1.0 GENERAL INTRODUCTION

1.1 Biology of Shrimps

Shrimps are swimming decapod crustaceans classified in the infraorder Caridea, found around the world in both fresh and salt water. Adult shrimps are filter feeding benthic animals living close to the bottom. They can live in schools and can swim rapidly backwards. Shrimp are an important food source for larger animals from fish to whales. They have a high tolerance to toxins in polluted areas and may contribute to high toxin levels in their predators. Together with prawns, shrimp are widely caught and farmed for human consumption. A number of more or less unrelated crustaceans share the word "shrimp" in their common name. Examples are the mantis shrimp and the opossum or mysid shrimp, both of which belong to the same class (Malacostraca) as decapods including shrimp, but constitute two different orders within it, the Stomatopoda and the Mysidacea. *Triops longicaudatus* and *Triops cancriformis* are also popular animals in freshwater aquaria and are often called shrimp, although they belong instead to the Notostraca, a quite unrelated group.
The class Malacostraca contains about half of the crustaceans. The members of this class have a primitive body plan that can be described as shrimp-like, consisting of a 5-8-7 body plan. They have a small carapace that encloses the head and the thorax, and have a muscular abdomen for swimming. They also have a thin exoskeleton to maintain a light weight. These general characteristics are common in all members of the class. The class can be further divided into the decapods, which are even still divided into the dendrobranchiates (prawns) and the carideans (shrimp and snapping shrimp) (http://en.wikipedia.org/wiki/Shrimp - cite_note-0).

The prawns have sequentially overlapping body segments (segment one covers the segment two, segment two covers segment three, etc.), chelate (claw like) first three leg pairs, and have a very basic larval body type. Biologists distinguish the true shrimp from the true prawn because of the differences in their gill structures. The gill structure is lamellar in shrimp but branching in prawns. The easiest practical way to separate true shrimps from true prawns is to examine the second abdominal segment. The second segment of a shrimp overlaps both the first and the third segment, while the second segment of a prawn overlaps only the third segment (Charles and Linda, 2008).
1.2 Shrimp Farming

The business of shrimp farming is widespread in almost all the important coastal regions and shorelines of the world. The shrimp farmers have successfully developed a variety of farming methods and strategies that work well for the whole industry. In the old days, the shrimp demand was quenched by the wild fisheries. Farmers used to catch the shrimp in traditional ways and sell them to the markets. As the years passed by the demand for the shrimp increased dramatically and it was hard to fulfill the demand with the wild catch. This situation made it imperative to switch to a more efficient shrimp farming methodology.

The initial shrimp farms were called extensive farms. Ponds spread across few hectares of area were used to breed shrimp. Some artificial ponds were also set up by clearing away the mangroves. With technology, farmers could now calculate and optimize the yield per area. This helped the issue of converting good agriculture land into artificial ponds. As more technical advancements were invented in the field of agriculture and livestock research, semi-intensive and intensive farms were introduced, where the shrimp were raised on artificial feeds. Water management and hygiene was also looked after in a better way.
With the increase in the import and export orders of shrimp, farmers rear shrimp in hatcheries and the postlarvae fishing methods. Such controlled breeding ensures good quality, uniform size, low mortality rate and accelerated growth of the shrimp. A food chain is established in the ponds, based on the growth of phytoplankton. Growth is accelerated by means of fertilizers and mineral conditioners. Most farms are producing one to three harvests a year.

There are three different stages in shrimp farming according to the life-stage of the shrimp. 1. Hatcheries breed shrimps and produce nauplii or even postlarvae, which they sell to farms. Shrimps are provided with a diet of algae, brine shrimp nauplii, animal proteins like krill along with antibiotics, if necessary. Small-scale hatcheries are very common in Southeast Asia. They use small tanks (less than ten tons) with low animal densities. Greenwater hatcheries are medium sized but still, they are used for low animal density farming. While the survival rate of shrimp in small-scale hatcheries is 90%, the greenwater hatcheries have only 40% survival rate. Galveston hatcheries are large scale industrial hatcheries with high densities in 15 to 30 ton tanks and the survival rates are as high as 80%. 2. Nurseries raise the postlarvae in large, rectangular tanks called raceways in order to make them fit for the marine conditions in the ponds growout. Usually, the
density is 150-200 animals per square meter. 3. Growout ponds take in the juvenile shrimp from the nurseries and grow them to the required marketable size. These ponds are usually around 100 hectares in area and have low densities of 2-3 animals per square meter. This process alone takes almost 3 to 6 months time.

