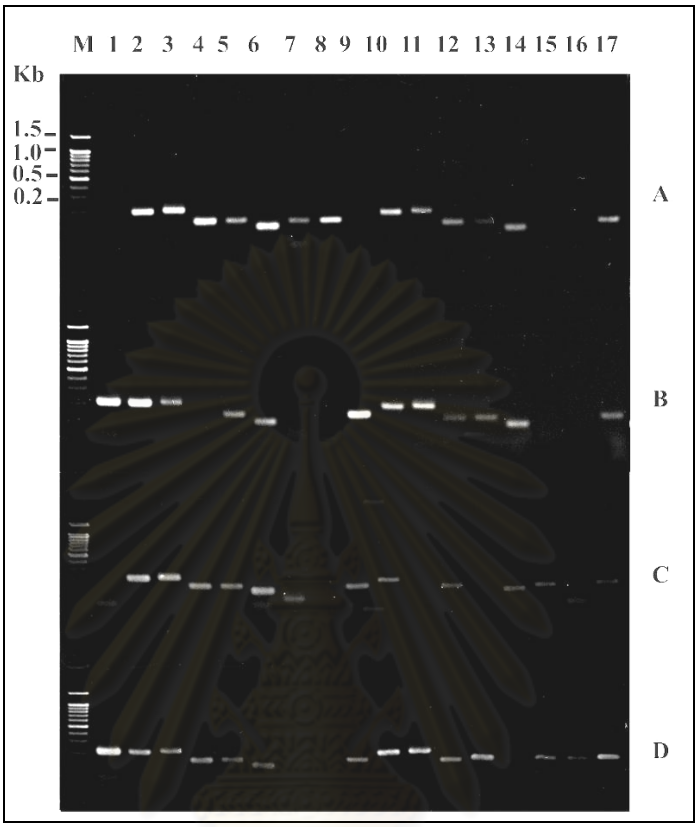

Figure B1 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	Initial #1 β-actin	**2B**	Initial #3 β-actin	**2C**	Control week1 #2 β-actin	**2D**	0.1 µg/L week1 #1 β-actin
Lane 3A	Initial #1 MT	**3B**	Initial #3 MT	**3C**	Control week1 #2 MT	**3D**	0.1 µg/L week1 #1 MT
Lane 4A	Initial #1 pvMT01	**4B**	Initial #3 pvMT01	**4C**	Control week1 #2 pvMT01	**4D**	0.1 µg/L week1 #1 pvMT01
Lane 5A	Initial #1 pvMT02	**5B**	Initial #3 pvMT02	**5C**	Control week1 #2 pvMT02	**5D**	0.1 µg/L week1 #1 pvMT02
Lane 6A	Initial #1 pvMT03	**6B**	Initial #3 pvMT03	**6C**	Control week1 #2 pvMT03	**6D**	0.1 µg/L week1 #1 pvMT03
Lane 7A	Initial #1 pvMT07	**7B**	Initial #3 pvMT07	**7C**	Control week1 #2 pvMT07	**7D**	0.1 µg/L week1 #1 pvMT07
Lane 8A	Initial #1 pvMT08	**8B**	Initial #3 pvMT08	**8C**	Control week1 #2 pvMT08	**8D**	0.1 µg/L week1 #1 pvMT08
Lane 9A	Initial #1 pvMT11	**9B**	Initial #3 pvMT11	**9C**	Control week1 #2 pvMT11	**9D**	0.1 µg/L week1 #1 pvMT11
Lane 10A	Initial #2 β-actin	**10B**	Control week1 #1 β-actin	**10C**	Control week1 #3 β-actin	**10D**	0.1 µg/L week1 #2 β-actin
Lane 11A	Initial #2 MT	**11B**	Control week1 #1 MT	**11C**	Control week1 #3 MT	**11D**	0.1 µg/L week1 #2 MT
Lane 12A	Initial #2 pvMT01	**12B**	Control week1 #1 pvMT01	**12C**	Control week1 #3 pvMT01	**12D**	0.1 µg/L week1 #2 pvMT01
Lane 13A	Initial #2 pvMT02	**13B**	Control week1 #1 pvMT02	**13C**	Control week1 #3 pvMT02	**13D**	0.1 µg/L week1 #2 pvMT02
Lane 14A	Initial #2 pvMT03	**14B**	Control week1 #1 pvMT03	**14C**	Control week1 #3 pvMT03	**14D**	0.1 µg/L week1 #2 pvMT03
Lane 15A	Initial #2 pvMT07	**15B**	Control week1 #1 pvMT07	**15C**	Control week1 #3 pvMT07	**15D**	0.1 µg/L week1 #2 pvMT07
Lane 16A	Initial #2 pvMT08	**16B**	Control week1 #1 pvMT08	**16C**	Control week1 #3 pvMT08	**16D**	0.1 µg/L week1 #2 pvMT08
Lane 17A	Initial #2 pvMT11	**17B**	Control week1 #1 pvMT11	**17C**	Control week1 #3 pvMT11	**17D**	0.1 µg/L week1 #2 pvMT11

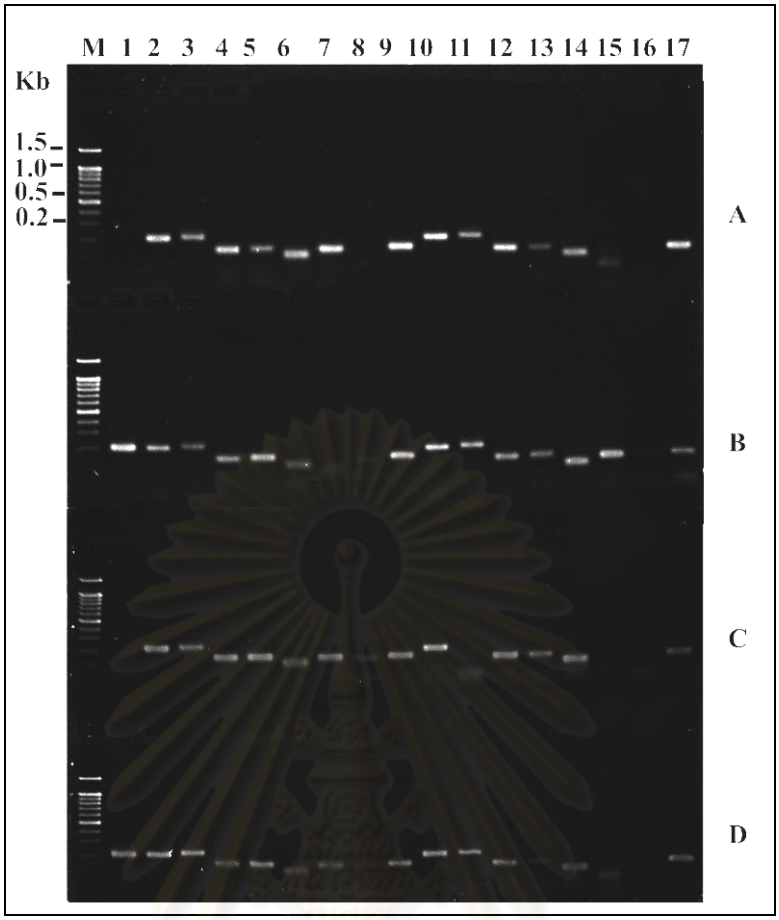

Figure B2 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.1 µg/L week1 #3 β-actin	**2B**	0.2 µg/L week1 #2 β-actin	**2C**	0.5 µg/L week1 #1 β-actin	**2D**	0.5 µg/L week1 #3 β-actin
Lane 3A	0.1 µg/L week1 #3 MT	**3B**	0.2 µg/L week1 #2 MT	**3C**	0.5 µg/L week1 #1 MT	**3D**	0.5 µg/L week1 #3 MT
Lane 4A	0.1 µg/L week1 #3 pvMT01	**4B**	0.2 µg/L week1 #2 pvMT01	**4C**	0.5 µg/L week1 #1 pvMT01	**4D**	0.5 µg/L week1 #3 pvMT01
Lane 5A	0.1 µg/L week1 #3 pvMT02	**5B**	0.2 µg/L week1 #2 pvMT02	**5C**	0.5 µg/L week1 #1 pvMT02	**5D**	0.5 µg/L week1 #3 pvMT02
Lane 6A	0.1 µg/L week1 #3 pvMT03	**6B**	0.2 µg/L week1 #2 pvMT03	**6C**	0.5 µg/L week1 #1 pvMT03	**6D**	0.5 µg/L week1 #3 pvMT03
Lane 7A	0.1 µg/L week1 #3 pvMT07	**7B**	0.2 µg/L week1 #2 pvMT07	**7C**	0.5 µg/L week1 #1 pvMT07	**7D**	0.5 µg/L week1 #3 pvMT07
Lane 8A	0.1 µg/L week1 #3 pvMT08	**8B**	0.2 µg/L week1 #2 pvMT08	**8C**	0.5 µg/L week1 #1 pvMT08	**8D**	0.5 µg/L week1 #3 pvMT08
Lane 9A	0.1 µg/L week1 #3 pvMT11	**9B**	0.2 µg/L week1 #2 pvMT11	**9C**	0.5 µg/L week1 #1 pvMT11	**9D**	0.5 µg/L week1 #3 pvMT11
Lane 10A	0.2 µg/L week1 #1 β-actin	**10B**	0.2 µg/L week1 #3 β-actin	**10C**	0.5 µg/L week1 #2 β-actin	**10D**	1.0 µg/L week1 #1 β-actin
Lane 11A	0.2 µg/L week1 #1 MT	**11B**	0.2 µg/L week1 #3 MT	**11C**	0.5 µg/L week1 #2 MT	**11D**	1.0 µg/L week1 #1 MT
Lane 12A	0.2 µg/L week1 #1 pvMT01	**12B**	0.2 µg/L week1 #3 pvMT01	**12C**	0.5 µg/L week1 #2 pvMT01	**12D**	1.0 µg/L week1 #1 pvMT01
Lane 13A	0.2 µg/L week1 #1 pvMT02	**13B**	0.2 µg/L week1 #3 pvMT02	**13C**	0.5 µg/L week1 #2 pvMT02	**13D**	1.0 µg/L week1 #1 pvMT02
Lane 14A	0.2 µg/L week1 #1 pvMT03	**14B**	0.2 µg/L week1 #3 pvMT03	**14C**	0.5 µg/L week1 #2 pvMT03	**14D**	1.0 µg/L week1 #1 pvMT03
Lane 15A	0.2 µg/L week1 #1 pvMT07	**15B**	0.2 µg/L week1 #3 pvMT07	**15C**	0.5 µg/L week1 #2 pvMT07	**15D**	1.0 µg/L week1 #1 pvMT07
Lane 16A	0.2 µg/L week1 #1 pvMT08	**16B**	0.2 µg/L week1 #3 pvMT08	**16C**	0.5 µg/L week1 #2 pvMT08	**16D**	1.0 µg/L week1 #1 pvMT08
Lane 17A	0.2 µg/L week1 #1 pvMT11	**17B**	0.2 µg/L week1 #3 pvMT11	**17C**	0.5 µg/L week1 #2 pvMT11	**17D**	1.0 µg/L week1 #1 pvMT11

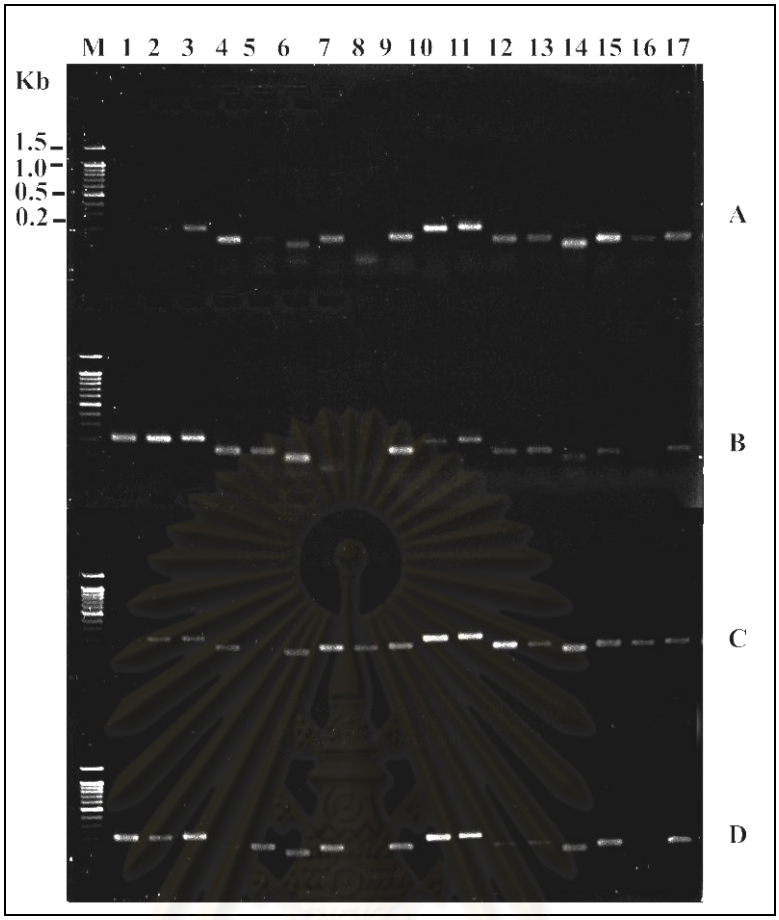

Figure B3 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	1.0 µg/L week1 #2 β-actin	**2B**	Control week2 #1 β-actin	**2C**	Control week2 #1 β-actin	**2D**	0.1 µg/L week2 #2 β-actin
Lane 3A	1.0 µg/L week1 #2 MT	**3B**	Control week2 #1 MT	**3C**	Control week2 #1 MT	**3D**	0.1 µg/L week2 #2 MT
Lane 4A	1.0 µg/L week1 #2 pvMT01	**4B**	Control week2 #1 pvMT01	**4C**	Control week2 #1 pvMT01	**4D**	0.1 µg/L week2 #2 pvMT01
Lane 5A	1.0 µg/L week1 #2 pvMT02	**5B**	Control week2 #1 pvMT02	**5C**	Control week2 #1 pvMT02	**5D**	0.1 µg/L week2 #2 pvMT02
Lane 6A	1.0 µg/L week1 #2 pvMT03	**6B**	Control week2 #1 pvMT03	**6C**	Control week2 #1 pvMT03	**6D**	0.1 µg/L week2 #2 pvMT03
Lane 7A	1.0 µg/L week1 #2 pvMT07	**7B**	Control week2 #1 pvMT07	**7C**	Control week2 #1 pvMT07	**7D**	0.1 µg/L week2 #2 pvMT07
Lane 8A	1.0 µg/L week1 #2 pvMT08	**8B**	Control week2 #1 pvMT08	**8C**	Control week2 #1 pvMT08	**8D**	0.1 µg/L week2 #2 pvMT08
Lane 9A	1.0 µg/L week1 #2 pvMT11	**9B**	Control week2 #1 pvMT11	**9C**	Control week2 #1 pvMT11	**9D**	0.1 µg/L week2 #2 pvMT11
Lane 10A	1.0 µg/L week1 #3 β-actin	**10B**	Control week2 #1 β-actin	**10C**	0.1 µg/L week2 #1 β-actin	**10D**	0.1 µg/L week2 #3 β-actin
Lane 11A	1.0 µg/L week1 #3 MT	**11B**	Control week2 #1 MT	**11C**	0.1 µg/L week2 #1 MT	**11D**	0.1 µg/L week2 #3 MT
Lane 12A	1.0 µg/L week1 #3 pvMT01	**12B**	Control week2 #1 pvMT01	**12C**	0.1 µg/L week2 #1 pvMT01	**12D**	0.1 µg/L week2 #3 pvMT01
Lane 13A	1.0 µg/L week1 #3 pvMT02	**13B**	Control week2 #1 pvMT02	**13C**	0.1 µg/L week2 #1 pvMT02	**13D**	0.1 µg/L week2 #3 pvMT02
Lane 14A	1.0 µg/L week1 #3 pvMT03	**14B**	Control week2 #1 pvMT03	**14C**	0.1 µg/L week2 #1 pvMT03	**14D**	0.1 µg/L week2 #3 pvMT03
Lane 15A	1.0 µg/L week1 #3 pvMT07	**15B**	Control week2 #1 pvMT07	**15C**	0.1 µg/L week2 #1 pvMT07	**15D**	0.1 µg/L week2 #3 pvMT07
Lane 16A	1.0 µg/L week1 #3 pvMT08	**16B**	Control week2 #1 pvMT08	**16C**	0.1 µg/L week2 #1 pvMT08	**16D**	0.1 µg/L week2 #3 pvMT08
Lane 17A	1.0 µg/L week1 #3 pvMT11	**17B**	Control week2 #1 pvMT11	**17C**	0.1 µg/L week2 #1 pvMT11	**17D**	0.1 µg/L week2 #3 pvMT11

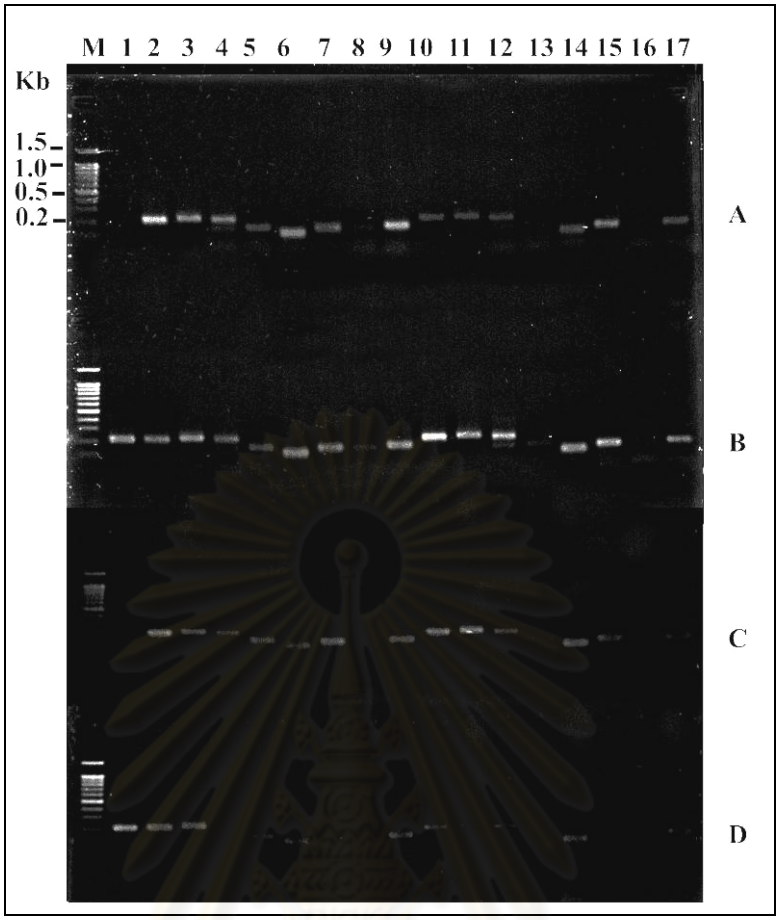

Figure B4 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.2 µg/L week2 #1 β-actin	**2B**	0.2 µg/L week2 #3 β-actin	**2C**	0.5 µg/L week2 #2 β-actin	**2D**	1.0 µg/L week2 #1 β-actin
Lane 3A	0.2 µg/L week2 #1 MT	**3B**	0.2 µg/L week2 #3 MT	**3C**	0.5 µg/L week2 #2 MT	**3D**	1.0 µg/L week2 #1 MT
Lane 4A	0.2 µg/L week2 #1 pvMT01	**4B**	0.2 µg/L week2 #3 pvMT01	**4C**	0.5 µg/L week2 #2 pvMT01	**4D**	1.0 µg/L week2 #1 pvMT01
Lane 5A	0.2 µg/L week2 #1 pvMT02	**5B**	0.2 µg/L week2 #3 pvMT02	**5C**	0.5 µg/L week2 #2 pvMT02	**5D**	1.0 µg/L week2 #1 pvMT02
Lane 6A	0.2 µg/L week2 #1 pvMT03	**6B**	0.2 µg/L week2 #3 pvMT03	**6C**	0.5 µg/L week2 #2 pvMT03	**6D**	1.0 µg/L week2 #1 pvMT03
Lane 7A	0.2 µg/L week2 #1 pvMT07	**7B**	0.2 µg/L week2 #3 pvMT07	**7C**	0.5 µg/L week2 #2 pvMT07	**7D**	1.0 µg/L week2 #1 pvMT07
Lane 8A	0.2 µg/L week2 #1 pvMT08	**8B**	0.2 µg/L week2 #3 pvMT08	**8C**	0.5 µg/L week2 #2 pvMT08	**8D**	1.0 µg/L week2 #1 pvMT08
Lane 9A	0.2 µg/L week2 #1 pvMT11	**9B**	0.2 µg/L week2 #3 pvMT11	**9C**	0.5 µg/L week2 #2 pvMT11	**9D**	1.0 µg/L week2 #1 pvMT11
Lane 10A	0.2 µg/L week2 #2 β-actin	**10B**	0.5 µg/L week2 #1 β-actin	**10C**	0.5 µg/L week2 #3 β-actin	**10D**	1.0 µg/L week2 #2 β-actin
Lane 11A	0.2 µg/L week2 #2 MT	**11B**	0.5 µg/L week2 #1 MT	**11C**	0.5 µg/L week2 #3 MT	**11D**	1.0 µg/L week2 #2 MT
Lane 12A	0.2 µg/L week2 #2 pvMT01	**12B**	0.5 µg/L week2 #1 pvMT01	**12C**	0.5 µg/L week2 #3 pvMT01	**12D**	1.0 µg/L week2 #2 pvMT01
Lane 13A	0.2 µg/L week2 #2 pvMT02	**13B**	0.5 µg/L week2 #1 pvMT02	**13C**	0.5 µg/L week2 #3 pvMT02	**13D**	1.0 µg/L week2 #2 pvMT02
Lane 14A	0.2 µg/L week2 #2 pvMT03	**14B**	0.5 µg/L week2 #1 pvMT03	**14C**	0.5 µg/L week2 #3 pvMT03	**14D**	1.0 µg/L week2 #2 pvMT03
Lane 15A	0.2 µg/L week2 #2 pvMT07	**15B**	0.5 µg/L week2 #1 pvMT07	**15C**	0.5 µg/L week2 #3 pvMT07	**15D**	1.0 µg/L week2 #2 pvMT07
Lane 16A	0.2 µg/L week2 #2 pvMT08	**16B**	0.5 µg/L week2 #1 pvMT08	**16C**	0.5 µg/L week2 #3 pvMT08	**16D**	1.0 µg/L week2 #2 pvMT08
Lane 17A	0.2 µg/L week2 #2 pvMT11	**17B**	0.5 µg/L week2 #1 pvMT11	**17C**	0.5 µg/L week2 #3 pvMT11	**17D**	1.0 µg/L week2 #2 pvMT11

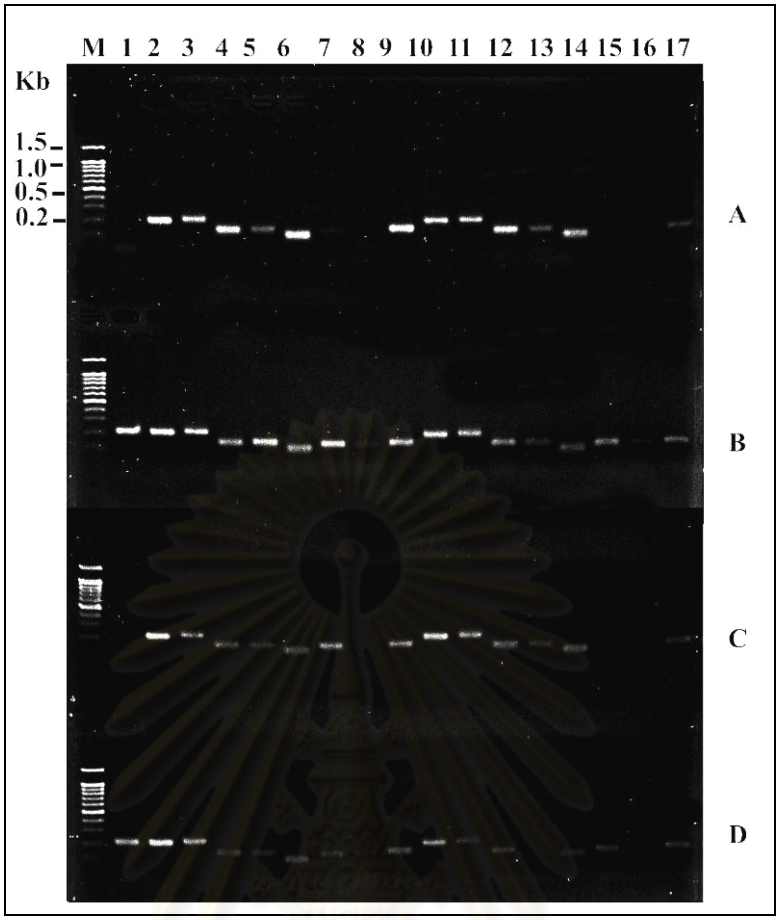

Figure B5 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	1.0 µg/L week2 #3 β-actin	**2B**	Control week3 #2 β-actin	**2C**	0.1 µg/L week3 #1 β-actin	**2D**	0.1 µg/L week3 #3 β-actin
Lane 3A	1.0 µg/L week2 #3 MT	**3B**	Control week3 #2 MT	**3C**	0.1 µg/L week3 #1 MT	**3D**	0.1 µg/L week3 #3 MT
Lane 4A	1.0 µg/L week2 #3 pvMT01	**4B**	Control week3 #2 pvMT01	**4C**	0.1 µg/L week3 #1 pvMT01	**4D**	0.1 µg/L week3 #3 pvMT01
Lane 5A	1.0 µg/L week2 #3 pvMT02	**5B**	Control week3 #2 pvMT02	**5C**	0.1 µg/L week3 #1 pvMT02	**5D**	0.1 µg/L week3 #3 pvMT02
Lane 6A	1.0 µg/L week2 #3 pvMT03	**6B**	Control week3 #2 pvMT03	**6C**	0.1 µg/L week3 #1 pvMT03	**6D**	0.1 µg/L week3 #3 pvMT03
Lane 7A	1.0 µg/L week2 #3 pvMT07	**7B**	Control week3 #2 pvMT07	**7C**	0.1 µg/L week3 #1 pvMT07	**7D**	0.1 µg/L week3 #3 pvMT07
Lane 8A	1.0 µg/L week2 #3 pvMT08	**8B**	Control week3 #2 pvMT08	**8C**	0.1 µg/L week3 #1 pvMT08	**8D**	0.1 µg/L week3 #3 pvMT08
Lane 9A	1.0 µg/L week2 #3 pvMT11	**9B**	Control week3 #2 pvMT11	**9C**	0.1 µg/L week3 #1 pvMT11	**9D**	0.1 µg/L week3 #3 pvMT11
Lane 10A	Control week3 #1 β-actin	**10B**	Control week3 #3 β-actin	**10C**	0.1 µg/L week3 #2 β-actin	**10D**	0.2 µg/L week3 #1 β-actin
Lane 11A	Control week3 #1 MT	**11B**	Control week3 #3 MT	**11C**	0.1 µg/L week3 #2 MT	**11D**	0.2 µg/L week3 #1 MT
Lane 12A	Control week3 #1 pvMT01	**12B**	Control week3 #3 pvMT01	**12C**	0.1 µg/L week3 #2 pvMT01	**12D**	0.2 µg/L week3 #1 pvMT01
Lane 13A	Control week3 #1 pvMT02	**13B**	Control week3 #3 pvMT02	**13C**	0.1 µg/L week3 #2 pvMT02	**13D**	0.2 µg/L week3 #1 pvMT02
Lane 14A	Control week3 #1 pvMT03	**14B**	Control week3 #3 pvMT03	**14C**	0.1 µg/L week3 #2 pvMT03	**14D**	0.2 µg/L week3 #1 pvMT03
Lane 15A	Control week3 #1 pvMT07	**15B**	Control week3 #3 pvMT07	**15C**	0.1 µg/L week3 #2 pvMT07	**15D**	0.2 µg/L week3 #1 pvMT07
Lane 16A	Control week3 #1 pvMT08	**16B**	Control week3 #3 pvMT08	**16C**	0.1 µg/L week3 #2 pvMT08	**16D**	0.2 µg/L week3 #1 pvMT08
Lane 17A	Control week3 #1 pvMT11	**17B**	Control week3 #3 pvMT11	**17C**	0.1 µg/L week3 #2 pvMT11	**17D**	0.2 µg/L week3 #1 pvMT11

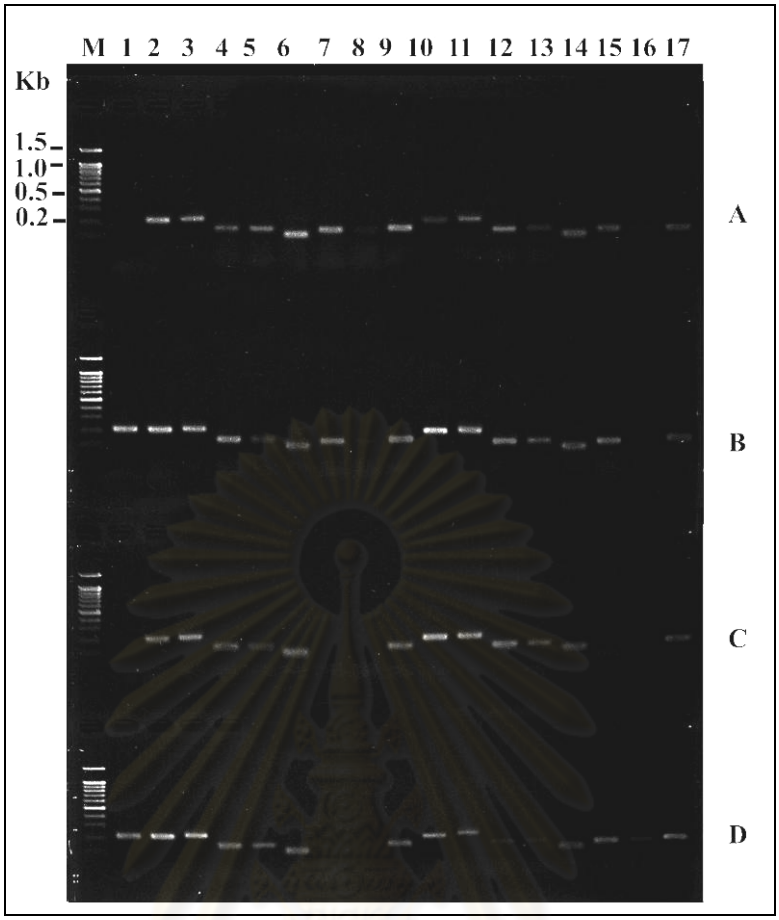

Figure B6 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.2 µg/L week3 #2 β-actin	**2B**	0.5 µg/L week3 #1 β-actin	**2C**	0.5 µg/L week3 #3 β-actin	**2D**	1.0 µg/L week3 #2 β-actin
Lane 3A	0.2 µg/L week3 #2 MT	**3B**	0.5 µg/L week3 #1 MT	**3C**	0.5 µg/L week3 #3 MT	**3D**	1.0 µg/L week3 #2 MT
Lane 4A	0.2 µg/L week3 #2 pvMT01	**4B**	0.5 µg/L week3 #1 pvMT01	**4C**	0.5 µg/L week3 #3 pvMT01	**4D**	1.0 µg/L week3 #2 pvMT01
Lane 5A	0.2 µg/L week3 #2 pvMT02	**5B**	0.5 µg/L week3 #1 pvMT02	**5C**	0.5 µg/L week3 #3 pvMT02	**5D**	1.0 µg/L week3 #2 pvMT02
Lane 6A	0.2 µg/L week3 #2 pvMT03	**6B**	0.5 µg/L week3 #1 pvMT03	**6C**	0.5 µg/L week3 #3 pvMT03	**6D**	1.0 µg/L week3 #2 pvMT03
Lane 7A	0.2 µg/L week3 #2 pvMT07	**7B**	0.5 µg/L week3 #1 pvMT07	**7C**	0.5 µg/L week3 #3 pvMT07	**7D**	1.0 µg/L week3 #2 pvMT07
Lane 8A	0.2 µg/L week3 #2 pvMT08	**8B**	0.5 µg/L week3 #1 pvMT08	**8C**	0.5 µg/L week3 #3 pvMT08	**8D**	1.0 µg/L week3 #2 pvMT08
Lane 9A	0.2 µg/L week3 #2 pvMT11	**9B**	0.5 µg/L week3 #1 pvMT11	**9C**	0.5 µg/L week3 #3 pvMT11	**9D**	1.0 µg/L week3 #2 pvMT11
Lane 10A	0.2 µg/L week3 #3 β-actin	**10B**	0.5 µg/L week3 #2 β-actin	**10C**	1.0 µg/L week3 #1 β-actin	**10D**	1.0 µg/L week3 #3 β-actin
Lane 11A	0.2 µg/L week3 #3 MT	**11B**	0.5 µg/L week3 #2 MT	**11C**	1.0 µg/L week3 #1 MT	**11D**	1.0 µg/L week3 #3 MT
Lane 12A	0.2 µg/L week3 #3 pvMT01	**12B**	0.5 µg/L week3 #2 pvMT01	**12C**	1.0 µg/L week3 #1 pvMT01	**12D**	1.0 µg/L week3 #3 pvMT01
Lane 13A	0.2 µg/L week3 #3 pvMT02	**13B**	0.5 µg/L week3 #2 pvMT02	**13C**	1.0 µg/L week3 #1 pvMT02	**13D**	1.0 µg/L week3 #3 pvMT02
Lane 14A	0.2 µg/L week3 #3 pvMT03	**14B**	0.5 µg/L week3 #2 pvMT03	**14C**	1.0 µg/L week3 #1 pvMT03	**14D**	1.0 µg/L week3 #3 pvMT03
Lane 15A	0.2 µg/L week3 #3 pvMT07	**15B**	0.5 µg/L week3 #2 pvMT07	**15C**	1.0 µg/L week3 #1 pvMT07	**15D**	1.0 µg/L week3 #3 pvMT07
Lane 16A	0.2 µg/L week3 #3 pvMT08	**16B**	0.5 µg/L week3 #2 pvMT08	**16C**	1.0 µg/L week3 #1 pvMT08	**16D**	1.0 µg/L week3 #3 pvMT08
Lane 17A	0.2 µg/L week3 #3 pvMT11	**17B**	0.5 µg/L week3 #2 pvMT11	**17C**	1.0 µg/L week3 #1 pvMT11	**17D**	1.0 µg/L week3 #3 pvMT11

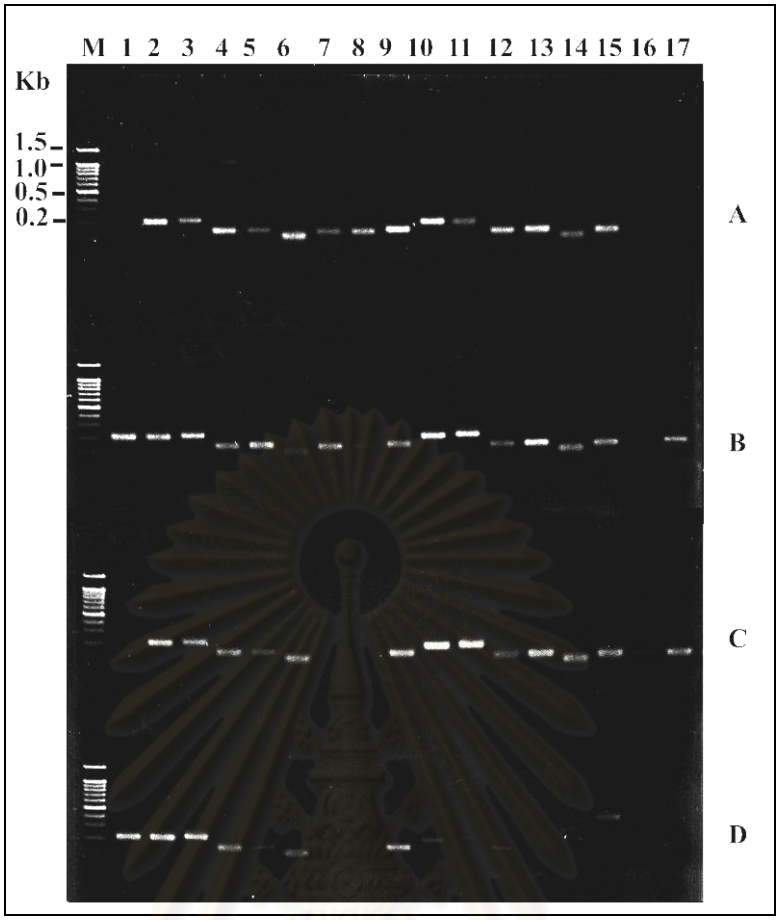

Figure B7 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	Control week4 #1 β-actin	**2B**	Control week4 #3 β-actin	**2C**	0.1 µg/L week4 #2 β-actin	**2D**	0.2 µg/L week4 #1 β-actin
Lane 3A	Control week4 #1 MT	**3B**	Control week4 #3 MT	**3C**	0.1 µg/L week4 #2 MT	**3D**	0.2 µg/L week4 #1 MT
Lane 4A	Control week4 #1 pvMT01	**4B**	Control week4 #3 pvMT01	**4C**	0.1 µg/L week4 #2 pvMT01	**4D**	0.2 µg/L week4 #1 pvMT01
Lane 5A	Control week4 #1 pvMT02	**5B**	Control week4 #3 pvMT02	**5C**	0.1 µg/L week4 #2 pvMT02	**5D**	0.2 µg/L week4 #1 pvMT02
Lane 6A	Control week4 #1 pvMT03	**6B**	Control week4 #3 pvMT03	**6C**	0.1 µg/L week4 #2 pvMT03	**6D**	0.2 µg/L week4 #1 pvMT03
Lane 7A	Control week4 #1 pvMT07	**7B**	Control week4 #3 pvMT07	**7C**	0.1 µg/L week4 #2 pvMT07	**7D**	0.2 µg/L week4 #1 pvMT07
Lane 8A	Control week4 #1 pvMT08	**8B**	Control week4 #3 pvMT08	**8C**	0.1 µg/L week4 #2 pvMT08	**8D**	0.2 µg/L week4 #1 pvMT08
Lane 9A	Control week4 #1 pvMT11	**9B**	Control week4 #3 pvMT11	**9C**	0.1 µg/L week4 #2 pvMT11	**9D**	0.2 µg/L week4 #1 pvMT11
Lane 10A	Control week4 #2 β-actin	**10B**	0.1 µg/L week4 #1 β-actin	**10C**	0.1 µg/L week4 #3 β-actin	**10D**	0.2 µg/L week4 #2 β-actin
Lane 11A	Control week4 #2 MT	**11B**	0.1 µg/L week4 #1 MT	**11C**	0.1 µg/L week4 #3 MT	**11D**	0.2 µg/L week4 #2 MT
Lane 12A	Control week4 #2 pvMT01	**12B**	0.1 µg/L week4 #1 pvMT01	**12C**	0.1 µg/L week4 #3 pvMT01	**12D**	0.2 µg/L week4 #2 pvMT01
Lane 13A	Control week4 #2 pvMT02	**13B**	0.1 µg/L week4 #1 pvMT02	**13C**	0.1 µg/L week4 #3 pvMT02	**13D**	0.2 µg/L week4 #2 pvMT02
Lane 14A	Control week4 #2 pvMT03	**14B**	0.1 µg/L week4 #1 pvMT03	**14C**	0.1 µg/L week4 #3 pvMT03	**14D**	0.2 µg/L week4 #2 pvMT03
Lane 15A	Control week4 #2 pvMT07	**15B**	0.1 µg/L week4 #1 pvMT07	**15C**	0.1 µg/L week4 #3 pvMT07	**15D**	0.2 µg/L week4 #2 pvMT07
Lane 16A	Control week4 #2 pvMT08	**16B**	0.1 µg/L week4 #1 pvMT08	**16C**	0.1 µg/L week4 #3 pvMT08	**16D**	0.2 µg/L week4 #2 pvMT08
Lane 17A	Control week4 #2 pvMT11	**17B**	0.1 µg/L week4 #1 pvMT11	**17C**	0.1 µg/L week4 #3 pvMT11	**17D**	0.2 µg/L week4 #2 pvMT11

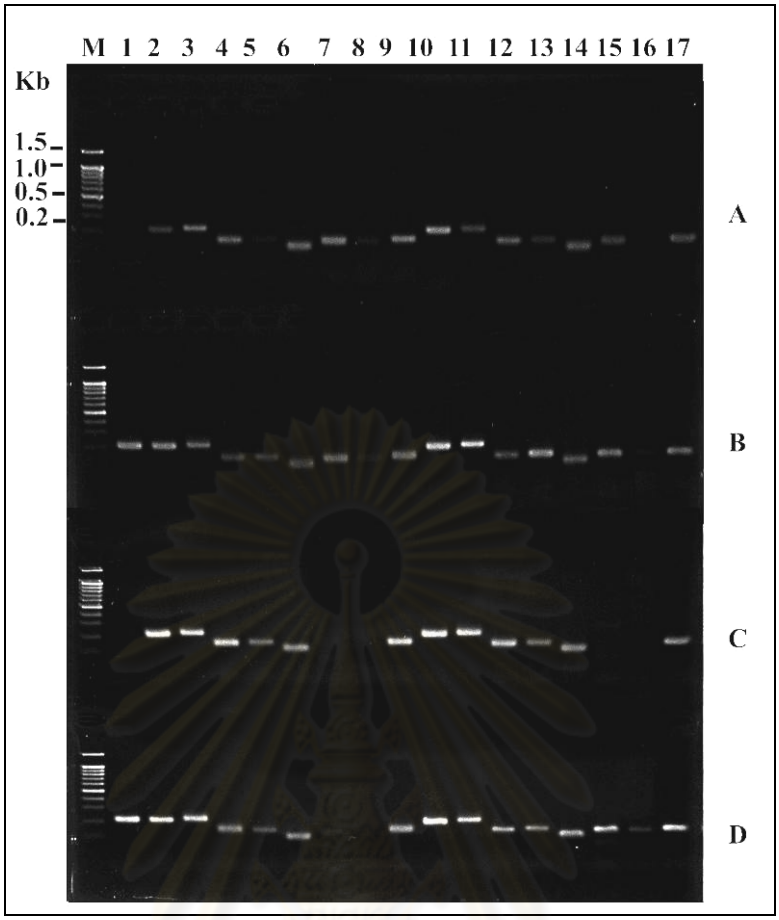

Figure B8 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.2 µg/L week4 #3 β-actin	**2B**	0.5 µg/L week4 #2 β-actin	**2C**	1.0 µg/L week4 #1 β-actin	**2D**	1.0 µg/L week4 #3 β-actin
Lane 3A	0.2 µg/L week4 #3 MT	**3B**	0.5 µg/L week4 #2 MT	**3C**	1.0 µg/L week4 #1 MT	**3D**	1.0 µg/L week4 #3 MT
Lane 4A	0.2 µg/L week4 #3 pvMT01	**4B**	0.5 µg/L week4 #2 pvMT01	**4C**	1.0 µg/L week4 #1 pvMT01	**4D**	1.0 µg/L week4 #3 pvMT01
Lane 5A	0.2 µg/L week4 #3 pvMT02	**5B**	0.5 µg/L week4 #2 pvMT02	**5C**	1.0 µg/L week4 #1 pvMT02	**5D**	1.0 µg/L week4 #3 pvMT02
Lane 6A	0.2 µg/L week4 #3 pvMT03	**6B**	0.5 µg/L week4 #2 pvMT03	**6C**	1.0 µg/L week4 #1 pvMT03	**6D**	1.0 µg/L week4 #3 pvMT03
Lane 7A	0.2 µg/L week4 #3 pvMT07	**7B**	0.5 µg/L week4 #2 pvMT07	**7C**	1.0 µg/L week4 #1 pvMT07	**7D**	1.0 µg/L week4 #3 pvMT07
Lane 8A	0.2 µg/L week4 #3 pvMT08	**8B**	0.5 µg/L week4 #2 pvMT08	**8C**	1.0 µg/L week4 #1 pvMT08	**8D**	1.0 µg/L week4 #3 pvMT08
Lane 9A	0.2 µg/L week4 #3 pvMT11	**9B**	0.5 µg/L week4 #2 pvMT11	**9C**	1.0 µg/L week4 #1 pvMT11	**9D**	1.0 µg/L week4 #3 pvMT11
Lane 10A	0.5 µg/L week4 #1 β-actin	**10B**	0.5 µg/L week4 #3 β-actin	**10C**	1.0 µg/L week4 #2 β-actin	**10D**	Control week5 #1 β-actin
Lane 11A	0.5 µg/L week4 #1 MT	**11B**	0.5 µg/L week4 #3 MT	**11C**	1.0 µg/L week4 #2 MT	**11D**	Control week5 #1 MT
Lane 12A	0.5 µg/L week4 #1 pvMT01	**12B**	0.5 µg/L week4 #3 pvMT01	**12C**	1.0 µg/L week4 #2 pvMT01	**12D**	Control week5 #1 pvMT01
Lane 13A	0.5 µg/L week4 #1 pvMT02	**13B**	0.5 µg/L week4 #3 pvMT02	**13C**	1.0 µg/L week4 #2 pvMT02	**13D**	Control week5 #1 pvMT02
Lane 14A	0.5 µg/L week4 #1 pvMT03	**14B**	0.5 µg/L week4 #3 pvMT03	**14C**	1.0 µg/L week4 #2 pvMT03	**14D**	Control week5 #1 pvMT03
Lane 15A	0.5 µg/L week4 #1 pvMT07	**15B**	0.5 µg/L week4 #3 pvMT07	**15C**	1.0 µg/L week4 #2 pvMT07	**15D**	Control week5 #1 pvMT07
Lane 16A	0.5 µg/L week4 #1 pvMT08	**16B**	0.5 µg/L week4 #3 pvMT08	**16C**	1.0 µg/L week4 #2 pvMT08	**16D**	Control week5 #1 pvMT08
Lane 17A	0.5 µg/L week4 #1 pvMT11	**17B**	0.5 µg/L week4 #3 pvMT11	**17C**	1.0 µg/L week4 #2 pvMT11	**17D**	Control week5 #1 pvMT11

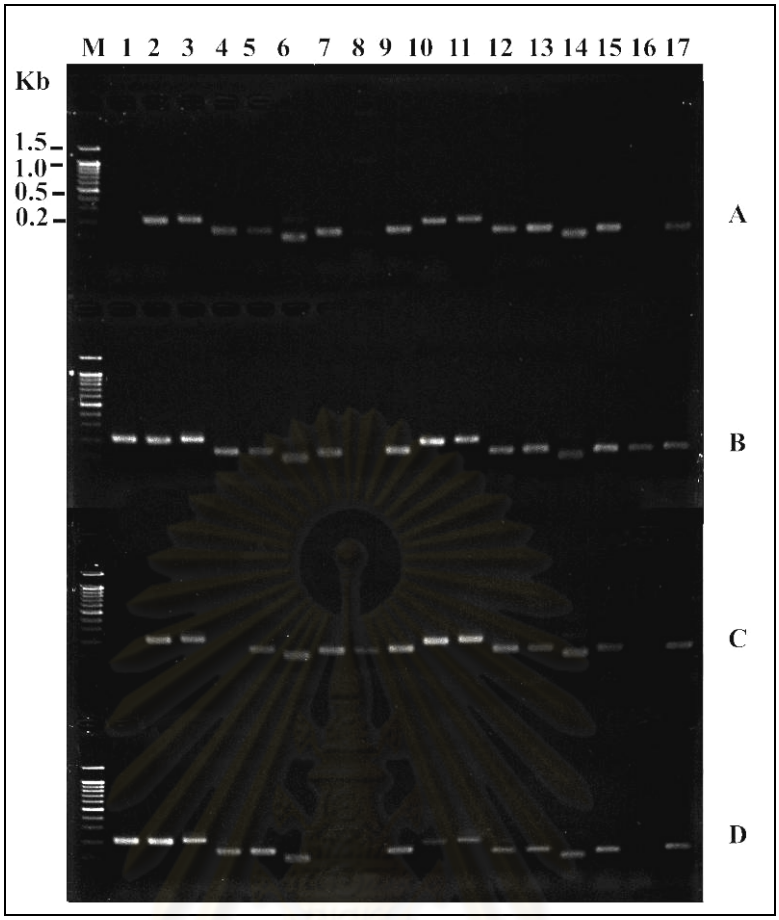

Figure B9 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	Control week5 #2 β-actin	**2B**	0.1 µg/L week5 #1 β-actin	**2C**	0.1 µg/L week5 #3 β-actin	**2D**	0.2 µg/L week5 #2 β-actin
Lane 3A	Control week5 #2 MT	**3B**	0.1 µg/L week5 #1 MT	**3C**	0.1 µg/L week5 #3 MT	**3D**	0.2 µg/L week5 #2 MT
Lane 4A	Control week5 #2 pvMT01	**4B**	0.1 µg/L week5 #1 pvMT01	**4C**	0.1 µg/L week5 #3 pvMT01	**4D**	0.2 µg/L week5 #2 pvMT01
Lane 5A	Control week5 #2 pvMT02	**5B**	0.1 µg/L week5 #1 pvMT02	**5C**	0.1 µg/L week5 #3 pvMT02	**5D**	0.2 µg/L week5 #2 pvMT02
Lane 6A	Control week5 #2 pvMT03	**6B**	0.1 µg/L week5 #1 pvMT03	**6C**	0.1 µg/L week5 #3 pvMT03	**6D**	0.2 µg/L week5 #2 pvMT03
Lane 7A	Control week5 #2 pvMT07	**7B**	0.1 µg/L week5 #1 pvMT07	**7C**	0.1 µg/L week5 #3 pvMT07	**7D**	0.2 µg/L week5 #2 pvMT07
Lane 8A	Control week5 #2 pvMT08	**8B**	0.1 µg/L week5 #1 pvMT08	**8C**	0.1 µg/L week5 #3 pvMT08	**8D**	0.2 µg/L week5 #2 pvMT08
Lane 9A	Control week5 #2 pvMT11	**9B**	0.1 µg/L week5 #1 pvMT11	**9C**	0.1 µg/L week5 #3 pvMT11	**9D**	0.2 µg/L week5 #2 pvMT11
Lane 10A	Control week5 #3 β-actin	**10B**	0.1 µg/L week5 #2 β-actin	**10C**	0.2 µg/L week5 #1 β-actin	**10D**	0.2 µg/L week5 #3 β-actin
Lane 11A	Control week5 #3 MT	**11B**	0.1 µg/L week5 #2 MT	**11C**	0.2 µg/L week5 #1 MT	**11D**	0.2 µg/L week5 #3 MT
Lane 12A	Control week5 #3 pvMT01	**12B**	0.1 µg/L week5 #2 pvMT01	**12C**	0.2 µg/L week5 #1 pvMT01	**12D**	0.2 µg/L week5 #3 pvMT01
Lane 13A	Control week5 #3 pvMT02	**13B**	0.1 µg/L week5 #2 pvMT02	**13C**	0.2 µg/L week5 #1 pvMT02	**13D**	0.2 µg/L week5 #3 pvMT02
Lane 14A	Control week5 #3 pvMT03	**14B**	0.1 µg/L week5 #2 pvMT03	**14C**	0.2 µg/L week5 #1 pvMT03	**14D**	0.2 µg/L week5 #3 pvMT03
Lane 15A	Control week5 #3 pvMT07	**15B**	0.1 µg/L week5 #2 pvMT07	**15C**	0.2 µg/L week5 #1 pvMT07	**15D**	0.2 µg/L week5 #3 pvMT07
Lane 16A	Control week5 #3 pvMT08	**16B**	0.1 µg/L week5 #2 pvMT08	**16C**	0.2 µg/L week5 #1 pvMT08	**16D**	0.2 µg/L week5 #3 pvMT08
Lane 17A	Control week5 #3 pvMT11	**17B**	0.1 µg/L week5 #2 pvMT11	**17C**	0.2 µg/L week5 #1 pvMT11	**17D**	0.2 µg/L week5 #3 pvMT11

