PROMOTING INQUIRY-BASED TEACHING PRACTICES THROUGH AN AQUATIC TOXICOLOGY LABORATORY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
(SCIENCE AND TECHNOLOGY EDUCATION)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2010

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Thesis
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ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and sincere gratitude to my major-advisor, Assoc. Prof. Dr. Bhinyo Panijpan, for his guidance, advice, encouragement and numerous opportunities that he has provided over the years throughout this research study. I also would like to thank my co-advisor, Assoc. Prof. Dr. Pintip Ruenwongsa, for valuable supervision, creative guidance, useful suggestions and extensive supports. My special appreciation is also extended to my committee, Dr. Watcharee Ketpichainarong, for her useful comments and advices. I am also very grateful to Dr. Pisarn Soydhurum for his variable time spent for serving as my chair of the dissertation committee and provided helpful comments and suggestions for improvement.

My special thanks go to Dr. Kevin J. Niemi, my great facilitator during my time at the Center for Biology Education, University of Wisconsin-Madison at Wisconsin, USA, for his warmly supports, useful guidance and suggestions on science education. I would like to express my sincere thanks to Dr. Terence P. Barry at the Laboratory of Fish Endocrinology and Aquaculture, School of Animal Sciences, University of Wisconsin-Madison, my wonderful science mentor, for his kindly supports, unfailing guidance, and grateful comments on endocrine disrupting chemicals (EDCs) research.

I am extremely thankful to the Office of Higher Education Commission, Ministry of Education for providing me a great opportunity to continue my doctoral study and the scholarship during my study. I am also particularly grateful to STAR program for a financial support on EDCs experiments. I would like to express my special thank you to Mrs. Kathy Knoeppel for her efforts on teaching at Mayville High School, Wisconsin and my co-researcher, Xuejiao, Tian, for her kindly assistance and valuable discussion. I would like to say a special thank you to all students involving in this research study.

I wish to express my deepest thank to my parents, and my families for their love, care and support, and for helping me to focus on my education with out any distractions. Finally, I dedicate this dissertation to my parents, my families, all teachers and friends.

Bunlung Nuangsaeng

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PROMOTING INQUIRY-BASED TEACHING PRACTICES THROUGH AN AQUATIC TOXICOLOGY LABORATORY

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ABSTRACT

This research aimed at promoting inquiry-based teaching practices through an aquatic toxicology laboratory for high school students in an environmental science course. In the joint development of the practical work, the effects of six steroid hormones on development of zebrafish (*Danio rerio*) embryos at the stage of 6-8 h post fertilization (hpf) to hatching were investigated. The results at 96h endpoint showed effects of hormones at lethal, sublethal, and teratogenic concentrations. Although the mortality percentage of the embryos was relatively low, the hormones at 5-500 ng/mL did apparently retard development. Deformation was observed in yolk sac, body in addition to pericardial edema.

The results from the scientific work were implemented as laboratory exercises in the seven-topic learning unit. The teacher was trained beforehand on how to carry out the scientific activities on aquatic toxicology. The educational instruments used to evaluate achievements of both students and the teacher were student projects and presentations, student reflection, teacher reflection, teacher interview, and classroom observation. The results showed that the inquiry-based learning unit successfully promoted the students' learning outcomes, when compared to the traditional teaching. The students' achievements in terms of content knowledge, scientific method, attitudes, inquiry skills, and science process skills significantly increased through the four-week period of the intervention. In addition, the students gained communication skills through collaborative teamwork. Teaching practices also changed through the intervention as evidenced from interviews of the teacher and her reflections. The results clearly indicated that the teacher in this study gained teaching efficacy from the experience; the teacher gradually became more confident in teaching and giving answers to students' queries.

KEY WORDS: AQUATIC TOXICOLOGY / INQUIRY / PROFESSIONAL DEVELOPMENT / TEACHING PRACTICES / ZEBRAFISH

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การเสริมสร้างแบบการสอนโดยกระบวนการสืบเสาะหาความรู้ด้วยบทปฏิบัติการมลพิษทางน้ำ

PROMOTING INQUIRY-BASED TEACHING PRACTICES THROUGH AN AQUATIC TOXICOLOGY LABORATORY

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บทคัดย่อ

การศึกษาวิจัยนี้มุ่งเน้นแผนการสอนแบบสืบเสาะหาความรู้โดยใช้บทปฏิบัติการด้านมลพิษทางน้ำที่ได้พัฒนาร่วมกับครูผู้สอนในวิชาวิทยาศาสตร์สิ่งแวดล้อมสำหรับนักเรียนมัธยมศึกษาตอนปลาย จากการศึกษาผลของฮอร์โมนเพศจำนวน 6 ชนิด ต่อการพัฒนาตัวอ่อนของปลา zebrafish (*Danio rerio*) จากตัวอ่อนอายุ 6-8 ชั่วโมงหลังจากการปฏิสนธิจนถึงระยะที่ลูกปลาฟักออกจากไข่ โดยจะสิ้นสุดการทดลองที่ 96 ชั่วโมงหลังจากไข่ได้รับการปฏิสนธิ ผลการศึกษาพบว่า ฮอร์โมนเพศมีผลต่อการพัฒนาตัวอ่อนจากระดับที่ทำให้การพัฒนาตัวอ่อนผิดปกติเล็กน้อยจนถึงระดับที่ทำให้ตัวอ่อนของปลาตายได้ แม้ว่าตัวอ่อนของปลา zebrafish จะมีอัตราการตายต่ำแต่ฮอร์โมนที่ระดับความเข้มข้นของ 5-500 ng/mL มีผลทำให้ตัวอ่อนมีพัฒนาการช้าลง ลักษณะการพัฒนาที่ผิดปกติของตัวอ่อนทำให้ตัวอ่อนมีรูปร่างของถุงไข่แดงและลำตัวเปลี่ยนแปลงไป และอาจพบหัวใจและส่วนของช่องอกมีขนาดใหญ่ขึ้นคล้ายอาการบวมน้ำ

ผลงานทดลองทางวิทยาศาสตร์ได้ถูกนำมาพัฒนาเป็นบทปฏิบัติการทดลองแบบสืบเสาะหาความรู้ในหน่วยการเรียนที่มี 7 บทเรียน โดยครูผู้สอนได้รับการฝึกอบรมทักษะการดำเนินกิจกรรมทางวิทยาศาสตร์ด้านมลพิษทางน้ำก่อนทำการสอน เครื่องมือที่ใช้สำหรับประเมินผลสัมฤทธิ์ของครูและนักเรียน ได้แก่ โครงงานวิทยาศาสตร์ของนักเรียน และการนำเสนอผลงาน ผลสะท้อนจากนักเรียนและครู การสัมภาษณ์ครูผู้สอน และการสังเกตกิจกรรมการเรียนการสอนในห้องเรียน ผลการศึกษาแสดงให้เห็นถึงความสำเร็จของหน่วยการเรียนแบบสืบเสาะหาความรู้ต่อผลสัมฤทธิ์ต่อการเรียนของนักเรียนเมื่อเปรียบเทียบการสอนแบบปกติ ผมสัมฤทธิ์ต่อการเรียนทั้งด้านความรู้ วิธีการทางวิทยาศาสตร์ เจตคติ ทักษะการเรียนแบบการสืบเสาะหาความรู้ และทักษะกระบวนการทางวิทยาศาสตร์เพิ่มขึ้นอย่างต่อเนื่องจากการดำเนินกิจกรรมเป็นเวลา 4 สัปดาห์ นอกจากนี้นักเรียนมีทักษะการสื่อสารมากขึ้นจากการทำงานเป็นกลุ่ม จากการสัมภาษณ์และผลสะท้อนจากครูผู้สอนพบว่ารูปแบบแผนการสอนมีการปรับเปลี่ยนตลอดช่วงเวลาทำการศึกษา ผลการศึกษาแสดงให้เห็นอย่างชัดเจนว่าครูผู้สอนมีความเชื่อมั่นเพิ่มมากขึ้นในการสอนแบบสืบเสาะหาความรู้โดยใช้บทปฏิบัติการทดลอง และครูมีความมั่นใจสูงขึ้นในการตอบคำถามต่อข้อสงสัยของนักเรียน

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LIST OF ABBREVIATIONS

AAAS	American Association for the Advancement of Science
ASTM	American Society for Testing and Materials
A4	4-androstene-3, 17-dione
cm	centimeter
CAFOs	concentrated animal feeding operations
° C	degree Celsius
DHP	17α, 20β-dihydroxyprogesterone
ELS	early life stage
EDCs	endocrine-disrupting chemicals
E1	estrogen
E2	17β-estradiol
EE2	17 α-ethinylestradiol
e.g.	for example
et al.	*et. alli* (Latin), and others
FAS	fetal alcohol syndrome
g	gram
h	hour
HGPs	hormonal growth promotants
hpf	hour post fertilization
i.e.	*ed est* (Latin), that is
ISO	International Standard Organization
kg	kilogram
L	liter
LC_{50}	median lethal concentration / lethal concentration, 50%
µL	microliter
mm	millimeter
MoA	mode of action

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LIST OF ABBREVIATIONS (cont.)

NRC	National Research Council
NSES	National Science Education Standards
OECD	Organization for Economic Co-operation and Development
P4	progesterone
RTG-2 cells	rainbow trout gonad-2 cell lines
STPs	sewage treatment plants
STWs	sewage treatments works
T	testosterone
Tb	17β-trenbolone
TbA	trenbolone acetate
Vtg	vitellogenin

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CHAPTER I
INTRODUCTION

Overview

This chapter introduces the overall details of the research including the scientific and the educational aspects containing background and rationale of the study, purpose of the study, significance of the study, and research questions. The chapter ends with the organization of the thesis which includes the details in chapters one to six.

1.1 Background and Rationale of the Study

Aquatic ecosystems that run through agricultural or industrial areas have high probability of being contaminated by runoff and groundwater leaching by a variety of chemicals. There is growing concern about the adverse effects of environmental contaminants on human and wild animal health. In particular, effects and risks associated with the presence in surface waters of chemicals having estrogenic activity (dominated as endocrine disrupters) and thus able to interfere with endocrine/reproductive functions in wild fish has been the object to studies and investigations in several countries. Starting from 1980, several conjectures and experiments with caged fishes have raised the possibility that effluents of sewage treatment plants (STPs) receiving primarily domestic inputs are one of the sources of endocrine disrupters. Desbrow and colleagues (1998) have indicated natural and synthetic estrogens as candidate compounds to contribute to estrogenic activity of effluents of STPs located in urban areas. *In vitro* studies have shown that exposure of fishes to 1-10 ng/L of 17β-estradiol (E2) and 0.1 ng/L of the synthetic birth control contraceptive 17 α-ethinylestradiol (EE2) provoke feminization in some species of male wild fishes (Routledge et al., 1998; Purdom et al., 1994). Another source of

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steroid estrogens is from the use of conjugated estrogens in the treatment of cancer, osteoporosis, the menopause and hypogonadism, with an estimated amount used in the USA of 1,690 kg per year. More diffuse sources of steroid estrogens come from agriculture (Arcand-Hoy & Benson, 1998; Arcand-Hoy, Nimrod, & Benson, 1998; Shore, Gurevitz, & Shemesh, 1993).

Currently, hazard assessment of chemicals for fish is based on international standards (American Society for Testing and Materials [ASTM] International) and guidelines (Organization for Economic Co-operation and Development [OECD] guidelines for testing of chemicals) based on global toxicology endpoints as mortality, growth, and reproduction impairments. For chemical industries and some countries the state of mind is now to reduce the cost of these experiments and the number of used organisms, in concern for animal welfare. A significant example is the awareness of the ecotoxicologist community of the pharmaceutical industry to apply the principles of replacement, reduction, and refinement (the 3Rs), established by Russell and Burch (1959), in the context of regulatory environmental assessments (Hutchinson et al., 2003). One of the alternatives proposed is to use the early life stage (ELS) of fish as an experimental model (Hutchinson et al., 2003; Nagal, 2002; Oberemm, 2000), because it is no longer necessary to demonstrate that the fish embryo and larva are generally the most sensitive stages in the life cycle of the teleost (Laale & Learner, 1981; Lele & Krone, 1996; McKim, 1985; von Westernhagen, 1988).

Different fish species may vary by orders of magnitude with respect to their sensitivity in acute tests to environmental contaminants. Several research studies reported the use of zebrafish embryos compared to other fishes and cells. For instance, Lange and colleagues (1995) reported that the zebrafish embryo test to those of cytotoxicity tests with the permanent cell line RTG-2 derived from rainbow trout (*Oncorhynchus mykiss*) for 10 selected compounds with different modes of action. In most cases the zebrafish embryo was more sensitive than adult zebrafish and the RTG-2 cells. This species is easily obtainable, inexpensive, readily maintainable and providing a large number of non-adherent and transparent eggs (Laale, 1977). In addition, the embryonic development was described in numerous studies and the basis for the interpretation of effects caused by environmental pollutants was studied in

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several research works (Ensenbach & Nagel, 1995; Hill, Teraoka, Heideman, & Peterson, 2005; Hisaoka & Battle, 1958; Kienle, Kohler, & Gerhardt, 2009; Kimmel et al., 1995; Laale, 1977; Thomas & Waterman, 1978; Todd & Van Leeuwan, 2002).

Since sex steroids and several hormones influence reproduction in aquatic vertebrates and may interact during larval development (Arcand-Hoy & Benson, 1998; Cyr & Eales, 1996). More diffuse sources of steroid estrogens come from agriculture (Arcand-Hoy, Nimrod, & Benson, 1998; Shore, Gurevitz, & Shemesh, 1993). To monitor some potentially health threatening products from agriculture, biological markers such as aquatic animals, their organs or cells are used. This study proposes the well-known zebrafish (*Danio rerio*) as a marker for detecting natural and synthetic steroid hormones in the environment. The six sex steroid hormones; trenbolone acetate, progesterone (P4), 17α, 20β-dihydroxyprogesterone (DHP), 17β-estradiol (E2), testosterone (T), and 4-androstene-3, 17-dione (A4) at various concentrations (0, 5, 50, 500, 5000 ng/ml) were used to test their effects on the development of zebrafish (*Danio rerio*) embryos at the stage of 6-8 hpf to hatching. The toxic effect is assessed through the analysis of various criteria, defined as endpoints described by Schulte and Nagel (1994) and Nagel (1998).

In science education, the laboratory exercise is one of the strategies that has been used to promote a better students' understanding of the scientific concepts (Cameselle et al., 2000). The laboratory exercise has been promoted as serving a wide variety of educational functions (Hofstein & Lunetta, 2004): developing and restructuring knowledge schemes, reflecting science as it is practiced by scientists, and developing skills in the process of science (Hofstein & Lunetta, 1982; Tobin, 1990). Therefore, some of the findings from this scientific study on effects of sex steroid hormones on zebrafish embryonic development were adapted for using as inquiry-based laboratory exercise in the learning unit for the high school students in environmental science course.

Regarding science education reform documents in several countries, including the United States, advocate changing science teaching in the classrooms from emphasis on using direct instruction to greater emphasis on inquiry-based one. According to the *National Science Education Standards* (NSES): *"Scientific inquiry refers to the diverse ways in which scientists study the natural world and propose*

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explanations based on the evidence derived from their works" (National Research Council [NRC], 1996, p.23). Hands-on activity and laboratory-based instruction can be designed to help students learn about the nature of scientific inquiry (American Association for the Advancement of Science [AAAS], 1989, 1993; Germann, Haskins, & Auls, 1996; Wilson & Chalmers-Neubauer, 1990).

Inquiry-based instruction as a model for pedagogy has the potential to enhance student understanding and engagement in science (Capps & Crawford, 2009; DeBoer, 2006). However, most teachers do not use inquiry-based instruction in the classroom due to a number of issues e.g., perceived time constraints regarding high-stake testing; unfamiliarity with how science is practiced; inadequate preparation in science; misunderstanding of the purposes of inquiry or misconception about exactly what inquiry is (Bybee, 2000; Costenson & Lawson, 1986; Welch, Klopfer, Aikenhead, & Robinson, 1981). To achieve student learning outcomes in science understanding, inquiry, and discourse, teachers need adequate knowledge of science content, and effective instructional strategies/teaching practice including teacher efficacy (Gusky, 2002; Keys & Bryan, 2001; Lee, Hart, Cuevas, & Enders, 2004).

Many studies on the professional development programs showed subsequent significant success stories in teaching and learning of science in the classroom. The trainee teachers are thereafter able to provide opportunities for their students to conduct the inquiry-based learning. The experience in project-based inquiry science instruction should help teachers in teaching science such as promoting science content understanding, relating it to daily life, science inquiry skills, science process skills, greater cognitive involvement and improved thinking ability including problem solving skills (Banilower, Heck, & Weiss, 2007; Geier et al., 2008; Jeanpierre, Oberhauser, & Freeman, 2005).

The classroom practice/instructional unit in this study emphasizes issues related students' everyday life and local problems which should pique their interest to study science more seriously because they can see the relevance. The experiment on using endocrine-disrupting chemicals (EDCs) is related to concentrated animal feeding operations (CAFOs) in dairy farms, a familiar scenario in the community of this study. The effect was tested on zebrafish embryonic development, an experiment, which was appropriately modified for the learning unit. The purpose of this study was to help the

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teacher in developing the inquiry-based laboratory learning unit on aquatic toxicology, and to promote the teaching practice in the inquiry-based curriculum. Student achievement was used as key success factor for adoption or adaptation for teacher professional development.

1.2 Objectives of the Study

This study aimed at promoting inquiry-based teaching practices through an aquatic toxicology laboratory.

1. To develop an inquiry-based learning unit with collaboratively efforts from the teacher, and researcher. The teacher in this study played an important role in designing the instructional unit while the scientific expertise was provided by the researcher.

2. To train the teacher on how to carry out the scientific activities on aquatic toxicology during the development of the learning unit.

3. To implement the learning unit in the environmental science course for high school students.

4. To investigate the effectiveness of the inquiry-based learning unit on the students' learning outcomes, compared to the conventional teaching. The students' achievements were assessed in terms of content knowledge, scientific method, attitudes, inquiry skills, and science process skills through the four-week period of the intervention. In addition, the students' communication skills through collaborative team working were investigated.

5. To observe the teacher's change in the teaching practices through the intervention from interviews and reflection. It will also be studied whether the teacher in this study gain teaching efficacy from the experience, become more confident in teaching, and become competent in guiding students in using scientific instruments and conducting the scientific experiments.

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1.3 Research Questions

1. To what extent the results from aquatic toxicology experiment in the scientific part can be used in developing the inquiry-based learning unit for the high school students?

2. Can the teacher promote student learning outcomes by using the newly developed inquiry-based learning unit on aquatic toxicology?

3. Would the teacher's view of the practice change while going through the unit?

4. What are the teacher attitudes toward the new experience on inquiry-based teaching and learning?

1.4 Significance of the Study

Intensive and large-scale animal husbandry generally involves the use of technological products such as medicines, hormones, feed supplements, which may be subsequently discharged into the immediate environment. Then products may affect the fauna, flora and even humans living near the farms. To monitor some potentially health threatening products, biological markers such as aquatic animals, their organs or cells are used. This study proposes the well-known zebrafish (*Danio rerio*) as a marker for detecting natural and synthetic steroid hormones in the environment of a high school near a farming community. Apart from being engaged by the local aquatic toxicology problem, students are expected to learn how to simply detect harmful chemicals by observing abnormal development of the fish from the fertilized egg to the larval stage. The newly developed inquiry-based learning unit through an aquatic toxicology laboratory may promote teachers to effectively develop their teaching practices and implement the learning unit, and how to perform the scientific experiment using the inquiry approach. This study should offer a guideline for further development of teaching professional in designing and conducting inquiry-based learning unit in the classroom.

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1.5 Organization of the Thesis

This study is divided into two main research aspects: the scientific and the educational. Some of the findings, ideas and laboratory techniques used in the scientific study of the effects of endocrine disrupting chemicals (EDCs) were adapted to develop the hands-on activities in a newly developed inquiry-based learning unit with the teacher participation in this study. The newly teaching practices in aquatic toxicology laboratory can thus be used not only to promote students' understanding, science process skills, and student attitudes and awareness on environmental impacts in aquatic ecosystems, but also to help the teacher develop her professional experiences. This thesis is organized in six sequential chapters as follows:

Chapter One provides the background of the research problems and the purpose of this study. This section also specifies the significance of the study, and research objectives and questions.

Chapter Two presents the literature reviews related to the scope of the present study in scientific and educational aspects. The scientific review covers the information of environmental risk assessment, the standards and guideline of aquatic toxicology/ecotoxicology, effects of endocrine disrupting chemicals on aquatic organisms, and then ends with a topic of fish embryo toxicity test. The educational review part provides the relevant literatures regarding professional development, inquiry-based approach, science process skills, and student learning outcomes.

Chapter Three describes the methodology and methods/tools used to conduct the research in both the scientific and educational aspects. The first part includes the methods employed to observe the effects of sex steroid hormones on zebrafish embryonic development. The latter part deals with the educational research design to develop the learning unit based on an inquiry approach to promote teaching practices emphasizing teacher and students learning outcomes.

Chapter Four reports the main findings of the scientific research regarding the effects of endocrine disrupting chemicals on the zebrafish embryonic development. The other part provides the results of the newly developed inquiry-based learning unit on both teacher and student achievement.

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Chapter Five presents a discussion of the major findings of the scientific and educational study in relation to other research studies. The limitations and implications of the study are also mentioned at the end of the chapter.

Chapter Six concludes the overall findings of the research study.

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CHAPTER II
LITERATURE REVIEW

Overview

This chapter presents a review of literature relevant to this research. It is divided into two parts: science and education. The scientific part begins with the background information of aquatic toxicology and the effects of steroid hormones, chemicals and toxicants on some organisms. This is followed by their role of living organisms, especially the zebrafish (*Danio rerio*) for toxicity testing. Then the methods for assay the endocrine disrupting chemicals are described.

The educational part begins with the theoretical basis of professional development and its application of teaching practices and learning outcomes. This is followed by the learning units and scientific inquiry which promote student science process skills.

2.1 Scientific Review

2.1.1 Aquatic Toxicology

General Introduction:

Many reports concerning adverse health effects of chemicals and population declines in fish have been published as reviewed by Norrgren and colleagues (1998). It has been proposed that these problems are mainly because of chemical contamination in many cases; declines in population size, low reproductive success, disruption of gonad development, and larval mortality. Reproduction and development of the offspring, as embryo and larva, are considered to be more sensitive stages in the life cycle to chemical exposure (von Westerhagen, 1988; Nagel, 2002). Intensive research on substances reported to disrupt endocrine system in fish, mainly

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focused on disruption of sex hormone production, sexual differentiation, and reproductive system (Anderson et al., 2006; Meucci & Arukwe, 2005; Vinggaard, Hnida, Breinholt, & Larsen, 2000).

The increasing number of reports about fish and other aquatic animal population declines makes it important to include these animals in test strategies that could be applied both for regulatory assessment and a support in connection with environmental monitoring programs. In OECD test Guidelines, a number of standard guidelines have been developed for use in aquatic ecotoxicology testing to assess potential effects of the chemicals. Efforts have been made in increasing sensitivity and specificity of such tests by the implementation of additional sublethal endpoints reflecting hormone disruption effects, behavior alterations, physiological effects and reproduction disorders (Darland & Dowling, 2001; Fort et al., 2004; Holbech et al, 2006; Örn, Holbech, Madsen, Norrgren, & Petersen, 2003; Peitsaro, Kaslin, Anichtchik, & Panula, 2003).

