1.0 INTRODUCTION

The shrimp fishery is a major global industry with more than 3.4 million tons caught per year, chiefly in Asia. Small-scale local fisheries for (shrimp) prawns have existed for centuries, and continue to form a large proportion of the world's shrimp fisheries. Fewer than 300 species of shrimps and prawns are of commercial importance, in which 20 of these species are commercially significant out of a total of 3000 species. Shrimps are an important type of seafood which are consumed worldwide.

The world’s production of shrimp is about 6 million tonnes of which approximately 3.4 million tonnes are contributed by capture fisheries and 2.4 tonnes by aquaculture. By economic value, shrimp is currently the most important seafood commodity worldwide, and these are often the subject of scientific research in shrimp farming countries (Leu et al., 2011).

1.1. Shrimp Nutrition Facts

- Glycemic Index (GI) Rating / Glycemic Load: As shrimp contain no carbohydrates, their Glycemic Index rating is 0.
- Calories: Shrimp are relatively low in calories, with 1 gram of shrimp containing 1 calorie.
- Macronutrients: Shrimp are made almost entirely of protein and water, but they do contain some fat and cholesterol, too.
- Vitamins & Minerals: Shrimp are supercharged with vitamin B12 and selenium. In addition, they provide a fair amount of vitamin A, vitamin E, vitamin B6, some vitamin C, iron, magnesium, sodium (salt), zinc and copper (www.healwithfood.org/health-benefits/eating-shrimp.php).
1.2. Shrimp
Scientific classification
Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Decapoda
Sub Order: Dendrobranchiata

1.2.1. Description: Shrimp have a cylindrical body that is laterally compressed. They have a well-developed abdomen region and slender legs. Some shrimp have chelipeds. Shrimp have a thin, flexible exoskeleton. They possess stalked eyes on their head that are sometimes covered by a head shield.

1.2.2. Size: To 23 cm (9.1 in.).

1.2.3. Locomotion: Shrimp use their appendages for walking or burrowing. The well-developed abdomen region of shrimp allows them to use a form of temporary burst swimming as an escape reaction. By rapidly contracting the lower abdominal muscles, the shrimp quickly shoots backwards, using the tail fan for propulsion.

1.2.4. Diet: Plankton and detritus.

1.2.5. Feeding: Scavengers and detritivores.

1.2.6. Reproduction: Shrimps are either male or female. Female shrimps can only be fertilized when they are newly molted. Some shrimp deposit their eggs in the water; others brood the eggs in a pouch protected by their pleopods.

1.2.7. Respiration: Gills are feathery expansions of the body wall and are located on the side of thoracic segments. The number of gills varies between species.

1.2.8. Life Span: 1 to 5 years.

1.2.9. Range: Temperate and tropical seas worldwide; from the intertidal zone to the deep sea.

1.2.10. Habitat: Benthic among algae, sea grasses, stones, and shells, also within the crevices of coral or rock. Some burrow in sand or mud. A few species are pelagic to depths of 1000 m (3280 ft.). Freshwater species occur in estuaries, rivers, and lakes (Ruppert et al., 1994).

1.3. Phylum: Arthropoda
An arthropod is an invertebrate animal having an exoskeleton (external skeleton), a segmented body, and jointed appendages. Arthropods include the insects, arachnids, and
crustaceans. Arthropods are characterized by their jointed limbs and cuticles. The rigid cuticle inhibits growth, so arthropods replace it periodically by molting. Their versatility has enabled them to become the most species-rich members of all ecological guilds in most environments. They have over a million described species, making up more than 80% of all described living animal species (Hegna et al., 2013).

1.4. Subphylum: Crustacea
Crustaceans (Crustacea) form a very large group of arthropods, usually treated as a subphylum, which includes animals such as crabs, lobsters, crayfish, shrimp, krill and barnacles. The 67,000 described species range in size from 0.1 mm (0.004 in), to 3.8 m (12.5 ft) and a mass of 20 kg (44 lb). Crustaceans are distinguished from other groups of arthropods, such as insects, myriapods and chelicerates, by the possession of biramous (two-parted) limbs, and by the nauplius form of the larvae. Most crustaceans are free-living aquatic animals. More than 10 million tons of crustaceans are produced by fishery or farming for human consumption, the majority of it being shrimp and prawns (Crustacean glossary, 2010).

1.5. Class: Malacostraca
Malacostraca is the largest of the six classes of crustaceans, containing over 25,000 extant species, divided among 16 orders. They are abundant in all marine environments and have also colonized freshwater and terrestrial habitats. They are united by a common Bauplan, comprising 20 body segments (rarely 21), divided into a head, thorax and abdomen. They are abundant in all marine ecosystems, and most species are scavengers. The Class Malacostraca includes over 25,000 species, are characterized by the presence of three tagmata – a five-segmented head, an eight-segmented thorax and an abdomen with six segments. (Little et al., 1983).

1.6. Order: Decapoda
The decapods or Decapoda -literally "ten-footed" are an order of crustaceans within the class Malacostraca, including many familiar groups, such as crayfish, crabs, lobsters, prawns and shrimp. Most decapods are scavengers. The order is estimated to contain nearly 15,000 species in around 2,700 genera, with approximately 3,300 fossil species. All decapods have ten legs in the form of five pairs of thoracic appendages on the last five thoracic segments. The front three pairs function as mouthparts and are generally referred to as maxillipeds; the remainders are pereiopods. In many decapods, one pair of legs has enlarged pincers called chelipeds. Further appendages are found on the abdomen, with each segment capable of
carrying a pair of biramous pleopods, the last of which form part of the tail fan together with the telson called uropods (Grave et al., 2009).

1.7. Sub Order: Dendrobranchiata

Dendrobranchiata is a suborder of decapod crustaceans, variously called shrimp or prawns. There are 540 extant species in seven families. They have branching form of the gills and by the fact that they do not brood their eggs, but release them directly into the water. They may reach a length of over 330 millimeters (13 in) and a mass of 450 grams (1.0 lb), and are widely fished and farmed for human consumption. The body is typically robust, and can be divided into a cephalothorax (head and thorax fused together) and a pleon (abdomen). The body is generally slightly flattened side-to-side (Martin et al., 2001).

The body of the prawns/shrimps is almost laterally compressed with a toothed rostrum and a longer abdomen than the cephalothorax or head. Antennules in most of the species bear small scales or spines, while the antennae have large plate like scales. The pereopods are usually slender sometimes large and chelated. The pleopods or the swimming legs are well developed and present on all the abdominal segments (Kannupandi et al.,) Based on the place where they are grown and harvested, shrimps are classified into the following types.

Cold water shrimps are the smaller varieties harvested in ocean waters in the northwest and northeast regions of the United States and Canada. They are known as Pandalid shrimp.

Warm water shrimps are harvested and farmed in tropical and sub-tropical regions around the world. They include Penaeus and Litopenaeus species that are more commonly sold by reference to basic shell colors (white, brown and pink shrimps).

Wild shrimp refers to either cold water or warm water varieties that are harvested from coastal ocean waters with traditional vessels. The harvesting of wild shrimp is regulated by management programs that set annual production limits.

Farmed shrimp refers to warm water varieties that are grown in open and closed pond systems supplemented with formulated feeds. Shrimp diets and pond waters can be controlled to influence production rates and sensory attributes of the shrimp. Over 90 percent of the shrimp eaten in the United States come from farmed sources grown in other countries around the world (seafoodhealthfacts.org).
The shrimp species cultured in Asian countries belong to the family Penaeidae; genera Penaeus and Metapenaeus (Kungvankij, 1984). Penaeid shrimps are usually marine and coastal crustaceans. This family includes the most valuable marine commercial species of shrimps and accounts for at least 95% of the total shrimp production.

Among the decapods crustaceans, Penaeids constitute a distinct group of commercially important species. Due to their nutritional value, they support a valuable, trade export market. The genus - Penaeus spawns offshore at depth of about 10-80m and the eggs that hatch within a few hours, release very small larvae, then nauplii. Usually there are 11 larval stages, which include 5 nauplii, 3 protozoa and 3 mysis. The larvae are planktonic and are carried by current towards shore, where they arrive as post-larvae. This occurs about 3 weeks after hatching when the shrimps are about 6 to 14 mm long. Then the post-larvae invade in shore water like mangroves and brackish water, and they grow in these nursery grounds, develop into juveniles and return to near shore areas, more gradually back towards as they become sub-adults. Soon these shrimps migrate offshore, continue growing, and finally as adults reach the spawning grounds, and the cycle is repeated. In general, the Penaeid shrimps and non-Penaeid shrimps can be identified by using their morphological character.

In Penaeid, the pleurae are arranged regularly, the third pereopods are chelated. Abdominal segment does not have a sharp bend in the Penaeids. In Penaeid, the distinguished identical characters are the rostral structure, rostral teeth, antenna coloration and body color with strips. In majority of the Penaeid shrimps, rostral teeth are important characters to distinguish the different species and also within the groups. For e.g., in Penaeus species the rostral teeth are present both in the upper and lower portions of the rostrum, but in the case of Metapenaeus species the rostral teeth are present only in the dorsal side of the rostrum, but not in the ventral side.

In the non-Penaeid shrimps the pleura of the second abdominal segment are seen overlapping those of the first and third segments, the third pereopods are not chelated. Abdominal segment has a sharp bend in the non-Penaeid prawns (Kannupandi et al.).

The lifecycle of the Penaeid shrimp is divided into 4 successive stages: larval, post-larval, juvenile and adult during which, morphological, behavioral, feeding, and habitat changes occur. It starts in the open sea, where the male and female shrimps mate. Spawning takes place at night, the female releases the eggs and at the same time the spermatozoa are within
spermatophores. The process of fertilization is completed in the sea. Depending on the size of the female and species, one spawn gives between 1,00,000 and 10,00,000 eggs. The average diameter of egg ranges 250–300μm. The eggs hatch between 12 and 16 hours after fertilization, in Nauplii larvae. At this stage the larvae are vitellotrophs: they feed on their own yolk (vitellus). Nauplii look more like tiny aquatic spiders than shrimps. Then they metamorphose into Zoeae larvae, which have feathery appendages and elongated bodies. The zoeae shape is still far away from the adult shrimp. It lasts six days, feeds on micro-algae and then metamorphoses into mysis larvae. Mysis have many of the characteristics of an adult shrimp, segmented body, stalked eyes, and shrimp-like tails. They feed on phytoplanktons and zooplanktons. This stage lasts another three days, and then the mysis metamorphoses into post-larvae. Post-larvae look like juveniles and adults. After metamorphosing into post-larvae, the shrimp migrates from the open sea, where they led a planktonic life, to bay or inland sea of lower salinity, by means of oceanic and tidal currents. Then, post-larvae settle down on the sea-bed and begin their benthic life. During this stage, shrimps feed on small benthos, detritus, and algae. Then shrimps gradually migrate back from the shallow sea to the open sea where they will be mature enough to breed (Chim et al.,).

1.8. Penaeidae

Penaeidae is a family of marine crustacean in the suborder dendrobranchiata, which are often referred to as Penaeid shrimp or Penaeid prawn. It contains many species of economic importance, such as the tiger prawn (Penaeus monodon), white leg shrimp, Atlantic white shrimp and Indian prawn. Many prawns are the subject of commercial fishery, and farming, both in marine settings, and in freshwater farms (Denton et al., 1985).

A number of the prominent tropical shrimp species that are farmed commercially have been placed on the seafood red list, including the white leg shrimp, Indian prawn and giant tiger shrimp (TNAU, 2008).

1.9. White leg shrimp

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Decapoda
Sub Order: Dendrobranchiata Family: Penaeidae
Genus: *Litopenaeus*
Species: *vannamei*

White leg shrimp (*Litopenaeus vannamei*, formerly *Penaeus vannamei*), also known as Pacific white shrimp, native to the Eastern Pacific Ocean, from the Mexican state of Sonora as far south as Northern Peru. It is restricted to areas where the water temperature remains above 20°C (68°F) throughout the year.

*Litopenaeus vannamei* grows to a maximum length of 230 millimeters (9.1 in), with a carapace length of 90 mm (3.5 in). Adults live in the ocean, at depths of up to 72 meters (236 ft), while juveniles live in estuaries. Production of *vannamei* is limited due to its susceptibility to various diseases, including white spot syndrome, taura syndrome, infectious hypodermal and hematopoietic necrosis, baculoviral midgut gland necrosis and vibrio infections. By 2004, global production of *L. vannamei* approached 1,116,000 tonnes, and exceeded that of *Penaeus monodon*. (Species Fact Sheets, 2011).

1.10. **Indian prawn**

Kingdom: Animalia Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Decapoda
Sub Order: Dendrobranchiata Family: Penaidae
Genus: *Fenneropenaeus*
Species: *indicus*

The Indian prawn, *Fenneropenaeus indicus* (formerly *Penaeus indicus*), is one of the major commercial prawn species of the world. It is found in the Indo-West Pacific from eastern and south-eastern Africa, through India, Malaysia and Indonesia to southern China and northern Australia. *F. indicus* is known by many common names around the world, including Indian white prawn, Tugela prawn, White prawn, Banana prawn, Indian banana prawn and Red leg banana prawn.

*F. indicus* is a marine decapod with estuarine juveniles. It prefers mud or sandy mud at depths of 2–90 meters (7–295 ft). It grows to 228 millimeters (9 in) and has a life span of about 18 months. Among shrimps, the contribution of *F. indicus* to global fisheries was around 2.4%,
and to global farmed shrimp production was 1.2% in 2005. In India \textit{F. indicus} farming declined from 5200 tonnes in 2000 to 1100 tonnes in 2005 due to preference \textit{P. monodon} by farmers (Edwards, 1837).

1.11. Giant Tiger Prawn

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Decapoda
Sub Order: Dendrobranchiata Family: Penaidae
Genus: \textit{Penaeus}
Species: \textit{monodon} \textit{Penaeus monodon} is a marine crustacean that is widely reared for food. Its natural distribution is the Indo-West-Pacific, ranging from the eastern coast of Africa, the Arabian Peninsula, as far as Southeast Asia, the Sea of Japan and northern Australia. It is an invasive species in the northern waters of the Gulf of Mexico (Holthuis, 1949).

Females can reach approximately 33 centimeters (13 in) in length, but are typically 25–30 cm (10–12 in) in length and weigh 200–320 grams (7–11 oz); males are slightly smaller at 20–25 cm (8–10 in) length and weigh 100–170 g (3.5–6.0 oz).

\textit{Penaeus monodon} is the most widely cultured prawn species in the world, although it is gradually losing ground to the White leg shrimp, \textit{Litopenaeus vannamei} (MPEDA 2005).

In recent years, the global capture of wild shrimp has been overtaken by the harvest from farmed shrimp. A shrimp farm is an aquaculture business for the cultivation of marine shrimps or prawns for human consumption. The total global production of farmed shrimp reached more than 1.6 million tonnes in 2003 and about 75% of farmed shrimp are produced in Asia. By 2010, the aquaculture harvest was 3.9 million tonnes, compared to 3.1 million tonnes for the capture of wild shrimp (Gillet, 2008).

1.12. Shrimp farming

The life cycle of shrimps are controlled for size, survival, and to speed up the harvest cycle. A good farm location can be described as one with quality brackish water with soil and high
clay content with no bedrock or hard layers within 3 feet of the surface. There are three different stages in shrimp farming - hatcheries, nurseries, and grow outs.

1.12.1. Hatcheries
Hatcheries are the first stage in shrimp farming and dependant on mated female shrimps to provide larvae. In a few weeks, shrimps are relocated to nurseries for their development and growth.

1.12.2. Nurseries
The transition from shrimp larvae to adults occur in nurseries. After 21 to 25 days in the hatcheries, they are transferred to the nurseries in which they are fed on a high protein diet. In 4 to 6 weeks, they are transferred to the grow out systems.

1.12.3. Grow-outs
The last stage in shrimp farming is grow-outs, where shrimps are grown to maturity and transferred into ponds. Depending on the shrimp species, it takes between 3 to 6 months of growing until they reach market size after which they are harvested for sale, thus ending the shrimp’s life cycle (Achuthankutty, 1998).

Shrimp culture systems are classified into three, namely, extensive, semi-intensive, and intensive. The classification is based mainly on pond facilities, stocking density, food supply, water management, and yield, technical knowhow and skill and other major inputs. While semi-intensive and intensive farming have gained some progress in recent years, the extensive system still remains the major practice possibly because of the relatively large landholdings (50-300 ha) per farmer. In order to shift to the semi-intensive or intensive system, large areas will require greater amount of inputs, risk, high-level technical knowhow, and supervision. On the other hand, increasing acquisition and development costs per unit area has led farmers with small landholdings to go into intensive operation. Intensified farming virtually increases the yield per hectare thereby absorbing the relatively higher capital investment and risk. Further progress and development of any farming operation will largely depend on the supply and cost of fry, technical knowhow, quality and cost of feed and economic viability of the operation. For purposes of this paper, discussion is confined to extensive and semi-intensive farming operations (Apud, 1985).

1.13. Extensive shrimp farms using traditional low-density methods are invariably located
on a coast and often in mangrove areas. The ponds range from just a few to more than 100 hectares; shrimp are stocked at low densities (2–3 animals per square meter, or 25,000/ha). The tides provide for some water exchange, and the shrimp feed on naturally occurring organisms. Prevalent in poorer or less developed countries where land prices are low, extensive farms produce annual yields from 50 to 500 kg/ha of shrimp (head-on weight).

1.14. **Semi-intensive farms** do not rely on tides for water exchange, but use pumps and a planned pond layout. They can therefore be built above the high tide line. Pond sizes range from 2 to 30 ha; the stocking densities range from 10 to 30/square meter (100,000–300,000/ha). At such densities, artificial feeding using industrially prepared shrimp feeds and fertilizing the pond to stimulate the growth of naturally occurring organisms become a necessity. Annual yields range from 500 to 5,000 kg/ha, with densities above 15 animals per square meter, aeration is often required to prevent oxygen depletion. Productivity varies depending upon water temperature, thus it is common to have larger sized shrimp in some seasons than in others.

1.15. **Intensive farms** use even smaller ponds (0.1–1.5 hectares or 0.25–3.7 acres) and even higher stocking densities. The ponds are actively managed: they are aerated, there is a high water exchange to remove waste products and maintain water quality, and the shrimp are fed on specially designed diets, typically in the form of formulated pellets. Such farms produce annual yields between 5,000 and 20,000 kg/ha; a few super-intensive farms can produce as much as 100,000 kg/ha. They require an advanced technical infrastructure and highly trained professionals for constant monitoring of water quality and other pond conditions (Tacon, 2002).

