3

Digestive Physiology of Fish and Shrimp

Digestion is the process of solubilizing and degrading nutrients into smaller components and elements that can be transported across the intestinal wall to support physiological processes. This chapter reviews comparative aspects of digestive function in fish and shrimp relevant to aquaculture. An understanding of the digestive processes and their limitations is necessary for the formulation of diets that can fulfill nutrient requirements. First, some considerations about feeding habits of species are presented. Then, knowledge about the structure of digestive organs, secretions of different parts of the digestive tract, enzyme activities, hydrolytic processes, and nutrient transport are addressed. The chapter focuses on the adult stages of fish and shrimp and their ability to digest the macronutrients proteins, lipids, and carbohydrates. Earlier stages of development are partly covered in other chapters. Sources of information for the present chapter include recent original papers as well as books, book chapters, and review papers such as Ceccaldi (1997), Carrillo-Farnes et al. (2007), Cyrino et al. (2008), Kuz'mina (2008), and Holmgren and Olsson (2009).

FISH

Knowledge about what an organism eats aids in understanding the diversity of the anatomical and physiological characteristics of the organism. Some fish species feed on dead items (scavengers), others on living material, some feed solely on microorganisms, others on larger plants and animals, and some are opportunistic eating whatever they can find. Food for fish in the wild comprises detritus, phytoplankton, zooplankton, micro- and macroalgae, aquatic plants, meiofauna, insects, crustaceans, mollusks, shellfish, fish, seeds and fruits, and even birds and mammals (Platell and Potter, 2001; Lundstedt et al., 2004; De Almeida et al., 2006). One way to classify fish is according to the primary ingredients of their natural diet; herbivores (milkfish and some carps), omnivores (channel catfish and some tilapia), and carnivores (salmonids, basses, seabreams, flounders, and groupers). Species that have a similar dietary selection may show great variation in intestinal anatomy, and within the same species there are differences among developmental stages.

Structural and Functional Aspects of Digestive Organs

Variation in anatomy and histomorphology of the digestive tract among fish species is greater than for any other phylum (Buddington and Kuz'mina, 2000a,b). The tract can be subdivided into the foregut with mouth, pharynx, esophagus, and stomach; the midgut with pyloric ceca; and the distal or hindgut terminating in the rectum. Figure 3-1 illustrates general characteristics of the digestive tract of fish grouped in four categories according to the anatomy of the tract. Figures 3-2 and 3-3 illustrate the organization of internal organs in a generalized fish with stomach and pyloric ceca and in Atlantic cod, respectively. The anatomy, particularly of the foregut, has presumably developed through evolution and been influenced by the nature of the food of the species to allow efficient intake and digestion. Bottom feeders have downward orientation of the mouth, whereas species eating food in the water column have the mouth oriented at the tip of the body (Jobling, 1995). There seems to be a relationship between mouth size of the fish and size of the food. However, this is not always the case (Platell and Potter, 2001). The second largest fish in the world, the basking shark (*Cetorhinus maximus*), is a filter feeder feeding on planktonic prey.

Most fish species start out at hatching with a straight simple digestive tract without a stomach. Through the larval and juvenile stages, the gastrointestinal (GI) tract develops into more complicated structures. Some fish continue to have a short and relatively simple tract, whereas others have long, more complex tracts. Some fish species, mostly herbivores, have no stomachs even as adults. Most fish without a true stomach belong to the microphagous, detrivorous,