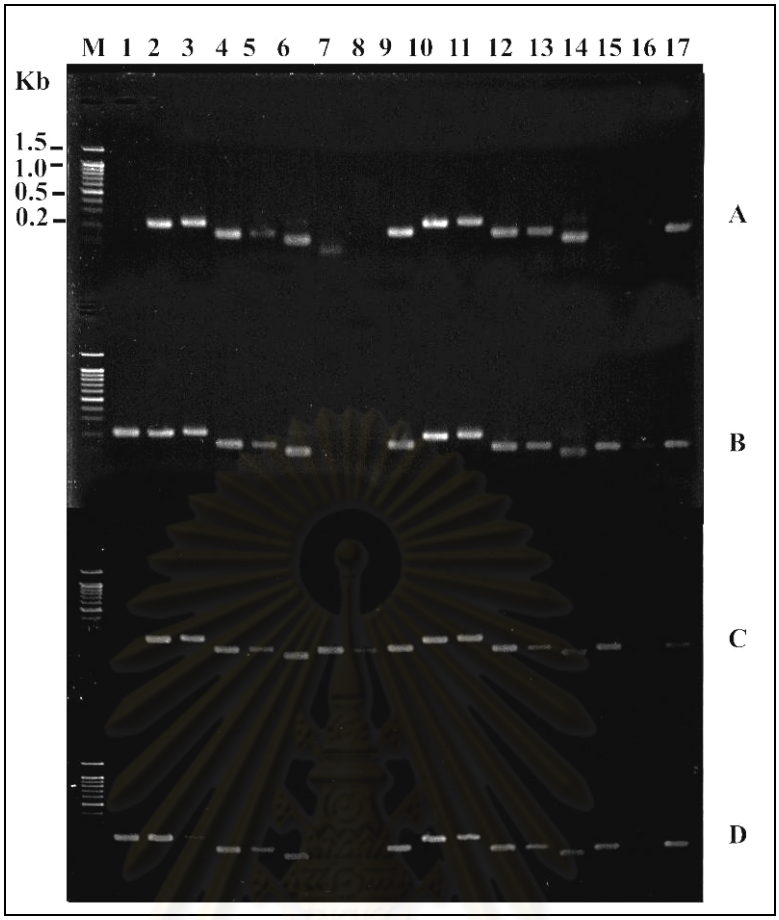

Figure B10 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.5 µg/L week5 #1 β-actin	**2B**	0.5 µg/L week5 #3 β-actin	**2C**	1.0 µg/L week5 #2 β-actin	**2D**	Control week6 #1 β-actin
Lane 3A	0.5 µg/L week5 #1 MT	**3B**	0.5 µg/L week5 #3 MT	**3C**	1.0 µg/L week5 #2 MT	**3D**	Control week6 #1 MT
Lane 4A	0.5 µg/L week5 #1 pvMT01	**4B**	0.5 µg/L week5 #3 pvMT01	**4C**	1.0 µg/L week5 #2 pvMT01	**4D**	Control week6 #1 pvMT01
Lane 5A	0.5 µg/L week5 #1 pvMT02	**5B**	0.5 µg/L week5 #3 pvMT02	**5C**	1.0 µg/L week5 #2 pvMT02	**5D**	Control week6 #1 pvMT02
Lane 6A	0.5 µg/L week5 #1 pvMT03	**6B**	0.5 µg/L week5 #3 pvMT03	**6C**	1.0 µg/L week5 #2 pvMT03	**6D**	Control week6 #1 pvMT03
Lane 7A	0.5 µg/L week5 #1 pvMT07	**7B**	0.5 µg/L week5 #3 pvMT07	**7C**	1.0 µg/L week5 #2 pvMT07	**7D**	Control week6 #1 pvMT07
Lane 8A	0.5 µg/L week5 #1 pvMT08	**8B**	0.5 µg/L week5 #3 pvMT08	**8C**	1.0 µg/L week5 #2 pvMT08	**8D**	Control week6 #1 pvMT08
Lane 9A	0.5 µg/L week5 #1 pvMT11	**9B**	0.5 µg/L week5 #3 pvMT11	**9C**	1.0 µg/L week5 #2 pvMT11	**9D**	Control week6 #1 pvMT11
Lane 10A	0.5 µg/L week5 #2 β-actin	**10B**	1.0 µg/L week5 #1 β-actin	**10C**	1.0 µg/L week5 #3 β-actin	**10D**	Control week6 #2 β-actin
Lane 11A	0.5 µg/L week5 #2 MT	**11B**	1.0 µg/L week5 #1 MT	**11C**	1.0 µg/L week5 #3 MT	**11D**	Control week6 #2 MT
Lane 12A	0.5 µg/L week5 #2 pvMT01	**12B**	1.0 µg/L week5 #1 pvMT01	**12C**	1.0 µg/L week5 #3 pvMT01	**12D**	Control week6 #2 pvMT01
Lane 13A	0.5 µg/L week5 #2 pvMT02	**13B**	1.0 µg/L week5 #1 pvMT02	**13C**	1.0 µg/L week5 #3 pvMT02	**13D**	Control week6 #2 pvMT02
Lane 14A	0.5 µg/L week5 #2 pvMT03	**14B**	1.0 µg/L week5 #1 pvMT03	**14C**	1.0 µg/L week5 #3 pvMT03	**14D**	Control week6 #2 pvMT03
Lane 15A	0.5 µg/L week5 #2 pvMT07	**15B**	1.0 µg/L week5 #1 pvMT07	**15C**	1.0 µg/L week5 #3 pvMT07	**15D**	Control week6 #2 pvMT07
Lane 16A	0.5 µg/L week5 #2 pvMT08	**16B**	1.0 µg/L week5 #1 pvMT08	**16C**	1.0 µg/L week5 #3 pvMT08	**16D**	Control week6 #2 pvMT08
Lane 17A	0.5 µg/L week5 #2 pvMT11	**17B**	1.0 µg/L week5 #1 pvMT11	**17C**	1.0 µg/L week5 #3 pvMT11	**17D**	Control week6 #2 pvMT11

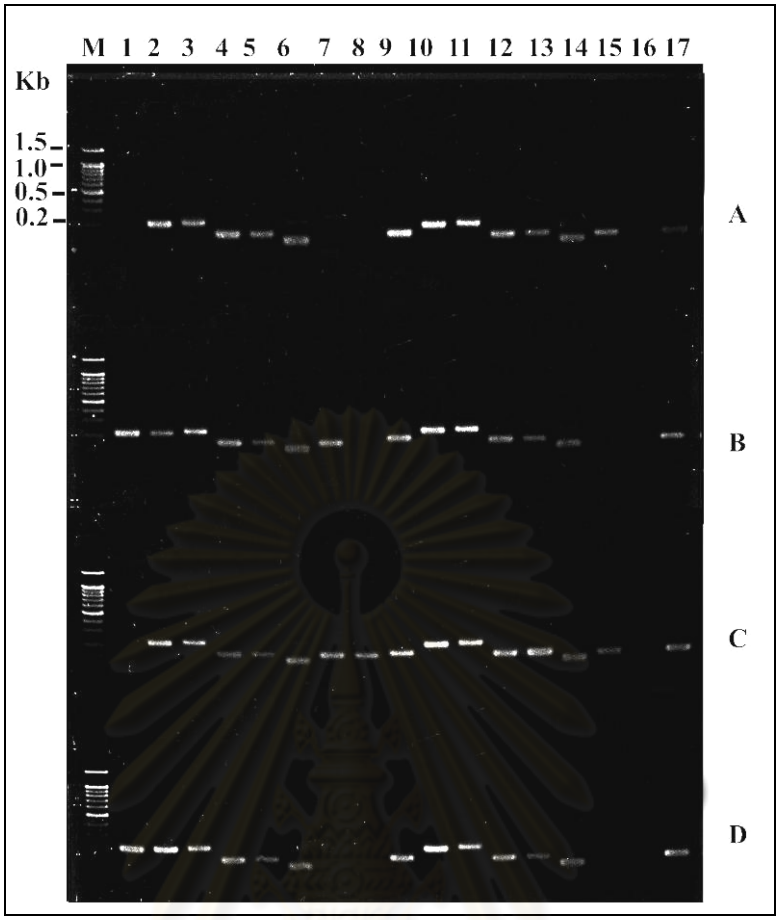

Figure B11 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	Control week6 #3 β-actin	**2B**	0.1 µg/L week6 #2 β-actin	**2C**	0.2 µg/L week6 #1 β-actin	**2D**	0.2 µg/L week6 #3 β-actin
Lane 3A	Control week6 #3 MT	**3B**	0.1 µg/L week6 #2 MT	**3C**	0.2 µg/L week6 #1 MT	**3D**	0.2 µg/L week6 #3 MT
Lane 4A	Control week6 #3 pvMT01	**4B**	0.1 µg/L week6 #2 pvMT01	**4C**	0.2 µg/L week6 #1 pvMT01	**4D**	0.2 µg/L week6 #3 pvMT01
Lane 5A	Control week6 #3 pvMT02	**5B**	0.1 µg/L week6 #2 pvMT02	**5C**	0.2 µg/L week6 #1 pvMT02	**5D**	0.2 µg/L week6 #3 pvMT02
Lane 6A	Control week6 #3 pvMT03	**6B**	0.1 µg/L week6 #2 pvMT03	**6C**	0.2 µg/L week6 #1 pvMT03	**6D**	0.2 µg/L week6 #3 pvMT03
Lane 7A	Control week6 #3 pvMT07	**7B**	0.1 µg/L week6 #2 pvMT07	**7C**	0.2 µg/L week6 #1 pvMT07	**7D**	0.2 µg/L week6 #3 pvMT07
Lane 8A	Control week6 #3 pvMT08	**8B**	0.1 µg/L week6 #2 pvMT08	**8C**	0.2 µg/L week6 #1 pvMT08	**8D**	0.2 µg/L week6 #3 pvMT08
Lane 9A	Control week6 #3 pvMT11	**9B**	0.1 µg/L week6 #2 pvMT11	**9C**	0.2 µg/L week6 #1 pvMT11	**9D**	0.2 µg/L week6 #3 pvMT11
Lane 10A	0.1 µg/L week6 #1 β-actin	**10B**	0.1 µg/L week6 #3 β-actin	**10C**	0.2 µg/L week6 #2 β-actin	**10D**	0.5 µg/L week6 #1 β-actin
Lane 11A	0.1 µg/L week6 #1 MT	**11B**	0.1 µg/L week6 #3 MT	**11C**	0.2 µg/L week6 #2 MT	**11D**	0.5 µg/L week6 #1 MT
Lane 12A	0.1 µg/L week6 #1 pvMT01	**12B**	0.1 µg/L week6 #3 pvMT01	**12C**	0.2 µg/L week6 #2 pvMT01	**12D**	0.5 µg/L week6 #1 pvMT01
Lane 13A	0.1 µg/L week6 #1 pvMT02	**13B**	0.1 µg/L week6 #3 pvMT02	**13C**	0.2 µg/L week6 #2 pvMT02	**13D**	0.5 µg/L week6 #1 pvMT02
Lane 14A	0.1 µg/L week6 #1 pvMT03	**14B**	0.1 µg/L week6 #3 pvMT03	**14C**	0.2 µg/L week6 #2 pvMT03	**14D**	0.5 µg/L week6 #1 pvMT03
Lane 15A	0.1 µg/L week6 #1 pvMT07	**15B**	0.1 µg/L week6 #3 pvMT07	**15C**	0.2 µg/L week6 #2 pvMT07	**15D**	0.5 µg/L week6 #1 pvMT07
Lane 16A	0.1 µg/L week6 #1 pvMT08	**16B**	0.1 µg/L week6 #3 pvMT08	**16C**	0.2 µg/L week6 #2 pvMT08	**16D**	0.5 µg/L week6 #1 pvMT08
Lane 17A	0.1 µg/L week6 #1 pvMT11	**17B**	0.1 µg/L week6 #3 pvMT11	**17C**	0.2 µg/L week6 #2 pvMT11	**17D**	0.5 µg/L week6 #1 pvMT11

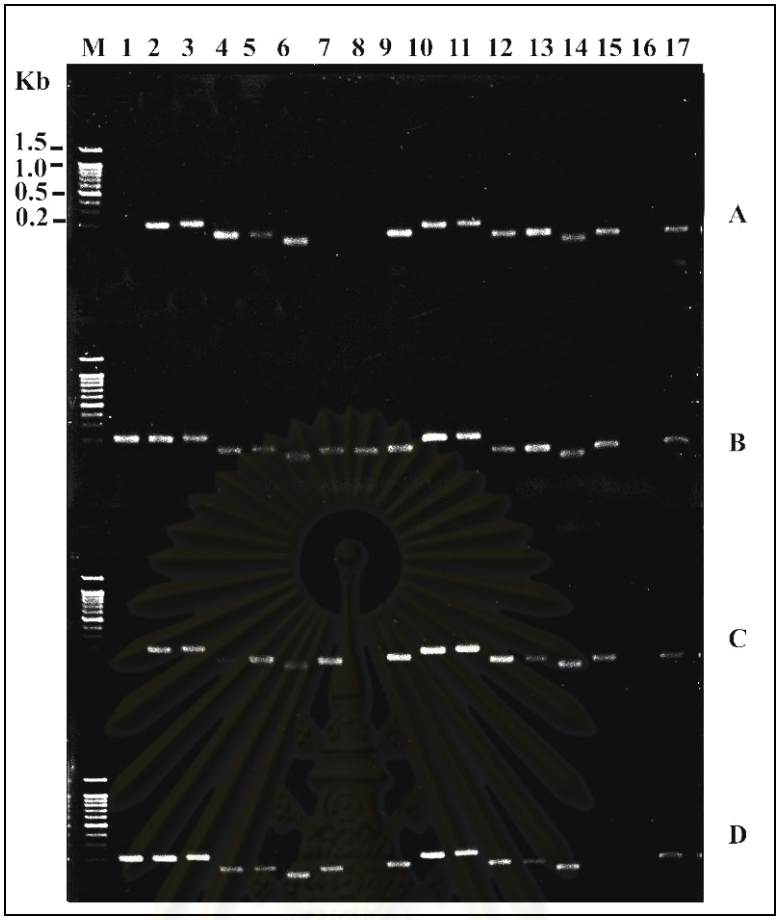

Figure B12 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.5 µg/L week6 #2 β-actin	**2B**	1.0 µg/L week6 #1 β-actin	**2C**	1.0 µg/L week6 #3 β-actin	**2D**	Control week7 #2 β-actin
Lane 3A	0.5 µg/L week6 #2 MT	**3B**	1.0 µg/L week6 #1 MT	**3C**	1.0 µg/L week6 #3 MT	**3D**	Control week7 #2 MT
Lane 4A	0.5 µg/L week6 #2 pvMT01	**4B**	1.0 µg/L week6 #1 pvMT01	**4C**	1.0 µg/L week6 #3 pvMT01	**4D**	Control week7 #2 pvMT01
Lane 5A	0.5 µg/L week6 #2 pvMT02	**5B**	1.0 µg/L week6 #1 pvMT02	**5C**	1.0 µg/L week6 #3 pvMT02	**5D**	Control week7 #2 pvMT02
Lane 6A	0.5 µg/L week6 #2 pvMT03	**6B**	1.0 µg/L week6 #1 pvMT03	**6C**	1.0 µg/L week6 #3 pvMT03	**6D**	Control week7 #2 pvMT03
Lane 7A	0.5 µg/L week6 #2 pvMT07	**7B**	1.0 µg/L week6 #1 pvMT07	**7C**	1.0 µg/L week6 #3 pvMT07	**7D**	Control week7 #2 pvMT07
Lane 8A	0.5 µg/L week6 #2 pvMT08	**8B**	1.0 µg/L week6 #1 pvMT08	**8C**	1.0 µg/L week6 #3 pvMT08	**8D**	Control week7 #2 pvMT08
Lane 9A	0.5 µg/L week6 #2 pvMT11	**9B**	1.0 µg/L week6 #1 pvMT11	**9C**	1.0 µg/L week6 #3 pvMT11	**9D**	Control week7 #2 pvMT11
Lane 10A	0.5 µg/L week6 #3 β-actin	**10B**	1.0 µg/L week6 #2 β-actin	**10C**	Control week7 #1 β-actin	**10D**	Control week7 #3 β-actin
Lane 11A	0.5 µg/L week6 #3 MT	**11B**	1.0 µg/L week6 #2 MT	**11C**	Control week7 #1 MT	**11D**	Control week7 #3 MT
Lane 12A	0.5 µg/L week6 #3 pvMT01	**12B**	1.0 µg/L week6 #2 pvMT01	**12C**	Control week7 #1 pvMT01	**12D**	Control week7 #3 pvMT01
Lane 13A	0.5 µg/L week6 #3 pvMT02	**13B**	1.0 µg/L week6 #2 pvMT02	**13C**	Control week7 #1 pvMT02	**13D**	Control week7 #3 pvMT02
Lane 14A	0.5 µg/L week6 #3 pvMT03	**14B**	1.0 µg/L week6 #2 pvMT03	**14C**	Control week7 #1 pvMT03	**14D**	Control week7 #3 pvMT03
Lane 15A	0.5 µg/L week6 #3 pvMT07	**15B**	1.0 µg/L week6 #2 pvMT07	**15C**	Control week7 #1 pvMT07	**15D**	Control week7 #3 pvMT07
Lane 16A	0.5 µg/L week6 #3 pvMT08	**16B**	1.0 µg/L week6 #2 pvMT08	**16C**	Control week7 #1 pvMT08	**16D**	Control week7 #3 pvMT08
Lane 17A	0.5 µg/L week6 #3 pvMT11	**17B**	1.0 µg/L week6 #2 pvMT11	**17C**	Control week7 #1 pvMT11	**17D**	Control week7 #3 pvMT11

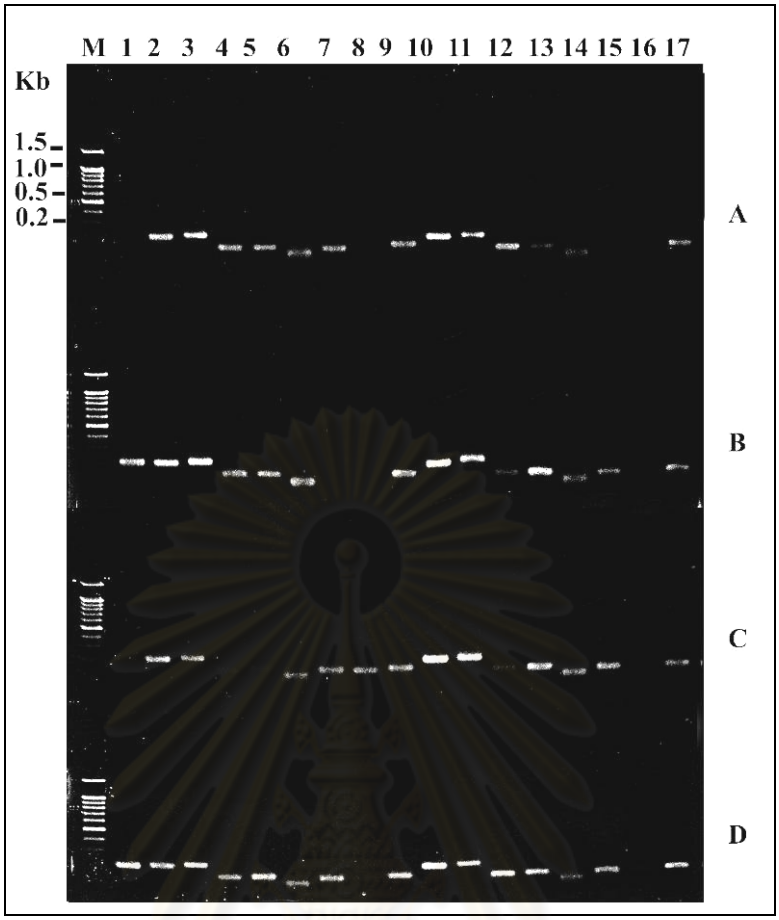

Figure B13 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.1 µg/L week7 #1 β-actin	**2B**	0.1 µg/L week7 #3 β-actin	**2C**	0.2 µg/L week7 #2 β-actin	**2D**	1.0 µg/L week7 #1 β-actin
Lane 3A	0.1 µg/L week7 #1 MT	**3B**	0.1 µg/L week7 #3 MT	**3C**	0.2 µg/L week7 #2 MT	**3D**	1.0 µg/L week7 #1 MT
Lane 4A	0.1 µg/L week7 #1 pvMT01	**4B**	0.1 µg/L week7 #3 pvMT01	**4C**	0.2 µg/L week7 #2 pvMT01	**4D**	1.0 µg/L week7 #1 pvMT01
Lane 5A	0.1 µg/L week7 #1 pvMT02	**5B**	0.1 µg/L week7 #3 pvMT02	**5C**	0.2 µg/L week7 #2 pvMT02	**5D**	1.0 µg/L week7 #1 pvMT02
Lane 6A	0.1 µg/L week7 #1 pvMT03	**6B**	0.1 µg/L week7 #3 pvMT03	**6C**	0.2 µg/L week7 #2 pvMT03	**6D**	1.0 µg/L week7 #1 pvMT03
Lane 7A	0.1 µg/L week7 #1 pvMT07	**7B**	0.1 µg/L week7 #3 pvMT07	**7C**	0.2 µg/L week7 #2 pvMT07	**7D**	1.0 µg/L week7 #1 pvMT07
Lane 8A	0.1 µg/L week7 #1 pvMT08	**8B**	0.1 µg/L week7 #3 pvMT08	**8C**	0.2 µg/L week7 #2 pvMT08	**8D**	1.0 µg/L week7 #1 pvMT08
Lane 9A	0.1 µg/L week7 #1 pvMT11	**9B**	0.1 µg/L week7 #3 pvMT11	**9C**	0.2 µg/L week7 #2 pvMT11	**9D**	1.0 µg/L week7 #1 pvMT11
Lane 10A	0.1 µg/L week7 #2 β-actin	**10B**	0.2 µg/L week7 #1 β-actin	**10C**	0.2 µg/L week7 #3 β-actin	**10D**	1.0 µg/L week7 #2 β-actin
Lane 11A	0.1 µg/L week7 #2 MT	**11B**	0.2 µg/L week7 #1 MT	**11C**	0.2 µg/L week7 #3 MT	**11D**	1.0 µg/L week7 #2 MT
Lane 12A	0.1 µg/L week7 #2 pvMT01	**12B**	0.2 µg/L week7 #1 pvMT01	**12C**	0.2 µg/L week7 #3 pvMT01	**12D**	1.0 µg/L week7 #2 pvMT01
Lane 13A	0.1 µg/L week7 #2 pvMT02	**13B**	0.2 µg/L week7 #1 pvMT02	**13C**	0.2 µg/L week7 #3 pvMT02	**13D**	1.0 µg/L week7 #2 pvMT02
Lane 14A	0.1 µg/L week7 #2 pvMT03	**14B**	0.2 µg/L week7 #1 pvMT03	**14C**	0.2 µg/L week7 #3 pvMT03	**14D**	1.0 µg/L week7 #2 pvMT03
Lane 15A	0.1 µg/L week7 #2 pvMT07	**15B**	0.2 µg/L week7 #1 pvMT07	**15C**	0.2 µg/L week7 #3 pvMT07	**15D**	1.0 µg/L week7 #2 pvMT07
Lane 16A	0.1 µg/L week7 #2 pvMT08	**16B**	0.2 µg/L week7 #1 pvMT08	**16C**	0.2 µg/L week7 #3 pvMT08	**16D**	1.0 µg/L week7 #2 pvMT08
Lane 17A	0.1 µg/L week7 #2 pvMT11	**17B**	0.2 µg/L week7 #1 pvMT11	**17C**	0.2 µg/L week7 #3 pvMT11	**17D**	1.0 µg/L week7 #2 pvMT11

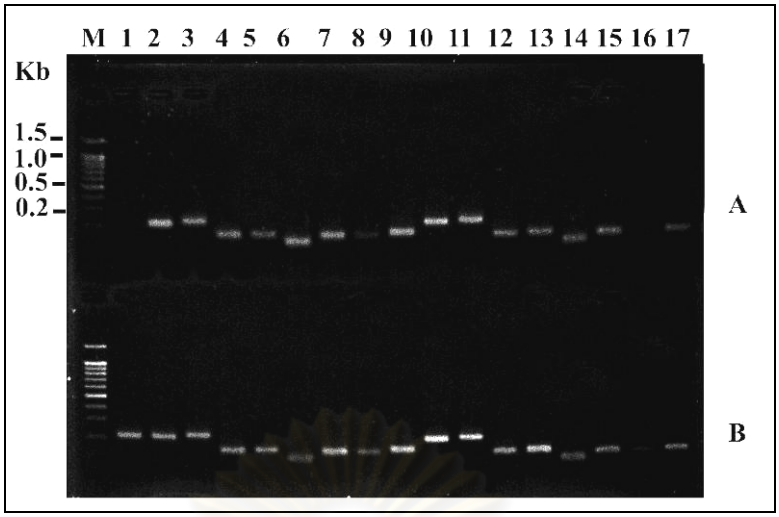

Figure B14 PCR product of MT, pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**					
Lane 1A	Negative Control	**1B**	Positive Control β-actin				
Lane 2A	1.0 µg/L week7 #3 β-actin	**2B**	0.1 µg/L week8 #2 β-actin				
Lane 3A	1.0 µg/L week7 #3 MT	**3B**	0.1 µg/L week8 #2 MT				
Lane 4A	1.0 µg/L week7 #3 pvMT01	**4B**	0.1 µg/L week8 #2 pvMT01				
Lane 5A	1.0 µg/L week7 #3 pvMT02	**5B**	0.1 µg/L week8 #2 pvMT02				
Lane 6A	1.0 µg/L week7 #3 pvMT03	**6B**	0.1 µg/L week8 #2 pvMT03				
Lane 7A	1.0 µg/L week7 #3 pvMT07	**7B**	0.1 µg/L week8 #2 pvMT07				
Lane 8A	1.0 µg/L week7 #3 pvMT08	**8B**	0.1 µg/L week8 #2 pvMT08				
Lane 9A	1.0 µg/L week7 #3 pvMT11	**9B**	0.1 µg/L week8 #2 pvMT11				
Lane 10A	0.1 µg/L week8 #1 β-actin	**10B**	0.1 µg/L week8 #3 β-actin				
Lane 11A	0.1 µg/L week8 #1 MT	**11B**	0.1 µg/L week8 #3 MT				
Lane 12A	0.1 µg/L week8 #1 pvMT01	**12B**	0.1 µg/L week8 #3 pvMT01				
Lane 13A	0.1 µg/L week8 #1 pvMT02	**13B**	0.1 µg/L week8 #3 pvMT02				
Lane 14A	0.1 µg/L week8 #1 pvMT03	**14B**	0.1 µg/L week8 #3 pvMT03				
Lane 15A	0.1 µg/L week8 #1 pvMT07	**15B**	0.1 µg/L week8 #3 pvMT07				
Lane 16A	0.1 µg/L week8 #1 pvMT08	**16B**	0.1 µg/L week8 #3 pvMT08				
Lane 17A	0.1 µg/L week8 #1 pvMT11	**17B**	0.1 µg/L week8 #3 pvMT11				

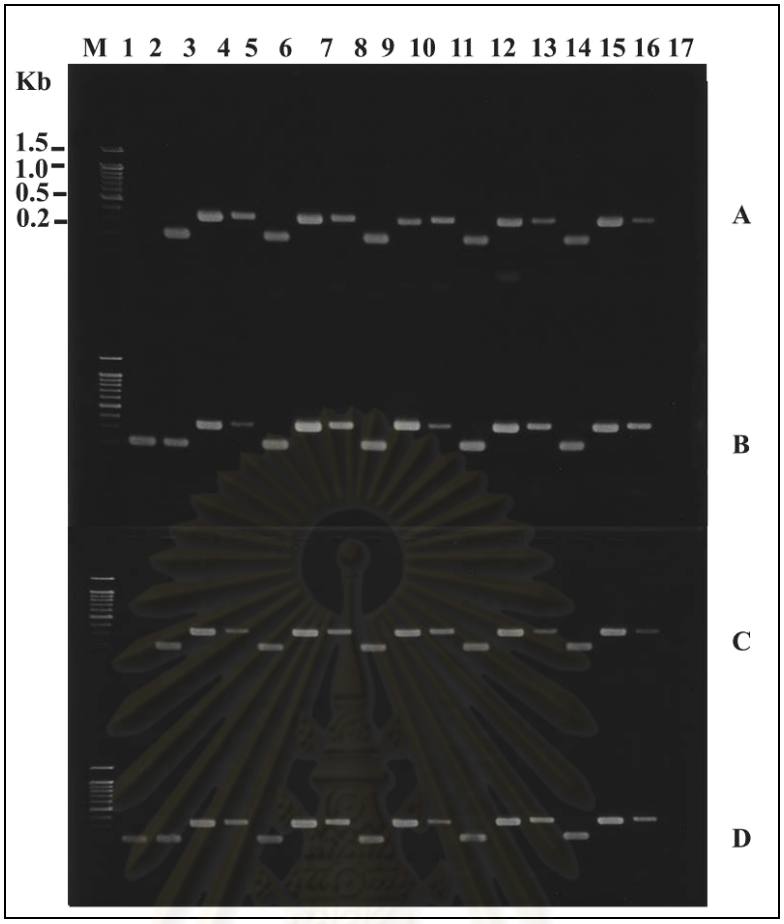

Figure B15 PCR products of HSP71 and CYP4 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	Control week1 #1 β-actin	**2B**	0.1 µg/L week1 #3 β-actin	**2C**	0.5 µg/L week1 #2 β-actin	**2D**	Control week2 #1 β-actin
Lane 3A	Control week1 #1 HSP71	**3B**	0.1 µg/L week1 #3 HSP71	**3C**	0.5 µg/L week1 #2 HSP71	**3D**	Control week2 #1 HSP71
Lane 4A	Control week1 #1 CYP450	**4B**	0.1 µg/L week1 #3 CYP450	**4C**	0.5 µg/L week1 #2 CYP450	**4D**	Control week2 #1 CYP450
Lane 5A	Control week1 #2 β-actin	**5B**	0.2 µg/L week1 #1 β-actin	**5C**	0.5 µg/L week1 #3 β-actin	**5D**	Control week2 #2 β-actin
Lane 6A	Control week1 #2 HSP71	**6B**	0.2 µg/L week1 #1 HSP71	**6C**	0.5 µg/L week1 #3 HSP71	**6D**	Control week2 #2 HSP71
Lane 7A	Control week1 #2 CYP450	**7B**	0.2 µg/L week1 #1 CYP450	**7C**	0.5 µg/L week1 #3 CYP450	**7D**	Control week2 #2 CYP450
Lane 8A	Control week1 #3 β-actin	**8B**	0.2 µg/L week1 #2 β-actin	**8C**	1.0 µg/L week1 #1 β-actin	**8D**	Control week2 #3 β-actin
Lane 9A	Control week1 #3 HSP71	**9B**	0.2 µg/L week1 #2 HSP71	**9C**	1.0 µg/L week1 #1 HSP71	**9D**	Control week2 #3 HSP71
Lane 10A	Control week1 #3 CYP450	**10B**	0.2 µg/L week1 #2 CYP450	**10C**	1.0 µg/L week1 #1 CYP450	**10D**	Control week2 #3 CYP450
Lane 11A	0.1 µg/L week1 #1 β-actin	**11B**	0.2 µg/L week1 #3 β-actin	**11C**	1.0 µg/L week1 #2 β-actin	**11D**	0.1 µg/L week2 #1 β-actin
Lane 12A	0.1 µg/L week1 #1 HSP71	**12B**	0.2 µg/L week1 #3 HSP71	**12C**	1.0 µg/L week1 #2 HSP71	**12D**	0.1 µg/L week2 #1 HSP71
Lane 13A	0.1 µg/L week1 #1 CYP450	**13B**	0.2 µg/L week1 #3 CYP450	**13C**	1.0 µg/L week1 #2 CYP450	**13D**	0.1 µg/L week2 #1 CYP450
Lane 14A	0.1 µg/L week1 #2 β-actin	**14B**	0.5 µg/L week1 #1 β-actin	**14C**	1.0 µg/L week1 #3 β-actin	**14D**	0.1 µg/L week2 #2 β-actin
Lane 15A	0.1 µg/L week1 #2 HSP71	**15B**	0.5 µg/L week1 #1 HSP71	**15C**	1.0 µg/L week1 #3 HSP71	**15D**	0.1 µg/L week2 #2 HSP71
Lane 16A	0.1 µg/L week1 #2 CYP450	**16B**	0.5 µg/L week1 #1 CYP450	**16C**	1.0 µg/L week1 #3 CYP450	**16D**	0.1 µg/L week2 #2 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	

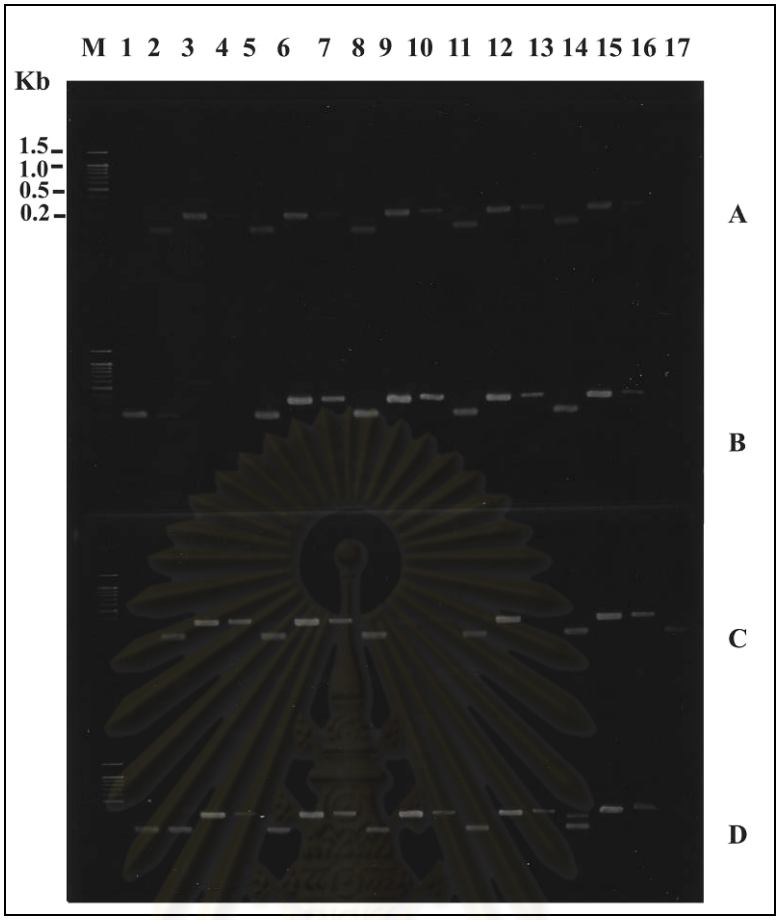

Figure B16 PCR products of HSP71 and CYP4 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.1 µg/L week2 #2 β-actin	**2B**	0.5 µg/L week2 #2 β-actin	**2C**	Control week3 #1 β-actin	**2D**	0.1 µg/L week3 #2 β-actin
Lane 3A	0.1 µg/L week2 #2 HSP71	**3B**	0.5 µg/L week2 #2 HSP71	**3C**	Control week3 #1 HSP71	**3D**	0.1 µg/L week3 #2 HSP71
Lane 4A	0.1 µg/L week2 #2 CYP450	**4B**	0.5 µg/L week2 #2 CYP450	**4C**	Control week3 #1 CYP450	**4D**	0.1 µg/L week3 #2 CYP450
Lane 5A	0.2 µg/L week2 #1 β-actin	**5B**	0.5 µg/L week2 #3 β-actin	**5C**	Control week3 #2 β-actin	**5D**	0.2 µg/L week3 #1 β-actin
Lane 6A	0.2 µg/L week2 #1 HSP71	**6B**	0.5 µg/L week2 #3 HSP71	**6C**	Control week3 #2 HSP71	**6D**	0.2 µg/L week3 #1 HSP71
Lane 7A	0.2 µg/L week2 #1 CYP450	**7B**	0.5 µg/L week2 #3 CYP450	**7C**	Control week3 #2 CYP450	**7D**	0.2 µg/L week3 #1 CYP450
Lane 8A	0.2 µg/L week2 #2 β-actin	**8B**	1.0 µg/L week2 #1 β-actin	**8C**	Control week3 #3 β-actin	**8D**	0.2 µg/L week3 #2 β-actin
Lane 9A	0.2 µg/L week2 #2 HSP71	**9B**	1.0 µg/L week2 #1 HSP71	**9C**	Control week3 #3 HSP71	**9D**	0.2 µg/L week3 #2 HSP71
Lane 10A	0.2 µg/L week2 #2 CYP450	**10B**	1.0 µg/L week2 #1 CYP450	**10C**	Control week3 #3 CYP450	**10D**	0.2 µg/L week3 #2 CYP450
Lane 11A	0.2 µg/L week2 #3 β-actin	**11B**	1.0 µg/L week2 #2 β-actin	**11C**	0.1 µg/L week3 #1 β-actin	**11D**	0.2 µg/L week3 #3 β-actin
Lane 12A	0.2 µg/L week2 #3 HSP71	**12B**	1.0 µg/L week2 #2 HSP71	**12C**	0.1 µg/L week3 #1 HSP71	**12D**	0.2 µg/L week3 #3 HSP71
Lane 13A	0.2 µg/L week2 #3 CYP450	**13B**	1.0 µg/L week2 #2 CYP450	**13C**	0.1 µg/L week3 #1 CYP450	**13D**	0.2 µg/L week3 #3 CYP450
Lane 14A	0.5 µg/L week2 #1 β-actin	**14B**	1.0 µg/L week2 #3 β-actin	**14C**	0.1 µg/L week3 #2 β-actin	**14D**	0.5 µg/L week3 #1 β-actin
Lane 15A	0.5 µg/L week2 #1 HSP71	**15B**	1.0 µg/L week2 #3 HSP71	**15C**	0.1 µg/L week3 #2 HSP71	**15D**	0.5 µg/L week3 #1 HSP71
Lane 16A	0.5 µg/L week2 #1 CYP450	**16B**	1.0 µg/L week2 #3 CYP450	**16C**	0.1 µg/L week3 #2 CYP450	**16D**	0.5 µg/L week3 #1 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	Blank

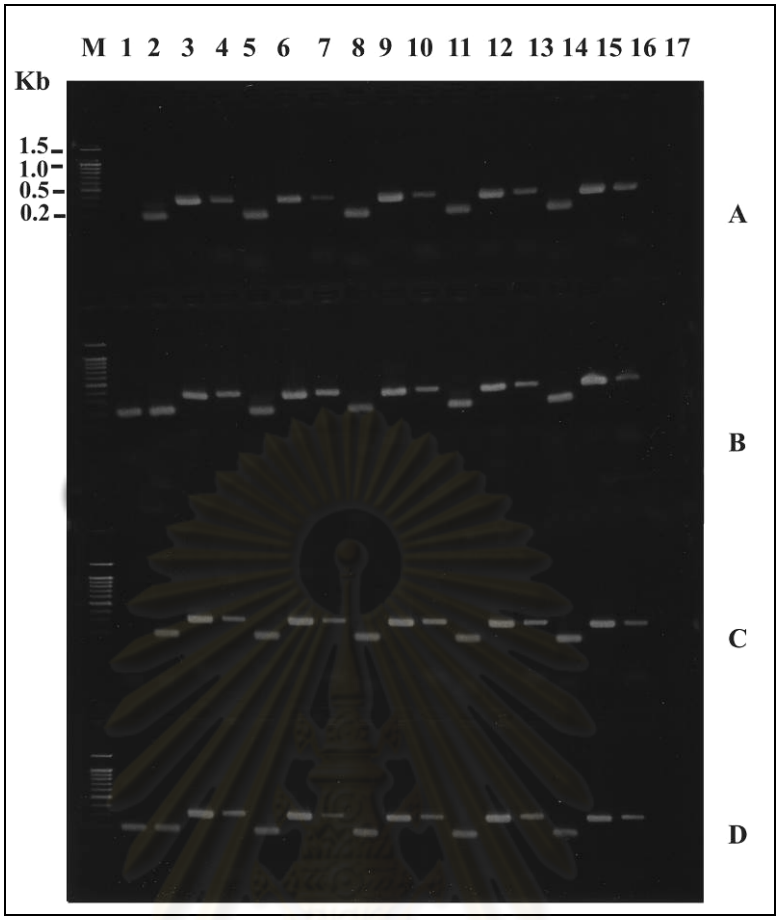

Figure B17 PCR products of HSP71 and CYP4 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.5 µg/L week3 #2 β-actin	**2B**	Control week4 #1 β-actin	**2C**	0.1 µg/L week4 #2 β-actin	**2D**	0.5 µg/L week4 #2 β-actin
Lane 3A	0.5 µg/L week3 #2 HSP71	**3B**	Control week4 #1 HSP71	**3C**	0.1 µg/L week4 #2 HSP71	**3D**	0.5 µg/L week4 #2 HSP71
Lane 4A	0.5 µg/L week3 #2 CYP450	**4B**	Control week4 #1 CYP450	**4C**	0.1 µg/L week4 #2 CYP450	**4D**	0.5 µg/L week4 #2 CYP450
Lane 5A	0.5 µg/L week3 #3 β-actin	**5B**	Control week4 #2 β-actin	**5C**	0.2 µg/L week4 #1 β-actin	**5D**	0.5 µg/L week4 #3 β-actin
Lane 6A	0.5 µg/L week3 #3 HSP71	**6B**	Control week4 #2 HSP71	**6C**	0.2 µg/L week4 #1 HSP71	**6D**	0.5 µg/L week4 #3 HSP71
Lane 7A	0.5 µg/L week3 #3 CYP450	**7B**	Control week4 #2 CYP450	**7C**	0.2 µg/L week4 #1 CYP450	**7D**	0.5 µg/L week4 #3 CYP450
Lane 8A	1.0 µg/L week3 #1 β-actin	**8B**	Control week4 #3 β-actin	**8C**	0.2 µg/L week4 #2 β-actin	**8D**	1.0 µg/L week4 #1 β-actin
Lane 9A	1.0 µg/L week3 #1 HSP71	**9B**	Control week4 #3 HSP71	**9C**	0.2 µg/L week4 #2 HSP71	**9D**	1.0 µg/L week4 #1 HSP71
Lane 10A	1.0 µg/L week3 #1 CYP450	**10B**	Control week4 #3 CYP450	**10C**	0.2 µg/L week4 #2 CYP450	**10D**	1.0 µg/L week4 #1 CYP450
Lane 11A	1.0 µg/L week3 #2 β-actin	**11B**	0.1 µg/L week4 #1 β-actin	**11C**	0.2 µg/L week4 #3 β-actin	**11D**	1.0 µg/L week4 #2 β-actin
Lane 12A	1.0 µg/L week3 #2 HSP71	**12B**	0.1 µg/L week4 #1 HSP71	**12C**	0.2 µg/L week4 #3 HSP71	**12D**	1.0 µg/L week4 #2 HSP71
Lane 13A	1.0 µg/L week3 #2 CYP450	**13B**	0.1 µg/L week4 #1 CYP450	**13C**	0.2 µg/L week4 #3 CYP450	**13D**	1.0 µg/L week4 #2 CYP450
Lane 14A	1.0 µg/L week3 #3 β-actin	**14B**	0.1 µg/L week4 #2 β-actin	**14C**	0.5 µg/L week4 #1 β-actin	**14D**	1.0 µg/L week4 #3 β-actin
Lane 15A	1.0 µg/L week3 #3 HSP71	**15B**	0.1 µg/L week4 #2 HSP71	**15C**	0.5 µg/L week4 #1 HSP71	**15D**	1.0 µg/L week4 #3 HSP71
Lane 16A	1.0 µg/L week3 #3 CYP450	**16B**	0.1 µg/L week4 #2 CYP450	**16C**	0.5 µg/L week4 #1 CYP450	**16D**	1.0 µg/L week4 #3 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	Blank

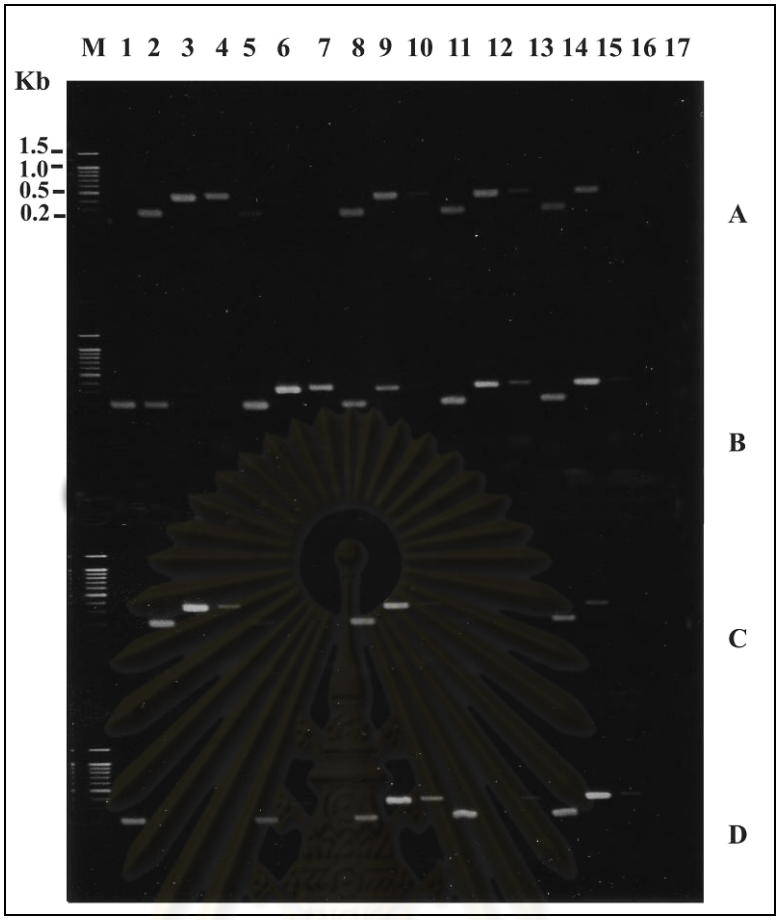

Figure B18 PCR products of HSP71 and CYP4 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	Control week5 #1 β-actin	**2B**	0.1 µg/L week5 #2 β-actin	**2C**	0.5 µg/L week5 #2 β-actin	**2D**	Control week6 #1 β-actin
Lane 3A	Control week5 #1 HSP71	**3B**	0.1 µg/L week5 #2 HSP71	**3C**	0.5 µg/L week5 #2 HSP71	**3D**	Control week6 #1 HSP71
Lane 4A	Control week5 #1 CYP450	**4B**	0.1 µg/L week5 #2 CYP450	**4C**	0.5 µg/L week5 #2 CYP450	**4D**	Control week6 #1 CYP450
Lane 5A	Control week5 #2 β-actin	**5B**	0.2 µg/L week5 #1 β-actin	**5C**	0.5 µg/L week5 #3 β-actin	**5D**	Control week6 #2 β-actin
Lane 6A	Control week5 #2 HSP71	**6B**	0.2 µg/L week5 #1 HSP71	**6C**	0.5 µg/L week5 #3 HSP71	**6D**	Control week6 #2 HSP71
Lane 7A	Control week5 #2 CYP450	**7B**	0.2 µg/L week5 #1 CYP450	**7C**	0.5 µg/L week5 #3 CYP450	**7D**	Control week6 #2 CYP450
Lane 8A	Control week5 #3 β-actin	**8B**	0.2 µg/L week5 #2 β-actin	**8C**	1.0 µg/L week5 #1 β-actin	**8D**	Control week6 #3 β-actin
Lane 9A	Control week5 #3 HSP71	**9B**	0.2 µg/L week5 #2 HSP71	**9C**	1.0 µg/L week5 #1 HSP71	**9D**	Control week6 #3 HSP71
Lane 10A	Control week5 #3 CYP450	**10B**	0.2 µg/L week5 #2 CYP450	**10C**	1.0 µg/L week5 #1 CYP450	**10D**	Control week6 #3 CYP450
Lane 11A	0.1 µg/L week5 #1 β-actin	**11B**	0.2 µg/L week5 #3 β-actin	**11C**	1.0 µg/L week5 #2 β-actin	**11D**	0.1 µg/L week6 #1 β-actin
Lane 12A	0.1 µg/L week5 #1 HSP71	**12B**	0.2 µg/L week5 #3 HSP71	**12C**	1.0 µg/L week5 #2 HSP71	**12D**	0.1 µg/L week6 #1 HSP71
Lane 13A	0.1 µg/L week5 #1 CYP450	**13B**	0.2 µg/L week5 #3 CYP450	**13C**	1.0 µg/L week5 #2 CYP450	**13D**	0.1 µg/L week6 #1 CYP450
Lane 14A	0.1 µg/L week5 #2 β-actin	**14B**	0.5 µg/L week5 #1 β-actin	**14C**	1.0 µg/L week5 #3 β-actin	**14D**	0.1 µg/L week6 #2 β-actin
Lane 15A	0.1 µg/L week5 #2 HSP71	**15B**	0.5 µg/L week5 #1 HSP71	**15C**	1.0 µg/L week5 #3 HSP71	**15D**	0.1 µg/L week6 #2 HSP71
Lane 16A	0.1 µg/L week5 #2 CYP450	**16B**	0.5 µg/L week5 #1 CYP450	**16C**	1.0 µg/L week5 #3 CYP450	**16D**	0.1 µg/L week6 #2 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	

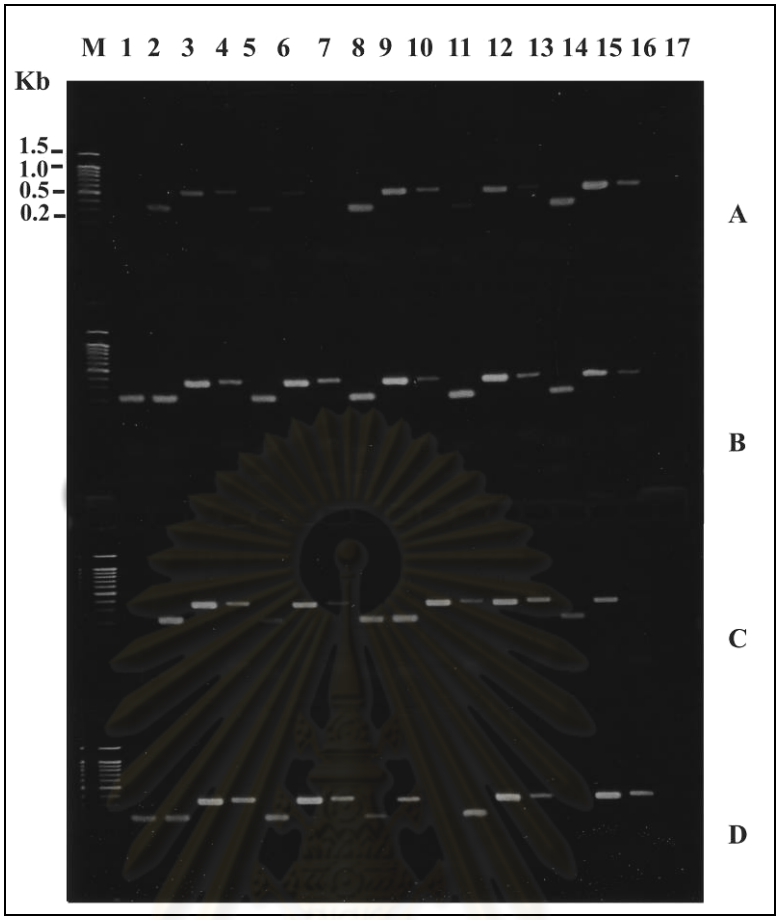

Figure B19 PCR products of HSP71 and CYP4 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	0.1 µg/L week6 #2 β-actin	**2B**	0.5 µg/L week6 #2 β-actin	**2C**	Control week7 #1 β-actin	**2D**	0.1 µg/L week7 #2 β-actin
Lane 3A	0.1 µg/L week6 #2 HSP71	**3B**	0.5 µg/L week6 #2 HSP71	**3C**	Control week7 #1 HSP71	**3D**	0.1 µg/L week7 #2 HSP71
Lane 4A	0.1 µg/L week6 #2 CYP450	**4B**	0.5 µg/L week6 #2 CYP450	**4C**	Control week7 #1 CYP450	**4D**	0.1 µg/L week7 #2 CYP450
Lane 5A	0.2 µg/L week6 #1 β-actin	**5B**	0.5 µg/L week6 #3 β-actin	**5C**	Control week7 #2 β-actin	**5D**	0.5 µg/L week7 #1 β-actin
Lane 6A	0.2 µg/L week6 #1 HSP71	**6B**	0.5 µg/L week6 #3 HSP71	**6C**	Control week7 #2 HSP71	**6D**	0.5 µg/L week7 #1 HSP71
Lane 7A	0.2 µg/L week6 #1 CYP450	**7B**	0.5 µg/L week6 #3 CYP450	**7C**	Control week7 #2 CYP450	**7D**	0.5 µg/L week7 #1 CYP450
Lane 8A	0.2 µg/L week6 #2 β-actin	**8B**	1.0 µg/L week6 #1 β-actin	**8C**	Control week7 #3 β-actin	**8D**	0.5 µg/L week7 #2 β-actin
Lane 9A	0.2 µg/L week6 #2 HSP71	**9B**	1.0 µg/L week6 #1 HSP71	**9C**	Control week7 #3 HSP71	**9D**	0.5 µg/L week7 #2 HSP71
Lane 10A	0.2 µg/L week6 #2 CYP450	**10B**	1.0 µg/L week6 #1 CYP450	**10C**	Control week7 #3 CYP450	**10D**	0.5 µg/L week7 #2 CYP450
Lane 11A	0.2 µg/L week6 #3 β-actin	**11B**	1.0 µg/L week6 #2 β-actin	**11C**	0.1 µg/L week7 #1 β-actin	**11D**	0.5 µg/L week7 #3 β-actin
Lane 12A	0.2 µg/L week6 #3 HSP71	**12B**	1.0 µg/L week6 #2 HSP71	**12C**	0.1 µg/L week7 #1 HSP71	**12D**	0.5 µg/L week7 #3 HSP71
Lane 13A	0.2 µg/L week6 #3 CYP450	**13B**	1.0 µg/L week6 #2 CYP450	**13C**	0.1 µg/L week7 #1 CYP450	**13D**	0.5 µg/L week7 #3 CYP450
Lane 14A	0.5 µg/L week6 #1 β-actin	**14B**	1.0 µg/L week6 #3 β-actin	**14C**	0.1 µg/L week7 #2 β-actin	**14D**	1.0 µg/L week7 #1 β-actin
Lane 15A	0.5 µg/L week6 #1 HSP71	**15B**	1.0 µg/L week6 #3 HSP71	**15C**	0.1 µg/L week7 #2 HSP71	**15D**	1.0 µg/L week7 #1 HSP71
Lane 16A	0.5 µg/L week6 #1 CYP450	**16B**	1.0 µg/L week6 #3 CYP450	**16C**	0.1 µg/L week7 #2 CYP450	**16D**	1.0 µg/L week7 #1 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	