**2.0 REVIEW OF LITERATURE**
Shrimps are the major commodity in Indian seafood exports since long. The export potential of cultured shrimp was recognized as early as 1979. Till 1988, contribution of shrimp to total export was mainly from marine capture fisheries. With the advent of scientific and commercial farming of shrimp, production increased gradually contributing over 32% in volume and 49% in value during 1988-1989, year from which data on cultured shrimps to exports are available. The export contribution of cultured shrimp was highest in 1994-95 but declined during 1996 to 1998 due to drop in production resulting in lower contribution by volume and value terms. The progress made in export front from 1998 onwards was highly promising with a significant rise in volume (49% to 60%) and value (75% to 86%). The cultured shrimp during 2001 - 02 contributed 59% in volume and 85.80% in value. The
contribution of cultured shrimp to total shrimp exports increased in a decade time (1990 to 2000) registering a growth of over 3 folds in volume and over 14 folds in value (Vasudevappa and Seenappa, 2002).

2.1. Penaeid shrimps

In 1934, Dr. Fujinaga, the world's acknowledged father of shrimp culture, successfully spawned and partially reared larvae of *Penaeus japonicus* in Japan. The success in larval rearing and subsequently in the grow-out of shrimp had brought the art to the point that mass culture was possible. In 1963, Mr. Harry Cook of the Galveston Laboratory in Texas, USA, in collaboration with Fujinaga, successfully spawned and reared the larvae of two American species, *P. setiferus* and *P. aztecus*. The technique was later adopted in Taiwan, Philippines, Thailand and Malaysia for local species such as *P. monodon, P. merguiensis, P. indicus* and *P. orientalis* (Kungvankij, 1984).

The genus - Penaeus spawns offshore at depths of about 10—80m and the eggs that hatch within a few hours, release very small larvae, the nauplii. Usually there are 11 larval stages, which include 5 nauplii, 3 protozoa and 3 mysis. The larvae are planktonic and are carried by current towards shore, where they arrive as post—larvae. This occurs about 3 weeks after hatching when the animals are about 6 to 14 mm long. Then the post—larvae invade inshore water like mangroves and brackish water, and they grow in these nursery grounds, develop into juveniles and return to near shore areas, more gradually back towards as they become sub—adults. Soon these shrimps migrate offshore, continue growing, and finally as adults reach the spawning grounds, and the cycle is repeated (Kannupandi *et al.*,).

The dietary protein requirement of Penaeid shrimps is an important nutritional consideration because it is a major limiting nutrient for growth. Four 28 day feeding trials were conducted to determine the maintenance requirement for protein (protein required to maintain body functions with all other nutrients provided in adequate amounts) by juvenile and sub-adult shrimp. Shrimps were offered practical diets containing 16 or 32% crude protein. Juvenile shrimps were found to have maintenance protein requirements in the range of 1.8-3.8 g dietary protein/kg body weight/day (g DP/ (kg BW * d)), and sub-adult shrimp were found to have maintenance protein requirements in the range of 1.5-2.1 g DP/ (kg BW * d). Protein requirement for maximum growth of juvenile shrimps were found to be 46.4 g DP/ (kg BW * d) when fed a 32% protein diet and 43.4 g DP/ (kg BW * d) when fed a 48% protein diet. Sub
adult shrimp exhibited a maximum protein requirement of 23.5 g DP/ (kg BW * d) when fed a 32% protein diet and 20.5 g DP/ (kg BW * d) when fed a 48% protein diet. FE was found to increase with the protein content of the diet and decreased with increasing feeding rates. Weight gain corresponded to daily protein intake. Based on these results a wide range of dietary protein levels could be used to produce maximum weight gain. However, due to restriction on feed intake and consequently protein intake, low protein diets may not support maximum growth (Kureshy and Davis, 2000).

Recent brood stock nutrition studies have contributed further to a better knowledge of the requirements of lipids, vitamins and carotenoids for the maturation process in Penaeid shrimp. Biochemical studies have detected high levels of TAG, phospholipids and cholesterol in the ovaries of mature shrimp, eggs and nauplii. Furthermore, ny3 HUFA were shown to be the predominant fatty acids in the tissues of shrimp broodstock and its offspring, and their importance was confirmed through diet studies. Biochemical and experimental work provided increasing evidence of the important role of natural anti-oxidants for optimal shrimp maturation, reproduction and larval quality, namely carotenoids and vitamins E and C (Wouters et al., 2001).

The Indian white prawn Penaeus indicus, a highly desirable species for culture, has been domesticated at the Marine Prawn Hatchery Laboratory of CMFRI, at Narakkal. Following the techniques developed at the Laboratory to induce them to breed in captivity and to rear them through all the stages of its life history in the farm itself, five successive penetrations were raised at the MPHL. The advantages of this kind of domestication in large-scale over the conventional culture of this prawn are briefly discussed in this note (Muthu et al., 1986).

Preliminary studies on the food and feeding habits of Penaeus monodon from Korapuzha estuary were undertaken. The food of the species consisted of crustaceans, fishes, molluscs, polychaetes and vegetable matter in order of importance. Mud and sand particles were also found among the food items. There was no appreciable variation in the nature of the food in the different size groups (Thomas, 1996).

Observations made on length-weight relationships, food, moults, sex ratio, breeding and migration in the case of Penaeus indicus and P. monodon of the Chilka lake were based on an eight-month study during May to December 1963. Within the Chilka lake the males and
females of both the species had different length-weight relationships. Both the species were found to feed to a large extent on detritus, crustacean remains, and plant matter and lamelli branches. The probable number of moults within a particular length range was given for both the species. By study of sex ratio the possibility of males migrating earlier to the sea than females was indicated. 93 mm and 155 mm were given as probable lengths attaining which *P. indicus* and *P. monodon* may start leaving the lake for the sea for breeding.

Observations on the availability of mature specimens of *P. indicus* in the coastal waters indicated the possibility of breeding in June, August and September (Prabhakararao, 1963).

2.2. **Nutrients of shrimp**

While shrimp may be small in size, they are rich in terms of nutritional value and the health benefits they offer. Loaded with protein, vitamin D, vitamin B3, and zinc, shrimp are an excellent, carbohydrate-free food. Zinc increases the levels of circulating leptin, a hormone that plays a key role in regulating the body's energy expenditure, fat storage, and appetite. Shrimp are loaded with selenium, which is believed to reduce cancer risk in two ways: First, selenium is an important constituent of glutathione peroxidase, an enzyme with anti-oxidant properties which can help protect the body from damaging effects of free radicals. Second, selenium is believed to prevent tumor growth by boosting the immune system and inhibiting the development of blood vessels to the tumor (www.healwithfood.org/health-benefits/eating-shrimp.php).

Physicochemical, proximate composition, microbiological and sensory analysis of farmed and wild harvested white shrimp *Litopenaeus vannamei* muscle were compared. The cultured white shrimp were obtained from two farms, whereas the wild shrimp were collected off the coasts of Sinaloa and Nayarit, Mexico. Both, the farmed and wild white shrimp muscle, supplied a good source of protein and polyunsaturated fatty acids. The physicochemical composition, microbiological and sensory properties could be associated to their origin and handling. The wild shrimp tended to have a better proximate composition than the farmed shrimp, due to the availability of a greater diet variety in their environment (Lopez et al., 2013).

The proximate chemical composition was determined in mid gut gland, ovary and muscle during different stages of ovarian development that was found to be varied during maturation among the different tissues of *Penaeus penicillatus* examined. The lipid content
was highest in the midgut gland as compared to ovary and muscle and the quantity was found to increase throughout the ovarian development in midgut gland and ovary while fluctuating in muscle. The increase in the protein content was not as significant in the midgut gland and muscle as that of the ovary during different stages of sexual maturation. The carbohydrate content did not show any significant change in any tissue though found to be highest in midgut gland as compared to ovary and muscle. The present study suggests that there was no mobilization of lipids from midgut gland to ovaries during ovarian maturation and the lipid increase was due to the intake through diet (Kherunnisa and Sultana, 2010).

Shrimps are an extremely good source of protein, yet are very low in fat and calories, making them a very healthy choice of food. The present was investigated the variation between the proximate and mineral compositions of the shell and flesh of *Penaeus indicus*. Shrimps were collected from the Vellar estuarine environment Southeast coast of India. The results of proximate composition shows that the percentage of protein in the flesh was higher (41.3%) than that of shell protein (32.5%). Likewise the carbohydrate content of flesh was higher (2.4) than the shell (1.5%). The highest amount of lipid was found in shell, the value being 9.8% where as in the higher level of moisture content was noticed in 14.7 % in flesh. The higher value of 26.6% of ash was noticed in the shell than that of flesh 18.5%. The results of the present study reported that *Penaeus indicus* is a good source of proteins and metabolically energy and average mineral supply (Ravichandran et al., 2009).

Black tiger shrimp harvested under controlled conditions had a shelf life of eight days in ice while shrimp purchased from open market broker system were unacceptable on the fourth day of storage. No bacterial contamination was found in either shrimp sample during ice storage. Total volatile base was found to be 20 mg/100g for open market shrimp and 25 mg/100g for controlled harvested shrimp. The pH of the shrimp increased from 6 – 7.3 during storage. The study demonstrates the benefit of controlled harvesting procedures temperature control during transportation to ensure optimal freshness and quality of black tiger shrimp (Hanpongkittikun et al., 1995).

2.3. Bycatch fisheries

Bycatch and discards are common problems faced by all fisheries globally. In tropical countries like India, bycatch issue is more complex due to the multi-species and multi-gear nature of the fisheries. Among the different fishing gears, trawling accounts for a higher rate of bycatch, due to comparatively low selectivity of the gear. The present study used shrimp
trawl in the traditional trawling grounds off Cochin in the southwest coast of India to reveal the quality, quantity and monthly variations of bycatch generated by trawlers. Mean monthly bycatch generated by shrimp trawling off Cochin ranged from 1.14 to 38.64 kg h$^{-1}$, in different months with an overall average of $12.85 \pm 1.97$ (SE, n=12) kg h$^{-1}$. Shrimp bycatch ratio ranged from 1:0.6 to 1:6908, during different months. The study identified 281 species including juveniles of commercially important fishes and shellfishes from the shrimp trawl bycatch (Gibinkumar et al., 2012).

Shrimp species of Gizan trawl fishing area were determined by commercial catch. *Penaeus semisulcatus* and *Metapenaeus monoceros* are the main species in the shrimp fishery. Analysis of length frequency data of females of these two species together with percentages of females with developed ovaries shows that both species have a prolonged spawning season with no trace of seasonal peaks. Remedy of fisheries problems related to overfishing is possible, but remedy of problems related to deterioration of nursery grounds is very difficult (Ghamrawy, 1990).

The present study makes it clear that nothing much from the shrimp trawler catches is wasted in India and almost all the fishes, which are termed trash fish and discarded over board the vessel in some of the developed countries, are utilized either for human consumption 12 or as fish meal and fish manure. However it is to be pointed out that the handling, processing and utilization of the fish catches, which form more than three fourths of the total landings, need further improvement (George et al., 1981).

### 2.4. Shrimp Farming

Aquaculture is the fastest growing form of food production in the world. Even though other products such as mollusks and carp are produced in greater numbers, shrimp dominates the aquaculture production by value. Also shrimp farming is one of the fastest growing aquaculture products in the world. Between 1982 and 1992 its production increased nine fold while its price remained consistent (Clay, 2004). This is encouraging for new shrimp farmers since prices don’t go down even though there is more competition. Unfortunately, with this increase in production, there are growing concerns about the environmental impacts, especially the destruction of mangrove swamps, pollution from antibiotics and pesticides used in intensive shrimp farming, and damage to the wild shrimp species. In recent years, the global capture of wild shrimp has been overtaken by the harvest from
farmed shrimp. A shrimp farm is an aquaculture business for the cultivation of marine shrimp or prawns for human consumption. The total global production of farmed shrimp reached more than 1.6 million tonnes in 2003; about 75% of farmed shrimp are produced in Asia. By 2010, the aquaculture harvest was 3.9 million tonnes, compared to 3.1 million tonnes for the capture of wild shrimp. (Gillet, 2008).

Most estimates figure that 90% of the shrimp sold in the United States comes from intensive or semi-intensive Asian or Latin American farms (Quick guide to shrimp).

Globally, shrimp farming is responsible for less than 10% of the loss of mangroves, yet the industry has borne the brunt of the criticism. This is because in certain regions the impact to mangroves has been much greater; Thailand for example cleared 64% of its mangroves for shrimp farms. Intensive shrimp farms, those with high stocking densities, are responsible for discharging effluents (pollutants) from fertilizers, feces, and excess artificial feed. These effluents can contribute to a high organic load which may pollute and eutrophication of the surrounding coastal waters. Chemicals used in shrimp culture are minimal, though the use of antibiotics is of concern because of the emergence of resistant bacteria. In some countries, post-larvae used to stock grow-out ponds are harvested from the wild as opposed to being grown in hatchery facilities. While the impacts of these practices are not documented, removal of wild larvae may have adverse effects on the recruitment of wild shrimp populations in those areas (Boyd and Clay, 1998).

Shrimp-farming operations are, however, highly varied. Extensive and improved extensive shrimp farms (i) stock shrimp larvae directly from the sea, in part or entirely, (ii) have a relatively low density of shrimp, and (iii) have overlapping generations of shrimp. On the other hand, intensive shrimp farms (i) stock shrimp post-larvae (PL) from hatcheries, and the brood stock can originate from geographic locations far from the farm, (ii) have a relatively high density of shrimp, and (iii) have non-overlapping generations of shrimp. Typically a pond is seeded with post larvae (PL) and they are subsequently harvested together, after which the pond is drained and cleaned. (Dieu et al., 2011).

Shrimp has emerged as an important item in the world seafood production. Asian countries like Taiwan, Indonesia, Thailand and India have emerged as global leaders in shrimp production. In order to bridge the gap between world demand and supply of shrimp, many
countries have undertaken intensive shrimp farming with intensive application of fertilizers and chemicals to boost the productivity. But such production system has led to degradation of the resource base which resulted in the massive disease outbreak in the shrimp industry especially in 1995-96 and caused subsequent drop in the world shrimp production. Thus, sustainability of shrimp farming is emerging as a major policy concern in the context of further development of shrimp farming as a money spinner. Inappropriate and excess use of chemicals, fertilizers and accumulation of excess feed in the pond bottom makes the soil acidic and unsuitable for any further use either for agriculture or other fish culture (Bhattacharya, 2009).

Performance evaluation of two different culture systems of *Penaeus* monodon namely modified extensive and semi-intensive farming in low saline water (>10 ppt saline soils) has been carried out in West Godavari district of Andhra Pradesh during a winter crop season. Total production (t/ha/crop) was found to be three times higher in the semi intensive farm than the modified extensive farm. However, growth rate of individual shrimps and final average body weight were more in the modified extensive farm. The average body weights at final harvest were $35 \pm 1.62$ g (105 days of culture) and $28 \pm 2.18$ g (112 days of culture) respectively for modified extensive and semi-intensive farms. Survival and FCR were similar in both the farming practices. Water quality parameters did not vary, but certain environmental indices such as total organic matter content and total nitrogen levels of soil, ammonia-nitrogen level in water were higher in semi intensive farm indicating its potential impact on environment (Laureatte *et al*., 2012).

Extension activities to disseminate the information on BMPs and adoption of these BMPs by farmers showed significant improvement in the success of farmers by resulting in better crop outcomes and reduced emergency harvests, which is indicative of a disease outbreak. Cluster farm management approach by promotion of farmer ‘Aquaclub’ proved that unity and farming discipline among shrimp farmers can effectively contain the diseases at village cluster level. The average crop outcomes in study ponds in terms of production, average body size, survival rate and crop duration were 1,366 kg/ha, 26.2 g, 72% and 116 days, respectively; while in non-study ponds were 764 kg/ha, 24.7 g, 46% and 98 days, respectively. Emergency harvests indicative of disease outbreaks was 2% in study ponds compared to 65% in surrounding non-study ponds and they were significantly different (p=0.00) (Padiyar *et al*., 2008).
2.5. Shrimp’s contamination with toxic heavy metals

Heavy metal pollutions are particularly hazardous contaminants in food and the environment. In general, they are not biodegradable and have long biological half-lives. According to the World Health Organization, 1995, heavy metals must be controlled in food sources in order to assure public safety. Excessive concentration of food heavy metals is associated with the etiology of a number of diseases, especially cardiovascular, renal, neurological, and bone diseases. A major reason to monitor levels of toxic metals in foods follows from the fact that contamination of the general environment has increased.

The level of the heavy metal in green tiger shrimp (Penaeus semisulcatus) and crab (Portunus pelagicus) caught off the Persian Gulf near Bushehr province were investigated. This study was performed to evaluate instrumental neutron activation analysis (INAA) to analyze heavy metal concentration in crab and shrimp whole body tissue. The order of the swimmer crab and shrimp heavy metal concentrations were Zn>Fe>As>Mn>Co and Fe>Zn>Mn>As>Co, respectively. The results showed swimmer crab (Portunus pelagicus) and shrimp (Penaeus semisulcatus) caught off Persian gulf, were contaminated with high level of As. High levels of As and Mn were noted in crabs and shrimp, respectively (Heidarieh et al., 2013).

Heavy metal pollution is one of the major types of pollution in coastal and marine environment. Heavy metals are conventionally defined as elements with metallic properties such as ductility, conductivity, stability as cations, ligand specificity, etc (Umamaheswari et al., 2011).

Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems. There is increasing concern about the quality of foods in several parts of world. The determination of toxic elements in food has prompted studies on toxicological effects of them in food. Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity and accumulation by marine organisms (Shrivastava et al., 2011).

In 2012, Balfour et al., done research work to determine chemical contaminants copper, zinc, cadmium, chromium, nickel and mercury in the marine shrimp (Penaeus spp.) Determination of all heavy metals was by flame atomic absorption spectroscopy except for mercury. The heavy metal findings in the marine shrimp were well below the United States Food and Drug
Administration, Canada’s Food Inspection Agency and, Trinidad and Tobago’s admissible limits for human consumption. Significant differences were observed only for copper and zinc by location and season interaction. The marine shrimp (Penaeus spp.) in Trinidad were safe to consume since the metal (Cu, Zn, Cd, Cr, Ni and Hg) concentrations were well below local and international admissible limits for human consumption. Some vending sites (locations) had higher levels of metal contamination mainly in the dry season which may have been due to anthropogenic metal contamination along the coastline or further in-land.

The presence of heavy metals in our environment has been of great concern because of their toxicity when their concentration is more than the permissible level. These metals enter in the environment by different ways like industrial activities etc. this study have used Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) technique for determination of Aluminum, Boron, Barium, Cadmium, Cobalt, Chromium, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Nickel, Lead, Antimony, Tin, Silicon, Phosphorus and Zinc in different species of fish tested. The concentrations of Ca, K, Mg, Na and P are too high as compared with other metals as these metals were not in the maximum permissible level set by World Health Organization (WHO) (Sen et al., 2011).