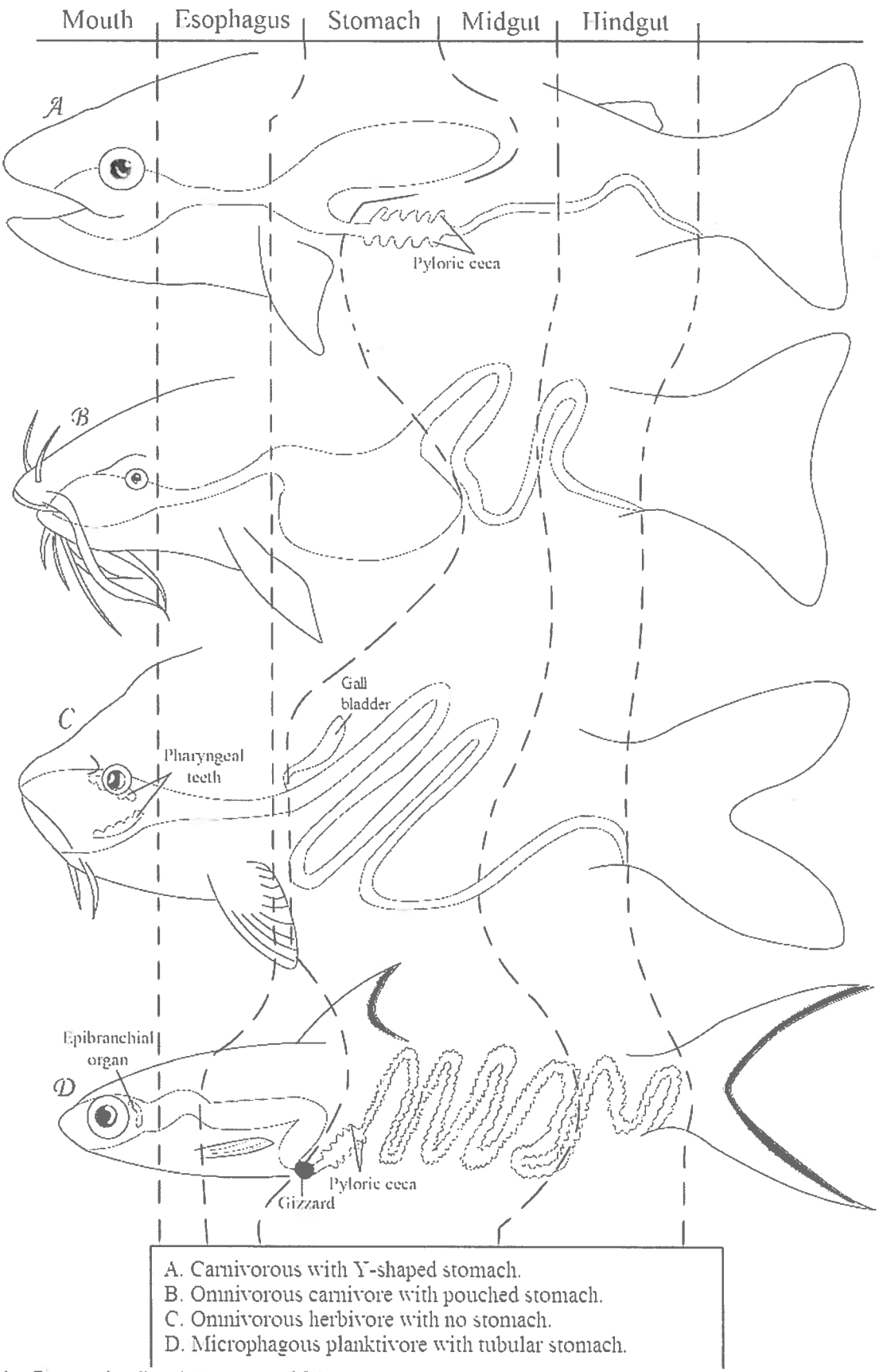

FIGURE 3-1 Comparative digestive anatomy of fish.
Illustration courtesy of Victoria Blondin, University of Guelph, Ontario.

