Introduction
1. INTRODUCTION

The revelation that numerous classes of environmental contaminants can act as either hormone agonists or antagonists has led to resurgence in the interest and need for reproductive and endocrine toxicology. Accelerated industrialization and economic development have caused various anthropogenic threats to the natural environment via different pathways triggering serious threats to environmental health. Estuarine and coastal ecosystems in particular face major challenges as these are the mixing zone between rivers and seas and act as natural sink for several environmental pollutants (Ternjej et al., 2009). From an ecotoxicological point of view, the concern over emerging endocrine disrupting compounds (EDCs) is on trek due its multiple effects which affect the health and reproduction of wildlife. They exert their physiological effects either by mimicking the natural hormone or by interfering with hormone’s production, release, metabolism and elimination (Tabb and Blumberg, 2006).

Increasing advances in science and lifestyle have led to the development of these EDCs and gained importance. The quantity of release of such substances into the aquatic environment is tremendous that they cause several impairments when living organisms are exposed to these compounds. A subclass of these new chemicals, Endocrine Disruptors (EDs), is critical as they are potentially harmful compounds affecting the endocrine function of the body (Mezuka et al., 2012). EDs are synthetic chemicals that were originally designed for a specific action such as pesticide, plasticizer, or solvent, that are more stable in the environment with potential toxicity.
Though the term exists only very recently, it has been known for centuries (Tamara et al., 2009).

The endocrine system integrates and directs many of the processes of growth, reproduction, and immunity through complex regulatory pathways. Many of the processes of endocrine signaling that are ubiquitous across the animal and plant phyla, representing potentially vulnerable targets for the action of diverse chemicals (Medesani et al., 2004). EDs are structurally similar to many hormones, function at extremely low concentrations, and many have lipophilic properties. EDs are capable of mimicking natural hormones and maintain similar modes of action, transport, and storage within tissues. The properties of these chemicals, while unintended, make them particularly well suited for activating or antagonizing nuclear hormone receptors. Thus, there is virtually no endocrine system immune to these substances, because of the shared properties and similarities of receptors and enzymes involved in the synthesis, release, and degradation of hormones (Schug et al., 2011). EDs were originally thought to exert their actions solely through nuclear hormone receptors, including estrogen receptors (ERs), androgen receptors (ARs), progesterone receptors (PRs), thyroid receptors (TRs), and retinoid receptors. However, recent evidences show that the mechanisms by which EDs act are much broader than originally recognized (Wei An et al., 2006).

A wide range of substances, both natural and man-made, were thought to cause endocrine disruption, including pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls, DDT and other pesticides, and components of plastics such as bisphenol A (BPA) and phthalates. EDCs are found in many everyday products
including plastic bottles, metal food cans, detergents, flame retardants, food additives, toys, cosmetics, and pesticides. EDCs interfere with the synthesis, secretion, transport, activity, or elimination of natural hormones. This interference can block or mimic hormone action, causing a wide range of effects (Thaddeus et al., 2011).

Among chemicals proven to be EDCs, there is growing concern worldwide, especially in developing countries, about a group of surface active agents, eg alkylphenols and alkylphenol polyethoxylates, the indiscriminate use and discharge of which result in environmental pollution (Satyanarayanan et al., 2010). Alkylphenol polyethoxylates (APEOs) belong to the group of non-ionic surfactants and are used as detergents, emulsifiers, solubilizers, wetting agents and dispersants. Alkylphenol polyethoxylates (APEOs) make up the world’s third largest group of surfactants in term of production (Greek and Layman, 1989). Nonylphenol polyethoxylates (NPEOs) account for about 80% of total APEOs and 20% of octylphenol polyethoxylates (OPEOs) (Renner, 1997). Nonylphenol (Np), degradation product of nonylphenol polyethoxylates (NPEOs) which are used as detergents, wetting agents, dispersing agents, lubricating oil and emulsifiers in various commercial, industrial and domestic applications (Renner, 1997; Ferguson et al., 2003). Nonylphenol possess severe aquatic toxicity effects than NPEOs and are more persistent and lipophilic (Servos, 1999). It has been recognized as an endocrine-disrupting chemical that causes estrogenic effects in fish and other aquatic organisms (Routledge and Sumpter, 1997).