Cd exhibits biochemical and physiological toxicity for crabs and shrimps, affecting on activity of antioxidant enzymes, affecting metabolic enzymes, affecting Na + -, K + - ATPase, etc. Cd showed noticeable effects on the reproduction of crabs and shrimps. Female crabs exposed to 0.50 mg/L Cd showed significantly lower the gonadal somatic index, oocyte diameter values and the ovary vitellin than controls. These proved certain concentration of Cd inhibited ovary development in E. sinensis. Cd stimulated the secretion of GIH, increased progesterone level and decreased estradiol level in haemolymph. The vesicles of the endoplasmic reticulum became swelled and dissolved; ribosomes on the endoplasmic reticulum gradually fell off by Cd toxicity. Cd restrained the proliferation of isolated spermatogenic cells from M. Nipponese (Kang et al., 2012).

Metallothioneins (MTs) have been widely considered for their potential use as specific biomarkers to reflect the existence of heavy metal pollution, because their induction has been observed to be obviously elevated after heavy metal exposure in a large number organism studied. However, relatively fewer efforts have been made in MT-related studies of prawn species, such as the white shrimp Litopenaeus vannamei, a globally important aquaculture species. With the results from gel filtration chromatography, we demonstrate the existence of
MTs or MT-like proteins in *L. vannamei*. We further studied the relationship between MT induction and metals accumulation after long-term exposure to the heavy metals Cd and Zn. From our results, it is very clear that the response of *L. vannamei* to Cd differs from that to Zn, and this should be considered when using MTs in field applications to monitor metals contamination (Wu and Chen, 2005).

2.6. Shrimp disease and veterinary drugs

Despite the explosive growth in the world production of cultivated shrimp, there have also been staggering periodic lose due to disease in which 60% loss due to viruses 20% due to bacteria (Flegel *et al.*, 2008). Disease outbreaks can be a fatal challenge to the shrimp species. Sometimes it can wipe out all of the farmed species. Shrimp farming businesses also have a heavy reliance on other countries for feed, brood stock and seed stock; creating a larger risk for new shrimp diseases.

Shrimp cultivation areas have expanded the most. However, this industry faces major problems with bacterial diseases, and large quantities of chemical and antibiotic products are frequently used to counteract this (Le *et al.*, 2005).

Antibiotics are commonly used in aquaculture during the production cycle, both in the larval and growth phases. The use of antibiotics in aquaculture is associated with environmental and human health problems, including bacterial resistance, persistence of the disease in the aquatic environment, and effects on the biogeochemical composition of the sediment. The accumulation of antibiotic residues in the edible tissues of shrimp may also alter human intestinal flora and cause food poisoning or allergy problems (Ma *et al.*, 2006).

In an attempt to stave off disease, shrimp in many foreign farms are given daily doses of antibiotics, either mixed in with feed pellets, dumped directly into pond water or both. Oxytetracycline and ciprofloxacin, both of which are used to treat human infections, are two of the most common drugs in shrimp farming. The use of chloramphenicol, penicillin and other antibiotics pose serious health risks – such as susceptibility to antibiotic-resistant bacteria – to consumers if residues of the drugs remain in the shrimp (Suspicious Shrimp, 2008).

Infectious diseases are a major concern in aquaculture both in terms of the potential negative impacts on production and the potential for disease impacts on wild populations. Outbreaks of disease are typically caused by widely distributed, opportunistic pathogens. In natural
systems they have a low prevalence and low intensity of infection, but in aquacultures or aquaculture hatcheries where stresses lower resistance and stocking density facilitates transmission of disease, impacts of disease outbreaks can be severe (Johnston and Santillo, 2002).

For decades, various diseases have devastated the shrimp industry throughout the producing nations by wiping out entire crops. One of the most damaging is the White Spot Syndrome Virus (WSSV), which has been the most widespread, causing high mortality rate in many shrimp species and other crustaceans. Unregulated processing, use, and disposal of infected imported shrimp or the use of contaminated larvae in farming have caused the rapid spread of WSSV from its endemic regions to wild and cultured stocks of shrimp throughout the world.

In efforts to protect their shrimp from the effects of WSSV and other pathogens, shrimp farmers worldwide turn to the chemical and pharmaceutical industries, although it is nearly impossible to control WSSV other than by destroying the entire infected crop. There are relatively few constraints on chemical usage in aquaculture in the countries where shrimp is farmed and many antibiotics are widely available from chemical and pharmaceutical suppliers. The U.S. is comparatively strict in this respect, limiting the use of antibiotics in aquaculture to three drugs: oxytetracycline, sulfamerazine, and a drug combination containing sulfadimethoxine and ormetoprim (The chemical cocktail, 2004).

Indian shrimp farmers prefer tiger prawns *P. monodon* due to its large size, faster growth and lucrative price. This may lead to indiscriminate explosion of wild spawning stock and over a long period of time this may also adversely affect the capture fisheries resources. Disease control management should receive utmost priority in India because the outbreak occurred in 94 caught us unawares and caused heavy loss financially. Cultured shrimps are susceptible to various types of infectious and non infectious disease. A number of viral, rickettesial, fungal, bacterial diseases have been reported. Diagnostic methods presently available are expensive, cumbersome and time consuming. By the time cause and its prophylaxis are identified, it spreads so easily to nearby areas that prevention and control become impossible (Achuthankutty, 1998).

The presence of 21 antibiotics in six different groups was investigated in coastal water of the Bohai Bay. Meantime, to illuminate the potential effects caused by the river discharge and aquaculture activities, wastewater from three breeding plants and surface water from six
rivers flowing into the Bohai Bay were also analyzed for the selected antibiotics. The result revealed that measured antibiotics in the North Bohai Bay were generally higher than those in the South, highlighting the remarkable effects of high density of human activities on the exposure of antibiotics in environment. The antibiotics found in the six rivers were generally higher than those in the Bohai Bay reflecting the important antibiotics source of river discharge. This study reveals that the high consumption of some antibiotics in aquaculture activities may pose high ecological risk to the bay (Zou et al., 2011).

This work summarizes the current methods for total malachite green (MG) detection which is known as a sum of MG and leuco-malachite green (LMG) that has been used extensively in aquaculture as fungicide, dye color in textile and other purposes in food industries. LMG is a reducing form of MG, where the MG is easily reduced due to the photo-oxidative de-methylation process. Nevertheless, the use of MG had become an issue due to its toxicity effects. Many analytical instruments such as HPLC, LC–MS/MS, GC–MS, and spectrometry have been widely used for detection of MG. However, these methods require long time sample preparation and analysis, expensive, use hazardous reagents and indirect measurements. Hence, other analytical methods which are more sensitive, safe, rapid, inexpensive and portable are required. Alternatively, biosensors promise a more sensitive and rapid detection method for MG and LMG (Hidayah et al., 2013).

Graslund et al., in 2003 documented the use of chemicals and biological products in marine and brackish water shrimp farming in Thailand, the world’s top producer of farmed shrimp. Interviews were conducted with 76 shrimp farmers who used on average 13 different chemicals and biological products. The most commonly used products were soil and water treatment products, pesticides and disinfectants. In the study, the use of more than 290 different chemicals and biological products was documented. Many of the pesticides, disinfectants and antibiotics used by the farmers could have negative effects on the cultured shrimps, cause a risk for food safety, occupational health, and/or have negative effects on adjacent ecosystems. Manufacturers and retailers of the products often neglected to provide farmers with necessary information regarding active ingredient and relevant instructions for safe and efficient use.

Shrimp culture is a very important economic activity in the world and the fastest growing industry in Northeastern Brazil. Problems with diseases are common in aquaculture and at the least seven species of Vibrio bacteria have been documented infecting shrimps during
different culture stages. Oxytetracycline (OTC) has been widely used against these pathogens. In the present study the distribution and residue depletion of OTC in *Litopenaeus vannamei* tissue were evaluated after 14 days of exposure to medicated feed containing 4 g/kg. Two trials were conducted: in indoor tanks under laboratory-controlled conditions and in cages installed in an outdoor commercial shrimp grow-out pond. Medicated animals were sampled periodically during the treatment period and thereafter. The tissue levels of OTC were measured by high-performance liquid chromatography (HPLC). Although conditions were similar for both treatments, OTC peak concentrations differed. After 7 and 12 days of exposure to OTC, the maximum tissue residue levels were 17.21 and 4.38 μg/g for shrimp reared in the laboratory and in the pond, respectively. The mean OTC tissue residue concentration observed for both experimental environments is within the limits of the minimum inhibitory concentration (MIC) determined for vibriosis treatment. Drug residue levels dropped quickly during the first 72 h after treatment termination, then slowly and steadily until a residue level of 0.1 μg/g, considered a safe limit by the European Community requirements, was attained at day 16 of OTC withdrawal. After 25 days of OTC withdrawal no drug residues could be detected. However, a longer withdrawal period is recommended to ensure complete drug depletion (Lima et al., 2005).

### 2.7. Cons of antibiotics in farmed shrimp

A primary concern for people who eat farmed shrimp, particularly those who consume substantial quantities over a long period of time, is the usage of a range of antibiotics to prevent and treat bacterial conditions common in shrimp farms. In efforts to protect their shrimp from the effects of diseases and other pathogens, shrimp farmers worldwide turn to the chemical and pharmaceutical industries, although it is nearly impossible to control the disease like WSSV other than by destroying the entire infected crop. The U.S. is comparatively strict in this respect, limiting the use of antibiotics in aquaculture to three drugs: oxy tetracycline, sulfa merazine, and a drug combination containing sulfa dimethoxine and ormetoprim. A host of antibiotics are broadly used in aquaculture to stimulate growth and to reduce the incidence and effects of diseases caused by crowded, factory-farm conditions. The more antibiotics used, however, the more rapidly bacterial resistance develops, and this problem is reaching crisis proportions today, where bacterial growth is no longer stopped by the antibiotic, and thus the antibiotic is no longer capable of treating or curing the disease. Increasingly more bacteria are becoming resistant not only to one, but many antibiotics, making it more difficult to combat bacteria that cause illnesses in humans.
As in other animal production sectors, antibacterial substances (antibiotics and sulphonamides) are utilized in aquaculture production with the purpose of prevention (prophylactic) and treatment (therapeutic use) of bacterial diseases (Lupin et al., 2003). Antibiotics have also been utilized as growth factors, even if this is not now considered to be a good practice. Antibiotics utilized in aquaculture are of the same type utilized to treat bacterial diseases in humans.

Despite the often-encountered claim that there are no antibiotics used for growth promotion in aquaculture, a National Seafood HACCP Alliance for Training and Education Compendium identifies ‘growth’ as one the reasons why producers administer antibiotics (Benbrook, 2002).

It is illegal to use antibiotics in U.S. shrimp farms, but because most of the shrimp eaten in the United States is produced elsewhere, this law does little to protect most consumers. In a 2003 survey of Thai shrimp producers, 74 percent reported using antibiotics on their shrimp. To make matters worse, producers knew little about applying the drugs, leading to serious overuse. Many tried using antibiotics to treat viruses – without knowing that antibiotics don’t kill viruses (www.foodandwaterwatch.org).

For the consumer, the primary safety aspects concern the prevention of hazards of consuming unsafe residues in the edible tissues of farmed animals. Primary control is exercised via Maximum Residue Limits (MRL), established by Council Regulation EEC/2377/90 (CEC, 1990). The MRL defines the maximum level of residues of any component of a veterinary medicine that may be present in foodstuffs of animal origin without presenting any harm to the consumer. The MRL for any substance is determined from data submitted to the Safety of Residues Working Party, a subcommittee of the Committee for Veterinary Medicinal Products (CVMP). This determination is ratified by the CVMP and adopted into law by a Regulatory Committee in the form of Commission Regulations. This means that, although an MRL is generic and not proprietary, commercial support for the generation of data is essential. The MRL is determined by an iterative process from a range of safety data, the most important of which is the Acceptable Daily Intake (ADI). The ADI is defined as the level of a substance that may be consumed daily without presenting a hazard to the consumer (Alderman, 2009).
The exposure of the consumer to antibiotic residues in seafood is of great importance for health. Information related to residues of oxytetracycline, enrofloxacin and its metabolite ciprofloxacin in *Litopenaeus vannamei* tissues is very scarce, even though this species is among the most highly valued for its commercialization and oxytetracycline and enrofloxacin are frequently employed when farming this species. The liquid chromatography is the most widely used method, for quantitative analysis of antibiotics. Antibiotics must not be used as a preventative measure, since bacteria very rapidly develop resistance to them, leading to their ineffectiveness. Chemical agents should only be applied if there is an appropriate diagnosis of the situation and always under previously established control protocols (Almada et al., 2012).

Administration of antibiotics appeared to down-regulate a number of immune-related genes. Prophenoloxidase (proPO), clottable protein and b-thymosin, Penaeidin, haemocyanin, whey acidic protein, and profilin were down-regulated by antibiotics. The expression of other immune-related genes meanwhile remained unchanged or even up-regulated including a-2 macroglobulin and TNF-induced protein. The shrimp lymphoid organ is a primary site of bacterial accumulation and bacteriostasis and plays a role in the elimination of viral particles and other infectious agents. Expression of several genes in the shrimp lymphoid organ including those that are involved in immune response was altered after administering antibiotics. Antibiotics used to treat and prevent microbial infections in shrimp affect the shrimp transcriptomic profile and down-regulate the expression of a few immune-related genes (Fagutao et al., 2009).

The use of antibiotics in aquaculture may cause development of antibiotic resistance among pathogens infecting cultured animals and humans. Antibiotics are commonly used in shrimp farming to prevent or treat disease outbreaks, but there is little published documentation on details of usage patterns. According to this study a large proportion of shrimp farmers along the Thai coast used antibiotics in their farms. Of the seventy-six farmers interviewed, 74% used antibiotics in shrimp pond management. Most farmers used them prophylactically, some on a daily basis, and at least thirteen different antibiotics were used. Many farmers were not well informed about efficient and safe application practices. A more restrictive use of antibiotics could have positive effects for the individual farmer and, simultaneously, decrease impacts on regional human medicine and adjacent coastal ecosystems.

Dissemination of information could contribute to a decreased use of antibiotics, without
decreasing the level of shrimp production (Holmstrom et al., 2003).

Among the substances that appear as hazards for consumers are malachite green (MG), and its metabolite leucomalachite green (LMG). MG is a synthetic fabric dye that has been found effective for the treatment for parasitic and fungal infections in fish and shellfish and is used in aquaculture in many countries around the world. It is relatively inexpensive and in many countries is freely available. A study performed recently by the National Toxicology Program of the United States (NTP, 2005) concluded that malachite green chloride might have caused tumours of the thyroid gland, liver and mammary gland in female rats (but did not cause cancer in female mice). LMG on the other hand might have caused cancers of the thyroid gland in male and female rats, of the testes in male rats and the liver in female rats (LMG also caused increased cancer in the liver of female mice).

MG and LMG are also suspected to be potential in vivo mutagens, which means with the possibility to damage genetic material, and they are therefore potentially carcinogenic to humans (Sriviastava et al., 2004). A study by the National Toxicology Program of the United States (NTP, 2004), concluded that MG was not mutagenic in any of several strains of Salmonella typhimurium. In addition to the discussions about toxicological data, MG has never been a licensed veterinary medicine in many countries, which in turn makes it difficult to analyze for possible MRL. The use of MG only in hatcheries raises the question that MG, and particularly LMG, could remain in sediments and be released when sediments are disturbed (Lupin 2009).

Pesticides and antibiotics can also impair shrimp health and can accumulate inside muscle and, therefore, should be forbidden for sale. A set of biomarkers, using proteomics and more classical biochemical and physiological approaches, should be developed as an early warning signal to prevent excess use of chemicals in shrimp farming. It is suggested that the problem of the use of antibiotics and pesticides should be dealt through unified local and global preventive approaches. Heavy use of antibiotics and pesticides must be reduced drastically and replaced with improved culture techniques. Future research should focus on the development of new biomarkers, with which information from several techniques and several levels of biological organization are used to provide a better assessment of contamination in the environment (Tu et al., 2010).

The present study demonstrates a complete method to rapidly and precisely determine residue
levels of malachite green and leucomalachite green in fish with the new Agilent 6410 LC/MS triple quadrupole system. Using positive mode electrospary ionization (ESI+) and multiple reaction monitoring (MRM), qualification and quantification were accomplished without the traditional tedious PbO2 oxidation process. The LC/MS/MS method’s LOQ is 0.01 μg/Kg, which easily meets the import requirement of 2 μg/Kg set by Japan and the EU. (Liang et al, 2006).

A sensitive and specific method is described for the simultaneous determination of oxytetracycline, tetracycline (TC), and chlortetracycline residues in edible swine tissues, by combining liquid chromatography with spectrofluorometric and mass spectrometry detection. The procedure involved a preliminary extraction with EDTA-McIlvaine buffer acidified at pH 4.0, followed by solid-phase extraction cleanup using a polymeric sorbent. The liquid chromatography analysis was performed with spectrofluorometric detection after postcolumn derivatization with magnesium ions. The limits of quantification were 50 μg/kg for muscle and 100 μg/kg for kidney tissues. The recovery values were greater than 77.8% for muscle and 65.1% for kidney. The method has been successfully used for the quantification of tetracyclines in swine tissues samples. The selective liquid chromatography mass spectrometric analysis for confirmation of oxytetracycline in one positive swine muscle sample was made by atmospheric pressure chemical ionization (APCI). The APCI mass spectra of the TCs gave the protonated molecular ion and two typical fragment ions, required for their confirmation in single ion monitoring scan mode in animal tissues (Pena et al., 2007).

Furazolidone is a broad-spectrum antibiotic that is frequently used in aquaculture on account of its excellent antibacterial properties. In this study, both the Enzyme-Linked Immunosorbent Assay (ELISA) and High-Performance Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) methods were used to analyze the content of residual 3-amino-2-oxazolidinone (AOZ), a metabolite of furazolidone in *Tilapia* tissue. Homogenized fish samples were spiked with various amounts of AOZ, and following combined acid-hydrolysis and derivatization of the homogenized tissue with 2-NBA(2-nitrobenzaldehyde), sample clean-up was performed and the derived 2-nitrophenylmethylene-3-amino-2-oxazolidinone (NPAOZ) was analyzed. Using the LC-MS/MS method, a linear correlation between measured concentration Y and spiked concentration X was observed: Y = 0.4518X + 0.0166, R2 = 0.9972. The linear equation for the ELISA method was Y = 0.9322X + 0.5168,
R2 = 0.9066. These results demonstrated that the ELISA method might overestimate the residual AOZ content at low concentrations. The detection limit and recovery of the known addition were 0.05 µg kg\(^{-1}\) and 108% for the LC-MS/MS method and 0.31 µg kg\(^{-1}\) and 305% for the ELISA method, respectively (Tsai \textit{et al.}, 2009).