Figure B20 PCR products of HSP71 and CYP4 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0% agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**					
Lane 1A	Negative Control	**1B**	Positive Control β-actin				
Lane 2A	1.0 µg/L week7 #3 β-actin	**2B**	Initial #2 β-actin				
Lane 3A	1.0 µg/L week7 #3 HSP71	**3B**	Initial #2 HSP71				
Lane 4A	1.0 µg/L week7 #3 CYP450	**4B**	Initial #2 CYP450				
Lane 5A	0.1 µg/L week8 #1 β-actin	**5B**	Initial #3 β-actin				
Lane 6A	0.1 µg/L week8 #1 HSP71	**6B**	Initial #3 HSP71				
Lane 7A	0.1 µg/L week8 #1 CYP450	**7B**	Initial #3 CYP450				
Lane 8A	0.1 µg/L week8 #2 β-actin						
Lane 9A	0.1 µg/L week8 #2 HSP71						
Lane 10A	0.1 µg/L week8 #2 CYP450						
Lane 11A	0.1 µg/L week8 #2 β-actin						
Lane 12A	0.1 µg/L week8 #2 HSP71						
Lane 13A	0.1 µg/L week8 #2 CYP450						
Lane 14A	Initial #1 β-actin						
Lane 15A	Initial #1 HSP71						
Lane 16A	Initial #1 CYP450						
Lane 17A	Blank						

PCR product of MTs, HSP71 and CYP4 gene (Field study)

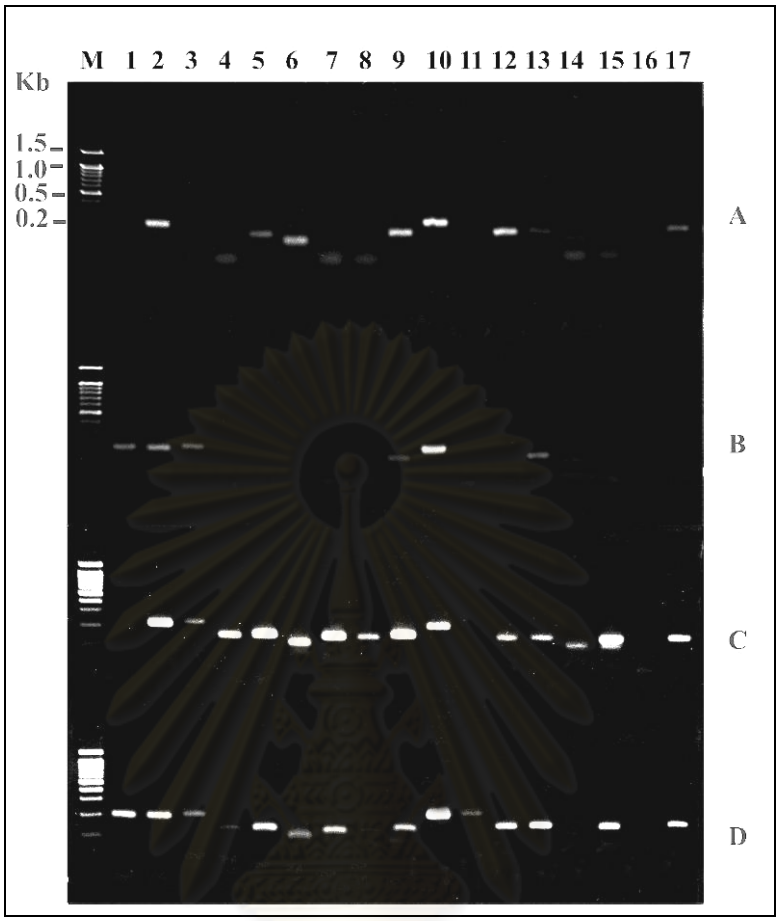

Figure B21 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT07 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.A 5M 30D # 1 β-actin	**2B**	St.A 5M 30D # 3 β-actin	**2C**	St.A 20M 30D # 2 β-actin	**2D**	St.A 40M 30D # 1 β-actin
Lane 3A	St.A 5M 30D # 1 MT	**3B**	St.A 5M 30D # 3 MT	**3C**	St.A 20M 30D # 2 MT	**3D**	St.A 40M 30D # 1 MT
Lane 4A	St.A 5M 30D # 1 pvMT01	**4B**	St.A 5M 30D # 3 pvMT01	**4C**	St.A 20M 30D # 2 pvMT01	**4D**	St.A 40M 30D # 1 pvMT01
Lane 5A	St.A 5M 30D # 1 pvMT02	**5B**	St.A 5M 30D # 3 pvMT02	**5C**	St.A 20M 30D # 2 pvMT02	**5D**	St.A 40M 30D # 1 pvMT02
Lane 6A	St.A 5M 30D # 1 pvMT03	**6B**	St.A 5M 30D # 3 pvMT03	**6C**	St.A 20M 30D # 2 pvMT03	**6D**	St.A 40M 30D # 1 pvMT03
Lane 7A	St.A 5M 30D # 1 pvMT07	**7B**	St.A 5M 30D # 3 pvMT07	**7C**	St.A 20M 30D # 2 pvMT07	**7D**	St.A 40M 30D # 1 pvMT07
Lane 8A	St.A 5M 30D # 1 pvMT08	**8B**	St.A 5M 30D # 3 pvMT08	**8C**	St.A 20M 30D # 2 pvMT08	**8D**	St.A 40M 30D # 1 pvMT08
Lane 9A	St.A 5M 30D # 1 pvMT11	**9B**	St.A 5M 30D # 3 pvMT11	**9C**	St.A 20M 30D # 2 pvMT11	**9D**	St.A 40M 30D # 1 pvMT11
Lane 10A	St.A 5M 30D # 2 β-actin	**10B**	St.A 20M 30D # 1 β-actin	**10C**	St.A 20M 30D # 3 β-actin	**10D**	St.A 40M 30D # 2 β-actin
Lane 11A	St.A 5M 30D # 2 MT	**11B**	St.A 20M 30D # 1 MT	**11C**	St.A 20M 30D # 3 MT	**11D**	St.A 40M 30D # 2 MT
Lane 12A	St.A 5M 30D # 2 pvMT01	**12B**	St.A 20M 30D # 1 pvMT01	**12C**	St.A 20M 30D # 3 pvMT01	**12D**	St.A 40M 30D # 2 pvMT01
Lane 13A	St.A 5M 30D # 2 pvMT02	**13B**	St.A 20M 30D # 1 pvMT02	**13C**	St.A 20M 30D # 3 pvMT02	**13D**	St.A 40M 30D # 2 pvMT02
Lane 14A	St.A 5M 30D # 2 pvMT03	**14B**	St.A 20M 30D # 1 pvMT03	**14C**	St.A 20M 30D # 3 pvMT03	**14D**	St.A 40M 30D # 2 pvMT03
Lane 15A	St.A 5M 30D # 2 pvMT07	**15B**	St.A 20M 30D # 1 pvMT07	**15C**	St.A 20M 30D # 3 pvMT07	**15D**	St.A 40M 30D # 2 pvMT07
Lane 16A	St.A 5M 30D # 2 pvMT08	**16B**	St.A 20M 30D # 1 pvMT08	**16C**	St.A 20M 30D # 3 pvMT08	**16D**	St.A 40M 30D # 2 pvMT08
Lane 17A	St.A 5M 30D # 2 pvMT11	**17B**	St.A 20M 30D # 1 pvMT11	**17C**	St.A 20M 30D # 3 pvMT11	**17D**	St.A 40M 30D # 2 pvMT11

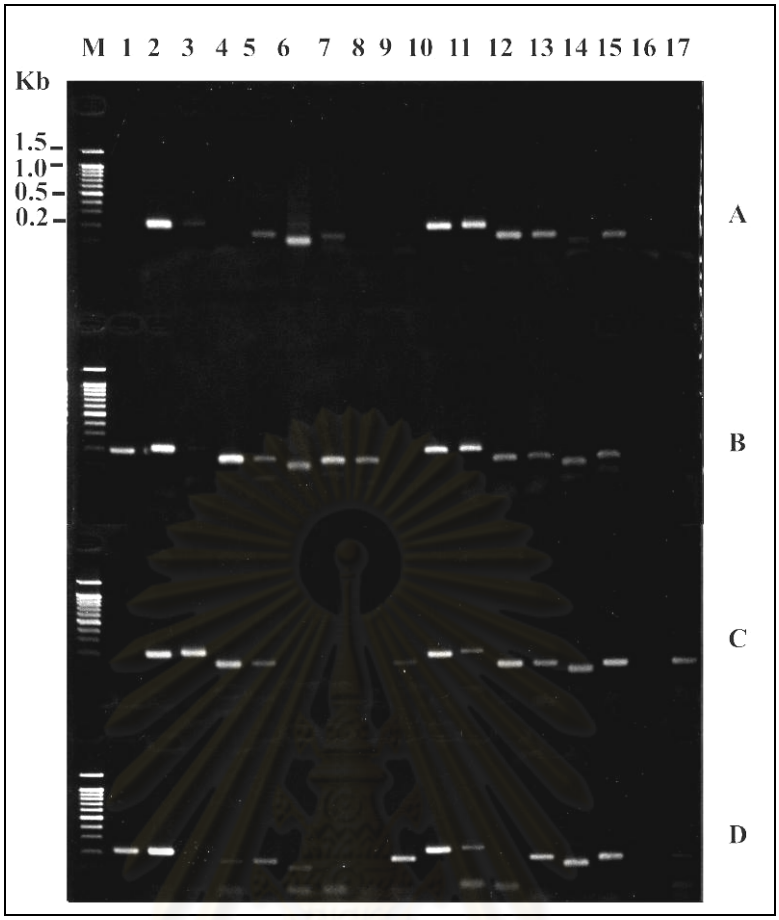

Figure B22 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT07 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.A 40M 30D # 3 β-actin	**2B**	St.A 5M 60D # 2 β-actin	**2C**	St.A 20M 60D # 1 β-actin	**2D**	St.A 20M 60D # 3 β-actin
Lane 3A	St.A 40M 30D # 3 MT	**3B**	St.A 5M 60D # 2 MT	**3C**	St.A 20M 60D # 1 MT	**3D**	St.A 20M 60D # 3 MT
Lane 4A	St.A 40M 30D # 3 pvMT01	**4B**	St.A 5M 60D # 2 pvMT01	**4C**	St.A 20M 60D # 1 pvMT01	**4D**	St.A 20M 60D # 3 pvMT01
Lane 5A	St.A 40M 30D # 3 pvMT02	**5B**	St.A 5M 60D # 2 pvMT02	**5C**	St.A 20M 60D # 1 pvMT02	**5D**	St.A 20M 60D # 3 pvMT02
Lane 6A	St.A 40M 30D # 3 pvMT03	**6B**	St.A 5M 60D # 2 pvMT03	**6C**	St.A 20M 60D # 1 pvMT03	**6D**	St.A 20M 60D # 3 pvMT03
Lane 7A	St.A 40M 30D # 3 pvMT07	**7B**	St.A 5M 60D # 2 pvMT07	**7C**	St.A 20M 60D # 1 pvMT07	**7D**	St.A 20M 60D # 3 pvMT07
Lane 8A	St.A 40M 30D # 3 pvMT08	**8B**	St.A 5M 60D # 2 pvMT08	**8C**	St.A 20M 60D # 1 pvMT08	**8D**	St.A 20M 60D # 3 pvMT08
Lane 9A	St.A 40M 30D # 3 pvMT11	**9B**	St.A 5M 60D # 2 pvMT11	**9C**	St.A 20M 60D # 1 pvMT11	**9D**	St.A 20M 60D # 3 pvMT11
Lane 10A	St.A 5M 60D # 1 β-actin	**10B**	St.A 5M 60D # 3 β-actin	**10C**	St.A 20M 60D # 2 β-actin	**10D**	St.A 40M 60D # 1 β-actin
Lane 11A	St.A 5M 60D # 1 MT	**11B**	St.A 5M 60D # 3 MT	**11C**	St.A 20M 60D # 2 MT	**11D**	St.A 40M 60D # 1 MT
Lane 12A	St.A 5M 60D # 1 pvMT01	**12B**	St.A 5M 60D # 3 pvMT01	**12C**	St.A 20M 60D # 2 pvMT01	**12D**	St.A 40M 60D # 1 pvMT01
Lane 13A	St.A 5M 60D # 1 pvMT02	**13B**	St.A 5M 60D # 3 pvMT02	**13C**	St.A 20M 60D # 2 pvMT02	**13D**	St.A 40M 60D # 1 pvMT02
Lane 14A	St.A 5M 60D # 1 pvMT03	**14B**	St.A 5M 60D # 3 pvMT03	**14C**	St.A 20M 60D # 2 pvMT03	**14D**	St.A 40M 60D # 1 pvMT03
Lane 15A	St.A 5M 60D # 1 pvMT07	**15B**	St.A 5M 60D # 3 pvMT07	**15C**	St.A 20M 60D # 2 pvMT07	**15D**	St.A 40M 60D # 1 pvMT07
Lane 16A	St.A 5M 60D # 1 pvMT08	**16B**	St.A 5M 60D # 3 pvMT08	**16C**	St.A 20M 60D # 2 pvMT08	**16D**	St.A 40M 60D # 1 pvMT08
Lane 17A	St.A 5M 60D # 1 pvMT11	**17B**	St.A 5M 60D # 3 pvMT11	**17C**	St.A 20M 60D # 2 pvMT11	**17D**	St.A 40M 60D # 1 pvMT11

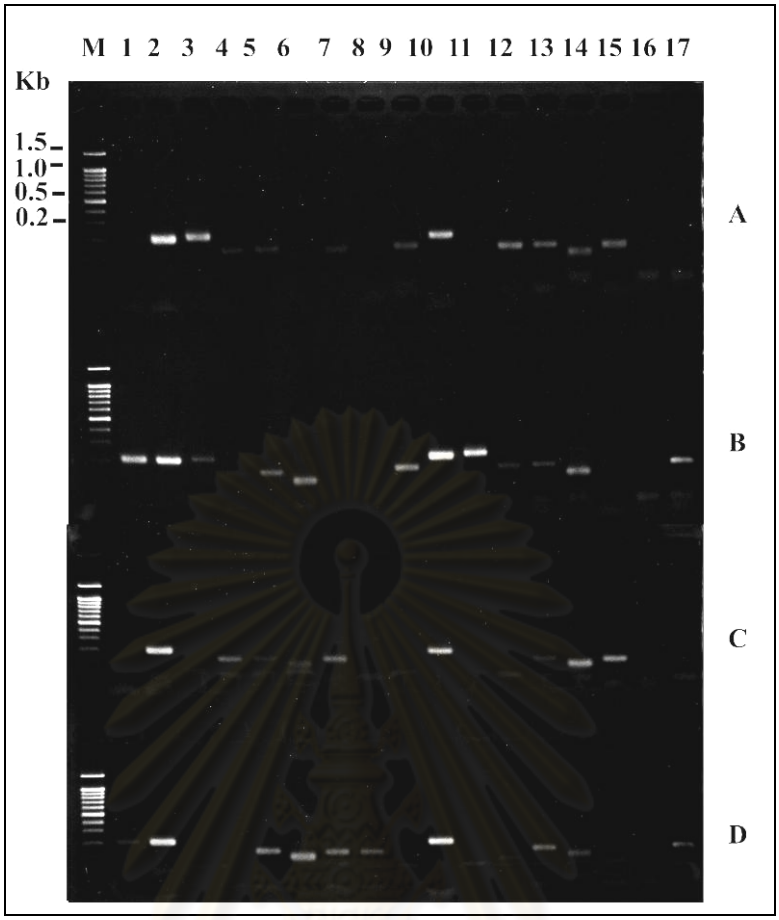

Figure B23 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT07 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.A 40M 60D # 2 β-actin	**2B**	St.A 5M 90D # 1 β-actin	**2C**	St.A 5M 90D # 3 β-actin	**2D**	St.A 20M 90D # 2 β-actin
Lane 3A	St.A 40M 60D # 2 MT	**3B**	St.A 5M 90D # 1 MT	**3C**	St.A 5M 90D # 3 MT	**3D**	St.A 20M 90D # 2 MT
Lane 4A	St.A 40M 60D # 2 pvMT01	**4B**	St.A 5M 90D # 1 pvMT01	**4C**	St.A 5M 90D # 3 pvMT01	**4D**	St.A 20M 90D # 2 pvMT01
Lane 5A	St.A 40M 60D # 2 pvMT02	**5B**	St.A 5M 90D # 1 pvMT02	**5C**	St.A 5M 90D # 3 pvMT02	**5D**	St.A 20M 90D # 2 pvMT02
Lane 6A	St.A 40M 60D # 2 pvMT03	**6B**	St.A 5M 90D # 1 pvMT03	**6C**	St.A 5M 90D # 3 pvMT03	**6D**	St.A 20M 90D # 2 pvMT03
Lane 7A	St.A 40M 60D # 2 pvMT07	**7B**	St.A 5M 90D # 1 pvMT07	**7C**	St.A 5M 90D # 3 pvMT07	**7D**	St.A 20M 90D # 2 pvMT07
Lane 8A	St.A 40M 60D # 2 pvMT08	**8B**	St.A 5M 90D # 1 pvMT08	**8C**	St.A 5M 90D # 3 pvMT08	**8D**	St.A 20M 90D # 2 pvMT08
Lane 9A	St.A 40M 60D # 2 pvMT11	**9B**	St.A 5M 90D # 1 pvMT11	**9C**	St.A 5M 90D # 3 pvMT11	**9D**	St.A 20M 90D # 2 pvMT11
Lane 10A	St.A 40M 60D # 3 β-actin	**10B**	St.A 5M 90D # 2 β-actin	**10C**	St.A 20M 90D # 1 β-actin	**10D**	St.A 20M 90D # 3 β-actin
Lane 11A	St.A 40M 60D # 3 MT	**11B**	St.A 5M 90D # 2 MT	**11C**	St.A 20M 90D # 1 MT	**11D**	St.A 20M 90D # 3 MT
Lane 12A	St.A 40M 60D # 3 pvMT01	**12B**	St.A 5M 90D # 2 pvMT01	**12C**	St.A 20M 90D # 1 pvMT01	**12D**	St.A 20M 90D # 3 pvMT01
Lane 13A	St.A 40M 60D # 3 pvMT02	**13B**	St.A 5M 90D # 2 pvMT02	**13C**	St.A 20M 90D # 1 pvMT02	**13D**	St.A 20M 90D # 3 pvMT02
Lane 14A	St.A 40M 60D # 3 pvMT03	**14B**	St.A 5M 90D # 2 pvMT03	**14C**	St.A 20M 90D # 1 pvMT03	**14D**	St.A 20M 90D # 3 pvMT03
Lane 15A	St.A 40M 60D # 3 pvMT07	**15B**	St.A 5M 90D # 2 pvMT07	**15C**	St.A 20M 90D # 1 pvMT07	**15D**	St.A 20M 90D # 3 pvMT07
Lane 16A	St.A 40M 60D # 3 pvMT08	**16B**	St.A 5M 90D # 2 pvMT08	**16C**	St.A 20M 90D # 1 pvMT08	**16D**	St.A 20M 90D # 3 pvMT08
Lane 17A	St.A 40M 60D # 3 pvMT11	**17B**	St.A 5M 90D # 2 pvMT11	**17C**	St.A 20M 90D # 1 pvMT11	**17D**	St.A 20M 90D # 3 pvMT11

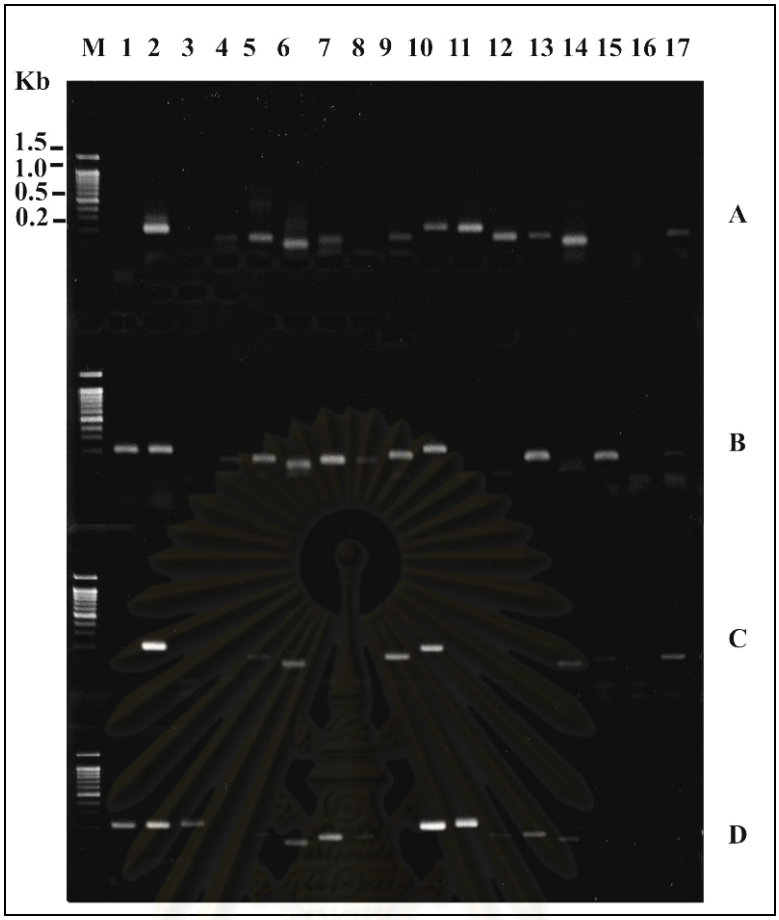

Figure B24 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.B 5M 30D # 1 β-actin	**2B**	St.B 5M 30D # 3 β-actin	**2C**	St.B 20M 30D # 2 β-actin	**2D**	St.B 40M 30D # 1 β-actin
Lane 3A	St.B 5M 30D # 1 MT	**3B**	St.B 5M 30D # 3 MT	**3C**	St.B 20M 30D # 2 MT	**3D**	St.B 40M 30D # 1 MT
Lane 4A	St.B 5M 30D # 1 pvMT01	**4B**	St.B 5M 30D # 3 pvMT01	**4C**	St.B 20M 30D # 2 pvMT01	**4D**	St.B 40M 30D # 1 pvMT01
Lane 5A	St.B 5M 30D # 1 pvMT02	**5B**	St.B 5M 30D # 3 pvMT02	**5C**	St.B 20M 30D # 2 pvMT02	**5D**	St.B 40M 30D # 1 pvMT02
Lane 6A	St.B 5M 30D # 1 pvMT03	**6B**	St.B 5M 30D # 3 pvMT03	**6C**	St.B 20M 30D # 2 pvMT03	**6D**	St.B 40M 30D # 1 pvMT03
Lane 7A	St.B 5M 30D # 1 pvMT07	**7B**	St.B 5M 30D # 3 pvMT07	**7C**	St.B 20M 30D # 2 pvMT07	**7D**	St.B 40M 30D # 1 pvMT07
Lane 8A	St.B 5M 30D # 1 pvMT08	**8B**	St.B 5M 30D # 3 pvMT08	**8C**	St.B 20M 30D # 2 pvMT08	**8D**	St.B 40M 30D # 1 pvMT08
Lane 9A	St.B 5M 30D # 1 pvMT11	**9B**	St.B 5M 30D # 3 pvMT11	**9C**	St.B 20M 30D # 2 pvMT11	**9D**	St.B 40M 30D # 1 pvMT11
Lane 10A	St.B 5M 30D # 2 β-actin	**10B**	St.B 20M 30D # 1 β-actin	**10C**	St.B 20M 30D # 3 β-actin	**10D**	St.B 40M 30D # 2 β-actin
Lane 11A	St.B 5M 30D # 2 MT	**11B**	St.B 20M 30D # 1 MT	**11C**	St.B 20M 30D # 3 MT	**11D**	St.B 40M 30D # 2 MT
Lane 12A	St.B 5M 30D # 2 pvMT01	**12B**	St.B 20M 30D # 1 pvMT01	**12C**	St.B 20M 30D # 3 pvMT01	**12D**	St.B 40M 30D # 2 pvMT01
Lane 13A	St.B 5M 30D # 2 pvMT02	**13B**	St.B 20M 30D # 1 pvMT02	**13C**	St.B 20M 30D # 3 pvMT02	**13D**	St.B 40M 30D # 2 pvMT02
Lane 14A	St.B 5M 30D # 2 pvMT03	**14B**	St.B 20M 30D # 1 pvMT03	**14C**	St.B 20M 30D # 3 pvMT03	**14D**	St.B 40M 30D # 2 pvMT03
Lane 15A	St.B 5M 30D # 2 pvMT07	**15B**	St.B 20M 30D # 1 pvMT07	**15C**	St.B 20M 30D # 3 pvMT07	**15D**	St.B 40M 30D # 2 pvMT07
Lane 16A	St.B 5M 30D # 2 pvMT08	**16B**	St.B 20M 30D # 1 pvMT08	**16C**	St.B 20M 30D # 3 pvMT08	**16D**	St.B 40M 30D # 2 pvMT08
Lane 17A	St.B 5M 30D # 2 pvMT11	**17B**	St.B 20M 30D # 1 pvMT11	**17C**	St.B 20M 30D # 3 pvMT11	**17D**	St.B 40M 30D # 2 pvMT11

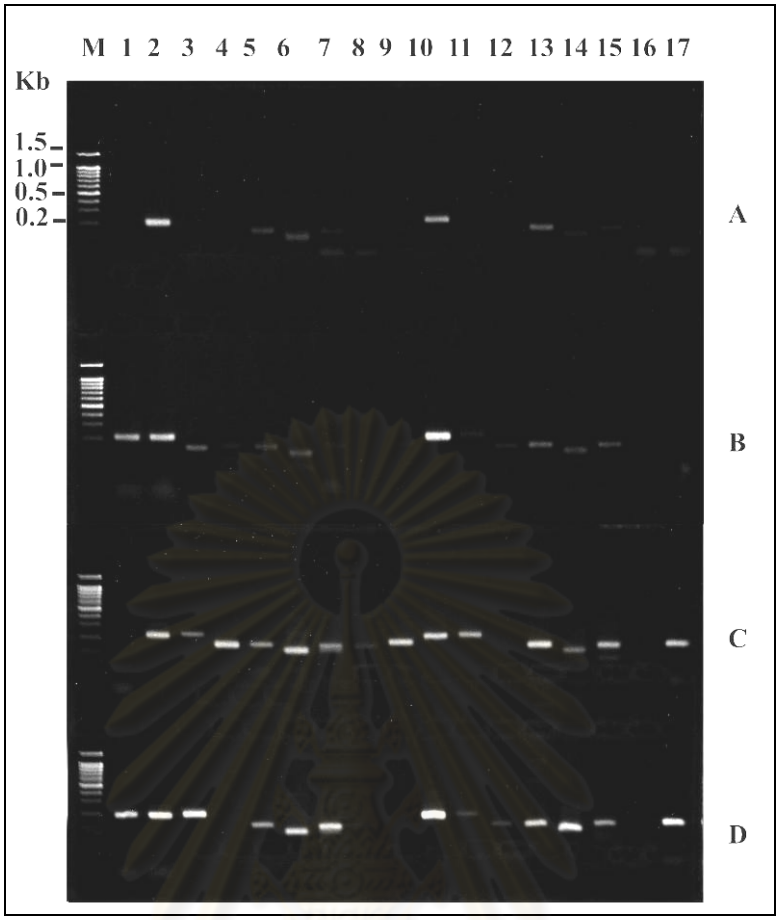

Figure B25 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.B 40M 30D # 3 β-actin	**2B**	St.B 5M 60D # 2 β-actin	**2C**	St.B 20M 60D # 1 β-actin	**2D**	St.B 20M 60D # 3 β-actin
Lane 3A	St.B 40M 30D # 3 MT	**3B**	St.B 5M 60D # 2 MT	**3C**	St.B 20M 60D # 1 MT	**3D**	St.B 20M 60D # 3 MT
Lane 4A	St.B 40M 30D # 3 pvMT01	**4B**	St.B 5M 60D # 2 pvMT01	**4C**	St.B 20M 60D # 1 pvMT01	**4D**	St.B 20M 60D # 3 pvMT01
Lane 5A	St.B 40M 30D # 3 pvMT02	**5B**	St.B 5M 60D # 2 pvMT02	**5C**	St.B 20M 60D # 1 pvMT02	**5D**	St.B 20M 60D # 3 pvMT02
Lane 6A	St.B 40M 30D # 3 pvMT03	**6B**	St.B 5M 60D # 2 pvMT03	**6C**	St.B 20M 60D # 1 pvMT03	**6D**	St.B 20M 60D # 3 pvMT03
Lane 7A	St.B 40M 30D # 3 pvMT07	**7B**	St.B 5M 60D # 2 pvMT07	**7C**	St.B 20M 60D # 1 pvMT07	**7D**	St.B 20M 60D # 3 pvMT07
Lane 8A	St.B 40M 30D # 3 pvMT08	**8B**	St.B 5M 60D # 2 pvMT08	**8C**	St.B 20M 60D # 1 pvMT08	**8D**	St.B 20M 60D # 3 pvMT08
Lane 9A	St.B 40M 30D # 3 pvMT11	**9B**	St.B 5M 60D # 2 pvMT11	**9C**	St.B 20M 60D # 1 pvMT11	**9D**	St.B 20M 60D # 3 pvMT11
Lane 10A	St.B 5M 60D # 1 β-actin	**10B**	St.B 5M 60D # 3 β-actin	**10C**	St.B 20M 60D # 2 β-actin	**10D**	St.B 40M 60D # 1 β-actin
Lane 11A	St.B 5M 60D # 1 MT	**11B**	St.B 5M 60D # 3 MT	**11C**	St.B 20M 60D # 2 MT	**11D**	St.B 40M 60D # 1 MT
Lane 12A	St.B 5M 60D # 1 pvMT01	**12B**	St.B 5M 60D # 3 pvMT01	**12C**	St.B 20M 60D # 2 pvMT01	**12D**	St.B 40M 60D # 1 pvMT01
Lane 13A	St.B 5M 60D # 1 pvMT02	**13B**	St.B 5M 60D # 3 pvMT02	**13C**	St.B 20M 60D # 2 pvMT02	**13D**	St.B 40M 60D # 1 pvMT02
Lane 14A	St.B 5M 60D # 1 pvMT03	**14B**	St.B 5M 60D # 3 pvMT03	**14C**	St.B 20M 60D # 2 pvMT03	**14D**	St.B 40M 60D # 1 pvMT03
Lane 15A	St.B 5M 60D # 1 pvMT07	**15B**	St.B 5M 60D # 3 pvMT07	**15C**	St.B 20M 60D # 2 pvMT07	**15D**	St.B 40M 60D # 1 pvMT07
Lane 16A	St.B 5M 60D # 1 pvMT08	**16B**	St.B 5M 60D # 3 pvMT08	**16C**	St.B 20M 60D # 2 pvMT08	**16D**	St.B 40M 60D # 1 pvMT08
Lane 17A	St.B 5M 60D # 1 pvMT11	**17B**	St.B 5M 60D # 3 pvMT11	**17C**	St.B 20M 60D # 2 pvMT11	**17D**	St.B 40M 60D # 1 pvMT11

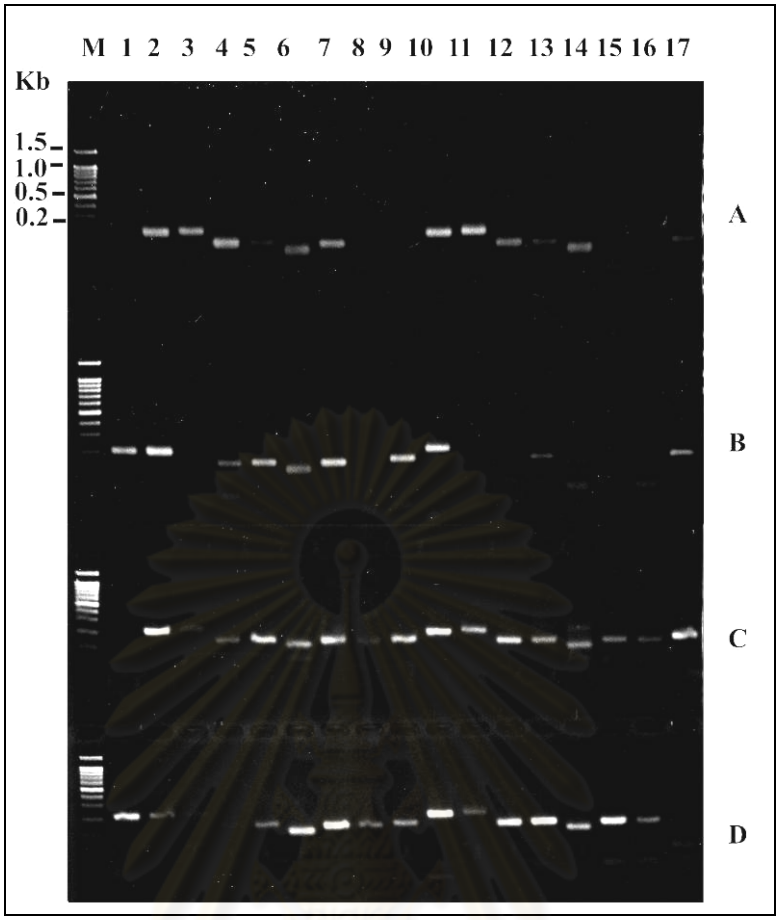

Figure B26 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.B 40M 60D # 2 β-actin	**2B**	St.B 5M 90D # 1 β-actin	**2C**	St.B 5M 90D # 3 β-actin	**2D**	St.B 20M 90D # 2 β-actin
Lane 3A	St.B 40M 60D # 2 MT	**3B**	St.B 5M 90D # 1 MT	**3C**	St.B 5M 90D # 3 MT	**3D**	St.B 20M 90D # 2 MT
Lane 4A	St.B 40M 60D # 2 pvMT01	**4B**	St.B 5M 90D # 1 pvMT01	**4C**	St.B 5M 90D # 3 pvMT01	**4D**	St.B 20M 90D # 2 pvMT01
Lane 5A	St.B 40M 60D # 2 pvMT02	**5B**	St.B 5M 90D # 1 pvMT02	**5C**	St.B 5M 90D # 3 pvMT02	**5D**	St.B 20M 90D # 2 pvMT02
Lane 6A	St.B 40M 60D # 2 pvMT03	**6B**	St.B 5M 90D # 1 pvMT03	**6C**	St.B 5M 90D # 3 pvMT03	**6D**	St.B 20M 90D # 2 pvMT03
Lane 7A	St.B 40M 60D # 2 pvMT07	**7B**	St.B 5M 90D # 1 pvMT07	**7C**	St.B 5M 90D # 3 pvMT07	**7D**	St.B 20M 90D # 2 pvMT07
Lane 8A	St.B 40M 60D # 2 pvMT08	**8B**	St.B 5M 90D # 1 pvMT08	**8C**	St.B 5M 90D # 3 pvMT08	**8D**	St.B 20M 90D # 2 pvMT08
Lane 9A	St.B 40M 60D # 2 pvMT11	**9B**	St.B 5M 90D # 1 pvMT11	**9C**	St.B 5M 90D # 3 pvMT11	**9D**	St.B 20M 90D # 2 pvMT11
Lane 10A	St.B 40M 60D # 3 β-actin	**10B**	St.B 5M 90D # 2 β-actin	**10C**	St.B 20M 90D # 1 β-actin	**10D**	St.B 20M 90D # 3 β-actin
Lane 11A	St.B 40M 60D # 3 MT	**11B**	St.B 5M 90D # 2 MT	**11C**	St.B 20M 90D # 1 MT	**11D**	St.B 20M 90D # 3 MT
Lane 12A	St.B 40M 60D # 3 pvMT01	**12B**	St.B 5M 90D # 2 pvMT01	**12C**	St.B 20M 90D # 1 pvMT01	**12D**	St.B 20M 90D # 3 pvMT01
Lane 13A	St.B 40M 60D # 3 pvMT02	**13B**	St.B 5M 90D # 2 pvMT02	**13C**	St.B 20M 90D # 1 pvMT02	**13D**	St.B 20M 90D # 3 pvMT02
Lane 14A	St.B 40M 60D # 3 pvMT03	**14B**	St.B 5M 90D # 2 pvMT03	**14C**	St.B 20M 90D # 1 pvMT03	**14D**	St.B 20M 90D # 3 pvMT03
Lane 15A	St.B 40M 60D # 3 pvMT07	**15B**	St.B 5M 90D # 2 pvMT07	**15C**	St.B 20M 90D # 1 pvMT07	**15D**	St.B 20M 90D # 3 pvMT07
Lane 16A	St.B 40M 60D # 3 pvMT08	**16B**	St.B 5M 90D # 2 pvMT08	**16C**	St.B 20M 90D # 1 pvMT08	**16D**	St.B 20M 90D # 3 pvMT08
Lane 17A	St.B 40M 60D # 3 pvMT11	**17B**	St.B 5M 90D # 2 pvMT11	**17C**	St.B 20M 90D # 1 pvMT11	**17D**	St.B 20M 90D # 3 pvMT11

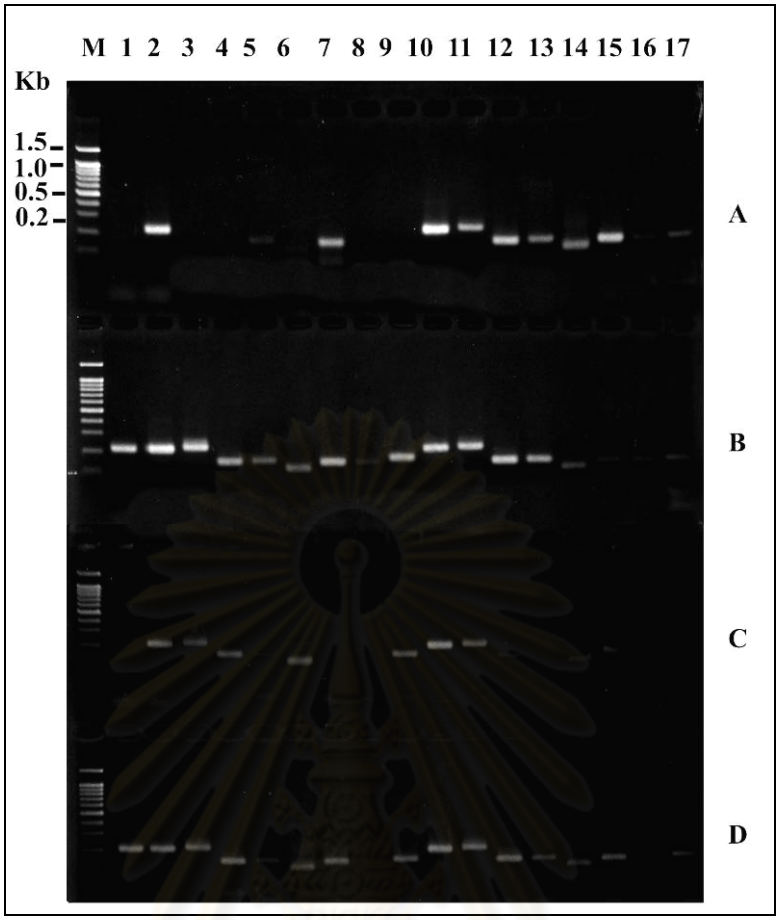

Figure B27 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.B 40M 90D # 1 β-actin	**2B**	St.B 40M 90D # 3 β-actin	**2C**	St.C 5M 30D # 2 β-actin	**2D**	St.C 20M 30D # 1 β-actin
Lane 3A	St.B 40M 90D # 1 MT	**3B**	St.B 40M 90D # 3 MT	**3C**	St.C 5M 30D # 2 MT	**3D**	St.C 20M 30D # 1 MT
Lane 4A	St.B 40M 90D # 1 pvMT01	**4B**	St.B 40M 90D # 3 pvMT01	**4C**	St.C 5M 30D # 2 pvMT01	**4D**	St.C 20M 30D # 1 pvMT01
Lane 5A	St.B 40M 90D # 1 pvMT02	**5B**	St.B 40M 90D # 3 pvMT02	**5C**	St.C 5M 30D # 2 pvMT02	**5D**	St.C 20M 30D # 1 pvMT02
Lane 6A	St.B 40M 90D # 1 pvMT03	**6B**	St.B 40M 90D # 3 pvMT03	**6C**	St.C 5M 30D # 2 pvMT03	**6D**	St.C 20M 30D # 1 pvMT03
Lane 7A	St.B 40M 90D # 1 pvMT07	**7B**	St.B 40M 90D # 3 pvMT07	**7C**	St.C 5M 30D # 2 pvMT07	**7D**	St.C 20M 30D # 1 pvMT07
Lane 8A	St.B 40M 90D # 1 pvMT08	**8B**	St.B 40M 90D # 3 pvMT08	**8C**	St.C 5M 30D # 2 pvMT08	**8D**	St.C 20M 30D # 1 pvMT08
Lane 9A	St.B 40M 90D # 1 pvMT11	**9B**	St.B 40M 90D # 3 pvMT11	**9C**	St.C 5M 30D # 2 pvMT11	**9D**	St.C 20M 30D # 1 pvMT11
Lane 10A	St.B 40M 90D # 2 β-actin	**10B**	St.C 5M 30D # 1 β-actin	**10C**	St.C 5M 30D # 3 β-actin	**10D**	St.C 20M 30D # 2 β-actin
Lane 11A	St.B 40M 90D # 2 MT	**11B**	St.C 5M 30D # 1 MT	**11C**	St.C 5M 30D # 3 MT	**11D**	St.C 20M 30D # 2 MT
Lane 12A	St.B 40M 90D # 2 pvMT01	**12B**	St.C 5M 30D # 1 pvMT01	**12C**	St.C 5M 30D # 3 pvMT01	**12D**	St.C 20M 30D # 2 pvMT01
Lane 13A	St.B 40M 90D # 2 pvMT02	**13B**	St.C 5M 30D # 1 pvMT02	**13C**	St.C 5M 30D # 3 pvMT02	**13D**	St.C 20M 30D # 2 pvMT02
Lane 14A	St.B 40M 90D # 2 pvMT03	**14B**	St.C 5M 30D # 1 pvMT03	**14C**	St.C 5M 30D # 3 pvMT03	**14D**	St.C 20M 30D # 2 pvMT03
Lane 15A	St.B 40M 90D # 2 pvMT07	**15B**	St.C 5M 30D # 1 pvMT07	**15C**	St.C 5M 30D # 3 pvMT07	**15D**	St.C 20M 30D # 2 pvMT07
Lane 16A	St.B 40M 90D # 2 pvMT08	**16B**	St.C 5M 30D # 1 pvMT08	**16C**	St.C 5M 30D # 3 pvMT08	**16D**	St.C 20M 30D # 2 pvMT08
Lane 17A	St.B 40M 90D # 2 pvMT11	**17B**	St.C 5M 30D # 1 pvMT11	**17C**	St.C 5M 30D # 3 pvMT11	**17D**	St.C 20M 30D # 2 pvMT11

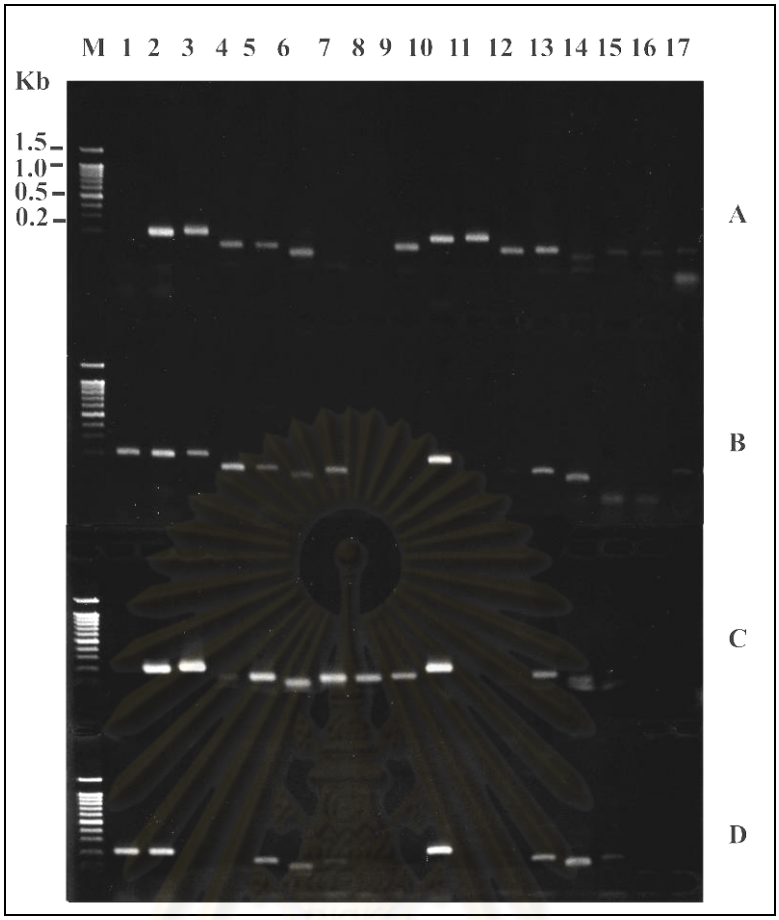

Figure B28 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.C 20M 30D # 3 β-actin	**2B**	St.C 40M 30D # 2 β-actin	**2C**	St.C 5M 60D # 1 β-actin	**2D**	St.C 5M 60D # 3 β-actin
Lane 3A	St.C 20M 30D # 3 MT	**3B**	St.C 40M 30D # 2 MT	**3C**	St.C 5M 60D # 1 MT	**3D**	St.C 5M 60D # 3 MT
Lane 4A	St.C 20M 30D # 3 pvMT01	**4B**	St.C 40M 30D # 2 pvMT01	**4C**	St.C 5M 60D # 1 pvMT01	**4D**	St.C 5M 60D # 3 pvMT01
Lane 5A	St.C 20M 30D # 3 pvMT02	**5B**	St.C 40M 30D # 2 pvMT02	**5C**	St.C 5M 60D # 1 pvMT02	**5D**	St.C 5M 60D # 3 pvMT02
Lane 6A	St.C 20M 30D # 3 pvMT03	**6B**	St.C 40M 30D # 2 pvMT03	**6C**	St.C 5M 60D # 1 pvMT03	**6D**	St.C 5M 60D # 3 pvMT03
Lane 7A	St.C 20M 30D # 3 pvMT07	**7B**	St.C 40M 30D # 2 pvMT07	**7C**	St.C 5M 60D # 1 pvMT07	**7D**	St.C 5M 60D # 3 pvMT07
Lane 8A	St.C 20M 30D # 3 pvMT08	**8B**	St.C 40M 30D # 2 pvMT08	**8C**	St.C 5M 60D # 1 pvMT08	**8D**	St.C 5M 60D # 3 pvMT08
Lane 9A	St.C 20M 30D # 3 pvMT11	**9B**	St.C 40M 30D # 2 pvMT11	**9C**	St.C 5M 60D # 1 pvMT11	**9D**	St.C 5M 60D # 3 pvMT11
Lane 10A	St.C 40M 30D # 1 β-actin	**10B**	St.C 40M 30D # 3 β-actin	**10C**	St.C 5M 60D # 2 β-actin	**10D**	St.C 20M 60D # 1 β-actin
Lane 11A	St.C 40M 30D # 1 MT	**11B**	St.C 40M 30D # 3 MT	**11C**	St.C 5M 60D # 2 MT	**11D**	St.C 20M 60D # 1 MT
Lane 12A	St.C 40M 30D # 1 pvMT01	**12B**	St.C 40M 30D # 3 pvMT01	**12C**	St.C 5M 60D # 2 pvMT01	**12D**	St.C 20M 60D # 1 pvMT01
Lane 13A	St.C 40M 30D # 1 pvMT02	**13B**	St.C 40M 30D # 3 pvMT02	**13C**	St.C 5M 60D # 2 pvMT02	**13D**	St.C 20M 60D # 1 pvMT02
Lane 14A	St.C 40M 30D # 1 pvMT03	**14B**	St.C 40M 30D # 3 pvMT03	**14C**	St.C 5M 60D # 2 pvMT03	**14D**	St.C 20M 60D # 1 pvMT03
Lane 15A	St.C 40M 30D # 1 pvMT07	**15B**	St.C 40M 30D # 3 pvMT07	**15C**	St.C 5M 60D # 2 pvMT07	**15D**	St.C 20M 60D # 1 pvMT07
Lane 16A	St.C 40M 30D # 1 pvMT08	**16B**	St.C 40M 30D # 3 pvMT08	**16C**	St.C 5M 60D # 2 pvMT08	**16D**	St.C 20M 60D # 1 pvMT08
Lane 17A	St.C 40M 30D # 1 pvMT11	**17B**	St.C 40M 30D # 3 pvMT11	**17C**	St.C 5M 60D # 2 pvMT11	**17D**	St.C 20M 60D # 1 pvMT11

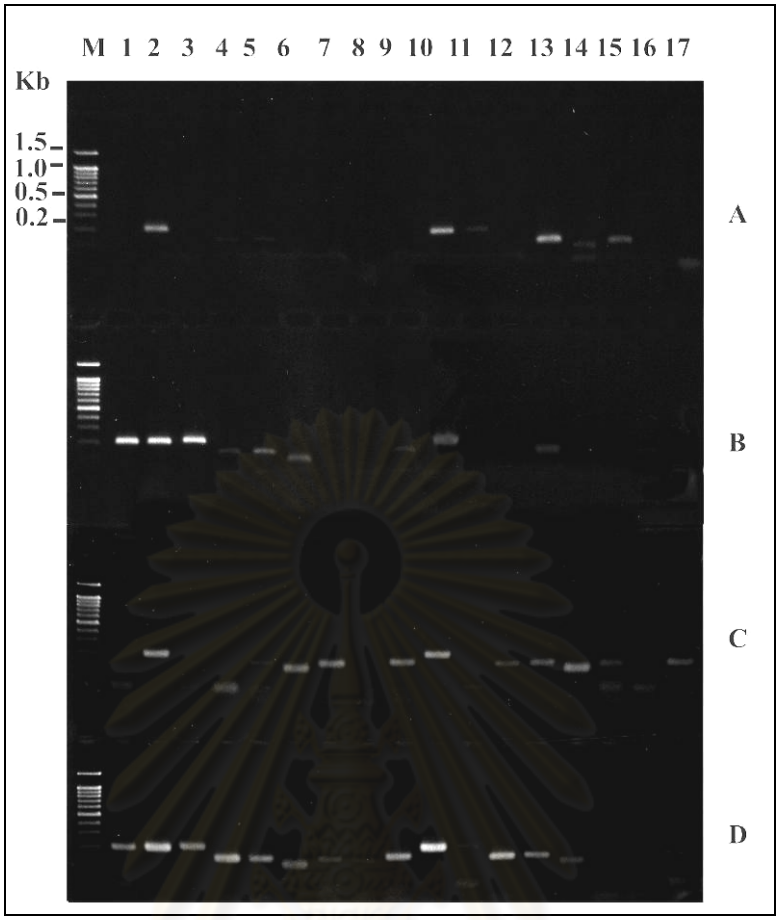

Figure B29 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.C 20M 60D # 2 β-actin	**2B**	St.C 40M 60D # 1 β-actin	**2C**	St.C 40M 60D # 3 β-actin	**2D**	St.C 5M 90D # 2 β-actin
Lane 3A	St.C 20M 60D # 2 MT	**3B**	St.C 40M 60D # 1 MT	**3C**	St.C 40M 60D # 3 MT	**3D**	St.C 5M 90D # 2 MT
Lane 4A	St.C 20M 60D # 2 pvMT01	**4B**	St.C 40M 60D # 1 pvMT01	**4C**	St.C 40M 60D # 3 pvMT01	**4D**	St.C 5M 90D # 2 pvMT01
Lane 5A	St.C 20M 60D # 2 pvMT02	**5B**	St.C 40M 60D # 1 pvMT02	**5C**	St.C 40M 60D # 3 pvMT02	**5D**	St.C 5M 90D # 2 pvMT02
Lane 6A	St.C 20M 60D # 2 pvMT03	**6B**	St.C 40M 60D # 1 pvMT03	**6C**	St.C 40M 60D # 3 pvMT03	**6D**	St.C 5M 90D # 2 pvMT03
Lane 7A	St.C 20M 60D # 2 pvMT07	**7B**	St.C 40M 60D # 1 pvMT07	**7C**	St.C 40M 60D # 3 pvMT07	**7D**	St.C 5M 90D # 2 pvMT07
Lane 8A	St.C 20M 60D # 2 pvMT08	**8B**	St.C 40M 60D # 1 pvMT08	**8C**	St.C 40M 60D # 3 pvMT08	**8D**	St.C 5M 90D # 2 pvMT08
Lane 9A	St.C 20M 60D # 2 pvMT11	**9B**	St.C 40M 60D # 1 pvMT11	**9C**	St.C 40M 60D # 3 pvMT11	**9D**	St.C 5M 90D # 2 pvMT11
Lane 10A	St.C 20M 60D # 3 β-actin	**10B**	St.C 40M 60D # 2 β-actin	**10C**	St.C 5M 90D # 1 β-actin	**10D**	St.C 5M 90D # 3 β-actin
Lane 11A	St.C 20M 60D # 3 MT	**11B**	St.C 40M 60D # 2 MT	**11C**	St.C 5M 90D # 1 MT	**11D**	St.C 5M 90D # 3 MT
Lane 12A	St.C 20M 60D # 3 pvMT01	**12B**	St.C 40M 60D # 2 pvMT01	**12C**	St.C 5M 90D # 1 pvMT01	**12D**	St.C 5M 90D # 3 pvMT01
Lane 13A	St.C 20M 60D # 3 pvMT02	**13B**	St.C 40M 60D # 2 pvMT02	**13C**	St.C 5M 90D # 1 pvMT02	**13D**	St.C 5M 90D # 3 pvMT02
Lane 14A	St.C 20M 60D # 3 pvMT03	**14B**	St.C 40M 60D # 2 pvMT03	**14C**	St.C 5M 90D # 1 pvMT03	**14D**	St.C 5M 90D # 3 pvMT03
Lane 15A	St.C 20M 60D # 3 pvMT07	**15B**	St.C 40M 60D # 2 pvMT07	**15C**	St.C 5M 90D # 1 pvMT07	**15D**	St.C 5M 90D # 3 pvMT07
Lane 16A	St.C 20M 60D # 3 pvMT08	**16B**	St.C 40M 60D # 2 pvMT08	**16C**	St.C 5M 90D # 1 pvMT08	**16D**	St.C 5M 90D # 3 pvMT08
Lane 17A	St.C 20M 60D # 3 pvMT11	**17B**	St.C 40M 60D # 2 pvMT11	**17C**	St.C 5M 90D # 1 pvMT11	**17D**	St.C 5M 90D # 3 pvMT11