1.3 Shrimp disease

According to FAO statistics world production of cultivated shrimp has increased steadily since the early 1980’s. It is also known that, the world shrimp fishery is not growing, while the demand for shrimp is increasing steadily. Only aquaculture can meet this increasing demand. Thailand represents a good example. Since a peak in 1982, captured shrimp production has declined slowly while aquaculture production has steadily grown. Despite the explosive growth in world production of cultivated shrimp, there have also been staggering, periodic losses due to disease. A global shrimp survey by the Global Aquaculture Alliance (GAA) in 2001 revealed a rough overall loss to disease of approximately 22% in a single year. Given a total production of 700,000 metric tons in 2001 valued at roughly US$8 per kg, this translated into an estimate of about US$1 billion loss in a single year. This was probably a conservative estimate, since farms with very bad results may not have responded to the survey. Thus, a
conservative estimate for the total loss to disease over the past 15 years may be in the order of US$15 billion. This illustrates the importance of disease control to the industry.

With respect to disease agents, the GAA survey revealed that, 58% of losses were attributed to viruses and about 22% to bacteria. Thus, the majority of the effort on disease control (80%) should clearly be focused on viral and bacterial pathogens. Indeed, that has been the case as the following review of disease control work will exemplify. The control effort has emphasized prevention, and this has required the development of good diagnostic tools, trained personnel and a better understanding of the hosts and their pathogens.
Diseases are one of the major constraints for the sustainable increase of shrimp production. Shrimp diseases can be divided into non-infectious and infectious in origin (Lightner and Redman, 1998). Infectious diseases are caused by viruses, bacteria, fungi and parasites. Biological factors such as microbial flora present in the pond play a major role on the susceptibility of shrimp to pathogens. Proper management of the microbial flora is done by biosecurity measures, aeration, reduction or elimination of pathogens and their carriers, application of probiotics, sludge management, waste treatment, reduction of the amount of water exchange and treatment of the incoming water. These are all important issues in prevention of shrimp disease (Horowitz and Horowitz, 2001). Viruses are considered to be the most important pathogens in shrimp. Different life stages of shrimp may be susceptible to certain viral infections causing mortality, slow growth and deformations. More than 20 viruses have been reported as pathogenic to shrimp.

In these early days, there were few disease control measures. Shrimp farmers used high rates of unfiltered water exchange and a wide range of chemicals and antibiotics, especially in the hatchery phase of production. There were few disease specialists available to help shrimp farmers and diagnostic capabilities in most regions were limited. This was a vulnerable
position as the industry grew exponentially with trends towards increasing farm densities in suitable farming areas and increasing rearing (stocking) intensity in individual ponds. Little was known of shrimp defense mechanisms, especially for viral pathogens. This eventually led to severe disease epidemics (epizootics) for which the industry was more or less unprepared.

The first widely reported shrimp disease epidemic was for Monodon Baculovirus (MBV) in Taiwan in the mid-1980’s (Lin, 1989; Liao et al., 1992). This was followed by epidemics caused by Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) in the Americas (Lightner et al., 1983; Lightner, 1996b), Yellow Head Virus (YHV) in Thailand (Chantanachookin et al., 1993; Flegel, 1997) and Taura Syndrome Virus (TSV) in the Americas (Hasson et al., 1995; Brock et al., 1995; Brock et al., 1997).