2.8 Molecular biology in Shrimp study

Molecular phylogeny of Penaeid shrimps has been reported based on nucleotide sequences of COI, 16S rDNA and COI and AFLP. Phylogenetic trees revealed close genetic relationships between \textit{P. monodon} and \textit{P. semisulcatus} (subgenera \textit{Penaeus}) but distant relationships were observed among economically important shrimps from different genera (\textit{P. monodon}, \textit{P. semisulcatus}, \textit{F. merguiensis}, \textit{L. vannamei} and \textit{Marsupenaeus japonicus}). Nevertheless, simple molecular markers for differentiation of these shrimps have not been reported (Khamnamtong \textit{et al.}, 2005).

The last decade of research and development in shrimp genomics and genetics has seen significant advancements. An abundance of sequence information from expressed genes is available in public databases, providing a first glimpse at the gene content of several Penaeid species. Researchers and aquaculture geneticists are only now beginning to exploit these resources, especially for the identification of genetic markers, candidate disease resistance genes, and genes linked to reproduction and other aquaculture-relevant processes. A growing number of initiatives are mining the available sequence data to implement markers and generate increasingly more extensive linkage maps. The number of shrimp genes for which at least some sequence information is now known is in the thousands, rather than the dozens, as was the case just a few years ago. The tools are also in place to refine the selection of candidate aquaculture-relevant genes through the characterization of two key aspects of the function of a gene: its expression and its loss-of-function phenotype (Robalino \textit{et al.}, 2012).

The goals of the present work was testing four protocols of DNA extraction, with slight changes, using samples of muscle tissue of \textit{F. paulensis}. We tested: saline DNA extraction (S), plant and fungal DNA protocol (A), and modified insect DNA protocol (B), in which variations were focused on different concentrations of reagents in the digestion solution. Other changes were performed in protocols A and B: addiction of (1) pure chloroform; (2) chloroform/iso amyllic alcohol; (3) pure chloroform and beta mercaptoethanol. The DNA extraction products were visualized in 0.8% agarose gel stained with ethidium bromide. DNA amplification using ISSR (Inter Simple Sequence Repeat) primers was also carried out in
order to verify the utility of DNA samples obtained by each method in PCR. No significant differences in the DNA concentrations according to each procedure were detected (Oliveira et al., 2011).

The use of polymerase chain reaction in combination with restriction enzymes digestion, RFLP or single stranded conformation polymorphism is favored for identifying and characterizing the species origin of shrimp products due to their convenient and cost effective (Khamnamtong et al., 2005).

Multiplex PCR assay based on the 16S rRNA mitochondrial gene were used to identify the Penaeid shrimp *Farfantepenaeus aztecus*, *Farfantepenaeus duorarum*, *Farfantepenaeus brasiliensis* and *Litopenaeus setiferus*, all native to the Gulf of Mexico, and the exotic *Litopenaeus vannamei*. The assay was validated using positively identified adult shrimp and confirmed by direct sequencing. Samples of post larvae and early juveniles collected in the eastern and western Gulf of Mexico were tested yielding 119 *F. aztecus*, 78 *F. duorarum* and five *L. setiferus*. Reliable identification of the morphologically similar early life stages of *F. aztecus* and *F. duorarum* has important implications for management and conservation. Similarly, the ability to identify *L. vannamei* is relevant as early detection could help minimize the ecological impact if this species escapes to the wild (Bremer et al., 2010).

The population genetics of many Penaeid shrimps have been well studied, since the knowledge of the genetic structure of fisheries stocks is crucial to shape policies that will ensure sustainable stock viability (reviewed in Benzie, 2000). However, genetic studies of natural populations of Brazilian shrimps have been conducted only for the genera *Farfantepenaeus* and *Litopenaeus*. In addition, until now, despite its economic importance, the genetic structure of populations of *X. kroyeri* has never been studied (Voloch and Cava, 2005).

*Artemia* is one of the most conspicuous invertebrates associated with aquaculture. It can be considered as a model organism, offering numerous advantages for comprehensive and multidisciplinary studies using morphologic or molecular methods. Since DNA extraction is an important step of any molecular experiment, a new and a rapid method of DNA extraction from adult *Artemia* was described in this study. Besides, the efficiency of this technique was compared with two widely used alternative techniques, namely Chelex® 100 resin and SDS-chloroform methods. Data analysis revealed that the new method is the easiest and the most
cost effective method among the other methods which allows a quick and efficient extraction of DNA from the adult animal (Manaffar et al., 2010).

Polymerase chain reaction-based methodologies have been developed for the identification of three commercially-relevant Penaeid shrimp species: *Litopenaeus vannamei*, *Penaeus monodon* and *Fenneropenaeus indicus* in food products. Preliminary sequencing of a mitochondrial sequence of ca. 530 bp in the 16S rRNA/tRNA Val mitochondrial region was performed in nearly 20 Penaeid shrimp species of commercial relevance. Careful analysis of such sequences allowed the design of primers PNVF/PNVR, which allowed the combined identification of *P. monodon* and *L. vannamei*, and PNIF/PNIR, which allowed the specific identification of *F. indicus*. In addition, *P. monodon* and *L. vannamei* could be easily differentiated by either restriction with TspE1 or by amplification with novel primers MPNF/MPNR, specific for *P. monodon*. The proposed specific methods improve current general identification methods of these species based on more general RFLP analyses (Pascoal et al., 2011).

DNA-based molecular markers for differentiation of five Penaeid shrimps (*Penaeus monodon*, *P. semisulcatus*, *Feneropenaeus merguiensis*, *Litopenaeus vannamei* and *Marsupenaeus japonicus*) were developed based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and single-stranded conformation polymorphism (SSCP) of 16S ribosomal (r) DNA. Differentiation of *P. monodon*, *P. semisulcatus* and *L. vannamei* can be unambiguously carried out by PCRRFLP of 16S rDNA560 whereas *P. semisulcatus* and *M. japonicus* shared a BABB mitotype. These shrimps were successfully discriminated by SSCP analysis of 16S rDNA560. 16S rDNA of an individual representing the most common mitotype of each species was cloned and sequenced. The new primer pair was designed and tested against the large sample sizes. The amplification success was consistent across all species. PCR-RFLP of 16S rDNA was as effective as that of 16S rDNA. Differentiation of all shrimp species were successfully carried out by SSCP analysis (Khamnamtong et al., 2005).

Expressed sequence tags (ESTs), were originally developed to study human genomics, but they are now available for studying other important organisms, including shrimp. ESTs are short sequences generated by sequencing randomly selected cDNA clones from a cDNA library. This is currently the most efficient and powerful method for providing transcriptomic data for organisms with an uncharacterized genome. EST data has been downloaded from
NCBI db EST for the four major Penaeid shrimp species and constructed a database to host all of these EST data. This database provided the shrimp aquaculture research community with an outline of the shrimp transcriptome as well as a tool for shrimp gene identification (Leu et al., 2011).

The present study describes a simple method of extraction of white spot syndrome viral DNA (WSSV) from infected shrimp for the polymerase chain reaction (PCR) detection of WSSV. The DNA preparation using this method was found to be free from the host DNA, RNA and protein, and is suitable for different PCR protocols such as single-step PCR, nested PCR and single-tube semi-nested PCR. This method of extraction has worked successfully for extracting the WSSV- DNA from different organs (haemolymph, eyestalk, carapace, head muscle, heart, gills, appendages, heptopancreas, stomach, intestine, abdominal muscle and tail muscle) of WSSV-infected adult shrimp, and WSSV-infected larvae and post larvae (Yoganandhan et al., 2003).

We report here the molecular cloning of new members of the Penaeidin family from two Atlantic Penaeids from Brazil, *L. schmitti* and *F. paulensis*. The presence of Penaeidins in the granular hemocytes of both shrimps was first evidenced by immune fluorescence, using polyclonal antibodies raised against *L. vannamei* Penaeidin Litvan PEN3-1. cDNAs from the hemocytes of both Brazilian species were obtained by reverse transcription and the sequences encoding Penaeidins were amplified by PCR, using primers based on Penaeidin consensus sequences. Five Penaeidin clones were obtained. According to the international Penaeidin classification, the deduced amino acid sequences of two clones from *L. schmitti* and two from *F. paulensis* belong to the PEN2 subgroup and one clone from *L. schmitti* to the PEN4 subgroup of Penaeidins. Surprisingly, no Penaeidin from the PEN3 subgroup was obtained in both shrimp species, even though this subgroup appears to be the most commonly expressed in the hemocytes of *Penaeids* (Barracco et al., 2005).

### 3.0 AIM OF THE STUDY

The present study is aimed at comparing Penaeid shrimps *Fenneropenaeus indicus* (Indian prawn), *Litopenaeus vannamei* (White leg shrimp), *Penaeus monodon* (Giant Tiger shrimp) collected from different sources with regard to the following techniques.

#### 3.1 Molecular characterization

Molecular characterization to confirm the species collected for the study, using PCR and bioinformatics tools.
Isolation of genomic DNA were carried out in all the three species and DNA bands were viewed by agarose gel electrophoresis.

DNA quantification is done in Penaeid shrimps.

Isolated genomic DNA is then amplified by PCR and sequenced to characterize the Penaeid species genetically.

3.2 Chemical analysis: Chemical analysis for the determination of nutrients of species and analysis of toxic metals and veterinary drugs in the edible tissue of the shrimps.

Nutritional parameters such as crude protein, fat, energy, total carbohydrates were determined in all three species and values compared among them.

Analysis of toxic heavy metals cadmium, lead, zinc, mercury is carried out in Penaeid species.

Important banned and non banned veterinary drugs by FDA were determined in all the three samples as per export council norms which are commonly done for the export of marine food.

4.0 MATERIALS AND METHODS

4.1 Shrimp species selected for study

*Penaeid* Species chosen for the study include

- *Fenneropenaeus indicus* (Indian prawn)
- *Litopenaeus vannamei* (White leg shrimp)
- *Penaeus monodon* (Giant Tiger shrimp)

4.2 Sample collection

Shrimp samples used for the current study were collected from different sources.

*Fenneropenaeus indicus* commonly known as Indian white shrimp was collected from Cuddalore sea port as wild shrimp origin (Plate 1: a & b).

*Penaeus monodon* (Tiger shrimp) was collected from ponds near seashore in the Pondicherry region as an extensive farming origin. (Plate 2 a & b).

*Litopenaeus vannamei* was collected from Meenjur shrimp farm in Thiruvallur district as an intensive farming origin (Plate 3, 4, 5 & 6).

4.3 Sample Preparation
Shrimp samples were washed thoroughly with deionized water to remove any adhering contamination and drained using filter paper. Samples were brought to the laboratory in ice using insulated containers. The washed shrimps were wrapped in aluminum foil and frozen at -4°C for two days before samples were prepared for Plate 1.

a) **Lateral view of *Fenneropenaeus indicus* caught from Cuddalore sea port**

![Lateral view of *Fenneropenaeus indicus* caught from Cuddalore sea port](image)

b) *Fenneropenaeus indicus* caught from Cuddalore sea port

Plate 2

a) *Penaeus monodon* caught from local ponds in the Pondicherry region – Lateral view
b) *Penaeus monodon* caught from local ponds in the Pondicherry region – Dorsal view

Plate 3: *Litopenaeus indicus* caught from the shrimp farm in the Thiruvallur district
Plate 4: Soil Plugging in the shrimp farm
Plate 5
a) Shrimp farm in Tiruvallur district separated by bunds

Plate 6
a) Shrimp harvesting
b) *Litopenaeus vannamei* caught from the shrimp farm

Plate 7

a) PCR used in the amplification of genomic DNA of the Penaeid shrimp species

b) Loading the amplified DNA samples of the Penaeid shrimp species into the well of Agarose Gel Electrophoresis
a) HPLC MS instrument used for the determination of the antibiotics and antifungal agents in the Penaeid shrimp species

Plate 9
a) CVAAS instrument used for determination of Heavy metals (Mercury) in the Penaeid shrimp species

b) ICP - MS instrument used for the determination of Heavy metals in the Penaeid shrimp species
Result of the genomic DNA bands isolated from Penaeid shrimps under UV transilluminator

Lane 1 *Fenneropenaeus indicus*
Lane 2 *Penaeus monodon*
Lane 3 *Litopenaeus vannamei*

**Plate 11**
Result of the PCR bands of the genomic DNA isolated from Penaeid shrimps under UV transilluminator along with 100bp DNA ladder

Lane 1 *Fenneropenaeus indicus*
Lane 2 *Penaeus monodon*
Lane 3 *Litopenaeus vannamei*
Lane 4 100bp DNA ladder
Analysis. 250 g of shrimp from each species was weighed for homogenization. The head and external shell of the shrimp samples were removed and the whole edible tissue was homogenized for the analysis. The amount of subsample required for an analytical procedure usually varies from a fraction of a gram to several grams (Curren et al, 2002).

4.4. Molecular characterization - DNA bar coding – COI gene sequence

4.4.1. Reagents and chemicals
DNA purification kit (Pure Fast® Tissue Genomic DNA purification kit), 2X Pfu PCR Master Mix, Pure fast PCR Clean-up kit, Agarose gel electrophoresis consumables and Primers purchased from HELINI Biomolecules, Chennai, India.

4.4.1. a. 2X Master Mix
2U of pfu Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl2, 1µl of 10mM dNTPs mix and PCR additives.

4.4.1. b. Agarose gel electrophoresis
Agarose, 50X TAE buffer, 6X gel loading buffer and Ethidium bromide were purchased from HELINI Biomolecules, Chennai.

4.4.2. Isolation of genomic DNA
1. 10 -50 mg of homogenized tissue was weighed and transferred into 1.5ml tube.
2. 400µl of lysis buffer added and grinded using micro pestle.
3. 40µl of Proteinase K [10mg/ml] was added and gently mixed well.
4. Incubated in water bath at 56°C for 45 min.
5. 200µl of isopropanol was added, mixed by inverting and incubated at room temperature for 5min.
6. Whole lysate was transferred into Pure Fast spin column and centrifuged at 10000rpm for 1min.
7. Flow through was discarded and 500µl of wash buffer was added and centrifuge at 10000 rpm 1 min.
8. Flow through was discarded and 500µl of wash buffer-2 was added and centrifuged at 10000rpm for 1min. Wash was repeated one more time.
9. Column was for additional 2 minute to remove any residual ethanol.
10. DNA was eluted by adding 100µl of elution buffer and centrifuged for 1min.
11. Quality and quantity of extracted DNA was checked by loading in 1% agarose gel and 1µl of extracted DNA is used for PCR amplification.
4.4.3. DNA Quantification
DNA was quantified using nano drop UV spectrophotometer. 1 µl of isolated DNA was placed in nano drop Spectrophotometer. Concentration of the DNA was read on the detector.

4.4.4. Agarose Gel Electrophoresis
1. 2% agarose was prepared. (2gm agarose in 100ml of 1X TAE buffer and melted using micro oven)
2. When the agarose gel temperature was around 60°C, 5µl of ethidium bromide of was added.
3. Warm agarose solution was slowly poured into the gel platform.
4. Gel set was kept undisturbed till the agarose solidifies.
5. 1X TAE buffer was poured into submarine gel tank.
6. Gel platform was carefully placed into the tank. The tank buffer level was maintained 0.5cm above than the gel.
7. 5µl of DNA samples were loaded into the wells after mixing with gel loading dye
8. Electrophoresis was run at 50V till the dye reaches three fourth distance of the gel.
9. Gel was viewed in UV Tran illuminator and observed the bands pattern.

4.4.5. Shrimp COI Primer: [Folmer]
Forward primer : TGAGCWCAYCATATATTACWGTAGG
Reverse primer : TGTTGGGGGARAGAATGTAATATT

4.4.6. PCR procedure
[25µl of Master Mix contains: 10X Taq buffer, 2mM Mgcl2, 0.4mM dNTPs mix, and 2U Proofreading Taq DNA polymerase]. (Plate 7a)
1. Reactions were set up as follows

Table 01: List of components to be used in the Polymerized Chain Reaction

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>In PCR vial</td>
<td></td>
</tr>
<tr>
<td>Master Mix</td>
<td>40µl</td>
</tr>
<tr>
<td>Primer Mix (5pmoles/µl)</td>
<td>2µl</td>
</tr>
<tr>
<td>Genomic DNA</td>
<td>2µl</td>
</tr>
<tr>
<td>Water, nuclease free</td>
<td>6µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>50µl</td>
</tr>
</tbody>
</table>
1. The components were mixed gently and spun down briefly.
2. Placed into PCR machine and program was set as follows;

<table>
<thead>
<tr>
<th>Reaction Type</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>94°C</td>
<td>3 min</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>53°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>30 sec</td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>5 min</td>
</tr>
</tbody>
</table>

**Initial Denaturation**

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>94°C</td>
<td>30 sec</td>
</tr>
</tbody>
</table>

**4.4.7. Pure Fast clean up**

1. Volumes of binding buffer was added to 1 volume of the PCR reaction and mixed well.
2. The entire sample was applied to the Pure Fast Spin column and centrifuged for 1 min.
3. Flow-through was discarded. The Pure Fast spin column was placed back into the same collection tube.
4. 500μl of wash buffer was added to the Pure Fast spin column for washing and centrifuged for 1 min. Flow-through discarded and the Pure Fast spin column was placed back in the same collection tube.
5. 250μl wash buffer was added to the Pure Fast spin column and centrifuged for 1 min. The flow-through discarded and the Pure Fast spin column was placed back in the same collection tube.
6. The column was centrifuged for an additional 2 min at maximum speed.
7. The Pure Fast spin column was placed in a clean 1.5 ml micro centrifuge tube. 20 - 50μl of elution buffer was added to the centre of the membrane to elute DNA; the column was left to stand for 1 min and then centrifuged for 1 min.

**4.4.8. Agarose Gel Electrophoresis**

1. 2% agarose gel was prepared as mentioned above (see 4.4.4) and used for viewing amplified DNA.
2. 5μl of amplified PCR product was loaded into the gel, along with 10μl HELINI 100bp DNA Ladder.
3. Electrophoresis was run at 50V till the dye reaches three fourth distances and the bands were observed under UV Tran illuminator.(Plate 7b).

**4.4.9. DNA Sequencing**

Quality and quantity of purified PCR product was verified and sent to DNA Sequencing. The
DNA sequencing was carried out using Big Dye Terminator sequencing kits. Complete sequencing process was carried out on an Applied Biosystems Automated Sequencer ABI13730XL – 1414 -008. The electropherogram data of the sequenced DNA was obtained in the sequence scanner.

4.4.10. Species confirmation using Bioinformatics tools

The sequences were then submitted to the National Center for Biotechnology Information for further identification and confirmation of the Penaied species. DNA Sequences were submitted to FASTA software for aligning the sequence. Aligned sequence was submitted to NCBI- BLAST for finding sequence similarity between the submitted sample and other closely related species for confirming the species. Similarity score is calculated by phylogenetic analysis using T-COFFEE.