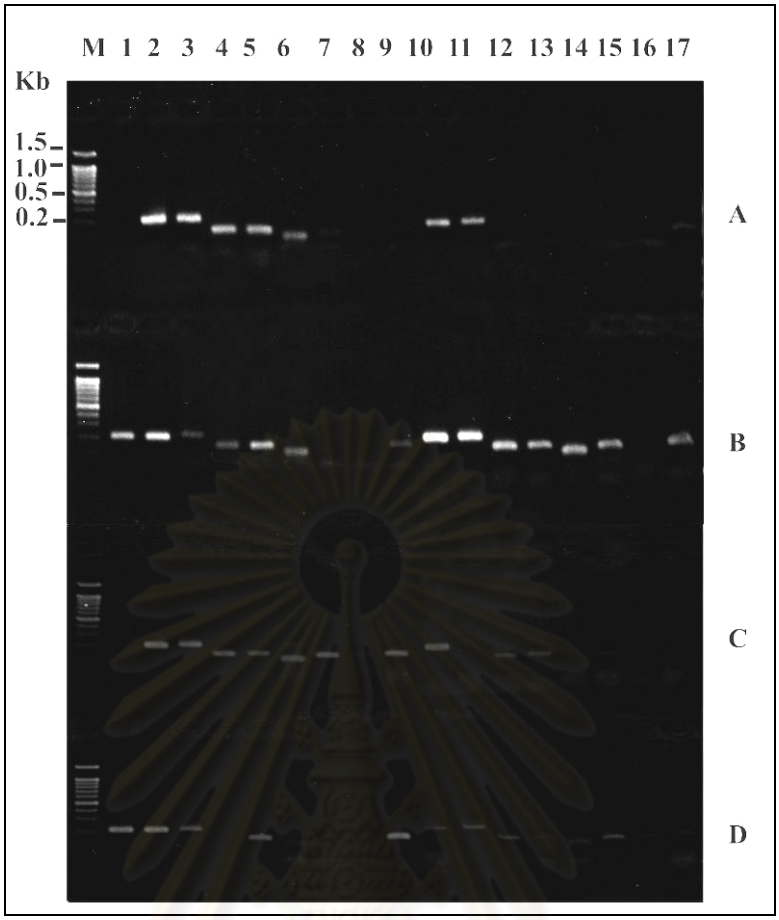

Figure B30 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.C 20M 90D # 1 β-actin	**2B**	St.C 20M 90D # 3 β-actin	**2C**	St.C 40M 90D # 2 β-actin	**2D**	St.D 5M 30D # 1 β-actin
Lane 3A	St.C 20M 90D # 1 MT	**3B**	St.C 20M 90D # 3 MT	**3C**	St.C 40M 90D # 2 MT	**3D**	St.D 5M 30D # 1 MT
Lane 4A	St.C 20M 90D # 1 pvMT01	**4B**	St.C 20M 90D # 3 pvMT01	**4C**	St.C 40M 90D # 2 pvMT01	**4D**	St.D 5M 30D # 1 pvMT01
Lane 5A	St.C 20M 90D # 1 pvMT02	**5B**	St.C 20M 90D # 3 pvMT02	**5C**	St.C 40M 90D # 2 pvMT02	**5D**	St.D 5M 30D # 1 pvMT02
Lane 6A	St.C 20M 90D # 1 pvMT03	**6B**	St.C 20M 90D # 3 pvMT03	**6C**	St.C 40M 90D # 2 pvMT03	**6D**	St.D 5M 30D # 1 pvMT03
Lane 7A	St.C 20M 90D # 1 pvMT07	**7B**	St.C 20M 90D # 3 pvMT07	**7C**	St.C 40M 90D # 2 pvMT07	**7D**	St.D 5M 30D # 1 pvMT07
Lane 8A	St.C 20M 90D # 1 pvMT08	**8B**	St.C 20M 90D # 3 pvMT08	**8C**	St.C 40M 90D # 2 pvMT08	**8D**	St.D 5M 30D # 1 pvMT08
Lane 9A	St.C 20M 90D # 1 pvMT11	**9B**	St.C 20M 90D # 3 pvMT11	**9C**	St.C 40M 90D # 2 pvMT11	**9D**	St.D 5M 30D # 1 pvMT11
Lane 10A	St.C 20M 90D # 2 β-actin	**10B**	St.C 40M 90D # 1 β-actin	**10C**	St.C 40M 90D # 3 β-actin	**10D**	St.D 5M 30D # 2 β-actin
Lane 11A	St.C 20M 90D # 2 MT	**11B**	St.C 40M 90D # 1 MT	**11C**	St.C 40M 90D # 3 MT	**11D**	St.D 5M 30D # 2 MT
Lane 12A	St.C 20M 90D # 2 pvMT01	**12B**	St.C 40M 90D # 1 pvMT01	**12C**	St.C 40M 90D # 3 pvMT01	**12D**	St.D 5M 30D # 2 pvMT01
Lane 13A	St.C 20M 90D # 2 pvMT02	**13B**	St.C 40M 90D # 1 pvMT02	**13C**	St.C 40M 90D # 3 pvMT02	**13D**	St.D 5M 30D # 2 pvMT02
Lane 14A	St.C 20M 90D # 2 pvMT03	**14B**	St.C 40M 90D # 1 pvMT03	**14C**	St.C 40M 90D # 3 pvMT03	**14D**	St.D 5M 30D # 2 pvMT03
Lane 15A	St.C 20M 90D # 2 pvMT07	**15B**	St.C 40M 90D # 1 pvMT07	**15C**	St.C 40M 90D # 3 pvMT07	**15D**	St.D 5M 30D # 2 pvMT07
Lane 16A	St.C 20M 90D # 2 pvMT08	**16B**	St.C 40M 90D # 1 pvMT08	**16C**	St.C 40M 90D # 3 pvMT08	**16D**	St.D 5M 30D # 2 pvMT08
Lane 17A	St.C 20M 90D # 2 pvMT11	**17B**	St.C 40M 90D # 1 pvMT11	**17C**	St.C 40M 90D # 3 pvMT11	**17D**	St.D 5M 30D # 2 pvMT11

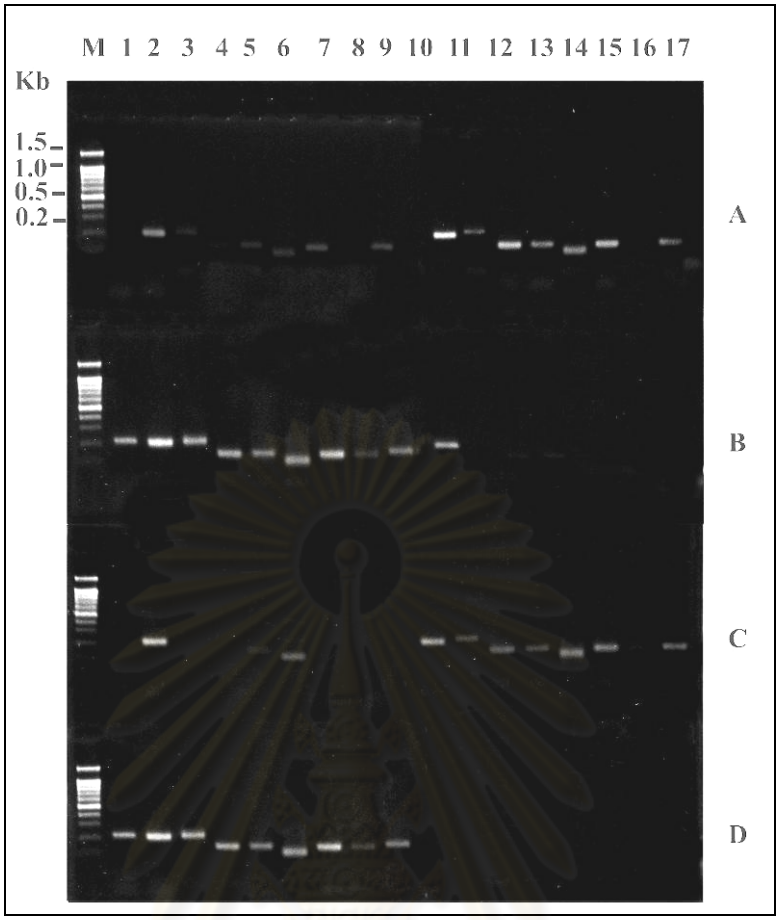

Figure B31 PCR product of MT , pvMT01, pvMT02, pvMT03, pvMT07, pvMT08 and pvMT11 using first strand cDNA from mussel gill , β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.D 5M 30D # 3 β-actin	**2B**	St.D 5M 60D # 2 β-actin	**2C**	St.D 5M 90D # 1 β-actin	**2D**	St.D 5M 90D # 3 β-actin
Lane 3A	St.D 5M 30D # 3 MT	**3B**	St.D 5M 60D # 2 MT	**3C**	St.D 5M 90D # 1 MT	**3D**	St.D 5M 90D # 3 MT
Lane 4A	St.D 5M 30D # 3 pvMT01	**4B**	St.D 5M 60D # 2 pvMT01	**4C**	St.D 5M 90D # 1 pvMT01	**4D**	St.D 5M 90D # 3 pvMT01
Lane 5A	St.D 5M 30D # 3 pvMT02	**5B**	St.D 5M 60D # 2 pvMT02	**5C**	St.D 5M 90D # 1 pvMT02	**5D**	St.D 5M 90D # 3 pvMT02
Lane 6A	St.D 5M 30D # 3 pvMT03	**6B**	St.D 5M 60D # 2 pvMT03	**6C**	St.D 5M 90D # 1 pvMT03	**6D**	St.D 5M 90D # 3 pvMT03
Lane 7A	St.D 5M 30D # 3 pvMT07	**7B**	St.D 5M 60D # 2 pvMT07	**7C**	St.D 5M 90D # 1 pvMT07	**7D**	St.D 5M 90D # 3 pvMT07
Lane 8A	St.D 5M 30D # 3 pvMT08	**8B**	St.D 5M 60D # 2 pvMT08	**8C**	St.D 5M 90D # 1 pvMT08	**8D**	St.D 5M 90D # 3 pvMT08
Lane 9A	St.D 5M 30D # 3 pvMT11	**9B**	St.D 5M 60D # 2 pvMT11	**9C**	St.D 5M 90D # 1 pvMT11	**9D**	St.D 5M 90D # 3 pvMT11
Lane 10A	St.D 5M 60D # 1 β-actin	**10B**	St.D 5M 60D # 3 β-actin	**10C**	St.D 5M 90D # 2 β-actin	**10D**	
Lane 11A	St.D 5M 60D # 1 MT	**11B**	St.D 5M 60D # 3 MT	**11C**	St.D 5M 90D # 2 MT	**11D**	
Lane 12A	St.D 5M 60D # 1 pvMT01	**12B**	St.D 5M 60D # 3 pvMT01	**12C**	St.D 5M 90D # 2 pvMT01	**12D**	
Lane 13A	St.D 5M 60D # 1 pvMT02	**13B**	St.D 5M 60D # 3 pvMT02	**13C**	St.D 5M 90D # 2 pvMT02	**13D**	
Lane 14A	St.D 5M 60D # 1 pvMT03	**14B**	St.D 5M 60D # 3 pvMT03	**14C**	St.D 5M 90D # 2 pvMT03	**14D**	
Lane 15A	St.D 5M 60D # 1 pvMT07	**15B**	St.D 5M 60D # 3 pvMT07	**15C**	St.D 5M 90D # 2 pvMT07	**15D**	
Lane 16A	St.D 5M 60D # 1 pvMT08	**16B**	St.D 5M 60D # 3 pvMT08	**16C**	St.D 5M 90D # 2 pvMT08	**16D**	
Lane 17A	St.D 5M 60D # 1 pvMT11	**17B**	St.D 5M 60D # 3 pvMT11	**17C**	St.D 5M 90D # 2 pvMT11	**17D**	

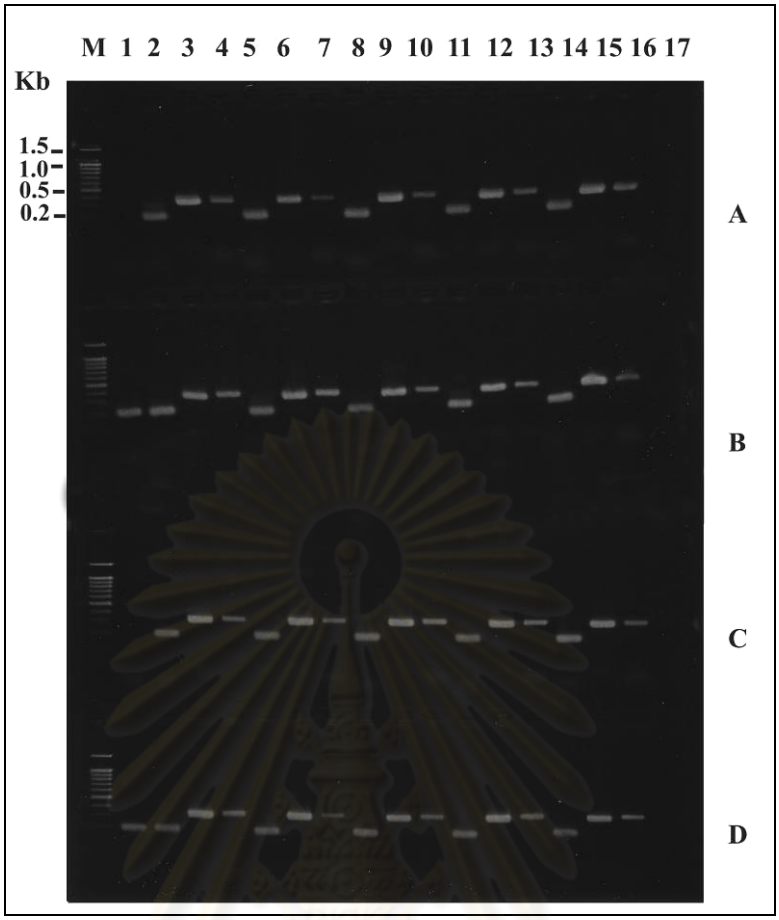

Figure B32 PCR products of HSP71 and CYP450 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.A 5M 30D # 1 β-actin	**2B**	St.A 20M 30D # 3 β-actin	**2C**	St.A 5M 60D # 2 β-actin	**2D**	St.A 40M 60D # 1 β-actin
Lane 3A	St.A 5M 30D # 1 HSP71	**3B**	St.A 20M 30D # 3 HSP71	**3C**	St.A 5M 60D # 2 HSP71	**3D**	St.A 40M 60D # 1 HSP71
Lane 4A	St.A 5M 30D # 1 CYP450	**4B**	St.A 20M 30D # 3 CYP450	**4C**	St.A 5M 60D # 2 CYP450	**4D**	St.A 40M 60D # 1 CYP450
Lane 5A	St.A 5M 30D # 2 β-actin	**5B**	St.A 40M 30D # 1 β-actin	**5C**	St.A 5M 60D # 3 β-actin	**5D**	St.A 40M 60D # 2 β-actin
Lane 6A	St.A 5M 30D # 2 HSP71	**6B**	St.A 40M 30D # 1 HSP71	**6C**	St.A 5M 60D # 3 HSP71	**6D**	St.A 40M 60D # 2 HSP71
Lane 7A	St.A 5M 30D # 2 CYP450	**7B**	St.A 40M 30D # 1 CYP450	**7C**	St.A 5M 60D # 3 CYP450	**7D**	St.A 40M 60D # 2 CYP450
Lane 8A	St.A 5M 30D # 3 β-actin	**8B**	St.A 40M 30D # 2 β-actin	**8C**	St.A 20M 60D # 1 β-actin	**8D**	St.A 40M 60D # 3 β-actin
Lane 9A	St.A 5M 30D # 3 HSP71	**9B**	St.A 40M 30D # 2 HSP71	**9C**	St.A 20M 60D # 1 HSP71	**9D**	St.A 40M 60D # 3 HSP71
Lane 10A	St.A 5M 30D # 3 CYP450	**10B**	St.A 40M 30D # 2 CYP450	**10C**	St.A 20M 60D # 1 CYP450	**10D**	St.A 40M 60D # 3 CYP450
Lane 11A	St.A 20M 30D # 1 β-actin	**11B**	St.A 40M 30D # 3 β-actin	**11C**	St.A 20M 60D # 2 β-actin	**11D**	St.A 5M 90D # 1 β-actin
Lane 12A	St.A 20M 30D # 1 HSP71	**12B**	St.A 40M 30D # 3 HSP71	**12C**	St.A 20M 60D # 2 HSP71	**12D**	St.A 5M 90D # 1 HSP71
Lane 13A	St.A 20M 30D # 1 CYP450	**13B**	St.A 40M 30D # 3 CYP450	**13C**	St.A 20M 60D # 2 CYP450	**13D**	St.A 5M 90D # 1 CYP450
Lane 14A	St.A 20M 30D # 2 β-actin	**14B**	St.A 5M 60D # 1 β-actin	**14C**	St.A 20M 60D # 3 β-actin	**14D**	St.A 5M 90D # 2 β-actin
Lane 15A	St.A 20M 30D # 2 HSP71	**15B**	St.A 5M 60D # 1 HSP71	**15C**	St.A 20M 60D # 3 HSP71	**15D**	St.A 5M 90D # 2 HSP71
Lane 16A	St.A 20M 30D # 2 CYP450	**16B**	St.A 5M 60D # 1 CYP450	**16C**	St.A 20M 60D # 3 CYP450	**16D**	St.A 5M 90D # 2 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	Blank

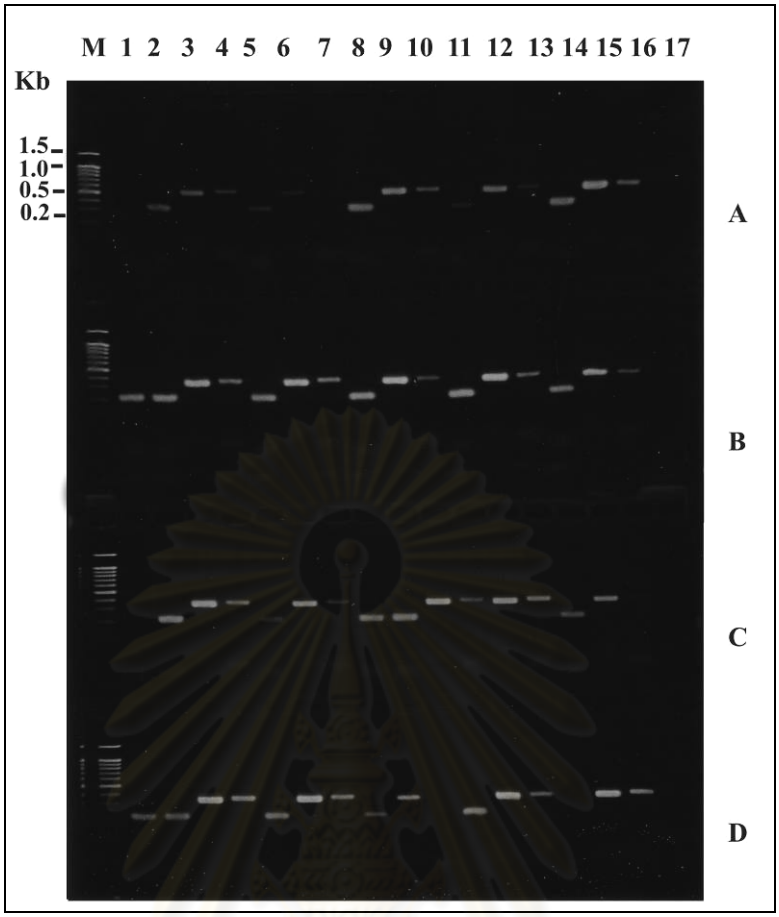

Figure B33 PCR products of HSP71 and CYP450 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.A 5M 90D # 3 β-actin	**2B**	St.B 5M 30D # 2 β-actin	**2C**	St.B 40M 30D # 1 β-actin	**2D**	St.B 5M 60D # 3 β-actin
Lane 3A	St.A 5M 90D # 3 HSP71	**3B**	St.B 5M 30D # 2 HSP71	**3C**	St.B 40M 30D # 1 HSP71	**3D**	St.B 5M 60D # 3 HSP71
Lane 4A	St.A 5M 90D # 3 CYP450	**4B**	St.B 5M 30D # 2 CYP450	**4C**	St.B 40M 30D # 1 CYP450	**4D**	St.B 5M 60D # 3 CYP450
Lane 5A	St.A 20M 90D # 1 β-actin	**5B**	St.B 5M 30D # 3 β-actin	**5C**	St.B 40M 30D # 2 β-actin	**5D**	St.B 20M 60D # 1 β-actin
Lane 6A	St.A 20M 90D # 1 HSP71	**6B**	St.B 5M 30D # 3 HSP71	**6C**	St.B 40M 30D # 2 HSP71	**6D**	St.B 20M 60D # 1 HSP71
Lane 7A	St.A 20M 90D # 1 CYP450	**7B**	St.B 5M 30D # 3 CYP450	**7C**	St.B 40M 30D # 2 CYP450	**7D**	St.B 20M 60D # 1 CYP450
Lane 8A	St.A 20M 90D # 2 β-actin	**8B**	St.B 20M 30D # 1 β-actin	**8C**	St.B 40M 30D # 3 β-actin	**8D**	St.B 20M 60D # 2 β-actin
Lane 9A	St.A 20M 90D # 2 HSP71	**9B**	St.B 20M 30D # 1 HSP71	**9C**	St.B 40M 30D # 3 HSP71	**9D**	St.B 20M 60D # 2 HSP71
Lane 10A	St.A 20M 90D # 2 CYP450	**10B**	St.B 20M 30D # 1 CYP450	**10C**	St.B 40M 30D # 3 CYP450	**10D**	St.B 20M 60D # 2 CYP450
Lane 11A	St.A 20M 90D # 3 β-actin	**11B**	St.B 20M 30D # 2 β-actin	**11C**	St.B 5M 60D # 1 β-actin	**11D**	St.B 20M 60D # 3 β-actin
Lane 12A	St.A 20M 90D # 3 HSP71	**12B**	St.B 20M 30D # 2 HSP71	**12C**	St.B 5M 60D # 1 HSP71	**12D**	St.B 20M 60D # 3 HSP71
Lane 13A	St.A 20M 90D # 3 CYP450	**13B**	St.B 20M 30D # 2 CYP450	**13C**	St.B 5M 60D # 1 CYP450	**13D**	St.B 20M 60D # 3 CYP450
Lane 14A	St.B 5M 30D # 1 β-actin	**14B**	St.B 20M 30D # 3 β-actin	**14C**	St.B 5M 60D # 2 β-actin	**14D**	St.B 40M 60D # 1 β-actin
Lane 15A	St.B 5M 30D # 1 HSP71	**15B**	St.B 20M 30D # 3 HSP71	**15C**	St.B 5M 60D # 2 HSP71	**15D**	St.B 40M 60D # 1 HSP71
Lane 16A	St.B 5M 30D # 1 CYP450	**16B**	St.B 20M 30D # 3 CYP450	**16C**	St.B 5M 60D # 2 CYP450	**16D**	St.B 40M 60D # 1 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	

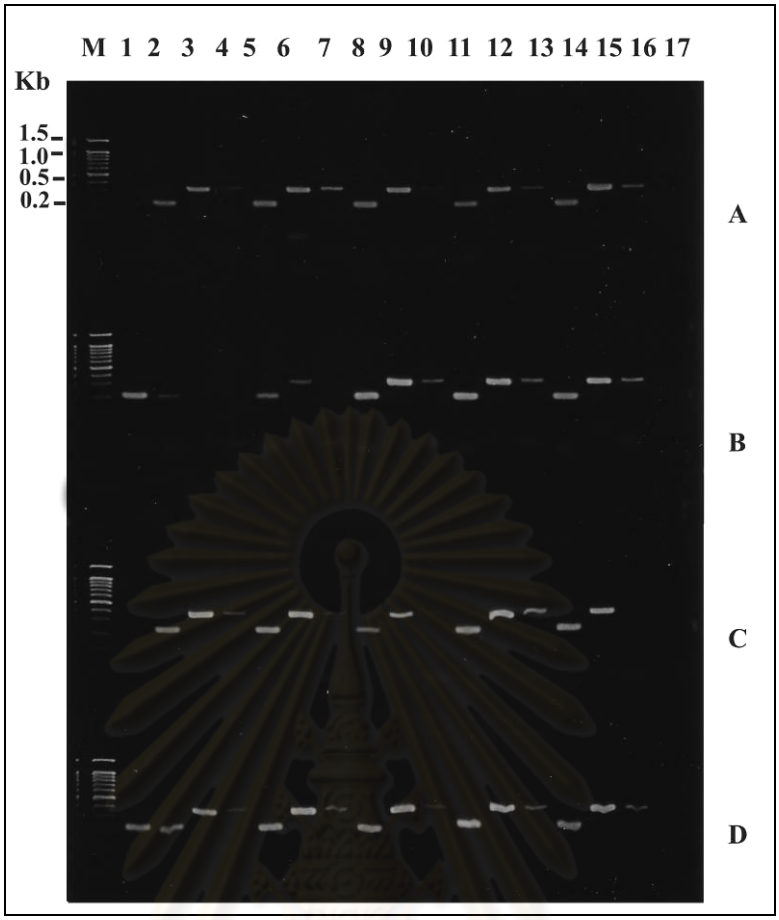

Figure B34 PCR products of HSP71 and CYP450 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.B 40M 60D # 2 β-actin	**2B**	St.B 20M 90D # 1 β-actin	**2C**	St.B 40M 90D # 3 β-actin	**2D**	St.C 20M 30D # 2 β-actin
Lane 3A	St.B 40M 60D # 2 HSP71	**3B**	St.B 20M 90D # 1 HSP71	**3C**	St.B 40M 90D # 3 HSP71	**3D**	St.C 20M 30D # 2 HSP71
Lane 4A	St.B 40M 60D # 2 CYP450	**4B**	St.B 20M 90D # 1 CYP450	**4C**	St.B 40M 90D # 3 CYP450	**4D**	St.C 20M 30D # 2 CYP450
Lane 5A	St.B 40M 60D # 3 β-actin	**5B**	St.B 20M 90D # 2 β-actin	**5C**	St.C 5M 30D # 1 β-actin	**5D**	St.C 20M 30D # 3 β-actin
Lane 6A	St.B 40M 60D # 3 HSP71	**6B**	St.B 20M 90D # 2 HSP71	**6C**	St.C 5M 30D # 1 HSP71	**6D**	St.C 20M 30D # 3 HSP71
Lane 7A	St.B 40M 60D # 3 CYP450	**7B**	St.B 20M 90D # 2 CYP450	**7C**	St.C 5M 30D # 1 CYP450	**7D**	St.C 20M 30D # 3 CYP450
Lane 8A	St.B 5M 90D # 1 β-actin	**8B**	St.B 20M 90D # 3 β-actin	**8C**	St.C 5M 30D # 2 β-actin	**8D**	St.C 40M 30D # 1 β-actin
Lane 9A	St.B 5M 90D # 1 HSP71	**9B**	St.B 20M 90D # 3 HSP71	**9C**	St.C 5M 30D # 2 HSP71	**9D**	St.C 40M 30D # 1 HSP71
Lane 10A	St.B 5M 90D # 1 CYP450	**10B**	St.B 20M 90D # 3 CYP450	**10C**	St.C 5M 30D # 2 CYP450	**10D**	St.C 40M 30D # 1 CYP450
Lane 11A	St.B 5M 90D # 2 β-actin	**11B**	St.B 40M 90D # 1 β-actin	**11C**	St.C 5M 30D # 3 β-actin	**11D**	St.C 40M 30D # 2 β-actin
Lane 12A	St.B 5M 90D # 2 HSP71	**12B**	St.B 40M 90D # 1 HSP71	**12C**	St.C 5M 30D # 3 HSP71	**12D**	St.C 40M 30D # 2 HSP71
Lane 13A	St.B 5M 90D # 2 CYP450	**13B**	St.B 40M 90D # 1 CYP450	**13C**	St.C 5M 30D # 3 CYP450	**13D**	St.C 40M 30D # 2 CYP450
Lane 14A	St.B 5M 90D # 3 β-actin	**14B**	St.B 40M 90D # 2 β-actin	**14C**	St.C 20M 30D # 1 β-actin	**14D**	St.C 40M 30D # 3 β-actin
Lane 15A	St.B 5M 90D # 3 HSP71	**15B**	St.B 40M 90D # 2 HSP71	**15C**	St.C 20M 30D # 1 HSP71	**15D**	St.C 40M 30D # 3 HSP71
Lane 16A	St.B 5M 90D # 3 CYP450	**16B**	St.B 40M 90D # 2 CYP450	**16C**	St.C 20M 30D # 1 CYP450	**16D**	St.C 40M 30D # 3 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	

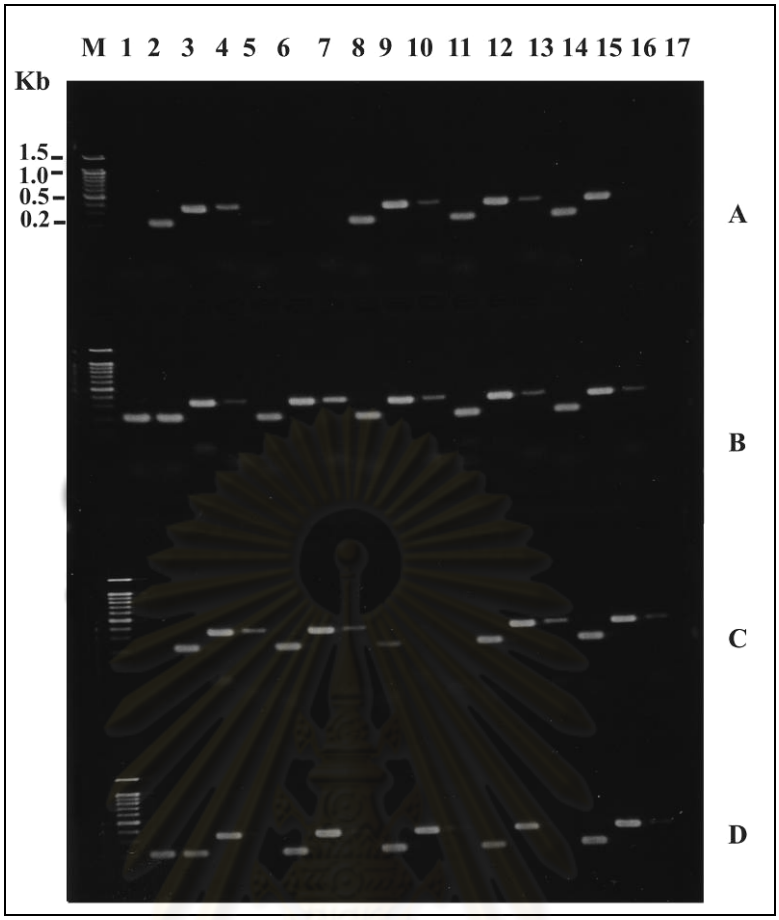

Figure B35 PCR products of HSP71 and CYP450 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B and 1D. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A and 1C. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**		**Lane**		**Lane**	
Lane 1A	Negative Control	**1B**	Positive Control β-actin	**1C**	Negative Control	**1D**	Positive Control β-actin
Lane 2A	St.C 5M 60D # 1 β-actin	**2B**	St.C 20M 60D # 3 β-actin	**2C**	St.C 5M 90D # 2 β-actin	**2D**	St.C 40M 90D # 1 β-actin
Lane 3A	St.C 5M 60D # 1 HSP71	**3B**	St.C 20M 60D # 3 HSP71	**3C**	St.C 5M 90D # 2 HSP71	**3D**	St.C 40M 90D # 1 HSP71
Lane 4A	St.C 5M 60D # 1 CYP450	**4B**	St.C 20M 60D # 3 CYP450	**4C**	St.C 5M 90D # 2 CYP450	**4D**	St.C 40M 90D # 1 CYP450
Lane 5A	St.C 5M 60D # 2 β-actin	**5B**	St.C 40M 60D # 1 β-actin	**5C**	St.C 5M 90D # 3 β-actin	**5D**	St.C 40M 90D # 2 β-actin
Lane 6A	St.C 5M 60D # 2 HSP71	**6B**	St.C 40M 60D # 1 HSP71	**6C**	St.C 5M 90D # 3 HSP71	**6D**	St.C 40M 90D # 2 HSP71
Lane 7A	St.C 5M 60D # 2 CYP450	**7B**	St.C 40M 60D # 1 CYP450	**7C**	St.C 5M 90D # 3 CYP450	**7D**	St.C 40M 90D # 2 CYP450
Lane 8A	St.C 5M 60D # 3 β-actin	**8B**	St.C 40M 60D # 2 β-actin	**8C**	St.C 20M 90D # 1 β-actin	**8D**	St.C 40M 90D # 3 β-actin
Lane 9A	St.C 5M 60D # 3 HSP71	**9B**	St.C 40M 60D # 2 HSP71	**9C**	St.C 20M 90D # 1 HSP71	**9D**	St.C 40M 90D # 3 HSP71
Lane 10A	St.C 5M 60D # 3 CYP450	**10B**	St.C 40M 60D # 2 CYP450	**10C**	St.C 20M 90D # 1 CYP450	**10D**	St.C 40M 90D # 3 CYP450
Lane 11A	St.C 20M 60D # 1 β-actin	**11B**	St.C 40M 60D # 3 β-actin	**11C**	St.C 20M 90D # 2 β-actin	**11D**	St.D 5M 30D # 1 β-actin
Lane 12A	St.C 20M 60D # 1 HSP71	**12B**	St.C 40M 60D # 3 HSP71	**12C**	St.C 20M 90D # 2 HSP71	**12D**	St.D 5M 30D # 1 HSP71
Lane 13A	St.C 20M 60D # 1 CYP450	**13B**	St.C 40M 60D # 3 CYP450	**13C**	St.C 20M 90D # 2 CYP450	**13D**	St.D 5M 30D # 1 CYP450
Lane 14A	St.C 20M 60D # 2 β-actin	**14B**	St.C 5M 90D # 1 β-actin	**14C**	St.C 20M 90D # 3 β-actin	**14D**	St.D 5M 30D # 2 β-actin
Lane 15A	St.C 20M 60D # 2 HSP71	**15B**	St.C 5M 90D # 1 HSP71	**15C**	St.C 20M 90D # 3 HSP71	**15D**	St.D 5M 30D # 2 HSP71
Lane 16A	St.C 20M 60D # 2 CYP450	**16B**	St.C 5M 90D # 1 CYP450	**16C**	St.C 20M 90D # 3 CYP450	**16D**	St.D 5M 30D # 2 CYP450
Lane 17A	Blank	**17B**	Blank	**17C**	Blank	**17D**	

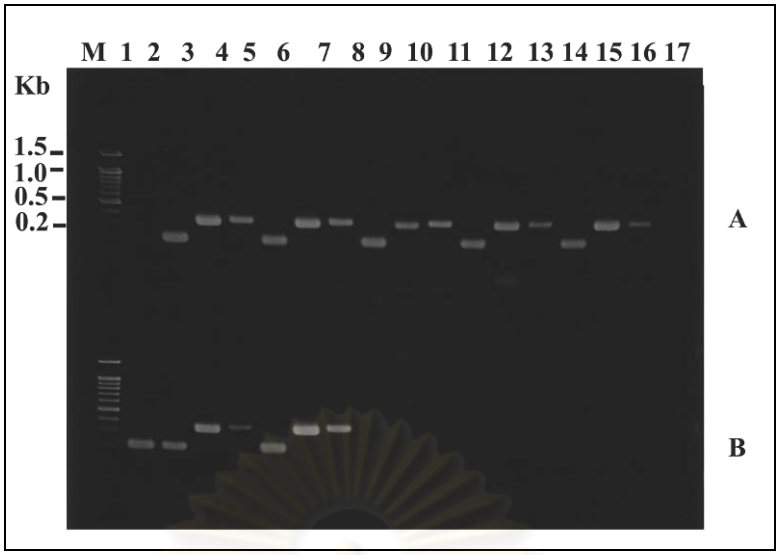

Figure B36 PCR products of HSP71 and CYP450 using first strand cDNA from mussel gill, β-actin used as positive control is shown in lane 1B. Lane M is 100 bp DNA ladder. Negative control is shown in Lane 1A. Analyzed on 2.0 % agarose gel electrophoresis and stain with ethidium bromide.

Lane M	100 base pair ladder	**Lane**					
Lane 1A	Negative Control	**1B**	Positive Control β-actin				
Lane 2A	St.D 5M 30D # 3 β-actin	**2B**	St.D 5M 90D # 2 β-actin				
Lane 3A	St.D 5M 30D # 3 HSP71	**3B**	St.D 5M 90D # 2 HSP71				
Lane 4A	St.D 5M 30D # 3 CYP450	**4B**	St.D 5M 90D # 2 CYP450				
Lane 5A	St.D 5M 60D # 1 β-actin	**5B**	St.D 5M 90D # 3 β-actin				
Lane 6A	St.D 5M 60D # 1 HSP71	**6B**	St.D 5M 90D # 3 HSP71				
Lane 7A	St.D 5M 60D # 1 CYP450	**7B**	St.D 5M 90D # 3 CYP450				
Lane 8A	St.D 5M 60D # 2 β-actin						
Lane 9A	St.D 5M 60D # 2 HSP71						
Lane 10A	St.D 5M 60D # 2 CYP450						
Lane 11A	St.D 5M 60D # 3 β-actin						
Lane 12A	St.D 5M 60D # 3 HSP71						
Lane 13A	St.D 5M 60D # 3 CYP450						
Lane 14A	St.D 5M 90D # 1 β-actin						
Lane 15A	St.D 5M 90D # 1 HSP71						
Lane 16A	St.D 5M 90D # 1 CYP450						
Lane 17A	Blank						

Table B1 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity MT	Ratio MT/AT	Remark
1	Initial # 1	4,614.1009	4,626.5590	1.0027	
2	Initial # 2	4,362.2049	3,697.4049	0.8476	
3	Initial # 3	4,470.4610	2,570.5151	0.5750	
4	Control week 1 #1	3,729.9440	3,833.2634	1.0277	
5	Control week 1 #2	4,132.5567	3,932.1277	0.9515	
6	Control week 1 #3	2,939.6814	2,029.8500	0.6905	
7	0.1µg/L week 1 #1	2,819.2144	2,566.0490	0.9102	
8	0.1µg/L week 1 #2	4,505.5442	4,296.9375	0.9537	
9	0.1µg/L week 1 #3	4,793.9111	4,527.3696	0.9444	
10	0.2µg/L week 1 #1	4,521.8023	3,777.0614	0.8353	
11	0.2µg/L week 1 #2	4,385.6091	3,822.4969	0.8716	
12	0.2µg/L week 1 #3	3,741.5767	3,487.8978	0.9322	
13	0.5µg/L week 1 #1	4,059.6151	3,764.0751	0.9272	
14	0.5µg/L week 1 #2	2,774.2561	1,644.3016	0.5927	
15	0.5µg/L week 1 #3	2,660.4727	2,489.4043	0.9357	
16	1.0µg/L week 1 #1	3,814.7958	3,572.9378	0.9366	
17	1.0µg/L week 1 #2	4,240.0034	5,006.1720	1.1807	
18	1.0µg/L week 1 #3	4,287.2744	4,163.8009	0.9712	

Table B2 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity MT	Ratio MT/AT	Remark
1	Control week 2 #1	4,024.6938	3,934.9431	0.9777	
2	Control week 2 #2	3,493.8203	3,844.9493	1.1005	
3	Control week 2 #3	4,000.2257	4,158.2347	1.0395	
4	0.1µg/L week 2 #1	3,083.5758	2,982.7428	0.9673	
5	0.1µg/L week 2 #2	2,907.9053	3,277.7909	1.1272	
6	0.1µg/L week 2 #3	4,284.1060	4,080.1826	0.9524	
7	0.2µg/L week 2 #1	4,877.0516	4,413.2439	0.9049	
8	0.2µg/L week 2 #2	4,305.4288	4,344.1777	1.0090	
9	0.2µg/L week 2 #3	3,920.9012	4,084.7948	1.0418	
10	0.5µg/L week 2 #1	3,740.3510	3,379.4071	0.9035	
11	0.5µg/L week 2 #2	4,069.0739	3,745.1757	0.9204	
12	0.5µg/L week 2 #3	4,068.2414	4,912.4015	1.2075	
13	1.0µg/L week 2 #1	3,062.4611	2,951.2937	0.9637	
14	1.0µg/L week 2 #2	4,076.4051	3,842.8271	0.9427	
15	1.0µg/L week 2 #3	3,814.7958	3,585.1451	0.9398	

Table B3 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity MT	Ratio MT/AT	Remark
1	Control week 3 #1	3,082.5593	2,940.4534	0.9539	
2	Control week 3 #2	3,017.1826	2,836.7550	0.9402	
3	Control week 3 #3	2,848.2378	2,734.8779	0.9602	
4	0.1µg/L week 3 #1	2,574.0440	2,276.7419	0.8845	
5	0.1µg/L week 3 #2	2,918.3688	2,676.4360	0.9171	
6	0.1µg/L week 3 #3	3,034.4658	2,755.9018	0.9082	
7	0.2µg/L week 3 #1	2,344.5554	2,081.0274	0.8876	
8	0.2µg/L week 3 #2	3,086.3942	2,941.9510	0.9532	
9	0.2µg/L week 3 #3	2,966.6725	3,252.3630	1.0963	
10	0.5µg/L week 3 #1	2,880.3089	2,763.3684	0.9594	
11	0.5µg/L week 3 #2	2,902.5039	2,746.9297	0.9464	
12	0.5µg/L week 3 #3	3,144.0750	3,380.8238	1.0753	
13	1.0µg/L week 3 #1	2,863.1308	2,773.2284	0.9686	
14	1.0µg/L week 3 #2	3,037.7491	2,942.9713	0.9688	
15	1.0µg/L week 3 #3	2,127.5958	1,969.7282	0.9258	

Table B4 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity MT	Ratio MT/AT	Remark
1	Control week 4 #1	2,592.5354	2,242.0246	0.8648	
2	Control week 4 #2	3,341.3874	2,180.2553	0.6525	
3	Control week 4 #3	3,404.5315	3,422.2351	1.0052	
4	0.1µg/L week 4 #1	3,081.7976	3,049.7469	0.9896	
5	0.1µg/L week 4 #2	2,765.4202	2,595.0703	0.9384	
6	0.1µg/L week 4 #3	2,837.3635	2,676.2013	0.9432	
7	0.2µg/L week 4 #1	2,935.9934	2,849.0880	0.9704	
8	0.2µg/L week 4 #2	2,370.9793	2,252.6674	0.9501	
9	0.2µg/L week 4 #3	3,283.8642	3,829.3140	1.1661	
10	0.5µg/L week 4 #1	3,532.2120	3,054.6569	0.8648	
11	0.5µg/L week 4 #2	3,221.1666	2,101.8112	0.6525	
12	0.5µg/L week 4 #3	2,937.3593	2,952.6336	1.0052	
13	1.0µg/L week 4 #1	3,044.7096	2,878.4685	0.9454	
14	1.0µg/L week 4 #2	2,666.5538	2,515.0936	0.9432	
15	1.0µg/L week 4 #3	3,054.0867	3,087.9871	1.0111	

Table B5 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity MT	Ratio MT/AT	Remark
1	Control week 5 #1	3,271.7227	2,888.2768	0.8828	
2	Control week 5 #2	3,248.4071	3,179.5409	0.9788	
3	Control week 5 #3	2,354.1545	2,285.6486	0.9709	
4	0.1µg/L week 5 #1	2,950.2865	3,006.0469	1.0189	
5	0.1µg/L week 5 #2	3,110.6768	2,836.6262	0.9119	
6	0.1µg/L week 5 #3	3,252.3404	3,229.2488	0.9929	
7	0.2µg/L week 5 #1	2,758.9107	2,674.4880	0.9694	
8	0.2µg/L week 5 #2	3,416.6640	3,118.7309	0.9128	
9	0.2µg/L week 5 #3	3,657.7959	4,007.1154	1.0955	
10	0.5µg/L week 5 #1	3,546.0033	3,513.3801	0.9908	
11	0.5µg/L week 5 #2	3,164.2350	2,827.5604	0.8936	
12	0.5µg/L week 5 #3	2,857.3082	2,819.5917	0.9868	
13	1.0µg/L week 5 #1	3,013.5030	2,881.5116	0.9562	
14	1.0µg/L week 5 #2	2,716.6498	2,642.4852	0.9727	
15	1.0µg/L week 5 #3	2,302.1478	2,261.3998	0.9823	

Table B6 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity MT	Ratio MT/AT	Remark
1	Control week 6 #1	2,791.7754	2,191.8228	0.7851	
2	Control week 6 #2	3,678.1178	3,369.1559	0.9160	
3	Control week 6 #3	3,357.4901	3,229.5697	0.9619	
4	0.1µg/L week 6 #1	3,144.3501	2,967.3232	0.9437	
5	0.1µg/L week 6 #2	3,293.4432	3,505.5409	1.0644	
6	0.1µg/L week 6 #3	3,059.6972	2,970.0481	0.9707	
7	0.2µg/L week 6 #1	2,449.5583	2,323.6510	0.9486	
8	0.2µg/L week 6 #2	2,438.6325	2,295.9725	0.9415	
9	0.2µg/L week 6 #3	3,159.6243	2,940.3464	0.9306	
10	0.5µg/L week 6 #1	2,709.5262	2,453.4759	0.9055	
11	0.5µg/L week 6 #2	2,968.1829	2,935.8297	0.9891	
12	0.5µg/L week 6 #3	2,891.1344	2,786.1862	0.9637	
13	1.0µg/L week 6 #1	2,927.7575	2,824.9933	0.9649	
14	1.0µg/L week 6 #2	2,956.9075	2,736.6179	0.9255	
15	1.0µg/L week 6 #3	2,977.7102	2,941.0843	0.9877	

Table B7 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity MT	Ratio MT/AT	Remark
1	Control week 7 #1	3,613.9686	3,564.8187	0.9864	
2	Control week 7 #2	3,580.6571	3,516.9214	0.9822	
3	Control week 7 #3	3,512.8680	3,333.0092	0.9488	
4	0.1µg/L week 7 #1	2,760.9605	2,757.3713	0.9987	
5	0.1µg/L week 7 #2	3,284.3780	3,310.9815	1.0081	
6	0.1µg/L week 7 #3	2,493.4878	2,480.7710	0.9949	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	3,395.9464	3,421.4160	1.0075	
11	0.5µg/L week 7 #2	3,329.0584	3,223.8602	0.9684	
12	0.5µg/L week 7 #3	3,030.7918	2,858.3397	0.9431	
13	1.0µg/L week 7 #1	3,236.8091	3,292.1585	1.0171	
14	1.0µg/L week 7 #2	2,242.8432	2,022.3717	0.9017	
15	1.0µg/L week 7 #3	2,142.8291	2,150.1147	1.0034	

Table B8 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity MT	Ratio MT/AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	2,933.1098	2,948.6553	1.0053	
5	0.1µg/L week 8 #2	3,396.6355	3,399.0131	1.0007	
6	0.1µg/L week 8 #3	3,351.7961	3,374.5883	1.0068	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B9 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01/AT	Remark
1	Initial # 1	4,614.1009	4,640.8627	1.0058	
2	Initial # 2	4,362.2049	3,673.8490	0.8422	
3	Initial # 3	4,470.4610	1,669.2701	0.3734	
4	Control week 1 #1	3,729.9440	2,602.0089	0.6976	
5	Control week 1 #2	4,132.5567	3,542.4276	0.8572	
6	Control week 1 #3	2,939.6814	2,730.0821	0.9287	
7	0.1µg/L week 1 #1	2,819.2144	2,627.7898	0.9321	
8	0.1µg/L week 1 #2	4,505.5442	3,562.5338	0.7907	
9	0.1µg/L week 1 #3	4,793.9111	5,116.5413	1.0673	
10	0.2µg/L week 1 #1	4,521.8023	4,386.1482	0.9700	
11	0.2µg/L week 1 #2	4,385.6091	4,365.8739	0.9955	
12	0.2µg/L week 1 #3	3,741.5767	3,453.4753	0.9230	
13	0.5µg/L week 1 #1	4,059.6151	4,118.0736	1.0144	
14	0.5µg/L week 1 #2	2,774.2561	2,664.3956	0.9604	
15	0.5µg/L week 1 #3	2,660.4727	2,294.6577	0.8625	
16	1.0µg/L week 1 #1	3,814.7958	3,542.0379	0.9285	
17	1.0µg/L week 1 #2	4,240.0034	5,668.4605	1.3369	
18	1.0µg/L week 1 #3	4,287.2744	3,783.5196	0.8825	

Table B10 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01/AT	Remark
1	Control week 2 #1	4,024.6938	3,787.2369	0.9410	
2	Control week 2 #2	3,493.8203	3,613.6584	1.0343	
3	Control week 2 #3	4,000.2257	4,241.4394	1.0603	
4	0.1µg/L week 2 #1	3,083.5758	3,045.9561	0.9878	
5	0.1µg/L week 2 #2	2,907.9053	2,435.0799	0.8374	
6	0.1µg/L week 2 #3	4,284.1060	3,059.2801	0.7141	
7	0.2µg/L week 2 #1	4,877.0516	4,306.4365	0.8830	
8	0.2µg/L week 2 #2	4,305.4288	4,275.7214	0.9931	
9	0.2µg/L week 2 #3	3,920.9012	3,795.4323	0.9680	
10	0.5µg/L week 2 #1	3,740.3510	3,411.2001	0.9120	
11	0.5µg/L week 2 #2	4,069.0739	3,485.1618	0.8565	
12	0.5µg/L week 2 #3	4,068.2414	4,243.5826	1.0431	
13	1.0µg/L week 2 #1	3,062.4611	2,423.9379	0.7915	
14	1.0µg/L week 2 #2	4,076.4051	3,904.3808	0.9578	
15	1.0µg/L week 2 #3	3,814.7958	3,831.5809	1.0044	

Table B11 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01/AT	Remark
1	Control week 3 #1	3,082.5593	3,186.7499	1.0338	
2	Control week 3 #2	3,017.1826	2,672.0169	0.8856	
3	Control week 3 #3	2,848.2378	2,565.6926	0.9008	
4	0.1µg/L week 3 #1	2,574.0440	2,203.3816	0.8560	
5	0.1µg/L week 3 #2	2,918.3688	2,560.8686	0.8775	
6	0.1µg/L week 3 #3	3,034.4658	2,451.5449	0.8079	
7	0.2µg/L week 3 #1	2,344.5554	2,097.2048	0.8945	
8	0.2µg/L week 3 #2	3,086.3942	2,756.1500	0.8930	
9	0.2µg/L week 3 #3	2,966.6725	3,339.2865	1.1256	
10	0.5µg/L week 3 #1	2,880.3089	2,701.4417	0.9379	
11	0.5µg/L week 3 #2	2,902.5039	2,515.3099	0.8666	
12	0.5µg/L week 3 #3	3,144.0750	3,016.7400	0.9595	
13	1.0µg/L week 3 #1	2,863.1308	2,579.1082	0.9008	
14	1.0µg/L week 3 #2	3,037.7491	2,649.2210	0.8721	
15	1.0µg/L week 3 #3	2,127.5958	1,705.6936	0.8017	

Table B12 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01/AT	Remark
1	Control week 4 #1	2,592.5354	2,503.8707	0.9658	
2	Control week 4 #2	3,341.3874	3,027.2970	0.9060	
3	Control week 4 #3	3,404.5315	3,197.5360	0.9392	
4	0.1µg/L week 4 #1	3,081.7976	2,516.9041	0.8167	
5	0.1µg/L week 4 #2	2,765.4202	2,587.3271	0.9356	
6	0.1µg/L week 4 #3	2,837.3635	2,073.8290	0.7309	
7	0.2µg/L week 4 #1	2,935.9934	2,623.6037	0.8936	
8	0.2µg/L week 4 #2	2,370.9793	2,251.7190	0.9497	
9	0.2µg/L week 4 #3	3,283.8642	3,894.6629	1.1860	
10	0.5µg/L week 4 #1	3,532.2120	3,411.4103	0.9658	
11	0.5µg/L week 4 #2	3,221.1666	2,918.3769	0.9060	
12	0.5µg/L week 4 #3	2,937.3593	2,758.7678	0.9392	
13	1.0µg/L week 4 #1	3,044.7096	2,950.0192	0.9689	
14	1.0µg/L week 4 #2	2,666.5538	2,439.3634	0.9148	
15	1.0µg/L week 4 #3	3,054.0867	2,898.9391	0.9492	