Currently, hazard assessment of chemicals for fish is based on American Society for Testing and Materials (ASTM) International standards and Organization for Economic Co-operation and Development (OECD) guidelines based on global toxicology endpoints as mortality, growth, and reproduction impairments. For chemical industries and some countries, the state of mind now is to reduce the cost of these experiments and the number of used organisms, in concern for animal welfare. A significant example is the awareness of the ecotoxicologist community of the pharmaceutical industry to apply the principles of replacement, reduction, and refinement (the 3Rs), established by Russell and Burch (1959), in the context of regulatory environmental assessments (Hutchinson et al., 2003). One of the alternatives proposed is to use the early life stage (ELS) of fish as an experimental model (Hutchinson et al., 2003; Nagal, 2002; Oberemm, 2000), because it is no longer necessary to demonstrate that the fish embryo and larva are generally the most sensitive stages in the life cycle of the teleost (Laale & Learner, 1981; Lele & Krone, 1996; McKim, 1985; von Westernhagen, 1988).

In recent years acute toxicity tests with fish have also aroused considerable ethical concern. Since acute toxicity to fish is determined in tests with juvenile or adult animals, intact fish are subjected to considerable pain and suffering, which is clearly in

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conflict with current Animal Rights Welfare legislation. Since results of LC_{50} tests are only of minor ecotoxicological significance, acute toxicity tests with fish should be replaced in terms of the 3Rs. Possible alternatives to the acute fish test might be the embryo test not only with cytotoxicology tests but also embryos of the well-known zebrafish.

Fish Embryo Test as a Model for Developmental Toxicity:

Based on the fish embryo test in previous research works, several research studies reported the use of zebrafish embryos compared to other fishes and cells. For instance, Lange and colleagues (1995) compared the zebrafish embryo test to those of cytotoxicity tests using cells from the permanent cell line RTG-2 derived from rainbow trout (*Oncorhynchus mykiss*) for 10 selected compounds with different modes of action. In most cases the zebrafish embryo was more sensitive than adult zebrafish and the RTG-2 cells. Schulte and Nagel (1994) suggested zebrafish is one of alternative species for toxicity test. The basic principle is to expose eggs from fertilization until completion of embryogenesis at 48 h post fertilization (hpf). Exposure is performed in well-plates in small volume of media. Individual monitoring is performed at regular time intervals, where various responses are recorded (Schulte & Nagel, 1994). The endpoints can be separated in two types, lethal and sub-lethal (Nagel, 2002) Lethal endpoints include coagulation of egg, tail not detached from yolk, lack of somites, and no heart beats. Further, sublethal endpoints that might indicate the mode of action of the toxic response can be measured such as completion of gastrulation, eye development, spontaneous movement, circulation, pigmentation, edema and heart rate.

Further expansion of the test can be made by prolonging the time of experiment and including measuring such as presence of spinal deformation (Hollert et al., 2003), pericardial areas (Fraysse, Mons, & Garric, 2006) and body or tail length (Fraysse, Mons, & Garric, 2006; Nagel, 2002). The fish embryo test has been standardized in Germany for the use in routine effluent monitoring. Standardization is under consideration in ISO 12890 (International Standard Organization [ISO], 1999) and OECD guideline 203 and 210 of the method for sewage water and chemical testing (Braunbeck et al., 2005). The methodology has been used, for example, in toxicology test of environmentally relevant chemicals (Cook, Paradise, & Lom, 2005;

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Fraysse, Mons, & Garric, 2006; Kapp, Kammann, Vobach, & Vetter, 2006) and tests of sediment and sediment extracts (Hallare et al., 2005; Kammann, et al., 2004). Braunbeck and colleagues (2005) showed that this protocol can also be adapted for two other fish species recommended by OECD, the Japanese medaka (*Oryzias latipes*) and fathead minnow (*Pimephales promelas*). Generally, this assay shows good correction with the conventional 96 h fish test as described in OECD guideline 203 and OECD guideline 210 (OECD, 1992a, 1992b). However, given its superior high number of eggs per spawning act, the rapid development, the perfect transparency of its eggs, and last but not least, the immense body of already existing information on zebrafish development (Braunbeck et al., 2005; Nagel, 2002), the zebrafish seems to be first choice for routine embryo toxicity testing.

2.1.2 Biology and Use of Zebrafish in Research

The growing demand for increasingly sophisticated information on the toxic hazards of potential water pollutants has focused attention on the need for a suitable 'standard' animal model which could be accepted internationally. The zebrafish (*Danio rerio*) is considered to be one of the most likely candidates. It is relatively easy to maintain and breed in laboratory aquaria and it has proved to be responsive to a wide range of mutagens, carcinogens and teratogens, as well as direct toxicants.

The zebrafish *Danio rerio* (Hamilton-Buchanan 1922; formerly *Brachydanio rerio*) is a small cyprinid found in tributaries and branches of the Ganges River in the South-East Asia (Eaton & Farley, 1974). This species measures 3-5 cm as an adult and thrives in both soft and hard waters. At 26 °C the zebrafish grows quickly and reaches maturity within three months. This species is easily obtainable, inexpensive, readily maintainable and provides a large number of non-adherent and transparent eggs (Laale, 1977). Zebrafish is insensitive to change in water quality and easily obtainable to a low cost. It is relatively also easily maintained in laboratories with simple equipment; it breeds all year round and eggs can be collected in large quantities. The life cycle is rapid under optimal rearing conditions. Embryos of zebrafish are transparent making it possible to monitor individual organs during developmental period (approximately 96 h at 26° C) using only a standard

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stereomicroscope. These advantages have led to the widespread use of this species in toxicology testing, including standardized protocols (Braunbeck et al., 1990; Carlsson, Örn, Andersson, Söderström, & Norrgren, 2000, Carlsson et al. 2000; Örn, Holbech, Madsen, Norrgren, & Petersen, 2003).

Under spawning conditions, males can easily be distinguished from females by their more slender body shape and an orange to reddish tint in the silvery bands along the body (Figure 2.1). Due to the large number of eggs produced, females can be recognized by their swollen bellies. One female spawns between 50 and 200 eggs on a daily basis. Egg production can be significantly stimulated by additional rations of natural food (*Artemia* nauplii; *Daphnia*). The fish used for producing eggs should be between 4-15 months of age.

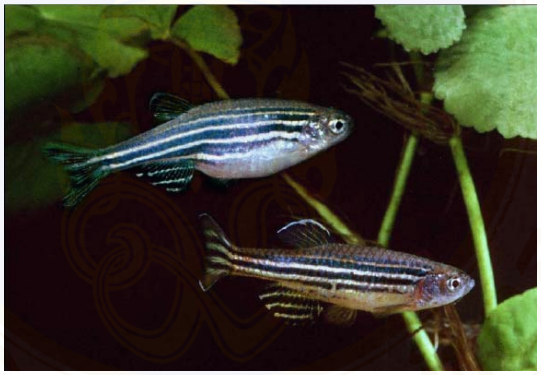

Figure 2.1 Adult zebrafish (*Danio rerio*) female (upper individual) can easily be differentiated from male (lower individual) by their extended bellies and the lack of reddish tint along the silver longitudinal lines. (Braunbeck & Lammer, 2006)

Larval zebrafish have been utilized to study the effects of many environmental pollutants including cadmium (Blechinger, Warren, Kuwada & Krone, 2002), insecticides (Levin, Swain, Donerly, & Linney, 2004), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Mattingly, McLAchlan, & Toscano, 2001). The adverse effects of Cadmium on developing zebrafish have been investigated at the whole-body, cellular, and molecular levels. These effects include ectopic apoptosis (Chan & Cheng, 2003), morphological deformities due to altered gene expression (Cheng, Wai, So, & Wu, 2000), abnormal somitogenesis (Chow & Cheng, 2003), and induction of heat shock protein 70 gene expression *(hsp70)* (Blechinger, Warren, Kuwada, &

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Krone, 2002). Currently, zebrafish has become a major model in neurobiology and toxicology as well as in general molecular and developmental biology (Cheng, Wai, So, & Wu, 2000; Dooley & Zon, 2000; Goolish, Okutake, & Lesure, 1999; Westerfield, 1995). Furthermore, as the zebrafish holds many similar cellular and physiological characteristics with higher vertebrates, the toxicological results of zebrafish embryonic development can be easily compared with those of mammalian development (Marguerie, Buckley, & Fleming, 2006; Reimers, Flockton, & Tanguay, 2004).

2.1.3 Effects of Endocrine Disrupting Chemicals (EDCs)

It has been clearly established that a number of chemical compounds and natural substances present in the aquatic environment are able to disturb the normal physiology and endocrinology of organisms (Arukwe & Goksøyr, 1998; Sumpter, 1998). Previous research has examined the effects of a variety of chemicals (heavy metals, insecticides, pesticides, fungicides, natural and synthetic hormones) on zebrafish development. These studies can be divided into several areas: acute toxicity, physiology of adult fish, reproductive behavior involving numbers and viability of eggs spawned by adult females, and developmental abnormalities in eggs and larvae (Strmac & Braunbeck, 1997; Todd & van Leeuwen, 2002).

The role of endocrine disrupting chemicals (EDCs) in the aquatic environment is a global concern. A number of compounds—including industrial chemicals, pesticides, and pharmaceuticals—have been considered as EDCs.

Some endocrine disrupting chemicals such as alkylphenol polyethoxylates, the natural steroid estrogens 17β-estradiol (E2), estrogen (E1) and, to a lesser extent, the synthetic estrogen, ethinylestradiol (EE2) have been measured in industrial and municipal sewage treatments works (STWs) effluents: these discharges represent the main source of synthetic and natural estrogens into the aquatic environment. It should also be realized however that surface runoff is another possible source of estrogenic contamination (Desbrow, Routledge, Brighty, Sumpter, & Waldock, 1998; Lai, Scrimshaw, & Lester, 2002). Concentrations of E2 and E1 in effluents from sewage treatment works from European countries ranged from low nanograms per liter up to hundreds of nanograms per liter (Desbrow, Routledge, Brighty, Sumpter, & Waldock,

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1998). The origin of the natural steroid estrogens is predominantly from humans where the daily production emanating from women ranges from tens micrograms up to 30 mg in pregnant women (Aldercruetz et al., 1994). Another source of steroid estrogens is from the use of conjugated estrogens in the treatment of cancer, osteoporosis, menopause and hypogonadism, with an estimated amount used in the USA of 1,690 kg per year (Arcand-Hoy, Nimrod, & Benson, 1998). More diffuse sources of steroid estrogens come from agriculture (Shore, Gurevitz, & Shemesh, 1993). One of the most potent and significant xenoestrogens, 17 α-ethinylestradiol (EE2), has been measured in sewage effluents at concentrations up to 7.0 ng/L in the United Kingdom (Desbrow, Routledge, Brighty, Sumpter, & Waldock, 1998), 15 ng/L in Germany, 42 ng/L in Canada (Ternes et al., 1999), and up to 4.5 ng/L in Sweden (Larsson et al., 1999). Estrogens are excreted in urine in an inactive conjugated form (Shackleton, 1986; Guengerich, 1990) but are possible reactivated through bacterial activity in sewage (Ternes, Kreckel, & Mueller, 1999; Legler et al., 2002).

Since the early 1990s, an international effort has focused on identifying possible adverse effects of endocrine-disrupting chemicals on reproduction and development in both human and wildlife, The hypothalamic-pituitary-gonadal (HPG) axis, especially aspects of the system directly related to steroid hormones (estrogen, androgens), has been of particular concern (Huet, 2000). A number of chemicals with potential to affect the HPG axis of animals enter aquatic systems through a variety of point and nonpoint source discharges. Not surprisingly, therefore, some of the better documented examples of adverse effects of EDCs in the environment are for aquatic animals, particularly fish (Ankley & Giesy, 1998; Tyler, Jobling, & Sumpter, 1998).

The HPG axis of the teleost fishes is, in general, similar to that found in other oviparous (egg laying) vertebrates. The principal components of the axis include the hypothalamus and hypophysis (pituitary gland) in the brain, the gonads (testis, ovary), and the liver. These tissues are structurally connected in one or both of the following mechanisms: a relatively fast neuronal linkage and a slower vascular linkage. The functional signals that affect the linkages between these components are somewhat diverse in chemical structure and how they travel among tissues. The target cells within target tissues respond to these signaling molecules in various ways, but in general the responses are agonistic (i.e., activation of cellular process) or antagonistic

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(i.e., inhibition of cellular processes). Depending on the developmental status of the organism, the ultimate effects of these signals on the whole animal can be categorized as organizational or activational. In other words, during the early developmental stages of fish and other vertebrates, the differentiation of tissues and cells into organs with the proper structure and capability of responding to external and internal cues is controlled, at least in part, by the hormones of the HPG axis. These responses are considered to be organizational. In later life-stages, these same tissues are capable of responding to one or more external signals (e.g., photoperiod, temperature) with responses such as the initiation of reproduction. These responses are termed activational (Ankley & Johnson, 2004).

Steroidal hormones potentially present in feedlot manure and effluent include endogenous (naturally occurring) hormones and some synthetic hormones applied in agriculture. Endogenous hormones are commonly identified in animal excretions including manure and urine (Hoffmann, dePinho, & Schuler, 1997). The levels of these residues vary considerable with sex, age, breed, castration, and pregnancy (Khan et al., 2008). Both natural and synthetic steroidal hormones are used in many countries as hormonal growth promotants (HGPs) in cattle (Mader & Lechtenberg, 2000; Preston, 1999; Song & Choi, 2001). They are used to improve feed efficiency, rates of weight gain and relative proportions of muscle and fat (Lefebvre, Malouin, Roy, Giguère, & Diarra, 2006). The use of HGPs may increase both the range and concentration of steroids present in livestock waste (Preston, 1999). HGPs are normally administered via a subcutaneous implant in the animal's ear. All such compounds are known to be present in animal tissue as well as in urinary and faecal excretions (Galbraith, 2002). In addition, commercially available hormonal products are also used to improve the reproductive performance of dairy cattle (Refsdal, 2000). The active hormones used in cattle include 17β-estradiol, estradiol valerate, estradiol benzoate, trenbolone acetate, zeranol, progesterone, and testosterone proprionate (Khan et al., 2008).

Partial feminization of male fish, described as intersex and increased levels of the yolk precursor protein vitellogenin (Vtg), have been observed in a number of fish species living in rivers contaminated by xenoestrogens (Jobling, Nolan, Tyler, Brighty, Sumpter, 1998; Van Aerle et al., 2001; Sole, Raldua, Piferrer, Barcelo, &

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Porte, 2003). Masculinization of fish has been described in mosquitofish (*Gumbusia* sp., *G. affins*, *G. holbrooki*) and eelpout (*Zoarces viviparous*) living in waters receiving pulp and paper mill effluents (Howell, Black, & Bortone, 1980; Cody & Bortone, 1997; Larsson, Hallman, & Forlin, 2000; Parks et al., 2001). Musculinization has also been reported in fathead minnow (*Pimephales promelas*) living in rivers downstream from cattle feedlots (Jegou et al., 2001). Androgenic activity in feedlot effluents has been observed in *in vitro* studies. This has been connected with exposure to major metabolites (17α-trenbolone, 17β-trenbolone [Tb], and triendione) of trenbolone acetate (TbA), which is used as a growth promoter in beef cattle production in North America (Durhan et al., 2006; Lange, Daxenberger, & Meyer, 2001; Wilson, Lambright, Ostby, & Gray, 2002). The metabolites of TbA can remain active for > 270 days in manure piles (Schiffer, Daxenberger, Meyer, & Meyer, 2001)

Zebrafish (*Danio rerio*) serve as an excellent model species for studies of developmental biology. A wealth of information exists on the well-orchestrated events that occur during its embryonic development, and a variety of genetic mutants are readily available for the analysis of genetic pathways (Rossant & Hopkins, 1992). An intricate network of developmental regulatory genes, which are expressed in dynamic and spatially restricted patterns in the developing fish embryo, are involved in the body pattern formation, cell proliferation, and cell death. Expression of regulatory genes, such as sonic hedgehog (*shh*), axial, evenskipped 1 (*eve1*), and notail (*ntl*), correlates with, and may be used as marker genes of, developmental events. Sonic hedgehog and axial, whose domain of expression is the central nervous system (CNS), are markers for neural development (Strähle & Blader, 1994). Both genes are involved in early neurogenesis, and their lack of expression in the generic mutant Cyclops, results in CNS malformations. Sonic hedgehog also plays a pivotal role in the induction of somite patterning and survival of myogenic lineages (Blagden, Currie, Ingham, & Hughes, 1997; Teillet et al., 1998). Evenskipped 1 is a marker for posterior cells and is expressed in the most posterior part of the extending tail tip (Joly, Joly, Schulte-Merker, Boulekbache, & Condamine, 1993). Absence of *eve1* expression in the caudal region of the genetic mutant *ntl*, during early somitogenesis, indicates the importance of this gene during tail extension. No tail expression is required for normal mesoderm development and extension of the body axis (Schulte-Merker et al., 1994).

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Furthermore, expression of *ntl* could be used as a marker for dorsoventral specification within the CNS and formation of the notochord.

2.2 Educational Review

Science education reform documents in several countries, including the United States, advocate changing science teaching in the classrooms from emphasis on using direct instruction to greater emphasis on inquiry-based one.

2.2.1 Scientific Inquiry

The *National Science Education Standards* reflect a vision of science learning as an active process of inquiry:

"Inquiry is a multifaceted activity that involves making observations; posing questions; examining books and other sources of information to see what is already known; planning investigations; reviewing what is already known in light of experimental evidence; using tools to gather, analyze, and interpret data; proposing answers, explanations, and predictions; and communicating the results. Inquiry requires identification of assumptions, use of critical and logical thinking, and consideration of alternative explanations." (National Research Council [NRC], 1996, p. 23)

Five essential features of classroom inquiry defined by the NSES can be used by teachers to evaluate in inquiry, specific to science, is occurring in their science classrooms. The learner should engage in scientifically oriented questions, give priority to evidence in responding to questions, formulate explanations from evidence, connect explanations to scientific knowledge, and communicate and justify explanations (NRC, 2000, p.29). For a science student, developing one's own question and the means to resolve the question suggests an inquiry experience that is profoundly different from the far more common task of science schooling which consist of answering questions prescribed in the curriculum using methods also preordained in the curriculum or by the classroom teacher (Windschitl, 2002).

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Science inquiry takes place when students generate questions, plan procedures, design and carry out investigations, analyze data, draw conclusions, and report findings (NRC, 1996; 2000). According to the *National Science Education Standards* (NSES): *"Scientific inquiry refers to the diverse ways in which scientists study the natural world and propose explanations based on the evidence derived from their works"* (NRC, 1996, p.23). Inquiry also involves higher order thinking about science such as searching for patterns, making models or simulations, and inventing original procedures. To promote science inquiry among students who may be less familiar with scientific practices, the instructional unit is designed to move progressively from more teacher explicit instruction to student-initiated exploration. To enable students to achieve the above the teacher needs adequate knowledge of science content and effective instructional strategies. Hands-on activity and laboratory-based instruction can be designed to help students learn about the nature of scientific inquiry (AAAS, 1989, 1993; Germann, Haskins, & Auls, 1996; Tamir, 1989). Hands-on, inquiry-based science instruction provides opportunities for students to develop scientific understanding, engage in inquiry, and construct shared meaning more effectively than traditional textbook-based instruction.

According to the NRC (1996, 2000), teachers of science must know that inquiry involves (a) the cognitive abilities that their students must develop; (b) an understanding of methods used by scientists to search for answers for their research questions; and (c) a variety of teaching strategies that help students to learn about scientific inquiry, develop their abilities of inquiry, and understand science concepts. However, there is a lack of agreement on the meaning of inquiry in the field of science education. (Martin-Hauser, 2002; Minstrell & van Zee, 2000 cites Barrow, 2006). For example, scientific inquiry is a way of investigating problems via the mind, the senses, and the mechanical or electronic extensions (Novak, 1964). Minstrell and van Zee (2000) cites Barrow, 2006 listed several different definitions of inquiry: encouraging inquisitiveness (habit of the mind), teaching strategy for motivating learning, hands-on and minds-on, manipulating materials to study particular phenomena, and stimulating questions by students.

To distinguish among various forms of inquiry practiced in classrooms, science education researchers have developed inquiry "continua," indexed by the

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degree of independence students have in asking and answering questions. The lowest levels of inquiry are confirmation experiences, often referred to as “cookbook labs,” in which students verify known scientific principles by following a given procedure. The next level is referred to as structured inquiry in which the teacher presents a question for which the students do not know the answer, and students are given a procedure to follow in order to complete the inquiry. In guided inquiry, teachers provide students with a problem to investigate but the methods for resolving the problem are left to the students. The open or independent inquiry teachers allow students to develop their own questions and design their own investigations (Germann, Haskins, & Auls, 1996; Herron, 1971; Schwab, 1962).

The deceptively minor differences between structured, guided, and open inquiry have, in fact, monumental implications for students’ practice. For example, guided inquiry in the classroom is far more intellectually challenging for learners and more pedagogically complex for teachers to manage than is structured inquiry. Because students who engage in guided inquiry must take the teacher’s question, design their own ways to collect data, and coordinate the data collection with analysis, the guided inquiry can be valuable experience in which learners come to understand, through first-hand experience, how evidence and argument are marshaled to support knowledge claims. In open inquiry experiences, the teacher may circumscribe a subject matter area for investigation, but otherwise the learner has a universe of possibilities from which to fashion a question. Crafting a question that is meaningful, consistent with existing theory, and testable is a complex. For this reason, open inquiry is yet a more challenging endeavor than guided inquiry for learners to participate in and for teachers to facilitate (Windschitl, 2002).

Inquiry was studied from two dimensions: the content for teachers and their students and the strategy used by science teachers to help their students learn science (Harms & Yager, 1981 cites Barrow, 2006). Nevertheless, there are some obstacles to using inquiry in science classrooms. Teachers perceive inquiry activities as assurance for textbooks, or as preparation for final exams, and these activities are teacher-centered rather than student-centered. When open types of inquiries were administered, results indicated that students did not usually progress to a higher level of process skills (Gabel, 2001). In the schools, science laboratories are being used less

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and less in the acquisition of genuine practical inquiry skills. Students work too often in the laboratory as technicians following "cookbook recipes," and they are unable to meaningfully summarize the important aspects of an experiment they have just completed (Bell, Blair, Crawford, & Lederman, 2003; Germann, Haskins, & Auls, 1996; Tamir & Lunetta, 1981). Welch and colleagues (1981) identified factors that prevented science teachers from using inquiry. These include ; teachers' lack of understanding of inquiry, time constraints, limited available teaching materials, lack of support, lack of classroom management skills, and curricula and syllabi. There are three factors identified by Eltinge and Roberts (1993) to explain teachers' exclusion of inquiry from their teaching; state documents emphasizing content, easier to access content, and textbooks' emphasis of science as a body of knowledge.

However, several studies show the benefits of using inquiry in science classrooms. Harms and Yager (1981) cites Barrow (2006) identified three benefits derived from teachers' implementation of inquiry into their teaching. These benefits are; (a) students could acquire and master science process skills, (b) students could develop their understanding of the nature of scientific inquiry, and (c) students could become proficient in their uses of an inquiry process. Siebert (2001) cites Barrow (2006) recommended that laboratory experience should foster inquiry, rather than being used to confirm scientific phenomena. This can be accomplished when the research-oriented laboratory experiences involve group work and are open ended and long term, rather than single period.

Inquiry-based instruction as a model for pedagogy has the potential to enhance student understanding and engagement in science (Capps & Crawford, 2009; DeBoer, 2006). However, most teachers do not use inquiry-based instruction in the classroom due to a number of issues e.g., perceived time constraints regarding high-stake testing; unfamiliarity with how science is practiced; inadequate preparation in science; misunderstanding of the purposes of inquiry or misconception about exactly what inquiry is (Bybee, 2000; Costenson & Lawson, 1986; Welch, Klopfer, Aikenhead, & Robinson, 1981). To achieve student learning outcomes in science understanding, inquiry, and discourse, teachers need adequate knowledge of science content, and effective instructional strategies/teaching practice including teacher efficacy (Gusky, 2002; Keys & Bryan, 2001; Lee, Hart, Cuevas, & Enders, 2004).

