INTRODUCTION
Marine fisheries are important to the economy and well being of coastal communities, providing food security, job opportunities and livelihood (Bell, 1978; Delgado et al., 2003). In world marine fisheries produced 81.5 million tonnes of fish in 2014 and directly employed 34 million peoples in fishing operations in 2014 (FAO, 2016). In India marine fisheries produced 3418821 tonnes of fish in 2014 (FAO, 2016).

Fish diseases constitute one of most important problems confronting the fishery biologist today. Diseases of marine and estuarine fish have been received considerable attention during the last few years. This is partly due to the fact that monitoring of diseases of marine fishes has been recommended as a useful tool for biological effects monitoring (McIntyre and Pearce, 1980). External diseases of marine fishes were recognized as having a potential for biological effects monitoring, among them fin rot, ulcerations and epidermal neoplasms with increasing interest in occurrence and abundance (Sindermann et al., 1980).

Neoplasia is the cellular proliferative disease that is factually defined as new growth. More specifically, a neoplasm is an abnormal mass of tissue which is not coordinated with the growth of normal tissue and exceeds the growth of normal tissues (Willis, 1967). In earlier, they are very few reports available on fish tumours in India (Sarkar and Datta Chaudhuri, 1953, 1958; Rao and Lakshmi, 1986; Selvaraj et al., 1973; Lakshmanaperumalsamy et al., 1976; Rao and Rao, 1979), but the situation has recently changed. In recent days frequent tumour incidence has been reported in both cultured and captured fish in India (Jithendran et al., 2011; Gopalakrishnan et al., 2011; Ananda Raja et al., 2012; Sahoo et al., 2012; Vijayakumar et al., 2014; Sinduja et al., 2014; Vijayakumar et al., 2015). Hence the
aim of the present study focused the tumour affection in marine food fishes with the following objectives.

The specific objectives of the present study are

1. To study the occurrence of tumours in fishes, their gross-morphology as well as their distribution (positioning) on the body, investigate the radiography, analyse their prevalence and intensity and length and weight relationship of *S. longiceps* and *S. jello*.

2. To study the histopathology and histochemistry of fish tumour lesions.

3. To study the tumour immunology and aetiology using transmission electron microscope.

MATERIALS AND METHODS

The fishes were collected from fishing boats, auction yard and merchants of Parangipettai fish landing centre (Lat. 11° 30’N; Long. 79° 46’E) Tamil Nadu, southwest Bay of Bengal for the period of two years since January 2014 to December 2015.

Fishes were identified based on FAO fish identification sheet and fish Base (De Bruin *et al.*, 1995; Froese and Pauly, 2013). Total length and weight of each fish was measured to the nearest millimeter (mm) and gram (g) by ruler and weighing balance, respectively, and the sex was determined. The body surface, fins and head of each fish inspected both visually and palpation by hand. Internal tumours suspected by necropsy examination. The prevalence was calculated the following method of Margolis *et al.* (1982) and Bush *et al.* (1997). The mean intensity of the tumour determined using the scale coverage method (Vainikka *et al.*, 2004). Fishes were radiographed at 100 mA in 45 kV, to confirm the bony nature and extent of the tumours in the body. Tumour associated scale deformities
were examined under phase contrast microscopy (Nikon eclipse TS 100 and Magnus MLX-DX). Then, the scales were dehydrated and coated with gold ions using automatic ion coater (JEOL JFC 1600) under: 0.1 torr pressure, 20 mA current and 80s coating time. Surface structure was visualized by SEM using a 15 kV accelerating voltage (JEOL JSM 6610LV).

The tumour lesions excised and fixed in 10 % neutral buffered formalin for 48 h. The ossified bone tumours were subsequently decalcified with 5% formic acid for histological assessments. Then, the biopsies that were dehydrated through graded alcohol series, cleared in xylene, and embedded in paraffin wax (m.p. 56 °C). The thin section of 4µm was incised with rotary microtome (Yorco YSI-115). Then, the sections deparaffinized and rehydrated with xylene and graded series of ethanol. The sections were then stained with Harris hematoxylin and eosin (H&E) and special stains such as Argyrophilic nucleolar organizer regions (AgNORs), Periodic Acid Schiff (PAS), Masson’s tri-chrome (MT), Van Gieson, Von Kossa, Alizarin red S, Azo dye and Toluidine blue (TB). The stained sections were examined under a light and phase contrast microscope and photomicrographs were taken (Nikon eclipse TS100; Magnus MLX-DX).