While the shrimp industry was still struggling with MBV, IHHNV, YHV and TSV outbreaks, it was hit by an even bigger disaster with the arrival of White Spot Syndrome Virus (WSSV). After its first appearance in China in 1992, it spread rapidly around Asia (Nakano et al., 1994; Flegel, 1997; Flegel and Alday-Sanz, 1998) and eventually to the Americas, first in 1996, but later with devastating losses from 1999 onwards. Total losses for
this virus alone have been estimated to be in the range of US$1 billion per year since the middle of the 1990’s

Serious viral disease outbreaks revealed that, the shrimp industry had to be better prepared with more knowledge about shrimp and their pathogens so that disease prevention methods could be improved. This need shifted attention to biosecurity, that is, possible methods of cultivating shrimp in restricted systems designed to prevent the entry of potential pathogens. The industry also realized that, a good number of disease outbreaks originated from careless transboundary movement of contaminated but grossly normal aquaculture stocks. More than any other problem, the WSSV pandemic served as a “wake up” call that shocked the industry into concerted actions. The catastrophic losses had serious impacts on whole national economies in Asia and the Americas. They resulted in increased support for research on shrimp diseases (including epidemiology) and in increased farmer awareness of the need for biosecurity. Research on shrimp defenses and shrimp pathogens increased sharply. Many diagnostic techniques were developed, particularly PCR and RT-PCR. Training programs were carried out (e.g. SEAFDEC and the University of Arizona) and shrimp domestication and breeding programs were started with *P. vannamei* and *P. stylirostris*.
1.4 White spot syndrome virus (WSSV)

WSSV is an enveloped, double stranded DNA virus, ovoid to bacilliform in shape with a tail like extension at one end (Van Hulten et al., 2001a; Yang et al., 2001b). A schematic diagram of the WSS virion is shown below. The virus is the only member of the family Nimaviridae, genus Whis povirus (Mayo 2002). WSSV is pathogenic to at least 78 species, mainly to decapod crustaceans including marine and freshwater shrimp, crab, crayfish and lobsters (Lightner, 1996; Flegel, 2006). The first outbreak due to WSSV was reported in shrimp farms in Taiwan in 1992 (Chou et al., 1995) followed by other shrimp farming countries of South East Asia, Middle East, North, Central and South America (Lightner, 1996; Rossenberry, 2002; Rodríguez et al., 2003; Flegel, 2006).
The genome of different WSSV isolates ranges from 292 kbp to 307 kbp in size (Van Hulten et al., 2001a; Yang et al., 2001; Chen et al., 2002). The virions are 70-138 nm x 240-340 nm in diameter. It contains a rod shaped nucleocapsid of 70-90 x 200-350 nm (Kasornchandra et al., 1998; Wang et al., 2000a). Genomic deletions were observed among isolates from different geographical areas (1 to 13 kb) (Marks et al., 2004) and from different host species of the same area (Lan et al., 2002). High variations in 54 bp DNA repeats were found in the samples from different regions in Thailand (Wongteerasupaya et al., 2003) and the intensity of hybridization signals varied between isolates by dot blot hybridization and some samples even failed to hybridize with some of the probes. This indicated the existence of WSSV mutants (Lo et al., 1999). WSSV is inactivated by various physical and chemical treatments including heat treatment at 55°C for 90 min, 70°C for 10 min, desiccation in filter paper within 3 h at 26°C, very acidic pH 1 for 10 min, pH 3 for 1 h, very alkaline pH 12 for 10 min, UV irradiation at $9 \times 10^5 \mu W \text{ cm}^{-2}$ for 60 min, different concentrations of disinfectants - ozone 0.5 and 0.8 µg/ml, formalin 200 parts per million (ppm), 25% sodium chloride within 24 h, chloroform within 15 min (Chang et al., 1998; Nakano et al., 1998; Balasubramanian et al., 2006). The effective concentrations of sodium

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hypochlorite, povidone iodine, benzalkonium chloride were between 75-200 ppm (Chang et al., 1998; Balasubramanian et al., 2006).