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Hands-on activity and laboratory-based instruction can be designed to help students learn about the nature of scientific inquiry (American Association for the Advancement of Science [AAAS], 1989, 1993; Germann, Haskins, & Auls, 1996; Wilson & Chalmers-Neubauer, 1990). Hands-on, inquiry-based science instruction provides opportunities for students to develop scientific understanding, engage in inquiry, and construct shared meaning more effectively than traditional textbook-based instruction; however, science supplies are not always available in elementary schools with limited funding and resources. Laboratory instruction is central to the teaching and learning of science. Shulman and Tamir (1973) have identified the goal of laboratory instruction as: (a) to arouse and maintain interests, attitude, satisfaction, open-mindedness, and curiosity in science; (b) to develop creative thinking and problem-solving ability; (c) to promote aspects of scientific thinking and the scientific methods; (d) to develop conceptual understanding and intellectual ability, and (e) to develop practical abilities, e.g., designing and executing investigations, observing, recording data, analyzing, and interpreting results. Based on the foregoing, it can be concluded that the laboratory provides a unique medium for teaching the student how the scientist works.

2.2.2 Science Process Skills

Science is more than a body of facts, a collection of principles, and a set of tools for measurement. Science is a structured and directed way of asking and answering questions. It is a pedagogical feat to teach students the facts of science and technology; it is a pedagogical triumph to teach students these facts in their relation to the procedures of scientific inquiry. The process of scientific inquiry learned not as a set of rigid rules but as a ways of finding answers, can be applied without limit. To be effective, methods for testing student achievement must provide students with hands-on materials and the opportunity to demonstrate their use of science process skills.

For the past several decades, science educators have focused attention on the basic and integrated science processes skills as important to an understanding of science as inquiry. There are many research studied on science process skills assessment instruments and rubrics for a performance assessment of science process skills regarding inquiry activity (Germann & Aram, 1996; Germann, Aram, & Burkel,

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1996; Tamir & Lunetta, 1981; Scharmann, Harty, & Holland, 1986). Classroom studies of scientific reasoning in science education have centered around the basic and integrated science process skills as key elements in inquiry. Research and development has included the identification of these skills (Dillashaw & Okey, 1980; Livermore, 1964; Tamir & Lunetta, 1981), development of strategies and curricula for implementing these skills in the teaching-learning process, evaluation of lab manual for inquiry and science process skills (Germann, Haskins & Auls, 1996), and assessment of student efforts in the laboratory and acquisition of process skills (Dillashaw & Okey, 1980). For example, Germann, Aram, and Burke (1996) developed the Science Process Skills Inventory (SPSI) from student responses to the Alternative Assessment of Science Process Skills (AASPS) developed by the Missouri Department of Education and the Department of Education Assessment. The SPSI was used to analyze student efforts at writing experimental designs. The results indicated that the integrated science process skills are considered to serve as scaffolding for the process of scientific inquiry.

Science process skills are inseparable in practice from the conceptual understanding that is involved in learning and applying science. Nevertheless, it is useful to identify and discuss the skills which can apply to different subject-matter because of their central role in learning with understanding, whether in formal education or throughout life (Harlen, 1999). However, several studies show that the lack of those skills is part of a more general and widespread problem. For example, Foulds and Rowe (1996) found that science process skills of primary teacher education students in Western Australia are often poorly developed. In a study of first year tertiary students Moneira (1980) claims that many students cannot identify the basic phenomena and even the basic question involved in experiments. He suggests that they see experiments merely as using equipment rather than as a process of generating information.

Despite the apparent failure of much of contemporary science teaching to impart science process skills to students there is evidence that the appropriate kind of instruction can be successful. Roth and Roychoudhury (1993) worked with year 8 general science students and year 11 and 12 physics students in what they describe as open-inquiry laboratory sessions. They found that students develop higher-order

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process skills through non-traditional laboratory experiences that provided the students with freedom to perform experiments of personal relevance in authentic contexts.

Radford and colleagues (1992) assess the science process skills achievement of preservice elementary teachers on their students. The results of the analysis of preliminary practical performance test and the attitude survey for preservice teachers show the statement that performance assessment gives a truer picture of a student's understanding of science concepts than does a paper-and-pencil test. Woolnough and Allsop (1985) argue that the development of science process skills is a valid aim for science laboratory work. The study on using experiential research projects to promote science learning facilitates not only the mastery of sophisticated subject mater, but also the development of process skills (DebBurman, 2002). Dirks and Cunningham (2006) enhance diversity in science by helping students succeed in the rigorous introductory biology classes and motivating them to engage in undergraduate research.

Classroom studies of scientific reasoning in science education have centered around the basic and integrated science process skills as key elements in inquiry (Dillashaw & Okey, 1980; Tamir & Lunetta, 1981). Research works have also included the development of strategies and curricula for implementing these skills in the teaching-learning process, evaluation of lab manual for inquiry and science process skills (Germann, Haskins, & Auls, 1996), and assessment of student efforts in the laboratory and acquisition of process skills (Dillashaw & Okey, 1980). In reality, science process skills are inseparable from the conceptual understanding involved in learning and applying science. Science process skills are defined as a set of broadly transferable abilities, appropriate to many science disciplines and reflective of the behavior of scientists. The AAAS (1975) categorized those of skills into two types, basic (observing, classifying, measuring, inferring, predicting, communicating, and using number relationships) and integrated (making models, defining operationally, collecting data, interpreting data, identifying and controlling variables, formulating hypotheses and experimenting). Several studies have differentiated basic and integrated skills. For example, Tomera (1974) defined basic skills as the skills that could be taught. More importantly, once students acquired and mastered these skills, students then could use such skills to underlie and develop integrated skills.

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Implementation of science process skills in teaching will make learning experiences not only richer but also more meaningful for students. This is because students will acquire both science skills and science content. As a consequence, they will acquire a deeper and better understanding of such a content, resulting in positive attitudes towards science. To accomplish these skills, science educators generally view that the inquiry approach is the most important strategy of science learning. This approach can be effective in developing problem-solving ability by training students to construct their own knowledge through investigation. The students should also be provided with hands-on materials and the opportunity to demonstrate their use of science process skills. It is important to note that while one process skill is being appraised for mastery in each activity, the student is often demonstrating mastery of several process skills at once (Marzano, Pickering, & McTighe, 1993; Ostlund, 1992).

2.2.3 Professional Development

Currently science education reform tends to emphasize the crucial role of professional development for the improvement of achievement in science students. There are professional development programs which have resulted in changes in the classroom practices of teachers, in their attitudes and beliefs, and in the learning outcomes of students (Capps & Crawford, 2009; Guskey, 2002; Supovitz & Turner, 2000). These learning outcomes encompass not only criteria about cognitive skills and achievements, but also a wide range of student behavior and attitudes. Also close collaboration between the program developer / researcher and teacher can greatly facilitate this process (Ward & Tikinoff, 1982). The evaluation of the impact of a program should not only be in terms of meeting the developers' objectives, but also the extent to which it moves teachers' practices towards those proved to lead to effective teaching. Four of the impacts were on: (a) teachers' knowledge; (b) teachers' practice; (c) student learning outcomes; and (d) teachers' efficacy (Ingvarson, Meiers, & Beavis, 2005).

Professional development programs are systematic efforts to bring about change in the classroom practices of teachers, in their attitudes and beliefs, and in the learning outcomes of students (Guskey, 2002). At stated earlier, the three major goals of professional development programs are change in the classroom practices of

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teachers, change in their attitudes and beliefs, and change in the learning outcomes of students. Professional development programs based on the assumption that change in attitudes and beliefs comes first are typically designed to gain acceptance, commitment, and enthusiasm from teachers and school administrators before the implementation of new practices or strategies.

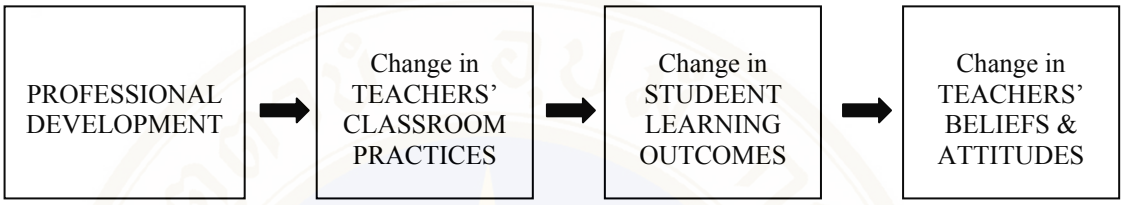

Figure 2.2 A Model of Teacher Change. (Guskey, 2002)

The 'Model of Teacher Change' shown in Figure 2.2 presents an alternative approach. This model suggests a different sequence among the three major outcomes of professional development. According to the model, significant change in teachers' attitudes and beliefs occurs primarily after they gain evidence of improvements in student learning. These improvements typically result from changes teachers have made in their classroom practices—a new instructional approach, the use of new materials or curricula, or simply a modification in teaching procedures or classroom format. Learning outcomes are broadly construed in the model to include not only cognitive and achievement indices, but also the wide range of student behavior and attitudes. In other words, learning outcomes include whatever kinds of evidence teachers use to judge the effectiveness of their teaching. Close collaboration between program developers / researchers and teachers can greatly facilitate this process and can be accomplished in a variety of ways (Ward & Tikinoff, 1982).

Effective professional-development activities concentrate both on teachers' knowledge of specific subject matter content and their understanding of how students learn that content. Innovative use of hands-on instruction in science is also deemed important (Garet, Porter, Desimone, Birman, & Yoon, 2001; Wilson & Chalmers-Neubauer, 1990). Several research works about teacher professional development programs emphasized teacher's initial beliefs and practices about inquiry-based science to assess the impact of the professional development intervention on gradual changes in teachers' beliefs and practices related to inquiry-

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based science (Crawford, 2000, 2007; Lee, Hart, Cuevas, & Enders, 2004; Supovitz, Mayer, & Kahle, 2000).

Many studies on the professional development programs showed subsequent significant success stories in teaching and learning of science in the classroom. The trainee teachers are thereafter able to provide opportunities for their students to conduct the inquiry-based learning. The experience in project-based inquiry science instruction should help teachers in teaching science such as promoting science content understanding, relating it to daily life, science inquiry skills, science process skills, greater cognitive involvement and improved thinking ability including problem solving skills (Banilower, Heck, & Weiss, 2007; Geier et al., 2008; Jeanpierre, Oberhauser, & Freeman, 2005).

2.2.4 Zebrafish Research in Education

Model organisms such as chicks, mice, and rats are difficult to work with in a classroom setting because they introduced prohibitive costs, require special equipment, and present complication regarding animal right issues. Zebrafish are ideal for experimental studies in the classroom because, in contrast to chicks or mammals (mice and rats), fish embryos are relatively easy and inexpensive to maintain, and embryonic development can be observed with common classroom equipment (Fields et al., 2009).

Zebrafish are now being used with more frequency in classroom to teach biological concepts, i.e. embryology, developmental biology, genetics, molecular and cell biology, comparative anatomy, physiology, neurobiology, and environmental science. Mostly, teaching and learning dealt with zebrafish are using the traditional way. Although there are several laboratory exercises related to those of concepts, they are only laboratory manual for conducting the experiment.

Currently, several works on using zebrafish in science classroom aim at promoting not only understand the scientific concepts, but also conduct science experiment using scientific method, science process skills including scientific inquiry (D' Costa & Shephred, 2009; Fields et al., 2009; McKeown, Downes, & Hutson, 2009; Schmoldt, et al., 2009). For example, Bagatto (2009) used two guided inquiry exercises for high school and/or college level courses. Fields and colleagues (2009)

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presented eight student-designed laboratory exercises that had been used successfully by students in middle school through high school. By designing their own experiments, making careful observations and hypotheses, and drawing conclusions from experimental results on zebrafish, students gained a deeper understanding of the scientific method than was possible from the following prescribed laboratory exercises. Moreover, Shen and colleagues (2009) demonstrated that zebrafish provide an excellent model system to teach engineering principles. A-seven member undergraduate team in a biomedical engineering class designed, built, and tested a zebrafish microfluidic bioreactor applying microfluidics, an emerging engineering technology, to study zebrafish development.

Furthermore, there are several outreach programs promote science education using zebrafish to students, teachers, and general public, i.e. Project BioEYES Outreach Program which fosters an enthusiasm for science education, promotes interest for future participation in a biology-related field, and allows students the opportunity to life science through a hands-on, student-centered approach to instruction (Aoki, 2009; Shuda & Kearns-Sixsmith, 2009).

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CHAPTER III
METHODOLOGY

Overview

This chapter describes methodology used to conduct the research to answer the research questions posed earlier. This chapter involves two aspects: science and education. It begins with the development of an aquatic toxicology experiment on the effects of endocrine-disrupting chemicals (EDCs) related to concentrated animals feeding operations (CAFOs) on zebrafish embryonic development. Then, the development of the learning unit as applied from scientific results, and the implementation of the unit are described. Next, the participants involving this research are specified. Finally, the methods/tools used for collecting and analyzing data are described.

3.1 Scientific Aspect

3.1.1 Development of an Aquatic Toxicology Experiment on Zebrafish Embryos: Effects of Endocrine Disrupting Chemicals (EDCs) on Zebrafish Embryonic Development

The experiment on the effects of endocrine-disrupting chemicals (EDCs) related to concentrated animals feeding operations (CAFOs) on zebrafish embryonic development was used in this study. The 4-day embryo-larval zebrafish test, from early gastrula to hatching stage, was developed. The observation on embryonic development was made at different stages, for which morphological, physiological, and behavioral endpoints were selected and quantified for unexposed and exposed embryos (Nagel, 2002; OECD 210, 1992b). The development of zebrafish embryos was observed until the 72-96 h post fertilization (hpf). Normally at this time all of the

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normal condition/non-exposed embryos are being hatched. Lethal, sublethal (heart/pericardial edema, spontaneous movements, and hatching rate/time disturbance), and teratogenic effects were detected for all hormones studied as described by Nagel (2002).

3.1.1.1 Zebrafish Maintenance and Embryo Collection

The zebrafish broodstock were obtained from the Pharmaceutical Science Division, Department of School of Pharmacy, University of Wisconsin-Madison, USA. They were reared and maintained as described by Westerfield (1995). The filtered flow-through water was supplied for the laboratory system. Fish were reared in aquaria containing 100 L of continuously flow through with filtered-water. The water temperature was kept at 28 °C. Adult fish were fed 3 times a day, 7 days a week, with dry flake and food supplemented with live brine shrimps nauplii (*Artemia salina*) in the evening. To ensure optimal water quality, remaining food should be removed daily. The 6-month to 1.5-year old broodstocks were used for spawning in this study (2 males and 1 female). Plastic containers (12 x 20 cm) equipped with a mesh bottom to protect the eggs from being eaten were placed at the bottom of 100 L spawning tank after group mating (8-10 males: 5 females). The spawning and fertilization take place within 30 minutes after light was turned on in the morning. The zebrafish eggs were collected and removed from the spawning tank about 30-60 min after spawning.

The fertilized eggs were separated from the unfertilized and placed in the 90 mm Petri dishes with a pasture pipette under a stereomicroscope. After eggs collection and rinsing with egg water (see Appendix A), the fertilized eggs were randomly distributed into 90 mm Petri dishes containing embryo medium (see Appendix B). Then, each embryo was transferred into each well of the 96-well plate. The zebrafish embryos at 6-8 hpf (30-60% epiboly or the early gastrula stage) were exposed to the tested hormones. The plates were kept in an incubator at constant temperature of 28 °C during the 4-day experiment period.

3.1.1.2 Embryo in the Medium Containing Steroid Hormones

The six sex-steroid hormones; trenbolone acetate (TbA), progesterone (P4), 17α, 20β-dihydroxyprogesterone (DHP), 17β-estradiol (E2),

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testosterone (T), and 4-androstene-3, 17-dione (A4) were administered to the fish embryos at the stage of 6-8 hpf. The tested concentrations were 0, 5, 50, 500, 5000 ng/mL. Then, solutions were stored at 4 °C in the dark for the duration of the experiment.

3.1.1.3 Toxicity Assay on Embryo

The embryo test procedure is as described by Schulte and Nagel (1994) and Nagel (1998). The toxicity of chemical substances was determined using 96-well plates. A stock solution of the test substance, at five concentrations (0, 5, 50, 500, 5000 ng/ml) were tested on 24 embryos for 4 days. The fertilized eggs were placed individually in 96-well plates filled up with 200 µL tested hormone solutions. Then, the plates were covered with a lid and incubated at 28 °C for 4 days. Lethal, sublethal and teratogenic endpoints were determined using a stereomicroscope within 96 hpf. In this study, the embryogenesis was divided into five observation periods (6-8, 24, 48, 72, and 96 hpf). The medium was totally changed daily. The embryos were classified into lethal, sublethal, and teratogenic as proposed by Nagal (2002). Developmental and morphological changes in terms of cell division, segmentation, and organ formation were recorded. The distinction between normal and abnormal development was compared using the zebrafish embryogenesis description published by Nagel (2002), Kimmel et al. (1995) and Westerfield, (1995) as shown in Table 3.1 and Figure 3.1-3.4. After each observation, dead embryos are counted and then removed.

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Table 3.1 Stages of Embryonic Development of the Zebrafish (*Danio rerio*) at 26±1 °C (Nagel, 2002)

Time (h)	Stage	Characterization
0	Fertilization	Zygote
0	Zygote periods	Cytoplasm accumulates at the animal pole, one-cell stage
0.75	Cleavage period	Discoidal partial cleavage;
		1. median vertical division: two-cell-stage
1		2. vertical division: four-cell-stage
1.25		3. vertical and parallel to the plane of the first: eight-cell-stage
1.5		4. vertical and parallel to the second plane of division: 16-cell-stage
2	Blastula period	Start of blastula stage
3		Late cleavage; blastodisc contains approximately 256 blastomeres
4		Flat interface between blastoderm and yolk
5.25	Gastrula period	50 % of epibolic movements; blastoderms thins and interface between periblast and blastoderm become curved
8		75% of epibolic movement
10		Epibolic movement ends, blastopore is nearly closed
10.5	Segmentation period	First somite furrow
12		Somites are developed, undifferentiated mesodermal component of the early trunk, tail segment or metamere
20		Muscular twitches; sacculus; tail well extended
22		Site to side flexures; otoliths
24	Pharyngula period	Phylotypic satge, spontaneous movement; tail is detached from the yolk; early pigmentation

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Table 3.1 (cont.) Stages of Embryonic Development of the Zebrafish (*Danio rerio*) at 26±1 °C (Nagel, 2002)

Time (h)	Stage	Characterization
30		Reduced spontaneous movement; retina pigmented, cellular degeneration of the tail end; circulation in the aortic arch 1
36		Tail pigmentation; strong circulation; single aortic arch pair, early mortality; heart beating starts
72-96	Hatching period	Heart-beat regularly; yolk extension beginning to taper; dorsal and ventral stripes meets at tail; segmental blood vessels: thickened sacculus with two chambers; foregut development; neuromasts

The normal development of zebrafish embryos in terms of developmental period / age, and characterization are described as follow:

Day0 (6-8 hpf): 50-60% epiboly-early stage of gastrula

Day1 (24 hpf): head, eyes, and otolith formed, extended tail, developed somites

Day2 (48 hpf): organ formation, yolk sac, heart beat, blood circulation, pigmentation-start hatching

Day3-4(72-96 hpf): newly hatched larvae; developmental of heart (well developed), heart rate, body and yolk sac (size and shape), larval size

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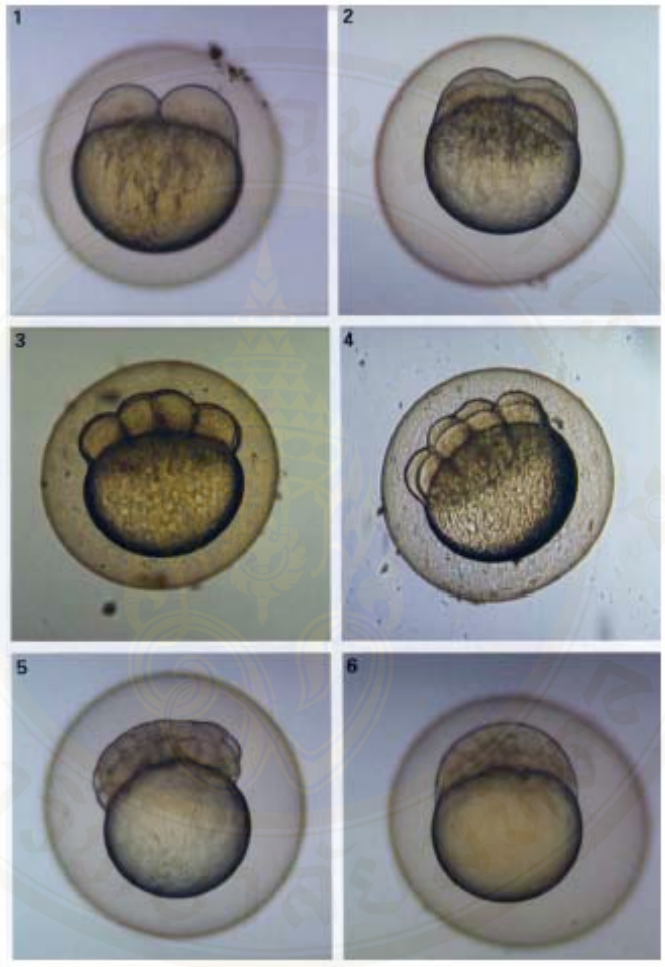

Figure 3.1 Normal development of zebrafish (*Danio rerio*) embryos I: (1) 0.75 h; (2) 1 h; (3) 1.2 h; (4) 1.5 h; (5) 4.7 h; (6) 5.3 h. (Braunbeck & Lammer, 2006)

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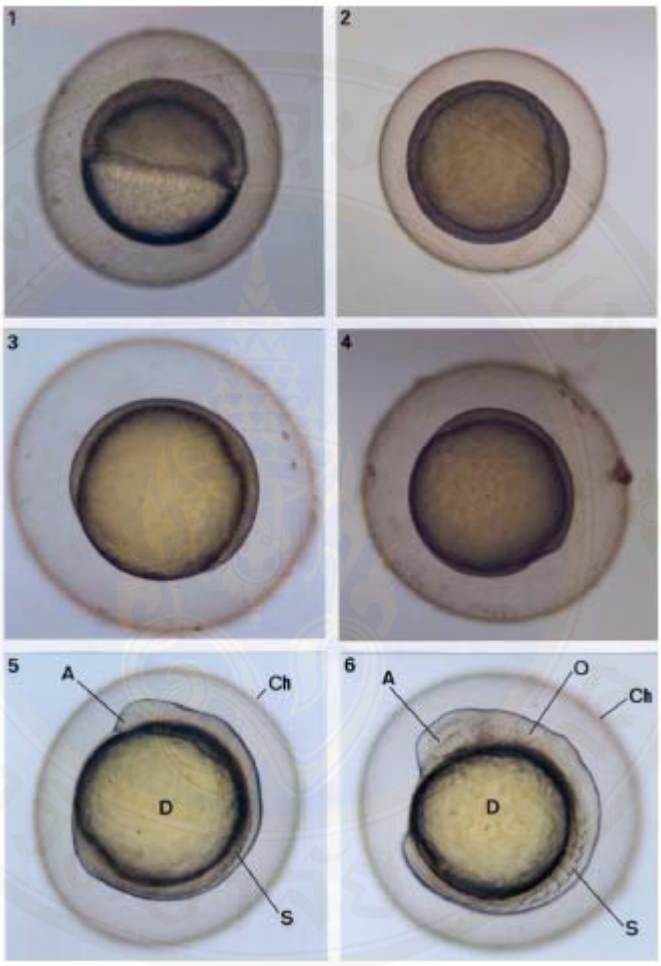

Figure 3.2 Normal development of zebrafish (*Danio rerio*) embryos II: (1) 6 h; (2) 6 h; (3) 8 h; (4) 9 h; (5) 12 h; (6) 14 h. A -- eye anlage; Ch – chorion; O – ear bud; S – somites (muscle segments). (Braunbeck & Lammer, 2006)