Inflammatory infiltrations were enumerated in 10 high power fields (× 400). The scores of inflammation were graded according to the K–M criteria method followed by Roxburgh et al. (2009). A score of ‘0’ indicated no inflammatory cells; score ‘1’ denoted a low, score ‘2’ a moderate, and score ‘3’ a high inflammatory infiltrate.

The finely minced small fragments of tumour biopsies were fixed in 3.0 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.6) for 2 h at 4 °C for ultrastructural assessment. After fixation, the tissue was soaked in cacodylate buffer for 2 h at 4 °C
and post-fixed in 1 % osmium tetraoxide for 2 h at 4 °C. The fragments were dehydrated through graded alcohol series, infiltrated with propylene oxide and epoxy resin mixture and embedded with epoxy resin. The ultra-thin sections (below 100 nm) were cut through ultra microtome (Leica ultracut UCT) with a diamond knife; the ultra-thin sections were collected on copper grids and stained with uranyl acetate (double metallic) and Reynold’s solution (sodium citrate + lead citrate). The sections were transmitted under transmission electron microscope (Philips Tecnai TR spirit) and photographed.

RESULTS

The extended tumour-like masses were observed in commercially important marine fishes such as Indian oil sardine (Sardinella longiceps Valenciennes, 1847), Pickhandle barracuda (Sphyraena jello Cuvier, 1829), Goldlined seabream (Rhabdosargus sarba (Forsskål, 1775)), Bartail flathead (Platycephalus indicus (Linnaeus, 1758)), Grey mullet (Mugil cephalus Linnaeus, 1758), Blackfin sea catfish (Arius jella Day, 1877), Indian mackerel (Rastrelliger kanagurta (Cuvier, 1816)), Spotted catfish (Arius maculates (Thunberg, 1792)) and Tall-fin goatfish (Upeneus indicus Uiblein and Heemstra, 2010). Among them, S. longiceps was most frequently affected with tumours, followed by S. jello. Remains were less frequent R. sarba (3 cases), P. indicus (2 cases), M. cephalus (2 cases), A. jella (1 case), R. kanagurta (1 case), A. maculates (1 case) and U. indicus (1 case).

The overall tumour prevalence in sardine was 0.44 %. Year wise, the maximum prevalence (0.46 %) was recorded during 2015, whereas the minimum (0.41 %) was recorded during 2014. Season wise, the maximum prevalence (0.81 %) was recorded during summer 2015, whereas the minimum (0.14 %) was noted during post-monsoon 2014. Month wise, the higher prevalence (1.14 %) was
observed during June 2014. The majority of tumours located on the external region (99.26 %), than internal organs (0.74 %). Within the external tumour, the majority was located on the head region (57.81 %), followed by fins (16.08) and body (7.88 %). Whereas, in internal organs, the stomach region frequently found to be affected with tumour and tumour in gonad was rare. Sex wise, the higher prevalence was observed in female (52.13 %), then male (42.93 %) and some were immature gonads (4.93 %).

The overall prevalence of neoplasms in barracuda was 0.37 %. Year wise, the maximum prevalence (0.40 %) was observed during 2014, whereas the minimum was (0.32 %) recorded during 2015. The higher seasonal prevalence (0.68 %) was observed during post-monsoon 2014, whereas the lower was (0.06 %) occurred during summer 2015. Among the oral tumour, 6 fishes also affected with lingual tumours.

The present study clearly indicated that there was a variation observed in the prevalence of tumour infection between seasons. The strong correlation found between the prevalence and mean intensity according to the seasons. The higher prevalence and mean intensity during summer could be the reason of both the temperature and hormones and migration patterns of sardine. The higher tumour prevalence in barracuda during post-monsoon, may be due to contaminated water influx and/or viral production.