Table B13 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01/AT	Remark
1	Control week 5 #1	3,271.7227	2,630.1379	0.8039	
2	Control week 5 #2	3,248.4071	3,001.5282	0.9240	
3	Control week 5 #3	2,354.1545	2,303.3047	0.9784	
4	0.1µg/L week 5 #1	2,950.2865	2,721.0492	0.9223	
5	0.1µg/L week 5 #2	3,110.6768	2,586.8389	0.8316	
6	0.1µg/L week 5 #3	3,252.3404	2,340.0589	0.7195	
7	0.2µg/L week 5 #1	2,758.9107	2,386.7336	0.8651	
8	0.2µg/L week 5 #2	3,416.6640	2,920.9061	0.8549	
9	0.2µg/L week 5 #3	3,657.7959	3,902.8682	1.0670	
10	0.5µg/L week 5 #1	3,546.0033	3,446.7152	0.9720	
11	0.5µg/L week 5 #2	3,164.2350	2,771.8698	0.8760	
12	0.5µg/L week 5 #3	2,857.3082	2,767.3030	0.9685	
13	1.0µg/L week 5 #1	3,013.5030	2,580.7640	0.8564	
14	1.0µg/L week 5 #2	2,716.6498	2,598.2038	0.9564	
15	1.0µg/L week 5 #3	2,302.1478	2,187.5008	0.9502	

Table B14 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01/AT	Remark
1	Control week 6 #1	2,791.7754	2,594.3969	0.9293	
2	Control week 6 #2	3,678.1178	3,351.1331	0.9111	
3	Control week 6 #3	3,357.4901	3,357.8258	1.0001	
4	0.1µg/L week 6 #1	3,144.3501	2,886.8278	0.9181	
5	0.1µg/L week 6 #2	3,293.4432	3,472.9358	1.0545	
6	0.1µg/L week 6 #3	3,059.6972	2,789.2200	0.9116	
7	0.2µg/L week 6 #1	2,449.5583	2,199.4584	0.8979	
8	0.2µg/L week 6 #2	2,438.6325	2,264.0265	0.9284	
9	0.2µg/L week 6 #3	3,159.6243	2,962.4637	0.9376	
10	0.5µg/L week 6 #1	2,709.5262	2,413.9169	0.8909	
11	0.5µg/L week 6 #2	2,968.1829	3,016.8611	1.0164	
12	0.5µg/L week 6 #3	2,891.1344	2,769.7067	0.9580	
13	1.0µg/L week 6 #1	2,927.7575	2,720.4723	0.9292	
14	1.0µg/L week 6 #2	2,956.9075	2,521.3550	0.8527	
15	1.0µg/L week 6 #3	2,977.7102	2,539.0935	0.8527	

Table B15 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01/AT	Remark
1	Control week 7 #1	3,613.9686	3,387.7342	0.9374	
2	Control week 7 #2	3,580.6571	3,142.3847	0.8776	
3	Control week 7 #3	3,512.8680	3,099.4035	0.8823	
4	0.1µg/L week 7 #1	2,760.9605	2,769.7956	1.0032	
5	0.1µg/L week 7 #2	3,284.3780	3,304.0843	1.0060	
6	0.1µg/L week 7 #3	2,493.4878	2,512.4383	1.0076	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	3,395.9464	3,467.9405	1.0212	
11	0.5µg/L week 7 #2	3,329.0584	2,877.9710	0.8645	
12	0.5µg/L week 7 #3	3,030.7918	2,312.4941	0.7630	
13	1.0µg/L week 7 #1	3,236.8091	3,150.0626	0.9732	
14	1.0µg/L week 7 #2	2,242.8432	2,052.8743	0.9153	
15	1.0µg/L week 7 #3	2,142.8291	2,161.0431	1.0085	

Table B16 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01/AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	2,933.1098	2,970.6536	1.0128	
5	0.1µg/L week 8 #2	3,396.6355	3,397.3148	1.0002	
6	0.1µg/L week 8 #3	3,351.7961	3,402.7434	1.0152	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B17 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02/AT	Remark
1	Initial # 1	4,614.1009	3,226.1794	0.6992	
2	Initial # 2	4,362.2049	2,883.8537	0.6611	
3	Initial # 3	4,470.4610	2,464.5651	0.5513	
4	Control week 1 #1	3,729.9440	2,763.5155	0.7409	
5	Control week 1 #2	4,132.5567	3,161.8192	0.7651	
6	Control week 1 #3	2,939.6814	2,203.5852	0.7496	
7	0.1µg/L week 1 #1	2,819.2144	2,259.3184	0.8014	
8	0.1µg/L week 1 #2	4,505.5442	3,831.0642	0.8503	
9	0.1µg/L week 1 #3	4,793.9111	4,403.2073	0.9185	
10	0.2µg/L week 1 #1	4,521.8023	3,240.3235	0.7166	
11	0.2µg/L week 1 #2	4,385.6091	4,812.3289	1.0973	
12	0.2µg/L week 1 #3	3,741.5767	3,114.8626	0.8325	
13	0.5µg/L week 1 #1	4,059.6151	4,267.0615	1.0511	
14	0.5µg/L week 1 #2	2,774.2561	2,341.1947	0.8439	
15	0.5µg/L week 1 #3	2,660.4727	2,500.0462	0.9397	
16	1.0µg/L week 1 #1	3,814.7958	2,681.4200	0.7029	
17	1.0µg/L week 1 #2	4,240.0034	4,360.8435	1.0285	
18	1.0µg/L week 1 #3	4,287.2744	3,545.5759	0.8270	

Table B18 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02/AT	Remark
1	Control week 2 #1	4,024.6938	3,575.9404	0.8885	
2	Control week 2 #2	3,493.8203	3,678.6434	1.0529	
3	Control week 2 #3	4,000.2257	3,578.2019	0.8945	
4	0.1µg/L week 2 #1	3,083.5758	2,400.5637	0.7785	
5	0.1µg/L week 2 #2	2,907.9053	2,966.0634	1.0200	
6	0.1µg/L week 2 #3	4,284.1060	3,119.6860	0.7282	
7	0.2µg/L week 2 #1	4,877.0516	3,917.7355	0.8033	
8	0.2µg/L week 2 #2	4,305.4288	3,925.2595	0.9117	
9	0.2µg/L week 2 #3	3,920.9012	3,558.2178	0.9075	
10	0.5µg/L week 2 #1	3,740.3510	2,642.9320	0.7066	
11	0.5µg/L week 2 #2	4,069.0739	3,673.1530	0.9027	
12	0.5µg/L week 2 #3	4,068.2414	3,881.1023	0.9540	
13	1.0µg/L week 2 #1	3,062.4611	2,539.0865	0.8291	
14	1.0µg/L week 2 #2	4,076.4051	3,421.7344	0.8394	
15	1.0µg/L week 2 #3	3,814.7958	3,162.4658	0.8290	

Table B19 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02/AT	Remark
1	Control week 3 #1	3,082.5593	2,581.0269	0.8373	
2	Control week 3 #2	3,017.1826	2,911.2794	0.9649	
3	Control week 3 #3	2,848.2378	2,246.9748	0.7889	
4	0.1µg/L week 3 #1	2,574.0440	2,041.2169	0.7930	
5	0.1µg/L week 3 #2	2,918.3688	2,300.5501	0.7883	
6	0.1µg/L week 3 #3	3,034.4658	2,345.3386	0.7729	
7	0.2µg/L week 3 #1	2,344.5554	1,840.2415	0.7849	
8	0.2µg/L week 3 #2	3,086.3942	2,867.5689	0.9291	
9	0.2µg/L week 3 #3	2,966.6725	2,853.9389	0.9620	
10	0.5µg/L week 3 #1	2,880.3089	2,234.5437	0.7758	
11	0.5µg/L week 3 #2	2,902.5039	2,268.3068	0.7815	
12	0.5µg/L week 3 #3	3,144.0750	2,719.3105	0.8649	
13	1.0µg/L week 3 #1	2,863.1308	2,226.0842	0.7775	
14	1.0µg/L week 3 #2	3,037.7491	2,505.5354	0.8248	
15	1.0µg/L week 3 #3	2,127.5958	1,659.7375	0.7801	

Table B20 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02/AT	Remark
1	Control week 4 #1	2,592.5354	2,082.5837	0.8033	
2	Control week 4 #2	3,341.3874	3,188.3518	0.9542	
3	Control week 4 #3	3,404.5315	3,477.7290	1.0215	
4	0.1µg/L week 4 #1	3,081.7976	2,966.2302	0.9625	
5	0.1µg/L week 4 #2	2,765.4202	2,271.5161	0.8214	
6	0.1µg/L week 4 #3	2,837.3635	2,418.0012	0.8522	
7	0.2µg/L week 4 #1	2,935.9934	2,193.1871	0.7470	
8	0.2µg/L week 4 #2	2,370.9793	2,109.2232	0.8896	
9	0.2µg/L week 4 #3	3,283.8642	2,952.1939	0.8990	
10	0.5µg/L week 4 #1	3,532.2120	2,837.4259	0.8033	
11	0.5µg/L week 4 #2	3,221.1666	3,073.6371	0.9542	
12	0.5µg/L week 4 #3	2,937.3593	3,000.5125	1.0215	
13	1.0µg/L week 4 #1	3,044.7096	2,461.3433	0.8084	
14	1.0µg/L week 4 #2	2,666.5538	2,081.2453	0.7805	
15	1.0µg/L week 4 #3	3,054.0867	2,516.5674	0.8240	

Table 21 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02/AT	Remark
1	Control week 5 #1	3,271.7227	2,509.0842	0.7669	
2	Control week 5 #2	3,248.4071	2,712.4200	0.8350	
3	Control week 5 #3	2,354.1545	2,494.9329	1.0598	
4	0.1µg/L week 5 #1	2,950.2865	2,441.6571	0.8276	
5	0.1µg/L week 5 #2	3,110.6768	2,681.4034	0.8620	
6	0.1µg/L week 5 #3	3,252.3404	2,932.9606	0.9018	
7	0.2µg/L week 5 #1	2,758.9107	2,155.2610	0.7812	
8	0.2µg/L week 5 #2	3,416.6640	3,059.2810	0.8954	
9	0.2µg/L week 5 #3	3,657.7959	3,952.2484	1.0805	
10	0.5µg/L week 5 #1	3,546.0033	2,709.1466	0.7640	
11	0.5µg/L week 5 #2	3,164.2350	2,523.4774	0.7975	
12	0.5µg/L week 5 #3	2,857.3082	2,414.9969	0.8452	
13	1.0µg/L week 5 #1	3,013.5030	2,402.0633	0.7971	
14	1.0µg/L week 5 #2	2,716.6498	2,312.9556	0.8514	
15	1.0µg/L week 5 #3	2,302.1478	1,893.2863	0.8224	

Table B22 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02/AT	Remark
1	Control week 6 #1	2,791.7754	2,364.9129	0.8471	
2	Control week 6 #2	3,678.1178	3,031.5047	0.8242	
3	Control week 6 #3	3,357.4901	3,101.9851	0.9239	
4	0.1µg/L week 6 #1	3,144.3501	2,646.9139	0.8418	
5	0.1µg/L week 6 #2	3,293.4432	3,176.5259	0.9645	
6	0.1µg/L week 6 #3	3,059.6972	2,545.9740	0.8321	
7	0.2µg/L week 6 #1	2,449.5583	2,118.1331	0.8647	
8	0.2µg/L week 6 #2	2,438.6325	2,299.6305	0.9430	
9	0.2µg/L week 6 #3	3,159.6243	2,682.2051	0.8489	
10	0.5µg/L week 6 #1	2,709.5262	2,169.5176	0.8007	
11	0.5µg/L week 6 #2	2,968.1829	2,634.2623	0.8875	
12	0.5µg/L week 6 #3	2,891.1344	2,941.7292	1.0175	
13	1.0µg/L week 6 #1	2,927.7575	2,612.7308	0.8924	
14	1.0µg/L week 6 #2	2,956.9075	2,737.2093	0.9257	
15	1.0µg/L week 6 #3	2,977.7102	2,971.1592	0.9978	

Table B23 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02/AT	Remark
1	Control week 7 #1	3,613.9686	2,929.8444	0.8107	
2	Control week 7 #2	3,580.6571	3,028.5198	0.8458	
3	Control week 7 #3	3,512.8680	2,802.9174	0.7979	
4	0.1µg/L week 7 #1	2,760.9605	2,780.0111	1.0069	
5	0.1µg/L week 7 #2	3,284.3780	3,336.9281	1.0160	
6	0.1µg/L week 7 #3	2,493.4878	2,512.6877	1.0077	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	3,395.9464	3,412.9262	1.0050	
11	0.5µg/L week 7 #2	3,329.0584	2,871.9787	0.8627	
12	0.5µg/L week 7 #3	3,030.7918	2,643.1535	0.8721	
13	1.0µg/L week 7 #1	3,236.8091	3,297.3374	1.0187	
14	1.0µg/L week 7 #2	2,242.8432	1,970.3377	0.8785	
15	1.0µg/L week 7 #3	2,142.8291	2,160.6146	1.0083	

Table B24 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02/AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	2,933.1098	2,973.2934	1.0137	
5	0.1µg/L week 8 #2	3,396.6355	3,414.6376	1.0053	
6	0.1µg/L week 8 #3	3,351.7961	3,371.2365	1.0058	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B25 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03/AT	Remark
1	Initial # 1	4,614.1009	4,553.6562	0.9869	
2	Initial # 2	4,362.2049	3,790.7561	0.8690	
3	Initial # 3	4,470.4610	2,655.9009	0.5941	
4	Control week 1 #1	3,729.9440	3,463.9990	0.9287	
5	Control week 1 #2	4,132.5567	3,938.3266	0.9530	
6	Control week 1 #3	2,939.6814	2,760.0669	0.9389	
7	0.1µg/L week 1 #1	2,819.2144	2,353.7621	0.8349	
8	0.1µg/L week 1 #2	4,505.5442	2,648.8094	0.5879	
9	0.1µg/L week 1 #3	4,793.9111	5,134.2787	1.0710	
10	0.2µg/L week 1 #1	4,521.8023	3,928.9940	0.8689	
11	0.2µg/L week 1 #2	4,385.6091	3,945.2940	0.8996	
12	0.2µg/L week 1 #3	3,741.5767	3,575.4507	0.9556	
13	0.5µg/L week 1 #1	4,059.6151	3,747.0248	0.9230	
14	0.5µg/L week 1 #2	2,774.2561	2,599.2006	0.9369	
15	0.5µg/L week 1 #3	2,660.4727	2,201.0091	0.8273	
16	1.0µg/L week 1 #1	3,814.7958	3,445.1421	0.9031	
17	1.0µg/L week 1 #2	4,240.0034	4,964.6200	1.1709	
18	1.0µg/L week 1 #3	4,287.2744	4,064.3361	0.9480	

Table B26 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03/AT	Remark
1	Control week 2 #1	4,024.6938	3,952.6518	0.9821	
2	Control week 2 #2	3,493.8203	3,444.9069	0.9860	
3	Control week 2 #3	4,000.2257	4,351.8456	1.0879	
4	0.1µg/L week 2 #1	3,083.5758	2,817.4632	0.9137	
5	0.1µg/L week 2 #2	2,907.9053	2,941.9278	1.0117	
6	0.1µg/L week 2 #3	4,284.1060	3,626.0673	0.8464	
7	0.2µg/L week 2 #1	4,877.0516	4,526.3915	0.9281	
8	0.2µg/L week 2 #2	4,305.4288	4,522.8530	1.0505	
9	0.2µg/L week 2 #3	3,920.9012	4,097.3417	1.0450	
10	0.5µg/L week 2 #1	3,740.3510	3,402.2233	0.9096	
11	0.5µg/L week 2 #2	4,069.0739	3,575.0884	0.8786	
12	0.5µg/L week 2 #3	4,068.2414	4,609.7243	1.1331	
13	1.0µg/L week 2 #1	3,062.4611	2,673.8348	0.8731	
14	1.0µg/L week 2 #2	4,076.4051	4,228.0474	1.0372	
15	1.0µg/L week 2 #3	3,814.7958	3,970.0580	1.0407	

Table B27 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03/AT	Remark
1	Control week 3 #1	3,082.5593	2,993.7816	0.9712	
2	Control week 3 #2	3,017.1826	2,718.7832	0.9011	
3	Control week 3 #3	2,848.2378	2,384.2598	0.8371	
4	0.1µg/L week 3 #1	2,574.0440	2,247.9126	0.8733	
5	0.1µg/L week 3 #2	2,918.3688	2,538.1053	0.8697	
6	0.1µg/L week 3 #3	3,034.4658	2,568.0684	0.8463	
7	0.2µg/L week 3 #1	2,344.5554	2,000.3746	0.8532	
8	0.2µg/L week 3 #2	3,086.3942	3,307.0714	1.0715	
9	0.2µg/L week 3 #3	2,966.6725	3,253.8464	1.0968	
10	0.5µg/L week 3 #1	2,880.3089	2,528.3352	0.8778	
11	0.5µg/L week 3 #2	2,902.5039	2,387.3095	0.8225	
12	0.5µg/L week 3 #3	3,144.0750	3,268.2660	1.0395	
13	1.0µg/L week 3 #1	2,863.1308	2,340.8957	0.8176	
14	1.0µg/L week 3 #2	3,037.7491	2,680.2060	0.8823	
15	1.0µg/L week 3 #3	2,127.5958	1,899.3048	0.8927	

Table B28 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03/AT	Remark
1	Control week 4 #1	2,592.5354	2,505.9447	0.9666	
2	Control week 4 #2	3,341.3874	2,700.5093	0.8082	
3	Control week 4 #3	3,404.5315	2,736.9029	0.8039	
4	0.1µg/L week 4 #1	3,081.7976	2,686.0948	0.8716	
5	0.1µg/L week 4 #2	2,765.4202	2,614.9813	0.9456	
6	0.1µg/L week 4 #3	2,837.3635	2,255.1365	0.7948	
7	0.2µg/L week 4 #1	2,935.9934	2,467.1153	0.8403	
8	0.2µg/L week 4 #2	2,370.9793	2,084.8021	0.8793	
9	0.2µg/L week 4 #3	3,283.8642	3,925.5313	1.1954	
10	0.5µg/L week 4 #1	3,532.2120	3,414.2361	0.9666	
11	0.5µg/L week 4 #2	3,221.1666	2,603.3468	0.8082	
12	0.5µg/L week 4 #3	2,937.3593	2,361.3431	0.8039	
13	1.0µg/L week 4 #1	3,044.7096	2,726.8419	0.8956	
14	1.0µg/L week 4 #2	2,666.5538	2,258.0378	0.8468	
15	1.0µg/L week 4 #3	3,054.0867	2,881.2254	0.9434	

Table B29 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03/AT	Remark
1	Control week 5 #1	3,271.7227	2,729.5983	0.8343	
2	Control week 5 #2	3,248.4071	3,075.2670	0.9467	
3	Control week 5 #3	2,354.1545	2,271.9945	0.9651	
4	0.1µg/L week 5 #1	2,950.2865	2,505.3833	0.8492	
5	0.1µg/L week 5 #2	3,110.6768	2,348.2499	0.7549	
6	0.1µg/L week 5 #3	3,252.3404	3,092.3253	0.9508	
7	0.2µg/L week 5 #1	2,758.9107	2,273.0665	0.8239	
8	0.2µg/L week 5 #2	3,416.6640	2,842.3228	0.8319	
9	0.2µg/L week 5 #3	3,657.7959	3,991.7526	1.0913	
10	0.5µg/L week 5 #1	3,546.0033	3,222.6078	0.9088	
11	0.5µg/L week 5 #2	3,164.2350	2,694.9789	0.8517	
12	0.5µg/L week 5 #3	2,857.3082	2,748.4447	0.9619	
13	1.0µg/L week 5 #1	3,013.5030	2,309.2474	0.7663	
14	1.0µg/L week 5 #2	2,716.6498	2,555.8241	0.9408	
15	1.0µg/L week 5 #3	2,302.1478	1,870.0346	0.8123	

Table B30 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03/AT	Remark
1	Control week 6 #1	2,791.7754	2,473.5130	0.8860	
2	Control week 6 #2	3,678.1178	3,017.8956	0.8205	
3	Control week 6 #3	3,357.4901	3,296.3837	0.9818	
4	0.1µg/L week 6 #1	3,144.3501	2,744.3887	0.8728	
5	0.1µg/L week 6 #2	3,293.4432	3,381.7074	1.0268	
6	0.1µg/L week 6 #3	3,059.6972	2,635.0112	0.8612	
7	0.2µg/L week 6 #1	2,449.5583	2,339.3282	0.9550	
8	0.2µg/L week 6 #2	2,438.6325	2,041.8670	0.8373	
9	0.2µg/L week 6 #3	3,159.6243	2,839.2384	0.8986	
10	0.5µg/L week 6 #1	2,709.5262	2,322.8768	0.8573	
11	0.5µg/L week 6 #2	2,968.1829	2,909.1161	0.9801	
12	0.5µg/L week 6 #3	2,891.1344	2,699.7413	0.9338	
13	1.0µg/L week 6 #1	2,927.7575	2,675.0921	0.9137	
14	1.0µg/L week 6 #2	2,956.9075	2,559.4991	0.8656	
15	1.0µg/L week 6 #3	2,977.7102	2,760.6351	0.9271	

Table B31 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03/AT	Remark
1	Control week 7 #1	3,613.9686	3,191.4957	0.8831	
2	Control week 7 #2	3,580.6571	3,261.2625	0.9108	
3	Control week 7 #3	3,512.8680	3,100.8086	0.8827	
4	0.1µg/L week 7 #1	2,760.9605	2,770.9000	1.0036	
5	0.1µg/L week 7 #2	3,284.3780	3,326.0896	1.0127	
6	0.1µg/L week 7 #3	2,493.4878	2,502.4644	1.0036	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	3,395.9464	3,447.5648	1.0152	
11	0.5µg/L week 7 #2	3,329.0584	3,157.2790	0.9484	
12	0.5µg/L week 7 #3	3,030.7918	2,454.9413	0.8100	
13	1.0µg/L week 7 #1	3,236.8091	3,138.4101	0.9696	
14	1.0µg/L week 7 #2	2,242.8432	1,833.5243	0.8175	
15	1.0µg/L week 7 #3	2,142.8291	2,143.2577	1.0002	

Table B32 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03/AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	2,933.1098	2,979.4529	1.0158	
5	0.1µg/L week 8 #2	3,396.6355	3,417.3549	1.0061	
6	0.1µg/L week 8 #3	3,351.7961	3,423.1894	1.0213	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B33 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07/AT	Remark
1	Initial # 1	4,614.1009	2,152.4781	0.4665	
2	Initial # 2	4,362.2049	3,250.7151	0.7452	
3	Initial # 3	4,470.4610	1,380.9254	0.3089	
4	Control week 1 #1	3,729.9440	1,275.6408	0.3420	
5	Control week 1 #2	4,132.5567	1,419.9465	0.3436	
6	Control week 1 #3	2,939.6814	1,204.0935	0.4096	
7	0.1µg/L week 1 #1	2,819.2144	818.1360	0.2902	
8	0.1µg/L week 1 #2	4,505.5442	4,883.5594	1.0839	
9	0.1µg/L week 1 #3	4,793.9111	2,441.0595	0.5092	
10	0.2µg/L week 1 #1	4,521.8023	7,319.4413	1.6187	
11	0.2µg/L week 1 #2	4,385.6091	4,672.4280	1.0654	
12	0.2µg/L week 1 #3	3,741.5767	4,928.7790	1.3173	
13	0.5µg/L week 1 #1	4,059.6151	4,405.4943	1.0852	
14	0.5µg/L week 1 #2	2,774.2561	4,055.9624	1.4620	
15	0.5µg/L week 1 #3	2,660.4727	2,223.3571	0.8357	
16	1.0µg/L week 1 #1	3,814.7958	933.8620	0.2448	
17	1.0µg/L week 1 #2	4,240.0034	3,995.7792	0.9424	
18	1.0µg/L week 1 #3	4,287.2744	3,333.7846	0.7776	

Table B34 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07/AT	Remark
1	Control week 2 #1	4,024.6938	1,106.7908	0.2750	
2	Control week 2 #2	3,493.8203	3,457.4846	0.9896	
3	Control week 2 #3	4,000.2257	4,049.4285	1.0123	
4	0.1µg/L week 2 #1	3,083.5758	1,104.5368	0.3582	
5	0.1µg/L week 2 #2	2,907.9053	2,738.6652	0.9418	
6	0.1µg/L week 2 #3	4,284.1060	4,394.6360	1.0258	
7	0.2µg/L week 2 #1	4,877.0516	3,353.9484	0.6877	
8	0.2µg/L week 2 #2	4,305.4288	3,594.1720	0.8348	
9	0.2µg/L week 2 #3	3,920.9012	3,113.9797	0.7942	
10	0.5µg/L week 2 #1	3,740.3510	5,769.1174	1.5424	
11	0.5µg/L week 2 #2	4,069.0739	3,992.9823	0.9813	
12	0.5µg/L week 2 #3	4,068.2414	3,861.1679	0.9491	
13	1.0µg/L week 2 #1	3,062.4611	3,730.9963	1.2183	
14	1.0µg/L week 2 #2	4,076.4051	3,624.7394	0.8892	
15	1.0µg/L week 2 #3	3,814.7958	2,988.5111	0.7834	

Table B35 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07/AT	Remark
1	Control week 3 #1	3,082.5593	763.5500	0.2477	
2	Control week 3 #2	3,017.1826	1,603.9342	0.5316	
3	Control week 3 #3	2,848.2378	2,354.3533	0.8266	
4	0.1µg/L week 3 #1	2,574.0440	3,748.3228	1.4562	
5	0.1µg/L week 3 #2	2,918.3688	2,949.5953	1.0107	
6	0.1µg/L week 3 #3	3,034.4658	2,995.0177	0.9870	
7	0.2µg/L week 3 #1	2,344.5554	2,153.2397	0.9184	
8	0.2µg/L week 3 #2	3,086.3942	2,851.2110	0.9238	
9	0.2µg/L week 3 #3	2,966.6725	2,440.3848	0.8226	
10	0.5µg/L week 3 #1	2,880.3089	4,255.0804	1.4773	
11	0.5µg/L week 3 #2	2,902.5039	2,359.4454	0.8129	
12	0.5µg/L week 3 #3	3,144.0750	3,195.6378	1.0164	
13	1.0µg/L week 3 #1	2,863.1308	6,771.0179	2.3649	
14	1.0µg/L week 3 #2	3,037.7491	2,729.1138	0.8984	
15	1.0µg/L week 3 #3	2,127.5958	2,107.5964	0.9906	

Table B36 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07/AT	Remark
1	Control week 4 #1	2,592.5354	2,125.8791	0.8200	
2	Control week 4 #2	3,341.3874	3,427.9293	1.0259	
3	Control week 4 #3	3,404.5315	3,350.7399	0.9842	
4	0.1µg/L week 4 #1	3,081.7976	4,401.4234	1.4282	
5	0.1µg/L week 4 #2	2,765.4202	3,025.9227	1.0942	
6	0.1µg/L week 4 #3	2,837.3635	2,734.9347	0.9639	
7	0.2µg/L week 4 #1	2,935.9934	1,731.3553	0.5897	
8	0.2µg/L week 4 #2	2,370.9793	2,367.8970	0.9987	
9	0.2µg/L week 4 #3	3,283.8642	3,593.5326	1.0943	
10	0.5µg/L week 4 #1	3,532.2120	1,554.5265	0.4401	
11	0.5µg/L week 4 #2	3,221.1666	3,292.0322	1.0220	
12	0.5µg/L week 4 #3	2,937.3593	4,770.2715	1.6240	
13	1.0µg/L week 4 #1	3,044.7096	1,171.6043	0.3848	
14	1.0µg/L week 4 #2	2,666.5538	3,000.9397	1.1254	
15	1.0µg/L week 4 #3	3,054.0867	3,255.6564	1.0660	

Table B37 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07/AT	Remark
1	Control week 5 #1	3,271.7227	5,455.9248	1.6676	
2	Control week 5 #2	3,248.4071	2,211.8404	0.6809	
3	Control week 5 #3	2,354.1545	1,641.5519	0.6973	
4	0.1µg/L week 5 #1	2,950.2865	2,263.4598	0.7672	
5	0.1µg/L week 5 #2	3,110.6768	1,968.7474	0.6329	
6	0.1µg/L week 5 #3	3,252.3404	2,569.0237	0.7899	
7	0.2µg/L week 5 #1	2,758.9107	5,558.1014	2.0146	
8	0.2µg/L week 5 #2	3,416.6640	3,483.2890	1.0195	
9	0.2µg/L week 5 #3	3,657.7959	3,618.2917	0.9892	
10	0.5µg/L week 5 #1	3,546.0033	2,981.4796	0.8408	
11	0.5µg/L week 5 #2	3,164.2350	5,773.1467	1.8245	
12	0.5µg/L week 5 #3	2,857.3082	4,594.5515	1.6080	
13	1.0µg/L week 5 #1	3,013.5030	2,836.3090	0.9412	
14	1.0µg/L week 5 #2	2,716.6498	3,278.1813	1.2067	
15	1.0µg/L week 5 #3	2,302.1478	2,384.1042	1.0356	

Table B38 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07/AT	Remark
1	Control week 6 #1	2,791.7754	2,136.8249	0.7654	
2	Control week 6 #2	3,678.1178	2,578.3606	0.7010	
3	Control week 6 #3	3,357.4901	2,314.3179	0.6893	
4	0.1µg/L week 6 #1	3,144.3501	2,641.8829	0.8402	
5	0.1µg/L week 6 #2	3,293.4432	3,891.5325	1.1816	
6	0.1µg/L week 6 #3	3,059.6972	2,133.8328	0.6974	
7	0.2µg/L week 6 #1	2,449.5583	2,242.0807	0.9153	
8	0.2µg/L week 6 #2	2,438.6325	2,592.5103	1.0631	
9	0.2µg/L week 6 #3	3,159.6243	4,066.1205	1.2869	
10	0.5µg/L week 6 #1	2,709.5262	3,553.0017	1.3113	
11	0.5µg/L week 6 #2	2,968.1829	3,539.5581	1.1925	
12	0.5µg/L week 6 #3	2,891.1344	3,186.3192	1.1021	
13	1.0µg/L week 6 #1	2,927.7575	3,330.9098	1.1377	
14	1.0µg/L week 6 #2	2,956.9075	3,362.2995	1.1371	
15	1.0µg/L week 6 #3	2,977.7102	3,105.4539	1.0429	

Table B39 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07/AT	Remark
1	Control week 7 #1	3,613.9686	2,119.9540	0.5866	
2	Control week 7 #2	3,580.6571	2,649.6863	0.7400	
3	Control week 7 #3	3,512.8680	2,416.5019	0.6879	
4	0.1µg/L week 7 #1	2,760.9605	1,611.5727	0.5837	
5	0.1µg/L week 7 #2	3,284.3780	2,351.9431	0.7161	
6	0.1µg/L week 7 #3	2,493.4878	1,929.7102	0.7739	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	3,395.9464	6,344.9863	1.8684	
11	0.5µg/L week 7 #2	3,329.0584	5,030.8731	1.5112	
12	0.5µg/L week 7 #3	3,030.7918	4,357.6724	1.4378	
13	1.0µg/L week 7 #1	3,236.8091	3,891.6156	1.2023	
14	1.0µg/L week 7 #2	2,242.8432	3,261.5425	1.4542	
15	1.0µg/L week 7 #3	2,142.8291	2,958.1756	1.3805	

Table B40 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07/AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	2,933.1098	1,768.6652	0.6030	
5	0.1µg/L week 8 #2	3,396.6355	3,624.2100	1.0670	
6	0.1µg/L week 8 #3	3,351.7961	3,559.2723	1.0619	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B41 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08/AT	Remark
1	Initial # 1	4,614.1009	3,883.6888	0.8417	
2	Initial # 2	4,362.2049	2,337.7056	0.5359	
3	Initial # 3	4,470.4610	1,650.0472	0.3691	
4	Control week 1 #1	3,729.9440	2,248.7832	0.6029	
5	Control week 1 #2	4,132.5567	2,158.4344	0.5223	
6	Control week 1 #3	2,939.6814	1,854.9390	0.6310	
7	0.1µg/L week 1 #1	2,819.2144	1,630.0698	0.5782	
8	0.1µg/L week 1 #2	4,505.5442	2,790.7341	0.6194	
9	0.1µg/L week 1 #3	4,793.9111	2,996.1944	0.6250	
10	0.2µg/L week 1 #1	4,521.8023	2,275.8231	0.5033	
11	0.2µg/L week 1 #2	4,385.6091	3,087.9074	0.7041	
12	0.2µg/L week 1 #3	3,741.5767	2,204.5370	0.5892	
13	0.5µg/L week 1 #1	4,059.6151	3,045.5233	0.7502	
14	0.5µg/L week 1 #2	2,774.2561	1,482.8399	0.5345	
15	0.5µg/L week 1 #3	2,660.4727	1,763.8934	0.6630	
16	1.0µg/L week 1 #1	3,814.7958	2,249.9666	0.5898	
17	1.0µg/L week 1 #2	4,240.0034	4,190.3954	0.9883	
18	1.0µg/L week 1 #3	4,287.2744	3,287.4820	0.7668	

Table B42 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08/AT	Remark
1	Control week 2 #1	4,024.6938	2,882.0832	0.7161	
2	Control week 2 #2	3,493.8203	2,914.1955	0.8341	
3	Control week 2 #3	4,000.2257	4,251.8399	1.0629	
4	0.1µg/L week 2 #1	3,083.5758	2,406.1142	0.7803	
5	0.1µg/L week 2 #2	2,907.9053	2,390.5890	0.8221	
6	0.1µg/L week 2 #3	4,284.1060	2,855.7851	0.6666	
7	0.2µg/L week 2 #1	4,877.0516	3,568.0509	0.7316	
8	0.2µg/L week 2 #2	4,305.4288	3,793.9439	0.8812	
9	0.2µg/L week 2 #3	3,920.9012	3,329.6293	0.8492	
10	0.5µg/L week 2 #1	3,740.3510	2,435.3425	0.6511	
11	0.5µg/L week 2 #2	4,069.0739	3,131.5593	0.7696	
12	0.5µg/L week 2 #3	4,068.2414	3,514.5537	0.8639	
13	1.0µg/L week 2 #1	3,062.4611	2,282.4522	0.7453	
14	1.0µg/L week 2 #2	4,076.4051	3,321.4549	0.8148	
15	1.0µg/L week 2 #3	3,814.7958	2,434.6027	0.6382	

Table B43 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08/AT	Remark
1	Control week 3 #1	3,082.5593	1,989.1755	0.6453	
2	Control week 3 #2	3,017.1826	2,110.8209	0.6996	
3	Control week 3 #3	2,848.2378	2,006.0139	0.7043	
4	0.1µg/L week 3 #1	2,574.0440	1,878.5373	0.7298	
5	0.1µg/L week 3 #2	2,918.3688	1,905.4030	0.6529	
6	0.1µg/L week 3 #3	3,034.4658	2,219.7117	0.7315	
7	0.2µg/L week 3 #1	2,344.5554	1,736.8466	0.7408	
8	0.2µg/L week 3 #2	3,086.3942	2,387.6346	0.7736	
9	0.2µg/L week 3 #3	2,966.6725	2,473.3148	0.8337	
10	0.5µg/L week 3 #1	2,880.3089	2,005.8471	0.6964	
11	0.5µg/L week 3 #2	2,902.5039	1,771.6884	0.6104	
12	0.5µg/L week 3 #3	3,144.0750	2,332.2748	0.7418	
13	1.0µg/L week 3 #1	2,863.1308	1,711.2933	0.5977	
14	1.0µg/L week 3 #2	3,037.7491	1,909.2253	0.6285	
15	1.0µg/L week 3 #3	2,127.5958	1,620.3770	0.7616	

Table B44 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08/AT	Remark
1	Control week 4 #1	2,592.5354	2,353.7629	0.9079	
2	Control week 4 #2	3,341.3874	2,125.4565	0.6361	
3	Control week 4 #3	3,404.5315	2,633.4051	0.7735	
4	0.1µg/L week 4 #1	3,081.7976	2,028.7474	0.6583	
5	0.1µg/L week 4 #2	2,765.4202	1,984.7420	0.7177	
6	0.1µg/L week 4 #3	2,837.3635	1,748.6671	0.6163	
7	0.2µg/L week 4 #1	2,935.9934	2,030.5331	0.6916	
8	0.2µg/L week 4 #2	2,370.9793	2,108.0377	0.8891	
9	0.2µg/L week 4 #3	3,283.8642	2,994.5558	0.9119	
10	0.5µg/L week 4 #1	3,532.2120	3,206.8953	0.9079	
11	0.5µg/L week 4 #2	3,221.1666	2,048.9841	0.6361	
12	0.5µg/L week 4 #3	2,937.3593	2,272.0474	0.7735	
13	1.0µg/L week 4 #1	3,044.7096	1,860.3176	0.6110	
14	1.0µg/L week 4 #2	2,666.5538	1,479.9374	0.5550	
15	1.0µg/L week 4 #3	3,054.0867	2,094.4927	0.6858	

Table B45 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08/AT	Remark
1	Control week 5 #1	3,271.7227	2,054.3147	0.6279	
2	Control week 5 #2	3,248.4071	2,470.7385	0.7606	
3	Control week 5 #3	2,354.1545	1,688.3996	0.7172	
4	0.1µg/L week 5 #1	2,950.2865	2,093.5233	0.7096	
5	0.1µg/L week 5 #2	3,110.6768	2,236.8877	0.7191	
6	0.1µg/L week 5 #3	3,252.3404	2,742.6987	0.8433	
7	0.2µg/L week 5 #1	2,758.9107	1,736.4584	0.6294	
8	0.2µg/L week 5 #2	3,416.6640	2,265.2483	0.6630	
9	0.2µg/L week 5 #3	3,657.7959	3,223.9813	0.8814	
10	0.5µg/L week 5 #1	3,546.0033	2,354.1916	0.6639	
11	0.5µg/L week 5 #2	3,164.2350	1,784.3121	0.5639	
12	0.5µg/L week 5 #3	2,857.3082	2,012.1164	0.7042	
13	1.0µg/L week 5 #1	3,013.5030	1,904.5339	0.6320	
14	1.0µg/L week 5 #2	2,716.6498	2,168.9732	0.7984	
15	1.0µg/L week 5 #3	2,302.1478	1,626.6976	0.7066	

Table B46 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT08/AT	Remark
1	Control week 6 #1	2,791.7754	2,094.3899	0.7502	
2	Control week 6 #2	3,678.1178	2,611.4636	0.7100	
3	Control week 6 #3	3,357.4901	2,593.9968	0.7726	
4	0.1µg/L week 6 #1	3,144.3501	2,097.2815	0.6670	
5	0.1µg/L week 6 #2	3,293.4432	2,862.6608	0.8692	
6	0.1µg/L week 6 #3	3,059.6972	2,138.4224	0.6989	
7	0.2µg/L week 6 #1	2,449.5583	2,297.1958	0.9378	
8	0.2µg/L week 6 #2	2,438.6325	1,633.8838	0.6700	
9	0.2µg/L week 6 #3	3,159.6243	2,300.5225	0.7281	
10	0.5µg/L week 6 #1	2,709.5262	1,834.0783	0.6769	
11	0.5µg/L week 6 #2	2,968.1829	2,318.7445	0.7812	
12	0.5µg/L week 6 #3	2,891.1344	2,205.9355	0.7630	
13	1.0µg/L week 6 #1	2,927.7575	2,728.9628	0.9321	
14	1.0µg/L week 6 #2	2,956.9075	2,154.9942	0.7288	
15	1.0µg/L week 6 #3	2,977.7102	2,494.7256	0.8378	

Table B47 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08/AT	Remark
1	Control week 7 #1	3,613.9686	2,409.0715	0.6666	
2	Control week 7 #2	3,580.6571	2,619.2507	0.7315	
3	Control week 7 #3	3,512.8680	2,370.8346	0.6749	
4	0.1µg/L week 7 #1	2,760.9605	2,823.0821	1.0225	
5	0.1µg/L week 7 #2	3,284.3780	3,396.7038	1.0342	
6	0.1µg/L week 7 #3	2,493.4878	2,562.8068	1.0278	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	3,395.9464	3,517.8609	1.0359	
11	0.5µg/L week 7 #2	3,329.0584	3,231.1841	0.9706	
12	0.5µg/L week 7 #3	3,030.7918	2,030.9336	0.6701	
13	1.0µg/L week 7 #1	3,236.8091	2,886.2627	0.8917	
14	1.0µg/L week 7 #2	2,242.8432	1,658.1339	0.7393	
15	1.0µg/L week 7 #3	2,142.8291	2,195.1141	1.0244	

Table B48 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08/AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	2,933.1098	3,057.7669	1.0425	
5	0.1µg/L week 8 #2	3,396.6355	3,426.8655	1.0089	
6	0.1µg/L week 8 #3	3,351.7961	3,495.5882	1.0429	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B49 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11/AT	Remark
1	Initial # 1	4,614.1009	1,859.9441	0.4031	
2	Initial # 2	4,362.2049	3,740.1545	0.8574	
3	Initial # 3	4,470.4610	4,103.4361	0.9179	
4	Control week 1 #1	3,729.9440	2,950.0127	0.7909	
5	Control week 1 #2	4,132.5567	3,203.5580	0.7752	
6	Control week 1 #3	2,939.6814	2,382.6118	0.8105	
7	0.1µg/L week 1 #1	2,819.2144	2,449.3335	0.8688	
8	0.1µg/L week 1 #2	4,505.5442	3,819.3498	0.8477	
9	0.1µg/L week 1 #3	4,793.9111	5,490.4663	1.1453	
10	0.2µg/L week 1 #1	4,521.8023	4,322.3908	0.9559	
11	0.2µg/L week 1 #2	4,385.6091	4,742.1591	1.0813	
12	0.2µg/L week 1 #3	3,741.5767	3,022.4457	0.8078	
13	0.5µg/L week 1 #1	4,059.6151	4,121.7272	1.0153	
14	0.5µg/L week 1 #2	2,774.2561	2,138.6740	0.7709	
15	0.5µg/L week 1 #3	2,660.4727	2,372.0775	0.8916	
16	1.0µg/L week 1 #1	3,814.7958	3,428.3570	0.8987	
17	1.0µg/L week 1 #2	4,240.0034	5,428.9004	1.2804	
18	1.0µg/L week 1 #3	4,287.2744	3,735.5022	0.8713	

Table B50 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11/AT	Remark
1	Control week 2 #1	4,024.6938	3,984.0444	0.9899	
2	Control week 2 #2	3,493.8203	3,493.4710	0.9999	
3	Control week 2 #3	4,000.2257	4,641.4619	1.1603	
4	0.1µg/L week 2 #1	3,083.5758	2,402.4139	0.7791	
5	0.1µg/L week 2 #2	2,907.9053	3,114.6574	1.0711	
6	0.1µg/L week 2 #3	4,284.1060	3,695.4699	0.8626	
7	0.2µg/L week 2 #1	4,877.0516	4,671.7277	0.9579	
8	0.2µg/L week 2 #2	4,305.4288	4,372.1630	1.0155	
9	0.2µg/L week 2 #3	3,920.9012	4,196.1484	1.0702	
10	0.5µg/L week 2 #1	3,740.3510	3,069.3320	0.8206	
11	0.5µg/L week 2 #2	4,069.0739	3,809.0601	0.9361	
12	0.5µg/L week 2 #3	4,068.2414	3,986.8766	0.9800	
13	1.0µg/L week 2 #1	3,062.4611	2,719.4654	0.8880	
14	1.0µg/L week 2 #2	4,076.4051	3,953.7053	0.9699	
15	1.0µg/L week 2 #3	3,814.7958	3,922.7546	1.0283	

Table B51 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11/AT	Remark
1	Control week 3 #1	3,082.5593	2,399.7725	0.7785	
2	Control week 3 #2	3,017.1826	2,792.7042	0.9256	
3	Control week 3 #3	2,848.2378	2,385.9688	0.8377	
4	0.1µg/L week 3 #1	2,574.0440	2,318.4414	0.9007	
5	0.1µg/L week 3 #2	2,918.3688	2,261.1521	0.7748	
6	0.1µg/L week 3 #3	3,034.4658	2,599.0199	0.8565	
7	0.2µg/L week 3 #1	2,344.5554	2,068.6012	0.8823	
8	0.2µg/L week 3 #2	3,086.3942	3,124.6655	1.0124	
9	0.2µg/L week 3 #3	2,966.6725	2,899.9223	0.9775	
10	0.5µg/L week 3 #1	2,880.3089	2,624.2494	0.9111	
11	0.5µg/L week 3 #2	2,902.5039	2,148.7237	0.7403	
12	0.5µg/L week 3 #3	3,144.0750	3,119.5512	0.9922	
13	1.0µg/L week 3 #1	2,863.1308	2,210.0506	0.7719	
14	1.0µg/L week 3 #2	3,037.7491	2,474.2466	0.8145	
15	1.0µg/L week 3 #3	2,127.5958	1,971.0048	0.9264	

Table B52 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11/AT	Remark
1	Control week 4 #1	2,592.5354	2,676.2743	1.0323	
2	Control week 4 #2	3,341.3874	2,054.9532	0.6150	
3	Control week 4 #3	3,404.5315	3,233.2836	0.9497	
4	0.1µg/L week 4 #1	3,081.7976	2,607.8172	0.8462	
5	0.1µg/L week 4 #2	2,765.4202	2,777.5880	1.0044	
6	0.1µg/L week 4 #3	2,837.3635	2,195.5519	0.7738	
7	0.2µg/L week 4 #1	2,935.9934	2,712.2707	0.9238	
8	0.2µg/L week 4 #2	2,370.9793	2,252.1932	0.9499	
9	0.2µg/L week 4 #3	3,283.8642	4,033.8988	1.2284	
10	0.5µg/L week 4 #1	3,532.2120	3,646.3024	1.0323	
11	0.5µg/L week 4 #2	3,221.1666	1,981.0174	0.6150	
12	0.5µg/L week 4 #3	2,937.3593	2,789.6101	0.9497	
13	1.0µg/L week 4 #1	3,044.7096	2,973.1589	0.9765	
14	1.0µg/L week 4 #2	2,666.5538	2,122.0435	0.7958	
15	1.0µg/L week 4 #3	3,054.0867	3,085.8492	1.0104	

Table 53 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11/AT	Remark
1	Control week 5 #1	3,271.7227	2,734.5059	0.8358	
2	Control week 5 #2	3,248.4071	3,252.3052	1.0012	
3	Control week 5 #3	2,354.1545	2,057.2956	0.8739	
4	0.1µg/L week 5 #1	2,950.2865	2,899.5416	0.9828	
5	0.1µg/L week 5 #2	3,110.6768	2,393.3548	0.7694	
6	0.1µg/L week 5 #3	3,252.3404	3,396.4191	1.0443	
7	0.2µg/L week 5 #1	2,758.9107	2,105.6006	0.7632	
8	0.2µg/L week 5 #2	3,416.6640	2,934.9144	0.8590	
9	0.2µg/L week 5 #3	3,657.7959	3,807.7655	1.0410	
10	0.5µg/L week 5 #1	3,546.0033	3,596.0020	1.0141	
11	0.5µg/L week 5 #2	3,164.2350	2,442.1566	0.7718	
12	0.5µg/L week 5 #3	2,857.3082	2,749.8734	0.9624	
13	1.0µg/L week 5 #1	3,013.5030	2,305.6312	0.7651	
14	1.0µg/L week 5 #2	2,716.6498	2,676.1717	0.9851	
15	1.0µg/L week 5 #3	2,302.1478	1,778.6394	0.7726	

Table B54 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11/AT	Remark
1	Control week 6 #1	2,791.7754	2,711.0931	0.9711	
2	Control week 6 #2	3,678.1178	3,111.3198	0.8459	
3	Control week 6 #3	3,357.4901	3,568.6762	1.0629	
4	0.1µg/L week 6 #1	3,144.3501	2,423.0362	0.7706	
5	0.1µg/L week 6 #2	3,293.4432	3,587.8770	1.0894	
6	0.1µg/L week 6 #3	3,059.6972	2,641.1306	0.8632	
7	0.2µg/L week 6 #1	2,449.5583	2,498.5495	1.0200	
8	0.2µg/L week 6 #2	2,438.6325	2,064.5463	0.8466	
9	0.2µg/L week 6 #3	3,159.6243	3,033.2393	0.9600	
10	0.5µg/L week 6 #1	2,709.5262	2,391.6987	0.8827	
11	0.5µg/L week 6 #2	2,968.1829	3,028.1402	1.0202	
12	0.5µg/L week 6 #3	2,891.1344	2,623.7044	0.9075	
13	1.0µg/L week 6 #1	2,927.7575	2,891.7461	0.9877	
14	1.0µg/L week 6 #2	2,956.9075	2,427.0297	0.8208	
15	1.0µg/L week 6 #3	2,977.7102	3,162.0304	1.0619	

Table B55 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11/AT	Remark
1	Control week 7 #1	3,613.9686	2,867.6841	0.7935	
2	Control week 7 #2	3,580.6571	3,239.7785	0.9048	
3	Control week 7 #3	3,512.8680	2,837.6948	0.8078	
4	0.1µg/L week 7 #1	2,760.9605	2,770.3478	1.0034	
5	0.1µg/L week 7 #2	3,284.3780	3,322.8053	1.0117	
6	0.1µg/L week 7 #3	2,493.4878	2,504.9579	1.0046	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	3,395.9464	3,458.4319	1.0184	
11	0.5µg/L week 7 #2	3,329.0584	3,302.0930	0.9919	
12	0.5µg/L week 7 #3	3,030.7918	2,398.8717	0.7915	
13	1.0µg/L week 7 #1	3,236.8091	3,328.7345	1.0284	
14	1.0µg/L week 7 #2	2,242.8432	1,925.9294	0.8587	
15	1.0µg/L week 7 #3	2,142.8291	2,138.5434	0.9980	

Table B56 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11/AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	2,933.1098	3,014.9435	1.0279	
5	0.1µg/L week 8 #2	3,396.6355	3,368.1037	0.9916	
6	0.1µg/L week 8 #3	3,351.7961	3,451.3444	1.0297	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B57 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71/AT	Remark
1	Initial # 1	4,948.9080	7,761.3724	1.5683	
2	Initial # 2	5,869.6804	7,099.3784	1.2095	
3	Initial # 3	6,391.2779	8,378.3263	1.3109	
4	Control week 1 #1	5,533.1721	7,276.1213	1.3150	
5	Control week 1 #2	5,744.7702	8,958.3947	1.5594	
6	Control week 1 #3	6,864.2663	9,275.6830	1.3513	
7	0.1µg/L week 1 #1	6,550.0303	8,069.6374	1.2320	
8	0.1µg/L week 1 #2	5,537.6524	7,020.0819	1.2677	
9	0.1µg/L week 1 #3	7,114.1081	9,693.6837	1.3626	
10	0.2µg/L week 1 #1	7,314.5163	9,584.2107	1.3103	
11	0.2µg/L week 1 #2	5,783.6662	4,375.9219	0.7566	
12	0.2µg/L week 1 #3	5,973.4150	6,316.2890	1.0574	
13	0.5µg/L week 1 #1	6,638.7972	7,134.7153	1.0747	
14	0.5µg/L week 1 #2	5,759.3939	9,428.7037	1.6371	
15	0.5µg/L week 1 #3	5,464.8281	7,824.5409	1.4318	
16	1.0µg/L week 1 #1	5,014.7358	7,932.3091	1.5818	
17	1.0µg/L week 1 #2	7,061.5204	9,004.1446	1.2751	
18	1.0µg/L week 1 #3	7,906.8951	9,951.6182	1.2586	

Table B58 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71/AT	Remark
1	Control week 2 #1	4,906.8794	6,726.8410	1.3709	
2	Control week 2 #2	7,745.7126	3,356.2173	0.4333	
3	Control week 2 #3	6,625.0768	2,222.0508	0.3354	
4	0.1µg/L week 2 #1	4,906.8794	5,637.5137	1.1489	
5	0.1µg/L week 2 #2	5,090.2482	3,078.0731	0.6047	
6	0.1µg/L week 2 #3	5,612.1038	604.4236	0.1077	
7	0.2µg/L week 2 #1	5,844.6554	8,465.9833	1.4485	
8	0.2µg/L week 2 #2	5,547.3226	5,477.4263	0.9874	
9	0.2µg/L week 2 #3	4,646.2849	4,661.1530	1.0032	
10	0.5µg/L week 2 #1	5,092.4202	6,750.0030	1.3255	
11	0.5µg/L week 2 #2	5,680.4334	7,378.3150	1.2989	
12	0.5µg/L week 2 #3	5,435.3515	6,986.0573	1.2853	
13	1.0µg/L week 2 #1	5,794.3042	6,042.3004	1.0428	
14	1.0µg/L week 2 #2	5,435.3515	3,782.4611	0.6959	
15	1.0µg/L week 2 #3	5,092.4202	5,310.3758	1.0428	