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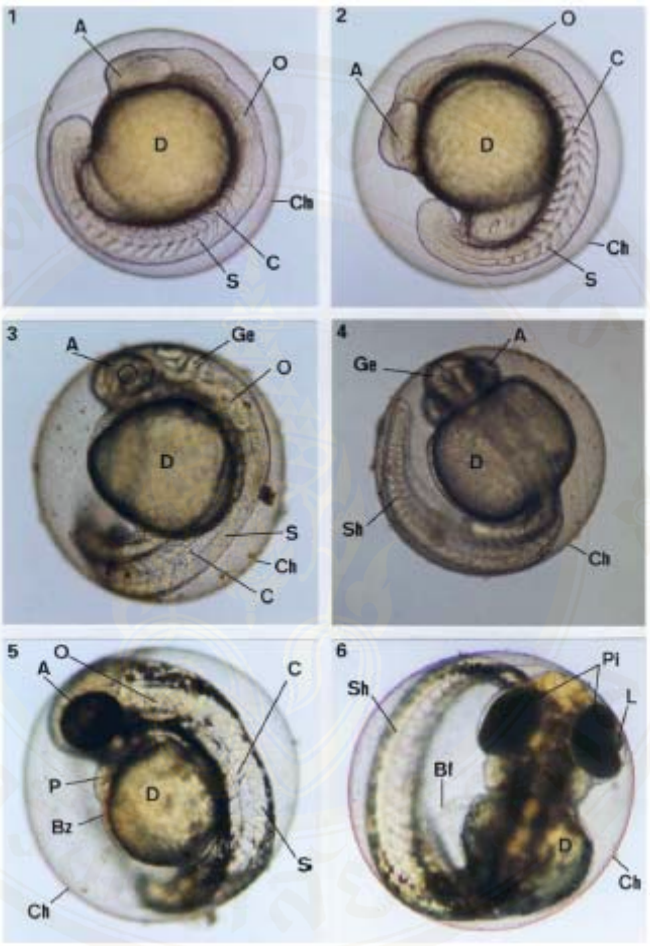

Figure 3.3 Normal development of zebrafish (*Danio rerio*) embryos III: (1) 16 h; (2) 18 h; (3) 25 h; (4) 25 h; (5) 48 h; (6) 72 h. A -- eye anlage; Bf – pectoral fin; Bz – blood cells; C – chorda; Ch—chorion; Ge—brain analage; L—optical lens; O—ear bud; P—pericard; S--somites. ; Sh—tail. (Braunbeck & Lammer, 2006)

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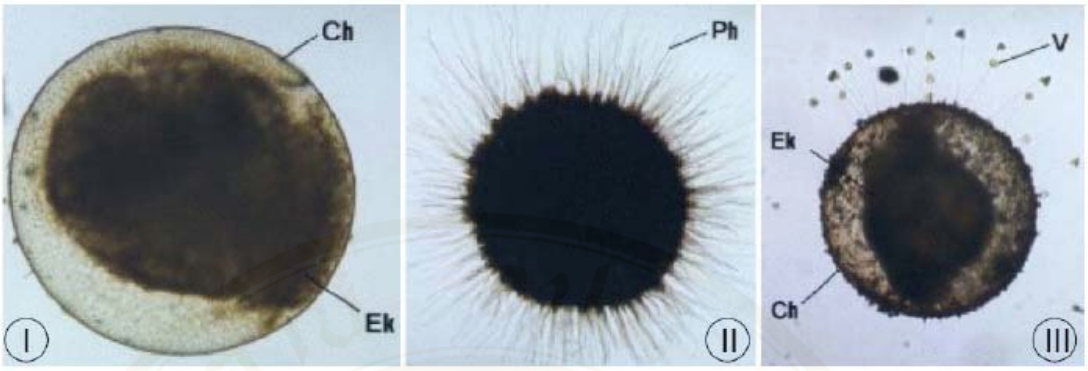

Figure 3.4 Mortality in early zebrafish (*Danio rerio*) eggs: coagulation (I), heavy infestation with fungi (II) and invasion of *Vorticella* spec. (V; III). Ch – chorion; Ek – coagulated egg; Ph – fungi. (Braunbeck & Lammer, 2006)

The observations were performed directly in each well using a stereomicroscope at x 60 magnification. Changes of embryonic development from the early stage of gastrula (6-8 hpf) to the hatching stage (72-96 hpf) was determined. The dead embryos were judged by detected lack of heart beat. The developmental status of zebrafish embryos were observed with a stereomicroscope at specific time after fertilization (time points = 8, 24, 48, 72 and 96 hpf). The various developmental endpoints used for assessing toxicity were recorded. Embryos mortality (the embryo with no heart beat), cardiac/pericardial edema, hatching rate, delayed hatching, body and yolk sac deformations were also described (Table 3.2).

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Table 3.2 Lethal and Sublethal Endpoints for Evaluating the Toxicity and Teratogenicity of Chemicals on the Embryo of *Danio rerio* (Schulte and Nagel 1994; Nagel, 1998)

Toxicological endpoints	**Exposure time (h)**			
	8	24	48	120
Lethal*				
Coagulation	•	•	•	
Tail not detached		•	•	
No somites		•	•	
No heart-beat			•	
Sublethal/Development				
Completion of gastrula	•			
Formation of somites		•		
Development of eyes		•	•	
Spontaneous movement		•	•	
Heart beat/blood circulation			•	
Pigmentation				
Edema			•	
Teratogenic				
Malformation of head		•	•	
Malformation of sacculi/otolith		•	•	
Malformation of tail		•	•	
Malformation of heart		•	•	
Modified structure of the corda		•	•	
Scoliosis		•	•	
Rachischisis		•	•	
Deformity of yolk		•	•	
Growth-retardation		•	•	
Length of tail**				•

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*After 48h the four endpoints were assessed to be lethal. Within the teratogenicity test for a better comparison with mammalian data only the endpoint “coagulated” is used as a lethal effect.

** There is the option to measure the length of tail after 120h. In this case the eggs are transferred into water without the test compound after 48h. After natural hatching the larvae is straightened and the length can be determined.

3.2 Educational Aspect

This research was designed to investigate changes in classroom practice of the teacher as a result of her participation in the project. The study comprised three phases: development of the inquiry-based learning unit, teacher training and implementation of the learning unit.

3.2.1 Participants

3.2.1.1 Students

The 12 students, eight females and four males, participating in this study were 11-12 graders from a high school located in the Midwest of the United States of America. They were divided into four groups of three students each. They carried out the inquiry-based experiment and the science project. The students were asked to respond freely through self-assessment on content understandings, skills, activities, and attitudes. They also presented their science project findings.

3.2.1.2 Teacher

The teacher in this study has a chemistry background. She has been teaching biology and environmental courses for a few years. She has no experience in using inquiry-based project for teaching science. However, she had a strong intention of providing her students with a conceptual understanding of science and tried to emphasize how it is applied to the real world situations. The researcher helped her in designing and evaluating the inquiry-based instructional unit on aquatic toxicology.

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3.2.2 Development of the Learning Unit

The researcher helped the teacher in developing the inquiry-based learning unit for environmental science course according to the Science Education Standards documents (AAAS, 1989, 1993; NRC, 1996, 2000). The learning unit (Table 3.3) consisted of seven topics to be covered in seven weeks (4-5 days per week). The first three topics were taught by traditional lecture and field trip whereas the other four topics were inquiry-based consisting of lecture, hands-on activity and science project. The experiment on the effects of endocrine-disrupting chemicals (EDCs) related to concentrated animals feeding operations (CAFOs) on zebrafish embryonic development was used in the hands-on activity.

3.2.3 Teacher Training

The teacher was trained to ensure effective conducting of the inquiry-based learning unit, both in pedagogy and content, including how to properly utilize the scientific tools in aquatic toxicology experiment e.g. micropipettes, a stereoscope. The training also included how to design the experiment on the effects of toxins and pollutants on zebrafish embryos. Moreover, the training focused on how to promote students initiatives and in conducting the inquiry, how to reduce the level of guidance for students (NRC, 1996, 2000).

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Table 3.3 Outline of the Teaching-Learning Sequence on Environmental Science

Duration	Content	Teaching and Learning Activity	Instrument/Assessment
3 weeks	1. Knowledge of water cycle 2. Knowledge of watershed 3. Environmental legislation	Traditional teaching :Lecture :Field trip	-Student self-assessment -Teacher reflection
1 week	4. Effects of agricultural practices on ecosystem	Guided inquiry-based learning: :Inquiry lecture -posing questions :Hands-on activity -using a stereoscope to observe normal and abnormal zebrafish embryonic development :Student discussion	-Student self-assessment -Teacher reflection -Classroom observation
1 week	5. Use of organisms to indicate the effects of pollutants	Guided inquiry-based learning: :Inquiry lecture -posing questions :Guided inquiry-based experiment on using of organisms to indicate the effects of pollutants :Student discussion	-Student self-assessment -Teacher reflection -Classroom observation
1 week	6. Effects of toxins and other factors on organisms	Guided inquiry-based teaching: :Inquiry lecture -posing question :Guided inquiry-based experiment on toxins and other factors affecting organisms :Student discussion	-Student self-assessment -Teacher reflection -Classroom observation
1 week	7. Effects of consumer products on ecosystem	Open inquiry: :Student designed and conducted their own science project :Students' presentation and discussion	-Student self-assessment -Student assessment by the teacher -Teacher reflection -Classroom observation

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3.2.4 Implementation of the Learning Unit

The 12 high school students enrolled in the environmental science course were given the guidelines for the aquatic toxicology laboratory. They were trained how to use certain scientific apparatuses and techniques in observing normal and abnormal zebrafish embryos. Then, the teacher encouraged students to apply the knowledge learned in designing their own projects on effects of chemicals/toxins commonly found in daily life on zebrafish embryos.

In the learning unit, the students have to observe abnormal development of zebrafish (*Danio rerio*) due to farm chemicals, e.g. sex hormones in their early life stages. They compared the normal and abnormal embryonic morphology and internal organs and functions by themselves with the teacher as a guide. They also tried various household products at selected concentrations on the early development of the zebrafish. The learning unit was divided into three experiments as following.

Experiment I: Students Observing Normal Development

The teacher asked the students to observe and learn from observation about the stages of the zebrafish embryonic development from fertilization (hpf) to hatching. Developmental and morphological changes in terms of cell division, segmentation, and organ formation were recorded in the laboratory note/report and compared with photographs in the textbooks and internet sources, e.g. Kimmel et al (1995) and Westerfield (1995).

Experiment II: Students Perceiving Abnormal Growths

Students were instructed to put the zebrafish embryos at gastrula stage (6-8 hpf) and segmentation period (18-24 hpf) into the wells containing various concentrations of sex hormones commonly used for promoting growth in cattle and/or detected in the waste water, e.g. 17β-trenbolone, 17 β-estradiol. For three to four days, students spent about 40-50 min period each day to observe the anatomical change in the control (without hormone) and the experimental fish (with 0-500 ng/mL of hormone). External and internal deformations (Figure 3.5 and 3.6) in the zebrafish embryos and larvae could be seen for those exposed to the hormone whereas normal body and internal organs were observed for the control ones. The part of experiment lasted one week during which the teacher also used educational instruments to monitor

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students' progress in laboratory skills, observation, communication and conceptualization.

Experiment III: Students Applying Knowledge Learned

Students used their knowledge about appearance of fish at different developmental stages and the effects of commercial animal hormones on external and internal parts of the fish to design their own experiments: in this they had to predict (based on their hypothesis), observe and explain. They brought household products, e.g. detergent, floor and window cleaner, diluted in water to see the latter's effects on growth, development and survival. The students followed the fish embryonic development in the same manner as in the Experiment II. Here they could observe better and compare the effects with the normal fish in the photographs. Apart from parts of the anatomy these fish were studied also for physiological function, e.g. heart function and swimming and even death.

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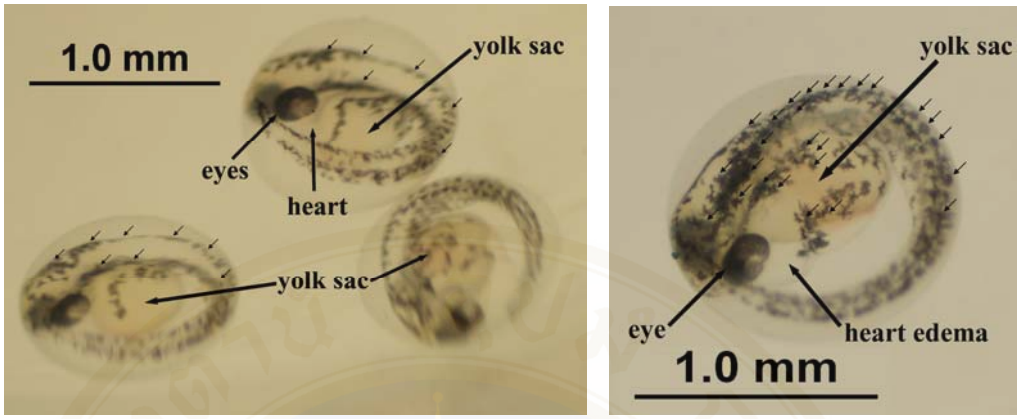
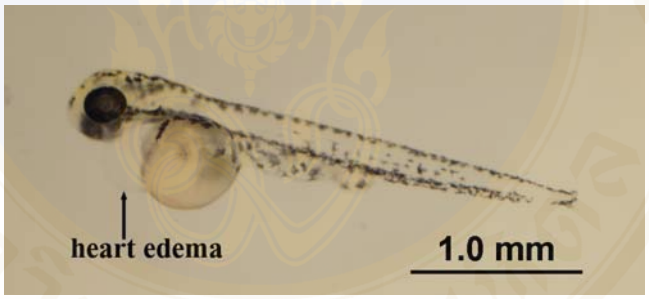
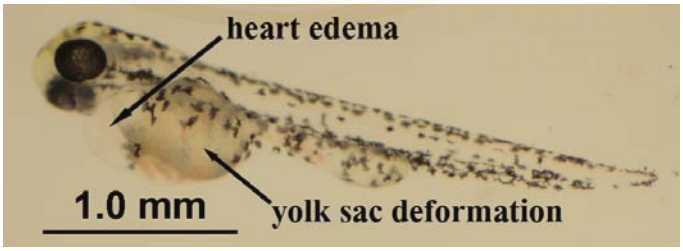


(a) (b)

Figure 3.5 Comparison of number of pigment spots (pointed by small arrows) on the body of zebrafish embryos before hatching (36 hpf) (a) normal (b) abnormal (many more spots)



(a)



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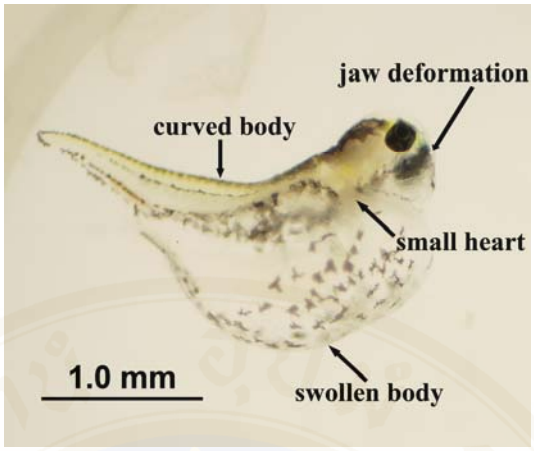

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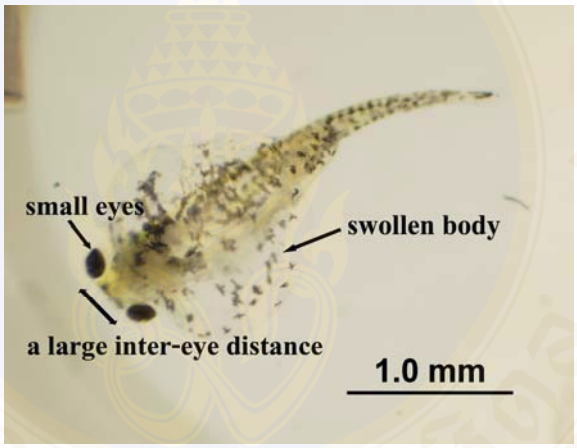

(d)

Figure 3.6 Newly hatched zebrafish larvae (6-8 h after)

(a) Nearly normal appearance except for a relatively larger heart

(b) A larva at 6-8 h after hatching from the eggs showing swollen pericardium and yolk sac deformation; head also abnormally large

(c) Side view of a larva (6-8 h after hatching) having abnormal body shape (curved or bent), abnormal appearance of upper and lower jaws which show deformation. Some fish are jawless, have a small heart where the two chambers cannot be distinguished clearly.

(d) Top view (dorsal view) of a larva (6-8 h after hatching) showing distorted and swollen body, and yolk sac deformation

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3.2.5 Data Collection and Analysis

The teacher achievement in this study was based on changes in her classroom practices, students learning outcomes and attitude. A mixed method was employed in this study; a quantitative approach to generate numerical data and a qualitative approach for in-depth data. Criteria for the students learning outcomes were student knowledge, scientific inquiry, science process skills, collaborative learning, and student attitudes. The instruments used were student self-assessment, students' science project quality, teacher assessment of student science process skills, teacher's and students' reflections, teacher interview and classroom observations. The data were analyzed using means statistics and descriptive. These multiple data sources enabled triangulation of the results.

3.2.5.1 Student Self-Assessment

The students were asked to respond to three sets of questions: understanding of unit contents, science process skills in terms of basic and integrated skills, and the attitude toward the learning unit. The students were asked to rate their own knowledge, skills, and attitudes by using a 5-point Likert system ranging from 5 (excellent) to 1 (poor). The questionnaire for students' reflection was administered at the end of the course.

3.2.5.2 Students' Science Project

Groups of three students designed and carried out their own inquiry-based science projects based on the knowledge learned in the previous sessions. Their results were shown by using both poster and oral presentation. They were encouraged to share scientific ideas and discuss to the class. The poster and oral presentations were evaluated by using scoring rubrics adapted from DebBurman (2002) and Allen and Tanner (2006). The 5-point Likert scale ranging from 5 (excellent) to 1 (poor) was used.

3.2.5.3 Teacher Reflection

The teacher was asked to answer open-ended questions on student understanding, students' learning attitude, the improvement of student performance, and teacher's attitude toward the teaching practices. These data were used to support the effectiveness of the learning unit as well as teacher professional development.

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3.2.5.4 Teacher Interview

The interview questions examined the teacher's conceptions of the goal of the learning unit, particularly on how to promote science inquiry with students. The interview focused on the teacher's experiences in inquiry strategy, her beliefs in teaching methods, her attitudes toward the new teaching experience, and how her idea change during the course. The teacher was interviewed weekly at the end of each topic. The four aspects of impact on professional development, teacher's knowledge, teacher's practice, student learning outcomes and teacher efficacy, were the main concern of the interviews (Guskey, 2002; Ingvarson, Meiers, & Beavis, 2005).

3.2.5.5 Classroom Observation

The classroom observation provided a way to evaluate how the inquiry-based learning unit was effectively used in a classroom. The classroom observation was designed to assess student learning outcomes and teaching practices (Abraham, 1982; Germann & Aram, 1996; Webb, 1997; Wilson & Chalmers-Neubauer, 1990).

3.3 Experimental Animal Ethics

In recent years acute toxicity tests with fish have also aroused considerable ethical concern. Since acute toxicity to fish is determined in tests with juvenile or adult animals, intact fish are subjected to considerable pain and suffering, which is clearly in conflict with current Animal Rights Welfare legislation (Nagel, 2002).

Currently, hazard assessment of chemicals for fish is based on international standards (ISO, ASTM) and guidelines (OECD) based on global toxicology endpoints as mortality, growth, and reproduction impairments. For chemical industries and some countries the state of mind is now to reduce the cost of these experiments and the number of used organisms, in concern for animal welfare. A significant example is the awareness of the ecotoxicologist community of the pharmaceutical industry to apply the principles of replacement, reduction, and refinement (the 3Rs), established by Russell and Burch (1959), in the context of

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regulatory environmental assessments (Hutchinson et al., 2003). One of the alternatives proposed is to use the early life stage (ELS) of fish as an experimental model (Oberemm, 2000; Nagal, 2002; Hutchinson et al., 2003), because it is no longer necessary to demonstrate that the fish embryo and larva are generally the most sensitive stages in the life cycle of the teleost (Laale & Learner, 1981; McKim, 1985; von Westernhagen, 1988; Lele & Krone, 1996). However, the use of zebrafish in research, testing and teaching should be concerned the ethical standards as described in several sources such as http://www.oie.int/boutique/extrait/gauthier735746.pdf, http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Fish/Fish_Guidelines_English.pdf.

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CHAPTER IV
RESULTS

Overview

This chapter presents the main findings of the research. It is divided into two parts: scientific and educational. The scientific part describes development of the experiment on aquatic toxicology which utilizes zebrafish embryonic development as a marker for testing toxicity of endocrine disrupting chemicals. The educational part describes the application of scientific experiment in developing an inquiry-based instructional unit for high school students. The results on effectiveness of the newly developed unit are presented.

4.1 Scientific Part

4.1.1 Effects of Endocrine Disrupting Chemicals (EDCs) on Zebrafish Embryonic Development

4.1.1.1 Effects of EDCs on Mortality in Zebrafish Embryos

The effects of six sex steroid hormones at concentrations 0-5000 ng/mL on mortality of zebrafish embryos at early gastrula stage were shown in Table 4.1. In the absence of hormones the percentage mortality of the embryo ranged from 8.3-20.8. At concentration up to 50 ng/mL, all six hormones tested seemed to have no effect on embryonic development, however, at 500 ng/mL progesterone gave higher percentage mortality, when compared to other hormones. At 5000 ng/mL, the percentage mortality in 17α, 20β-dihydroxyprogesterone and progesterone was about 90%, while that in trenbolone was only 45%.

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Table 4.1 4-Day Cumulative Mortality in Response to Sex Steroid Hormones in Zebrafish Embryos at Early Gastrula Stage (6 hpf) to Hatching

Dose	4-Day Cumulative Mortality (%) (Mean ± SD)					
(ng/mL)	Trenbolone	17α, 20β	Progesterone	Estradiol	Testosterone	Androgen
0	20.83±14.43	8.33±7.22	8.33±7.22	11.11±19.25	16.67±7.22	16.67±7.22
5	-	-	12.50±12.50	28.57±0.00	16.67±7.22	25.00±12.50
50	33.33±14.43	20.83±7.22	20.83±14.43	23.81±8.25	12.50±12.50	20.83±7.22
500	29.17±19.09	33.33±19.09	45.83±19.09	33.33±7.22	29.17±14.43	33.33±19.09
5000	45.83±19.09	87.50±12.50	91.67±7.22	-	-	-

4.1.1.2 Effects of EDCs on Hatching Rate in Zebrafish Embryos

The hatching rate in the presence of the six hormones tested was shown in Table 4.2. 100% hatching rate was found in the absence of hormones, and in the presence of 5 ng/mL progesterone and estradiol. About 94% hatching was observed at 5 ng/mL testoseterone and androgen. At 50 and 500 ng/mL, trenbolone had less effect on the hatching rate, compared to other hormones.

Table 4.2 4-Day Cumulative Hatching Rate in Response to Sex Steroid Hormones in Zebrafish Embryos

Dose	4-Day Cumulative Hatching Rate (%) (Mean ± SD)					
(ng/mL)	Trenbolone	17α, 20β	Progesterone	Estradiol	Testosterone	Androgen
0	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
5	-	-	100.00±0.00	100.00±0.00	94.44±9.62	93.33±11.55
50	93.33±11.55	89.68±9.01	68.81±7.84	75.56±7.70	86.31±14.32	52.38±17.17
500	87.78±10.72	66.67±28.87	72.22±25.46	68.89±10.18	50.48±27.00	75.48±4.31
5000	43.49±12.28	27.78±34.69	0.00±0.00	-	-	-

4.1.1.3 Effects of EDCs on Embryonic Deformation in Zebrafish Embryos

The effects of the tested hormones on embryonic deformation are illustrated in Table 4.3. The embryos showed normal development in the absence of hormones. However, deformations were observed even at 5 ng/mL of the hormones. Among the six hormones tested, trenbolone seemed to have less effect on embryonic

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deformation, with 23 and 55% deformation at 50 and 500 ng/mL. The percentage deformation ranged from 30% to 49% at 50 ng/mL of the other five hormones. At 5000 ng/mL, all hormones caused 100% deformation of the embryos. The results showed that 17α, 20β-dihydroxyprogesterone, estradiol and testosterone seemed to have similar results in causing embryonic deformation.