The length of the normal sardines ranged between 14.0 and 19.9 cm and weight varied from 28.0 to 83.0 g. The $b$ value of the pooled normal fish showed $b=2.36$, negative allometric growth. The $b$ value of the both male and female normal fish showed $b=2.43$ and $b= 2.27$, negative allometric growth. The length of the tumour infected fishes ranged between 16.9 and 20.5 cm and weight ranged
between 30 and 90 g. The \( b \) value of the pooled tumour affected fish showed \((b=1.7)\) negative allometric growth. The \( b \) value of the male and female tumour affected fish showed \( b=1.65 \) and \( b=1.41 \) negative allometric growth. Length and weight relationship of sardine fish indicate that the normal fish population is under negative allometric growth, moreover, tumour infection also affect the population growth which indicated lower \( b \) value than normal fish.

In the present study, based on the histopathological and histochemical diagnosis the epithelial and mesenchymal cell tumours are classified as; papilloma, hepatocellular carcinoma, nodular fasciitis, elastofibroma, desmoplastic fibroma, fibrosarcoma, myxoma, psammomatoid ossifying fibroma, myxoinflammatory fibroblastic sarcoma, leiomyoma, leiomyosarcoma, sarcomatoid epithelioma, cavernous hemangioma, lymphoma, lipoma, chondroma, enchondroma, chondrosarcoma, osteoma, osteosarcoma, paraosteal osteosarcoma, erythrophoroma, melanophoroma, iridophoroma, leucophoroma, branchioblastoma, compound odontoma, complex odontoma, melanotic osteoblastoma, chondroblastic iridophoroma and giant cell tumour in *Sardinella longiceps*, *Sphyraena jello*, *Rhabdosargus sarba*, *Platycephalus indicus*, *Mugil cephalus*, *Arius jella*, *Rastrelliger kanagurta*, *Arius maculates* and *Upeneus indicus*. A total of 31 different types of tumours were recorded from nine species of food fish. Among them, 24 were first time reported in the examined nine species. In addition, the histomorphic neoplastic lesions were clearly indicates their behavior such as benign, pre-malignant and malignant. The histomorphic neoplastic cells were consist pleomorphic and hyperchromatic nuclei, which are clearly indicate the cellular anaplasia and dysplasia. However, the lesions were mostly benign nature as behaviorally.
Most of the neoplastic lesions consist with infiltrating inflammatory cells. The preoperative systemic inflammatory response was graded as score 0 in 29 %, 1 in 39 %, 2 in 15 % and 3 in 17 %. The K–M criteria graded the tumour inflammatory cell response as ‘low grade’ in 76 biopsies (76%) and ‘high grade’ in 24 biopsies (24 %). The cells identified were following; mast cells, lymphocytes, monocytes and melano-macrophages.

Chronic inflammation is a promoting force of the tumor micro-environment. Moderate to abundant of inflammatory cells infiltrated with neoplastic lesions. They contain mast cells, lymphocytes, monocytes and melano-macrophage. Blood capillaries were frequently observed in mast cells infiltrated lesions. The ultrastructure of tumour lesion comprised with epithelial, fibroblast, osteoblast and immune cells, where associated with extracellular matrix of collagen fibrils. The cells size ranged between 4.5 and 35.0 µm in diameter. Inflammatory cells, granulocytes were most frequently present, than mononuclear cells. They consist of mast cells, lymphocytes and monocytes, but neutrophils were rarely observed. The results of the present study clearly show that the higher mast cells aggregation may enhance the formation of neo-angiogenesis and fibroblastic mitosis. The higher lymphocytes infiltrated lesions clearly indicating the tumour cell necrosis and rare vascular formation. Lymphocytes are suppressing other immune activities and promote cytotoxic activity against malignant cells. In addition, higher lymphocytes infiltration inhibits the mast cell aggregation. In contrast, higher mast cell infiltrated lesion also exhibit the lower lymphocytes aggregation. Both pathological and chronic inflammation of the fish tumours attributes the aggregation of melano-macrophage.
No virus particles were evident in the examined lesions, except seabream. Viral particles were spherical to polyhedral in shape with size ranged between 100 and 250 nm in diameter, enveloped with electron dense well-defined protein capsid with prominent core nucleo-capsid.