Nonylphenol have also been reported to be used in contact adhesive applications and, to a lesser extent, in coatings (Kirk, 1996). Contact adhesives are
blends of rubber, phenolic resin and additives either in a solvent or in an aqueous form. The phenolic resins promote adhesion and act as tackifiers. They are usually present at a concentration of 20-40%. In coating applications, the alkyl group on nonylphenol increases the compatibility with resins and varnishes (Kirk, 1996).

Due to its physico–chemical characteristics, such as low solubility and high hydrophobicity, nonylphenol accumulates in environmental compartments that are characterized by high organic content, typically sewage sludge and river sediments, where it persists. The occurrence of nonylphenol in the environment is clearly correlated with anthropogenic activities such as wastewater treatment, landfilling and sewage sludge recycling. Nonylphenol is found often in matrices such as sewage sludge, effluents from sewage treatment works, river water, sediments, soil and groundwater. The impact of nonylphenol in the environment includes feminization of aquatic organisms, decrease in male fertility and the survival of juveniles at concentrations as low as 8.2μg/l (Soares et al., 2008). Due to the harmful effects of the degradation products of nonylphenol ethoxylates in the environment, the use and production of such compounds have been banned in EU countries and strictly monitored in many other countries such as Canada and Japan. Although it has been shown that the concentration of nonylphenol in the environment is decreasing, it is still found at concentrations of 4.1μg/l in river waters and 1mg/kg in sediments. Nonylphenol has been referred to in the list of priority substances in the Water Frame Directive and in the 3rd draft Working Document on Sludge of the EU (Soares et al., 2008).
Nonylphenol has more severe aquatic toxicity effects than NPEOs and also more persistent and lipophilic chemical properties (Servos, 1999). Half-lives of biodegradation ranged from a few days to almost one hundred years (Zhen et al., 2011). Ever since nonylphenol was first synthesized in 1940, its use and production have been increasing almost exponentially (Manzano et al., 1998; Anonymous, 2001). The annual production of nonylphenol reached 154,200 tons in the USA (Anonymous, 2001), 73,500 tons in Europe (HELCOM, 2002), 16,500 tons in Japan (JME, 2001) and 16,000 tons in China (Anonymous, 2004). Np has a log \( K_{ow} \) of 4.48 (Ahel and Giger, 1993) and may consequently be taken up and bioaccumulated by aquatic organisms. Estrogenic effects of nonylphenol in fishes and other aquatic organisms first emerged in 1983–84 when Giger and Co-workers from Switzerland established that nonylphenol ethoxylates and products of degradation were more toxic to aquatic life than their precursors (Giger et al., 1984). Subsequently Soto et al. (1991) observed inadvertently that nonylphenol was capable of inducing breast tumour cell proliferation (Soto et al., 1991). Nonylphenol was found to mimic the natural hormone 17\( \beta \)-estradiol by competing for the binding site of the receptor for the natural estrogen (White et al., 1994; Lee and Lee, 1996).

Np is a compound which has numerous isomers, the side chain has nine carbons and can be attached to phenol at different points on the ring, thus producing different isomers (Zhen et al., 2011). Among NPs, 4-nonylphenol has been identified as the most critical metabolite because of its high resistance to biodegradation, toxicity and strong estrogenic effects (Taylor and Harrison, 1999). Besides, 4- Nonylphenol is able to
mimic the action of endogenous estrogens by binding to estrogenic receptors (Madigou et al., 2001). NPEOs being an extremely useful industrial surfactant, the wisdom of continued use is now in question because their biodegradation intermediates, which persist in the environment (Stephanou et al., 1982; Giger et al., 1984; Marcomini et al., 1990; Ahel et al., 1995) possess estrogen mimicking effects (Sumpter et al., 1993; White et al., 1994; Bicknell et al., 1995) that have been linked with perceived changes in the reproductive ability of aquatic and human populations (Jobling, 1996).

