CHAPTER I

INTRODUCTION

The discharge of synthetic chemicals into the environment is of great concern all over the world. Thousand of chemical compounds including pesticides, drugs, plastizicers, detergens and fuel which are currently registered for use in industry, agriculture, and households. Some of these chemicals have been shown to disrupt activity of natural estrogen hormones in adult female of wild life. These substances are called xenoestrogens as they can interact with estrogen receptor and can result in induction of protein transcription and translation like the activity of estrogen hormones. These effects lead to reproductive impairment, inhibiting sexual development, reducing egg production, and decreasing fertility of aquatic species especially fish (Kime et al.,1999).

The well known and important responses to xenoestrogen are the induction of vitellogenin (VTG) and zona radiata protein (ZRP) syntheses in fish. VTG, a phospholipoglycoprotein, is a precursor of egg yolk while ZRP, a glycoprotein, is a precursor of egg envelope. These proteins are synthesized in the liver, normally occurs only in sexually mature female in most fish. VTG and ZRP are almost completely absent in male and juveniles fish because estrogen concentrations are too low to induce the expression of these proteins. However, induction of VTG and ZRP synthesis can occur in male and juveniles fish when these fish are exposed to xenoestrogens. Thus, the abnormal production of VTG and ZRP in male and juvenile fish can be used as biomarkers of exposure to xenoestrogens. VTG and ZRP are relatively easy to mesure in blood plasma by using immunosorbent assay techniques such as enzyme–linked immunosorbent assay (ELISA).

Several polyclonal antibodies specific to VTG have already been developed for ELISA methods but only a few monoclonal antibodies have been produced. VTG assays based on polyclonal antibodies are generally for use with the homologous species, even though some antibodies do cross-react with VTG in other species (Arukwe et al., 2003). There have been reported that ZRP may be a more sensitive biomarker for xenoestrogens (Arukwe et al.,1997a). Although polyclonal antibodies for ZRP have been developed and used for some time, monoclonal antibodies to ZRP have only recently become available (Berg et al., 2001). Moreover, both of these antibodies are specific to the VTG and ZRP in fish species from differrent regions which are not found in Thai waters.

Greenback Mullet, *Liza subviridis*, is an omnivorous species, feeding on algae, plants, and small crustaceans. This fish is a local species commonly found along the coastline and the brackish water areas of the rivers where the pollutants potentially contaminates the environment. Therefore, the responses of Greenback Mullet can be used as a new model for evaluation of the xenoestrogenic effects in the wild environments of Thailand.

The aims of this study were to develop efficient methods for detecting xenoestrogenic activities in the aquatic environment. This included the immunological assay commonly used for detecting biomarkers in many aquatic organisms. Monoclonal antibody against VTG and ZRP of Greenback Mullet, *Liza subviridis*, were constructed and applied to the sensitive competitive ELISA methods. These method will be applied for the detection of xenoestrogen in aquatic environment and will be useful for the water quality monitoring and early warning for the toxic compounds that affect animal health and ecological system of aquatic environment.

Objectives:

1) To produce monoclonal antibody against VTG and ZRP of Greenback Mullet, *Liza subviridis*
2) To develop a sensitive ELISA methods for determining the VTG and ZRP levels in experiment fish