CONCLUSIONS

Tumour prevalence in *S. longiceps* and *S. jello* are an indicator of environmental stress. These neoplasms can also be useful as an bio-indicator of environmental degradation that has serious direct effects on aquatic ecosystems, including fish populations. The higher tumour incidences of sardine and barracuda populations indicate the high genetic instability and predisposition, because tumour is not reported in closely related species of sardine and barracuda. Temperature and hormonal patterns has important role on development and regression of neoplasms. Differential pathological diagnosis confirmed that the types of tumours and their behavior are benign, pre-malignant and malignant. Frequent and rapid regression suggests that there are changes in the immune system or that promote growth of neoplastic cells. The virus-like particles present in tumour tissue of *R. sarba* concludes that the retrovirus-like particles may be the causative agent for the tumour.
The ocean is a vast ecosystem and has enormous fish diversity. The fish has highly economic value and fulfill the food shortage. Marine fisheries are important to the economy and well being of coastal communities, providing food security, job opportunities and livelihood (Bell, 1978; Delgado et al., 2003). World marine fisheries produced 81.5 million tonnes of fish in 2014 and directly employed 34 million peoples in fishing operations in 2014 (FAO, 2016). In India marine fisheries produced 34,18,821 tonnes of fish in 2014 (FAO, 2016).

Ecological and environmental parameters such as biotic and abiotic factors play an important role in the fishery biomass. The abiotic factors such as seasonal variations, ocean current, water temperature, oxygen level, nutrients, pollution, sewage, industrial effluents and toxic metals, and biotic factors are predator, diseases, pathogen and parasites affect the fish diversity and biomass. The marine environments include a wide variety of physico-chemical and biological parameters, if it is more than acceptable level, which may stress to the fish leading to disease outbreaks (Roberts, 1989). Due to increase in air and water temperature which can enhance the breeding of vector organism (Freed et al., 2005) and increase the growth pathogen population (Woodhams et al., 2008) and also accelerate the transmission rates by causing proliferation of infective stages (Freed et al., 2005). Fish diseases constitute one of most important problems confronting the fishery biologist today.

Pathology

Pathology is the scientific study of disease, is a branch of medical science. Pathology embraces the structural and functional changes in disease, primarily
concerning to the examination of organs, tissues and body fluids. The ultimate goal of pathology is diagnosis of diseases and etiology, a fundamental objective leading to successful therapy and to disease prevention (Underwood, 2004). It is commonly divided into two main branches clinical pathology and anatomical pathology or a combination of these two, referred to as general pathology. Clinical pathology: the laboratory analysis of blood, urine and tissue samples to examine and diagnose disease. Anatomical pathology: the study and diagnosis of disease based on the examination of surgically removed biopsy or autopsy.

**Fish pathology**

Fish pathology is the study of diseases in fin and shellfishes and it is a branch of veterinary medical science. Disease, in fish, is closely linked to environmental stress. In the wild, they generally have some degree of freedom to modify their environment. The anatomy and physiology of fish are modified principally towards the two major ecological factors which control their existence: the aquatic environment and poikilotherm’s inability to control its temperature. These factors are also overriding significance in dictating the chain of events following any pathological change such as microbial infection, traumatic damage or nutritional deficiency.

Diseases of marine and estuarine fish have been received considerable attention during the last few years. This is partly due to the fact that the monitoring of diseases of marine fishes has been recommended as a useful tool for biological effects monitoring (McIntyre and Pearce, 1980). The disease and parasitism are threats to fish diversity, and it is important to identify the pathogens and parasites, which create risk to the biodiversity (Smith et al., 2006). External diseases of marine fishes were recognized as having a potential for biological effects
monitoring, among them fin rot, ulcerations and epidermal neoplasms with increasing interest in occurrence and abundance (Sindermann et al., 1980).

**Tumour**

Tumour is a cellular proliferative disease in which cells are infiltrative, aggressive, invasive and sometimes metastatic. The term “neoplasia” and “neoplasms” particularly as they concern lower animal, are difficult to define precisely. Meissner and Warren (1971) defined a neoplasm as “a disturbance of growth characterized by primarily by an unceasing, abnormal, and excessive proliferation of cells”. Prehn (1971) defined neoplasia as “that form of hyperplasia which is caused, at least in part, by an intrinsically heritable abnormality in the involved cells”.