A wide variety of aquatic animals including fishes, molluscs and crustaceans are affected by nonylphenol toxicity, especially due to its estrogenic-like behavior (Flouriot et al., 1995; Cox, 1996). Np and NPEs may induce vitellogenin (vg) synthesis in freshwater and marine bivalves (Gagne et al., 2001) and in crustaceans (Zapata-Perez et al., 2005). The induction of vitellogenin (Vg) synthesis in male tissues has already been successfully used as an indicator as the presence of xenoestrogens in the environment, in fish (Hiramatsu et al., 2006; Orrego et al., 2006), bivalves (Marin and Matozzo, 2004; Matozzo and Marin, 2005), crabs and other crustaceans (Martin-Diaz et al., 2004, 2005). Exposure to nonylphenol significantly induced vitellin levels even at lowest exposure concentration (0.01µg/l) in the mysid Neomysis integer (Ghekiere et al., 2005). 4-nonylphenol and octylphenol caused intersex in males and resulted in enlargement of oocytes in both females and in the ovo-testis of intersex individuals of clams (Langston et al., 2007). Dose independent potential endocrine disruptor on ovo-testis induction is documented in the Japanese quail embryo (Atsushi et al., 2012). Nonylphenol treated pacific oyster (Crassostrea gigas) at 100µg/l showed significant
reduction in the number of oysters with motile sperm. Np not only causes gonadal architecture abnormalities, but also impairs sperm production and spermatozoan motility in teleost fishes in a relatively short time (Kawana et al., 2003).

In addition Np mimics estradiol (E2) in inducing the synthesis of hepatic estrogen receptor (ER) in Atlantic salmon (Yadetie et al., 2002). Ren et al. (1996) demonstrated significant increase in the vitellogenin production in rainbow trout (Oncorhynchus mykiss) exposed to nonylphenol at 100μg/l for 72 hr. Recent studies on individual or cumulative effects of Np concluded that the 4-nonylphenol caused genotoxicity in erythrocytes with many malformations in shape and number indicated with other blood parameters (Imam et al., 2011). Nonylphenol can alter the structures and biochemical constituents within non-endocrine tissue of fish and these changes may be mediated via destroying membrane structure and inducing cell necrosis (Haimanti et al., 2008). Low concentrations of Np generally caused malformations in the skeletal system. High concentrations (18.74μg Np/ l, 160μg Op/l) were found to inhibit the growth of embryos in the early life stages by preventing mitosis in sea urchin (Cakal et al., 2007). Nonylphenol significantly affects the expression of genes related to immune response in zebrafish embryos following oxidative stress (Xu et al., 2013). Studies on the effect of nonylphenol and octylphenol on Clarias gariepinus showed significant increase in haemoglobin and haematocrit levels exposed to 200 and 500μg/l of nonylphenol. Similarly biochemical constituents also showed significant increase based on dose-dependent manner. Tissue damage enzyme activities (AST, ALT, ALP, ACP and LDH) showed a mixed trend of both increase and decrease with Np and Op
exposure. Similarly exposure of Atlantic salmon smolts to a mixture of 4-Np and atrazine at concentrations of 5.0/1.0 and 10.0/2.0µg/l showed significant increase in gill Na⁺K⁺ATPase activity, plasma Cl⁻ and Na+ and increased mortalities.

Accumulation of alkylphenol ethoxylates (APEOs) has been noted at various trophic levels in the marine food chain including plankton, algae, crustaceans and fishes indicating that APEOs impact is continuously felt in the marine ecosystem. The most common route of nonylphenol entry into the environment is through wastewater. The nonionic surfactant group, NPEO, is typically used in domestic liquid laundry detergents, industrial liquid soaps and cleaners, cosmetics, paints, and as the dispersing agents in pesticides and herbicides (APE Research Council, 2001). Due to the extensive use of nonylphenol ethoxylates, they reach sewage treatment works in substantial amounts where they are incompletely degraded to nonylphenol (Ahel et al., 1994; Shao et al., 2003; Johnson et al., 2005; Koh et al., 2005; Nakada et al., 2006). NPEs are released most often to sewage treatment plants, and are degraded to shorter-chain NPEs, including NP1Eo and NP2Eo in active sewage sludge; these short-chain NPEs are then further degraded to Np (EPA, 2005). Nonylphenol is expected to adsorb strongly to soils and sediments. In sewage treatment plants, Np is expected to partition to sludge, and when released to the aquatic environment, is expected to partition mainly between water and sediment (EPA, 2005).