Table 4.3 4-Day Cumulative of Embryonic Deformation in Response to Sex Steroid Hormones in Zebrafish Embryos

Dose	4-Day Cumulative Embryonic Deformation (%) (Mean ± SD)					
(ng/mL)	Trenbolone	17α, 20β	Progesterone	Estradiol	Testosterone	Androgen
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5	-	-	33.73±8.94	33.33±11.55	19.84.±7.65	29.21±13.34
50	23.33±25.17	30.16±2.75	49.01±15.53	34.29±12.45	33.73±8.94	42.86±20.76
500	55.71±5.15	75.17±22.41	72.22±34.69	68.89±10.18	67.62±21.44	55.71±5.15
5000	100.00±0.00	100.00±0.00	100.00±0.00	-	-	-

The results in Tables 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9 showed types of embryonic deformation caused by trenbolone, 17α, 20β-dihydroxyprogesterone, progesterone, estradiol, testosterone, androgen, respectively. The types of embryonic deformation observed were deformation of yolk sac, pericardial edema, deformation of body, deformation of yolk sac and pericardial edema, deformation of yolk sac and body, and deformation of yolk sac, body and pericardial edema.

In the presence of trenbolone (Table 4.4) 50 ng/mL, about 55% deformation was found in yolk sac and 11% was pericardial edema. At higher concentration of 500 ng/mL, 77% deformation was in the yolk sac. 22% of the embryos showed both yolk sac deformation and pericardial edema, 16% showed yolk sac deformation and body deformation, and 16% showed yolk sac deformation as well as pericardial edema and body deformation.

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Table 4.4 Types of Embryonic Deformation in Response to Trenbolone in Zebrafish Embryos at 96 hpf (Mean ± SD)

Types of Embryonic Deformation	Dose				
	0 ng/mL	5 ng/mL	50 ng/mL	500 ng/mL	5000 ng/mL
% Deformation (Total)	0.00±0.00	-	23.33±25.17	55.71±5.15	100.33±0.00
Yolk Sac Deformation	0.00±0.00	-	55.56±50.92	77.78±19.25	50.00±8.61
Pericardial Edema	0.00±0.00	-	11.11±19.25	0.00±0.00	0.00±0.00
Body Deformation	0.00±0.00	-	0.00±0.00	0.00±0.00	0.00±0.00
Yolk Sac Deformation and Pericardial Edema	0.00±0.00	-	0.00±0.00	22.22±19.25	30.00±26.46
Yolk Sac Deformation and Body Deformation	0.00±0.00	-	0.00±0.00	16.67±28.87	4.76±0.00
Yolk Sac Deformation , Body Deformation, and Pericardial Edema	0.00±0.00	-	0.00±0.00	16.67±28.87	16.19±14.66

Table 4.5 Types of Embryonic Deformation in Response to 17α, 20β-dihydroxyprogesterone in Zebrafish Embryos at 96 hpf (Mean ± SD)

Types of Embryonic Deformation	Dose				
	0 ng/mL	5 ng/mL	50 ng/mL	500 ng/mL	5000 ng/mL
% Deformation (Total)	0.00±0.00	-	30.16±2.75	74.17±22.41	100.00±0.00
Yolk Sac Deformation	0.00±0.00	-	83.33±28.87	28.89±7.70	83.33±14.43
Pericardial Edema	0.00±0.00	-	16.67±28.87	0.00±0.00	0.00±0.00
Body Deformation	0.00±0.00	-	0.00±0.00	0.00±0.00	16.67±14.43
Yolk Sac Deformation and Pericardial Edema	0.00±0.00	-	0.00±0.00	17.78±16.78	0.00±0.00
Yolk Sac Deformation and Body Deformation	0.00±0.00	-	0.00±0.00	36.67±21.86	0.00±0.00
Yolk Sac Deformation, Pericardial Edema and Body Deformation	0.00±0.00	-	0.00±0.00	11.11±19.25	0.00±0.00

17α, 20β-dihydroxyprogesterone (Table 4.5) caused similar deformations to that of trenbolone but at a higher percentage of cases. At 50 ng/mL 83% yolk sac deformation and 16% pericardial edema were observed. At 500 ng/mL, the deformation occurred in both yolk sac and body, and the pericardial edema.

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Table 4.6 Types of Embryonic Deformation in Response to Progesterone in Zebrafish Embryos at 96 hpf (Mean ± SD)

Types of Embryonic Deformation	Dose				
	0 ng/mL	5 ng/mL	50 ng/mL	500 ng/mL	5000 ng/mL
% Deformation (Total)	0.00±0.00	33.73±8.94	49.01±15.53	72.22±34.69	100.00±0.00
Yolk Sac Deformation	0.00±0.00	88.89±19.25	38.89±9.62	21.67±20.21	5.56±9.62
Pericardial Edema	0.00±0.00	0.00±0.00	0.00±0.00	11.11±19.25	0.00±0.00
Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Yolk Sac Deformation and Pericardial Edema	0.00±0.00	11.11±19.25	61.11±9.62	0.00±0.00	0.00±0.00
Yolk Sac Deformation and Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	55.00±39.69	0.00±0.00
Yolk Sac Deformation, Pericardial Edema and Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	15.00±13.23	94.44±9.62

In the presence of progesterone (Table 4.6), deformation occurred first at yolk sac at low concentration of the hormone (5ng/mL) and then deformations in both yolk sac and body and pericardial edema were observed at higher concentrations.

Table 4.7 Types of Embryonic Deformation in Response to Estradiol in Zebrafish Embryos at 96 hpf (Mean ± SD)

Types of Embryonic Deformation	Dose			
	0 ng/mL	5 ng/mL	50 ng/mL	500 ng/mL
% Deformation (Total)	0.00±0.00	33.73±11.55	34.29±12.45	68.89±10.18
Yolk Sac Deformation	0.00±0.00	83.33±28.87	22.22±38.42	19.44±17.35
Pericardial Edema	0.00±0.00	16.67±28.87	0.00±0.00	8.33±14.43
Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	8.33±14.43
Yolk Sac Deformation and Pericardial Edema	0.00±0.00	0.00±0.00	44.44±50.92	52.78±20.97
Yolk Sac Deformation and Body Deformation	0.00±0.00	0.00±0.00	33.33±57.74	0.00±0.00
Yolk Sac Deformation, Pericardial Edema and Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	11.11±19.25

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Estradiol (Table 4.7) caused similar types of deformation to that of progesterone although the extent of deformation was a little bit less than that of progesterone.

Table 4.8 Types of Embryonic Deformation in Response to Testosterone in Zebrafish Embryos at 96 hpf (Mean ± SD)

Types of Embryonic Deformation	Dose			
	0 ng/mL	5 ng/mL	50 ng/mL	500 ng/mL
% Deformation (Total)	0.00±0.00	19.84±7.65	33.73±8.94	67.62±21.44
Yolk Sac Deformation	0.00±0.00	100.00±0.00	88.89±19.25	0.00±0.00
Pericardial Edema	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	11.11±19.25
Yolk Sac Deformation and Pericardial Edema	0.00±0.00	0.00±0.00	0.00±0.00	38.89±24.06
Yolk Sac Deformation and Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	8.33±14.43
Yolk Sac Deformation, Pericardial Edema and Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	41.67±38.19

The results in Table 4.8 show that at 50ng/mL the observed deformation in the presence of testosterone was in found only in the yolk sac. Pericardial edema and body deformations were observed at 500 ng/mL.

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Androgen (Table 4.9) at 5 ng/mL caused only yolk sac deformation, while the hormone at 50 ng/mL deformation was seemed in both yolk sac and body in addition pericardial edema. At 500 ng/mL types of deformation were similar to those other hormones: a higher percentage of was observed in embryos, pericardial edema and two types of deformation.

Table 4.9 Types of Embryonic Deformation in Response to Androgen in Zebrafish Embryos at 96 hpf (Mean ± SD)

Types of Embryonic Deformation	Dose			
	0 ng/mL	5 ng/mL	50 ng/mL	500 ng/mL
% Deformation (Total)	0.00±0.00	29.21±13.34	42.86±20.76	55.71±5.15
Yolk Sac Deformation	0.00±0.00	100.00±0.00	53.33±14.43	0.00±0.00
Pericardial Edema	0.00±0.00	0.00±0.00	8.33±14.43	0.00±0.00
Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	8.33±14.43
Yolk Sac Deformation and Pericardial Edema	0.00±0.00	0.00±0.00	0.00±0.00	33.33±28.87
Yolk Sac Deformation and Body Deformation	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Yolk Sac Deformation, Pericardial Edema and Body Deformation	0.00±0.00	0.00±0.00	33.33±28.87	33.33±28.87

4.2 Educational Part

4.2.1 Student Knowledge and Skills

The learning unit in the environmental science course comprised seven topics. The first three topics were taught by traditional lecture and field trip, and the rest by inquiry-based lecture cum hands-on activities. After completing the learning unit activities, the students were asked to do self-assessment for knowledge and skills by the 5-point Likert rating scale in the questionnaire. As shown in Table 4.10, the mean score of student knowledge in the traditional teaching (3.56 ± 0.56) in the first part was significantly lower than that of the inquiry-based activities (4.08 ± 0.07) in the second part. The scores in the traditional lecture part ranged from 2.92 to 3.83

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while those in the inquiry-based activities were from 4.0 to 4.17. The results also suggested that most students were able to grasp concepts from the latter four topics as evidenced by the narrow range of the standard deviation.

Table 4.10 Means and Standard Deviations for Student Knowledge from Self Assessment

Topic	Mean ± SD	Total
Traditional teaching:		
Knowledge of water cycle	3.20 ± 0.79	3.56 ± 0.56
Knowledge of watershed	3.83 ± 0.84	
Environmental legislation	2.92 ± 0.90	
Inquiry-based lecture, hands-on activities, and student projects:		
Effects of agricultural practices on ecosystem	4.08 ± 0.90	
Use of organisms to indicate the effects of pollutants	4.08 ± 0.79	4.08 ± 0.07
Effects of toxins and other factors on organisms	4.00 ± 0.74	
Effects of consumer products on ecosystem	4.17 ± 0.94	

The study also investigated the effects of the inquiry-based hands-on experiment on students' skills by asking the students to rate two aspects of their science process skills: basic and integrated skills. As shown in Table 4.11, the seven items on basic skills were rated from 3.17 to 4.0 with a mean score of 3.64 ± 0.33 (of total of 5), and the seven items of integrated skills were rated from 3.17 to 4.17 with mean score of 3.81 ± 0.42. In terms of basic skills, the students had high scores in their abilities to observe, predict and communicate. As for integrated skills, the students rated themselves high in experimentation, hypothesis formulation, data collection and data interpretation.

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Table 4.11 Means and Standard Deviations for Student Science Process Skills from Self Reflection

Science Process Skills	Mean ± SD
Basic skills:	
Observing	4.00 ± 0.74
Classifying	3.42 ± 0.67
Measuring	3.17 ± 0.84
Inferring	3.50 ± 0.91
Predicting	3.92 ± 0.10
Communicating	4.00 ± 0.85
Using number relationships	3.50 ± 0.91
Average of basic skills	3.64 ± 0.33
Integrated skills:	
Making models	3.33 ± 1.23
Defining things operationally	3.17 ± 0.84
Collecting data	4.17 ± 0.72
Interpreting data	4.08 ± 0.79
Identifying and controlling variables	3.67 ± 0.78
Formulating hypothesis	4.08 ± 0.90
Experimenting	4.17 ± 0.72
Average of integrated skills	3.81 ± 0.42

At the last session of the learning unit, each group of students carried out the project of their own design and then presented the results to the class. Most of the projects were on effects of the household cleaner on embryonic development of zebrafish. The results in Table 4.12 show the mean scores of students' projects as assessed by the teacher. The projects were rated by the 5-point Likert scale for their scientific concepts, scientific method, organization of posters, problem solving skills and presentation skills. The mean scores of each group ranged from 3.80 to 4.40. The results suggested that each group of students possessed similar ability in exploiting the knowledge learned to design, conduct and present the project. However, they differed in their abilities in organizing the knowledge and results into a well-presented poster.

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Regarding the students' development during the 4-week period of the inquiry-based instructional unit, the teacher evaluated improvement in skills of the students as illustrated in Table 4.13. The average mean scores of all skills gradually increased from 2.33 in the first week to 3.25, 3.69 and 4.50 in the second, third and fourth week, respectively. Of all the skills examined, there was a large improvement in the ability to use experimental tools which increased from 1.00 in the first week to 4.25 in the fourth week. The extent of improvement in other skills (as shown in Table 5) was similar, although there seemed to be more development in oral communication skills, understanding of scientific methodology and working in the team

Table 4.12 Teacher Evaluation of Student Science Projects

Group	Scientific knowledge	Scientific method	Problem solving	Oral presentation	Poster organization	Total (Mean $\pm$ SD)
1	5	5	4	5	3	4.40 ± 0.89
2	4	4	4	4	3	3.80 ± 0.48
3	4	5	4	4	4	4.20 ± 0.45
4	4	4	4	4	5	4.20 ± 0.45

Note. Group1: Effects of liquid detergent on zebrafish embryos
Group2: Effects of all-purpose cleaner on zebrafish embryos
Group3: Effects of dish soap on zebrafish embryos
Group4: Effects of cleaner and degreaser on zebrafish embryos

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Table 4.13 Teacher Evaluation of Student Achievement During the 4-Week Period of the Inquiry-Based Learning Unit.

Target skills	Mean ± SD			
	Week1	Week2	Week3	Week4
Contents -Understanding of basic contents	2.25 ± 0.50	3.25 ± 0.50	3.75 ± 0.50	4.75 ± 0.50
Scientific concepts -Understanding of scientific concepts	2.75 ± 0.50	3.25 ± 0.50	3.75 ± 0.50	4.50 ± 0.58
Scientific methods -Understanding of scientific methods	2.75 ± 0.50	3.50 ± 0.58	4.25 ± 0.50	4.75 ± 0.50
Scientific inquiry -Development of inquiry skills	2.50 ± 0.58	3.25 ± 0.50	3.50 ± 0.58	4.25 ± 0.50
Scientific process skills -Basic and integrated skills (experimental design, observation, collecting and reporting data etc.)	2.50 ± 0.58	3.25 ± 0.50	3.75 ± 0.50	4.50 ± 0.58
Use of scientific instruments -Ability to use scientific instruments	1.00 ± 0.00	2.75 ± 0.50	3.50 ± 0.58	4.25 ± 0.50
Communication skills -Communication with each other and teacher	2.50 ± 0.58	3.25 ± 0.50	3.25 ± 0.50	4.50 ± 0.58
Problem solving -Analysis of problem; application of relevant knowledge; feasibility of problem solving	2.25 ± 0.50	3.25 ± 0.50	3.25 ± 0.50	4.25 ± 0.50
Team work -Cooperation with other group members	2.50 ± 0.58	3.50 ± 0.58	4.25 ± 0.50	4.75 ± 0.50
Average	2.33 ± 0.53	3.25 ± 0.22	3.69 ± 0.37	4.50 ± 0.22

4.2.2 Student Attitudes

The student attitudes on the inquiry-based instructional unit as well as on environmental awareness were investigated by a questionnaire having 5-point rating scale with space for additional comments and suggestions. Student satisfaction on the learning unit was at very good level i.e., score of 4.08 from total of 5 (data not shown). Most of the students commented that they preferred learning by inquiry because this method helped them gain both content knowledge and linking it to environmental problems. Excerpts from student comments are as follows:

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"I like the zebrafish experiments a lot."

"The experiments help me understand aquatic toxicology."

"I realize that bigger dairy farms will result in more substances being put into the water system."

"Use of artificial hormones in animal feeds may cause more hormones to enter the water through runoffs and thus cause deaths and birth defects in the fish."

4.2.3 Teacher Reflection

The role of teacher in this study was to help increase learning outcomes of the students. The teacher commented that the newly developed unit provided students not only with knowledge and skills but also environmental awareness, as shown in the following excerpts:

"The zebrafish inquiry project took my students out of their comfort zones in many ways. In short order they learned about model species, some basic aspects of the science of toxicology, embryology and the use laboratory tools that they previously had no exposure to."

"The inquiry experiments provided students with a window on environmental issues. The nearby agriculture area provided the opportunity to discuss what we were seeing in the classroom with the broader real life watershed issues."

The teacher was glad to see development of the students throughout the 4-week period of the inquiry-based learning unit as following excerpts:

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"All of my students definitely had a learning curve when it came to learning the test procedures and adapting to the discipline required to record observations over weeks."

"I was pleased to see the growth in all of my students. Over time they mastered new laboratory techniques and began to see how scientists use observation, reasoning, and communication."

"I saw growth socially and I would have liked to start out the year with an inquiry project because of the team building qualities that I observed."

Regarding the students' learning attitudes, the teacher noticed a shift from just asking questions to finding the answers by themselves. The following are excerpts from teacher reflection.

"I think the inquiry projects helped the students construct their own knowledge and develop critical thinking skills and communication skills in the students."

"The students liked coming into the class and seeing the changes that were occurring and being able to explain our unique project to others in the school."

"One of my students often stated that this inquiry-based learning was the highlight of the year. She especially liked the fact that, no one knew the answer or the expected result."

4.2.4 Teacher Interview

The teacher was interviewed in the four main aspects according to the professional development: teaching experiences based on the inquiry laboratory, teacher beliefs, changes in the teacher during the course, and teacher efficacy.

Looking back at her teaching experiences and her beliefs, the teacher commented as below:

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"At first, I was frustrated in my search for materials that I could use to give my class's inquiry experiences. Nevertheless, I could find materials that allowed me to guide students to ask their own questions and create hypotheses."

"Before joining this activity, there was a big gap between the theoretical and the practical aspects of inquiry-based science that made it difficult to do inquiry science."

Teacher attitude toward students' abilities as well as in grading the students changed as shown in the following excerpts:

"Surprisingly, the students were able to use the sophisticated scientific tools to examine the development of fish embryos. This means students would be able to perform techniques that they have no experience before if they are appropriately trained."

"Now I do not grade based on results but on what the students are learning from the experience."

Concerning teacher efficacy, the teacher voiced as follows:

"From a teacher perspective I liked the fact that there was no specific answer, it gave me an opportunity to use prompts and have discussions about what they were observing."

"Overall, I believe my students were motivated and took the project very seriously. For many it was a glimpse into education at a major university and possible career paths."

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CHAPTER V
DISCUSSION

Overview

This chapter aims to present the interpretation of the research findings and discuss them in relation to other studies. It is divided into two aspects: scientific aspect and educational aspect. The implications arisen from this research study are given. The limitations and recommendations for further research are discussed.

5.1 Scientific Aspect

The results from this study clearly indicate that at least six sex steroid hormones, i.e. trenbolone acetate (TbA), progesterone (P4), 17α, 20β-dihydroxyprogesterone (DHP), 17β-estradiol (E2), testosterone (T), and 4-androstene-3, 17-dione (A4) had significant effects on development of zebrafish embryos. The hormones increased the mortality rate, delayed the hatching time, decreased the hatching rate. Most importantly, the hormones caused abnormalities in the zebrafish which included deformation of the yolk sac and body as well as pericardial edema. Our results are in agreement with several other studies that reported disturbance the normal physiology and endocrinology of organisms by a number of chemical compounds and natural substances present in the aquatic environment (Arukwe & Goksøyr, 1998; Sumpter, 1998). Different fish species may vary by orders of magnitude with respect to their sensitivity in acute tests to environmental contaminants. This study selected zebrafish as the tested organism because the zebrafish embryo was more sensitive than other species such as the fathead minnow (*Pimephales promelas*), the Japanese medaka (*Oryzias latipes*), adult zebrafish and some cell lines (Braunbeck et al., 2005; Lange, Gebauer, Markl, & Nagel, 1995). Furthermore, as the zebrafish holds many similar cellular and physiological

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characteristics with higher vertebrates, the toxicological results of zebrafish embryonic development can be easily compared with those of mammalian development (Marguerie, Buckley, & Fleming, 2006; Reimers, Flockton, & Tanguay, 2004). Zebrafish embryos and larvae have been utilized to study the effects of many environmental pollutants including cadmium (Blechinger, Warren, Kuwada, & Krone, 2002), insecticides (Levin, Swain, Donerly, & Linney, 2004), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Mattingly, McLAchan, & Toscano, 2001).

The stage of embryonic to be used in the acute fish test is very important. This study employed the zebrafish embryo at early gastrula stage, 72-96 h post fertilization (hpf) because at this time all of the normal embryos should be hatched. This is supported from a review on toxicological studies using different life-stage of fish, e.g., McKim (1985) arrived at the conclusion that in at least 80% of the cases long-term toxicity could be predicted by results from studies with the early life-stage. This conclusion was later corroborated by other studies, e.g. by Woltering (1984). In a study comparing the fish cytotoxicity test with the permanent fish cell line RTG-2 and an early version of the embryo toxicity test with zebrafish (*Danio rerio*) as two competing alternatives to the acute fish toxicity test, the embryo test proved to be more sensitive (Lange, Gebauer, Markl, & Nagel, 1995). The findings from this study show that the effects of steroid hormones on mortality rate and hatching rate in zebrafish embryos depend on the concentration of the exposed hormones. The control eggs hatched at first 72 hpf, which is the normal hatching time of zebrafish. However, hatching of eggs in presence of sex steroid hormones was delayed until 96-120 h. There was no hatching in some of the eggs in the presence of various hormones.

Sex steroids and several hormones influence reproduction in aquatic vertebrates and may interact during larval development (Arcand-Hoy & Benson, 1998; Cyr & Eales, 1996). However, the mortality percentage for all hormones in this experiment (0-500 ng/mL) was relatively low (29-45%), because these hormones do not directly kill the embryos. At high concentrations though, they retarded the development, affected embryo organ formation, shape and size, in addition to delaying hatching. Under the normal condition of 28.5 °C, zebrafish embryos take 48-72 h to develop from time of spawning to hatching (Kimmel et al., 1995; Westerfield, 1995).

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All the steroid hormones tested in this study caused the yolk sac deformation and abdomen malformation. The treated zebrafish embryos showed reduced spontaneous movement (heart and tail), slow development, deformed/distorted body, reduction of the size of the embryo. Death as an endpoint in toxicological research represents an unambiguous parameter for the individual. The accumulation of clear fluid (edema) in the coelomic cavity and pericardial areas in developing embryos exposed to higher concentrations indicated vascular or osmoregulatory dysfunction.

The abnormalities of the embryos found in this study are similar to those found in several other research works. For instance, Cheng and colleagues (2000) used zebrafish embryos as a model system to investigate cadmium-induced toxicities and observed six major types of deformities: head and eye hypoplasia, hypopigmentation, cardiac edema, yolk sac abnormalities, altered axial curvature, and tail malformations. The frequency of malformations increased with cadmium concentration. The hypothalamic-pituitary gonadal (HPG) axis and developmental regulatory genes in the central nervous system (CNS) play the importance role on developing patterns in the zebrafish embryo related to the adverse effects of endocrine-disrupting chemicals (Ankley & Johnson, 2004; Strahle & Blader, 1994). The principal components of the HPG axis include the hypothalamus and pituitary gland in the brain, the gonads (testis and ovary) and the liver. During the early developmental stages of fish, the differentiation of tissues and cells into organs with the proper structure and capability of responding to external and internal cues is controlled, at least in part, by the hormones of the HPG axis (Ankley & Johnson, 2004). An intricate network of developmental regulatory genes, which are expressed in dynamic and spatially restricted patterns in the developing fish embryo, are involved in the body pattern formation, cell proliferation, and cell death (Boulekbache, & Condamine, 1993; Blagden, Currie, Ingham, & Hughes, 1997; Strahle & Blader, 1994; Joly, Joly, Schulte-Merker, Schulte-Merker et al., 1994; Teillet et al., 1998). In this study, the zebrafish embryos exposed by sex steroid hormones which are categorized as endocrine disrupting chemicals showed abnormalities embryos might be affected from the adverse effects on HPG axis and the regulatory genes during the developmental stages.