Neoplasia is a disease in which genetically altered cells escape from normal growth regulations. Important concepts in the definition of neoplasia include: (i) the presence of an abnormal mass with growth that is uncoordinated with normal tissues; and (ii) persistence of excessive growth after cessation of the stimulus evoking the lesion (Willis, 1967). The abnormal growth is to some extent structurally and functionally independent of the host because neoplastic cells are partially free of the controls that act to regulate and limit growth of normal cells (Sirica et al., 1989). Persistence of growth after removal of the factor evoking the neoplasm indicates that the neoplastic trait is a change in structure or expression of DNA that is inherited by succeeding generations of cells.

Several morphological features distinguish neoplasms from normal tissues and from other types of lesions. Neoplastic growth is not controlled by the same mechanisms as that of controlling normal tissues. This results in a persistent, expanding or infiltrating growth without the architecture of normal tissue.
Neoplasms commonly form grossly visible masses, but this is not an essential part of the concept of the neoplasia. Neoplasms have varying degrees of abnormality in cellular appearance and growth rates, and functional differences are usually apparent between neoplastic tissue and related normal tissues.

Tumour is a common disease in the entire eukaryotic multi-cellular organisms. The tumours have been reported only in human beings, and later all the major varieties of tumors that occur in all the domestic and wild animals, including fish. Neoplasms have been reported in many species of aquatic organisms including copepods (Crisafi and Crescenti, 1975; Vanderploeg, 1998; Bridgeman et al., 2000; Jagadeesan and Jothibabu, 2016), sea lion (Moore and Stackhouse, 1978; Sato et al., 2002), seal (Brown et al., 1975), dolphins (Sanchez et al., 2002), water lizard (Schmidt, 1977), turtles (Herbst et al., 1999; Work et al., 2004), sea horse (Boylan et al., 2014), crab (Brock and Lightner, 1990), shrimps (Overstreet and Devender, 1978), lobster (Shields and Small, 2013) and fish (Bell, 1793; Schlumberger and Lucke, 1948; Wellings, 1969; Groff, 2004).

**Fish tumour**

Fish oncology is important not only because of the effect of neoplasms on individual fish and fish populations, but also because fish are good models for furthering our understanding of neoplasia. Neoplasms are growing on the skin and other visceral organs of fish. Neoplasms occur in many fish species, both in farmed and feral fish. Epidermal neoplasm is found throughout the world in freshwater and marine environments (Harshbarger and Clark 1990; Dethlefsen et al. 2000). The first incidence of tumour in fish reported as early as 1563, when farmed carp were afflicted by papillomatosis in Europe (Hofer, 1906). The first known report of tumor in feral fish appeared much later in 1793. Therefore, the first scientific
documents of a diseased fish concerned a Chaetodon species with tumour-like growths in the bones (Bell, 1793). More than 50 types of tumours have been reported in more than 1000 species of fish in worldwide.

Occurrence and high prevalence of tumours in cultured and captured fish populations have been reported in almost all tissue systems of the organs, and have been widely reported by scientist and naturalists (Wellings, 1969; Mawdesley-Thomas, 1971; Harshbarger, 1977; Moore et al., 1996). Because of tumours distinctive appearance and obvious pathological nature more than 50% of fish tumours are related to the skin (Anders and Yoshimizu, 1994; Mawdesley-Thomas, 1971). Prevalence of papilloma in specific fish populations may be very high. In one investigation as many as 55 % of the Pleuronectidae examined from the Pacific coast of North America had one or more papillomas. These occurred on younger fish mainly on the pigmented side (Stich et al., 1977). These are considered by some researchers to be degenerate parasites but the evidence suggests that in the case of Pacific flatfish papillomas, they are most probably transmitted by virus (Peters et al., 1983).

In fish, tumours of the skin, being visible externally, are among those most frequently reported. Epidermal neoplasm is highly found throughout the world in freshwater and marine environments, than tumours in visceral organs (Harshbarger and Clark, 1990; Dethlefsen et al., 2000). Although epidermal neoplasm is common in many fish species and the appearance of tumours similar across the species, there are many differences in the disease etiology across the species studied. The etiology of tumours is generally complex and many of the factors which contribute to tumour initiation and growth remain unknown. Evidence supports a multiplicity of causes in mammalian tumours and there is no reason to assume that fish tumours
differ in this respect. Known and suspected factors contributing to tumour formation in fish include viruses, chemical or biological toxins, physical agents, hormones, age, sex, genetic predisposition and immunological competence of the host. A genetic like may be closely associated with geographical location, which in turn may facilitate transmission of an infectious agent or aid the effects of a carcinogenic chemical. Carcinogens stimulate mutations in specific genes which become oncogenes which are active only in certain mutant form; many different carcinogens can induce the same mutation.