More than 50 structural proteins and one non-structural protein VP9 (Liu, et al., 2006) have been detected in WSSV till now. They were named according to the estimated molecular weights of the protein bands in SDS-PAGE or the number of amino acids. Proteins located in the envelope are: VP12, VP19, VP22, VP24, VP28, VP31, VP36B, VP38A, VP39, VP41, VP41A, VP41B, VP51B, VP52A, VP52B, VP53, VP53A, VP68, VP110, VP124, VP150, VP187, VP281, VP292, VP466 (Van Hulten et al., 2000; Van Hulten et al., 2001b; Huang et al., 2002a; Huang et al., 2002b; Van Hulten et al., 2002; Zhang et al., 2002; Tsai et al., 2004; Yi et al., 2004; Wu et al., 2005; Zhu et al., 2005; Li, et al., 2006; Tsai et al., 2006; Xie and Yang, 2006; Xie et al., 2006; Zhu et al., 2006), in the nucleocapsid: VP15, VP35, VP51C, VP60B, VP388, VP664 (Tsai et al., 2004; Leu et al., 2005; Witteveldt et al., 2005; Tsai et al., 2006; Xiao et al., 2006) and in the tegument: VP36A, VP39A, VP95 (Tsai et al., 2006). Locations of other proteins are not known. The functions of most of these proteins have not been fully elucidated. VP15 appears to be a DNA binding protein (Witteveldt et al., 2005). Neutralization assays suggested that, envelope proteins VP24, VP28, VP31, VP36B, VP68, VP76, VP281 and VP466 are involved in early stages of WSSV replication (Van Hulten et al., 2001b;
Huang et al., 2005; Li et al., 2005; Wu et al., 2005; Li et al., 2006; Xie and Yang, 2006). VP28 is involved in attachment and penetration into cells (Yi et al., 2004) and systemic infection (Van Hulten et al., 2001b; Wu et al., 2005). Primers designed against VP28 gene or antibodies produced against VP28 (Poulos et al., 2001) were found to be suitable to detect different isolates (Musthaq et al., 2006).

1.5 Natural products and drug discovery

During the last two decades, there has been an upsurge in the search for new plant derived drugs. This process has facilitated to produce remarkably a diverse array of over 1,39,000 natural products, containing medicinally useful terpenoid derivatives, alkaloids, glycosides, polyphenolics and steroids (Buckingham, 2001). The antiinfectives (antibacterial, antifungal, Parasitic and Viral), close to 70% are naturally derived, while in the cancer treatment, 67% are in this category. Several drugs are under clinical trials wherein most of them are anticancer drugs.

Most of the ayurvedic medicines are in the form of crude extracts which are a mixture of several ingredients and the active principles when isolated individually fail to give desired activity. This implies that, the activity of the extract is the synergistic effect of its various components. In the absence of pharmacopoeia data on the various plant extracts, it is not
possible to isolate or standardise the active contents having the desired effects.

Inventorisation of herbal drugs used in traditional and modern medicines for a country like India, appears to be a stupendous task, where a number of well established indigenous or traditional systems, including Ayurveda, Unani, Siddha, Homoeopathy, Tibetan, Amchi, Yoga and Naturopathy are practised along with modern medicine for the management of total health care system. In all these systems, a large number of plant drugs are used, although there may be some common plants. Another problem in correct identification of plants is that, the plant drugs in those systems of medicine are known by their classical, Shastriya or vernacular names. It is not easy to correlate these names with acceptable scientific names. One plant species can have many vernacular classical names and one name may refer to different plant species.

Chinese, Indian, Arabian and other traditional systems of medicines make extensive use of about 5000 plants. India is proud to be rich in biological diversity and tenth among the plant rich countries of Asia, sixth as far as centres of diversity especially agrodiversity are concerned. Nearly three fourth of the drugs and perfumery products used in the world are available in natural state in the country. India possesses almost 8% of the
estimated biodiversity of the world with around 1,26,000 species. It is one of the 12 mega biodiversity centres with 2 hot spots of biodiversity in Western Ghats and North-Eastern region. The sacred groves are a miniature ecosystem conserving biodiversity in its pristine form. There are about 400 families in the world of flowering plants, at least 315 are represented in India. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. About 5000 species have been studied. There are at least 121 major plant drugs of known structure, but none of them is currently produced through synthetic means. For developing phytomedicines as a major area of concern, it would be essential to adopt a holistic interdisciplinary approach, have a scientific basis of the understanding of the plant systems, new innovations and their conservation for utilisation in future on a sustainable basis (Sharma, 1997).