Owing to their wide usage, the occurrence of nonylphenol ethoxylates and nonylphenol has been reported around the world in rivers, lakes and coastal waters (Ying et al., 2002). Blackburn et al. (1990) reported concentrations from, 0.2 to 5.8mg/l
of nonylphenol and from, 0.6 to 76mg/l in estuaries and offshore areas of England and Wales Water. Nonylphenol concentrations ranked below detection level to significantly high concentration of 644µg/l in Spanish waters (Blackburn et al., 1995). Levels up to 53µg/l and 95µg/l of nonylphenol was detected in U.K and U.S.A respectively (Vazquez et al., 2005). Nonylphenol have been found in water samples from numerous locations worldwide (Blackburn and Waldock, 1995; Ahel et al., 1996; Rudel et al., 1998; Snyder et al., 1999; Potter et al., 1999; Kuch and Ballschmiter, 2001). The presence of Np has been reported in various other media including sediments (Bennie et al., 1997; Bennett and Metcalfe, 1998; Marcomini et al., 1990), air (Dachs et al., 1999; Van Ry et al., 2000), fish and mollusks (Lye et al., 1999; Keith et al., 2001; Ferrara et al., 2001) and even human food (Guenther et al., 2002). Nonylphenol ethoxylates were found in more than 70% of water samples in concentrations up to 11mg/l in coastal waters and sediments of Spain (Mira et al., 2002).

Besides, nonylphenol was also detected in different concentrations in microphytic algae of Glatt river (Switzerland) viz: Cladophora glomerata (38mg/kg dry weight), Fontinalis antipyretica (4.2mg/kg dry weight) and Potamogeton crispus (2.5mg/kg dry weight). The average concentration of nonylphenol in the river was 3.9µg/l. Concentrations of nonylphenol, detected in fish organs are as follows; muscle 0.18 mg/kg dry weight, gut 0.46-1.2mg/kg dry weight, liver 1.0-1.4mg/kg dry weight, gills 0.98-1.4mg/kg dry weight; Barbus barbus L., muscle 0.38mg/kg dry weight, gut 0.05mg/kg dry weight, liver 0.98mg/kg dry weight, gills <0.03mg/kg dry weight, heart 0.30mg/kg dry weight, roe 0.09mg/kg dry weight; Oncorhynchus mykiss, muscle
0.15mg/kg dry weight, gut 1.6mg/kg dry weight. Np has also been found in high levels in seafood from Singapore, especially in shrimps (Basheer et al., 2004) and at even higher levels in field-collected mussels, clams, and squid from Italy (Ferrara et al., 2001). These data indicate a potential pathway for human exposure through consumption of market seafood stuffs. NPs are, therefore, persistent, bioaccumulative, toxic to aquatic organisms, and estrogenic (Soto et al., 1998).

Backdrop review on literature strongly demonstrates that there is a concern that discharges of the wastewater effluents with high concentrations of APEOs and their degradation products (especially Np) into marine environments. This contamination can induce estrogen-like activity and exert cumulative action with other endocrine-disrupting compounds. Therefore, these substances should be monitored in the food chain of aquatic animals. Crustaceans are one of the most ubiquitous groups of invertebrates, inhabiting all types of aquatic animals. Hormonally regulated functions affected by pollutants have been previously reviewed in crustaceans (Fingerman et al., (1998); Zou and Fingerman (2003)). Evidences in animal models suggest that the EDs may affect not only the exposed animals but also the subsequent generations. Formation of ovo-testis in the first generation offsprings of the nonylphenol exposed adults (Lahnsteiner et al., 2011) and TBT-induced imposex in fresh water prawn Macrobrachium rosenbergii (Revathi et al., 2013) are of common examples. The mechanism of transmission involves non-genomic modifications of the germ line such as changes in the DNA methylation and histone acetylation. A major problem for the development of crustacean aquaculture is the control of vitellogenesis. Although not
fully elucidated, the process is known to be controlled by hormonal factors and neuroendocrine (Van Herp and Payen, 1991; Laufer and Biggers, 2001). While strong evidence on the effects of these substances has been published, reports on the presence of vertebrate like steroids in the hemolymph and other decapods crustacean organs are scarce (Couch et al., 1987). Several studies on the biological activity of these substances reported positive results with the stimulation of vitellogenesis in fresh water prawns by progesterone, estrone and 17β estradiol.

