General Introduction

The anatomy of the gastrointestinal tract of fishes follows the same basic plan as in other vertebrates with a degree of variation reflected in phylogeny and ontogeny, diet, and environment. Morphological studies provide us with a context for understanding the spatial organization and relationship of physiological and biochemical data, and the molecular machinery that is rapidly being elucidated through molecular techniques directed at the genome, transcriptome, and proteome. Morphological data are also key to understanding fish nutrition in ecology and aquaculture, and during development as well as mechanisms for physiological adaptations to a changing environment.

The properties of the gut and stomach of fishes are similar to other vertebrates, that is they have a stomach single and simple but variable in shape according to species and the whole length of the gut is simple, and if it have a reduplication or kink it loosens out again into a simple form. (Aristotle 345 BC1)

From this unassuming first description of the gross anatomy of the fish gut by Aristotle over 2000 years ago in his work Historium Animalia, the story of the investigation of fish gut morphology begins. However, it was not until the 19th century with the advent of comparative anatomy that significant effort was put toward determining the anatomical diversity and understanding of its significance in the fishes and vertebrates in general, starting with the works of Cuvier (1805), Rathke (1820, 1837), Edinger (1877), Oppel (1896), and Hopkins (1895).
With advances in technology over the past 200 years, from light to electron microscopy and staining techniques from histo-chemistry, to enzyme histo-chemistry, immune histo-chemistry and in situ hybridization of RNA, the level of detail has progressed from organ, to tissue, to cell and finally to the molecular level. The “back-end” of the gut where digestion and absorption take place as opposed to the “front-end” which is associated with the headgut and food capture and initial mechanical processing (Horn, 1997; Clements and Raubenheimer, 2005; Schwenk and Rubega, 2005). Also since the literature is extensive, spanning over 200 years, a number of reviews of the gastrointestinal tract of fishes are referred to (e.g. Jacobshagen, 1937; Pernkopf and Lehner, 1937; Barrington, 1957; Bertin, 1958; Adam, 1963; Kapoor et al., 1975; Harder, 1975; Fange and Grove, 1979; Youson, 1981).

The gastrointestinal tract of fishes can be subdivided into four topographical regions, the headgut, foregut, midgut and hindgut (Harder, 1975). Further morphofunctional subdivisions can be superimposed on this basic plan. The headgut is composed of the mouth and pharynx, and its function is to acquire food and mechanically process it (Horn, 1997; Clements and Raubenheimer, 2005). The foregut follows and is comprised of the oesophagus and stomach, where chemical digestion of food begins. In some fishes, the mechanical breakdown of food may also occur partially or fully in the stomach. The midgut or intestine accounts for the greatest proportion of the gut length and is where chemical digestion is continued and absorption mainly occurs. The hindgut is the final section of the gut, which includes the rectum; although in some cases there is no clear
morphological distinction between midgut and hindgut. The foregut epithelium is of ectodermal and the midgut of endodermal origin. Radially the gut wall from foregut to hindgut consists of four concentric layers. The mucosa consisting of the mucosal epithelium and tunica propria, vascularized connective tissue containing nerves and leukocytes. The sub-mucosa is an additional connective tissue layer. The muscularis consists of circular and longitudinal layers of either striated or smooth muscle. However, the lampreys only have a layer of oblique muscle. The serosa is only present within the coelomic cavity and corresponds to mesothelial cells and loose connective tissue containing blood vessels.

The oesophagus of fishes is generally a short and straight thick-walled tube, connecting the pharynx to the stomach, or intestine in agastric fishes. Morphologically the oesophagus is designed primarily for the passage of food. The mucosa is arranged in longitudinal folds or papillae that allow distension of the lumen for the passage of large food items during swallowing.

Unlike the rest of the gut, the muscularis of the oesophagus is composed of skeletal muscle instead of smooth muscle. The circular muscle layer dominates and is found exterior to the longitudinal muscle layer when present; a relationship that is reversed in the rest of the digestive tract (Harder, 1975).