Table B59 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71/AT	Remark
1	Control week 3 #1	3,552.3372	4,786.7744	1.3475	
2	Control week 3 #2	3,642.8747	4,908.7736	1.3475	
3	Control week 3 #3	3,473.7997	4,231.7828	1.2182	
4	0.1µg/L week 3 #1	4,339.0740	5,815.6609	1.3403	
5	0.1µg/L week 3 #2	1,094.4997	1,300.0467	1.1878	
6	0.1µg/L week 3 #3	4,174.2428	4,592.9194	1.1003	
7	0.2µg/L week 3 #1	5,278.5274	6,377.5168	1.2082	
8	0.2µg/L week 3 #2	5,780.5981	7,497.4358	1.2970	
9	0.2µg/L week 3 #3	5,037.0082	4,733.2766	0.9397	
10	0.5µg/L week 3 #1	5,301.9396	7,341.5957	1.3847	
11	0.5µg/L week 3 #2	6,864.2663	10,201.6725	1.4862	
12	0.5µg/L week 3 #3	6,550.0303	10,617.5992	1.6210	
13	1.0µg/L week 3 #1	5,537.6524	13,046.7090	2.3560	
14	1.0µg/L week 3 #2	7,114.1081	13,822.0006	1.9429	
15	1.0µg/L week 3 #3	7,314.5163	17,429.7609	2.3829	

Table B60 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	Control week 4 #1	4,895.2647	7,483.8806	1.5288	
2	Control week 4 #2	6,105.9066	8,545.8269	1.3996	
3	Control week 4 #3	5,812.1026	7,221.5375	1.2425	
4	0.1µg/L week 4 #1	4,094.2165	4,456.5547	1.0885	
5	0.1µg/L week 4 #2	5,844.3786	6,116.1422	1.0465	
6	0.1µg/L week 4 #3	3,992.4232	5,250.0366	1.3150	
7	0.2µg/L week 4 #1	3,925.4352	5,448.5040	1.3880	
8	0.2µg/L week 4 #2	5,030.4201	3,878.4539	0.7710	
9	0.2µg/L week 4 #3	3,886.1920	1,866.5380	0.4803	
10	0.5µg/L week 4 #1	4,419.5982	4,684.7741	1.0600	
11	0.5µg/L week 4 #2	4,948.9080	4,776.1911	0.9651	
12	0.5µg/L week 4 #3	5,869.6804	6,264.7099	1.0673	
13	1.0µg/L week 4 #1	6,391.2779	9,727.5250	1.5220	
14	1.0µg/L week 4 #2	5,533.1721	6,561.2355	1.1858	
15	1.0µg/L week 4 #3	5,744.7702	8,449.9825	1.4709	

Table 61 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	Control week 5 #1	2,153.4869	2,528.4089	1.1741	
2	Control week 5 #2	2,213.1355	2,186.1352	0.9878	
3	Control week 5 #3	2,354.1245	3,484.1042	1.4800	
4	0.1µg/L week 5 #1	2,063.9602	2,745.6862	1.3303	
5	0.1µg/L week 5 #2	1,536.8286	1,910.1242	1.2429	
6	0.1µg/L week 5 #3	3,697.7778	3,698.1476	1.0001	
7	0.2µg/L week 5 #1	4,255.7910	5,290.3738	1.2431	
8	0.2µg/L week 5 #2	3,558.9752	4,808.5314	1.3511	
9	0.2µg/L week 5 #3	3,140.5961	2,419.8293	0.7705	
10	0.5µg/L week 5 #1	2,054.3975	2,357.8320	1.1477	
11	0.5µg/L week 5 #2	253.7845	321.6210	1.2673	
12	0.5µg/L week 5 #3	1,960.1109	2,087.5181	1.0650	
13	1.0µg/L week 5 #1	1,617.9180	1,661.7636	1.0271	
14	1.0µg/L week 5 #2	1,045.7399	1,433.0820	1.3704	
15	1.0µg/L week 5 #3	1,583.5310	1,857.7986	1.1732	

Table B62 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	Control week 6 #1	2,491.1923	2,787.6442	1.1190	
2	Control week 6 #2	3,032.9762	3,590.7405	1.1839	
3	Control week 6 #3	1,626.4986	2,121.1169	1.3041	
4	0.1µg/L week 6 #1	2,410.1602	2,812.4159	1.1669	
5	0.1µg/L week 6 #2	2,682.1382	2,988.4384	1.1142	
6	0.1µg/L week 6 #3	2,848.4461	3,381.1055	1.1870	
7	0.2µg/L week 6 #1	2,612.2243	2,691.1135	1.0302	
8	0.2µg/L week 6 #2	2,665.1991	3,691.0343	1.3849	
9	0.2µg/L week 6 #3	2,567.2854	3,464.5516	1.3495	
10	0.5µg/L week 6 #1	2,568.4180	2,808.3082	1.0934	
11	0.5µg/L week 6 #2	3,823.1324	4,987.6585	1.3046	
12	0.5µg/L week 6 #3	2,563.3857	3,049.9163	1.1898	
13	1.0µg/L week 6 #1	3,511.4126	4,913.5196	1.3993	
14	1.0µg/L week 6 #2	2,522.7908	2,864.8812	1.1356	
15	1.0µg/L week 6 #3	2,125.5729	2,295.4062	1.0799	

Table B63 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	Control week 7 #1	5,425.6526	6,363.2053	1.1728	
2	Control week 7 #2	6,259.5219	7,379.3504	1.1789	
3	Control week 7 #3	6,690.2881	7,490.4466	1.1196	
4	0.1µg/L week 7 #1	6,278.3319	7,724.2318	1.2303	
5	0.1µg/L week 7 #2	6,924.6604	9,245.1141	1.3351	
6	0.1µg/L week 7 #3	4,916.2485	5,253.9948	1.0687	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	4,702.2705	5,241.6209	1.1147	
11	0.5µg/L week 7 #2	3,900.7953	4,373.5717	1.1212	
12	0.5µg/L week 7 #3	4,263.2821	4,395.4438	1.0310	
13	1.0µg/L week 7 #1	4,276.7157	4,282.7031	1.0014	
14	1.0µg/L week 7 #2	4,827.5104	5,392.3291	1.1170	
15	1.0µg/L week 7 #3	4,949.5030	5,122.2406	1.0349	

Table B64 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	3,125.2484	3,575.9093	1.1442	
5	0.1µg/L week 8 #2	3,248.5385	3,600.6801	1.1084	
6	0.1µg/L week 8 #3	3,637.7851	4,168.9017	1.1460	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B65 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Laboratory study (Week 1)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4/AT	Remark
1	Initial # 1	4,948.9080	4,825.1853	0.9750	
2	Initial # 2	5,869.6804	959.1058	0.1634	
3	Initial # 3	6,391.2779	2,540.5330	0.3975	
4	Control week 1 #1	5,533.1721	3,781.9231	0.6835	
5	Control week 1 #2	5,744.7702	940.9934	0.1638	
6	Control week 1 #3	6,864.2663	3,955.1902	0.5762	
7	0.1µg/L week 1 #1	6,550.0303	5,728.0015	0.8745	
8	0.1µg/L week 1 #2	5,537.6524	4,271.7450	0.7714	
9	0.1µg/L week 1 #3	7,114.1081	5,701.9576	0.8015	
10	0.2µg/L week 1 #1	7,314.5163	5,664.3614	0.7744	
11	0.2µg/L week 1 #2	5,783.6662	3,135.9038	0.5422	
12	0.2µg/L week 1 #3	5,973.4150	1,072.8253	0.1796	
13	0.5µg/L week 1 #1	6,638.7972	3,569.6812	0.5377	
14	0.5µg/L week 1 #2	5,759.3939	2,652.7768	0.4606	
15	0.5µg/L week 1 #3	5,464.8281	1,194.6114	0.2186	
16	1.0µg/L week 1 #1	5,014.7358	4,946.0339	0.9863	
17	1.0µg/L week 1 #2	7,061.5204	3,715.0659	0.5261	
18	1.0µg/L week 1 #3	7,906.8951	4,426.2799	0.5598	

Table B66 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Laboratory study (Week 2)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4/AT	Remark
1	Control week 2 #1	4,906.8794	2,681.1189	0.5464	
2	Control week 2 #2	7,745.7126	3,706.3235	0.4785	
3	Control week 2 #3	6,625.0768	2,233.9759	0.3372	
4	0.1µg/L week 2 #1	4,906.8794	3,411.7532	0.6953	
5	0.1µg/L week 2 #2	5,090.2482	4,132.7725	0.8119	
6	0.1µg/L week 2 #3	5,612.1038	2,661.2596	0.4742	
7	0.2µg/L week 2 #1	5,844.6554	4,954.5143	0.8477	
8	0.2µg/L week 2 #2	5,547.3226	4,310.2697	0.7770	
9	0.2µg/L week 2 #3	4,646.2849	1,804.6171	0.3884	
10	0.5µg/L week 2 #1	5,092.4202	3,948.6627	0.7754	
11	0.5µg/L week 2 #2	5,680.4334	3,821.2276	0.6727	
12	0.5µg/L week 2 #3	5,435.3515	2,319.2645	0.4267	
13	1.0µg/L week 2 #1	5,794.3042	2,152.0046	0.3714	
14	1.0µg/L week 2 #2	5,435.3515	1,018.5849	0.1874	
15	1.0µg/L week 2 #3	5,092.4202	1,891.3249	0.3714	

Table B67 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Laboratory study (Week 3)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4/AT	Remark
1	Control week 3 #1	3,552.3372	4,255.7000	1.1980	
2	Control week 3 #2	3,642.8747	1,801.0372	0.4944	
3	Control week 3 #3	3,473.7997	1,954.7071	0.5627	
4	0.1µg/L week 3 #1	4,339.0740	2,885.0503	0.6649	
5	0.1µg/L week 3 #2	1,094.4997	303.6142	0.2774	
6	0.1µg/L week 3 #3	4,174.2428	1,606.6661	0.3849	
7	0.2µg/L week 3 #1	5,278.5274	1,559.8049	0.2955	
8	0.2µg/L week 3 #2	5,780.5981	2,690.8684	0.4655	
9	0.2µg/L week 3 #3	5,037.0082	664.8851	0.1320	
10	0.5µg/L week 3 #1	5,301.9396	1,679.1243	0.3167	
11	0.5µg/L week 3 #2	6,864.2663	5,744.0180	0.8368	
12	0.5µg/L week 3 #3	6,550.0303	5,138.4988	0.7845	
13	1.0µg/L week 3 #1	5,537.6524	8,088.2950	1.4606	
14	1.0µg/L week 3 #2	7,114.1081	5,758.8705	0.8095	
15	1.0µg/L week 3 #3	7,314.5163	827.2718	0.1131	

Table B68 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Laboratory study (Week 4)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	Control week 4 #1	4,895.2647	2,240.0731	0.4576	
2	Control week 4 #2	6,105.9066	3,991.4312	0.6537	
3	Control week 4 #3	5,812.1026	764.8727	0.1316	
4	0.1µg/L week 4 #1	4,094.2165	2,337.3882	0.5709	
5	0.1µg/L week 4 #2	5,844.3786	3,281.6186	0.5615	
6	0.1µg/L week 4 #3	3,992.4232	931.0331	0.2332	
7	0.2µg/L week 4 #1	3,925.4352	1,452.8036	0.3701	
8	0.2µg/L week 4 #2	5,030.4201	959.8042	0.1908	
9	0.2µg/L week 4 #3	3,886.1920	1,111.0623	0.2859	
10	0.5µg/L week 4 #1	4,419.5982	977.1732	0.2211	
11	0.5µg/L week 4 #2	4,948.9080	1,403.5103	0.2836	
12	0.5µg/L week 4 #3	5,869.6804	2,429.4607	0.4139	
13	1.0µg/L week 4 #1	6,391.2779	1,289.1208	0.2017	
14	1.0µg/L week 4 #2	5,533.1721	1,373.3333	0.2482	
15	1.0µg/L week 4 #3	5,744.7702	1,980.7968	0.3448	

Table B69 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Laboratory study (Week 5)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	Control week 5 #1	2,153.4869	1,019.0300	0.4732	
2	Control week 5 #2	2,213.1355	465.6437	0.2104	
3	Control week 5 #3	2,354.1245	322.7505	0.1371	
4	0.1µg/L week 5 #1	2,063.9602	521.5627	0.2527	
5	0.1µg/L week 5 #2	1,536.8286	590.9106	0.3845	
6	0.1µg/L week 5 #3	3,697.7778	652.6578	0.1765	
7	0.2µg/L week 5 #1	4,255.7910	863.5000	0.2029	
8	0.2µg/L week 5 #2	3,558.9752	2,184.4990	0.6138	
9	0.2µg/L week 5 #3	3,140.5961	1,569.3559	0.4997	
10	0.5µg/L week 5 #1	2,054.3975	455.4599	0.2217	
11	0.5µg/L week 5 #2	253.7845	82.1500	0.3237	
12	0.5µg/L week 5 #3	1,960.1109	658.9893	0.3362	
13	1.0µg/L week 5 #1	1,617.9180	242.5259	0.1499	
14	1.0µg/L week 5 #2	1,045.7399	881.0359	0.8425	
15	1.0µg/L week 5 #3	1,583.5310	598.4164	0.3779	

Table B70 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Laboratory study (Week 6)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	Control week 6 #1	2,491.1923	702.0180	0.2818	
2	Control week 6 #2	3,032.9762	674.5339	0.2224	
3	Control week 6 #3	1,626.4986	775.6772	0.4769	
4	0.1µg/L week 6 #1	2,410.1602	654.1175	0.2714	
5	0.1µg/L week 6 #2	2,682.1382	803.3004	0.2995	
6	0.1µg/L week 6 #3	2,848.4461	1,078.1368	0.3785	
7	0.2µg/L week 6 #1	2,612.2243	405.4172	0.1552	
8	0.2µg/L week 6 #2	2,665.1991	486.6654	0.1826	
9	0.2µg/L week 6 #3	2,567.2854	264.1737	0.1029	
10	0.5µg/L week 6 #1	2,568.4180	721.2118	0.2808	
11	0.5µg/L week 6 #2	3,823.1324	669.8128	0.1752	
12	0.5µg/L week 6 #3	2,563.3857	598.5506	0.2335	
13	1.0µg/L week 6 #1	3,511.4126	2,929.9226	0.8344	
14	1.0µg/L week 6 #2	2,522.7908	1,094.1344	0.4337	
15	1.0µg/L week 6 #3	2,125.5729	1,038.9801	0.4888	

Table B71 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Laboratory study (Week 7)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	Control week 7 #1	5,425.6526	4,019.8660	0.7409	
2	Control week 7 #2	6,259.5219	5,061.4494	0.8086	
3	Control week 7 #3	6,690.2881	3,441.4842	0.5144	
4	0.1µg/L week 7 #1	6,278.3319	4,305.6800	0.6858	
5	0.1µg/L week 7 #2	6,924.6604	5,806.3278	0.8385	
6	0.1µg/L week 7 #3	4,916.2485	2,798.8203	0.5693	
7	0.2µg/L week 7 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 7 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 7 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 7 #1	4,702.2705	3,300.0534	0.7018	
11	0.5µg/L week 7 #2	3,900.7953	2,136.4656	0.5477	
12	0.5µg/L week 7 #3	4,263.2821	1,831.0796	0.4295	
13	1.0µg/L week 7 #1	4,276.7157	2,439.4387	0.5704	
14	1.0µg/L week 7 #2	4,827.5104	1,815.1439	0.3760	
15	1.0µg/L week 7 #3	4,949.5030	1,903.0839	0.3845	

Table B72 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Laboratory study (Week 8)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	Control week 8 #1	NA	NA	NA	Lost of sample
2	Control week 8 #2	NA	NA	NA	Lost of sample
3	Control week 8 #3	NA	NA	NA	Lost of sample
4	0.1µg/L week 8 #1	3,125.2484	2,327.9976	0.7449	
5	0.1µg/L week 8 #2	3,248.5385	1,514.1438	0.4661	
6	0.1µg/L week 8 #3	3,637.7851	1,555.1531	0.4275	
7	0.2µg/L week 8 #1	NA	NA	NA	Lost of sample
8	0.2µg/L week 8 #2	NA	NA	NA	Lost of sample
9	0.2µg/L week 8 #3	NA	NA	NA	Lost of sample
10	0.5µg/L week 8 #1	NA	NA	NA	Lost of sample
11	0.5µg/L week 8 #2	NA	NA	NA	Lost of sample
12	0.5µg/L week 8 #3	NA	NA	NA	Lost of sample
13	1.0µg/L week 8 #1	NA	NA	NA	Lost of sample
14	1.0µg/L week 8 #2	NA	NA	NA	Lost of sample
15	1.0µg/L week 8 #3	NA	NA	NA	Lost of sample

Table B73 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity MT	Ratio MT /AT	Remark
1	St. A 5 m. 30 D # 1	8,645.3427	2,819.2462	0.3261	
2	St. A 5 m. 30 D # 2	7,689.3353	6,277.5733	0.8164	
3	St. A 5 m. 30 D # 3	7,713.9057	2,071.1837	0.2685	
4	St. A 5 m. 60 D # 1	6,176.4749	4,242.6206	0.6869	
5	St. A 5 m. 60 D # 2	7,136.0990	7,111.8363	0.9966	
6	St. A 5 m. 60 D # 3	7,796.3429	5,256.2944	0.6742	
7	St. A 5 m. 90 D # 1	5,140.0396	6,881.4850	1.3388	
8	St. A 5 m. 90 D # 2	7,953.7543	10,531.5661	1.3241	
9	St. A 5 m. 90 D # 3	9,351.6848	2,910.2443	0.3112	
10	St. A 20 m. 30 D # 1	6,883.0467	1,556.9452	0.2262	
11	St. A 20 m. 30 D # 2	4,268.5360	965.5429	0.2262	
12	St. A 20 m. 30 D # 3	7,396.9588	6,402.8075	0.8656	
13	St. A 20 m. 60 D # 1	5,039.6338	3,313.0553	0.6574	
14	St. A 20 m. 60 D # 2	4,557.2385	4,256.9164	0.9341	
15	St. A 20 m. 60 D # 3	4,663.9016	1,618.3739	0.3470	
16	St. A 20 m. 90 D # 1	8,836.0504	7,837.5767	0.8870	
17	St. A 20 m. 90 D # 2	8,711.9107	7,012.2169	0.8049	
18	St. A 20 m. 90 D # 3	8,086.6887	1,742.6814	0.2155	
19	St. A 40 m. 30 D # 1	7,256.2690	5,028.5944	0.6930	
20	St. A 40 m. 30 D # 2	7,004.6322	1,790.3840	0.2556	
21	St. A 40 m. 30 D # 3	9,850.4621	2,208.4736	0.2242	
22	St. A 40 m. 60 D # 1	7,594.5678	1,521.1919	0.2003	
23	St. A 40 m. 60 D # 2	7,557.3311	5,607.5397	0.7420	
24	St. A 40 m. 60 D # 3	7,425.0832	3,453.4062	0.4651	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B74 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity MT	Ratio MT /AT	Remark
1	St. B 5 m. 30 D # 1	6,211.5898	3,579.1180	0.5762	
2	St. B 5 m. 30 D # 2	6,337.6929	1,836.0296	0.2897	
3	St. B 5 m. 30 D # 3	5,282.9348	1,423.2226	0.2694	
4	St. B 5 m. 60 D # 1	8,786.3905	4,961.6747	0.5647	
5	St. B 5 m. 60 D # 2	9,508.5596	2,379.0416	0.2502	
6	St. B 5 m. 60 D # 3	8,415.0986	7,582.8454	0.9011	
7	St. B 5 m. 90 D # 1	9,116.4300	8,204.7870	0.9000	
8	St. B 5 m. 90 D # 2	8,444.1335	2,687.7677	0.3183	
9	St. B 5 m. 90 D # 3	6,798.2050	1,742.3799	0.2563	
10	St. B 20 m. 30 D # 1	6,877.8125	6,404.6190	0.9312	
11	St. B 20 m. 30 D # 2	9,410.1542	6,840.2411	0.7269	
12	St. B 20 m. 30 D # 3	6,243.3234	3,967.0077	0.6354	
13	St. B 20 m. 60 D # 1	9,630.3849	2,686.8774	0.2790	
14	St. B 20 m. 60 D # 2	6,465.5505	1,406.2572	0.2175	
15	St. B 20 m. 60 D # 3	9,702.4652	2,110.2862	0.2175	
16	St. B 20 m. 90 D # 1	9,484.3576	3,709.3323	0.3911	
17	St. B 20 m. 90 D # 2	6,411.8919	1,300.3317	0.2028	
18	St. B 20 m. 90 D # 3	7,122.5406	1,728.6406	0.2427	
19	St. B 40 m. 30 D # 1	8,069.4263	2,630.6330	0.3260	
20	St. B 40 m. 30 D # 2	5,746.6361	1,165.4178	0.2028	
21	St. B 40 m. 30 D # 3	5,560.2206	1,525.7245	0.2744	
22	St. B 40 m. 60 D # 1	9,726.9608	2,316.9621	0.2382	
23	St. B 40 m. 60 D # 2	8,981.9906	5,717.9352	0.6366	
24	St. B 40 m. 60 D # 3	8,207.4043	1,315.6469	0.1603	
25	St. B 40 m. 90 D # 1	8,212.1626	8,123.4712	0.9892	
26	St. B 40 m. 90 D # 2	7,068.9784	5,635.3896	0.7972	
27	St. B 40 m. 90 D # 3	6,300.3348	3,659.8645	0.5809	

Table B75 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity MT	Ratio MT /AT	Remark
1	St. C 5 m. 30 D # 1	6,397.6185	6,365.6304	0.9950	
2	St. C 5 m. 30 D # 2	4,638.7242	1,083.6060	0.2336	
3	St. C 5 m. 30 D # 3	5,194.6838	1,698.1421	0.3269	
4	St. C 5 m. 60 D # 1	4,240.8082	3,961.3389	0.9341	
5	St. C 5 m. 60 D # 2	4,760.0214	3,790.8811	0.7964	
6	St. C 5 m. 60 D # 3	6,859.8407	5,077.6541	0.7402	
7	St. C 5 m. 90 D # 1	7,490.6541	1,852.4388	0.2473	
8	St. C 5 m. 90 D # 2	6,196.3638	1,909.0997	0.3081	
9	St. C 5 m. 90 D # 3	7,054.8009	3,467.4346	0.4915	
10	St. C 20 m. 30 D # 1	6,070.2033	1,607.3898	0.2648	
11	St. C 20 m. 30 D # 2	6,156.3653	1,568.6419	0.2548	
12	St. C 20 m. 30 D # 3	7,313.1038	2,229.7654	0.3049	
13	St. C 20 m. 60 D # 1	6,790.7105	5,304.2240	0.7811	
14	St. C 20 m. 60 D # 2	6,495.0784	5,819.5902	0.8960	
15	St. C 20 m. 60 D # 3	8,478.2315	2,098.3623	0.2475	
16	St. C 20 m. 90 D # 1	7,826.1923	5,601.2058	0.7157	
17	St. C 20 m. 90 D # 2	8,743.7875	2,066.1570	0.2363	
18	St. C 20 m. 90 D # 3	6,444.5242	1,607.9088	0.2495	
19	St. C 40 m. 30 D # 1	7,867.2218	7,281.1137	0.9255	
20	St. C 40 m. 30 D # 2	8,838.1920	8,681.7560	0.9823	
21	St. C 40 m. 30 D # 3	8,147.8342	6,851.5138	0.8409	
22	St. C 40 m. 60 D # 1	6,071.8885	2,569.0160	0.4231	
23	St. C 40 m. 60 D # 2	6,782.1438	2,832.9015	0.4177	
24	St. C 40 m. 60 D # 3	8,244.9591	2,050.5213	0.2487	
25	St. C 40 m. 90 D # 1	5,825.3692	5,446.1377	0.9349	
26	St. C 40 m. 90 D # 2	6,956.1669	6,417.7596	0.9226	
27	St. C 40 m. 90 D # 3	4,130.1569	3,862.1097	0.9351	

Table B76 Intensity of band and ratio MT expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity MT	Ratio MT /AT	Remark
1	St. D 5 m. 30 D # 1	5,886.6648	2,896.8278	0.4921	
2	St. D 5 m. 30 D # 2	5,534.5675	2,279.1349	0.4118	
3	St. D 5 m. 30 D # 3	6,681.7459	2,842.4147	0.4254	
4	St. D 5 m. 60 D # 1	6,595.9673	4,576.2821	0.6938	
5	St. D 5 m. 60 D # 2	5,854.2557	1,505.7146	0.2572	
6	St. D 5 m. 60 D # 3	9,043.0346	3,315.1765	0.3666	
7	St. D 5 m. 90 D # 1	4,098.3793	3,016.4072	0.7360	
8	St. D 5 m. 90 D # 2	5,600.8017	5,110.1715	0.9124	
9	St. D 5 m. 90 D # 3	6,774.3701	7,008.0859	1.0345	

Table B77 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01 /AT	Remark
1	St. A 5 m. 30 D # 1	8,645.3427	2,019.5520	0.2336	
2	St. A 5 m. 30 D # 2	7,689.3353	1,865.4327	0.2426	
3	St. A 5 m. 30 D # 3	7,713.9057	2,677.4967	0.3471	
4	St. A 5 m. 60 D # 1	6,176.4749	5,516.2098	0.8931	
5	St. A 5 m. 60 D # 2	7,136.0990	8,005.2759	1.1218	
6	St. A 5 m. 60 D # 3	7,796.3429	6,774.2423	0.8689	
7	St. A 5 m. 90 D # 1	5,140.0396	6,458.9737	1.2566	
8	St. A 5 m. 90 D # 2	7,953.7543	10,566.5626	1.3285	
9	St. A 5 m. 90 D # 3	9,351.6848	2,853.1990	0.3051	
10	St. A 20 m. 30 D # 1	6,883.0467	3,123.5266	0.4538	
11	St. A 20 m. 30 D # 2	4,268.5360	1,937.0617	0.4538	
12	St. A 20 m. 30 D # 3	7,396.9588	4,298.3727	0.5811	
13	St. A 20 m. 60 D # 1	5,039.6338	3,403.7687	0.6754	
14	St. A 20 m. 60 D # 2	4,557.2385	4,275.1454	0.9381	
15	St. A 20 m. 60 D # 3	4,663.9016	1,596.9199	0.3424	
16	St. A 20 m. 90 D # 1	8,836.0504	8,289.0989	0.9381	
17	St. A 20 m. 90 D # 2	8,711.9107	7,075.8139	0.8122	
18	St. A 20 m. 90 D # 3	8,086.6887	1,727.3167	0.2136	
19	St. A 40 m. 30 D # 1	7,256.2690	5,474.8549	0.7545	
20	St. A 40 m. 30 D # 2	7,004.6322	1,728.7432	0.2468	
21	St. A 40 m. 30 D # 3	9,850.4621	2,173.0119	0.2206	
22	St. A 40 m. 60 D # 1	7,594.5678	1,365.5033	0.1798	
23	St. A 40 m. 60 D # 2	7,557.3311	6,127.4841	0.8108	
24	St. A 40 m. 60 D # 3	7,425.0832	1,552.5849	0.2091	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B78 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01 /AT	Remark
1	St. B 5 m. 30 D # 1	6,211.5898	3,868.5781	0.6228	
2	St. B 5 m. 30 D # 2	6,337.6929	3,770.2935	0.5949	
3	St. B 5 m. 30 D # 3	5,282.9348	1,208.7355	0.2288	
4	St. B 5 m. 60 D # 1	8,786.3905	8,462.1727	0.9631	
5	St. B 5 m. 60 D # 2	9,508.5596	4,569.8137	0.4806	
6	St. B 5 m. 60 D # 3	8,415.0986	6,209.5013	0.7379	
7	St. B 5 m. 90 D # 1	9,116.4300	6,239.2847	0.6844	
8	St. B 5 m. 90 D # 2	8,444.1335	5,788.4535	0.6855	
9	St. B 5 m. 90 D # 3	6,798.2050	6,505.8822	0.9570	
10	St. B 20 m. 30 D # 1	6,877.8125	5,613.6706	0.8162	
11	St. B 20 m. 30 D # 2	9,410.1542	2,528.5084	0.2687	
12	St. B 20 m. 30 D # 3	6,243.3234	4,023.8219	0.6445	
13	St. B 20 m. 60 D # 1	9,630.3849	8,055.8170	0.8365	
14	St. B 20 m. 60 D # 2	6,465.5505	1,580.1806	0.2444	
15	St. B 20 m. 60 D # 3	9,702.4652	2,359.6395	0.2432	
16	St. B 20 m. 90 D # 1	9,484.3576	2,139.6711	0.2256	
17	St. B 20 m. 90 D # 2	6,411.8919	1,765.8350	0.2754	
18	St. B 20 m. 90 D # 3	7,122.5406	1,653.1417	0.2321	
19	St. B 40 m. 30 D # 1	8,069.4263	2,587.8650	0.3207	
20	St. B 40 m. 30 D # 2	5,746.6361	1,582.6236	0.2754	
21	St. B 40 m. 30 D # 3	5,560.2206	2,671.6860	0.4805	
22	St. B 40 m. 60 D # 1	9,726.9608	2,814.0098	0.2893	
23	St. B 40 m. 60 D # 2	8,981.9906	7,263.7358	0.8087	
24	St. B 40 m. 60 D # 3	8,207.4043	1,431.3713	0.1744	
25	St. B 40 m. 90 D # 1	8,212.1626	5,787.1110	0.7047	
26	St. B 40 m. 90 D # 2	7,068.9784	1,619.5029	0.2291	
27	St. B 40 m. 90 D # 3	6,300.3348	3,564.7294	0.5658	

Table B79 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01 /AT	Remark
1	St. C 5 m. 30 D # 1	6,397.6185	1,716.4810	0.2683	
2	St. C 5 m. 30 D # 2	4,638.7242	1,081.2866	0.2331	
3	St. C 5 m. 30 D # 3	5,194.6838	4,406.1308	0.8482	
4	St. C 5 m. 60 D # 1	4,240.8082	3,021.1518	0.7124	
5	St. C 5 m. 60 D # 2	4,760.0214	3,385.3272	0.7112	
6	St. C 5 m. 60 D # 3	6,859.8407	5,146.2525	0.7502	
7	St. C 5 m. 90 D # 1	7,490.6541	1,862.9257	0.2487	
8	St. C 5 m. 90 D # 2	6,196.3638	1,925.2102	0.3107	
9	St. C 5 m. 90 D # 3	7,054.8009	3,470.2565	0.4919	
10	St. C 20 m. 30 D # 1	6,070.2033	1,626.2075	0.2679	
11	St. C 20 m. 30 D # 2	6,156.3653	1,460.2898	0.2372	
12	St. C 20 m. 30 D # 3	7,313.1038	2,205.6321	0.3016	
13	St. C 20 m. 60 D # 1	6,790.7105	3,552.8997	0.5232	
14	St. C 20 m. 60 D # 2	6,495.0784	4,836.2353	0.7446	
15	St. C 20 m. 60 D # 3	8,478.2315	2,138.2100	0.2522	
16	St. C 20 m. 90 D # 1	7,826.1923	7,201.6621	0.9202	
17	St. C 20 m. 90 D # 2	8,743.7875	2,034.6794	0.2327	
18	St. C 20 m. 90 D # 3	6,444.5242	1,657.5316	0.2572	
19	St. C 40 m. 30 D # 1	7,867.2218	5,895.6960	0.7494	
20	St. C 40 m. 30 D # 2	8,838.1920	8,352.9753	0.9451	
21	St. C 40 m. 30 D # 3	8,147.8342	1,522.8302	0.1869	
22	St. C 40 m. 60 D # 1	6,071.8885	2,561.1226	0.4218	
23	St. C 40 m. 60 D # 2	6,782.1438	2,864.7775	0.4224	
24	St. C 40 m. 60 D # 3	8,244.9591	2,895.6297	0.3512	
25	St. C 40 m. 90 D # 1	5,825.3692	4,624.1781	0.7938	
26	St. C 40 m. 90 D # 2	6,956.1669	2,240.5814	0.3221	
27	St. C 40 m. 90 D # 3			0.7938	

Table B80 Intensity of band and ratio pvMT01 expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity pvMT01	Ratio pvMT01 /AT	Remark
1	St. D 5 m. 30 D # 1	5,886.6648	2,865.6284	0.4868	
2	St. D 5 m. 30 D # 2	5,534.5675	2,145.1984	0.3876	
3	St. D 5 m. 30 D # 3	6,681.7459	2,476.9232	0.3707	
4	St. D 5 m. 60 D # 1	6,595.9673	5,248.4112	0.7957	
5	St. D 5 m. 60 D # 2	5,854.2557	4,769.4621	0.8147	
6	St. D 5 m. 60 D # 3	9,043.0346	4,141.7098	0.4580	
7	St. D 5 m. 90 D # 1	4,098.3793	1,870.5003	0.4564	
8	St. D 5 m. 90 D # 2	5,600.8017	3,842.7101	0.6861	
9	St. D 5 m. 90 D # 3	6,774.3701	1,968.6319	0.2906	

Table B81 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02 /AT	Remark
1	St. A 5 m. 30 D # 1	8,645.3427	3,164.1954	0.3660	
2	St. A 5 m. 30 D # 2	7,689.3353	2,156.0896	0.2804	
3	St. A 5 m. 30 D # 3	7,713.9057	5,726.0322	0.7423	
4	St. A 5 m. 60 D # 1	6,176.4749	4,855.9446	0.7862	
5	St. A 5 m. 60 D # 2	7,136.0990	8,612.5579	1.2069	
6	St. A 5 m. 60 D # 3	7,796.3429	4,252.9050	0.5455	
7	St. A 5 m. 90 D # 1	5,140.0396	5,753.2463	1.1193	
8	St. A 5 m. 90 D # 2	7,953.7543	10,102.8587	1.2702	
9	St. A 5 m. 90 D # 3	9,351.6848	4,929.2730	0.5271	
10	St. A 20 m. 30 D # 1	6,883.0467	4,208.2947	0.6114	
11	St. A 20 m. 30 D # 2	4,268.5360	2,596.9773	0.6084	
12	St. A 20 m. 30 D # 3	7,396.9588	3,288.6879	0.4446	
13	St. A 20 m. 60 D # 1	5,039.6338	3,612.9135	0.7169	
14	St. A 20 m. 60 D # 2	4,557.2385	4,230.9402	0.9284	
15	St. A 20 m. 60 D # 3	4,663.9016	3,459.6822	0.7418	
16	St. A 20 m. 90 D # 1	8,836.0504	3,820.7082	0.4324	
17	St. A 20 m. 90 D # 2	8,711.9107	4,410.8404	0.5063	
18	St. A 20 m. 90 D # 3	8,086.6887	2,933.8506	0.3628	
19	St. A 40 m. 30 D # 1	7,256.2690	6,034.3133	0.8316	
20	St. A 40 m. 30 D # 2	7,004.6322	4,903.9430	0.7001	
21	St. A 40 m. 30 D # 3	9,850.4621	2,172.0269	0.2205	
22	St. A 40 m. 60 D # 1	7,594.5678	2,148.5032	0.2829	
23	St. A 40 m. 60 D # 2	7,557.3311	4,207.1662	0.5567	
24	St. A 40 m. 60 D # 3	7,425.0832	1,864.4384	0.2511	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B82 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02 /AT	Remark
1	St. B 5 m. 30 D # 1	6,211.5898	5,119.5923	0.8242	
2	St. B 5 m. 30 D # 2	6,337.6929	6,252.7678	0.9866	
3	St. B 5 m. 30 D # 3	5,282.9348	1,767.6700	0.3346	
4	St. B 5 m. 60 D # 1	8,786.3905	5,918.5126	0.6736	
5	St. B 5 m. 60 D # 2	9,508.5596	4,662.0468	0.4903	
6	St. B 5 m. 60 D # 3	8,415.0986	4,999.4101	0.5941	
7	St. B 5 m. 90 D # 1	9,116.4300	6,208.2888	0.6810	
8	St. B 5 m. 90 D # 2	8,444.1335	4,093.7159	0.4848	
9	St. B 5 m. 90 D # 3	6,798.2050	2,864.0838	0.4213	
10	St. B 20 m. 30 D # 1	6,877.8125	3,006.2918	0.4371	
11	St. B 20 m. 30 D # 2	9,410.1542	2,787.2877	0.2962	
12	St. B 20 m. 30 D # 3	6,243.3234	4,779.2640	0.7655	
13	St. B 20 m. 60 D # 1	9,630.3849	5,445.9827	0.5655	
14	St. B 20 m. 60 D # 2	6,465.5505	2,398.7192	0.3710	
15	St. B 20 m. 60 D # 3	9,702.4652	3,599.6146	0.3710	
16	St. B 20 m. 90 D # 1	9,484.3576	6,513.8568	0.6868	
17	St. B 20 m. 90 D # 2	6,411.8919	2,255.0624	0.3517	
18	St. B 20 m. 90 D # 3	7,122.5406	2,541.3225	0.3568	
19	St. B 40 m. 30 D # 1	8,069.4263	2,930.8156	0.3632	
20	St. B 40 m. 30 D # 2	5,746.6361	2,021.0919	0.3517	
21	St. B 40 m. 30 D # 3	5,560.2206	1,784.8308	0.3210	
22	St. B 40 m. 60 D # 1	9,726.9608	5,771.9785	0.5934	
23	St. B 40 m. 60 D # 2	8,981.9906	4,374.2294	0.4870	
24	St. B 40 m. 60 D # 3	8,207.4043	1,836.8171	0.2238	
25	St. B 40 m. 90 D # 1	8,212.1626	8,307.4237	1.0116	
26	St. B 40 m. 90 D # 2	7,068.9784	7,487.4619	1.0592	
27	St. B 40 m. 90 D # 3	6,300.3348	4,875.8291	0.7739	

Table B83 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02 /AT	Remark
1	St. C 5 m. 30 D # 1	6,397.6185	5,122.5731	0.8007	
2	St. C 5 m. 30 D # 2	4,638.7242	2,349.5138	0.5065	
3	St. C 5 m. 30 D # 3	5,194.6838	4,684.0464	0.9017	
4	St. C 5 m. 60 D # 1	4,240.8082	4,441.3984	1.0473	
5	St. C 5 m. 60 D # 2	4,760.0214	2,928.8412	0.6153	
6	St. C 5 m. 60 D # 3	6,859.8407	5,688.8659	0.8293	
7	St. C 5 m. 90 D # 1	7,490.6541	6,868.1808	0.9169	
8	St. C 5 m. 90 D # 2	6,196.3638	2,050.3768	0.3309	
9	St. C 5 m. 90 D # 3	7,054.8009	3,892.1336	0.5517	
10	St. C 20 m. 30 D # 1	6,070.2033	4,110.1347	0.6771	
11	St. C 20 m. 30 D # 2	6,156.3653	3,319.5121	0.5392	
12	St. C 20 m. 30 D # 3	7,313.1038	2,773.8603	0.3793	
13	St. C 20 m. 60 D # 1	6,790.7105	3,080.2663	0.4536	
14	St. C 20 m. 60 D # 2	6,495.0784	5,064.8621	0.7798	
15	St. C 20 m. 60 D # 3	8,478.2315	4,701.1794	0.5545	
16	St. C 20 m. 90 D # 1	7,826.1923	3,812.1383	0.4871	
17	St. C 20 m. 90 D # 2	8,743.7875	4,727.7659	0.5407	
18	St. C 20 m. 90 D # 3	6,444.5242	2,951.5921	0.4580	
19	St. C 40 m. 30 D # 1	7,867.2218	3,662.1917	0.4655	
20	St. C 40 m. 30 D # 2	8,838.1920	7,026.3626	0.7950	
21	St. C 40 m. 30 D # 3	8,147.8342	2,709.9696	0.3326	
22	St. C 40 m. 60 D # 1	6,071.8885	2,576.3023	0.4243	
23	St. C 40 m. 60 D # 2	6,782.1438	2,862.7429	0.4221	
24	St. C 40 m. 60 D # 3	8,244.9591	6,215.0502	0.7538	
25	St. C 40 m. 90 D # 1	5,825.3692	4,159.8962	0.7141	
26	St. C 40 m. 90 D # 2	6,956.1669	2,363.7055	0.3398	
27	St. C 40 m. 90 D # 3	4,130.1569	2,896.4790	0.7013	

Table B84 Intensity of band and ratio pvMT02 expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity pvMT02	Ratio pvMT02 /AT	Remark
1	St. D 5 m. 30 D # 1	5,886.6648	3,100.5064	0.5267	
2	St. D 5 m. 30 D # 2	5,534.5675	2,674.8565	0.4833	
3	St. D 5 m. 30 D # 3	6,681.7459	6,495.9934	0.9722	
4	St. D 5 m. 60 D # 1	6,595.9673	4,222.0787	0.6401	
5	St. D 5 m. 60 D # 2	5,854.2557	2,994.4518	0.5115	
6	St. D 5 m. 60 D # 3	9,043.0346	5,135.5393	0.5679	
7	St. D 5 m. 90 D # 1	4,098.3793	3,241.8180	0.7910	
8	St. D 5 m. 90 D # 2	5,600.8017	3,106.2046	0.5546	
9	St. D 5 m. 90 D # 3	6,774.3701	2,774.7820	0.4096	

Table B85 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03 /AT	Remark
1	St. A 5 m. 30 D # 1	8,645.3427	4,560.4183	0.5275	
2	St. A 5 m. 30 D # 2	7,689.3353	3,384.0764	0.4401	
3	St. A 5 m. 30 D # 3	7,713.9057	3,506.7415	0.4546	
4	St. A 5 m. 60 D # 1	6,176.4749	5,673.7099	0.9186	
5	St. A 5 m. 60 D # 2	7,136.0990	8,048.0925	1.1278	
6	St. A 5 m. 60 D # 3	7,796.3429	4,219.3808	0.5412	
7	St. A 5 m. 90 D # 1	5,140.0396	6,227.6720	1.2116	
8	St. A 5 m. 90 D # 2	7,953.7543	10,402.7152	1.3079	
9	St. A 5 m. 90 D # 3	9,351.6848	9,773.4458	1.0451	
10	St. A 20 m. 30 D # 1	6,883.0467	1,221.7408	0.1775	
11	St. A 20 m. 30 D # 2	4,268.5360	764.0680	0.1790	
12	St. A 20 m. 30 D # 3	7,396.9588	3,661.4946	0.4950	
13	St. A 20 m. 60 D # 1	5,039.6338	3,923.3549	0.7785	
14	St. A 20 m. 60 D # 2	4,557.2385	4,732.2364	1.0384	
15	St. A 20 m. 60 D # 3	4,663.9016	1,713.9838	0.3675	
16	St. A 20 m. 90 D # 1	8,836.0504	6,139.2878	0.6948	
17	St. A 20 m. 90 D # 2	8,711.9107	3,926.4582	0.4507	
18	St. A 20 m. 90 D # 3	8,086.6887	2,174.5106	0.2689	
19	St. A 40 m. 30 D # 1	7,256.2690	6,384.0654	0.8798	
20	St. A 40 m. 30 D # 2	7,004.6322	5,466.4149	0.7804	
21	St. A 40 m. 30 D # 3	9,850.4621	3,799.3232	0.3857	
22	St. A 40 m. 60 D # 1	7,594.5678	1,496.8893	0.1971	
23	St. A 40 m. 60 D # 2	7,557.3311	4,297.0985	0.5686	
24	St. A 40 m. 60 D # 3	7,425.0832	3,310.1021	0.4458	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B86 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03 /AT	Remark
1	St. B 5 m. 30 D # 1	6,211.5898	5,820.2596	0.9370	
2	St. B 5 m. 30 D # 2	6,337.6929	3,852.6835	0.6079	
3	St. B 5 m. 30 D # 3	5,282.9348	3,620.3952	0.6853	
4	St. B 5 m. 60 D # 1	8,786.3905	5,493.2513	0.6252	
5	St. B 5 m. 60 D # 2	9,508.5596	3,886.1483	0.4087	
6	St. B 5 m. 60 D # 3	8,415.0986	2,596.0579	0.3085	
7	St. B 5 m. 90 D # 1	9,116.4300	6,099.8033	0.6691	
8	St. B 5 m. 90 D # 2	8,444.1335	4,196.7344	0.4970	
9	St. B 5 m. 90 D # 3	6,798.2050	3,697.5437	0.5439	
10	St. B 20 m. 30 D # 1	6,877.8125	1,863.8872	0.2710	
11	St. B 20 m. 30 D # 2	9,410.1542	2,387.3561	0.2537	
12	St. B 20 m. 30 D # 3	6,243.3234	5,684.5459	0.9105	
13	St. B 20 m. 60 D # 1	9,630.3849	6,049.8078	0.6282	
14	St. B 20 m. 60 D # 2	6,465.5505	1,783.8454	0.2759	
15	St. B 20 m. 60 D # 3	9,702.4652	2,678.8506	0.2761	
16	St. B 20 m. 90 D # 1	9,484.3576	6,467.3835	0.6819	
17	St. B 20 m. 90 D # 2	6,411.8919	1,909.4614	0.2978	
18	St. B 20 m. 90 D # 3	7,122.5406	2,615.3969	0.3672	
19	St. B 40 m. 30 D # 1	8,069.4263	4,739.1741	0.5873	
20	St. B 40 m. 30 D # 2	5,746.6361	1,711.3482	0.2978	
21	St. B 40 m. 30 D # 3	5,560.2206	2,017.2480	0.3628	
22	St. B 40 m. 60 D # 1	9,726.9608	6,706.7395	0.6895	
23	St. B 40 m. 60 D # 2	8,981.9906	8,478.9991	0.9440	
24	St. B 40 m. 60 D # 3	8,207.4043	3,422.4876	0.4170	
25	St. B 40 m. 90 D # 1	8,212.1626	7,130.6208	0.8683	
26	St. B 40 m. 90 D # 2	7,068.9784	3,827.8518	0.5415	
27	St. B 40 m. 90 D # 3	6,300.3348	3,965.4307	0.6294	

Table B87 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03 /AT	Remark
1	St. C 5 m. 30 D # 1	6,397.6185	4,354.2191	0.6806	
2	St. C 5 m. 30 D # 2	4,638.7242	1,856.8813	0.4003	
3	St. C 5 m. 30 D # 3	5,194.6838	3,140.1864	0.6045	
4	St. C 5 m. 60 D # 1	4,240.8082	4,322.2317	1.0192	
5	St. C 5 m. 60 D # 2	4,760.0214	3,253.4746	0.6835	
6	St. C 5 m. 60 D # 3	6,859.8407	6,576.5293	0.9587	
7	St. C 5 m. 90 D # 1	7,490.6541	2,157.3084	0.2880	
8	St. C 5 m. 90 D # 2	6,196.3638	4,394.4612	0.7092	
9	St. C 5 m. 90 D # 3	7,054.8009	3,910.4761	0.5543	
10	St. C 20 m. 30 D # 1	6,070.2033	3,268.8045	0.5385	
11	St. C 20 m. 30 D # 2	6,156.3653	3,768.9268	0.6122	
12	St. C 20 m. 30 D # 3	7,313.1038	5,786.8591	0.7913	
13	St. C 20 m. 60 D # 1	6,790.7105	3,580.0626	0.5272	
14	St. C 20 m. 60 D # 2	6,495.0784	2,231.0594	0.3435	
15	St. C 20 m. 60 D # 3	8,478.2315	5,579.5241	0.6581	
16	St. C 20 m. 90 D # 1	7,826.1923	3,335.5231	0.4262	
17	St. C 20 m. 90 D # 2	8,743.7875	4,644.6999	0.5312	
18	St. C 20 m. 90 D # 3	6,444.5242	2,254.2946	0.3498	
19	St. C 40 m. 30 D # 1	7,867.2218	3,894.2748	0.4950	
20	St. C 40 m. 30 D # 2	8,838.1920	3,450.4302	0.3904	
21	St. C 40 m. 30 D # 3	8,147.8342	2,071.9942	0.2543	
22	St. C 40 m. 60 D # 1	6,071.8885	2,542.9069	0.4188	
23	St. C 40 m. 60 D # 2	6,782.1438	2,830.8668	0.4174	
24	St. C 40 m. 60 D # 3	8,244.9591	6,195.2623	0.7514	
25	St. C 40 m. 90 D # 1	5,825.3692	2,924.3354	0.5020	
26	St. C 40 m. 90 D # 2	6,956.1669	3,276.3546	0.4710	
27	St. C 40 m. 90 D # 3	4,130.1569	2,088.6203	0.5057	

Table B88 Intensity of band and ratio pvMT03 expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity pvMT03	Ratio pvMT03 /AT	Remark
1	St. D 5 m. 30 D # 1	5,886.6648	3,055.7677	0.5191	
2	St. D 5 m. 30 D # 2	5,534.5675	2,201.6510	0.3978	
3	St. D 5 m. 30 D # 3	6,681.7459	2,835.7330	0.4244	
4	St. D 5 m. 60 D # 1	6,595.9673	3,292.0473	0.4991	
5	St. D 5 m. 60 D # 2	5,854.2557	2,063.0397	0.3524	
6	St. D 5 m. 60 D # 3	9,043.0346	5,166.2856	0.5713	
7	St. D 5 m. 90 D # 1	4,098.3793	2,208.6166	0.5389	
8	St. D 5 m. 90 D # 2	5,600.8017	3,203.0985	0.5719	
9	St. D 5 m. 90 D # 3	6,774.3701	2,789.6856	0.4118	

Table B89 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07 /AT	Remark
1	St. A 5 m. 30 D # 1	8,645.3427	2,016.0939	0.2332	
2	St. A 5 m. 30 D # 2	7,689.3353	1,648.5935	0.2144	
3	St. A 5 m. 30 D # 3	7,713.9057	5,186.0588	0.6723	
4	St. A 5 m. 60 D # 1	6,176.4749	5,957.8277	0.9646	
5	St. A 5 m. 60 D # 2	7,136.0990	8,903.7107	1.2477	
6	St. A 5 m. 60 D # 3	7,796.3429	3,259.6510	0.4181	
7	St. A 5 m. 90 D # 1	5,140.0396	5,361.5753	1.0431	
8	St. A 5 m. 90 D # 2	7,953.7543	10,529.1799	1.3238	
9	St. A 5 m. 90 D # 3	9,351.6848	12,109.4966	1.2949	
10	St. A 20 m. 30 D # 1	6,883.0467	3,380.2642	0.4911	
11	St. A 20 m. 30 D # 2	4,268.5360	2,086.8873	0.4889	
12	St. A 20 m. 30 D # 3	7,396.9588	3,544.6226	0.4792	
13	St. A 20 m. 60 D # 1	5,039.6338	3,431.9906	0.6810	
14	St. A 20 m. 60 D # 2	4,557.2385	4,651.5733	1.0207	
15	St. A 20 m. 60 D # 3	4,663.9016	1,832.9133	0.3930	
16	St. A 20 m. 90 D # 1	8,836.0504	7,415.2135	0.8392	
17	St. A 20 m. 90 D # 2	8,711.9107	5,208.8514	0.5979	
18	St. A 20 m. 90 D # 3	8,086.6887	2,369.3998	0.2930	
19	St. A 40 m. 30 D # 1	7,256.2690	6,079.3021	0.8378	
20	St. A 40 m. 30 D # 2	7,004.6322	3,477.7999	0.4965	
21	St. A 40 m. 30 D # 3	9,850.4621	2,711.8322	0.2753	
22	St. A 40 m. 60 D # 1	7,594.5678	5,572.8938	0.7338	
23	St. A 40 m. 60 D # 2	7,557.3311	5,911.3444	0.7822	
24	St. A 40 m. 60 D # 3	7,425.0832	5,845.7680	0.7873	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B90 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07 /AT	Remark
1	St. B 5 m. 30 D # 1	6,211.5898	3,636.8858	0.5855	
2	St. B 5 m. 30 D # 2	6,337.6929	5,899.1246	0.9308	
3	St. B 5 m. 30 D # 3	5,282.9348	1,696.3504	0.3211	
4	St. B 5 m. 60 D # 1	8,786.3905	4,929.1651	0.5610	
5	St. B 5 m. 60 D # 2	9,508.5596	10,995.6983	1.1564	
6	St. B 5 m. 60 D # 3	8,415.0986	4,109.0927	0.4883	
7	St. B 5 m. 90 D # 1	9,116.4300	6,069.7191	0.6658	
8	St. B 5 m. 90 D # 2	8,444.1335	4,700.0047	0.5566	
9	St. B 5 m. 90 D # 3	6,798.2050	5,058.5444	0.7441	
10	St. B 20 m. 30 D # 1	6,877.8125	1,854.9460	0.2697	
11	St. B 20 m. 30 D # 2	9,410.1542	2,377.9460	0.2527	
12	St. B 20 m. 30 D # 3	6,243.3234	4,438.3786	0.7109	
13	St. B 20 m. 60 D # 1	9,630.3849	6,853.9450	0.7117	
14	St. B 20 m. 60 D # 2	6,465.5505	1,371.9898	0.2122	
15	St. B 20 m. 60 D # 3	9,702.4652	2,038.4879	0.2101	
16	St. B 20 m. 90 D # 1	9,484.3576	5,909.7033	0.6231	
17	St. B 20 m. 90 D # 2	6,411.8919	1,381.7627	0.2155	
18	St. B 20 m. 90 D # 3	7,122.5406	1,752.8572	0.2461	
19	St. B 40 m. 30 D # 1	8,069.4263	2,689.5398	0.3333	
20	St. B 40 m. 30 D # 2	5,746.6361	1,238.4001	0.2155	
21	St. B 40 m. 30 D # 3	5,560.2206	2,756.7574	0.4958	
22	St. B 40 m. 60 D # 1	9,726.9608	3,970.5454	0.4082	
23	St. B 40 m. 60 D # 2	8,981.9906	2,223.0427	0.2475	
24	St. B 40 m. 60 D # 3	8,207.4043	1,428.0883	0.1740	
25	St. B 40 m. 90 D # 1	8,212.1626	7,662.7689	0.9331	
26	St. B 40 m. 90 D # 2	7,068.9784	5,878.5624	0.8316	
27	St. B 40 m. 90 D # 3	6,300.3348	3,874.7059	0.6150	