It is well known that xenobiotics can disrupt reproductive function at the behavioural, anatomical and physiological level is fish. Crustaceans are found to accumulate contaminants from both water and food reaching levels of contamination higher than those measured in mussels (Micheletti et al., 2007). Hence a controlled experimental approach is necessary to focus on xenobiotic effects in any animal model. Although, a few experimental reports are available on the effects on nonylphenol on the reproductive aspects, still there exists a lacuna on the effects of environmental relevant concentrations of xenobiotics on the aquatic organisms.

Aquatic organisms living in polluted water may not be lethal immediately. However, the toxicants can damage the tissues by altering the structure of cells and their functions. Histological study appears to be a very sensitive parameter and is crucial in determining the cellular changes that may occur in target organs, such as gills, liver and gonads (Dutta, 1996). Hence a histological investigation may, therefore, prove to be an effective tool to determine the health of organisms. Besides, it serves as a model for studying the interactions between environmental factors which include biotoxins and
pollutants such as hydrocarbons, poly chlorinated biphenyls (PCBs) and heavy metals (Brusle and Gonzalez, 1996). Therefore, histopathological studies are necessary for the description and evaluation of potential lesions in aquatic animals exposed to various toxicants (Mayers and Hendricks, 1986).

Furthermore, biochemical parameters are the suitable indicators of stress conditions caused by any toxicants. Protein and lipid are excessively utilized by the exposed organisms in an attempt to challenge the toxic effects of any xenobiotic chemicals (Mohan Raj, 2007) which lead to probable degradation in the biochemical contents. Since the stress conditions lead to alteration in the metabolic cycles, it is necessary to understand the significance of these variations in the tissues (Kharat et al., 2009).

Biomarkers are valuable tools to study the effect of environmental pollutants upon the organisms. Vitellogenin, precursor to the yolk protein vitellin in female vertebrates and invertebrates serves as an indicator of exposure to endocrine disruptors (Billinghurst et al., 2000; Fenske et al., 2001; Tsukimura, 2001; Versonnen and Janssen, 2004). Vitellogenesis involves the production of yolk proteins that act as nutrient sources for developing embryos. Consequently, any event that affects the synthesis of the yolk precursor vitellogenin will also modify reproductive success. Abnormally high or low levels of vitellogenin are indicators of disruption at multiple sites of the reproductive endocrine system, or of early sexual development and differentiation. Hence biomarker studies serves as a potential tool to study the effects of estrogenic environmental pollutants.
In contrast to the extensive literature dealing with the occurrence of nonylphenol and its effects, low- and middle-income countries often still lack the awareness on environmental monitoring. Though there are reports indicating the environmental relevant concentration of nonylphenol, the information is restricted to very few Indian freshwater systems. However such studies are inadequate in marine water, especially coastal system which acts as dilution zone of anthropogenic pollutants. Chennai, the 4th largest metropolitan city, housing more than 8.6 million residing, as well as 1.5 floating population remains as one of the polluted cities in India. The city is richly supplied with water as it is drained by, Adyar river in the south and by Cooum and Kosasthalaiyar in the middle and north respectively. However these rivers remain as a no use for humans other than serving as recipient of innumerable contaminants released from the city all of them ultimately discharging into Bay of Bengal thus exterminating the marine life. Therefore, the present study documents the environmental relevant concentration of nonylphenol, prevalent in the surface water and sediment, along the Chennai coast and its effect on the reproductive physiology of the Indian white shrimp *Fenneropenaeus indicus*. 
Thus the present investigation includes:

- Extraction and quantification of nonylphenol (Np) from water and sediment samples from different sampling sites along the Chennai coast.

- Bioassay studies using the marine shrimp *Fenneropenaeus indicus* with environmentally detected levels of nonylphenol to augment its impact on the reproductive physiology of the marine shrimp.

- Evaluation of biochemical components in various tissues associated with chemical treatment.

- Quantitative detection of vitellogenin (Vg) and qualitative analysis of Vitellin (Vn) as biomarker of vitellogenesis in both control and treated groups and

- Finally the DNA integrity of control and nonylphenol treated shrimp.