4.5. Proximate analysis

4.5.1. Determination of moisture content

4.5.1.a. Instruments and Chemicals

- Analytical balance
- Dessicator
- Crucible with lid
- Oven, set at 105°C
- Tongs

4.5.1.b. Procedure

1. The crucible with the lid was dried in the oven at 105°C overnight and was transferred to the dessicator for cooling for approx 30 min.
2. The crucible was weighed.
3. 5 g sample was weighed in the crucible. The homogenized shrimp sample was spread.
4. The lid was replaced and the crucible was re weighed with its contents (WI).
5. The crucible was placed with its lid slipped to one side. The setup was then dried for 16h or overnight at 105°C.
6. After drying, a pair of tongs was used to transfer the crucible and lid to the dessicator for cooling approx 45 min. The crucible, lid and its dried content was reweighed.
7. The crucible with its lid partially covered was replaced in the oven for 1h. Then transferred to the dessicator for cooling, the dish and its content were then weighed
again. If the weight obtained at this step is less than that obtained at Step vi, it indicates that the sample was not sufficiently dried. Repeat for concordant values.

8. The experiment was performed in duplicates. (Pearson, 1976).

4.5.2. Determination of ash content

4.5.2.a. Apparatus

- Crucible (or similar porcelain or metal dishes)
- Muffled furnace
- Sample
- Hot plate

4.5.2. b. Procedure

1. 5 g of representative sample was weighed and dried in a crucible in an oven at 130°C for overnight. The sample was charred on an electric hot plate or over a low flame in a fume cupboard until it ceased smoking.

2. The above crucibles with charred sample were placed in a cold muffle oven and the temperature was brought to 550°C.

3. The samples were ignited to 12-18 h (or overnight) at 550°C.

4. The muffle furnace was turned off and waited until the temperature has dropped to at least 250°C, preferably lower. The door was opened carefully to avoid loss of ash that might be light and fluffy.

5. The safety tongs were used to transfer the crucibles quickly to a dessicator with a porcelain plate and desiccant. The crucibles were covered, and allowed to cool prior to weighing (Pearson, 1976).

4.5.3. Determination of Fat content

4.5.3.a. Instruments and Chemicals

- Mechanical blender
- Analytical balance
- Desiccator, with drying agent, e.g. Blaugel
- Soxtherm micro/macro and multistate
- Drying oven
- Cotton Wool, chemically clean and fat-free.
4.5.3.b. Procedure

i. Preparation of the extraction beakers

3-5 boiling stones were put into each extraction beaker. The beakers were then dried in a drying oven for about an hour at 103°C ± 2°C. After cooling off in the desiccator to room temperature, the beaker was weighed with a precision of +/- 0.1mg.

ii. Sample Preparation

200 g of representative sample were used. The samples were mixed and homogenized in the mixer at least twice. The samples were stored in an airtight container to prevent decay and any change in content. Prior to the analysis, 10g of the samples were weighed on a filter paper and folded into a predried extraction thimble. The thimble was covered with cotton wool.

iii. Extraction

The thimble was placed in the specified beaker. Remaining fat traces on the watch glass were removed with some cotton wool, damped with extraction agent, and was put into the extraction thimble as well. After adding 140-ml extraction agent the samples were extracted in the Soxtherm automatic using the following program:

Solvent : Petrol ether 40/60
Boiling Point : Boiling range 40 - 60 °C
Amount of Solvent : micro100ml/ macro 150 ml

iv. Parameter for the Program

Table 03: Reaction parameters for extraction procedure

<table>
<thead>
<tr>
<th>Program Step</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-Classification</td>
<td>200°C</td>
</tr>
<tr>
<td>Extraction Temperature</td>
<td>150°C</td>
</tr>
<tr>
<td>Reduction Interval</td>
<td>4 min</td>
</tr>
<tr>
<td>Reduction Pulse</td>
<td>4 s</td>
</tr>
<tr>
<td>Hot Extraction</td>
<td>30 min</td>
</tr>
<tr>
<td>Evaporation @</td>
<td>5 Intervals</td>
</tr>
<tr>
<td>Rinsing Time</td>
<td>70 min</td>
</tr>
</tbody>
</table>

After the program was finished, the extraction beakers were dried in the drying oven in an upright position for 60 min at 103°C ± 2°C. Then they were placed in a desiccator, left to cool down to room temperature and were weighed with a precision of +/- 1 mg. In order to check the consistency of the weight, the samples were left to dry for another 30 min and
The procedure was repeated as long as two successive weights showed no more than 1 mg difference. Extraction, drying, and weighing have to be done immediately after each other. (Nielen, 2003).

4.5.4. Determination of Protein content

The Kjeldahl procedure was divided into three parts: (1) digestion, (2) distillation, (3) titration. In the digestion step, organic nitrogen was converted to an ammonium in the presence of a catalyst at approximately 370ºC. In this experiment, the sample was digested in H₂SO₄, using Copper-based catalyst, converting N to NH₃ which was distilled and titrated.

\[
\text{Protein} \xrightarrow{\text{Sulfuric acid}} (\text{NH₄})₂\text{SO₄} \xrightarrow{\text{Heat, catalyst}} \text{ammonium}
\]

In the distillation step the digested sample was made alkaline with NaOH and the nitrogen was distilled off as NH₃. This NH₃ was trapped in a boric acid solution.

\[
\begin{align*}
(\text{NH₄})₂\text{SO₄} + 2\text{NaOH} & \rightarrow 2\text{NH₃} + \text{Na₂SO₄} + 2\text{H₂O} \\
\text{NH₃} + \text{H₃BO₃} \rightarrow \text{NH₄} + \text{H₃BO₃}²⁻ (\text{borate ion})
\end{align*}
\]

The amount of ammonia nitrogen in the solution was quantified by titration with a standard HCl solution. A reagent blank was carried throughout the analysis and the volume of HCl titrant required for the blank was subtracted from each determination.

\[
\text{H₂BO₃}⁻ + \text{H⁺} \rightarrow \text{H₃BO₃}
\]

4.5.4.a. Instruments and Chemicals

- Sulfuric acid 98% min.
- Catalyst tablets to be used: Kjeltabs CX
- Caustic soda 32%
- Boric acid solution 2%
- Indicator Solution M5 (Merck) or similar
- Standard acid 0.1N or c=0.1 mol/l, alternatively sulfuric acid 0.1N or c=0.05 mol/l
- Mechanical comminuting instrument
- Analytical balance (0.001 g)
- Kjeldahl digestion block Kjeldatherm, Turbotherm, flask heater for Kjeldahl flask with wide neck opening
- Vapodest distillation System
• Burette, 50 ml nominal capacity, with a scale on 0.05 ml or titration system (not with the Vap 50) or pH meter with combined electrode.

4.5.4. b. Procedure

i. Sample Preparation
• 2g of sample was weighed accurately on a piece of a filter paper.
• The sample was stored air tight so as to avoid any changes or decay of the composition. Prior to the analysis the sample should be at room temperature. The examination of the prepared sample has to be done within the 24 h.

ii. Chemicals
• Sulfuric acid was used to wash down any sample residue, which might remain at the glass walls.

Table 04: List of chemicals for protein estimation.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfuric acid</td>
<td>20 ml</td>
</tr>
<tr>
<td>Kjeltabs</td>
<td>2</td>
</tr>
<tr>
<td>Indicator solution M5</td>
<td>Few drops</td>
</tr>
<tr>
<td>Standard acid 0.1N or c=0.1mol/L; alternatively sulfuric acid 0.1N or c=0.05mol/L</td>
<td></td>
</tr>
</tbody>
</table>

iii. Parameters for digestion

Table 05: Time and Temperature conditions for digestion process.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>400</td>
<td>Digestion tubes were put into the preheated block and time it takes for the sample to become translucent</td>
</tr>
<tr>
<td>30</td>
<td>400</td>
<td>The sample was dehydrated</td>
</tr>
</tbody>
</table>

• Care should be taken to avoid the foaming higher than 2/3rd of the glass.
• The black particles remaining at the glass wall were washed back with condensing sulfuric acid.
• The sample glass has to be translucent after the digestion in order to obtain good results.

iv. Suction
• During the entire digestion period the scrubber should be on. About 1200 ml of a 15% caustic soda was used for the washing bottle; this amount is sufficient to neutralize digestion gases of about 60 digestions.
The cooling off period after the lifting of the insert rack or the cooling off period after turning off the heating is about 30 minutes.

The scrubber should be left on during the entire period for good results.

v. Distillation

After the digested sample has cooled off, the water steam distillation was done according to the following program:

Table 06: Parameters for water steam distillation.

<table>
<thead>
<tr>
<th>Program parameter</th>
<th>Vap 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water addition in s</td>
<td>9</td>
</tr>
<tr>
<td>NaOH addition in s</td>
<td>8</td>
</tr>
<tr>
<td>Reaction time in s</td>
<td>0</td>
</tr>
<tr>
<td>Distillation time in s</td>
<td>240</td>
</tr>
<tr>
<td>Steam output in %</td>
<td>100</td>
</tr>
<tr>
<td>Suction sample in s</td>
<td>25</td>
</tr>
<tr>
<td>Boric acid addition in s</td>
<td>6 s</td>
</tr>
<tr>
<td>Suction receiver in s</td>
<td>25</td>
</tr>
<tr>
<td>Titration</td>
<td>Auto</td>
</tr>
<tr>
<td>Calculation</td>
<td>Auto</td>
</tr>
</tbody>
</table>

vi. Titration

- 4 drops of an indicator mixture M 5 was added to the receiving solution and it was then titrated with 0.1 N titration acids till the color changes from green to grey/violet.

- If the determination of the endpoint was done with a pH-meter or a titrator, the addition of the indicator mixture was obsolete.

vii. Blank Value

- For the determination of the blank value the analysis (digestion and distillation) was run with chemicals alone.

- The consumption of those chemicals has taken into account while doing calculation. (AOAC, 2005).

4.6. Veterinary Drug Analysis

4.6.1. Determination of Chloramphenicol - HPLC-MSMS Method (Plate 8)

4.6.1.a. Chemicals

- Ethyl Acetate
- Acetonitrile
• Carbon Tetrachloride
• Hexane

4.6.1. b. Extraction

1. 1-5 gm of homogenized edible portion was weighed in a centrifuge tube.
2. 10 ml of ethyl acetate was added and stirred thoroughly for 10 min on a vortex/ rotary shaker.
3. Centrifuged at about 5,000 g for 15 minutes.
4. The upper ethyl acetate layer was transferred in a clean turbovap tube concentrator under nitrogen.
5. The extraction was repeated with another 5 ml ethyl acetate & all ethyl acetate layers was collected in the same turbovap tube and dried under nitrogen at about 45°C.
6. The dried residue was dissolved in 1 ml Hexane: Carbon Tetrachloride (1:1, v/v) by using a vortex mixer.
7. 1 ml of water was added, vortexed thoroughly and centrifuged at about 5,000 gravity for 15 min for layers separation.
8. The upper layer was filtered through 0.22 μm nylon membrane and transferred quantitatively into LC vial for injection in HPLC-MSMS.

4.6.1.c. HPLCMS-MS Analysis

Appropriate and equal volumes of final extracts of samples as well as calibration dilutions of standards were injected into LC system and thus obtained the MS Chromatogram (AFSSA; Commission Decision 2002; FAO/WHO 2003).

4.6.2. Determination of Nitrofuran Metabolites – HPLC-MSMS Method

4.6.2.a. Chemicals

• Ethyl acetate
• Acetonitrile
• HCl 32%
• 2- Nitrobenzaldehyde
• Carbon Tetrachloride
• Tri-sodium-phosphate-do-deca-hydrate
• Sodium Hydroxide pellets
• Ammonium Acetate
• Brand Sigma and gradient grade water

4.6.2. b. Extraction
1. 4 gm of homogenized edible portion was weighed in a centrifuge tube.
2. 10 ml of 0.2M HCl & 250 μl of 100mM, 2-NBA solution in methanol were added, the tubes were screw capped & vortexed for about a minute.
3. Incubated overnight (at least 16 hrs.) at 37°C ± 2°C, alternatively 50°C ± 2°C for four hours in an incubator cum rotary shaker.
4. The tubes were cooled to room temperature after incubation.
5. 250μl of 0.3 M Tri-sodium-phosphate solution was added to the tubes and the pH was adjusted to neutral using 2M NaOH solutions if required.
6. 10 ml ethyl acetate was added and hand mixed for 5 minutes each, ensuring no emulsion formation occurred.
7. Centrifuged at about 4000g for 10 minutes.
8. The ethyl acetate layer was transferred into a clean concentrator tube and re extracted with 5ml ethyl acetate by vortexing for 5 minute.
9. After centrifuging at 4000g for 10 minutes, ethyl acetate layer was collected to the same concentrator tube & dried under nitrogen in Turbovap concentrator at about 45ºC.
10. A wash with hexane: carbon tetrachloride (v/v: 50/50), might be required if coloration or fat content was observed. 1 ml of this mix was vortexed for a minute.
11. 1 ml water was added in the same tube and vortexed properly, and then centrifuged at 8500 rpm for 10-20 minutes.
12. The residue was filtered through 0.22 μm nylon membrane and upper water layer was transferred quantitatively into LC vial for injection in HPLC- MSMS.

4.6.2.c. HPLCMS-MS Analysis
Appropriate and equal volumes of final extract of samples and calibration dilutions of standards for calibration curve were injected into LC system and thus obtained the MS Chromatogram (RIKILT; Hancock et al., Commission Decision 2002; FAO/WHO 2003).

4.6.3. Determination of Tetracyclines – HPLC- MSMS Method
4.6.3.a. Chemicals
• Citric Acid monohydrate
• Disodium hydrogen phosphate
Phosphoric Acid
Oxalic Acid
Acetonitrile
Methanol
Water

4.6.3. b. Reagents
Mcllvaine Buffer pH 4.0
- 0.1M Citric acid monohydrate – 21.01 g/L (If anhydrous – 19.213 g/L) in water
- 0.2 M Na2HPO4 – 28.4 g/L.
- M methanolic oxalic acid.

4.6.3. c. Extraction
i. 5.0 g of well ground homogenized edible portion was weighed into the centrifuge tube.
ii. The tissue was blended with 20 ml, 20 ml; 10 ml of Mcllvaine buffer repeatedly each for at least 30 sec, collecting the extract after each addition.
iii. Centrifuged at 2500g for 10 minutes.
iv. The supernatant was filtered through GF/B filter paper in a Buchner funnel and moistened with Mcllvaine buffer- EDTA solution.
v. The filtrate was collected into a 125ml sidearm flask, applying gentle vacuum to side arm.

4.6.3. d. Column Chromatographic Separation (SPE)
i. The C18 cartridge tube was fit on to the SPE extractor.
ii. Conditioned with 20 ml of methanol following 20 ml buffer
iii. The sample filtrate was added to the tube (maintaining a flow rate not exceeding 4 ml/minute).
iv. Washed with 20 ml distilled water
v. Samples were eluted with two 5 ml portions of 0.1 m oxalic acid
vi. The elute was collected and evaporated to less than 1 ml under nitrogen with water bath at 40ºC.
vii. The residue was made up to 1 ml with methanol and filtered through 0.22 μm syringe filter into a vial for injection.
4.6.3.e. HPLCMS-MS Analysis
Appropriate and equal volumes of final extract of samples and calibration dilutions of standards for calibration curve were injected into LC system and thus obtained the MS Chromatogram( AOAC,2005; Agilent applications, 2009;Commission decision, 2002).

4.6.4.Determination of Sulphonamides - HPLC-MSMS Method
4.6.4.a. Chemicals
- Sodium di-hydrogen orthophosphate
- Sodium chloride
- Sodium hydroxide
- Chloroform
- Water
- Methanol

4.6.4.b. Extraction
1. 2.5 g of each thawed sample was weighed into a 50 ml centrifuge tube.
2. 6.0 ml of ethyl acetate was added to the sample and vortexed for 2 minutes. The samples were left to stand for at least 10 minutes.
3. The ethyl acetate was filtered through a fast flow filter column and collected into a clean 15-ml centrifuge tube.
4. 1.0 ml of 3.2M HCl was added. Vortexed for 30 seconds and left to stand for at least 5 minutes.
5. The ethyl acetate was aspirated to waste.
6. 5.0 ml of hexane was added and vortexed for 30 sec. allowed to stand for 5 minutes.
7. The hexane layer was discarded.
8. 2.0 ml of 3.5 M sodium acetate was added.
9. 3.0 ml of ethyl acetate was added, vortexed for 30 sec and left to stand for at least 5 minutes.
10. The ethyl acetate layer was transferred to a clean centrifuge tube.
11. The final extracts were evaporated to dryness under nitrogen with a water bath temperature set to 40 ± 5°C.
12. Reconstituted with 1 ml of methanol, vortexed and centrifuged at approximately 3000 rpm for 5 to 10 minutes.
13. The residue was filtered through 0.22 μm syringe filter for LC analysis.
4.6.4. HPLCMS-MS Analysis

Appropriate and equal volumes of final extract of samples and calibration dilutions of standards for calibration curve were injected into LC system and thus obtained the MS Chromatogram (USFDA method, 2011; Agilent applications, 2009;Commission decision, 2002).

4.6.5. Determination of Malachite Green and Leuco Malachite Green - HPLC- MSMS Method

4.6.5.a. Chemicals

- hydroxylamine hydrochloride
- p-toluene sulfonic acid
- 0.1M ammonium acetate buffer pH 4.5
- Acetonitrile
- dichloromethane
- Formic acid
- Methanol

4.6.5.b. Extraction

i. 5g of homogenized sample was weighed and added 1 ml of 0.25 g/l hydroxylamine hydrochloride.

ii. P-toluene sulfonic acid, 2 ml of 0.1mol/l ammonium acetate buffer (pH 4.5), and 40 ml acetonitrile were added to the sample.

iii. Homogenized for 2 min.

iv. The mixture was centrifuged at 3000 rpm for 3 min.

v. The supernatants were collected into a 250-ml separation funnel.

vi. The sample was re extracted with 20 ml acetonitrile.

4.6.5.c. Liquid-Liquid Extraction

1. To the acetonitrile crude extract in the separation funnel, 30 ml of dichloromethane and 35 ml of distilled water was added and shaken for 2 min.

2. The DCM layer was collected separately.

3. The aqueous phase was extracted one more time with 20 ml of DCM.

4. The combined DCM solvent was evaporated to dryness, and reconstituted in 3 ml of formic acid/acetonitrile (2:98).
4.6.5.d. Solid Phase Extraction (SPE)

i. The Oasis 60 mg/3 cc MCX cartridge was conditioned with 3 ml acetonitrile, and 3 ml 2% v/v formic acid aqueous solution.

ii. The sample was loaded (at ~0.2 ml/min).

iii. Washed with 2 ml formic acid: acetonitrile (2:98) and 6 ml of acetonitrile.

iv. The sample was eluted with 4 ml NH4Ac/ methanol (5:95).

v. The methanol layer was evaporated at 45°C under reduced pressure.

vi. The evaporate was dilute to 1.0 ml with initial mobile phase of water (0.1% v/v formic acid)/MeOH (70:30)

vii. The sample was filtered with a 0.45 μm syringe filter before injection to LC- MS.