A viral and chemical etiology for tumours in feral fish was already suspected (Russell and Kotin, 1957). Nowadays epidermal neoplasms are known to be one of the most frequently occurring tumours in fish (Harshbarger and Clark, 1990; Harshbarger and Slatick, 2001). The cause of papillomatosis seems to be most likely viral in some species, though the viral agent varies between species. In some species, no viral etiology has been found, though papillomas seem in other cases to be more affected by chemical contaminants (Harshbarger and Clark, 1990). Epidermal papillomatosis does not usually cause mortality in adult fish. Moreover, epidermal papillomatosis is easy and cost-effective to study in most of the fish species. For these reasons, epidermal papillomatosis could serve as a bioindicator of environmental stressors for many fish species in which papillomatosis is known to be promoted by contaminants and other environmental stressors (Vethaak et al., 1992; Baumann et al., 1996). Typically, these earlier field studies have compared prevalence of papillomatosis in populations sampled from contaminated and more pristine reference sites (Baumann et al., 1996).
Histopathology

Histopathological examination is usually used to assess the manifestation of diseases and health of organisms and evaluating the organs structure, which reflect the morphological structure of the cells and tissues (Yevich and Barszcz, 1983). Tumour diagnosis is predominantly based on the histological pattern (Fenoglio-Preiser et al., 2002). Similarly, neoplasms of lower animals are also classified based on higher animals (Groff, 2004). Fish tumours are also classified according to the histogenesis and tissue origin of the neoplasm and the benign or malignant nature of the neoplasm, although the histologic characterization of a neoplasm as benign or malignant may be difficult to determine. Despite, benign neoplasms are generally well-differentiated neoplasms that exhibit a slow rate of growth and have been described as a consistent distensible mass that is often delineated by a fibrous capsule, whereas malignant neoplasms generally exhibit more rapid growth than benign neoplasms and have a variable cellular differentiation that may be well differentiated or lack differentiated (Cotran et al., 1999). The lack of differentiation of malignant neoplasms is often due to cellular anaplasia that is characterized by pleomorphism or heterogeneity of the neoplastic cells, and cellular dysplasia that is characterized by a loss of the normal uniformity and polarity or orientation of the neoplastic cells. In the more anaplastic tumours, the cells are difficult to identify and this is complicated further when a combination of tissues occurs.

Tumours of the epithelial and mesenchymal tissue, whether internal or external surfaces or deep margins, or glandular tissues, are characterized by their ability to grow in clusters or sheets of similar cells which can be used to indicate an epithelial or mesenchymal origin in even the most anaplastic of neoplasms. They
are also characterized by their ability to stimulate the production of local proliferation capillaries and a supporting stroma of connective tissues.

**Tumour immunology**

The tumour microenvironment comprises tumour cells, extracellular matrix, immune cells, cytokines and other factors, which is play an important role in tumour formation, growth, invasion and metastasis (Liotta and Kohn, 2001). However, chronic inflammation is a promoting force in the tumor microenvironment (Coussens and Werb, 2002), emphasizing the significance of an effective immune response in controlling tumour initiation, promotion and progression (Coussens and Werb, 2001; 2002; Schreiber et al., 2011). Immune cells, particularly mast cells, lymphocytes, macrophages and null cells serve as regulatory factor in the tumour microenvironment (Huang et al., 2008). Therefore, mast cell infiltration into tumor may possibly remodel tumor microenvironment and profoundly influence tumor behavior (Coussens and Werb, 2001; 2002). However, although some studies have state that mast cells promote tumor angiogenesis and tumor growth. The tumour infiltrating mononuclear cells (MNCs) are suppressing the various immune activities and cytotoxic activity against malignant cells (Miescher et al., 1986). The degree of mononuclear cell infiltration plays an important role in the prognosis of the cancer (Svennevig et al., 1984). The tumour microenvironment associated mast cells and mononuclear cells are promotes and inhibits, respectively, the tumour cell propagation, angiogenesis and metastasis in higher vertebrates.