Table B91 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07 /AT	Remark
1	St. C 5 m. 30 D # 1	6,397.6185	5,074.5910	0.7932	
2	St. C 5 m. 30 D # 2	4,638.7242	992.2231	0.2139	
3	St. C 5 m. 30 D # 3	5,194.6838	4,274.7053	0.8229	
4	St. C 5 m. 60 D # 1	4,240.8082	3,837.0833	0.9048	
5	St. C 5 m. 60 D # 2	4,760.0214	3,964.1458	0.8328	
6	St. C 5 m. 60 D # 3	6,859.8407	5,043.3549	0.7352	
7	St. C 5 m. 90 D # 1	7,490.6541	6,068.1789	0.8101	
8	St. C 5 m. 90 D # 2	6,196.3638	4,418.6270	0.7131	
9	St. C 5 m. 90 D # 3	7,054.8009	3,573.9621	0.5066	
10	St. C 20 m. 30 D # 1	6,070.2033	1,975.2442	0.3254	
11	St. C 20 m. 30 D # 2	6,156.3653	1,755.7954	0.2852	
12	St. C 20 m. 30 D # 3	7,313.1038	5,100.1586	0.6974	
13	St. C 20 m. 60 D # 1	6,790.7105	1,667.1194	0.2455	
14	St. C 20 m. 60 D # 2	6,495.0784	2,337.5787	0.3599	
15	St. C 20 m. 60 D # 3	8,478.2315	4,195.0289	0.4948	
16	St. C 20 m. 90 D # 1	7,826.1923	4,858.5002	0.6208	
17	St. C 20 m. 90 D # 2	8,743.7875	2,100.2578	0.2402	
18	St. C 20 m. 90 D # 3	6,444.5242	1,580.8418	0.2453	
19	St. C 40 m. 30 D # 1	7,867.2218	6,239.4936	0.7931	
20	St. C 40 m. 30 D # 2	8,838.1920	2,092.8839	0.2368	
21	St. C 40 m. 30 D # 3	8,147.8342	1,418.5379	0.1741	
22	St. C 40 m. 60 D # 1	6,071.8885	2,451.2214	0.4037	
23	St. C 40 m. 60 D # 2	6,782.1438	2,734.5604	0.4032	
24	St. C 40 m. 60 D # 3	8,244.9591	8,574.7575	1.0400	
25	St. C 40 m. 90 D # 1	5,825.3692	1,444.6916	0.2480	
26	St. C 40 m. 90 D # 2	6,956.1669	2,217.6260	0.3188	
27	St. C 40 m. 90 D # 3	4,130.1569	1,023.0399	0.2477	

Table B92 Intensity of band and ratio pvMT07 expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity pvMT07	Ratio pvMT07 /AT	Remark
1	St. D 5 m. 30 D # 1	5,886.6648	2,758.4911	0.4686	
2	St. D 5 m. 30 D # 2	5,534.5675	2,620.0643	0.4734	
3	St. D 5 m. 30 D # 3	6,681.7459	3,834.6540	0.5739	
4	St. D 5 m. 60 D # 1	6,595.9673	2,419.4008	0.3668	
5	St. D 5 m. 60 D # 2	5,854.2557	1,307.2553	0.2233	
6	St. D 5 m. 60 D # 3	9,043.0346	5,679.0257	0.6280	
7	St. D 5 m. 90 D # 1	4,098.3793	1,997.1402	0.4873	
8	St. D 5 m. 90 D # 2	5,600.8017	2,727.0304	0.4869	
9	St. D 5 m. 90 D # 3	6,774.3701	1,693.5925	0.2500	

Table B93 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08 /AT	Remark
1	St. A 5 m. 30 D # 1	8,645.3427	1,986.6997	0.2298	
2	St. A 5 m. 30 D # 2	7,689.3353	1,603.9953	0.2086	
3	St. A 5 m. 30 D # 3	7,713.9057	2,021.0433	0.2620	
4	St. A 5 m. 60 D # 1	6,176.4749	4,791.7092	0.7758	
5	St. A 5 m. 60 D # 2	7,136.0990	8,921.5510	1.2502	
6	St. A 5 m. 60 D # 3	7,796.3429	2,472.2203	0.3171	
7	St. A 5 m. 90 D # 1	5,140.0396	6,912.8392	1.3449	
8	St. A 5 m. 90 D # 2	7,953.7543	10,598.3776	1.3325	
9	St. A 5 m. 90 D # 3	9,351.6848	4,907.7642	0.5248	
10	St. A 20 m. 30 D # 1	6,883.0467	1,077.1968	0.1565	
11	St. A 20 m. 30 D # 2	4,268.5360	670.5870	0.1571	
12	St. A 20 m. 30 D # 3	7,396.9588	1,874.3894	0.2534	
13	St. A 20 m. 60 D # 1	5,039.6338	3,074.1766	0.6100	
14	St. A 20 m. 60 D # 2	4,557.2385	4,086.0200	0.8966	
15	St. A 20 m. 60 D # 3	4,663.9016	1,542.8187	0.3308	
16	St. A 20 m. 90 D # 1	8,836.0504	2,795.7264	0.3164	
17	St. A 20 m. 90 D # 2	8,711.9107	1,798.1384	0.2064	
18	St. A 20 m. 90 D # 3	8,086.6887	1,435.3872	0.1775	
19	St. A 40 m. 30 D # 1	7,256.2690	5,223.0624	0.7198	
20	St. A 40 m. 30 D # 2	7,004.6322	1,099.7273	0.1570	
21	St. A 40 m. 30 D # 3	9,850.4621	2,095.1933	0.2127	
22	St. A 40 m. 60 D # 1	7,594.5678	1,216.6498	0.1602	
23	St. A 40 m. 60 D # 2	7,557.3311	1,163.0733	0.1539	
24	St. A 40 m. 60 D # 3	7,425.0832	1,922.3540	0.2589	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B94 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08 /AT	Remark
1	St. B 5 m. 30 D # 1	6,211.5898	3,626.9473	0.5839	
2	St. B 5 m. 30 D # 2	6,337.6929	2,471.0665	0.3899	
3	St. B 5 m. 30 D # 3	5,282.9348	1,188.1320	0.2249	
4	St. B 5 m. 60 D # 1	8,786.3905	4,758.7091	0.5416	
5	St. B 5 m. 60 D # 2	9,508.5596	2,259.2338	0.2376	
6	St. B 5 m. 60 D # 3	8,415.0986	2,141.6426	0.2545	
7	St. B 5 m. 90 D # 1	9,116.4300	6,115.3012	0.6708	
8	St. B 5 m. 90 D # 2	8,444.1335	2,660.7465	0.3151	
9	St. B 5 m. 90 D # 3	6,798.2050	3,356.2738	0.4937	
10	St. B 20 m. 30 D # 1	6,877.8125	1,785.4801	0.2596	
11	St. B 20 m. 30 D # 2	9,410.1542	2,312.0749	0.2457	
12	St. B 20 m. 30 D # 3	6,243.3234	4,244.8356	0.6799	
13	St. B 20 m. 60 D # 1	9,630.3849	2,496.1958	0.2592	
14	St. B 20 m. 60 D # 2	6,465.5505	1,339.0155	0.2071	
15	St. B 20 m. 60 D # 3	9,702.4652	2,004.5293	0.2066	
16	St. B 20 m. 90 D # 1	9,484.3576	2,051.4666	0.2163	
17	St. B 20 m. 90 D # 2	6,411.8919	1,543.9836	0.2408	
18	St. B 20 m. 90 D # 3	7,122.5406	1,601.8594	0.2249	
19	St. B 40 m. 30 D # 1	8,069.4263	2,539.4485	0.3147	
20	St. B 40 m. 30 D # 2	5,746.6361	1,383.7900	0.2408	
21	St. B 40 m. 30 D # 3	5,560.2206	1,608.5718	0.2893	
22	St. B 40 m. 60 D # 1	9,726.9608	2,173.9757	0.2235	
23	St. B 40 m. 60 D # 2	8,981.9906	2,289.5094	0.2549	
24	St. B 40 m. 60 D # 3	8,207.4043	1,275.4306	0.1554	
25	St. B 40 m. 90 D # 1	8,212.1626	6,209.2161	0.7561	
26	St. B 40 m. 90 D # 2	7,068.9784	1,533.2614	0.2169	
27	St. B 40 m. 90 D # 3	6,300.3348	3,354.2983	0.5324	

Table B95 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08 /AT	Remark
1	St. C 5 m. 30 D # 1	6,397.6185	4,182.1232	0.6537	
2	St. C 5 m. 30 D # 2	4,638.7242	940.7333	0.2028	
3	St. C 5 m. 30 D # 3	5,194.6838	1,822.8146	0.3509	
4	St. C 5 m. 60 D # 1	4,240.8082	3,351.0866	0.7902	
5	St. C 5 m. 60 D # 2	4,760.0214	1,940.6607	0.4077	
6	St. C 5 m. 60 D # 3	6,859.8407	4,601.5812	0.6708	
7	St. C 5 m. 90 D # 1	7,490.6541	1,773.7869	0.2368	
8	St. C 5 m. 90 D # 2	6,196.3638	1,886.1731	0.3044	
9	St. C 5 m. 90 D # 3	7,054.8009	3,538.6881	0.5016	
10	St. C 20 m. 30 D # 1	6,070.2033	1,683.8744	0.2774	
11	St. C 20 m. 30 D # 2	6,156.3653	1,192.4879	0.1937	
12	St. C 20 m. 30 D # 3	7,313.1038	2,180.7676	0.2982	
13	St. C 20 m. 60 D # 1	6,790.7105	1,571.3704	0.2314	
14	St. C 20 m. 60 D # 2	6,495.0784	1,932.9353	0.2976	
15	St. C 20 m. 60 D # 3	8,478.2315	3,418.4229	0.4032	
16	St. C 20 m. 90 D # 1	7,826.1923	2,254.7260	0.2881	
17	St. C 20 m. 90 D # 2	8,743.7875	1,997.0811	0.2284	
18	St. C 20 m. 90 D # 3	6,444.5242	1,569.8861	0.2436	
19	St. C 40 m. 30 D # 1	7,867.2218	2,006.9283	0.2551	
20	St. C 40 m. 30 D # 2	8,838.1920	1,990.3608	0.2252	
21	St. C 40 m. 30 D # 3	8,147.8342	1,152.1038	0.1414	
22	St. C 40 m. 60 D # 1	6,071.8885	2,417.2188	0.3981	
23	St. C 40 m. 60 D # 2	6,782.1438	2,695.9022	0.3975	
24	St. C 40 m. 60 D # 3	8,244.9591	3,121.5415	0.3786	
25	St. C 40 m. 90 D # 1	5,825.3692	1,273.4257	0.2186	
26	St. C 40 m. 90 D # 2	6,956.1669	2,173.1065	0.3124	
27	St. C 40 m. 90 D # 3	4,130.1569	900.7872	0.2181	

Table B96 Intensity of band and ratio pvMT08 expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity pvMT08	Ratio pvMT08 /AT	Remark
1	St. D 5 m. 30 D # 1	5,886.6648	2,611.3245	0.4436	
2	St. D 5 m. 30 D # 2	5,534.5675	1,979.7148	0.3577	
3	St. D 5 m. 30 D # 3	6,681.7459	2,378.0334	0.3559	
4	St. D 5 m. 60 D # 1	6,595.9673	1,881.8295	0.2853	
5	St. D 5 m. 60 D # 2	5,854.2557	1,218.2706	0.2081	
6	St. D 5 m. 60 D # 3	9,043.0346	3,259.1097	0.3604	
7	St. D 5 m. 90 D # 1	4,098.3793	2,043.8618	0.4987	
8	St. D 5 m. 90 D # 2	5,600.8017	916.2912	0.1636	
9	St. D 5 m. 90 D # 3	6,774.3701	1,593.3318	0.2352	

Table B97 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11 /AT	Remark
1	St. A 5 m. 30 D # 1	8,645.3427	4,979.7174	0.5760	
2	St. A 5 m. 30 D # 2	7,689.3353	3,853.1259	0.5011	
3	St. A 5 m. 30 D # 3	7,713.9057	5,433.6752	0.7044	
4	St. A 5 m. 60 D # 1	6,176.4749	5,426.0332	0.8785	
5	St. A 5 m. 60 D # 2	7,136.0990	8,679.6372	1.2163	
6	St. A 5 m. 60 D # 3	7,796.3429	6,097.5198	0.7821	
7	St. A 5 m. 90 D # 1	5,140.0396	5,520.4025	1.0740	
8	St. A 5 m. 90 D # 2	7,953.7543	9,489.6242	1.1931	
9	St. A 5 m. 90 D # 3	9,351.6848	6,331.0906	0.6770	
10	St. A 20 m. 30 D # 1	6,883.0467	2,987.9306	0.4341	
11	St. A 20 m. 30 D # 2	4,268.5360	1,855.1058	0.4346	
12	St. A 20 m. 30 D # 3	7,396.9588	1,918.0314	0.2593	
13	St. A 20 m. 60 D # 1	5,039.6338	3,227.3815	0.6404	
14	St. A 20 m. 60 D # 2	4,557.2385	4,168.5060	0.9147	
15	St. A 20 m. 60 D # 3	4,663.9016	1,499.9108	0.3216	
16	St. A 20 m. 90 D # 1	8,836.0504	7,139.5288	0.8080	
17	St. A 20 m. 90 D # 2	8,711.9107	3,446.4319	0.3956	
18	St. A 20 m. 90 D # 3	8,086.6887	1,415.1705	0.1750	
19	St. A 40 m. 30 D # 1	7,256.2690	5,986.4219	0.8250	
20	St. A 40 m. 30 D # 2	7,004.6322	5,807.5405	0.8291	
21	St. A 40 m. 30 D # 3	9,850.4621	3,886.9923	0.3946	
22	St. A 40 m. 60 D # 1	7,594.5678	1,319.1764	0.1737	
23	St. A 40 m. 60 D # 2	7,557.3311	2,087.3349	0.2762	
24	St. A 40 m. 60 D # 3	7,425.0832	1,537.7347	0.2071	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B98 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11 /AT	Remark
1	St. B 5 m. 30 D # 1	6,211.5898	6,148.2316	0.9898	
2	St. B 5 m. 30 D # 2	6,337.6929	6,513.2470	1.0277	
3	St. B 5 m. 30 D # 3	5,282.9348	1,258.3951	0.2382	
4	St. B 5 m. 60 D # 1	8,786.3905	6,691.7150	0.7616	
5	St. B 5 m. 60 D # 2	9,508.5596	7,219.8493	0.7593	
6	St. B 5 m. 60 D # 3	8,415.0986	2,162.6803	0.2570	
7	St. B 5 m. 90 D # 1	9,116.4300	7,677.8573	0.8422	
8	St. B 5 m. 90 D # 2	8,444.1335	2,691.9898	0.3188	
9	St. B 5 m. 90 D # 3	6,798.2050	1,655.3629	0.2435	
10	St. B 20 m. 30 D # 1	6,877.8125	2,246.9813	0.3267	
11	St. B 20 m. 30 D # 2	9,410.1542	3,198.5114	0.3399	
12	St. B 20 m. 30 D # 3	6,243.3234	6,057.2723	0.9702	
13	St. B 20 m. 60 D # 1	9,630.3849	4,881.6421	0.5069	
14	St. B 20 m. 60 D # 2	6,465.5505	4,197.4354	0.6492	
15	St. B 20 m. 60 D # 3	9,702.4652	6,295.9297	0.6489	
16	St. B 20 m. 90 D # 1	9,484.3576	2,330.3067	0.2457	
17	St. B 20 m. 90 D # 2	6,411.8919	1,669.6566	0.2604	
18	St. B 20 m. 90 D # 3	7,122.5406	1,614.6799	0.2267	
19	St. B 40 m. 30 D # 1	8,069.4263	2,718.5897	0.3369	
20	St. B 40 m. 30 D # 2	5,746.6361	1,496.4240	0.2604	
21	St. B 40 m. 30 D # 3	5,560.2206	1,517.3842	0.2729	
22	St. B 40 m. 60 D # 1	9,726.9608	3,791.5693	0.3898	
23	St. B 40 m. 60 D # 2	8,981.9906	3,847.8848	0.4284	
24	St. B 40 m. 60 D # 3	8,207.4043	4,510.7894	0.5496	
25	St. B 40 m. 90 D # 1	8,212.1626	8,578.4250	1.0446	
26	St. B 40 m. 90 D # 2	7,068.9784	6,312.5977	0.8930	
27	St. B 40 m. 90 D # 3	6,300.3348	3,242.7823	0.5147	

Table B99 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11 /AT	Remark
1	St. C 5 m. 30 D # 1	6,397.6185	3,653.0402	0.5710	
2	St. C 5 m. 30 D # 2	4,638.7242	933.7752	0.2013	
3	St. C 5 m. 30 D # 3	5,194.6838	1,015.5607	0.1955	
4	St. C 5 m. 60 D # 1	4,240.8082	4,365.9121	1.0295	
5	St. C 5 m. 60 D # 2	4,760.0214	2,843.1608	0.5973	
6	St. C 5 m. 60 D # 3	6,859.8407	4,568.6539	0.6660	
7	St. C 5 m. 90 D # 1	7,490.6541	2,462.1780	0.3287	
8	St. C 5 m. 90 D # 2	6,196.3638	4,779.2554	0.7713	
9	St. C 5 m. 90 D # 3	7,054.8009	3,680.4896	0.5217	
10	St. C 20 m. 30 D # 1	6,070.2033	1,680.2323	0.2768	
11	St. C 20 m. 30 D # 2	6,156.3653	1,248.5109	0.2028	
12	St. C 20 m. 30 D # 3	7,313.1038	2,072.5336	0.2834	
13	St. C 20 m. 60 D # 1	6,790.7105	4,132.8264	0.6086	
14	St. C 20 m. 60 D # 2	6,495.0784	2,144.0254	0.3301	
15	St. C 20 m. 60 D # 3	8,478.2315	2,089.0362	0.2464	
16	St. C 20 m. 90 D # 1	7,826.1923	2,230.4648	0.2850	
17	St. C 20 m. 90 D # 2	8,743.7875	2,261.1435	0.2586	
18	St. C 20 m. 90 D # 3	6,444.5242	2,217.5608	0.3441	
19	St. C 40 m. 30 D # 1	7,867.2218	6,105.7508	0.7761	
20	St. C 40 m. 30 D # 2	8,838.1920	2,451.7145	0.2774	
21	St. C 40 m. 30 D # 3	8,147.8342	1,161.0664	0.1425	
22	St. C 40 m. 60 D # 1	6,071.8885	2,438.4704	0.4016	
23	St. C 40 m. 60 D # 2	6,782.1438	2,727.1000	0.4021	
24	St. C 40 m. 60 D # 3	8,244.9591	7,130.2407	0.8648	
25	St. C 40 m. 90 D # 1	5,825.3692	1,354.3983	0.2325	
26	St. C 40 m. 90 D # 2	6,956.1669	2,494.4814	0.3586	
27	St. C 40 m. 90 D # 3	4,130.1569	961.0875	0.2327	

Table B100 Intensity of band and ratio pvMT11 expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity pvMT11	Ratio pvMT11 /AT	Remark
1	St. D 5 m. 30 D # 1	5,886.6648	3,157.0183	0.5363	
2	St. D 5 m. 30 D # 2	5,534.5675	3,529.9472	0.6378	
3	St. D 5 m. 30 D # 3	6,681.7459	2,393.4014	0.3582	
4	St. D 5 m. 60 D # 1	6,595.9673	4,788.6723	0.7260	
5	St. D 5 m. 60 D # 2	5,854.2557	1,361.6999	0.2326	
6	St. D 5 m. 60 D # 3	9,043.0346	5,315.4957	0.5878	
7	St. D 5 m. 90 D # 1	4,098.3793	3,872.5586	0.9449	
8	St. D 5 m. 90 D # 2	5,600.8017	2,197.7546	0.3924	
9	St. D 5 m. 90 D # 3	6,774.3701	1,821.6281	0.2689	

Table B101 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	St. A 5 m. 30 D # 1	6,219.9019	7,303.4089	1.1742	
2	St. A 5 m. 30 D # 2	7,301.4196	8,892.3989	1.2179	
3	St. A 5 m. 30 D # 3	5,203.9182	7,369.2686	1.4161	
4	St. A 5 m. 60 D # 1	6,684.5605	10,252.1105	1.5337	
5	St. A 5 m. 60 D # 2	6,299.7082	7,948.9718	1.2618	
6	St. A 5 m. 60 D # 3	5,222.4057	6,624.0993	1.2684	
7	St. A 5 m. 90 D # 1	5,299.2442	6,573.7125	1.2405	
8	St. A 5 m. 90 D # 2	5,846.1392	7,252.1357	1.2405	
9	St. A 5 m. 90 D # 3	6,074.7481	8,757.3569	1.4416	
10	St. A 20 m. 30 D # 1	4,136.2214	5,535.0915	1.3382	
11	St. A 20 m. 30 D # 2	4,257.1817	4,222.2728	0.9918	
12	St. A 20 m. 30 D # 3	6,541.1773	8,582.0246	1.3120	
13	St. A 20 m. 60 D # 1	6,177.6296	9,123.1234	1.4768	
14	St. A 20 m. 60 D # 2	5,078.5851	5,320.3258	1.0476	
15	St. A 20 m. 60 D # 3	5,443.9536	7,292.7203	1.3396	
16	St. A 20 m. 90 D # 1	7,280.8103	10,341.6629	1.4204	
17	St. A 20 m. 90 D # 2	5,980.8988	6,876.2394	1.1497	
18	St. A 20 m. 90 D # 3	7,261.3551	7,642.5763	1.0525	
19	St. A 40 m. 30 D # 1	7,380.7814	10,277.7381	1.3925	
20	St. A 40 m. 30 D # 2	6,096.7337	9,061.5753	1.4863	
21	St. A 40 m. 30 D # 3	5,639.7238	5,621.6767	0.9968	
22	St. A 40 m. 60 D # 1	6,316.7835	7,849.8668	1.2427	
23	St. A 40 m. 60 D # 2	5,995.6228	7,395.0012	1.2334	
24	St. A 40 m. 60 D # 3	6,140.2103	7,968.7650	1.2978	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B102 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	St. B 5 m. 30 D # 1	5,396.1974	6,996.1700	1.2965	
2	St. B 5 m. 30 D # 2	3,817.0966	4,982.0744	1.3052	
3	St. B 5 m. 30 D # 3	5,123.0797	6,686.6436	1.3052	
4	St. B 5 m. 60 D # 1	6,868.0082	4,943.5923	0.7198	
5	St. B 5 m. 60 D # 2	5,818.1178	6,661.1630	1.1449	
6	St. B 5 m. 60 D # 3	6,427.7295	8,849.0552	1.3767	
7	St. B 5 m. 90 D # 1	7,703.6697	10,779.7450	1.3993	
8	St. B 5 m. 90 D # 2	6,271.8660	9,280.4801	1.4797	
9	St. B 5 m. 90 D # 3	7,095.3268	8,459.0486	1.1922	
10	St. B 20 m. 30 D # 1	7,593.7272	9,476.2122	1.2479	
11	St. B 20 m. 30 D # 2	6,192.2819	7,784.3176	1.2571	
12	St. B 20 m. 30 D # 3	3,760.2508	5,846.4379	1.5548	
13	St. B 20 m. 60 D # 1	4,913.4242	6,362.8843	1.2950	
14	St. B 20 m. 60 D # 2	2,476.4540	3,225.8290	1.3026	
15	St. B 20 m. 60 D # 3	5,571.4229	3,246.4681	0.5827	
16	St. B 20 m. 90 D # 1	4,689.4389	5,427.0877	1.1573	
17	St. B 20 m. 90 D # 2	2,940.2739	4,497.7369	1.5297	
18	St. B 20 m. 90 D # 3	4,969.7610	3,967.8572	0.7984	
19	St. B 40 m. 30 D # 1	5,065.1371	5,499.7259	1.0858	
20	St. B 40 m. 30 D # 2	6,426.4750	9,946.8980	1.5478	
21	St. B 40 m. 30 D # 3	4,184.6514	3,081.1588	0.7363	
22	St. B 40 m. 60 D # 1	3,999.8202	6,503.7077	1.6260	
23	St. B 40 m. 60 D # 2	4,713.6396	6,846.5615	1.4525	
24	St. B 40 m. 60 D # 3	5,130.6352	7,612.3235	1.4837	
25	St. B 40 m. 90 D # 1	4,979.9545	7,851.3963	1.5766	
26	St. B 40 m. 90 D # 2	5,211.4594	9,367.5984	1.7975	
27	St. B 40 m. 90 D # 3	4,304.1422	6,115.7557	1.4209	

Table B103 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	St. C 5 m. 30 D # 1	3,465.6779	5,624.7952	1.6230	
2	St. C 5 m. 30 D # 2	8,349.7759	10,123.2684	1.2124	
3	St. C 5 m. 30 D # 3	8,043.1744	9,751.5446	1.2124	
4	St. C 5 m. 60 D # 1	6,662.6896	8,820.7348	1.3239	
5	St. C 5 m. 60 D # 2	7,111.4199	7,968.3460	1.1205	
6	St. C 5 m. 60 D # 3	5,695.2721	7,946.6132	1.3953	
7	St. C 5 m. 90 D # 1	4,820.8724	6,135.0422	1.2726	
8	St. C 5 m. 90 D # 2	4,820.8724	6,435.8646	1.3350	
9	St. C 5 m. 90 D # 3	3,832.6951	5,363.8568	1.3995	
10	St. C 20 m. 30 D # 1	3,438.3661	4,122.9448	1.1991	
11	St. C 20 m. 30 D # 2	5,110.1621	5,884.3517	1.1515	
12	St. C 20 m. 30 D # 3	2,888.1521	2,310.8105	0.8001	
13	St. C 20 m. 60 D # 1	6,198.7622	8,959.0710	1.4453	
14	St. C 20 m. 60 D # 2	7,118.3303	8,388.2404	1.1784	
15	St. C 20 m. 60 D # 3	6,703.1268	8,967.4430	1.3378	
16	St. C 20 m. 90 D # 1	4,335.6905	5,859.6858	1.3515	
17	St. C 20 m. 90 D # 2	6,068.3682	8,195.3312	1.3505	
18	St. C 20 m. 90 D # 3	3,125.2484	4,737.5641	1.5159	
19	St. C 40 m. 30 D # 1	6,678.9333	8,526.3263	1.2766	
20	St. C 40 m. 30 D # 2	5,276.2048	6,196.9026	1.1745	
21	St. C 40 m. 30 D # 3	6,570.1450	6,750.1670	1.0274	
22	St. C 40 m. 60 D # 1	3,248.5385	3,982.7082	1.2260	
23	St. C 40 m. 60 D # 2	4,405.3606	4,821.6672	1.0945	
24	St. C 40 m. 60 D # 3	4,949.5030	7,220.3349	1.4588	
25	St. C 40 m. 90 D # 1	6,092.1961	6,980.4383	1.1458	
26	St. C 40 m. 90 D # 2	7,360.4159	7,611.4060	1.0341	
27	St. C 40 m. 90 D # 3	4,260.4792	6,052.0107	1.4205	

Table B104 Intensity of band and ratio HSP71 expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity HSP71	Ratio HSP71 /AT	Remark
1	St. D 5 m. 30 D # 1	4,009.9779	6,096.3694	1.5203	
2	St. D 5 m. 30 D # 2	4,351.0668	5,046.3673	1.1598	
3	St. D 5 m. 30 D # 3	4,847.1662	4,364.8732	0.9005	
4	St. D 5 m. 60 D # 1	3,971.5049	5,665.7488	1.4266	
5	St. D 5 m. 60 D # 2	4,500.8802	5,627.4505	1.2503	
6	St. D 5 m. 60 D # 3	4,263.2821	5,441.2269	1.2763	
7	St. D 5 m. 90 D # 1	4,276.7157	5,458.3723	1.2763	
8	St. D 5 m. 90 D # 2	3,716.7096	4,068.6821	1.0947	
9	St. D 5 m. 90 D # 3	4,827.5104	4,762.8217	0.9866	

Table B105 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Field study (Station A)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	St. A 5 m. 30 D # 1	6,219.9019	4,711.5757	0.7575	
2	St. A 5 m. 30 D # 2	7,301.4196	6,139.7637	0.8409	
3	St. A 5 m. 30 D # 3	5,203.9182	3,120.2694	0.5996	
4	St. A 5 m. 60 D # 1	6,684.5605	6,326.2681	0.9464	
5	St. A 5 m. 60 D # 2	6,299.7082	5,428.4586	0.8617	
6	St. A 5 m. 60 D # 3	5,222.4057	2,633.6592	0.5043	
7	St. A 5 m. 90 D # 1	5,299.2442	2,301.4618	0.4343	
8	St. A 5 m. 90 D # 2	5,846.1392	2,538.9783	0.4343	
9	St. A 5 m. 90 D # 3	6,074.7481	5,533.4880	0.9109	
10	St. A 20 m. 30 D # 1	4,136.2214	2,849.8565	0.6890	
11	St. A 20 m. 30 D # 2	4,257.1817	2,126.4623	0.4995	
12	St. A 20 m. 30 D # 3	6,541.1773	4,567.7041	0.6983	
13	St. A 20 m. 60 D # 1	6,177.6296	4,182.8730	0.6771	
14	St. A 20 m. 60 D # 2	5,078.5851	4,480.8357	0.8823	
15	St. A 20 m. 60 D # 3	5,443.9536	3,512.4389	0.6452	
16	St. A 20 m. 90 D # 1	7,280.8103	5,767.8579	0.7922	
17	St. A 20 m. 90 D # 2	5,980.8988	5,077.7831	0.8490	
18	St. A 20 m. 90 D # 3	7,261.3551	3,929.1193	0.5411	
19	St. A 40 m. 30 D # 1	7,380.7814	5,193.1178	0.7036	
20	St. A 40 m. 30 D # 2	6,096.7337	4,926.1608	0.8080	
21	St. A 40 m. 30 D # 3	5,639.7238	2,503.4734	0.4439	
22	St. A 40 m. 60 D # 1	6,316.7835	3,483.7061	0.5515	
23	St. A 40 m. 60 D # 2	5,995.6228	3,967.9032	0.6618	
24	St. A 40 m. 60 D # 3	6,140.2103	4,414.8112	0.7190	
25	St. A 40 m. 90 D # 1	NA	NA	NA	Lost of sample
26	St. A 40 m. 90 D # 2	NA	NA	NA	Lost of sample
27	St. A 40 m. 90 D # 3	NA	NA	NA	Lost of sample

Table B106 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Field study (Station B)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	St. B 5 m. 30 D # 1	5,396.1974	4,536.5832	0.8407	
2	St. B 5 m. 30 D # 2	3,817.0966	3,395.3074	0.8895	
3	St. B 5 m. 30 D # 3	5,123.0797	4,556.9794	0.8895	
4	St. B 5 m. 60 D # 1	6,868.0082	5,340.5632	0.7776	
5	St. B 5 m. 60 D # 2	5,818.1178	3,583.3787	0.6159	
6	St. B 5 m. 60 D # 3	6,427.7295	3,382.2713	0.5262	
7	St. B 5 m. 90 D # 1	7,703.6697	3,598.3841	0.4671	
8	St. B 5 m. 90 D # 2	6,271.8660	6,184.0599	0.9860	
9	St. B 5 m. 90 D # 3	7,095.3268	3,104.9150	0.4376	
10	St. B 20 m. 30 D # 1	7,593.7272	5,440.1462	0.7164	
11	St. B 20 m. 30 D # 2	6,192.2819	4,611.3923	0.7447	
12	St. B 20 m. 30 D # 3	3,760.2508	3,846.3605	1.0229	
13	St. B 20 m. 60 D # 1	4,913.4242	3,330.8103	0.6779	
14	St. B 20 m. 60 D # 2	2,476.4540	1,059.4270	0.4278	
15	St. B 20 m. 60 D # 3	5,571.4229	2,156.6978	0.3871	
16	St. B 20 m. 90 D # 1	4,689.4389	2,282.8189	0.4868	
17	St. B 20 m. 90 D # 2	2,940.2739	1,481.3100	0.5038	
18	St. B 20 m. 90 D # 3	4,969.7610	2,482.3956	0.4995	
19	St. B 40 m. 30 D # 1	5,065.1371	2,742.2653	0.5414	
20	St. B 40 m. 30 D # 2	6,426.4750	5,727.2745	0.8912	
21	St. B 40 m. 30 D # 3	4,184.6514	2,863.5570	0.6843	
22	St. B 40 m. 60 D # 1	3,999.8202	1,641.1262	0.4103	
23	St. B 40 m. 60 D # 2	4,713.6396	4,033.9328	0.8558	
24	St. B 40 m. 60 D # 3	5,130.6352	2,778.2390	0.5415	
25	St. B 40 m. 90 D # 1	4,979.9545	4,899.2793	0.9838	
26	St. B 40 m. 90 D # 2	5,211.4594	3,904.4254	0.7492	
27	St. B 40 m. 90 D # 3	4,304.1422	3,010.3171	0.6994	

Table B107 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Field study (Station C)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	St. C 5 m. 30 D # 1	3,465.6779	4,001.1251	1.1545	
2	St. C 5 m. 30 D # 2	8,349.7759	6,793.3777	0.8136	
3	St. C 5 m. 30 D # 3	8,043.1744	6,543.9267	0.8136	
4	St. C 5 m. 60 D # 1	6,662.6896	3,923.6579	0.5889	
5	St. C 5 m. 60 D # 2	7,111.4199	6,597.9754	0.9278	
6	St. C 5 m. 60 D # 3	5,695.2721	5,585.3534	0.9807	
7	St. C 5 m. 90 D # 1	4,820.8724	3,258.4276	0.6759	
8	St. C 5 m. 90 D # 2	4,820.8724	4,367.7104	0.9060	
9	St. C 5 m. 90 D # 3	3,832.6951	1,610.1152	0.4201	
10	St. C 20 m. 30 D # 1	3,438.3661	2,361.8137	0.6869	
11	St. C 20 m. 30 D # 2	5,110.1621	4,254.2100	0.8325	
12	St. C 20 m. 30 D # 3	2,888.1521	2,229.9422	0.7721	
13	St. C 20 m. 60 D # 1	6,198.7622	5,790.2638	0.9341	
14	St. C 20 m. 60 D # 2	7,118.3303	6,237.0810	0.8762	
15	St. C 20 m. 60 D # 3	6,703.1268	6,030.8032	0.8997	
16	St. C 20 m. 90 D # 1	4,335.6905	2,929.1925	0.6756	
17	St. C 20 m. 90 D # 2	6,068.3682	3,229.5855	0.5322	
18	St. C 20 m. 90 D # 3	3,125.2484	3,508.4039	1.1226	
19	St. C 40 m. 30 D # 1	6,678.9333	5,356.5045	0.8020	
20	St. C 40 m. 30 D # 2	5,276.2048	4,630.9250	0.8777	
21	St. C 40 m. 30 D # 3	6,570.1450	5,359.2673	0.8157	
22	St. C 40 m. 60 D # 1	3,248.5385	3,121.8455	0.9610	
23	St. C 40 m. 60 D # 2	4,405.3606	2,923.3973	0.6636	
24	St. C 40 m. 60 D # 3	4,949.5030	4,451.5830	0.8994	
25	St. C 40 m. 90 D # 1	6,092.1961	4,103.7033	0.6736	
26	St. C 40 m. 90 D # 2	7,360.4159	3,986.4012	0.5416	
27	St. C 40 m. 90 D # 3	4,260.4792	3,473.9947	0.8154	

Table B108 Intensity of band and ratio CYP4 expression gene in gill of mussel *P.viridis* Field study (Station D)

No.	Sample	Intensity AT	Intensity CYP4	Ratio CYP4 /AT	Remark
1	St. D 5 m. 30 D # 1	4,009.9779	4,335.9891	1.0813	
2	St. D 5 m. 30 D # 2	4,351.0668	3,858.9612	0.8869	
3	St. D 5 m. 30 D # 3	4,847.1662	4,437.0960	0.9154	
4	St. D 5 m. 60 D # 1	3,971.5049	4,871.4479	1.2266	
5	St. D 5 m. 60 D # 2	4,500.8802	3,573.2488	0.7939	
6	St. D 5 m. 60 D # 3	4,263.2821	4,050.1180	0.9500	
7	St. D 5 m. 90 D # 1	4,276.7157	4,062.8799	0.9500	
8	St. D 5 m. 90 D # 2	3,716.7096	3,323.1101	0.8941	
9	St. D 5 m. 90 D # 3	4,827.5104	4,003.4543	0.8293	

APPENDIX C

Blast X result for MT gene

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AAF22487.1	metallothionein 2 [Perna viridis]	104	104	85%	4e-21	98%
Q9U568.2	RecName: Full=Metallothionein; Short=MT >gb|AAF22486.1|AF092971_1 metallothionein 1 [Perna viridis]	104	104	85%	4e-21	100%
AAD02054.1	metallothionein [Perna viridis]	99.4	99.4	85%	1e-19	90%
CAE11861.1	metallothionein [Mytilus edulis]	84.0	84.0	85%	5e-15	66%
P80246.2	RecName: Full=Metallothionein 10-Ia; Short=MT-10-Ia >emb|CAA06548.1| metallothionein 10 Ia [Mytilus edulis]	83.6	83.6	85%	7e-15	65%
O62554.3	RecName: Full=Metallothionein 10-Ib; Short=MT-10-Ib >emb|CAA06549.1| metallothionein 10 Ib [Mytilus edulis]	83.6	83.6	85%	7e-15	63%
ABM30214.1	metallothionein 10 [Mytilus sp. KL-2006]	82.4	82.4	85%	2e-14	65%
P80247.3	RecName: Full=Metallothionein 10-II; Short=MT-10-II >emb|CAA06550.1| metallothionein 10 II [Mytilus edulis]	82.4	82.4	85%	2e-14	63%
AAB29061.1	MT-10-I=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	81.6	81.6	83%	3e-14	64%
P80248.2	RecName: Full=Metallothionein 10-III; Short=MT-10-III >emb|CAA06551.1| metallothionein 10 III [Mytilus edulis] >gb|AAT72936.1| metallothionein 10-III [Mytilus galloprovincialis]	81.3	81.3	85%	4e-14	63%
P80249.2	RecName: Full=Metallothionein 10-IV; Short=MT-10-IV >emb|CAA07546.1| metallothionein 10IV [Mytilus edulis]	79.7	79.7	85%	1e-13	63%
CAF34421.1	metallothionein, isoform MT-10a [Bathymodiolus azoricus]	79.3	79.3	85%	1e-13	61%
CAF34422.1	metallothionein, isoform MT-10b [Bathymodiolus azoricus] >emb|CAF34423.1| metallothionein, isoform MT-10c [Bathymodiolus azoricus]	79.3	79.3	85%	1e-13	60%
AAB29060.1	MT-10-IV=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	77.8	77.8	83%	4e-13	62%
CAE11855.1	metallothionein [Mytilus edulis]	76.6	76.6	85%	9e-13	62%
P80252.2	RecName: Full=Metallothionein 20-II; Short=MT-20-II >emb|CAA06553.1| metallothionein 20 II [Mytilus edulis] >gb|ABM30215.1| metallothionein 20-II [Mytilus sp. KL-2006]	75.1	75.1	82%	3e-12	83%
CAI94401.1	metallothionein [Bathymodiolus azoricus]	74.3	74.3	81%	4e-12	61%
CAE11860.1	metallothionein [Bathymodiolus thermophilus]	73.6	73.6	81%	7e-12	70%
CAE11856.1	metallothionein [Mytilus edulis]	73.6	73.6	85%	7e-12	60%
CAE11859.1	metallothionein [Bathymodiolus thermophilus]	73.6	73.6	81%	7e-12	70%
CAD56896.1	metallothionein 10 [Bathymodiolus sp. FD-2002]	73.6	73.6	81%	7e-12	70%
P69153.2	RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA >sp|P69154.2|MT23A_MYTGA RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA; Short=MT-I >gb|AAG28538.1|AF199020_1 metallothionein isoform [Mytilus galloprovincialis]	73.6	73.6	82%	7e-12	80%
P80258.1	RecName: Full=Metallothionein 20-III isoform B; Short=MT-20-IIIB	71.6	71.6	81%	3e-11	80%
P80251.1	RecName: Full=Metallothionein 20-I isoforms A and B; AltName: Full=MT-20-IA and MT-20-IB >gb|AAB29062.1| MT-20-I=20 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 71 aa]	71.2	71.2	81%	4e-11	81%
CAE11862.1	metallothionein [Mytilus edulis]	68.9	68.9	82%	2e-10	78%
ABI30643.1	metallothionein-10B [Mytilus galloprovincialis]	65.9	65.9	79%	2e-09	49%
CAF34424.1	metallothionein [Bathymodiolus azoricus] >emb|CAF34425.1| metallothionein [Bathymodiolus thermophilus]	65.9	65.9	77%	2e-09	82%
AAT72935.1	metallothionein 20-IV [Mytilus galloprovincialis]	64.3	64.3	82%	4e-09	75%
ABH03633.1	metallothionein 10a [Laternula elliptica]	62.8	62.8	85%	1e-08	58%
ABH03634.1	metallothionein 10b [Laternula elliptica]	62.8	62.8	85%	1e-08	57%
CAE11857.1	metallothionein [Mytilus edulis]	62.8	62.8	79%	1e-08	47%
CAE11858.1	metallothionein [Mytilus edulis]	61.6	61.6	79%	3e-08	47%
AAS92877.1	metallothionein [Meretrix lusoria]	59.3	59.3	85%	1e-07	45%
AAK39563.1	metallothionein-like protein [Tegillarca granosa]	56.2	56.2	81%	1e-06	60%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AAK15581.1	metallothionein [Crassostrea angulata]	56.2	56.2	83%	1e-06	59%
AAQ23908.1	metallothionein IB [Crassostrea virginica]	55.5	55.5	85%	2e-06	37%
CAB85588.1	metallothionein [Crassostrea gigas] >emb|CAC48045.1| metallothionein [Crassostrea gigas]	55.5	55.5	83%	2e-06	58%
ABC69708.1	metallothionein [Crassostrea ariakensis]	54.7	54.7	83%	4e-06	58%
P23038.3	RecName: Full=Metallothionein; Short=MT >emb|CAA42522.1| metallothionein [Crassostrea virginica] >gb|AAQ23904.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23905.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23906.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23907.1| metallothionein IA [Crassostrea virginica]	54.3	54.3	85%	5e-06	37%
ABP01350.1	metallothionein [Unio tumidus]	53.5	53.5	85%	8e-06	61%
CAB64869.1	metallothionein [Crassostrea gigas]	53.5	53.5	83%	8e-06	56%
CAB96402.1	metallothionein [Venerupis (Ruditapes) decussatus]	53.1	53.1	78%	1e-05	48%
CAB96403.1	metallothionein [Venerupis (Ruditapes) philippinarum]	53.1	53.1	78%	1e-05	60%
ACB05816.1	metallothionein [Cerastoderma glaucum]	52.4	52.4	81%	2e-05	41%
ABP57063.1	metallothionein [Venerupis philippinarum]	51.2	51.2	85%	4e-05	40%
AAM90257.1	metallothionein [Crassostrea virginica]	49.7	49.7	85%	1e-04	35%
ABS20116.1	metallothionein [Venerupis decussatus]	49.3	49.3	81%	1e-04	40%
CAC82788.1	metallothionein [Crassostrea gigas]	49.3	49.3	85%	1e-04	40%
ACH99846.1	metallothionein [Scapharca broughtonii]	48.9	48.9	82%	2e-04	36%
AAS75318.1	metallothionein [Tegillarca granosa]	48.1	48.1	82%	3e-04	36%
AAZ76545.1	metallothionein [Scapharca inaequivalvis]	47.4	47.4	82%	6e-04	37%
ABM55725.1	metallothionein [Corbicula fluminea]	47.0	47.0	85%	7e-04	54%
CAC83770.1	metallothionein [Ostrea edulis]	47.0	47.0	85%	7e-04	53%
Q94550.1	RecName: Full=Metallothionein; Short=MT >gb|AAB07548.1| metallothionein [Dreissena polymorpha]	46.2	46.2	85%	0.001	44%
ACT53273.1	metallothionein [Pisidium coreanum]	45.4	45.4	78%	0.002	40%
AAQ23913.1	metallothionein IIE [Crassostrea virginica]	44.7	132	78%	0.004	55%
AAQ23911.1	metallothionein IIC [Crassostrea virginica]	44.7	88.6	78%	0.004	55%
AAQ23916.1	metallothionein IIH [Crassostrea virginica]	44.7	174	78%	0.004	53%
AAQ23914.1	metallothionein IIF [Crassostrea virginica]	43.9	131	36%	0.006	55%
AAQ23909.1	metallothionein IIA [Crassostrea virginica]	43.9	43.9	36%	0.006	55%
AAQ23915.1	metallothionein IIG [Crassostrea virginica]	43.9	175	36%	0.006	55%
AAQ23912.1	metallothionein IID [Crassostrea virginica]	43.9	131	36%	0.006	55%
AAK50565.1	metallothionein [Crassostrea rhizophorae]	43.9	43.9	32%	0.006	58%
ABN68955.1	metallothionein [Cerastoderma edule]	43.5	43.5	36%	0.008	51%
ACS44750.1	metallothionein [Hyriopsis cumingii]	43.1	43.1	85%	0.011	41%
ACU46012.1	metallothionein [Mactra veneriformis]	42.0	42.0	85%	0.024	34%
CAB96419.1	metallothionein [Venerupis pullastra]	41.2	41.2	31%	0.041	56%
ABP57066.2	metallothionein [Cerastoderma edule] >gb|ACT66292.1| metallothionein 1 [Cerastoderma edule] >gb|ACT66293.1| metallothionein 1 [Cerastoderma edule]	40.4	40.4	82%	0.070	43%
AAQ23910.1	metallothionein IIB [Crassostrea virginica]	39.7	39.7	36%	0.12	48%
AAK56498.1	putative metallothionein [Littorina littorea]	39.3	39.3	82%	0.16	44%
CAK22381.1	metallothionein IV [Crassostrea gigas]	38.1	38.1	85%	0.35	40%

Blast X result for pvMT01 gene

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AAF22487.1	metallothionein 2 [Perna viridis]	69.7	69.7	97%	1e-10	95%
Q9U568.2	RecName: Full=Metallothionein; Short=MT >gb|AAF22486.1|AF092971_1 metallothionein 1 [Perna viridis]	69.7	69.7	97%	1e-10	97%
AAD02054.1	metallothionein [Perna viridis]	64.7	64.7	97%	3e-09	86%
P80248.2	RecName: Full=Metallothionein 10-III; Short=MT-10-III >emb|CAA06551.1| metallothionein 10 III [Mytilus edulis] >gb|AAT72936.1| metallothionein 10-III [Mytilus galloprovincialis]	60.8	60.8	49%	5e-08	92%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AAB29061.1	MT-10-I=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	59.7	59.7	49%	1e-07	92%
AAB29060.1	MT-10-IV=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	59.7	59.7	49%	1e-07	92%
CAE11861.1	metallothionein [Mytilus edulis]	59.7	59.7	49%	1e-07	92%
CAE11856.1	metallothionein [Mytilus edulis]	59.7	59.7	49%	1e-07	92%
CAE11855.1	metallothionein [Mytilus edulis]	59.7	59.7	49%	1e-07	92%
P80246.2	RecName: Full=Metallothionein 10-Ia; Short=MT-10-Ia >emb|CAA06548.1| metallothionein 10 Ia [Mytilus edulis]	59.7	59.7	49%	1e-07	92%
O62554.3	RecName: Full=Metallothionein 10-Ib; Short=MT-10-Ib >emb|CAA06549.1| metallothionein 10 Ib [Mytilus edulis]	59.7	59.7	49%	1e-07	92%
P80249.2	RecName: Full=Metallothionein 10-IV; Short=MT-10-IV >emb|CAA07546.1| metallothionein 10IV [Mytilus edulis]	59.7	59.7	49%	1e-07	92%
CAI94401.1	metallothionein [Bathymodiolus azoricus]	59.3	59.3	49%	1e-07	88%
CAF34421.1	metallothionein, isoform MT-10a [Bathymodiolus azoricus]	59.3	59.3	49%	1e-07	88%
CAF34422.1	metallothionein, isoform MT-10b [Bathymodiolus azoricus] >emb|CAF34423.1| metallothionein, isoform MT-10c [Bathymodiolus azoricus]	59.3	59.3	49%	1e-07	88%
P80247.3	RecName: Full=Metallothionein 10-II; Short=MT-10-II >emb|CAA06550.1| metallothionein 10 II [Mytilus edulis]	58.5	58.5	49%	2e-07	88%
CAE11860.1	metallothionein [Bathymodiolus thermophilus]	58.2	58.2	49%	3e-07	84%
CAE11859.1	metallothionein [Bathymodiolus thermophilus]	58.2	58.2	49%	3e-07	84%
CAD56896.1	metallothionein 10 [Bathymodiolus sp. FD-2002]	58.2	58.2	49%	3e-07	84%
CAF34424.1	metallothionein [Bathymodiolus azoricus] >emb|CAF34425.1| metallothionein [Bathymodiolus thermophilus]	57.4	57.4	49%	6e-07	88%
P80251.1	RecName: Full=Metallothionein 20-I isoforms A and B; AltName: Full=MT-20-IA and MT-20-IB >gb|AAB29062.1| MT-20-I=20 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 71 aa]	57.4	57.4	49%	6e-07	88%
P80252.2	RecName: Full=Metallothionein 20-II; Short=MT-20-II >emb|CAA06553.1| metallothionein 20 II [Mytilus edulis] >gb|ABM30215.1| metallothionein 20-II [Mytilus sp. KL-2006]	57.4	57.4	49%	6e-07	88%
ABM30214.1	metallothionein 10 [Mytilus sp. KL-2006]	56.6	56.6	49%	9e-07	88%
CAE11862.1	metallothionein [Mytilus edulis]	55.8	55.8	49%	2e-06	84%
P69153.2	RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA >sp|P69154.2|MT23A_MYTGA RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA; Short=MT-I >gb|AAG28538.1|AF199020_1 metallothionein isoform [Mytilus galloprovincialis]	55.8	55.8	49%	2e-06	84%
P80258.1	RecName: Full=Metallothionein 20-III isoform B; Short=MT-20-IIIB	55.8	55.8	49%	2e-06	84%
ABI30643.1	metallothionein-10B [Mytilus galloprovincialis]	54.7	54.7	47%	4e-06	83%
CAE11857.1	metallothionein [Mytilus edulis]	51.6	51.6	47%	3e-05	79%
CAE11858.1	metallothionein [Mytilus edulis]	50.8	50.8	45%	5e-05	82%
AAT72935.1	metallothionein 20-IV [Mytilus galloprovincialis]	50.4	50.4	49%	7e-05	76%
AAK39563.1	metallothionein-like protein [Tegillarca granosa]	41.6	41.6	49%	0.031	52%
AAK50565.1	metallothionein [Crassostrea rhizophorae]	41.2	41.2	45%	0.041	56%
AAK15581.1	metallothionein [Crassostrea angulata]	41.2	41.2	45%	0.041	56%
CAB96419.1	metallothionein [Venerupis pullastra]	41.2	41.2	45%	0.041	56%
AAQ23914.1	metallothionein IIF [Crassostrea virginica]	39.7	118	45%	0.12	56%
AAQ23913.1	metallothionein IIE [Crassostrea virginica]	39.7	118	45%	0.12	56%
AAQ23909.1	metallothionein IIA [Crassostrea virginica]	39.7	39.7	45%	0.12	56%
AAQ23911.1	metallothionein IIC [Crassostrea virginica]	39.7	79.3	45%	0.12	56%
AAQ23915.1	metallothionein IIG [Crassostrea virginica]	39.7	158	45%	0.12	56%
AAQ23912.1	metallothionein IID [Crassostrea virginica]	39.7	118	45%	0.12	56%
AAQ23916.1	metallothionein IIH [Crassostrea virginica]	39.7	156	45%	0.12	54%
AAM90257.1	metallothionein [Crassostrea virginica]	39.7	39.7	45%	0.12	52%
AAS92877.1	metallothionein [Meretrix lusoria]	39.7	39.7	49%	0.12	53%
P23038.3	RecName: Full=Metallothionein; Short=MT >emb|CAA42522.1| metallothionein [Crassostrea virginica] >gb|AAQ23904.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23905.1|	39.7	39.7	45%	0.12	56%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	metallothionein IA [Crassostrea virginica] >gb|AAQ23906.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23907.1| metallothionein IA [Crassostrea virginica]					
ABN68955.1	metallothionein [Cerastoderma edule]	39.3	39.3	45%	0.16	52%
CAB96402.1	metallothionein [Venerupis (Ruditapes) decussatus]	39.3	39.3	47%	0.16	53%
CAB85588.1	metallothionein [Crassostrea gigas] >emb|CAC48045.1| metallothionein [Crassostrea gigas]	39.3	39.3	45%	0.16	52%
CAB96403.1	metallothionein [Venerupis (Ruditapes) philippinarum]	39.3	39.3	45%	0.16	52%
ABP01350.1	metallothionein [Unio tumidus]	38.9	38.9	49%	0.20	60%
AAQ23908.1	metallothionein IB [Crassostrea virginica]	38.9	38.9	45%	0.20	52%
ABC69708.1	metallothionein [Crassostrea ariakensis]	38.5	38.5	45%	0.26	52%
CAB64869.1	metallothionein [Crassostrea gigas]	37.7	37.7	45%	0.45	47%
ACB05816.1	metallothionein [Cerastoderma glaucum]	35.8	35.8	45%	1.7	54%
ABS20116.1	metallothionein [Venerupis decussatus]	35.8	35.8	45%	1.7	54%
ABH03633.1	metallothionein 10a [Laternula elliptica]	35.8	35.8	45%	1.7	56%
ABH03634.1	metallothionein 10b [Laternula elliptica]	35.8	35.8	45%	1.7	56%
ACT53273.1	metallothionein [Pisidium coreanum]	35.4	35.4	45%	2.2	50%
AAQ23910.1	metallothionein IIB [Crassostrea virginica]	35.4	35.4	45%	2.2	47%
CAC82788.1	metallothionein [Crassostrea gigas]	34.3	34.3	45%	5.0	54%
XP_002592563.1	hypothetical protein BRAFLDRAFT_68885 [Branchiostoma floridae] >gb|EEN48574.1| hypothetical protein BRAFLDRAFT_68885 [Branchiostoma floridae]	33.9	67.8	65%	6.5	40%
AAS75318.1	metallothionein [Tegillarca granosa]	33.5	33.5	43%	8.5	45%
AAX07723.1	unknown [Magnaporthe grisea]	33.5	33.5	39%	8.5	55%