The junction of the oesophagus and stomach is generally not clearly demarcated anatomically with the exception of a few elasmobranchs, which have an esogastric valve (Harder, 1975; Kapoor et al., 1975; Fange and Grove, 1979). However,
histologically and topographically, the beginning of the stomach is obvious with the abrupt change in the epithelium to the columnar mucous cells of the stomach and the appearance of gastric glands. Since this transition can be gradual the term esogaster is sometimes applied to the transition zone between these two organs (Kapoor et al., 1975). The striated muscle fibers of the oesophagus are gradually replaced by smooth muscle in the cardiac stomach and the circular muscle layer will now be internal to the new longitudinal muscle layer (Pernkopf and Lehner, 1937; Harder, 1975).

In terms of gross morphology the stomach of fishes can be classified according to shape as either straight (I), siphonal (U or J) or cecal (Y) (Pernkopf and Lehner, 1937; Suyehiro, 1942; Harder, 1975). The straight stomach is relatively rare (Esox) and in some cases is actually more indicative of the absence of the stomach (e.g. Tetraodontiformes; Suyehiro, 1942), while the siphonal shape is the most common among the osteichthyes and elasmobranchii. The cecal stomach allows the storage of larger quantities of food. The overall size of the stomach can also be quite variable from very large in gluttonous fishes such as *Gadus macrocephalus* and the Lophiidae to very small as in the Salangidae and Oplegnathidae (Pernkopf and Lehner, 1937; Suyehiro, 1942).

The intestine follows the pylorus or oesophagus in gastric and agastric fishes, respectively. In some stomachless fishes, the anterior intestine may bulge to form an intestinal bulb or pseudogaster (Petromyzon, Cyprinidae) and functions in temporary food storage; however, gastric glands and a pylorus are lacking. Some parrotfish have a spherically shaped
caecal chamber extending from the anterior intestine just after the oesophagus to serve a similar function (Al-Hussaini, 1946b).

The primary function of the intestine is the completion of the digestive processes started in the stomach and the absorption of nutrients. Central to this is optimizing intestinal surface area within the constraints of the coelomic cavity, which has a marked impact on intestinal morphology. Intestinal surface area is increased in all fish by folding (primary, secondary and tertiary) of the mucosa and by apical plasma membrane amplification through brush border microvilli.

In the lampreys, chondrosteans, chondrichtheans and dipnoids which all have short intestines, the mucosa forms a spiral valve whereas in the teleost fishes, surface area is increased by lengthening the intestine through convolution (loops). A wide range of looping and coiled arrangements can be seen within the teleosts with long intestines (e.g. Cyprinidae, Loricadae). However, some fish just have short, straight intestines with no looping or spiral valve (e.g. Cobitidae; Salmonidae, Agnathans). The intestine in some Scarides is constricted at short intervals giving a sacculated structure forming irregular internal pouches (Al-Hussaini, 1946b). Blindended appendages (pyloric caeca) are found in ostichthys (teleosts and chondrosteans) and elasmobranchs, although rarely in the latter.

Intestine length is used as a morphological indicator of trophic level in nutritional ecology (Horn, 1997). However, intestinal length is influenced by a number of other factors apart from diet, which include fish size (mass and length) and
body shape, recent feeding history (starved versus fed), ontogeny, and phylogeny (Horn, 1989, 1997; Clements and Raubenheimer, 2005). The length and relative mass of the intestine is generally greater in herbivores relative to carnivores, which is thought to allow for additional processing of relatively difficult to digest items (Horn, 1997; Clements and Raubenheimer, 2005); although within omnivorous fishes there is no clear relationship with degree of herbivory or carnivory (Kramer and Bryant, 1995). Kramer and Bryant (1995) caution that gut length as a reflection of diet should be applied only to identifying broad catagories Al-Hussaini (1949) estimated the entire surface area of the intestine (however, excluding the contribution of microvilli) in three species having different feeding habits: *Rutilus rutilus* (omnivore), *Gobio gobio* (carnivore) and *Cyprinus carpio* (herbivore). In a comparison of size-matched individuals he found no differences in total surface area even though intestinal lengths between species varied greatly indicating compensation through increased folding. Buddington and Diamond (1987) found similar results including fish with pyloric ceca.