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The result in this study agrees with the finding of Bilotta and colleagues (2002) that exposure of 1.5% ethanol to zebrafish embryos affected the visual function particularly when the exposure occurred during eye development. These findings illustrated the usefulness of the zebrafish as a viable animal model for studying fetal alcohol syndrome (FAS). It is believed that low concentrations and/or short durations of alcohol exposure can produce more subtle effects, e.g. small eye diameter and lower heart rate.

Based on the specific appearance from stage to stage of development, steroid hormones affected embryonic development by retarding stage progression. This is corroborated by several research works on effects of estrogenic hormones on different life stages of zebrafish. For instance, Brion and colleagues (2004) studied the impacts of 17β-estradiol (E2) on reproduction after exposure during embryo-larval-, juvenile-, and adult-life stages in zebrafish (*Danio rerio*) for 3 weeks. The results showed that the effective concentration for vitellogenin induction in zebrafish early life stage was 100 ng E2/L, and in adult male zebrafish the effective concentration for vitellogenin induction (between 5 and 25 ng/L) was lower than for the early life stage of fish.

In this study, the concentrations of the tested steroid hormones in this study that caused deformation of the zebrafish embryo was about 5-50 ng/mL. Progesterone, estradiol, testosterone and androgen at 5 ng/mL caused 85-100% deformation in yolk sac while the mortality rate was only 20-33%. The environmental relevant concentrations of estrogenic chemicals present in sewage treatment work (STW) effluent at measured concentrations range from 1 ng/L (0.001 ng/mL) to almost 50-80 ng/L (0.05-0.08 ng/mL) (Desbrow, Routledge, Brighty, Sumpter, & Waldock, 1998). The results suggested that in certain area where the effluent from the factory (industrial), domestic or sewage treatment work containing less than 5 ng/mL, the steroid hormone may not kill the fish. However, the previous research work reported that the concentrations measured at the effluent area (0.05-0.08 ng/mL) may be responsible for the observed induction of vitellogenin synthesis in male fish. Fraysse, Mons, and Garric (2006) developed a zebrafish 4-day embryo-larval bioassay to assess three model toxicants with well-known toxic effects (propranolol, malathion, cadmium). The sensitivity and ability of these endpoints to inform about mode of

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action (MoA) were established in testing three model toxicants with well-known toxic effects (propranolol, malathion, cadmium). The bioassay allows characterization of impairments at different biological levels: neuromuscular, physiological, morphological, and behavioral, and provides useful information about the toxic MoA of the chemicals on nontarget organisms.

5.2 Educational Aspect

The results of the scientific research in this study were applied in the environmental science course for the high school students. The laboratory exercise was developed into three experiments: Experiment I, Students Observing Normal Development; Experiment II, Students Perceiving Abnormal Growths; and Experiment III, Students Applying Knowledge Learned. The teacher was trained on these experiments to ensure effective teaching practice.

Apart from becoming aware of the care and attention needed for growing healthy fish, from Experiment I, the students developed their own science process skills such as observing and concluding about normal and abnormal growth of the whole body and internal parts very well. They also learned to appreciate the dose-dependency of the effects of the hormone on fish: a type of knowledge useful for the future practicing investigator. In fact many students were so impressed by the simplicity of the fish model that they wanted to explore the use of the fish further with other chemical products from industry. Certainly most of them could see the relevance of this type of scientific experiments to their daily life because they lived near the big farms. Students showed gradually more profound learning by their increasingly sophisticated response to questions and their better conceptual understanding.

The teacher had to tell the students that the large effects they saw in Experiment II might be caused by a rather large doses of hormone used in the experiment; smaller doses have effects that are not as visible or drastic, but harmful just the same. The importance of using a proper stage of development for certain tests was also emphasized. More generally, the class went on to discuss the general risks of

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the industrial and agricultural chemicals. They could extrapolate from what they learned to concepts about persistence of residues of toxic substances.

In experiment III students could exploit concepts gained in the two previous experiments. They used their judgment about fish at certain stage of development to test their ideas and also the safer concentration of the potentially toxic substance used. Since abnormalities or even deaths were observed, students had to explain why things happened as observed. They became more mature learners.

The findings of this study showed the success of the newly developed learning unit that is because the students conducted their experiments and science projects using knowledge learned through the inquiry activities. The teacher in this study also developed her professional experience through the developed teaching practices. Supporting details are as described in the following educational aspect.

This study was successful in achieving its goal in promoting the practice of inquiry-based instruction. The teacher, facilitated by the researcher, was able to develop a 4-week inquiry-based unit on aquatic toxicology. Most importantly the students in this study showed gradual achievement in their learning outcomes in both knowledge and skills. The knowledge gained, albeit measured by student self-assessment and not by objective pretest/posttest, provided useful information. The students came to realize that learning by inquiry-strategy helped gain better knowledge than traditional teaching. The newly developed unit enabled the students to actively participate in activities with several essential features of the inquiry process. They were engaged by scientifically oriented questions. Importantly they learned to respond to questions by using evidence either from laboratory results or from literature. They were also able to connect the explanation to the real life situation. The students automatically built their team through the inquiry-based activity resulting in better understanding on scientific knowledge. This was supported by results from students' self reflection and from teacher reflection and interviews.

The teacher was pleased to see development in the students. Over time the students mastered new laboratory techniques and began to see how scientists use observation, reasoning, and communication. The students found out first-hand how science is really used and applied in research. The students applied the knowledge learned about environmental toxins to real problems in their community. This is

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because students were able to construct their own knowledge through the inquiry-based laboratory which provided students with an eye-opener on environmental issues in their community.

Several previous research studies have shown that science laboratory in secondary schools performs a wide variety of educational functions. These functions included developing and restructuring knowledge schemes, promoting inquiry and problem solving, reflecting science as it is practiced by scientists, and developing skills in the process of science (DebBurman, 2002; Driver, Asoko, Leach, Mortimer, & Scott, 1994; Mattheis & Nakayama, 1988; Tobin, 1986). Our results are in agreement with other research works that the open inquiry laboratory gives students even more independence in conducting inquiry by enabling them to identify their own problem and develop their own solutions (Roth & Roychoudhury, 1993; Zion et al., 2004; Tamir & Lunetta, 1981).

Scientific inquiry, especially when it involves direct hands-on investigations, has often been promoted as a way to support an understanding of scientific content (Deboer, 2006; Stohr-Hunt, 1996). Giving the opportunity for the students to participate in the inquiry laboratory, from the guided to the open one, also helps them acquire the science process skills, both basic and integrated. Learning by inquiry activities results in gradual improvement of students' skills. This agrees with Lawson and Wollman (1976) and Karplus (1977) that laboratory activities play a key role in cognitive development. Both the concrete learning experiences and the reasoning skills acquired in inquiry are crucial to intellectual development.

From the classroom investigation, the hands-on activity did help the students to perform and develop science process skills, both basic and integrated skills. Since the inquiry-based instruction was specifically designed for students to combine the content knowledge and skills to perform their science projects, the improvement in student performances as well as their good attitude toward the learning process was therefore observed. With practice, students can gain appropriate prerequisite knowledge and experience, ask the right question and ask it right, and design experiments to test their hypothesis (Germann & Aram, 1996; Germann, Aram, & Burke, 1996). The use of project-based inquiry science units has been shown to

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increase science content understanding and process skills as well as higher pass rates on a statewide test (Geier et al., 2008).

The result in this study agrees with the finding of Germann and Aram (1996) that if students make the connection between the domain-specific context of the laboratory and the more general context of the real world, then students will be able to apply science processes outside the classroom. In addition, the content on aquatic toxicology in the laboratory was close to their everyday life and helped to enhance student attention. The result is in congruence with those of Palmer (2009) who studied students' interests generated during situated inquiry lesson and found that interest arousal depends on the type of activities involved.

The teacher in this study commented that although the inquiry-based science project was a sophisticated piece of work requiring continuous collaboration within the team and attention on detail, it nevertheless helped develop critical thinking skills and communication skills in the students. The results clearly showed a much more positive social interaction among students that were not in their usual groups of friends. This cooperative aspect benefited students. The teacher commented that she saw growth in social terms and thus liked to start out the following year with an inquiry project because of the team building qualities that she observed.

The results demonstrated changes in teacher's classroom practices, in students' outcomes, and in teacher's belief and attitude. These are features of teacher change in the professional development as suggested by Gusky (2002). Instead of the conventional lecture, the teacher used teaching-learning strategies appropriate to the content which proved to be more effective. The inquiry-based experiment on aquatic toxicology was challenging and engaging to the students. These activities also led students to acquire several higher science process skills including high order thinking. The teacher allowed students to conduct investigation on problems of their own design allowing exploration on a broader front in which they had a personal interest. Changes in the teacher's practice resulted in the better students' outcomes. As mentioned earlier, the students were actively engaged in the learning activity and thus learned more purposefully. The teacher reflected that the students were able to construct their own knowledge and shifted from just asking questions to forming hypotheses and finding answers.

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Regarding changes in teacher's belief and attitude, some research studies pointed out the belief and attitude should occur first before changes in classroom practice whereas other works suggested that practices that result in promoting students' learning outcome will lead to belief and attitude (Gusky, 2002; Lee, Hart, Cuevas, & Enders, 2004). The teacher in this study, however, had no experience with the project-based inquiry teaching, but she had a strong drive to make her students have better understanding of science. She was interested in changing her normal classroom practice into an inquiry-based one. Enhancement of student knowledge and skills as well as the positive attitude of the students after the intervention thus changed the teacher's belief. This is evidenced by the teacher reflection and interview. The teacher was now trying to provide students with a conceptual understanding of science through exploration or self-designed projects. The teacher showed a strong commitment to using inquiry-based strategy in her teaching of other topics, and she had already approached the principal for support. Furthermore, she had successfully developed and implemented more inquiry-based instructional units by herself in the following semester. This shows that the ability to meet the learning needs of the students can lead to positive ramifications, in other words, teacher efficacy. The confidence in teaching science has also increased. These results agree with several research works. For example, Lee, Hart, Cuevas, and Enders (2004) reported enhanced knowledge of science content and stronger belief about the importance of science instruction after one-year of professional development. However, the actual practice of these teachers did not change significantly. The success of the teacher in this study is partly due to the background of the teacher who happens to have good content knowledge in the subject taught. Adequate science content knowledge and effective instructional strategy are required for giving rise to better science understanding in students (Garet, Porter, Desimone, Birman, & Yoon, 2001).

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5.3 Implication of the Study

5.3.1 Scientific Aspect

Sex steroid hormones have been shown to cause retarded development, organ malformation, body abnormality, reduction of hatching rate of the zebrafish embryos. This study although used higher concentrations than those found in the effluent discharge from sewage treatment work (STW). The results can be used as a guideline for development of toxicity test for sex steroid hormones. Eventhough the concentration of pollutants/endocrine disrupting chemicals (EDCs) in the environment is in the sublethal range, the chronic effects resulting from long-term exposure to low concentration of the hormones should be investigated.

The zebrafish embryo test could also be an alternative to the fish acute toxicity test of waste water. This is because zebrafish offers many advantages for toxicological assessment of embryonic and larval stages including the small size, high reproductive potential, transparent embryos, and well-described development. The data presented above may raise further concerns about the effects of steroid hormones in the environment on fish reproduction and human health.

5.3.2 Educational Aspect

The results in this study are only preliminary and should be confirmed with other teachers and other groups of students. Nevertheless, they can be used as a guideline for educators for short-term training of teachers in certain topics. Better results would be obtained if trainee in-service teachers have adequate content knowledge. Otherwise these teachers should be trained to have deeper content knowledge as well as the pedagogical knowledge so that they can successfully implement inquiry in the classroom. The enhancement of students' outcomes would have an impact on teacher confidence and lead to teacher's belief and attitude in using appropriate teaching strategies for better learning outcomes of students.

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5.4 Limitation of this Study

5.4.1 Scientific Aspect

In the scientific part, although the results demonstrated the effects of sex steroid hormones on zebrafish embryonic development, the extent to which such a mechanism plays a role remains unclear without further supportive evidence. Moreover, this research work needs further studies to be developed to bioassay of steroid hormone as contaminant (toxicant) in water surrounding the industrial site. Nevertheless, it should be noted, however, that for a small set of particular substances, cytotoxicity tests may be more sensitive than fish embryo tests.

5.4.2 Educational Aspect

In the educational aspects, the results indicate that the teacher changed the classroom practice to the extent that the students were motivated to learn resulting in enhanced learning outcomes. However, this study used only student self-assessment as indicator of knowledge gained. A more conclusive result should be derived from teacher's own assessment of students by using test for knowledge or another kind of standard test i.e., conceptual knowledge test, content knowledge test and test of science process skills . The students should be examined whether they have met the objectives either in the science content or science process skills listed in curriculum framework.

5.5 Suggestions for Further Study

5.5.1 Scientific Aspect

Because mortality is the most important parameter in the tests, relatively high concentrations must be used. In the environment, however, fish are usually exposed to sublethal concentrations of toxicants for a long time. The experiment should be reinvestigated using the hormone concentration that found in the environment near the industrial site. The exposure time and/or the observation period

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should be extended. More sophisticated method at cellular/molecular levels should also be done to obtain in-depth data. Full-life cycle tests with a variety of developmental and reproductive endpoints are ideal for detecting all possible effects of EDCs on fish.

5.5.2 Educational Aspect

The results from this study should be confirmed with different groups of students and teachers. As a matter of fact, this study was tried with high school students and a teacher the Midwest of the United States of America. It should also be tried out with students in Thailand. It should also be used a training program for teacher development in Thailand, after receiving comments, suggestion from pilot studies. The development of standard instruments on science process skills should be studied in a research work emphasizing hands-on activity and/or conducting the scientific experiment. Pre-test and post test of the conceptual knowledge and content knowledge could be the evidence of student learning outcomes and effectiveness of teaching practices. The teacher belief and attitude are important aspects supporting teacher change and teacher efficacy in using the developmental learning unit.

The nature of laboratory exercise could also be modified to suit the environmental condition in Thailand. Zebrafish experiment can be either adopted in both types of toxicants test, i.e. the heavy metal problems in many areas. Other types of fish could also be tested for efficacy in the bioassay for certain pollutant.

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CHAPTER VI
CONCLUSION

Overview

This chapter concludes the main findings of the research. It is divided into two parts: scientific part and educational part, and then are summarized at the end of the chapter.

6.1 Scientific Aspect

The zebrafish embryo has the potential to be used for testing the presence of steroid hormones in the environment. The exposure of zebrafish embryos to sex steroid hormones can result in mortality, delayed hatching, body deformation, and organ malformation. At higher concentrations the sex hormones have higher deleterious effects. Since the concentration of all sex steroid hormone studied was relatively low, most zebrafish embryos were not killed. However, at higher concentrations retarded development, embryo organ malformation and abnormality in shape, reduction of size, and delay hatching were more apparent.

The simple zebrafish experiments were integrated with an inquiry approach (from guided to open) and developed in the learning unit for high school students in environmental science course. The learning unit composed of three experiments: Experiment I, Students Observing Normal Development; Experiment II, Students Perceiving Abnormal Growths; and Experiment III, Students Applying Knowledge Learned.

Experiment I: the students developed their own science process skills such as observing and concluding about normal and abnormal growth of the whole body and internal parts very well. They also learned to appreciate the dose-dependency of the effects of the hormone on fish: a type of knowledge useful for the future practicing investigator.

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Experiment II: Students learned that the large effects they saw in the experiment that might be caused by a rather large doses of hormone used; smaller doses have effects that are not as visible or drastic, but harmful just the same. The importance of using a proper stage of development for certain tests was also emphasized.

Experiment III: students could exploit concepts gained in the two previous experiments. They used their judgment about fish at certain stage of development to test their ideas and also the safer concentration of the potentially toxic substance used.

6.2 Educational Aspect

The findings of this study show the success of the newly developed learning unit because the students could conduct their experiments and science projects using knowledge learned through the inquiry activities. The teacher in this study also developed professionally through the teaching experience.

This inquiry-based unit on the environmental impact of modern agricultural practices for students living in affected areas has two educational results. First, although it was primarily aimed at teacher development, the student learned to relate science to reality, i.e., everyday life. They became active learners, acquiring science process skills and content knowledge, and along the way they learned to be team workers and to have more awareness of environmental issues related to the use of common fertilizers, hormones, chemicals in farming and husbandry. The teacher, who was aided by the researcher, was involved in the inquiry-based pedagogy and saw gradual improvement of the students in many aspects, and thus became convinced of the value of the method. In addition, she had moved beyond this classroom research and showed further changes in the teacher efficacy. Overall, classroom outcomes and practice, changes in teacher's belief and attitude that the teacher had witnessed and experienced in this research can be used as a model for teacher professional development. However, this piece of research needs refinement. Nevertheless, as it stands it can be a model for use in short-term training of science teachers.

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APPENDICES

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APPENDIX A
EGG WATER

Egg Water:

1.5 mL stock salts added to 1 L distilled water = 60 µg/mL final concentration

Stock Salts:

40 g “instant ocean” sea salt added to 1 L distilled water

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APPENDIX B
EMBRYO MEDIUM

Embryo Medium:

1.0 mL	Hank's Stock # 1
0.1 mL	Hank's Stock # 2
1.0 mL	Hank's Stock # 4
95.9 mL dd H_2O	
1.0 mL	Hank's Stock # 5
1.0 mL	fresh Hank's Stock # 6

Hank's Stock Solutions:

Stock # 1:

8.0 g NaCl
0.4 g KCl
in 100 mL dd H_2O

Stock # 2:

0.358 g Na_2HPO_4 Anhydrous
0.60 g KH_2PO_4
in 100 mL dd H_2O

Stock # 4:

0.72 g $CaCl_2$
in 50 mL dd H_2O

Stock # 5:

1.23 g $MgSO_4$. 7 H_2O
in 50 mL dd H_2O

Stock # 6:

0.35 g $NaHCO_3$
in 10 mL dd H_2O

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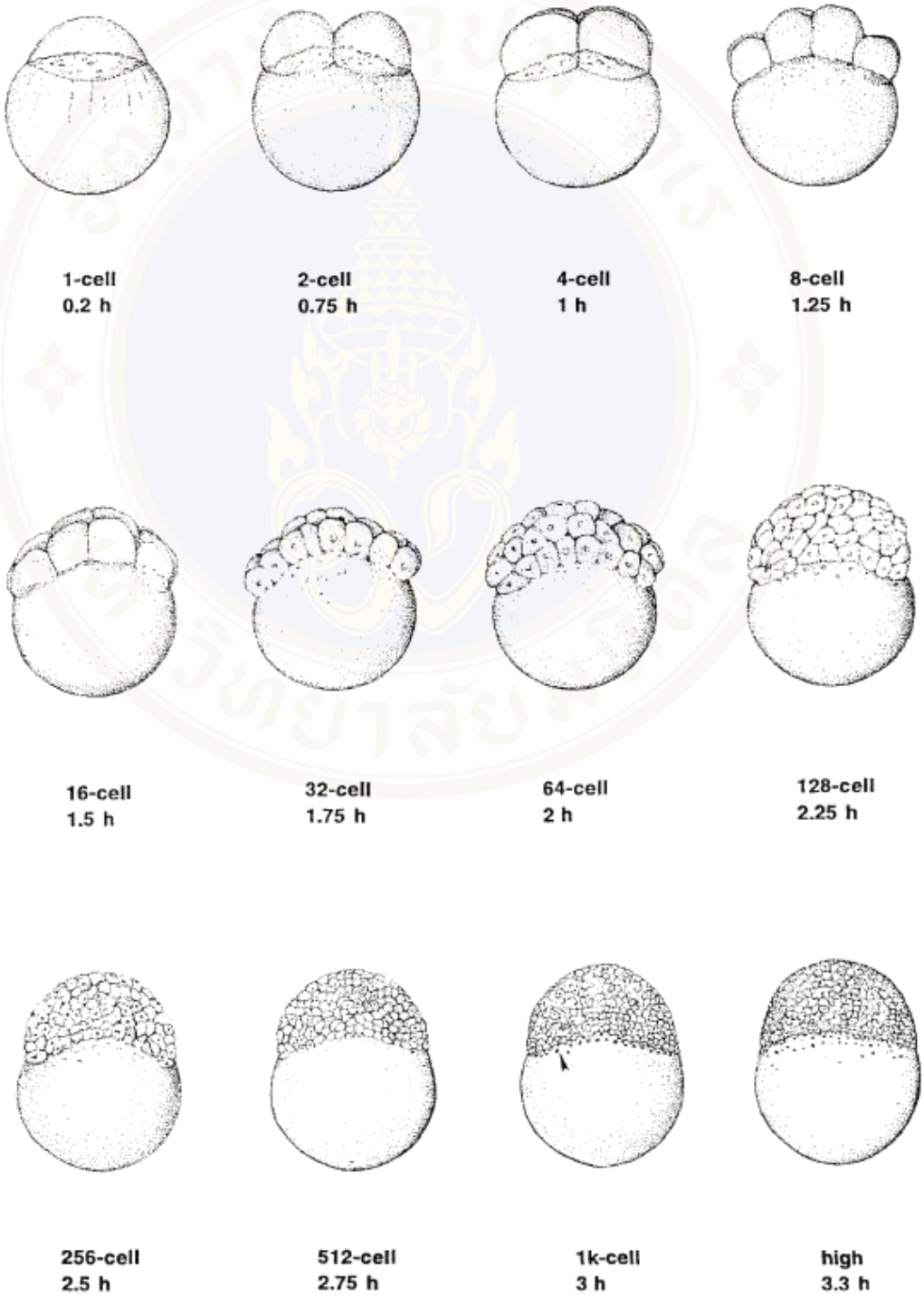
APPENDIX C
ZEBRAFISH EMBRYONIC DEVELOPMENT