Blast X result for pvMT02 gene

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AAF22487.1	metallothionein 2 [Perna viridis]	76.3	76.3	97%	1e-12	88%
Q9U568.2	RecName: Full=Metallothionein; Short=MT >gb|AAF22486.1|AF092971_1 metallothionein 1 [Perna viridis]	76.3	76.3	97%	1e-12	90%
AAD02054.1	metallothionein [Perna viridis]	71.2	71.2	97%	4e-11	71%
CAE11861.1	metallothionein [Mytilus edulis]	53.1	53.1	93%	1e-05	56%
AAB29061.1	MT-10-I=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	52.8	52.8	93%	1e-05	54%
P80246.2	RecName: Full=Metallothionein 10-Ia; Short=MT-10-Ia >emb|CAA06548.1| metallothionein 10 Ia [Mytilus edulis]	52.8	52.8	93%	1e-05	54%
O62554.3	RecName: Full=Metallothionein 10-Ib; Short=MT-10-Ib >emb|CAA06549.1| metallothionein 10 Ib [Mytilus edulis]	52.8	52.8	93%	1e-05	52%
ABP01350.1	metallothionein [Unio tumidus]	51.6	51.6	97%	3e-05	62%
ABM30214.1	metallothionein 10 [Mytilus sp. KL-2006]	51.6	51.6	93%	3e-05	54%
CAE11860.1	metallothionein [Bathymodiolus thermophilus]	51.6	51.6	93%	3e-05	53%
CAE11859.1	metallothionein [Bathymodiolus thermophilus]	51.6	51.6	93%	3e-05	53%
CAD56896.1	metallothionein 10 [Bathymodiolus sp. FD-2002]	51.6	51.6	93%	3e-05	53%
P80247.3	RecName: Full=Metallothionein 10-II; Short=MT-10-II >emb|CAA06550.1| metallothionein 10 II [Mytilus edulis]	51.6	51.6	93%	3e-05	52%
P80258.1	RecName: Full=Metallothionein 20-III isoform B; Short=MT-20-IIIB	51.2	51.2	97%	4e-05	68%
P80252.2	RecName: Full=Metallothionein 20-II; Short=MT-20-II >emb|CAA06553.1| metallothionein 20 II [Mytilus edulis] >gb|ABM30215.1| metallothionein 20-II [Mytilus sp. KL-2006]	51.2	51.2	97%	4e-05	72%
CAE11856.1	metallothionein [Mytilus edulis]	50.4	50.4	93%	7e-05	51%
CAE11855.1	metallothionein [Mytilus edulis]	50.4	50.4	93%	7e-05	51%
AAK39563.1	metallothionein-like protein [Tegillarca granosa]	49.7	49.7	93%	1e-04	64%
CAF34424.1	metallothionein [Bathymodiolus azoricus] >emb|CAF34425.1| metallothionein [Bathymodiolus thermophilus]	49.3	49.3	97%	1e-04	70%
P80251.1	RecName: Full=Metallothionein 20-I isoforms A and B; AltName: Full=MT-20-IA and MT-20-IB >gb|AAB29062.1| MT-20-I=20 kda class I	49.3	49.3	97%	1e-04	70%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 71 aa]					
CAI94401.1	metallothionein [Bathymodiolus azoricus]	48.9	48.9	93%	2e-04	52%
ABH03633.1	metallothionein 10a [Laternula elliptica]	48.9	48.9	97%	2e-04	54%
ABH03634.1	metallothionein 10b [Laternula elliptica]	48.9	48.9	97%	2e-04	52%
CAF34421.1	metallothionein, isoform MT-10a [Bathymodiolus azoricus]	48.9	48.9	93%	2e-04	52%
CAF34422.1	metallothionein, isoform MT-10b [Bathymodiolus azoricus] >emb|CAF34423.1| metallothionein, isoform MT-10c [Bathymodiolus azoricus]	48.9	48.9	93%	2e-04	50%
AAB29060.1	MT-10-IV=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	48.9	48.9	93%	2e-04	52%
P80249.2	RecName: Full=Metallothionein 10-IV; Short=MT-10-IV >emb|CAA07546.1| metallothionein 10IV [Mytilus edulis]	48.9	48.9	93%	2e-04	52%
P69153.2	RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA >sp|P69154.2|MT23A_MYTGA RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA; Short=MT-I >gb|AAG28538.1|AF199020_1 metallothionein isoform [Mytilus galloprovincialis]	48.9	48.9	97%	2e-04	68%
P80248.2	RecName: Full=Metallothionein 10-III; Short=MT-10-III >emb|CAA06551.1| metallothionein 10 III [Mytilus edulis] >gb|AAT72936.1| metallothionein 10-III [Mytilus galloprovincialis]	48.1	48.1	93%	3e-04	52%
AAK56498.1	putative metallothionein [Littorina littorea]	47.8	47.8	95%	4e-04	46%
AAS92877.1	metallothionein [Meretrix lusoria]	46.6	46.6	91%	0.001	46%
ACS44750.1	metallothionein [Hyriopsis cumingii]	46.2	46.2	93%	0.001	43%
AAT72935.1	metallothionein 20-IV [Mytilus galloprovincialis]	45.8	45.8	97%	0.002	66%
ADB29127.1	metallothionein [Physa acuta]	45.4	45.4	97%	0.002	38%
CAE11862.1	metallothionein [Mytilus edulis]	45.1	45.1	97%	0.003	66%
ABM66449.1	metallothionein [Helix aspersa]	44.7	44.7	97%	0.004	34%
AAK84863.1	Cd-metallothionein isoform [Helix pomatia] >gb|ACN66299.1| Cd-specific metallothionein [Helix pomatia] >gb|ACS91928.1| cadmium-metallothionein [Biomphalaria glabrata]	44.3	44.3	97%	0.005	34%
P33187.1	RecName: Full=Cadmium-metallothionein; Short=CD-MT	44.3	44.3	97%	0.005	34%
ACC17831.1	metallothionein [Nesiohelix samarangae]	42.7	42.7	97%	0.014	46%
ABP57063.1	metallothionein [Venerupis philippinarum]	42.7	42.7	89%	0.014	41%
ABM55725.1	metallothionein [Corbicula fluminea]	42.7	42.7	97%	0.014	54%
AAQ23908.1	metallothionein IB [Crassostrea virginica]	42.7	42.7	97%	0.014	50%
ACB05816.1	metallothionein [Cerastoderma glaucum]	42.0	42.0	89%	0.024	41%
CAB85588.1	metallothionein [Crassostrea gigas] >emb|CAC48045.1| metallothionein [Crassostrea gigas]	41.6	78.9	93%	0.031	58%
CAA06552.1	metallothionein 10 IV [Mytilus edulis]	41.6	41.6	87%	0.031	48%
ABC69708.1	metallothionein [Crassostrea ariakensis]	41.2	41.2	97%	0.041	54%
CAB64869.1	metallothionein [Crassostrea gigas]	41.2	41.2	93%	0.041	56%
P23038.3	RecName: Full=Metallothionein; Short=MT >emb|CAA42522.1| metallothionein [Crassostrea virginica] >gb|AAQ23904.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23905.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23906.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23907.1| metallothionein IA [Crassostrea virginica]	41.2	41.2	97%	0.041	49%
ABL73910.1	cadmium-metallothionein [Helix aspersa]	40.8	40.8	97%	0.053	34%
AAK15581.1	metallothionein [Crassostrea angulata]	40.4	40.4	93%	0.069	56%
ABS20116.1	metallothionein [Venerupis decussatus]	40.0	40.0	93%	0.091	37%
CAC82788.1	metallothionein [Crassostrea gigas]	40.0	40.0	93%	0.091	37%
Q94550.1	RecName: Full=Metallothionein; Short=MT >gb|AAB07548.1| metallothionein [Dreissena polymorpha]	40.0	40.0	89%	0.091	44%
ACU46012.1	metallothionein [Mactra veneriformis]	39.3	39.3	33%	0.15	64%
AAK84864.1	Cu-metallothionein isoform [Helix pomatia] >gb|ACS91927.1| copper-metallothionein [Biomphalaria glabrata]	39.3	39.3	97%	0.15	37%
P55947.1	RecName: Full=Copper-metallothionein; AltName: Full=Cu-MT	39.3	39.3	97%	0.15	37%
P55946.1	RecName: Full=Metallothionein; Short=MT >gb|AAB47141.1| metallothionein [Arianta arbustorum, Peptide Partial, 66 aa]	39.3	39.3	97%	0.15	30%
ACH99846.1	metallothionein [Scapharca broughtonii]	38.9	38.9	93%	0.20	31%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AAZ94899.1	metallothionein IVC [Crassostrea virginica]	38.5	38.5	40%	0.26	47%
AAS75318.1	metallothionein [Tegillarca granosa]	38.5	38.5	93%	0.26	34%
AAB47142.1	metallothionein [Arianta arbustorum, Peptide Partial, 66 aa] >prf||2123233A metallothionein	38.5	38.5	97%	0.26	30%
CAC83770.1	metallothionein [Ostrea edulis]	38.5	38.5	93%	0.26	54%
AAZ94897.1	metallothionein IVA [Crassostrea virginica]	38.1	38.1	40%	0.34	47%
AAZ94898.1	metallothionein IVB [Crassostrea virginica]	38.1	38.1	40%	0.34	47%
CAK22381.1	metallothionein IV [Crassostrea gigas]	37.7	37.7	93%	0.45	36%
ABO16370.1	metallothionein [Argopecten irradians]	37.7	37.7	93%	0.45	33%
AAM90257.1	metallothionein [Crassostrea virginica]	37.7	37.7	97%	0.45	48%
ABI30643.1	metallothionein-10B [Mytilus galloprovincialis]	36.2	36.2	93%	1.3	35%
CAE11857.1	metallothionein [Mytilus edulis]	36.2	36.2	93%	1.3	35%
CAE11858.1	metallothionein [Mytilus edulis]	35.0	35.0	93%	2.9	35%

Blast X result for pvMT07 gene

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
ABM30214.1	metallothionein 10 [Mytilus sp. KL-2006]	45.4	45.4	42%	0.002	76%
CAE11861.1	metallothionein [Mytilus edulis]	44.7	44.7	42%	0.004	76%
ACS44750.1	metallothionein [Hyriopsis cumingii]	43.5	43.5	42%	0.008	71%
ABP01350.1	metallothionein [Unio tumidus]	43.5	43.5	42%	0.008	71%
P80247.3	RecName: Full=Metallothionein 10-II; Short=MT-10-II >emb|CAA06550.1| metallothionein 10 II [Mytilus edulis]	43.5	43.5	42%	0.008	71%
AAB29061.1	MT-10-I=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	42.7	42.7	42%	0.014	71%
P80246.2	RecName: Full=Metallothionein 10-Ia; Short=MT-10-Ia >emb|CAA06548.1| metallothionein 10 Ia [Mytilus edulis]	42.7	42.7	42%	0.014	71%
O62554.3	RecName: Full=Metallothionein 10-Ib; Short=MT-10-Ib >emb|CAA06549.1| metallothionein 10 Ib [Mytilus edulis]	42.7	42.7	42%	0.014	71%
ABH03633.1	metallothionein 10a [Laternula elliptica]	42.4	42.4	42%	0.018	71%
ABH03634.1	metallothionein 10b [Laternula elliptica]	42.4	42.4	42%	0.018	71%
ABP57063.1	metallothionein [Venerupis philippinarum]	42.0	42.0	42%	0.024	61%
ACU46012.1	metallothionein [Mactra veneriformis]	41.6	41.6	38%	0.031	73%
ACB05816.1	metallothionein [Cerastoderma glaucum]	41.6	41.6	42%	0.031	61%
ABM55725.1	metallothionein [Corbicula fluminea]	41.6	41.6	42%	0.031	66%
CAC83770.1	metallothionein [Ostrea edulis]	41.6	41.6	42%	0.031	71%
AAD02054.1	metallothionein [Perna viridis]	41.2	41.2	42%	0.041	72%
AAF22487.1	metallothionein 2 [Perna viridis]	41.2	41.2	42%	0.041	72%
Q9U568.2	RecName: Full=Metallothionein; Short=MT >gb|AAF22486.1|AF092971_1 metallothionein 1 [Perna viridis]	41.2	41.2	42%	0.041	72%
P80252.2	RecName: Full=Metallothionein 20-II; Short=MT-20-II >emb|CAA06553.1| metallothionein 20 II [Mytilus edulis] >gb|ABM30215.1| metallothionein 20-II [Mytilus sp. KL-2006]	40.4	40.4	42%	0.070	66%
P80248.2	RecName: Full=Metallothionein 10-III; Short=MT-10-III >emb|CAA06551.1| metallothionein 10 III [Mytilus edulis] >gb|AAT72936.1| metallothionein 10-III [Mytilus galloprovincialis]	40.0	40.0	42%	0.091	66%
CAI94401.1	metallothionein [Bathymodiolus azoricus]	39.7	39.7	42%	0.12	66%
CAF34421.1	metallothionein, isoform MT-10a [Bathymodiolus azoricus]	39.7	39.7	42%	0.12	66%
AAB29060.1	MT-10-IV=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	39.7	39.7	42%	0.12	66%
CAE11856.1	metallothionein [Mytilus edulis]	39.7	39.7	42%	0.12	66%
CAE11855.1	metallothionein [Mytilus edulis]	39.7	39.7	42%	0.12	66%
CAA06552.1	metallothionein 10 IV [Mytilus edulis]	39.7	39.7	42%	0.12	66%
P80249.2	RecName: Full=Metallothionein 10-IV; Short=MT-10-IV >emb|CAA07546.1| metallothionein 10IV [Mytilus edulis]	39.7	39.7	42%	0.12	66%
P80258.1	RecName: Full=Metallothionein 20-III isoform B; Short=MT-20-IIIB	39.3	39.3	42%	0.16	61%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
CAE11860.1	metallothionein [Bathymodiolus thermophilus]	38.9	38.9	42%	0.20	68%
CAE11859.1	metallothionein [Bathymodiolus thermophilus]	38.9	38.9	42%	0.20	68%
CAD56896.1	metallothionein 10 [Bathymodiolus sp. FD-2002]	38.9	38.9	42%	0.20	68%
ABS20116.1	metallothionein [Venerupis decussatus]	38.5	38.5	42%	0.27	57%
CAF34424.1	metallothionein [Bathymodiolus azoricus] >emb|CAF34425.1| metallothionein [Bathymodiolus thermophilus]	38.5	38.5	42%	0.27	61%
CAF34422.1	metallothionein, isoform MT-10b [Bathymodiolus azoricus] >emb|CAF34423.1| metallothionein, isoform MT-10c [Bathymodiolus azoricus]	38.5	38.5	40%	0.27	70%
AAK39563.1	metallothionein-like protein [Tegillarca granosa]	38.5	38.5	42%	0.27	61%
CAC82788.1	metallothionein [Crassostrea gigas]	38.5	38.5	42%	0.27	57%
AAT72935.1	metallothionein 20-IV [Mytilus galloprovincialis]	38.5	38.5	42%	0.27	61%
P69153.2	RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA >sp|P69154.2|MT23A_MYTGA RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA; Short=MT-I >gb|AAG28538.1|AF199020_1 metallothionein isoform [Mytilus galloprovincialis]	38.5	38.5	42%	0.27	61%
P80251.1	RecName: Full=Metallothionein 20-I isoforms A and B; AltName: Full=MT-20-IA and MT-20-IB >gb|AAB29062.1| MT-20-I=20 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 71 aa]	38.5	38.5	42%	0.27	61%
ABC69708.1	metallothionein [Crassostrea ariakensis]	38.1	38.1	42%	0.35	57%
AAS75318.1	metallothionein [Tegillarca granosa]	38.1	38.1	42%	0.35	65%
CAB85588.1	metallothionein [Crassostrea gigas] >emb|CAC48045.1| metallothionein [Crassostrea gigas]	38.1	76.2	42%	0.35	57%
AAK15581.1	metallothionein [Crassostrea angulata]	38.1	38.1	42%	0.35	57%
CAB64869.1	metallothionein [Crassostrea gigas]	37.7	37.7	42%	0.45	57%
ABP57066.2	metallothionein [Cerastoderma edule] >gb|ACT66292.1| metallothionein 1 [Cerastoderma edule] >gb|ACT66293.1| metallothionein 1 [Cerastoderma edule]	37.4	37.4	42%	0.59	57%
ADB29127.1	metallothionein [Physa acuta]	37.0	37.0	38%	0.77	70%
ACH99846.1	metallothionein [Scapharca broughtonii]	37.0	37.0	42%	0.77	57%
XP_002345698.1	PREDICTED: hypothetical protein, partial [Homo sapiens]	36.6	36.6	87%	1.0	39%
ABM66449.1	metallothionein [Helix aspersa]	36.2	36.2	38%	1.3	65%
AAK84863.1	Cd-metallothionein isoform [Helix pomatia] >gb|ACN66299.1| Cd-specific metallothionein [Helix pomatia] >gb|ACS91928.1| cadmium-metallothionein [Biomphalaria glabrata]	36.2	36.2	38%	1.3	65%
P33187.1	RecName: Full=Cadmium-metallothionein; Short=CD-MT	36.2	36.2	38%	1.3	65%
CAE11862.1	metallothionein [Mytilus edulis]	35.8	35.8	42%	1.7	61%
Q94550.1	RecName: Full=Metallothionein; Short=MT >gb|AAB07548.1| metallothionein [Dreissena polymorpha]	35.8	35.8	42%	1.7	52%
AAS92877.1	metallothionein [Meretrix lusoria]	35.4	35.4	42%	2.2	54%
ABL73910.1	cadmium-metallothionein [Helix aspersa]	35.0	35.0	38%	2.9	65%
AAZ76545.1	metallothionein [Scapharca inaequivalvis]	35.0	35.0	36%	2.9	66%
XP_001135604.1	PREDICTED: hypothetical protein [Pan troglodytes]	34.7	34.7	71%	3.8	37%
ACC17831.1	metallothionein [Nesiohelix samarangae]	34.3	34.3	38%	5.0	60%
CAB96402.1	metallothionein [Venerupis (Ruditapes) decussatus]	34.3	34.3	36%	5.0	61%
CAB96403.1	metallothionein [Venerupis (Ruditapes) philippinarum]	34.3	34.3	36%	5.0	61%
AAQ23908.1	metallothionein IB [Crassostrea virginica]	33.9	33.9	42%	6.5	52%

Blast X result for pvMT08 gene

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
ABM30214.1	metallothionein 10 [Mytilus sp. KL-2006]	45.1	45.1	45%	0.003	72%
CAE11861.1	metallothionein [Mytilus edulis]	44.3	44.3	45%	0.005	72%
ACS44750.1	metallothionein [Hyriopsis cumingii]	43.1	43.1	45%	0.011	68%
ABP01350.1	metallothionein [Unio tumidus]	43.1	43.1	45%	0.011	68%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
P80247.3	RecName: Full=Metallothionein 10-II; Short=MT-10-II >emb|CAA06550.1| metallothionein 10 II [Mytilus edulis]	43.1	43.1	45%	0.011	68%
AAB29061.1	MT-10-I=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	42.4	42.4	45%	0.018	68%
P80246.2	RecName: Full=Metallothionein 10-Ia; Short=MT-10-Ia >emb|CAA06548.1| metallothionein 10 Ia [Mytilus edulis]	42.4	42.4	45%	0.018	68%
O62554.3	RecName: Full=Metallothionein 10-Ib; Short=MT-10-Ib >emb|CAA06549.1| metallothionein 10 Ib [Mytilus edulis]	42.4	42.4	45%	0.018	68%
ABP57063.1	metallothionein [Venerupis philippinarum]	41.6	41.6	45%	0.031	59%
ACB05816.1	metallothionein [Cerastoderma glaucum]	41.2	41.2	45%	0.041	59%
ABM55725.1	metallothionein [Corbicula fluminea]	41.2	41.2	45%	0.041	63%
ACU46012.1	metallothionein [Mactra veneriformis]	40.8	40.8	45%	0.054	63%
AAD02054.1	metallothionein [Perna viridis]	40.8	40.8	45%	0.054	69%
AAF22487.1	metallothionein 2 [Perna viridis]	40.8	40.8	45%	0.054	69%
Q9U568.2	RecName: Full=Metallothionein; Short=MT >gb|AAF22486.1|AF092971_1 metallothionein 1 [Perna viridis]	40.8	40.8	45%	0.054	69%
ABH03633.1	metallothionein 10a [Laternula elliptica]	40.0	40.0	43%	0.091	66%
ABH03634.1	metallothionein 10b [Laternula elliptica]	40.0	40.0	43%	0.091	66%
P80252.2	RecName: Full=Metallothionein 20-II; Short=MT-20-II >emb|CAA06553.1| metallothionein 20 II [Mytilus edulis] >gb|ABM30215.1| metallothionein 20-II [Mytilus sp. KL-2006]	40.0	40.0	45%	0.091	63%
CAC83770.1	metallothionein [Ostrea edulis]	39.7	39.7	45%	0.12	63%
P80248.2	RecName: Full=Metallothionein 10-III; Short=MT-10-III >emb|CAA06551.1| metallothionein 10 III [Mytilus edulis] >gb|AAT72936.1| metallothionein 10-III [Mytilus galloprovincialis]	39.7	39.7	45%	0.12	63%
CAI94401.1	metallothionein [Bathymodiolus azoricus]	39.3	39.3	45%	0.16	63%
CAF34421.1	metallothionein, isoform MT-10a [Bathymodiolus azoricus]	39.3	39.3	45%	0.16	63%
AAK39563.1	metallothionein-like protein [Tegillarca granosa]	39.3	39.3	45%	0.16	59%
AAB29060.1	MT-10-IV=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	39.3	39.3	45%	0.16	63%
CAE11856.1	metallothionein [Mytilus edulis]	39.3	39.3	45%	0.16	63%
CAE11855.1	metallothionein [Mytilus edulis]	39.3	39.3	45%	0.16	63%
CAA06552.1	metallothionein 10 IV [Mytilus edulis]	39.3	39.3	45%	0.16	63%
P80249.2	RecName: Full=Metallothionein 10-IV; Short=MT-10-IV >emb|CAA07546.1| metallothionein 10IV [Mytilus edulis]	39.3	39.3	45%	0.16	63%
P80258.1	RecName: Full=Metallothionein 20-III isoform B; Short=MT-20-IIIB	38.9	38.9	45%	0.20	59%
CAE11860.1	metallothionein [Bathymodiolus thermophilus]	38.5	38.5	45%	0.27	65%
CAE11859.1	metallothionein [Bathymodiolus thermophilus]	38.5	38.5	45%	0.27	65%
CAD56896.1	metallothionein 10 [Bathymodiolus sp. FD-2002]	38.5	38.5	45%	0.27	65%
ABS20116.1	metallothionein [Venerupis decussatus]	38.1	38.1	45%	0.35	54%
CAF34424.1	metallothionein [Bathymodiolus azoricus] >emb|CAF34425.1| metallothionein [Bathymodiolus thermophilus]	38.1	38.1	45%	0.35	59%
CAC82788.1	metallothionein [Crassostrea gigas]	38.1	38.1	45%	0.35	54%
AAT72935.1	metallothionein 20-IV [Mytilus galloprovincialis]	38.1	38.1	45%	0.35	59%
P69153.2	RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA >sp|P69154.2|MT23A_MYTGA RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA; Short=MT-I >gb|AAG28538.1|AF199020_1 metallothionein isoform [Mytilus galloprovincialis]	38.1	38.1	45%	0.35	59%
P80251.1	RecName: Full=Metallothionein 20-I isoforms A and B; AltName: Full=MT-20-IA and MT-20-IB >gb|AAB29062.1| MT-20-I=20 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 71 aa]	38.1	38.1	45%	0.35	59%
ABC69708.1	metallothionein [Crassostrea ariakensis]	37.7	37.7	45%	0.45	54%
CAF34422.1	metallothionein, isoform MT-10b [Bathymodiolus azoricus] >emb|CAF34423.1| metallothionein, isoform MT-10c [Bathymodiolus azoricus]	37.7	37.7	45%	0.45	63%
AAS75318.1	metallothionein [Tegillarca granosa]	37.7	37.7	45%	0.45	62%
CAB85588.1	metallothionein [Crassostrea gigas] >emb|CAC48045.1| metallothionein [Crassostrea gigas]	37.7	75.5	45%	0.45	54%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AAK15581.1	metallothionein [Crassostrea angulata]	37.7	37.7	45%	0.45	54%
CAB64869.1	metallothionein [Crassostrea gigas]	37.4	37.4	45%	0.59	54%
ABP57066.2	metallothionein [Cerastoderma edule] >gb|ACT66292.1| metallothionein 1 [Cerastoderma edule] >gb|ACT66293.1| metallothionein 1 [Cerastoderma edule]	35.0	35.0	43%	2.9	52%
ADB29127.1	metallothionein [Physa acuta]	34.7	34.7	39%	3.8	65%
ACH99846.1	metallothionein [Scapharca broughtonii]	34.7	34.7	43%	3.8	52%
XP_001135604.1	PREDICTED: hypothetical protein [Pan troglodytes]	34.7	34.7	71%	3.8	37%
XP_002345698.1	PREDICTED: hypothetical protein, partial [Homo sapiens]	33.9	33.9	59%	6.6	44%
ABM66449.1	metallothionein [Helix aspersa]	33.9	33.9	39%	6.6	60%
AAK84863.1	Cd-metallothionein isoform [Helix pomatia] >gb|ACN66299.1| Cd-specific metallothionein [Helix pomatia] >gb|ACS91928.1| cadmium-metallothionein [Biomphalaria glabrata]	33.9	33.9	39%	6.6	60%
CAE11862.1	metallothionein [Mytilus edulis]	33.9	33.9	45%	6.6	54%
P33187.1	RecName: Full=Cadmium-metallothionein; Short=CD-MT	33.9	33.9	39%	6.6	60%
Q94550.1	RecName: Full=Metallothionein; Short=MT >gb|AAB07548.1| metallothionein [Dreissena polymorpha]	33.5	33.5	43%	8.6	47%

Blast X result for pvMT11 gene

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AAF22487.1	metallothionein 2 [Perna viridis]	82.4	82.4	100%	2e-14	96%
Q9U568.2	RecName: Full=Metallothionein; Short=MT >gb|AAF22486.1|AF092971_1 metallothionein 1 [Perna viridis]	82.4	82.4	100%	2e-14	98%
AAD02054.1	metallothionein [Perna viridis]	77.4	77.4	100%	5e-13	87%
P80248.2	RecName: Full=Metallothionein 10-III; Short=MT-10-III >emb|CAA06551.1| metallothionein 10 III [Mytilus edulis] >gb|AAT72936.1| metallothionein 10-III [Mytilus galloprovincialis]	68.6	68.6	54%	2e-10	89%
CAE11861.1	metallothionein [Mytilus edulis]	67.4	67.4	54%	5e-10	89%
CAE11855.1	metallothionein [Mytilus edulis]	67.4	67.4	54%	5e-10	89%
P80246.2	RecName: Full=Metallothionein 10-Ia; Short=MT-10-Ia >emb|CAA06548.1| metallothionein 10 Ia [Mytilus edulis]	67.4	67.4	54%	5e-10	89%
O62554.3	RecName: Full=Metallothionein 10-Ib; Short=MT-10-Ib >emb|CAA06549.1| metallothionein 10 Ib [Mytilus edulis]	67.4	67.4	54%	5e-10	89%
P80249.2	RecName: Full=Metallothionein 10-IV; Short=MT-10-IV >emb|CAA07546.1| metallothionein 10IV [Mytilus edulis]	67.4	67.4	54%	5e-10	89%
CAF34421.1	metallothionein, isoform MT-10a [Bathymodiolus azoricus]	67.0	67.0	54%	7e-10	86%
CAF34422.1	metallothionein, isoform MT-10b [Bathymodiolus azoricus] >emb|CAF34423.1| metallothionein, isoform MT-10c [Bathymodiolus azoricus]	67.0	67.0	54%	7e-10	86%
P80247.3	RecName: Full=Metallothionein 10-II; Short=MT-10-II >emb|CAA06550.1| metallothionein 10 II [Mytilus edulis]	66.2	66.2	54%	1e-09	86%
AAB29061.1	MT-10-I=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	65.5	65.5	52%	2e-09	89%
AAB29060.1	MT-10-IV=10 kda class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 72 aa]	65.5	65.5	52%	2e-09	89%
P80252.2	RecName: Full=Metallothionein 20-II; Short=MT-20-II >emb|CAA06553.1| metallothionein 20 II [Mytilus edulis] >gb|ABM30215.1| metallothionein 20-II [Mytilus sp. KL-2006]	64.7	64.7	54%	3e-09	86%
ABM30214.1	metallothionein 10 [Mytilus sp. KL-2006]	64.3	64.3	54%	4e-09	86%
CAE11856.1	metallothionein [Mytilus edulis]	64.3	64.3	54%	4e-09	86%
CAE11862.1	metallothionein [Mytilus edulis]	63.2	63.2	54%	1e-08	82%
P69153.2	RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA >sp|P69154.2|MT23A_MYTGA RecName: Full=Metallothionein 20-III isoform A; Short=MT-20-IIIA; Short=MT-I >gb|AAG28538.1|AF199020_1 metallothionein isoform [Mytilus galloprovincialis]	63.2	63.2	54%	1e-08	82%
P80251.1	RecName: Full=Metallothionein 20-I isoforms A and B; AltName: Full=MT-20-IA and MT-20-IB >gb|AAB29062.1| MT-20-I=20 kda	62.8	62.8	52%	1e-08	85%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
	class I metallothionein [Mytilus edulis=common sea mussels, cytosol, Peptide, 71 aa]					
ABI30643.1	metallothionein-10B [Mytilus galloprovincialis]	62.4	62.4	52%	2e-08	82%
CAI94401.1	metallothionein [Bathymodiolus azoricus]	62.0	62.0	49%	2e-08	88%
P80258.1	RecName: Full=Metallothionein 20-III isoform B; Short=MT-20-IIIB	61.2	61.2	52%	4e-08	82%
CAE11860.1	metallothionein [Bathymodiolus thermophilus]	60.8	60.8	49%	5e-08	84%
CAE11859.1	metallothionein [Bathymodiolus thermophilus]	60.8	60.8	49%	5e-08	84%
CAD56896.1	metallothionein 10 [Bathymodiolus sp. FD-2002]	60.8	60.8	49%	5e-08	84%
CAE11857.1	metallothionein [Mytilus edulis]	59.3	59.3	52%	1e-07	78%
CAE11858.1	metallothionein [Mytilus edulis]	58.5	58.5	50%	2e-07	81%
CAF34424.1	metallothionein [Bathymodiolus azoricus] >emb|CAF34425.1| metallothionein [Bathymodiolus thermophilus]	57.4	57.4	47%	5e-07	88%
AAT72935.1	metallothionein 20-IV [Mytilus galloprovincialis]	54.7	54.7	54%	4e-06	72%
AAK15581.1	metallothionein [Crassostrea angulata]	45.4	45.4	50%	0.002	55%
ABH03633.1	metallothionein 10a [Laternula elliptica]	44.7	44.7	50%	0.004	62%
ABH03634.1	metallothionein 10b [Laternula elliptica]	44.7	44.7	50%	0.004	62%
AAK39563.1	metallothionein-like protein [Tegillarca granosa]	44.3	44.3	49%	0.005	53%
AAQ23914.1	metallothionein IIF [Crassostrea virginica]	43.9	131	50%	0.006	55%
AAQ23913.1	metallothionein IIE [Crassostrea virginica]	43.9	131	50%	0.006	55%
AAQ23909.1	metallothionein IIA [Crassostrea virginica]	43.9	43.9	50%	0.006	55%
AAQ23911.1	metallothionein IIC [Crassostrea virginica]	43.9	87.8	50%	0.006	55%
AAQ23915.1	metallothionein IIG [Crassostrea virginica]	43.9	175	50%	0.006	55%
AAQ23912.1	metallothionein IID [Crassostrea virginica]	43.9	131	50%	0.006	55%
AAQ23916.1	metallothionein IIH [Crassostrea virginica]	43.9	173	50%	0.006	53%
AAK50565.1	metallothionein [Crassostrea rhizophorae]	43.9	43.9	45%	0.006	58%
AAM90257.1	metallothionein [Crassostrea virginica]	43.9	43.9	50%	0.006	51%
AAS92877.1	metallothionein [Meretrix lusoria]	43.9	43.9	54%	0.006	52%
P23038.3	RecName: Full=Metallothionein; Short=MT >emb|CAA42522.1| metallothionein [Crassostrea virginica] >gb|AAQ23904.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23905.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23906.1| metallothionein IA [Crassostrea virginica] >gb|AAQ23907.1| metallothionein IA [Crassostrea virginica]	43.9	43.9	50%	0.006	55%
ABN68955.1	metallothionein [Cerastoderma edule]	43.5	43.5	50%	0.008	51%
AAQ23908.1	metallothionein IB [Crassostrea virginica]	43.5	43.5	50%	0.008	51%
CAB85588.1	metallothionein [Crassostrea gigas] >emb|CAC48045.1| metallothionein [Crassostrea gigas]	43.5	43.5	50%	0.008	51%
ABP01350.1	metallothionein [Unio tumidus]	43.1	43.1	54%	0.011	58%
ABC69708.1	metallothionein [Crassostrea ariakensis]	42.7	42.7	50%	0.014	51%
CAB64869.1	metallothionein [Crassostrea gigas]	42.0	42.0	50%	0.024	48%
CAB96402.1	metallothionein [Venerupis (Ruditapes) decussatus]	42.0	42.0	47%	0.024	54%
CAB96403.1	metallothionein [Venerupis (Ruditapes) philippinarum]	42.0	42.0	45%	0.024	54%
CAB96419.1	metallothionein [Venerupis pullastra]	41.2	41.2	43%	0.040	56%
AAQ23910.1	metallothionein IIB [Crassostrea virginica]	39.7	39.7	50%	0.12	48%
ACB05816.1	metallothionein [Cerastoderma glaucum]	38.5	38.5	45%	0.26	56%
ABS20116.1	metallothionein [Venerupis decussatus]	38.5	38.5	45%	0.26	56%
CAC82788.1	metallothionein [Crassostrea gigas]	38.5	38.5	50%	0.26	53%
ACT53273.1	metallothionein [Pisidium coreanum]	38.1	38.1	98%	0.34	37%
AAS75318.1	metallothionein [Tegillarca granosa]	37.7	37.7	49%	0.45	46%
Q94550.1	RecName: Full=Metallothionein; Short=MT >gb|AAB07548.1| metallothionein [Dreissena polymorpha]	37.0	37.0	54%	0.76	51%
ABP57063.1	metallothionein [Venerupis philippinarum]	36.6	36.6	50%	1.00	50%
ACH99846.1	metallothionein [Scapharca broughtonii]	36.2	36.2	54%	1.3	44%
ABM55725.1	metallothionein [Corbicula fluminea]	36.2	36.2	50%	1.3	50%
AAZ76545.1	metallothionein [Scapharca inaequivalvis]	35.8	35.8	54%	1.7	41%
CAC83770.1	metallothionein [Ostrea edulis]	35.0	35.0	49%	2.9	46%
ACH73074.1	metallothionein 1 [Epinephelus coioides]	34.7	34.7	43%	3.8	47%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
XP_002107650.1	expressed hypothetical protein [Trichoplax adhaerens] >gb|EDV28448.1| expressed hypothetical protein [Trichoplax adhaerens]	34.7	34.7	49%	3.8	46%
ABF50549.1	metallothionein 1 [Anguilla anguilla]	34.7	34.7	43%	3.8	47%
CAE45770.1	metallothionein [Trichoplax adhaerens]	34.7	34.7	49%	3.8	46%
O93571.1	RecName: Full=Metallothionein; Short=MT >gb|AAC36348.1| metallothionein [Ictalurus punctatus]	34.7	34.7	43%	3.8	47%
P51902.1	RecName: Full=Metallothionein; Short=MT >gb|AAA74418.1| metallothionein [Gadus morhua]	34.7	34.7	43%	3.8	47%
XP_002592563.1	hypothetical protein BRAFLDRAFT_68885 [Branchiostoma floridae] >gb|EEN48574.1| hypothetical protein BRAFLDRAFT_68885 [Branchiostoma floridae]	33.9	67.8	62%	6.5	40%
AAX07723.1	unknown [Magnaporthe grisea]	33.5	33.5	37%	8.4	55%

Blast X result for heat shock protein HSP71 gene

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
ABJ98722.1	heat shock protein 71 [Perna viridis] >gb|ABQ11278.1| heat shock protein 71 [Perna viridis]	221	221	98%	2e-56	100%
CAH04109.1	heat shock cognate 71 [Mytilus galloprovincialis]	217	217	98%	4e-55	98%
ABM92345.1	heat shock protein 70 [Laternula elliptica]	215	215	98%	2e-54	95%
ABJ97378.1	heat shock protein 70 [Pinctada fucata]	214	214	98%	2e-54	96%
ABE77386.1	HSP70 [Chlamys farreri]	214	214	98%	3e-54	95%
AAS17724.1	heat shock protein 70 [Mizuhopecten yessoensis]	214	214	98%	3e-54	95%
AAO38780.1	heat shock protein 70 [Chlamys farreri]	214	214	98%	3e-54	95%
AAR11487.1	heat shock protein 70 [Mizuhopecten yessoensis]	214	214	98%	3e-54	95%
ACO36047.1	heat shock cognate protein 70 [Haliotis diversicolor]	213	213	98%	4e-54	95%
ABJ97377.1	heat shock protein 70 [Pteria penguin]	213	213	98%	4e-54	95%
CAK95236.1	71kDa heat shock protein [Haliotis tuberculata]	213	213	98%	4e-54	95%
ABC54952.1	heat shock protein 70 [Haliotis discus hannai]	213	213	98%	4e-54	95%
ACF31553.1	heat shock protein 70 [Pinctada fucata]	213	213	98%	8e-54	95%
AAW52766.1	HSP70 [Mytilus galloprovincialis]	213	213	98%	8e-54	96%
AAO41703.1	heat shock protein 70 [Crassostrea ariakensis]	211	211	98%	3e-53	93%
CAC83009.1	heat shock protein 70 [Crassostrea gigas]	211	211	98%	3e-53	93%
AAD31042.1	heat shock protein 70 [Crassostrea gigas] >dbj|BAD15287.1| 71kDa heat shock connate protein [Crassostrea gigas]	211	211	98%	3e-53	93%
ABU63809.1	heat shock protein 70 form 2 [Paralvinella grasslei]	210	210	98%	5e-53	94%
CAC83683.1	HSC70 protein [Crassostrea gigas]	209	209	98%	8e-53	93%
AAY40792.1	heat shock cognate protein 70 [Oligocottus maculosus]	209	209	98%	8e-53	93%
NP_001036892.1	heat shock cognate protein [Bombyx mori] >dbj|BAB92074.1| heat shock cognate protein [Bombyx mori]	209	209	98%	8e-53	91%
XP_002724720.1	PREDICTED: heat shock cognate 71 kDa protein-like [Rattus norvegicus]	209	209	98%	1e-52	93%
XP_002716475.1	PREDICTED: heat shock 70kDa protein 8 [Oryctolagus cuniculus]	209	209	98%	1e-52	93%
ACJ03596.1	heat shock protein 70 [Ctenopharyngodon idella]	209	209	98%	1e-52	93%
ACJ03595.1	heat shock protein 70 [Hypophthalmichthys molitrix]	209	209	98%	1e-52	93%
ACC93993.2	heat shock cognate 70 [Megalobrama amblycephala] >gb|ACS74754.1| heat shock cognate 70 [Megalobrama amblycephala]	209	209	98%	1e-52	93%
3FZF_A	Chain A, Crystal Structure Of Hsc70BAG1 IN COMPLEX WITH ATP >pdb|3FZH|A Chain A, Crystal Structures Of Hsc70BAG1 IN COMPLEX WITH SMALL Molecule Inhibitors >pdb|3FZK|A Chain A, Crystal Structures Of Hsc70BAG1 IN COMPLEX WITH SMALL Molecule Inhibitors >pdb|3FZL|A Chain A, Crystal Structures Of Hsc70BAG1 IN COMPLEX WITH SMALL Molecule Inhibitors >pdb|3FZM|A Chain A, Crystal Structures Of Hsc70BAG1 IN COMPLEX WITH SMALL Molecule Inhibitors	209	209	98%	1e-52	93%
XP_002195736.1	PREDICTED: similar to heat shock protein 70B [Taeniopygia guttata]	209	209	98%	1e-52	93%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
3CQX_A	Chain A, Chaperone Complex >pdb|3CQX|B Chain B, Chaperone Complex	209	209	98%	1e-52	93%
XP_002407132.1	heat shock protein, putative [Ixodes scapularis] >gb|EEC03688.1| heat shock protein, putative [Ixodes scapularis]	209	209	98%	1e-52	90%
ACA53150.1	heat shock cognate 70 protein [Pteromalus puparum]	209	209	98%	1e-52	91%
BAG53212.1	unnamed protein product [Homo sapiens]	209	209	98%	1e-52	93%
ACD84945.1	heat shock cognate protein 70 [Macrocentrus cingulum]	209	209	98%	1e-52	91%
3C7N_B	Chain B, Structure Of The Hsp110:hsc70 Nucleotide Exchange Complex	209	209	98%	1e-52	93%
2QW9_A	Chain A, Crystal Structure Of Bovine Hsc70 (1-394aa)in The Apo State >pdb|2QW9|B Chain B, Crystal Structure Of Bovine Hsc70 (1-394aa)in The Apo State >pdb|2QWL|A Chain A, Crystal Structure Of Bovine Hsc70 (1-394aa)in The Adp State >pdb|2QWL|B Chain B, Crystal Structure Of Bovine Hsc70 (1-394aa)in The Adp State >pdb|2QWM|A Chain A, Crystal Structure Of Bovine Hsc70 (1-394aa)in The AdpVi State >pdb|2QWM|B Chain B, Crystal Structure Of Bovine Hsc70 (1-394aa)in The AdpVi State	209	209	98%	1e-52	93%
BAF94143.1	heat shock protein 70B [Alligator mississippiensis]	209	209	98%	1e-52	93%
NP_001166228.1	heat shock cognate 70 [Nasonia vitripennis]	209	209	98%	1e-52	91%
XP_001510947.1	PREDICTED: similar to heat shock protein [Ornithorhynchus anatinus]	209	209	98%	1e-52	93%
EDL95233.1	rCG57965, isoform CRA_b [Rattus norvegicus]	209	209	98%	1e-52	93%
Q5NVM9.2	RecName: Full=Heat shock cognate 71 kDa protein; AltName: Full=Heat shock 70 kDa protein 8	209	209	98%	1e-52	93%
EDL25524.1	mCG5074, isoform CRA_a [Mus musculus]	209	209	98%	1e-52	93%
P19120.2	RecName: Full=Heat shock cognate 71 kDa protein; AltName: Full=Heat shock 70 kDa protein 8 >gb|ABQ12927.1| heat shock 70kDa protein 8 [Bos taurus]	209	209	98%	1e-52	93%
XP_001380093.1	PREDICTED: similar to heat shock protein [Monodelphis domestica]	209	209	98%	1e-52	93%
BAE87166.1	unnamed protein product [Macaca fascicularis]	209	209	98%	1e-52	93%
BAD96348.1	heat shock 70kDa protein 8 isoform 2 variant [Homo sapiens]	209	209	98%	1e-52	93%
AAH07276.2	HSPA8 protein [Homo sapiens]	209	209	98%	1e-52	93%
BAD12572.1	heat shock protein [Numida meleagris] >dbj|BAF37041.1| heat shock protein 70kDa [Coturnix japonica] >dbj|BAF38392.1| heat shock protein 70kDa [Coturnix japonica]	209	209	98%	1e-52	93%
AAQ97970.1	heat shock 70kDa protein 8 [Danio rerio]	209	209	98%	1e-52	93%
CAC83010.1	heat shock protein 70 [Ostrea edulis]	209	209	98%	1e-52	93%
BAB69718.1	hypothetical protein [Macaca fascicularis]	209	209	98%	1e-52	93%
AAH66191.1	Heat shock protein 8 [Mus musculus]	209	209	98%	1e-52	93%
AAO43731.1	heat shock cognate 70 kDa protein [Carassius gibelio]	209	209	98%	1e-52	93%
AAH45841.1	Heat shock protein 8 [Danio rerio] >gb|AAI65717.1| Hspa8 protein [Danio rerio]	209	209	98%	1e-52	93%
ABB29585.1	cytoplasmic heat shock 70 kDa protein [Platynereis dumerilii]	209	209	98%	1e-52	93%
AAI06170.1	Hspa8 protein [Mus musculus]	209	209	98%	1e-52	93%
XP_859437.1	PREDICTED: similar to heat shock protein 8 isoform 3 [Canis familiaris]	209	209	98%	1e-52	93%
NP_001103873.1	heat shock protein 8 [Danio rerio] >gb|AAH63228.1| Heat shock protein 8 [Danio rerio] >gb|AAH66491.1| Heat shock protein 8 [Danio rerio] >emb|CAK03640.1| heat shock 70kDa protein 8 [Danio rerio] >gb|AAI54756.1| Similar to Heat shock protein 8 [Danio rerio]	209	209	98%	1e-52	93%
AAA37869.1	heat shock protein 70 cognate [Mus musculus] >gb|AAB18391.1| heat shock 70 protein [Mus musculus]	209	209	98%	1e-52	93%

Blast X result for Cytochrome P450 CYP4 gene

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
ABZ81919.1	cytochrome P450 family 4 [Perna viridis]	249	249	99%	6e-65	100%
AAC32835.1	cytochrome p450 CYP4Y1 [Mytilus galloprovincialis]	167	167	95%	3e-40	61%
ACM16804.2	CYP450 family 4 [Venerupis philippinarum]	142	142	99%	1e-32	55%
AAR88241.2	CYP4BB1 [Neanthes virens]	140	140	98%	4e-32	55%
ACD75826.1	cytochrome P450 family 4 [Cyphoma gibbosum]	136	136	99%	9e-31	52%
ACD75825.1	cytochrome P450 family 4 [Cyphoma gibbosum]	133	133	99%	8e-30	50%
NP_001071376.1	cytochrome P450, family 4, subfamily A, polypeptide 11 [Bos taurus] >gb|AAI18406.1| Cytochrome P450, family 4, subfamily A, polypeptide 11 [Bos taurus]	133	133	99%	8e-30	50%
XP_002609390.1	hypothetical protein BRAFLDRAFT_59660 [Branchiostoma floridae] >gb|EEN65400.1| hypothetical protein BRAFLDRAFT_59660 [Branchiostoma floridae]	132	132	97%	1e-29	50%
XP_002609363.1	hypothetical protein BRAFLDRAFT_236272 [Branchiostoma floridae] >gb|EEN65373.1| hypothetical protein BRAFLDRAFT_236272 [Branchiostoma floridae]	132	132	97%	2e-29	51%
BAF64511.1	cytochrome 4A35 [Balaenoptera acutorostrata]	132	132	99%	2e-29	50%
NP_001092460.1	cytochrome P450, family 4, subfamily A, polypeptide 22 [Bos taurus] >gb|AAI42396.1| CYP4A22 protein [Bos taurus]	131	131	99%	3e-29	50%
CAE52533.1	fatty acid hydroxylase [Sus scrofa]	131	131	99%	3e-29	49%
XP_002606950.1	hypothetical protein BRAFLDRAFT_200913 [Branchiostoma floridae] >gb|EEN62960.1| hypothetical protein BRAFLDRAFT_200913 [Branchiostoma floridae]	130	130	97%	4e-29	51%
AAR88242.2	CYP342A1 [Neanthes virens]	130	130	97%	5e-29	51%
NP_067010.3	cytochrome P450, family 4, subfamily F, polypeptide 11 [Homo sapiens] >ref|NP_001122404.1| cytochrome P450, family 4, subfamily F, polypeptide 11 [Homo sapiens]	130	130	99%	7e-29	47%
BAF82113.1	unnamed protein product [Homo sapiens]	130	130	99%	7e-29	47%
EAW84511.1	cytochrome P450, family 4, subfamily F, polypeptide 11, isoform CRA_a [Homo sapiens]	130	130	99%	7e-29	47%
AAG15889.1	CYP4F11 [Homo sapiens]	130	130	99%	7e-29	47%
EAW84512.1	cytochrome P450, family 4, subfamily F, polypeptide 11, isoform CRA_b [Homo sapiens] >gb|EAW84513.1| cytochrome P450, family 4, subfamily F, polypeptide 11, isoform CRA_b [Homo sapiens]	130	130	99%	7e-29	47%
Q9HBI6.2	RecName: Full=Cytochrome P450 4F11; AltName: Full=CYPIVF11 >gb|AAH16853.1| CYP4F11 protein [Homo sapiens] >gb|ABM82393.1| cytochrome P450, family 4, subfamily F, polypeptide 11 [synthetic construct] >gb|ABM85576.1| cytochrome P450, family 4, subfamily F, polypeptide 11 [synthetic construct]	130	130	99%	7e-29	47%
XP_001172581.1	PREDICTED: similar to cytochrome P450, family 4, subfamily F, polypeptide 11 isoform 6 [Pan troglodytes]	129	129	99%	1e-28	48%
XP_001172556.1	PREDICTED: similar to cytochrome P450, family 4, subfamily F, polypeptide 11 isoform 5 [Pan troglodytes] >ref|XP_001172593.1| PREDICTED: similar to cytochrome P450, family 4, subfamily F, polypeptide 11 isoform 7 [Pan troglodytes]	129	129	99%	1e-28	48%
Q8SPK0.1	RecName: Full=Cytochrome P450 4A25; AltName: Full=CYPIVA25; AltName: Full=Fatty acid omega-hydroxylase >emb|CAC85663.1| cytochrome P450 [Sus scrofa]	129	129	99%	1e-28	49%
AAZ29444.1	cytochrome P450 4F45 [Macaca fascicularis]	128	128	99%	2e-28	48%
AAZ09199.1	cytochrome P450 family 4 subfamily A [Bos taurus]	128	128	99%	2e-28	49%
CAE52532.1	taurochenodeoxycholic acid 6 alpha-hydroxylase [Sus scrofa]	128	128	99%	2e-28	47%
NP_999590.1	taurochenodeoxycholic 6 alpha-hydroxylase [Sus scrofa] >sp|Q9GJX5.1|CP4AL_PIG RecName: Full=Taurochenodeoxycholic 6 alpha-hydroxylase; AltName: Full=Cytochrome P450 4A21; AltName: Full=CYPIVA21 >emb|CAC19358.1| cytochrome P450 [Sus scrofa]	128	128	99%	2e-28	47%

BIOGRAPHY

Mr. Chatree Ritthong was born on July 10, 1978 in Bangkok, Thailand. He graduated with the degree of Master of Science in Marine Biology from Chulalongkorn University. He has carried out this research as apart of studied for a doctoral degree of Doctor of Philosophy Program in Environmental Management at graduate school, Chulalongkorn University. Under the management of National Center of Excellence for Environmental and Hazardous Waste Management.

Publications from this thesis as of May 2010, 2 papers have been produced from these studies as follows.

1. Chatree Ritthong, Narongsak Puanglarp, Wantaka Boonprasetpol, Somkiat Piyatiratitivorakul, and Piamsak Menasvate. 2008. Use of Metallothionein in green mussel, *Pernaviridis*, As biomarker for determining mercury contamination. **Proceeding of 2008 International Conference on Environmental Quality Concern, Control and Conservation (EQC 2008).** pp. IIA-1_51 – IIA-1_59. Chia Nan University, Taiwan, May 23-24, 2008.

2. Chatree Ritthong, Narongsak Puanglarp, Somkiat Piyatiratitivorakul, and Piamsak Menasvate 2009. Expression analysis of various subunit of Metallothionein gene in green mussel, *Perna viridis*, Located at Petroleum Platforms in the Gulf of Thailand. **The 35th Congress on Science and technology of Thailand (STT35)** pp. 88. The Tide Resort, Chonburi, Thailand, Oct 15-17, 2009.