4.6.5.e. HPLCMS-MS Analysis

Appropriate and equal volumes of final extract of samples and calibration dilutions of standards for calibration curve were injected into LC system and thus obtained the MS Chromatogram (Ding et al, 2007).

4.6.6. Determination of Flumiquine - HPLC-MSMS Method

4.6.6. a. Chemicals

• Sodium hydroxide
• Ultra pure sodium sulfate anhydrate,
• Formic acid
• Acetonitrile

4.6.6. b. Extraction

i. 2g of homogenized shrimp sample was taken in a 50-ml polypropylene centrifuge tube.

ii. 20 ml acetonitrile containing 1% formic acid was added to the sample, which was then reversely shaken for 5 min at high setting.

iii. 2g of sodium sulfate anhydrate was added to tube and shaken again for another 5 min, followed by centrifugation for 10 min (6000 rpm, 4°C).

iv. The acetonitrile extract was evaporated in a rotary evaporator at 40°C.

v. The residue was resuspended in 2 ml of 10% acetonitrile containing 0.1% formic acid, poured into a 16 × 12 mm glass tube and defatted by extraction with 4 ml hexane while mixing on a vortex mixer in 15 sec Twice.

vi. The mixture was centrifuged for 5 min (4000 rpm, 4°C) and the aqueous supernatant was transferred and filtered through 0.22 μm Nylon membrane before injecting into LCMSMS.
system.

4.6.6. c. HPLCMS-MS Analysis
Appropriate and equal volumes of final extract of samples and calibration dilutions of standards for calibration curve were injected into LC system and thus obtained the MS Chromatogram (JFDA, 2010; Method CR, 2006; USFDA method, 2011; Commission decision, 2002).

4.7. Determination of Toxic Heavy Metals – ICP MS Method
i. 0.5-1.0g of well homogenized sample weighed into the Teflon vessel.
ii. 1ml of milli-q water was first added to control exothermic reactions during acid addition.
iii. 8ml of Conc. HNO₃ was added.
iv. Acid should be added drop wise for the first few ml until it is established that the sample does not react violently.
v. If reaction with the acid was observed, the vessels were left to be in a fume hood for 20 minutes or until the reaction subsides
vi. Digested in microwave ramp power over 25 minutes until 200°C was reached. This temperature was maintained for 15 minutes.
vii. The sample was cooled to below 50°C and removed from oven.
viii. Diluted to 25 ml with reagent grade-1 water. For Hg analysis 0.5ml HCl was added for stabilizing the mercury.
ix. At least one standard was processed for each analyte with each batch.
x. At least one fortified sample was included.
xii. Sample was then quantified using ICP-MS instrument (FSSAI, 2012) (Plate 9).

5.0 RESULTS
In the present study the Penaeid shrimp samples were collected from different places. *Fenneropenaeus indicus* as sea caught origin from Cuddalore port. *Penaeus monodon* was collected from Pondicherry region which was grown in extensive form. *Litopenaeus vannamei* collected from Thiruvallur district grown in intensive form. The shrimp samples were analyzed for various biochemical parameters and compared.

5.1. Molecular Characterization of the Penaeid Shrimps
The Penaeid species used for the analysis in the current study were confirmed genetically.
5.1.1. Genomic DNA isolation

Genomic DNA was isolated from all the three Penaeid species *L. vannamei*.

*F. indicus, P. monodon.* Three isolated DNA was run on agarose gel electrophoresis to confirm the isolation. DNA bands were shown in plate 10.

5.1.2. DNA quantification

Isolated DNA was quantified using nana drop spectrophotometer and the results were given in table. *L. vannamei* has the high amount of DNA concentration with 160 ng/µl, *F. indicus* has 120 ng/µl and *P. monodon* has 90 ng/µl (Table 7).

5.1.3. Amplification and Sequencing

Three isolated genomic DNA were amplified and run on agarose gel electrophoresis along with the molecular marker. The PCR product size of the *Penned* shrimps was 425 bp. Amplified PCR bands were shown in plate 11.

The amplified DNA was then sequenced and electropherogram of the sequence were shown in figure 1, 2 & 3. The sequenced DNA was then aligned using FASTA software. FASTA format sequence was shown in figure 4, 5 and 6.

5.1.4. Bioinformatics Study

FASTA format is then submitted to NCBI – nucleotide BLAST. Most accurate similarity was identified for all the three species.

Fig 7 & 8 shows that the submitted DNA sequence of the *Fenneropenaeus indicus* has high homology with the cytochrome oxidase gene of *F. indicus* with maximum high indent score. Thus confirming the sample collected from sea origin to be *F. indicus*. Tax BLAST also confirms the genus *Fenneropenaeus* with maximum hits (Fig 9). Also when submitted to TCOFFEE tool, sample shows maximum sequence similarity with the *F. indicus* in Fig 10.

In Fig 11 &12, the DNA sequence of the sample collected from Pondicherry region as an extensive farming origin showed high homology with the COI gene of *P. monodon* with maximum score. In fig 13 Tax BLAST result shows greater amount of 74 hits with that of *P. monodon*. TCOFFEE alignment also confirms the *P. monodon* with maximum sequence similarity (Fig 14).
DNA sequence of the sample from intensive farming shows high homology with COI gene of *Litopenaeus vannamei* having high indent score (Fig 15 & 16). Tax BLAST with 38 hits shows similarity with *L. vannamei* (Fig 17). In TCOFFEE *L. vannamei* shows high sequence similarity with that of the submitted sample thus confirming it to be *L. vannamei* (Fig 18).

In TCOFFEE Multiple sequence alignment the score of *Fenneropenaeus indicus, Penaeus monodon* and *Litopenaeus vannamei* were found to be 98, 62 and 95.

Thus all the *Penaeid* species were confirmed by molecular characterization using bioinformatics tools.

### 5.2. Moisture Content

Moisture content of the Penaeid shrimps was analyzed by dry air oven method (Table 8). Moisture value was 79.5 g/100g in *F. indicus*, 77.58 g/100g in *P. monodon* and 78.44g/100g in *L. vannamei*. Moisture content of *F.indicus* was found to be slightly higher than the other two Penaeid species. Moisture content is an important tool to indicate the quality and freshness of the sea food.

### 5.3. Ash Content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Ash content was determined by ashing the sample in muffled furnace at 525ºC. Ash content of *P. monodon* with 1.28 g/100g was higher than the other two species 1.10g/100g in *L. vannamei* and 0.83 *F. indicus* (Table 9). Ash value of the substance indicates the overall mineral content of the species.

### 5.4. Protein Content

Shrimps are the good source of protein. *F. indicus* has the protein value of 18.83 g/100g (Table 10), *P. monodon* has 20.62 g/100g and *L. vannamei* has 19.59 g/100g. The protein content of the shrimps determined by the Kjeldahl method in the present study indicates the crude protein content. All the three species have no much difference in the values in which *P. monodon* has the slightly greater protein value than the other two species.

### 5.5. Fat Content

Fat content of the *F. indicus* was 0.21 g/100g; *P. monodon* was 0.08 g/100g and *L. vannamei* 0.25g/100g. Among three species fat value was higher in *L. vannamei*. *P. monodon* has the
least fat content (Table 11). Shrimps are generally the low fat content seafood.

5.6. Carbohydrate and Energy
Carbohydrate and energy values were obtained by calculation. Shrimps are generally low in food energy.

*F. indicus* has the carbohydrate value of 0.63 g/100g which is found to be nearly equal to 0.62g/100g in *L. Vannamei*. *P. monodon* found to be low in carbohydrate with the value of 0.44 g/100g when comparing with the other two species (Table 12).

*L. vannamei* has the highest energy value among three species chosen for the present study with the value of 91.9 Kcal/100g. Second highest goes to *P. monodon* with 85 Kcal/100 g and *F. indicus* have the low energy with 75.7 Kcal/100g (Table 13).

In the present study overall proximate composition of the three Penaeid species shows no much big difference in their values except for energy value. Moisture content and carbohydrate was high in *F. indicus*, ash content and crude protein content was high in *P. monodon*, total fat content and energy value scores high in *L. vannamei*.

5.7. Veterinary Drug Residue
Veterinary drugs are used in shrimp farming to prevent bacterial diseases for healthy growth of the shrimps. Wild shrimps will not have any traces of veterinary drugs in their body. In the present study *F. indicus* was collected from sea. None of the drugs selected for the determination in the present study were detected in *F. indicus*. Shrimps without drugs and chemicals contamination after harvesting, indicates that they are good and safe quality food for human consumption.

Six antibiotics were monitored for the present study. Oxolinic acid and Flumequine comes under quinolone group was analysed for their presence in the *P. monodon* and *L. vannamei* only, which was not detected in both of them. (Table 14). Chromatograms of the quinolone compounds shown in Figure 63 & 64.

Chloramphenicol was found to be 0.094 µg/kg in *P. monodon* which was nearly equal to 0.097 µg/kg in *L. vannamei* (Table 15). Chloramphenicol is banned antibiotic and it should not be present in the shrimp and if present it should be below 1 µg/kg for safe level of human consumption. MRM chromatograms and Chromatogram along with calibration and shown in
Nitro furan parent compounds are metabolised in the shrimp body and its metabolites AOZ, AMOZ, AHD, and SEM were detected for its presence (Fig 22,23 &24). In spite nitrofuran metabolites being banned compound in European country its traces were found in many farmed shrimps. AHD and SEM were not detected in P. monodon and L. vannamei whereas AMOZ was found to be 0.048 µg/kg in L. vannamei and absent in P. monodon. AOZ was found to be 0.192 µg/kg in P. monodon and was higher than 0.088µg/kg in L. vannamei (Table 16). Chromatogram along with calibration graph shown in Figure 33 to 40.

Tetracyclines and sulphonamide groups are the approved antibiotics till date in shrimp farming. Among tetracycline group, tetracycline was not detected in P. monodon and L. vannamei. Oxytetracycline was absent in P. monodon and 0.917 µg/kg in L. vannamei. Chlorotetracycline was found to be higher in L. vannamei with 0.216 µg/kg when compared with 0.088 µg/kg in P. monodon. Maximum residual level for Tetracycline and sulphonamide is 100 µg/kg (Table 17). Doxycycline was not detected in any of the species in the present study. MRM and Calibration chromatogram along with the sample shown in Figure 25, 26 & 27; 41 to 48.

Six sulphonamide compounds from sulphonamide groups were monitored for the present study (Figure 28, 29 & 30). Sulpha diazine, sulpha dimethoxine, sulpha merazine and sulpha pyridine were not detected in L. vannamei. 0.087 µg/kg of sulpha methazine and 0.07 µg/kg of sulpha thiazole were detected in L. vannamei. Out of six five sulphonamide compounds were detected in P. monodon. 0.178 µg/kg of Sulpha diazine, 0.103 µg/kg of sulpha dimethoxine, 0.051 µg/kg of sulpha merazine, 0.141 µg/kg of sulpha pyridine and 0.480 µg/kg of sulpha thiazole were obtained. Sulpha methazin was absent in P. monodon (Table 18). Calibration chromatogram along with the sample in figure 49-51.

Malachite green is a dye used as an antifungal agent in aquaculture. Malachite green metabolise into Leuco malachite green inside the tissue. Thus both the compounds were determined to detect its use in aquaculture. Antifungal compounds namely Malachite green, Leuco malachite green and Crystal violet was analysed for their presence in the P. monodon and L. vannamei. Fig 60, 61 & 62.
P. monodon has slightly greater amount of malachite green with 0.763 µg/kg than L. vannamei with 0.609 µg/kg. Leuco malachite green was absent in both P. monodon and L. vannamei (Table 19).

Overall veterinary drugs were found in P. monodon and L. vannamei in traceable concentration. P. monodon was from extensive farming where rarely producers provide additional feed and antibiotics and chemicals contamination is possible. L. vannamei was collected from intensive farming where veterinary drugs were used during production cycle for shrimp growth. In the present study F. indicus was found to be good quality for human consumption with no veterinary drugs and antifungal agents in them.

5.8. Toxic Heavy Metals

Four toxic heavy metals namely lead, cadmium, mercury and zinc were analysed in the present study for its presence. Heavy metals are the widespread environmental contaminant found in marine and aquatic species.

Among four toxic metals determined in present study, zinc was found in high concentration than the other three metals in all the three Penaeid species. 7.01 µg/kg in F. indicus, 15.46 µg/kg in P. monodon and 12.16 µg/kg in L. vannamei were detected. Mercury was not present in any of the three species. Lead was present in equal concentration with 0.05 µg/kg in both P. monodon and L. vannamei. Lead was absent in F. indicus. Cadmium was absent in F. indicus and L. vannamei. 0.06 µg/kg of cadmium was found in P. monodon (Table 20).

Except for zinc, other toxic metals were present only in negligible amount in all the three species. Among three Penaeid species, F. indicus was free of any traceable concentration of toxic metals except for zinc which was also low when compared with other two species.

From overall biochemical analysis point of view, F. indicus was found to be best for human consumption which is naturally caught from sea. Even though F. indicus lacks in some nutritional parameters like protein content and energy value it is negotiable as the difference is 1 or 2 g with that of the other two species.

F. indicus was free of any traces of antibiotics, antifungal agents and toxic metal contamination which are the good sign of best quality when compared with other two species.

P. monodon has the high protein and ash content than the other two species but it lacks with
the presence of antibiotic residues in the tissue and has the high concentration of zinc. Out of six, five sulphonamide compounds were present in traceable amount but they are very below permissible level.

Even though *L. vannamei* leads with high energy value it also lacks behind *F. indicus* because of the presence of veterinary drugs and antifungal agents. Quality and safe wise both *P. monodon* and *L. vannamei* scores same place.

From over all comparison, *F. indicus* of sea caught origin was found to be the best species with good nutrition. *F. indicus* is found to be safe for human consumption with absence of antibiotics and antifungal in them. Even though veterinary drugs and toxic metals were present very below permissible level and negligible amount in *P. monodon* and *L. vannamei*, presence of traceable concentration of drugs drag them back in quality on comparison with *F. indicus*.

Table 07: DNA concenetrations of the Penaeid shrimps.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>DNA Concentration NG/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fenneropenaeus indicus</em></td>
<td>120</td>
</tr>
<tr>
<td><em>Penaeus monodon</em></td>
<td>90</td>
</tr>
<tr>
<td><em>Litopenaeus vannamei</em></td>
<td>160</td>
</tr>
</tbody>
</table>

Table 08: Moisture content of the Penaeid shrimps.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Moisture Content in (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fenneropenaeus indicus</em></td>
<td>79.50</td>
</tr>
<tr>
<td><em>Penaeus monodon</em></td>
<td>77.58</td>
</tr>
<tr>
<td><em>Litopenaeus vannamei</em></td>
<td>78.44</td>
</tr>
</tbody>
</table>

Table 09: Total ash content of the Penaeid shrimps.

<table>
<thead>
<tr>
<th>SAMPLE NAME</th>
<th>ASH CONTENT IN (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fenneropenaeus indicus</em></td>
<td>0.83</td>
</tr>
<tr>
<td><em>Penaeus monodon</em></td>
<td>1.28</td>
</tr>
<tr>
<td><em>Litopenaeus vannamei</em></td>
<td>1.10</td>
</tr>
</tbody>
</table>

Table 10: Total Crude protein content of the Penaeid shrimps.

<table>
<thead>
<tr>
<th>SAMPLE NAME</th>
<th>CRUDE PROTEIN CONTENT IN (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fenneropenaeus indicus</em></td>
<td>18.83</td>
</tr>
<tr>
<td><em>Penaeus monodon</em></td>
<td>20.62</td>
</tr>
<tr>
<td><em>Litopenaeus vannamei</em></td>
<td>19.59</td>
</tr>
</tbody>
</table>
### Table 11: Total Fat content of the Penaeid shrimps.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Crude Fat Content In (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenneropenaeus indicus</td>
<td>0.21</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>0.08</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>0.25</td>
</tr>
</tbody>
</table>

### Table 12: Carbohydrate value of the Penaeid shrimps.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Carbohydrate In (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenneropenaeus indicus</td>
<td>0.63</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>0.44</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>0.62</td>
</tr>
</tbody>
</table>

### Table 13: Calorific value of the Penaeid shrimps.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Energy In (Kcal/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenneropenaeus indicus</td>
<td>79.7</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>85.0</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>91.9</td>
</tr>
</tbody>
</table>

### Table 14: Veterinary drug analysis of the Penaeid shrimps – Quinolones.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Quinolones IN µG/KG</th>
<th>Flumequine</th>
<th>Oxolinic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenneropenaeus indicus</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

### Table 15: Veterinary drug analysis of the Penaeid shrimps – Chloramphenicol.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Chloramphenicol In µG/KG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenneropenaeus indicus</td>
<td>ND</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>0.094</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>0.097</td>
</tr>
</tbody>
</table>

### Table 16: Veterinary drug analysis of the Penaeid shrimps - Nitrofuran Metabolites.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Nitrofuran Metabolites In µg/kg</th>
<th>AMOZ</th>
<th>AOZ</th>
<th>AHD</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenneropenaeus indicus</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>ND</td>
<td>0.192</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>0.048</td>
<td>0.088</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
Table 17: Veterinary drug analysis of the Penaeid shrimps - Tetracycline group.

<table>
<thead>
<tr>
<th>SAMPLE NAME</th>
<th>TETRACYCLINES GROUP IN µg/kg</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxy tetracycline</td>
<td>Tetracycline</td>
<td>Chloro tetracycline</td>
<td>Doxycycline</td>
<td></td>
</tr>
<tr>
<td>Fenneropenaeus indicus</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>ND</td>
<td>ND</td>
<td>0.088</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>0.917</td>
<td>ND</td>
<td>0.216</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Table 18: Veterinary drug analysis of the Penaeid shrimps - Sulphonamides group.

<table>
<thead>
<tr>
<th>SAMPLE NAME</th>
<th>SULPHONAMIDES IN µg/kg</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-diazine</td>
<td>S-dimet</td>
<td>S-merazin</td>
<td>S-methazin</td>
<td>S-pyridine</td>
</tr>
<tr>
<td>Fenneropenaeus indicus</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>0.178</td>
<td>0.103</td>
<td>0.051</td>
<td>ND</td>
<td>0.141</td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.087</td>
<td>ND</td>
</tr>
</tbody>
</table>

Table 19: Veterinary drug analysis of the Penaeid shrimps - Antifungal agents.