The spiral valve not only increases the surface area of the intestine without increasing intestinal length, but also is associated with a slow food passage rate (Parker, 1880; Jacobshagen, 1937; Wetherbee and Gruber, 1993). The intestine is twisted along its longitudinal axis giving the spiral or screw-like appearance with the number of turns and height of the fold being variable between species (Parker, 1880).

The pyloric caeca are blind-ended sphincterless ducts associated with the anterior intestine in osteichthys and infrequently in chondrichthys (Jacobshagen 1937; Buddington
and Diamond, 1987). In species with high numbers of caecae, greater individual variation is seen and the numbers of openings to the intestine are limited and thus the caeca appear as tufts (Suyehiro, 1942; Harder, 1975).

The start of the hindgut is usually indicated by the presence of an “ileorectal” valve or a thickening of the circular muscle layer, a sudden change in gut diameter, and the mucosal folding pattern (Harder, 1975; Kapoor et al., 1975). The hindgut is generally short and subdivisions are usually not noted in fishes (Harder, 1975) although caecal chambers are present in some fishes for example kyphosids, (Rimmer and Wiebe, 1987). The rectum ends in a muscular sphincter that empties into either the cloaca or vent.

The main function of the alimentary tract of any animal is the acquisition of food with subsequent assimilation of vital nutrients. The natural diet of fishes varies tremendously between fish species and their natural habitats. Likewise, the structure of the digestive tract varies in the vastly diverse order Pisces. Despite this, the functional characteristics of nutrient assimilation show general consistency and include: capturing of food; puncturing, crushing, grinding and mixing; secretion of digestive enzymes and other components; and digestion and absorption of nutrients. Other functions of the gastrointestinal tract are intrinsically associated with nutrient assimilation from food, including osmoregulation, secretion of hormones not only involved in regulation of digestion, but also metabolism and other bodily functions and protection of the organisms from alien compounds and pathogens that may reach the alimentary tract with the food and water. Thus, the
digestive tract represents a critical interface between the internal and external environments of the fish.

Knowledge of diet and feeding habits is essential for the understanding of various aspects of fish biology and for developing aquaculture feeds and feeding methods. During the last several decades more than a hundred publications have documented the stomach contents from different fish species. The range of nutrient sources of fish is diverse, ranging from detritus, phytoplankton, zooplankton, micro and macroalgae, aquatic plants and meiofauna to insects, crustaceans, mollusks, shellfish, fish, birds and mammals. From these studies fish have been categorized as herbivores, omnivores, or carnivores or piscivores. Some studies add insectivorous and detrivorous groups. In general, omnivores with a preference for carnivory are the most numerous group. Many fish species are opportunistic feeders, feeding on whatever food they can find in their habitat. The geographical location, ecology and climate will account for the available biotope, the prey and also the possible predators (Starck, 1999; Clements et al., 2009). For many fish species, the choice of nutrient sources varies during their lifecycles from hatch to mature stages, from season to season, and also throughout the day. As larvae, most fish species are considered carnivores, dependent on zooplankton for normal development. Tilapia (Oreochromis mossambicus) may switch seasonally between zoophagy, phytophagy and detrivory (Maitipe and De Silva, 1985). Moreover, fish may feed intensively in some periods and fast in others. For example, anadromous salmonids feed while at sea and cease feeding as they reach rivers for spawning. Rapid adaptation to variations in diet quality and availability is possible because the
gastrointestinal tract is functionally dynamic and the epithelial cells are quickly renewed. Despite differences in natural diets, fish, as other animals of both higher and lower phyla, show the ability to select food and utilize its components to fulfill their energy and nutrient requirements (Dabrowski, 1993; Jobling, 1995; Choat and Clements, 1998; de la Higuera, 2001; Houlihan et al., 2001; Raubenheimer et al., 2009). For piscivorous fishes, nutrient requirements, especially regarding protein and lipids, are relatively easily met, with well balanced amino and fatty acid compositions in their diets. Herbivorous fish species, on the other hand, may have more difficulty in meeting their amino acid, protein requirements when feeding on plants with low protein content and an unbalanced amino acid profile. However, herbivorous fish species may be supplied with nutrients from symbiotic microbiota, either directly as sources of nutrients themselves or indirectly by microbial fermentation of food components otherwise non-degradable by the fishes endogenous enzymes; for example supplying absorbable carbohydrates and short chain fatty acids (Jobling, 1995; Kihara and Sakata, 1997; Clements et al., 2009).