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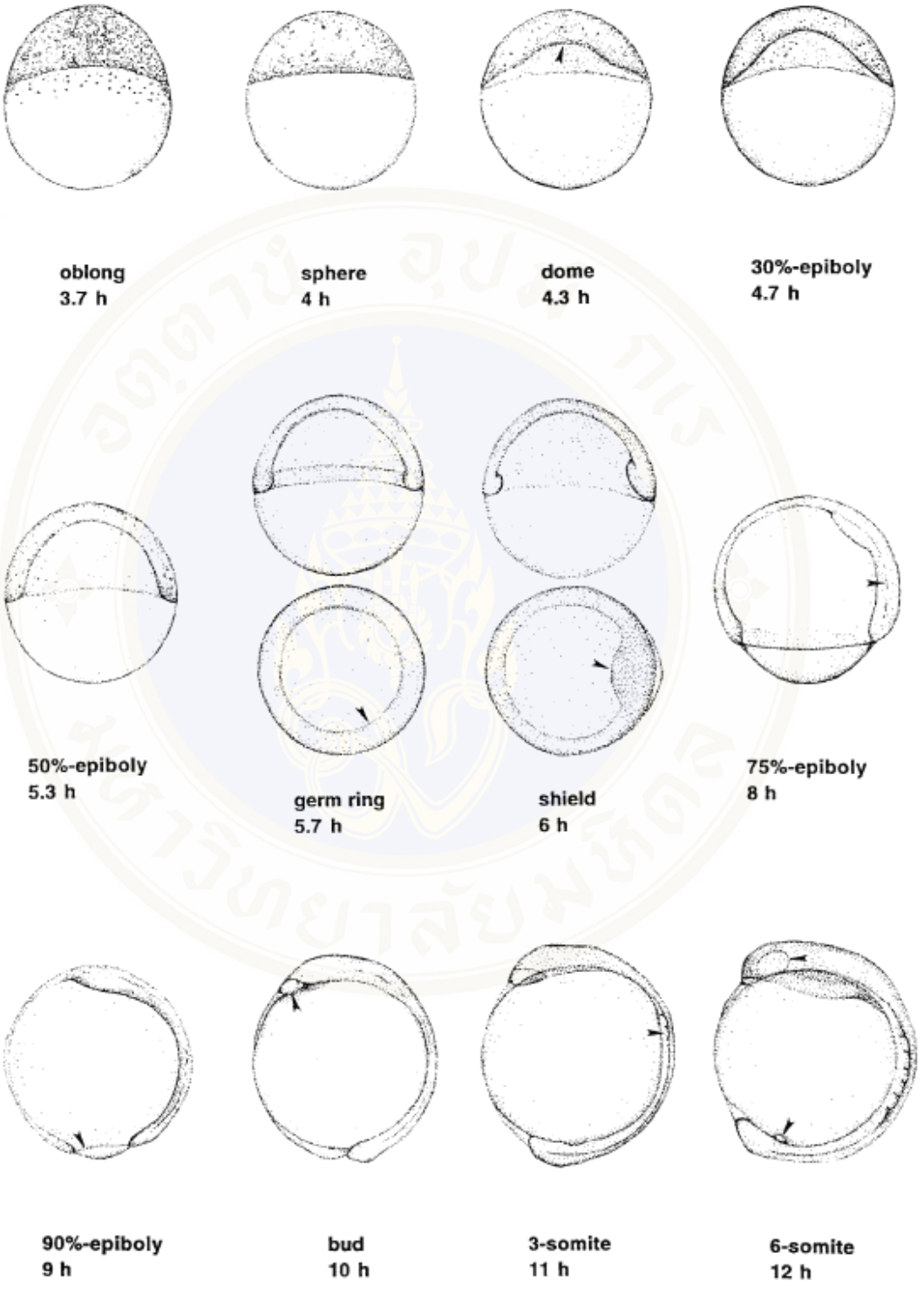

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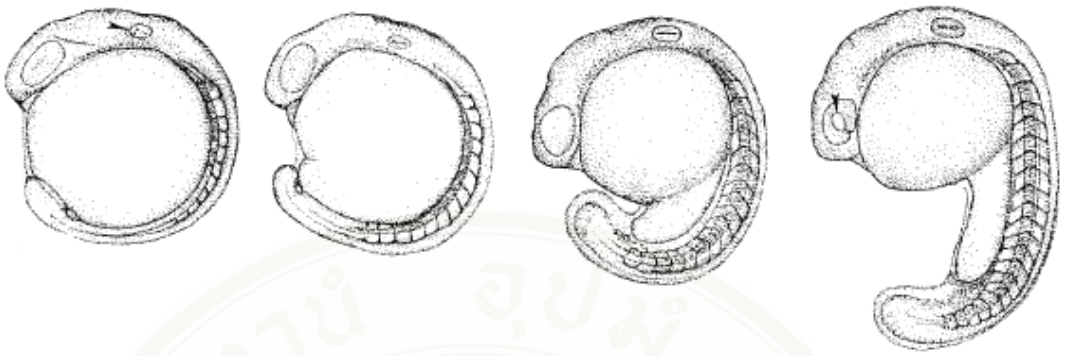
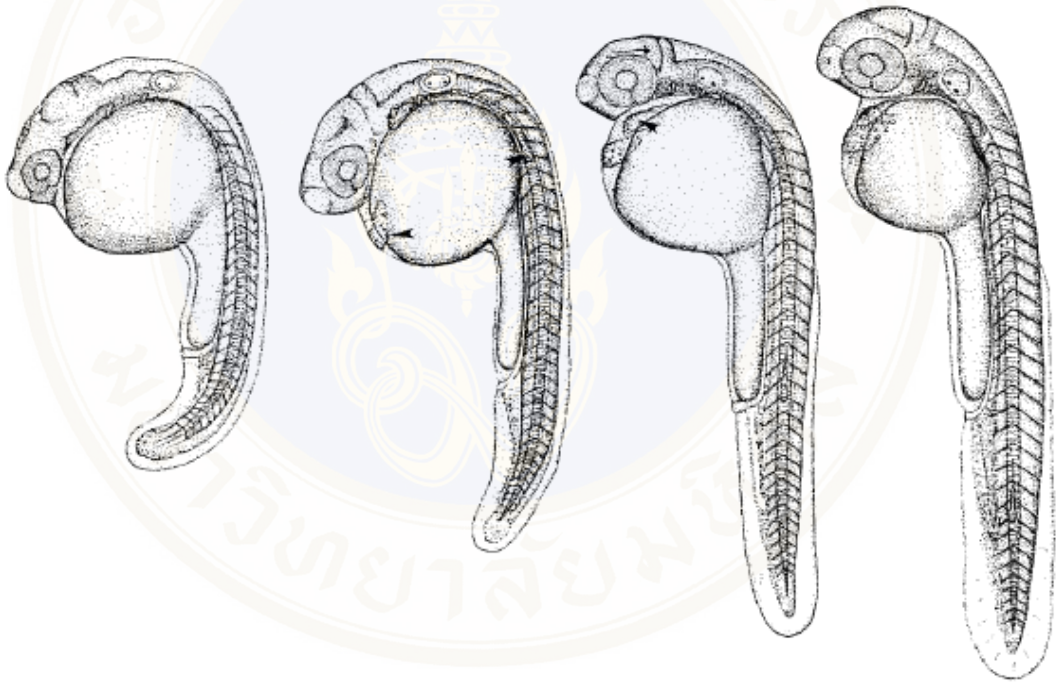


10-somite
14 h

14-somite
16 h

18-somite
18 h

21-somite
19.5 h



26-somite
22 h

prim-6
25 h

prim-16
31 h

prim-22
35 h

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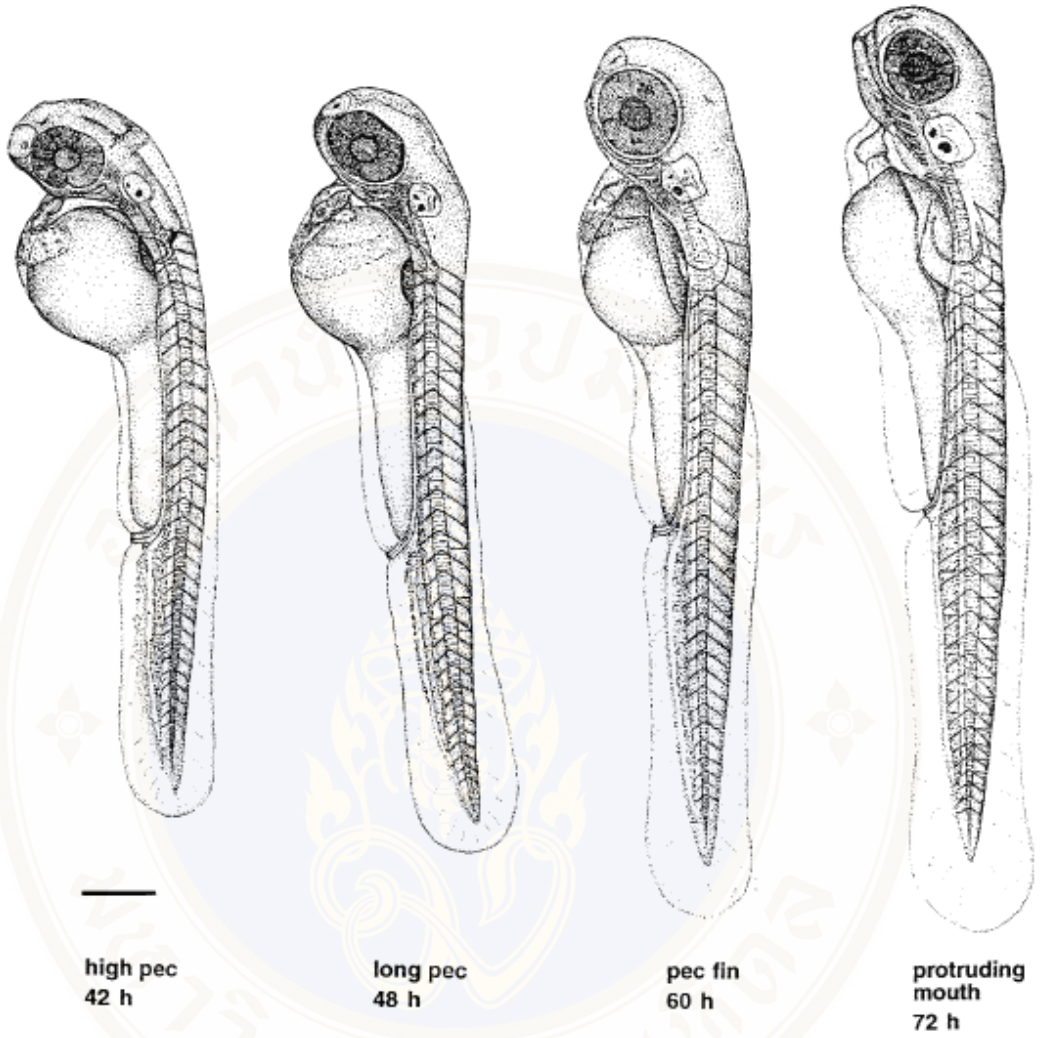

Stages of Embryonic Development of the Zebrafish. Camera lucida sketches of the embryo at selected stages. The animal pole is to the top for the early stages, and anterior is to the top later, except for the two animal polar (AP) views shown below their side view counterparts for germ-ring and shield gastrulas. Face views are shown during cleavage and blastula stages. After shield stage, the views are of the embryo's left side, but before the shield arises one cannot reliably ascertain which side is which. Pigmentation is omitted. Arrowheads indicate the early appearance of some key diagnostic features at the following stages: 1k-cell: YSL nuclei, Dome: the doming yolk syncytium, Germ ring: germ ring, Shield: embryo shield, 75%-epiboly; Brachet's cleft. 90%-epiboly: blastoderm margin closing over the yolk plug, Bud: poister, 3-somite: third somite, 6-somite: eye primordium (upper arrow), Kupffer's vesicle (lower), 10-somite: otic placode, 21-somite: lens primordium. Prim-6: primordium of the posterior lateral line (on the dorsal side), hatching gland (on the yolk ball), Prim-16: heart, High-pec: pectoral fin bud. Scale bar = 250 µm.

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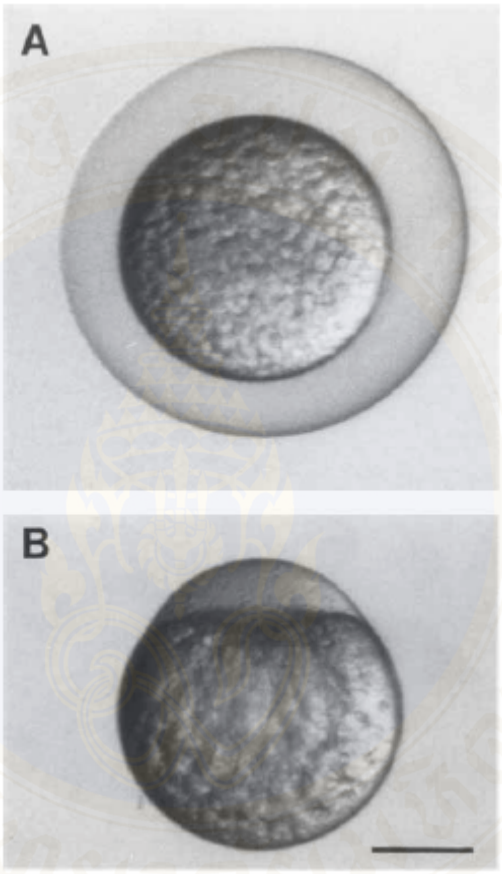

Stages During the Zygote Period. The zygote period. **A:** The zygote within its uplifted chorion, a few minutes after fertilization. **B:** The dechorionated zygote with the animal pole to the top, about 10 min after fertilization. The Yolk-free cytoplasm has begun to segregate to the animal pole. Scale bar = 250 µm.

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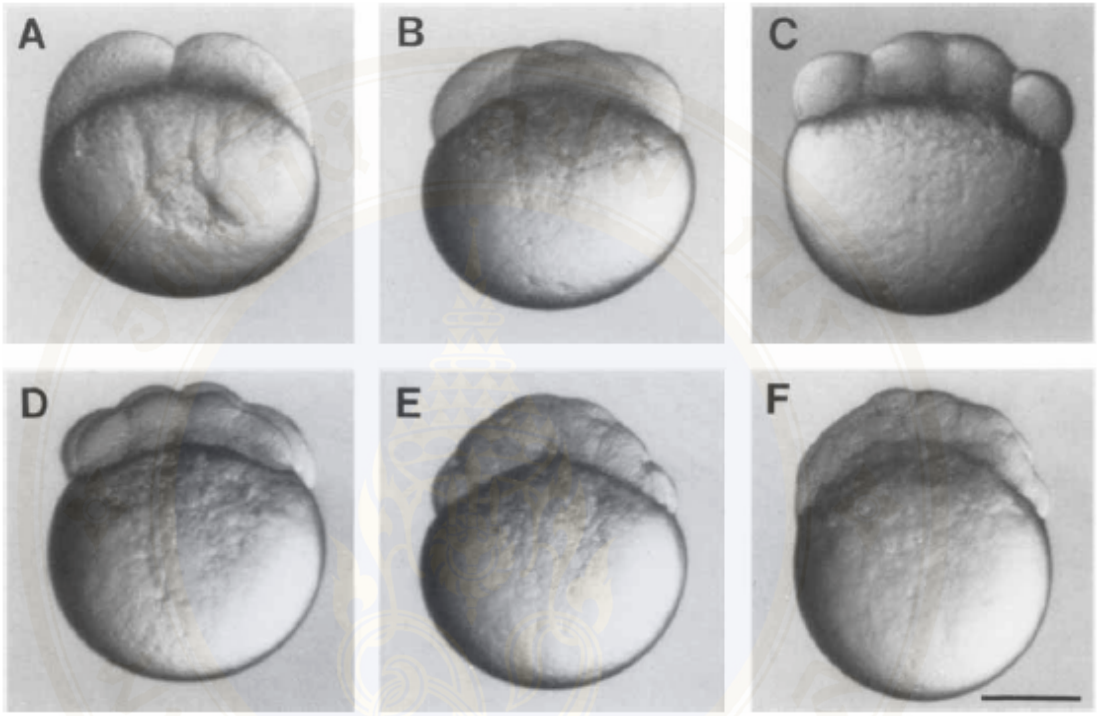

Stages During the Cleavage Period. Embryos during the cleavage period. Face views, except for B, which shows the embryo twisted about the animal-vegetal axis, roughly 45° from the face view. **A:** Two-cell stage (0.75 h). **B:** Four-cell stage (1 h). **C:** Eight-cell stage (1.25 h). **D:** Sixteen-cell stage (1.5 h). **E:** Thirty-two cell stage (1.75 h). **F:** Sixty-four cell stage (2 h), Scale bar = 250 µm.

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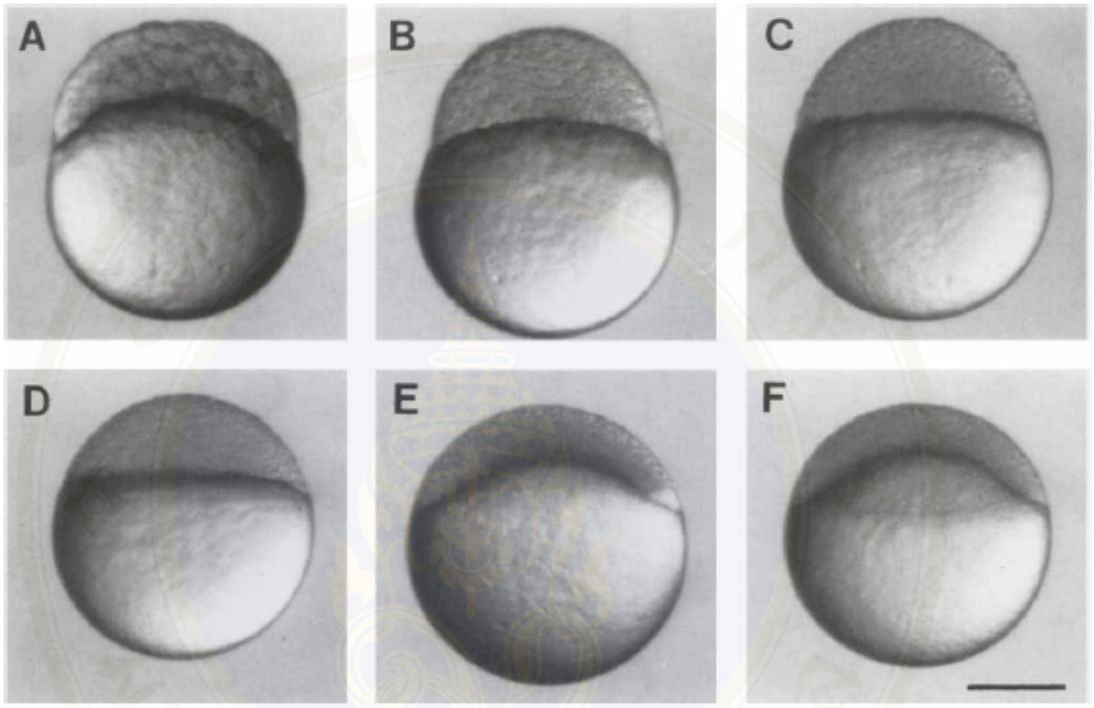

Stages During the Blastula Period. Face views of embryos during the blastula period. **A:** 256-cell stage (2.5 h). **B:** High stage (3.3 h). **C:** Transition between the high and oblong stages (3.5 h). **D:** Transition between the oblong and sphere stages (3.8 h). **E:** Dome stage (4.3 h). **F:** 30%-epiboloy stage (4.7 h). Scale bar = 250 µm.

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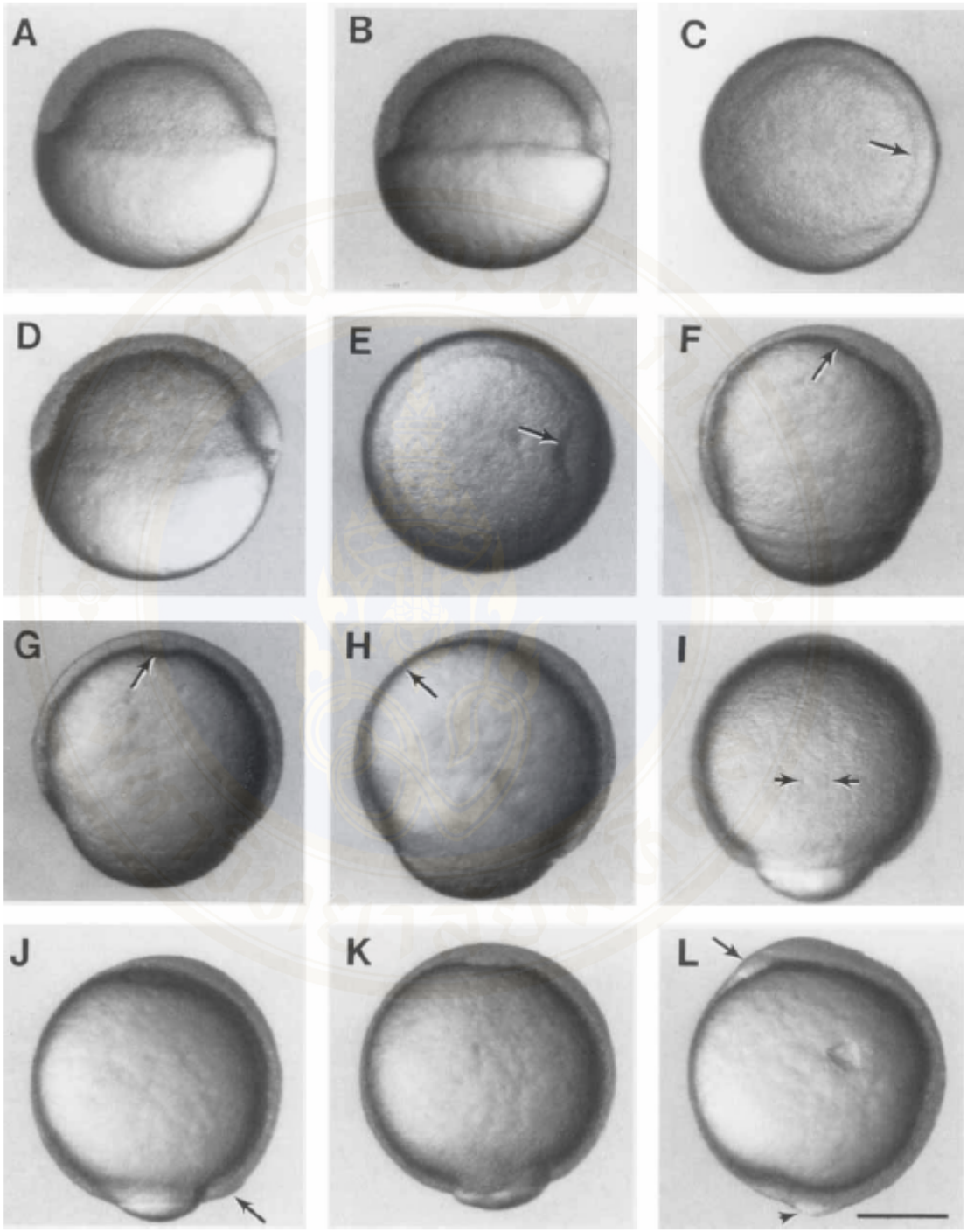

Stages During the Gastrula Period. Development during the gastrula period. Left side views, except where noted, with anterior up and dorsal to the left. **A:** 50%-epiboly stage (5.25 h). **B:** Germ ring stage (5.7 h). **C:** Animal pole view of the germ ring stage; the arrow indicates the germ ring; the embryonic shield will probably

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develop from the flattened region of the ring at the lower right. **D:** Shield stage (6 h). The embryonic shield, marking the dorsal side, is visible as a thickening of the germ ring to the left. **E:** Animal pole view of the shield stage; the arrow indicates the embryonic shield. **F:** 70%-epiboloy stage (7.7 h). The dorsal side of the blastoderm, to the left is thicker than the ventral side, to the right. The anterior axial hypoblast, or prechordal plate (arrow), extends nearly to the animal pole. **G:** 70%-epiboly stage, ventral view, but tipped slightly forward anteriorly to reveal the now well-delineated axial hypoblast (arrow) of the prechordal plate. **H:** 75%-epiboly stage (8 h). The arrow indicates the thin evacuation zone on the ventral side. **I:** 80%-epiboly stage (8.4 h), dorsal view. The arrows indicate the boundaries between axial mesoderm in the midline, and the paraxial mesoderm flanking to either side. **J:** 90%-epiboly stage (9 h). The tail bud (arrow) becomes visible in some embryos at this stage. **K:** 90%-epiboly stage, ventral view. The anterior prechordal plate (compare with G) enlarges as the polster. **L:** Bud stage (10 h). The arrow shows the polster, and the arrowhead shows the tail bud. A distinctive region just ventral to the tail bud (i.e., just to the left in this view) shows where the yolk disappears as epiboly ends. Scale bar = 250 µm.