<table>
<thead>
<tr>
<th>SAMPLE NAME</th>
<th>ANTIFUNGAL AGENTS IN µg/kg</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Malachite green</td>
<td>Leuco malachite green</td>
<td>Crystal violet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fenneropenaeus indicus</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>0.763</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>0.609</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 20: Heavy metals analysis of the Penaeid shrimps.

<table>
<thead>
<tr>
<th>SAMPLE NAME</th>
<th>HEAVY METALS CONCENTRATION IN µg/kg</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lead (Pb)</td>
<td>Cadmium(Cd)</td>
<td>Mercury(Hg)</td>
<td>Zinc(Zn)</td>
<td></td>
</tr>
<tr>
<td>Fenneropenaeus indicus</td>
<td>BLQ</td>
<td>BLQ</td>
<td>BLQ</td>
<td>7.01</td>
<td></td>
</tr>
<tr>
<td>Penaeus monodon</td>
<td>0.05</td>
<td>0.06</td>
<td>BLQ</td>
<td>15.46</td>
<td></td>
</tr>
<tr>
<td>Litopenaeus vannamei</td>
<td>0.05</td>
<td>BLQ</td>
<td>BLQ</td>
<td>12.16</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Sequence result of the *Fenneropenaeus indicus* in the chromatogram.
Figure 2: Sequence result of the *Penaeus monodon* in the chromatogram.

Figure 3: Sequence result of the *Litopenaeus vannamei* in the chromatogram.
Figure 4: FASTA format of the *Fenneropenaeus indicus* DNA sequence.

Figure 5: FASTA format of the *Penaeus monodon* DNA sequence.

Figure 6: FASTA format of the *Litopenaeus vannamei* DNA sequence.
Figure 7: Nucleotide BLAST Result of *Fenneropenaeus indicus*.

Figure 8: Some of the most accurate significant alignment with that of the submitted *Fenneropenaeus indicus* DNA sequence.
Figure 9: Result of the Taxonomy BLAST report for *Fenneropenaeus indicus*.
Figure 10: T-COFFEE multiple sequence alignment between the test sample of *Fenneropenaeus indicus* with that of other closely related species showing maximum similarity.
Figure 11: Nucleotide BLAST result of *Penaeus monodon*.

Figure 12: Some of the most accurate significant alignment with that of the submitted *Penaeus monodon* DNA sequence.
Figure 13: Result of the Taxonomy BLAST report of the *Penaeus monodon*. 
Figure 14: T-COFFEE multiple sequence alignment between the test sample of *Penaeus monodon* with that of other closely related species showing maximum similarity.
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Figure 16: Some of the most accurate significant alignment with that of the submitted *Litopenaeus vannamei* DNA sequence.
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Figure 20: MRM chromatogram for the Chloramphenicol in the Penaeus monodon.
Figure 21: MRM chromatogram for the Chloramphenicol in the *Litopenaeus vannamei* the Fenneropenaeus indicus.

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Figure 23: MRM chromatogram for the Nitrofuran metabolite compounds in the *Penaeus monodon* the *Litopenaeus vannamei*.

Figure 24: MRM chromatogram for the Nitrofuran metabolite compounds in the *Litopenaeus vannamei*.
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Figure 26: MRM chromatogram for the Tetracycline compounds in the *Penaeus monodon*.
Figure 27: MRM chromatogram for the Tetracycline compounds in the *Litopenaeus vannamei*.

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Figure 32: LCMS MS chromatogram for the Chloramphenicol in Penaeus monodon and Litopenaeus vannamei along with calibration graph.
Figure 33: LCMS MS chromatogram for the Nitrofuran compound - AMOZ in the *Fenneropenaeus indicus* along with calibration graph.

Figure 34: LCMS MS chromatogram for the Nitrofuran compound - AMOZ in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 35: LCMS MS chromatogram for the Nitrofuran compound - AOZ in the *Fenneropenaeus indicus* along with calibration graph.

Figure 36: LCMS MS chromatogram for the Nitrofuran compound - AOZ in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 37: LCMS MS chromatogram for the Nitrofuran compound - AHD in the *Fenneropenaeus indicus* along with calibration graph.

Figure 38: LCMS MS chromatogram for the Nitrofuran compound - AHD in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
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Figure 40: LCMS MS chromatogram for the Nitrofuran compound - SEM in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
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Figure 42: LCMS MS chromatogram for the Tetracycline compound in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 43: LCMS MS chromatogram of the Oxy tetracycline compound in the *Fenneropenaeus indicus* along with calibration graph.

Figure 44: LCMS MS chromatogram for the Oxy tetracycline compound in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 45: LCMS MS chromatogram of the Chlor tetracycline compound in the *Fenneropenaeus indicus* along with calibration graph.

Figure 46: LCMS MS chromatogram for the Chlor tetracycline compound in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 47: LCMS MS chromatogram of the Doxycycline compound in the *Fenneropenaeus indicus* along with calibration graph.

Figure 48: LCMS MS chromatogram for the Doxycycline compound in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 49: LCMS MS chromatogram of the Sulfa diazine compound in the *Fenneropenaeus indicus* along with calibration graph.

Figure 50: LCMS MS chromatogram for the Sulfa diazine in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 51: LCMS MS chromatogram of the Sulfa dimethoxine compound in the *Fenneropenaeus indicus* along with calibration graph.

Figure 52: LCMS MS chromatogram for the Sulfa dimethoxine in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 53: LCMS MS chromatogram of the Sulfa merazin compound in the *Fenneropenaeus indicus* along with calibration graph.

Figure 54: LCMS MS chromatogram for the Sulfa merazin in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 55: LCMS MS chromatogram of the Sulfa methazine compound in the *Fenneropenaeus indicus* along with calibration graph.

Figure 56: LCMS MS chromatogram for the Sulfa methazin in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
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Figure 58: LCMS MS chromatogram for the Sulfa pyridine in the *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 59: LCMS MS chromatogram for the Sulfa thiazole in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.

Figure 60: LCMS MS chromatogram for the Antifungal agent compound - Malachite green in the *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
Figure 61: LCMS MS chromatogram for the Antifungal agent compound - Leuco Malachite green in *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.

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Figure 63: LC MS MS chromatogram for the Quinolone compound - Oxolinic acid in the *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.

Figure 64: LCMS MS chromatogram for the Quinolone compound - Flumequine in the *Penaeus monodon* and *Litopenaeus vannamei* along with calibration graph.
6.0 DISCUSSION

6.1. Morphological features of the shrimps

Penaeid shrimps were collected from three different regions *Fenneropenaeus indicus* as sea caught origin from Cuddalore seaport, *Penaeus monodon* as extensive farming from pond areas in Pondicherry region, *Litopenaeus vannamei* as intensive farming origin from Meenjur shrimp farm. Kannupandi *et al* in his study mentioned the identification of Penaeid shrimps with the following features. The pleurae on either side of the second abdominal segment overlap the pleurae of the third segment; the first three pairs of perepods are chelated in the shrimps for Penaeid shrimps, which were similar to the penaeid shrimps in the present study. *Vannamei* shrimp was morphologically confirmed due to the presence of straight rostrum. Upper rostrum had 7 – 8 teeth while the lower rostrum has 2 - 3 teeth. The pair of antennae was pink. The swimmer let was white in the work of Sutabutr, 2007.

Brock *et al*, 1992 in their work found that *Penaeus indicus* had non banded antennae, spotted antennules, semi translucent body with olive green to grey-blue speckles, pink or red pleopods and pereopods with same color as that of the body. They also had a slender and long rostrum, 7 to 9 teeth on the dorsal and 4 to 6 teeth on the ventral margin.

Mature tiger shrimp had overall rusty brown color and the distinctive black and white banding across their back and on their tail. There was also a rarely seen color variant of the species with a conspicuous, wide, reddish-orange stripe along its back for *Penaeus monodon* in the work of Knott *et al*, 2012.

The Penaeid species collected as *P. indicus* from Cuddalore seaport had pink color pleopods and the body was semi transparent which correlated with the work of Brock *et al*, 1992. *L. vannamei* from Meenjur shrimp farm had white colored swimming legs and had straight rostrum which reinstated the findings of Sutabutr, 2007. Dark bands like tiger stripes were seen on the shell of the shrimp collected from Puducherry region which indicates that it is *Penaeus monodon* as mentioned by Knott *et al*, 2012.

6.2. Molecular characterization

Three penaeid species collected from three different origins were confirmed by molecular characterization. Genomic DNA was isolated from them and amplification was done by PCR. Amplified products sequenced and submitted to NCBI BLAST. The resulted sequences were
compared with already existing sequence of penaeid species. Sequence having maximum similarity with that of the submitted sequence confirmed the species.

Khamnamtong et al., 2005, in their study, used reliable PCR-based methods for identifying species origins of morphological similar shrimps (between \textit{P. monodon} and \textit{P. semisulcatus} and between \textit{L. vannamei} and \textit{F. merguiensis}).

Single barcoding DNA segment chosen for taxological studies, the COI gene-based identification system has been proven superior within taxonomic groups of Protista and animals, including gastropods, ants, butterflies, birds, spiders, fish, worms, Crustacea, and very recently primates. The high accuracy, throughput, and efficiency make the identification of genome sequences unique to different species and life forms easy.

Zhang et al., 2011, reported the comparative studies among different Artemia species which revealed complex sequence diversities that are expected to have functional relevance, such as energy metabolism and environmental adaptation. Consequently, results on DNA barcoding and comparative analysis reveal the current distribution of Artemia species in China and phylogenetic relationship among them, providing insights into the adaptive evolution of DNA sequences of Artemia.

6.3. **Biochemical determination**

6.3.1. **Moisture content**

Water is the major component of most foods and serves a variety of functions. It influences the structure, appearance and taste of food. The moisture content of food also influences its spoilage process. Each food has its own characteristic water content. The determination of moisture content is one of the most fundamental and important analytical procedures in food analysis.

Moisture content in the three species tested ranged from 77 to 80 g/100g approximately. Among the three Penaeid species, \textit{F. indicus} had high moisture content at 79.5g/100g than \textit{P. monodon} and \textit{L. vannamei} which had values of 77.58 and 78.44 g/100g respectively.

In 2013, Lopez et al., compared the proximate composition between wild and farmed shrimp of \textit{Litopenaeus vannamei}. It was found to have moisture content between 73.14 to 73.91 g/100g without any significant difference between farmed and wild shrimp, whereas Kherunnisa et al., 2010 reported the moisture content of \textit{Fenneropenaeus penicillatus} at 77
g/100g in the muscle. Ehigiator et al. in 2011 compared the moisture content in the three body parts namely whole prawn, flesh and exoskeleton of Macrobrachium vollenhovenii and Macrobrachium macrobrachion. The overall moisture content ranged from 5.57 to 7.63 g/100g.

Gunalan et al., 2013, in his study, estimated the concentration of moisture at 76.2g/100g in the flesh of L. vannamei which was slightly lower than the moisture value of L. vannami in the present study. Adeyeye et al., in 2008, reported a value of 13.3 g/100 in the shell and 15.4 g/100g in the flesh of P. notabilis. In 1973, Sidwell et al., 1973, compared the chemical and nutritive values of several fresh and canned finfish, crustacean mollusks and their moisture value ranged from 76.2 to 84 g/100g for shrimps obtained in different parts of the world like Alaska, Asia, Mexico, South Atlanta and Gulf country.

Moisture content of the penaeids used in the present study correlates with a small difference with those that of the Lopez et al., 2013 and Kherunnisa et al., 2010, but on comparison the present work shows a significant increase of value. The species in the current study shows a higher range of moisture value than those studied by Adeyeye et al., 2008 and Ehigiator et al., 2011.

6.3.2. Ash content
Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals (the “analyte”) can be distinguished from all the other components (the “matrix”) within a food in some measurable way. P. monodon was found to have slightly increased amount of ash content 1.28 g/100g among three tested Penaeid species. F. indicus had 0.83 g/100g of ash value which was slightly lower than the other two species. Ash content of shrimp is generally 1 to 1.5%.

Ash value of L. vannamei in the present study was found to be 1.10g/100g which showed no significant variation with that of 1.26 g/100g in L. vannamei collected from Andhrapradesh farm by Gunalan et al., 2013. Kherunnisa et al., 2010, recorded the value of ash ranging from 1.46 to 2.20 g/100g at different satges of maturity in F. penicillatus. Whereas the value ranged from 1.40 to 1.86 g/100g in the raw crustaceans of Penaeus family studied by Sidwell et al 1973, these values were very close to the findings of present study.
The present study disagrees with the result of Ehigiator et al., 2011 and Ekpenyong et al., 2013, who showed the vast variation in the value of ash with the range of 20 – 40.67 and 4.3 – 7.14 g/100g for the same species *M. macrobranchion*. They both have studied the same body parts of the same species but have obtained high dissimilarity in their ash values. Sample collection from the different places doesn’t seem to influence the ash value with the vast variation for the same species. The present work goes in contradiction with Adeyeye et al., 2008, who has reported high value of ash 19.9 g/100g in *Penaeus notabilis*. No much work has been carried out in this species.

### 6.3.3. Protein content

Shrimps are very good source of protein. The protein value of the present investigation was found to be 18.83 g/100g in *F. indicus*, 20.62 g/100g in *P. monodon* and 19.59 g/100g in *L. vannamei*.

According to the study of Sriraman (1978), the protein content of crustaceans and mollusks were around 20%. The protein value ranging from 18 -19 g/100g was reported by Kherunnisa et al, 2010 in different stages of maturity for *F. pencillatus*. Shrimp species from different parts of the world tested by Sidwell et al., 1973, showed protein value between 13 and 22 g/100g. It has been reported that protein content of shrimp ranged between 7- 21 % depending on species (Sriket et al., 2007 and Yanar et al., 2006). Protein value of 20 and 14.2 % in the flesh of *P. longirostris* and *P. martia* was recorded by Abdullah et al.,2009. Lopez et al in 2013, reported the protein value as 20g/100g in wild shrimp and 19.96 g/100g in farmed shrimp of *L. vannamei*.

The results of proximate composition by Ravichandran et al.,2009, reported that the percentage of protein in the flesh was higher (41.3%) than that of shell protein (32.5%) for *Penaeus indicus*. Similarly a high protein value of 35.69 % was recorded by Gunalan et al., 2013. The present study used Kjeldahl procedure for the determination of protein which uses protein factor for the conversion of nitrogen to protein, whereas Gunalan et al.,2013 and Ravichandran et al.,2009, used lowry’s method for the determination of protein which cannot be considered for the high protein value variation with that of the present study.

According to Everette et al., 2010, Lowry method involves reduction of the Folin reagent and oxidation of aromatic residues mainly tryptophan and tyrosine involving the color change of the sample solution in proportion to protein concentration, which can then be measured using
colorimetric techniques. As a result the total concentration of protein in the sample can be deduced from the concentration of Trp and Tyr residues that reduce the Folin reagent.

Fresh water prawns *Macrobrachium* species recorded very high protein value ranging from 38.50 - 40.56 g/100g by Ehigiator *et al.*, 2011 which showed controversy with the result of Ekpenyong *et al.*, 2013, ranging from 14.02 - 22.3 g/100g for the same species and same body parts. As no much work has been studied in the *P. noatbilis*, the increased protein value of 36.6 and 44.7 % in the shell and flesh of *P. notabilis* reported by Adeyeye *et al.*, 2008, was not much considered for their high protein value with that of the present work. Variation in the values might also be due to species difference species when compared with present work.

In spite of some contradictions with above study, the overall comparison of the previous studies with the results of protein content of *F. indicus*, *P. monodon* and *L. vannamei* in the present work, the findings were in good agreement.

### 6.3.4. Fat content

The term ‘lipid’ refers to a group of compounds that are sparingly soluble in water, but show variable solubility in a number of organic solvents (e.g., ethyl ether, petroleum ether, acetone, ethanol, methanol, benzene).

Shrimps are the good source of nutritional food with low fat content. Crude fat value of 0.21, 0.08 and 0.25 g/100g in *F. indicus*, *P. monodon* and *L. vannamei* was recorded in the present work. Abdullah *et al.* 2009, resulted with 1.1 and 2.6 g/100g fat content in *Parapenaeus longirostris* and *Plesionika martia* in their work.

Sidwell *et al.*, 1973 reported a range of fat value between 0.53 – 1.86 g/100g in shrimp species collected from different parts of the world. Curude fat value of 0.9 g/100g was found in the *Penaeus setiferus* species studied by Peplow *et al.* 1975. Lopez *et al.*, 2013 reported a fat value between 1.27 and 1.34 g/100g in the wild and farmed shrimp of *L. vannamei* species. These results showed good correlation with the present work.

High value of fat content was reported by Adeyeye *et al.*, 2008, with 10.3 and 8.5g/100g in the shell and flesh of *P. notabilis*. Similarly a high value was also obtained by Gunalan *et al.*, 2013, with 19 % in *L. vannamei* and Ravichandran *et al.*, 2009, with 7.6 and 9.8 g/100 in the flesh and shell of *P. indicus*. This significant increase in fat value of the above study in comparison with present might be due to method difference used for the determination. Crude
fat value more or less similar to the above findings were also seen in the species of *Macrobrachium* studied by Ehigiator *et al.*, 2011 and Ekpenyong *et al.*, 2013, ranging from 6.67 -15.67 and 4.4 – 7 g/100g. High fat content findings in the above studies with that of the present work might be due to the species differences.

Even if the fat level of the present findings were lower than the above findings, the amount of crude lipid may not be providing enough fat for human diet. Also fat content of the shrimp may not be a good source of human diet. (Abdullah *et al.*, 2009.)

**6.3.5. Carbohydrate and Energy**

Shrimps have low energy and very low carbohydrate. *F. indicus* and *L. vannamei* in the present study had approximately same value with 0.63 and 0.62 g/100g for carbohydrate. *P.mondon* was found to have 0.44 g/100 of carbohydrate value. Energy value of the penaeid species was found to be 79.7, 85 and 91.9 Kcal/100g in *F. indicus*, *P. monodon* and *L. vannamei* respectively.

Adeyeye *et al.*, 2008, noted the carbohydrate value of 1.6 and 2.6 g/100g and energy value of 1032 and 1119.5 KJ in the shell and flesh of *P. notabilis*. Gunalan *et al.*, 2013 and Ravichandran *et al.*, 2009, recorded the value of crude carbohydrate as 3.20 and 2.4 g/100g in the *L. vannamei* and *P. indicus* in their study. These findings of the carbohydrate values go in similarity with the present study.