The importance of studies on the food of fishes in fishery biological investigations is well known, and considerable attention is being paid at the moment to this problem in India. Several publications on the subject have appeared recently. To obtain a correct picture of the nutrition and feeding adaptations of a fish, a comprehensive study of all available age groups, covering all the seasons and environments of its occurrence, is essential. The seasonal variations in the nature of the food of fishes can be interpreted properly only if we have
knowledge of the availability of the constituents of their dietary in the habitats. The value of such an ecological investigation to elucidate the food chains that exist in our waters and to explain the behaviour of animals cannot be overstressed (Elton, 1927). Among the few detailed investigations on the food of fishes conducted in India, the more important ones are those of Hornell and Naidu (1923), Job (1940, 1941a and 1941b), John and Menon (1942), Mookerjee et al., (1946b) and Bapat and Bal (1950). For a correct understanding of the feeding habits of a fish, a study of the anatomy of the organs of feeding and digestion is necessary. ‘An examination of the special relations of food and feeding structures gives clues, not only to the present significance of fishes but also their past effect on life at large, showing how they must have modified the course of evolution’ (Forbes, 1888). The feeding apparatus exhibits one of the most significant examples of correlation, and the investigation of the food of a fish will be incomplete without a study of its alimentation. Al-Hussaini (1945) has given an excellent review of the previous work relating to the correlation of the structure of alimentary tracts with the feeding habits of fishes. The contributions of Al-Hussaini (1945, 1946 and 1949) and that of Angelescu and Gneri (1949) have served to elucidate some of the most interesting morphological adaptations among fishes. Practically very few works on these lines has been done in any detail in India. Rahimullah (1939 and 1943), Dharmarajan (1936), Vanajakshi (1938), Sarbahi (1940) and Mohsin (1944-46) have all dealt with the structure of the alimentary tract of fishes or of their appendages; but have not made simultaneously a detailed study of the food of these fishes or correlated the nature of the
food and the feeding habit with the morphology of the alimentary tract. The present work deals with a study of the food, feeding and alimentation of *Mastacembelus armatus*, *Wallago attu* and *Clarias batrachus* on the lines indicated above.

Food is a major axis along which co-occurring fish species are segregated (Ross, 1986). Differences in trophic adaptations among species have the effect of physical feeding segregation while differences in diet by coexisting species reflect underlying morphological diversities. Therefore, variation in morphology leads to variation in feeding success on food resources, thereby influencing diet (Wainwright and Richard, 1995). Morphological features, specifically those related to the capture and intake of prey, evolved to maximize feeding performance, and have been strongly correlated with diet (Gatz, 1979; Wikramanayake, 1990; Piet, 1998; Hugueny and Pouilly, 1999). Individual species are adapted not only to feed on a specific component of the broader resource base, but also on food particles of a particular size. Matthews *et al.*, (1982) showed a strong direct relationship between mouth width and prey size. Prey size was always less than the theoretical maximum prey size allowed by mouth width. In accordance with optimal foraging theory, benefits from prey increase directly with size, provided there is little or no corresponding increase in the energetic cost associated with prey handling (Werner and Hall, 1974). With fluctuations of predator and prey populations in natural environments, fishes, as predators may favour the most frequently encountered prey, rather than actively seeking the most energetically profitable (Bence and Murdoch, 1986).
The three species in this study were chosen for their
great variation in morphology. All three species are found fairly
commonly in rivers throughout Maharashtra.

The present study is an attempt to examined the feeding
ecology of these coexisting species Mastacembelus armatus,
Wallago attu and Clarias batrachus, based on their
comparative morphological features and stomach contents.
The working hypothesis employed was that differences in
morphology among the three species influence use of the
common food resource base through fundamental
composition.