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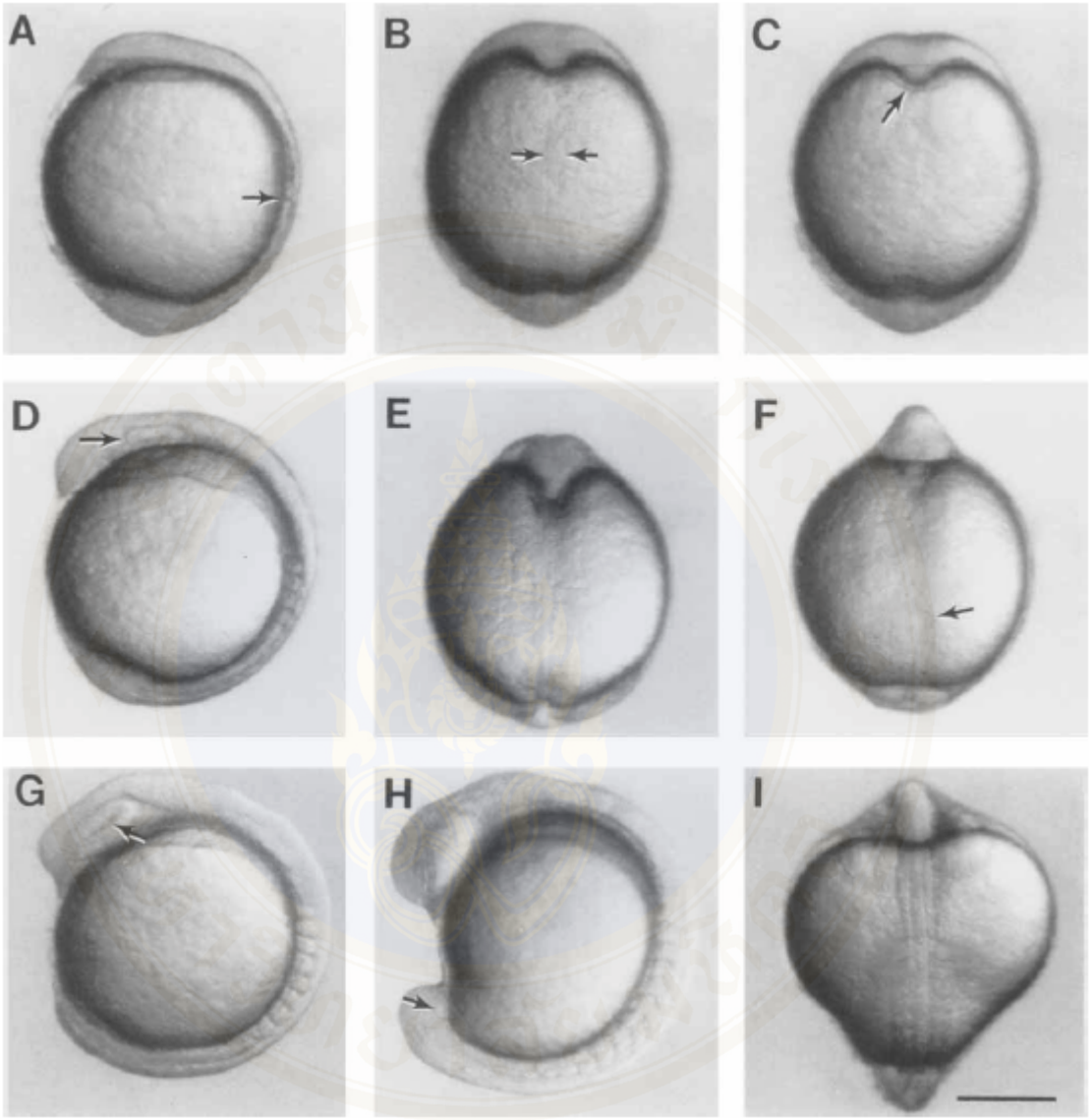

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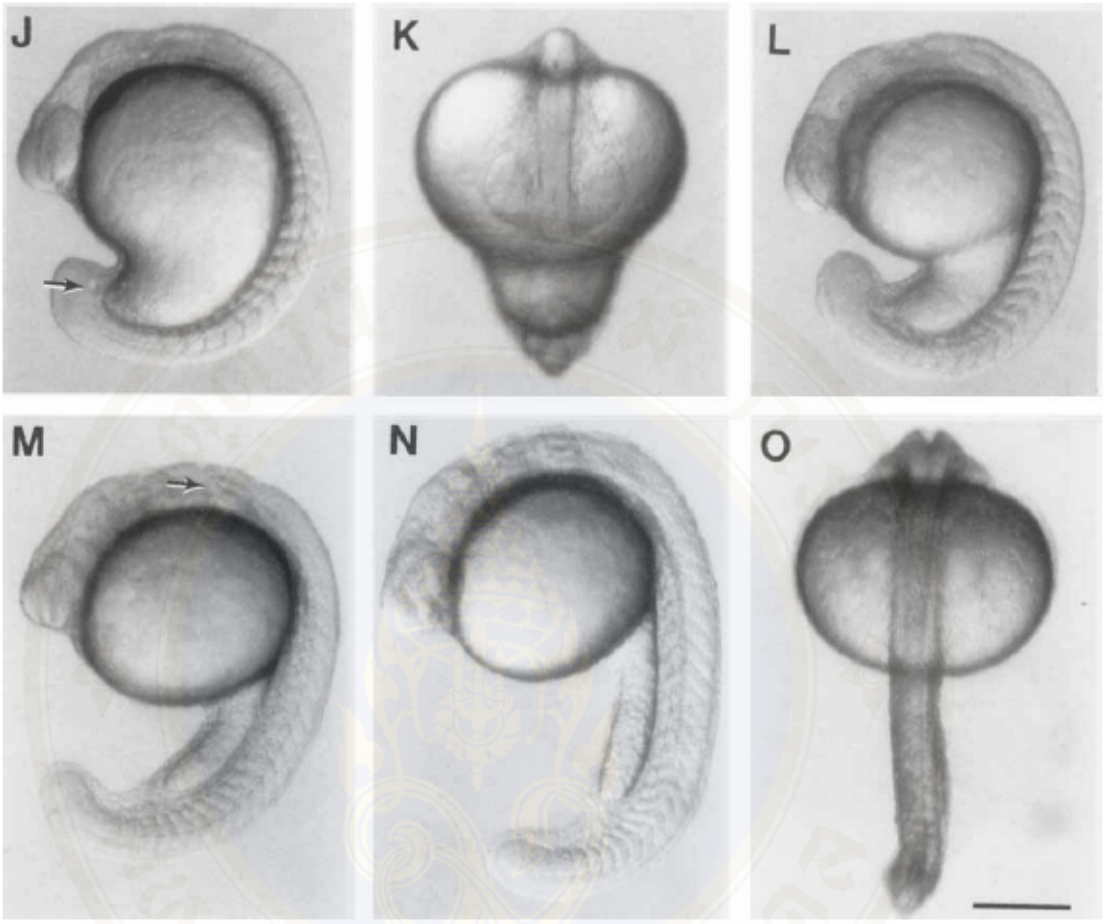

Stages During the Segmentation Period. Development during the segmentation period. Left side views, except where noted, with anterior up and dorsal to the left. **A:** Two-somite stage (10.7 h). Somite 2 is the only one entirely pinched off at this time, the arrow indicates its posterior boundary. Somite 1 is just developing a clear anterior boundary at this stage. **B:** Two-somite stage, dorsal view. The notochord rudiment shows between the arrows, just anterior to the level of somite 1. **C:** Two-somite stage, ventral view. The arrow indicates the polster. **D:** Four-somite stage (11.3 h). Somite 1 now has an anterior boundary. The optic primordium begins to show (arrow). **E:** Four-somite stage, dorsal view, focus is on the notochord at the level of the boundary between somites 2 and 3. Note at the top how the brain rudiment and underlying axial mesoderm prominently indent the yolk cell in the midline. **F:** Five-somite stage (11.7 h), ventral view, focus is on the newly forming Kupffer's vesicle (arrow). **G:** Eight-somite stage (13 h). The optic primordium has a prominent

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horizontal crease (arrow). The midbrain rudiment lies just dorsal and posterior to optic primordium. The segmental plate, developing paraxial mesoderm posterior to the somite row, is clearly delineated. **H:** Thirteen-somite stage (15.5 h). Somites begin to take on a chevron shape. The yolk cell begins to look like a kidney-bean, heralding formation of the yolk extension. The tail bud becomes more prominent and Kupffer's vesicle shows from the side (arrow). **I:** Fourteen-somite stage (16 h), dorsal view, and positioned so that the first somite pair is at the center. Note at the top the shape of the brain primordium, at the level of the midbrain, **J:** Fifteen-somite stage (16.5 h). The arrow shows Kupffer's vesicle. **K:** Fifteen-somite stage from a dorsal view to show the optic primordia. Kupffer's vesicle is also nearly in focus. **L:** Seventeen-somite stage (17.5 h). The otic placode begins to hollow. The yolk extension is now clearly delimited from the yolk ball as the tail straightens out. **M:** Twenty-somite stage (19 h). The arrow indicates the otic vesicle. **N:** Twenty-five-somite stage (21.5 h). The telencephalon is prominent dorsally, at the anterior end of the neuraxis. **O:** Twenty-five-somite stage, dorsal view. The hindbrain's fourth ventricle shows at the top. Scale bars = 250 μm.

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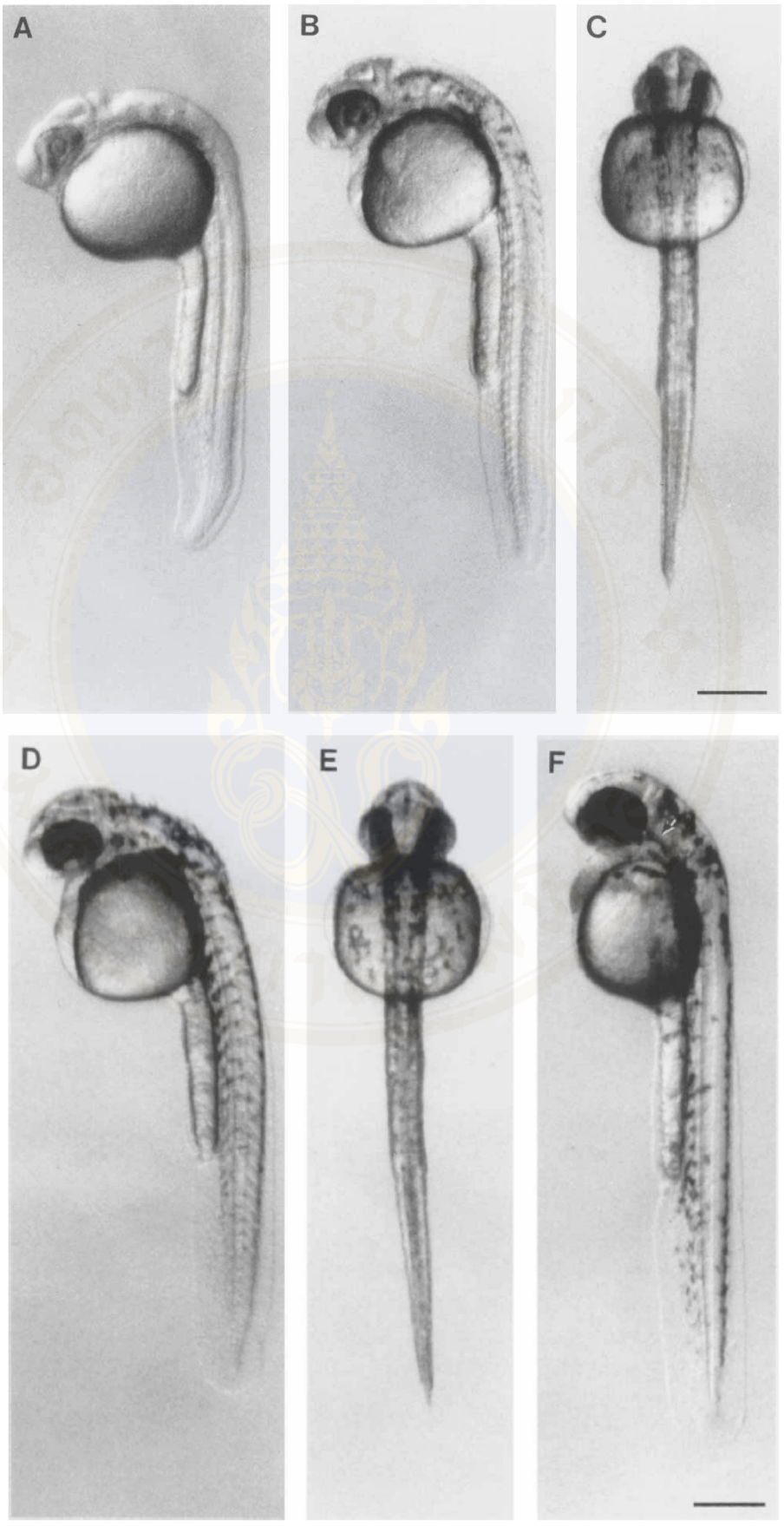

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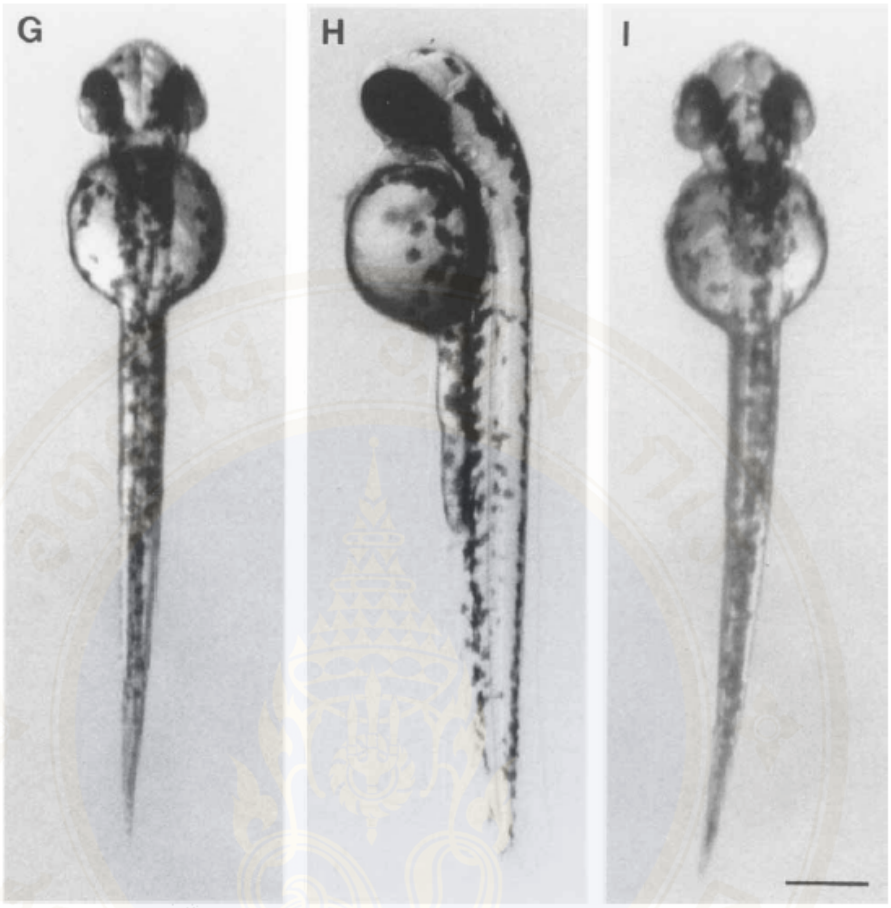

Development During the Pharyngula Period. Left-side and dorsal views (except for the prim-5 stage) of the same embryo at the given stage. **A:** Left-side view at the prim-5 stage (24 h). The brain is prominently sculptured. Melanogenesis has begun, but is not yet evident at this low magnification. **B,C:** The prim-12 stage (28 h). Melanophores extend from the level of the hindbrain to about the middle of the yolk ball. **D,E:** The prim-20 stage (33 h). A few pigment cells are now present along the axis dorsal to the yolk extension and on the dorsal part of the yolk ball. **F,G:** The prim-25 stage (36 h). Pigment extends almost to the end of the tail. The arrow in F indicates the ventral horn of melanophores. **H,I:** The high-pec stage (42 h). Pigment now extends the whole length of the embryo. The dorsal and ventral pigment body stripes are filled in, but not so neatly as they will be later. The lateral stripe is not yet evident. Scale bars = 250 µm.

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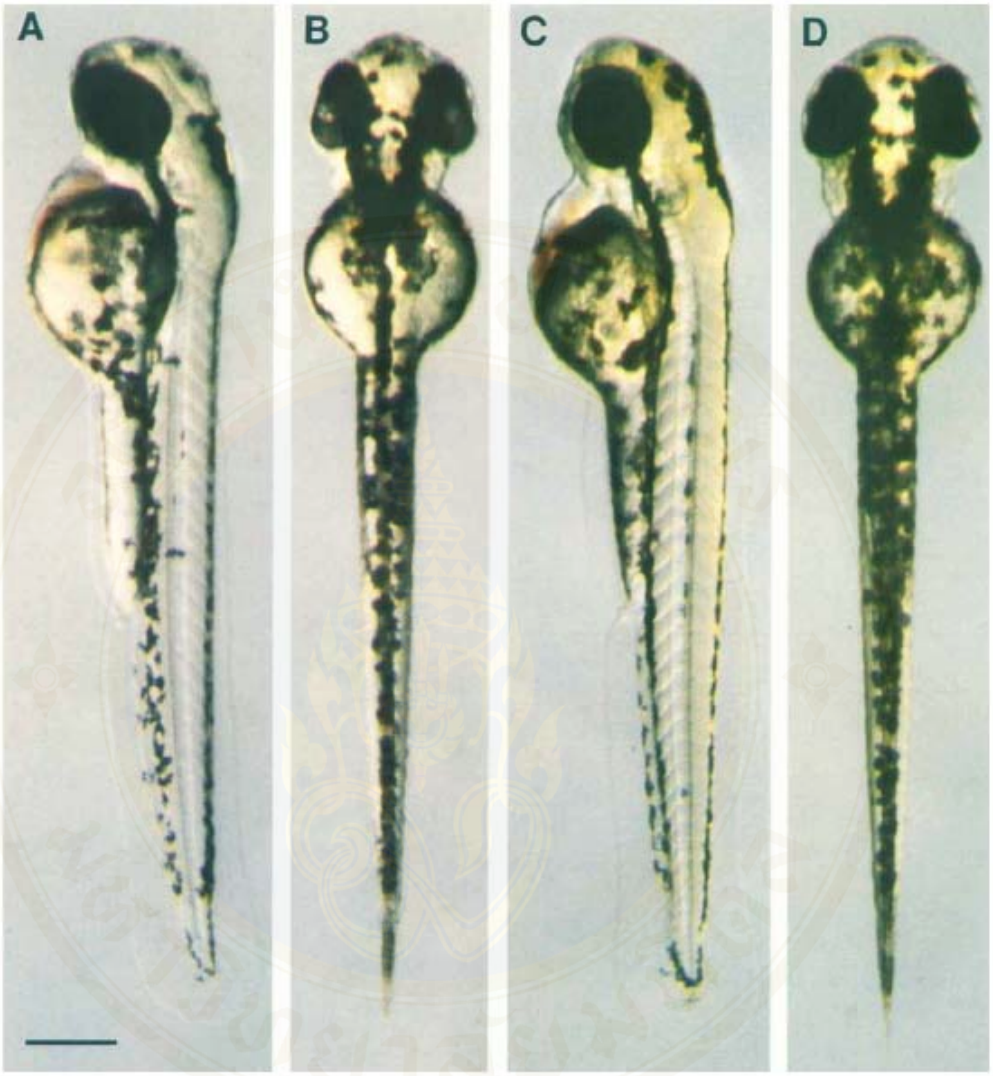

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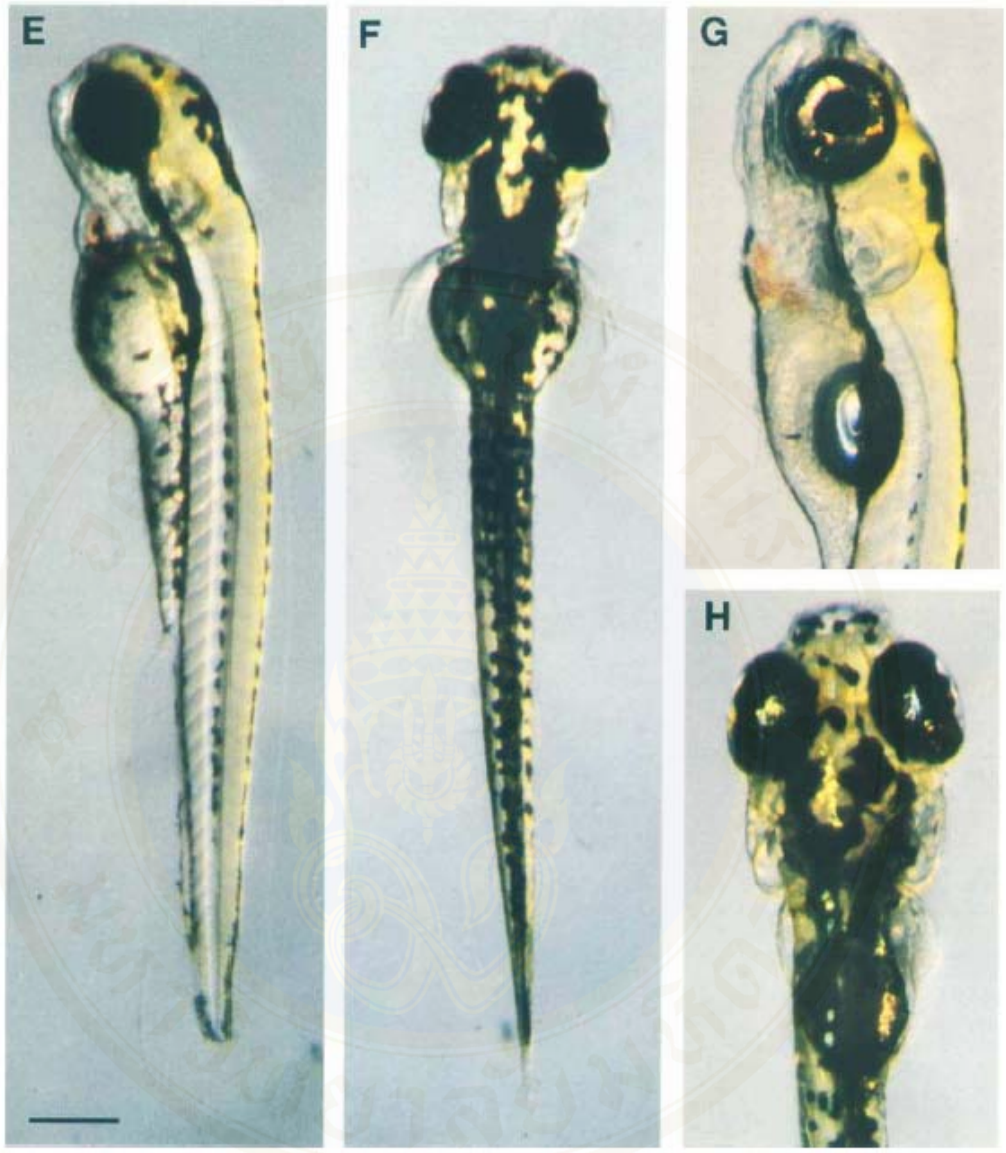

Development During the Hatching Period of Embryogenesis. (A-F), and the Early Larva (G,H). Left-side and dorsal views of the same embryo are paired for each time point. **A,B:** Long-pec stage (48 h). **C,D:** Pec-fin stage (60 h). **E,F:** Protruding-mouth stage (72 h). Note the progressive increase dorsally in yellow pigmentation owing to xanthophore development, and the progressive filling of melanophores into the lateral stripe. **G,H:** The early larva (120 h) is photographed with a combination of transmitted and incident illumination, the latter revealing reflective iridophores. The swim bladder is inflated at this stage. Continued development of the lower jaw, protruding it more anteriorly, brings the lower and upper jaws close together in front of the eyes. Scale bars = 250 µm.

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BIOGRAPHY

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