**6.4. Veterinary drugs**

The presence of residues of banned substances/ substances permitted but exceeding the prescribed limits by the regulatory authorities in case of veterinary drugs, pharmaceutical products and pharmaceutically active substances in products of animal origin (like muscle, liver, kidney, fish-flesh, egg, milk, honey etc) and from various species of the shrimps are matters of concern for public health. The presence of these substances may lead to allergies, suspected to be carcinogens, mutagens or may lead to emergence of resistant microbes. As a consequence, national food safety authorities and regulatory authorities have banned the use and strictly regulated its use in veterinary practice or established legal guidance to ensure proper use of these veterinary drugs, pharmaceutical products and pharmaceutically active substances. The successful implementation of national regulation and surveillance monitoring depends on availability of reliable analytical techniques.
In the present study the veterinary drugs namely chloramphenicol, tetracyclines, sulphonamides, nitrofuran metabolites, quinolones and antifungal agents were determined for its presence in the Penaeid shrimps. Among three species, *F. indicus* contained no traces of any antibiotics and antifungal agents, while *P. monodon* and *L. vannamei* was found to have some traces of antibiotics and antifungal in them.

In the present study chloramphenicol was found with concentration of 0.094 µg/kg in *P. monodon* and 0.097 µg/kg in *L. vannamei*. Raffi in 2011, screened for the presence of chloramphenicol in wild and farmed species of *P. monodon* in which the farmed samples displayed a wide range of 0.01µg/Kg to 0.3µg/Kg concentration. These values showed similarity with the concentration of chloramphenicol in the present study.

Flumequine and Oxolinic acid is a quinolone compound tested for their presence in the *P. monodon* and *L. vannamei*. Quinolone compounds were found to be absent in both the species tested in the present work. Among four Nitrofuran metabolites AMOZ with 0.048 µg/kg and AOZ with 0.088µg/kg were found in *L. vannamei*. Only AOZ was found in *P. monodon* with 0.192 µg/kg concentration which was higher than that in *L. vannamei*.

A brand naming survey carried out by the Veterinary Medicines Directorate (VMD) on behalf of the Veterinary Residues Committee (VRC) found evidence of the use of nitrofurans in three samples of imported warm water prawns. A total of 102 samples of warm water prawns were analyzed for residues of nitrofurans.

Fourteen samples tested non-compliant using an initial screening test. Of these three samples contained confirmed residues of nitrofurans in excess of the MRPL. A sample of raw black tiger prawns imported from India and collected at a UK Border Inspection Post was found to contain residues of AOZ at a concentration of 14 µg/kg. One sample of prawns imported from Thailand, via Italy, and purchased from Billingsgate Market contained residues of SEM at a concentration of 1.6 µg/kg. A sample of prawns imported from Thailand, via Italy, and purchased from Billingsgate Market contained residues of SEM at a concentration of 1.6 µg/kg and residues of AOZ at a concentration of 1.1 µg/kg.

In the present study, Oxy tetracycline was absent in *P. monodon* and *F. indicus* but present in *L. vannamei* with 0.917 µg/kg. Chlor tetracycline was higher in *L. vannamei* with 0.216 µg/kg than *P. monodon*. Doxycycline and Tetracycline was not present in any of the three species.
Oxytetracycline concentrations in the positive samples of catfish ranged between 234.3 and 987.5 µg kg in the liver and 22.5 and 553.2 µg kg in the fillets. Forty three (26.9%) liver and 30 (18.8%) fillets contained oxytetracycline residues above the Codex Alimentarius Commission established maximum residue limit of 600 and 200 µg kg respectively in the study of Olatoye, et al. 2013. The present investigation of the tetracyclines were very much low on comparison with above findings. The difference in the values might be due to species.

Five Sulphonamide compounds out of six were detected in P.monodon in the present work, in which sulfa thiazole was found to be in high concentration with value of 0.480 µg/kg. other compounds of sulphonamide with caoncentration of 0.178,0.103,0.051 and 0.141 µg/kg in S-diazone,S-dimethoxin,S-merazin,and S- pyridine. L.vannamei had the presence of only two sulphonamides compound namely, sulfa thiazole and sulfa methazine with 0.087 and 0.07 µg/kg. Detectable concentrations of sulphonamides were found to be present in the sample of poultry meat studied by Mehtabuddin, A et al 2012 in which the amount ranged from 0.02 to 0.8 µg/g and 0.02 to 0.6 µg/g between two different places.

Theresa A. G., et al in 2006, studied liquid chromatographic (LC) method for determining 14 sulfonamide residues in tissues of catfish, shrimp and salmon which was dosed with six selected sulfonamides namely sulfadiazine, sulfamerazine, sulfamethazine, sulfachloropyridazine, sulfadimethoxine and sulfaphinoxaline). All assays were within U.S. Food and Drug Administration guidelines for recovery and intra-assay variability. The above method was similar to the method used in the present study.

Residual Level of antibiotics viz: Chloramphenicol, Sulphonamide, Tetracycline, Erythromycin, Streptomycin and -Lactams were determined in farmed shrimps collected from major fish farms of Kerala, Tamil Nadu, Karnataka and Andhra Pradesh studied by Swapna et al., 2012. Sulfonamides and Erythromycin were detected in farmed shrimps at a level 100 ppb. The results of Hakem et al in 2013 revealed that 124 out of 145 poultry meat samples were positive to antibiotic residues of tetracyclines and sulfonamides with the percentage of 85.51% and 36.29% for Sulfonamides.

Raw and ready to eat chicken and beef samples were tested for antibiotic analysis. Fifty one out of 130 samples (39 %) found to be antibiotic residue positive for at least one of the tested antibiotic groups. Tetracycline (28%) was the leading group of antibiotics presenting in more number of samples followed by sulfonamide (23%) and Penicillin (20%) in the work of...
Gebre 2012. Similarly in the work of Shareef et al., 2009, out of 75 poultry samples 39 samples revealed positive for the antibiotic residues of oxytetracycline, sulfadiazine, neomycin, and gentamycin. Oxytetracycline was the most predominant antibiotic detected (28%), among the four studied antibiotics and followed by Sulfadiazine (24%). The above studies showed contradiction with the present work due to increased amount of antibiotic residues which might be because of the difference in the samples tested.

Antifungal compounds namely Malachite green, Leuco Malachite green and Crystal violet were analyzed in the *P. monodon* and *L. vannamei*. Leuco malachite green a metabolite of malachite green was absent in both the species *P. monodon* and *L. vannamei* tested in the current study. *P. monodon* had a greater amount of malachite green than the *L. vannamei* with 0.763 µg/kg. Liquid chromatographic methods are presented for the quantitative and confirmatory determination of malachite green (MG) and leucomalachite green (LMG) in catfish, trout, tilapia, basa, salmon, and shrimp Andersen et al., 2005.

Comparison of the present work with those of the above studies reveals that high concentration of antibiotics were seen in the majority of the poultry and other meat samples. Whereas comparison of similar types of samples also showed that only famred shrimps had the presence and traceability of the antibiotic residues in the edible part. Positive results for chloramphenicol residues in the *Penaeus monodon* collected from the estuarine ponds of Pondicherry region might be due to the release of disease affected shrimps from the cultured farms at their early stages.

6.5. Toxic metals

Toxic pollutants analyzed in the present study were cadmium, zinc, mercury and lead. *F. indicus* caught from sea origin was free of above metals except for zinc with 7.01µg/kg, which was below the safe level of human consumption.

*P. monodon* resulted with high amount of zinc having 15.46 µg/kg on comparison with other species in the present study, cadmium and lead were present in negligible concentration with 0.06 µg/kg and 0.05 µg/kg. *L. vannamei* of intensive farm origin was found to have 12.16 µg/kg of zinc and 0.05 µg/kg of lead. In the present study except for zinc other metals were present only in traceable amount which does not have much effect on human consumption.
Levent et al., 2013, in their study measured the heavy metal concentrations of Cd, Pb, Cu, Zn, Co, Mn, Ni and Fe with the range of 0.228-0.481, 0.291-0.491, 5.85-14.77, 18-36, 0.24-0.61, 6-15, 2-6 and 30-58 µg.g-1 wet weight, respectively in the _Crangon crangon_. Mitra et al., 2012, in their work recorded the concentrations of Zn, Cu, Pb and Cd, in muscle tissue of 5 commercially important crustacean species like _Penaeus monodon_, _Penaeus indicus_, _Penaeus semisulcatus_, _Penaeus marguensis_ and _Metapenaeus brevicornis_.

The distribution of trace metals accumulated in all the muscle tissues of the above shrimp species followed the order Zn > Cu> Pb> Cd. Concentration of Zn with the high range between 2 to 1184 ppm were found in all the shrimp species of the above study, when compared to concentration of other metals in the same animals.

Cu, Cd and Zn were found as 2.2, 0.7 and 6.1 mg/kg for _P. longirostris_ and 2.8, 0.14 and 5.8 mg/kg for _P. martia_ in the study of Abdullah et al 2009. Sophia Balfour et al 2012, the heavy metal findings (Cu, Zn, Cd, Cr, Ni and Hg) in the Penaeus spp. of marine shrimp were well below the United States Food and Drug Administration, Canada’s Food Inspection Agency and, Trinidad and Tobago’s admissible limits for human consumption. Significant differences were observed only for copper by location and season interaction and; zinc by location and season respectively. Cu with range of 1.66 to 2.38 ppm in wet season and 1.87 to 3.82 ppm in dry season was observed and zinc value ranged from 8.99 to 10.61 ppm. In 2013, Heidarieh., et al observed high level of As 8.28 ppm and Mn 25.43 ppm in the _Penaeus semisulcatus_ caught off Persian Gulf. The order of the heavy metal concentrations in the green tiger shrimp was Fe>Zn>Mn>As>Co. The concentrations of the heavy metals reported in these studies were too high when compared with the measurements in the present work.

All the heavy metals measure in the present work was only in the traceable amount with the unit of ppb whereas the concentrations of the heavy metals in the above studies were present in measurable amount with unit of ppm. The high variation in the heavy metal concentration of the above study with that of the present study might be due to environmental factors, location and species differences. The probable traceability of the heavy metals in the penaeid shrimps of the present work might be caused by various industries outlet which comes to the river and sea affecting the human health.
From overall conclusion, the result of the present study shows that *F.indicus* from wild source was found to be a better species among three species studied, with positive beneficial facts like good source of nutrition’s, free of veterinary drugs tested and free of toxic metals except for zinc with below safe level. The wild shrimp tended to have a better proximate composition than the ponded and farmed shrimp, due to the availability of a greater diet variety in their environment.

A study of this kind will form a base line data for such vivid investigations in future. The rules and regulations are to be fortified pertain to antibiotic residues in seafood’s, since antibiotic residue traces in the edible tissues were not considered to be a choice of good quality food for human consumption.

7.0 SUMMARY

*Fenneropenaeus indicus*, *Penaeus monodon* and *Litopenaeus vannamei* are the most common shrimps used for human consumption in India. These three species were compared for the better quality in the aspects of nutritious and any contamination free nature. Three *Penaeid* species were collected from three different regions indicating three different origins. The species collected for the present study by its name from three regions. *Fenneropenaeus indicus* collected from Cuddalore sea port. *Penaeus monodon* was collected from ponds near the sea area in the Pondicherry region. Finally *Litopenaeus vannamei* was collected from the Meenjur farm.

The *Penaeid* species collected for the current study were confirmed by molecular methods which include Genomic DNA isolation, DNA quantification using nano drop spectrophotometer, amplification using PCR, purification of the PCR products, sequencing and species confirmation using Bioinformatics tools.

All the three *Penaeid* species were subjected to Biochemical analysis for nutritional and veterinary drug analysis. They were subjected to the detection of toxic heavy metals contamination.

Nutritional parameters tested for the present study were moisture, ash, crude protein, crude fiber, and carbohydrate and energy value determination. Moisture content was found to be high in *F. indicus*. Ash value which indirectly denotes the total amount of mineral content was high in *P. monodon*. Out of three species *P. monodon* had high amount of protein in
comparison with the other two species. Shrimps are generally characterized with a poor source of fat content, *L. vannamei* had slightly high amount of fat than the other two species. Carbohydrate was nearly equal in concentration in *F. indicus* and *P. monodon*. *L. vannamei* had greater amount of energy value.

Nutritional values of three *Penaeid* species did not show much difference.

All the three species were good with one or the other parameters.

Chloramphenicol, nitrofuran metabolites, sulphonamides, tetracyclines, flumequine and anti fungal agents were analyzed for its residue traces in the *Penaeid* species. Chloramphenicol and nitrofuran metabolites are the banned antibiotics in European Union, Canada and US. Export council of India has set maximum residual level for permitted veterinary drugs and minimum residual permissible level for banned antibiotics. Chloramphenicol was absent in *F. indicus* and was present in both *P. monodon* and *L. vannamei* at trace quantities. Flumequine, a quinolone compound was not present in any of the three species. Nitro furan metabolites were absent in *F. indicus* and was found in concentrations that were well below the permitted level in *P. monodon* and *L. vannamei*.

*F. indicus* does not have any traces of Tetracyclines, sulphonamides and anti fungal agents. *P. monodon* and *L. vannamei* were found to have traces of tetracycline and sulphonamide compounds.

Leucomalachite green was absent in all three species. Malachite green was absent in *F. indicus*, but was present in traceable amounts in *P. monodon* and *L. vannmaei*.

Information on the distribution pattern of toxic heavy metal pollutants in aquatic environment becomes important so as to know the accumulation of such pollutants in the organisms and final transfer to man through sea foods.

Four toxic metals namely, Zinc, Lead, Cadmium, Mercury were determined for its contamination in the *Penaeid* shrimp. Except for Zinc the other metals were absent in *F. indicus*. *L. vannamei* had only zinc and lead in traceable quantities whereas *P. monodon* was found to have Lead, cadmium and zinc in amounts well below the permitted levels.
From over all comparisons, *F. indicus* was found to be the best shrimp in all the aspects with good quality of nutrients, veterinary drug and anti fungal contamination free nature. Except for Zinc other metals were not found even in traceable amount. Zinc was also found to be low in concentration than other two species. Thus *F. indicus* collected as wild shrimp sea caught origin from seaport was determined as a best quality shrimp among three *Penaeid* species studied in the present study. Even though *P. monodon* and *L. vannamei* had slightly greater nutritional value in protein, ash and energy value, presence of antibiotic residues in traces pushed them back in safe quality shrimp for human consumption. In spite of the presence of antibiotic residues and toxic metals which were well below the permitted level in both *P. monodon* and *L. vannamei*, it is to be taken into consideration that were present in traceable concentrations which over time could accumulate and lead to many detrimental effects on the consumers. The complete absence of any residues in *F. indicus* makes it a safe and encouraging option for human consumption.

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ABBREVIATION

% : Percentage
@ : at the rate of
° : Degree
µg : Microgram
µl : Micro Liter
µm : Micrometer
A : Adenine
ADI : Acceptable Daily Intake
AFLP : Amplification Fragment Length Polymorphism
AHD : 1-aminohydantoin
AMOZ : 3-amino-5-morpholino-methyl-1,3-oxazolidinone
AOAC : Official Analysis of Methods
AOZ : 3-Amino-2-Oxazolidinone
APCI : Atmospheric Pressure Chemical Ionization
As : Arsenic
ATP : Adenosine Triphosphate
BLAST : Basic Local Alignment Search Tool
BLQ : Below Level of Quantification
BMP : Better Management Practices
bp : Base Pair
BW : Body Weight
°C : degree Celcius
C : Cytocine
Ca : Calcium
Cd : cadmium
cDNA : cloned Dioxyribo Nucleic Acid
Cm : Centimeter
CMFRI : Central Marine Fisheries Research Institute
Co : Cobalt
COI : Cytochrome Oxidase
Conc : Concentrated
Cr : Chromium
Cu : Copper
CVMP : Committee for Veterinary Medicinal Product
d : Day
db : Dry Basis
DNA : Deoxyribo nucleic acid
dNTP : Deoxy Nuclease Tri Phosphate
DP : Dietary Protein
EC : European Commission
EDTA : Ethylene diamine tetraacetic acid
ELISA : Enzyme-Linked Immunosorbent Assay
ESI : Electro spray ionization
EST : Expressed Sequence Tags
EU : European Union
F : Fahrenheit
FAO : Food and Agricultural commission
FASTA : fast A
FCR : Feed Conversion Rate
FDA : Food and Drug Administration
FE : Feed Efficiency
Fe : Iron
FSSAI : Food Safety and Standard Authority of India
Ft : Feet
g : Grams
G : Guanine
GC : Gas Chromatography
GF/B : Gabor Filter Bank
GI : Glycemic index
GIH : Gastrointestinal Hemorrhage
H : Hour
h⁻¹ : per hour
ha : Hectare
HCl : Hydrochloric acid
Hg : Mercury
HNO₃ : Nitric Acid
HPLC : High Performance Liquid Chromatography
HUFA : Highly Unsaturated Fatty Acid
ICP-OES : Inductively Coupled Plasma-Optical Emission Spectroscopy in Inches
INAA : Instrumental Neutron Activation Analysis
K : Pottassium
Kcal : Kilo Calorie
Kg : Kilograms
KJ : Kilo joules
L : Liter
lb : Libra (pound mass)
LCMS : Liquid Chromatography Mass Spectrophotometry
LMG : Leuco Malachite Green
LOQ : Level of Quantification
m : Meter
M : Molar
MCX : Multi Commodity Exchange
mg : Milligram
Mg : Magnesium
MG : Malachite Green
MgCl : Magnesium Chloride
MIC : Minimum Inhibitory Concentration
Min : Minute
MI : Milli Liter
mm : Millimeter
mM : Milli Molar
Mn : Manganese
MPEDA : Marine Products Export Development Authority
MPHL : Marine Prawn Hatchery Laboratory
MRL : Maximum Residual Level
MRM : Multiple Residue Monitoring
MRPL : Minimum Residual Permissible Level
MTs : Metallothioneins
N : Normality
Na : Sodium
Na$_2$HPO$_4$ : Disodium Hydrogen Phosphate
NaOH : Sodium Hydroxide
NBA : 2-Nitrobenzaldehyde
NCBI : National Center for Biotechnology Information
ND : Not detected
ng : Nano Gram
NH$_4$Ac : Ammonium Acetate
Ni : Nickel
NPAOZ) : 2-Nitrophenylmethylene-3-Amino-2-Oxazolidinone
NTP : National Toxicology Program
OTC : Oxy Tetracycline
oz : Ounce
P : Phosphorous
Pb : Lead
PCR : Polymerase Chain Reaction
pfu : Plaque forming unit, *Pyrococcus furiosus*
PH : Power of Hydrogen
PL : Post Larvae
pMoles : Pico Moles
ppb : Parts per billion
ppm : Parts per million
ppt : Parts per trillion
proPO : Prophenoloxidase
RFLP : Restriction Fragment Length Polymorphism
RNA : Ribonucleic Acid
rpm : Rotation Per Minute
rRNA : ribosomal Ribonucleic Acid
s : Seconds
SEM : Semicarbacide
SPE : Solid Phase Extraction
spp : Species
SSCP : Single-Stranded Conformation Polymorphism
T : Thiamine
TAE : Tris Acetate EDTA
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