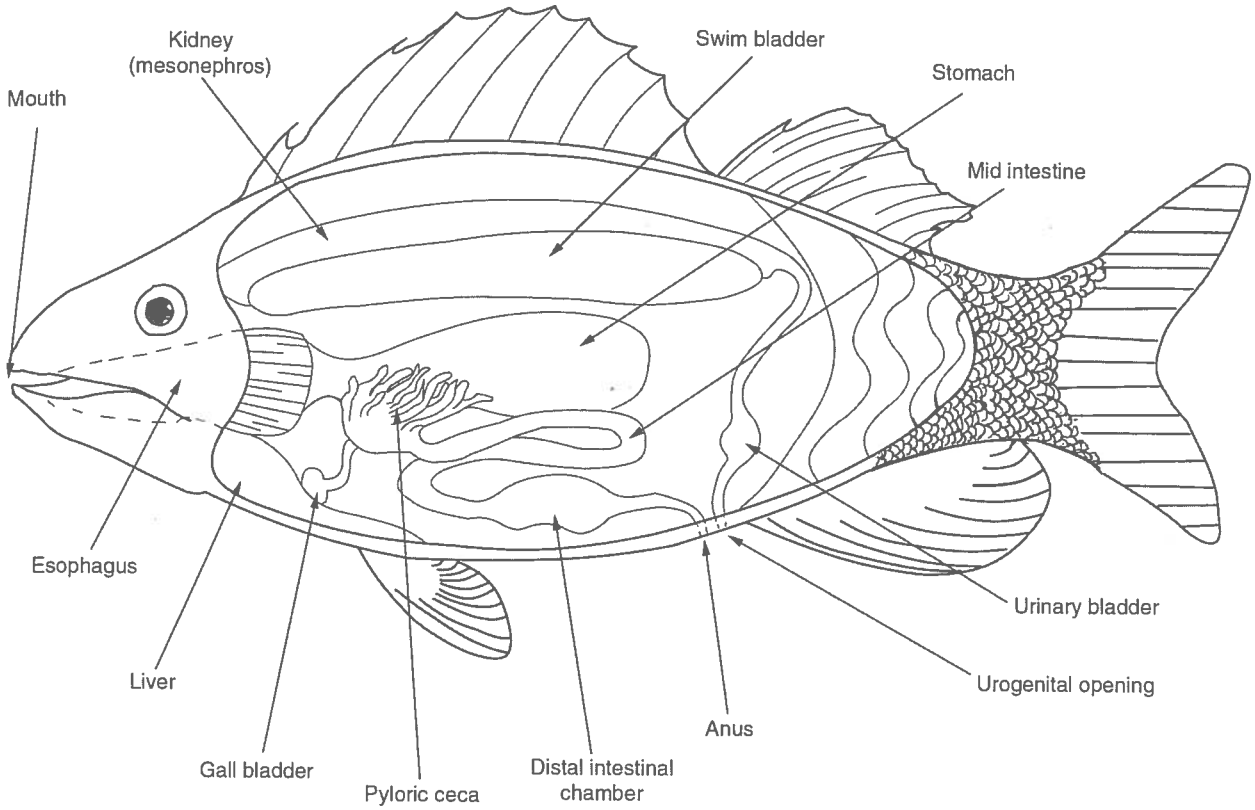

FIGURE 3-2 Organization of internal organs in a generalized fish.
Illustration courtesy of Victoria Blondin, University of Guelph, Ontario.

and herbivorous species. There are, however, examples of carnivorous fish without a stomach such as the cyprinid Colorado squawfish (*Ptychocheilus oregoensis*) (Jobling, 1995).

Fish vary greatly in the manner they catch food. Some, such as the great white shark (*Carcharodon carcharias*), use their teeth to hold and tear their prey. Salmonids suck in their prey with water into the foregut, whereas pacu, such as the cultivated *Piaractus mesopotamicus*, chew and grind plants with teeth that resemble human teeth. Many species, such as the silver carp (*Hypophthalmichthys molitrix*), blue tilapia (*Oreochromic aurea*), and Nile tilapia (*Oreochromis niloticus*), are filter feeders, collecting plankton by filtering large volumes of water and collecting food with gill rakers (Sims, 2008).

Fish stomachs show variation in anatomy, ranging from straight, to U- and T-shaped (Suyehiro, 1941). Some fish without a stomach have a gizzard-like structure in the foregut that aids in grinding of the food. Likewise, the intestines vary from short and straight to long and complex. The long intestines can have different three-dimensional organizations such as spirals or balls with various twists and turns. In some species of fish with short intestines, such as cartilaginous species, the surface is increased by luminal spiral valve formations. Some species are equipped with pyloric appendages that can number from one to several hundred. The distal intestinal structures usually differ from the more proximal compartments and can be very complex (Suyehiro, 1941). The functional morphology and biochemistry of the distal structures indicate that, not only water and minerals, but also protein, lipids, and carbohydrates are hydrolyzed and absorbed in this region—in contrast to the situation in mammals. Absorption of macromolecules also takes place in this region, which seems to be of great importance in antigen presentation and immune function (McLean and Ash, 1986, 1987a,b; Sire and Vernier, 1992; Amthauer et al., 2000a,b). In some species (e.g., the Atlantic cod, *Gadus morhua*) the distal intestinal regions have a section with a holding capacity allowing fermentation of dietary fiber components. However, in most fish species passage rate through the intestine is rapid, limiting the quantitative importance of fermentation for the nutrient supply of the fish (Kuz'mina, 2008).

The anatomy and histomorphology of the accessory organs of the digestive tract, the exocrine pancreas, and the liver also show high variability among fish species. Greatest variation is seen for pancreatic tissue. The pancreas is a distinct organ in some species, such as the sturgeon (*Acipenser*

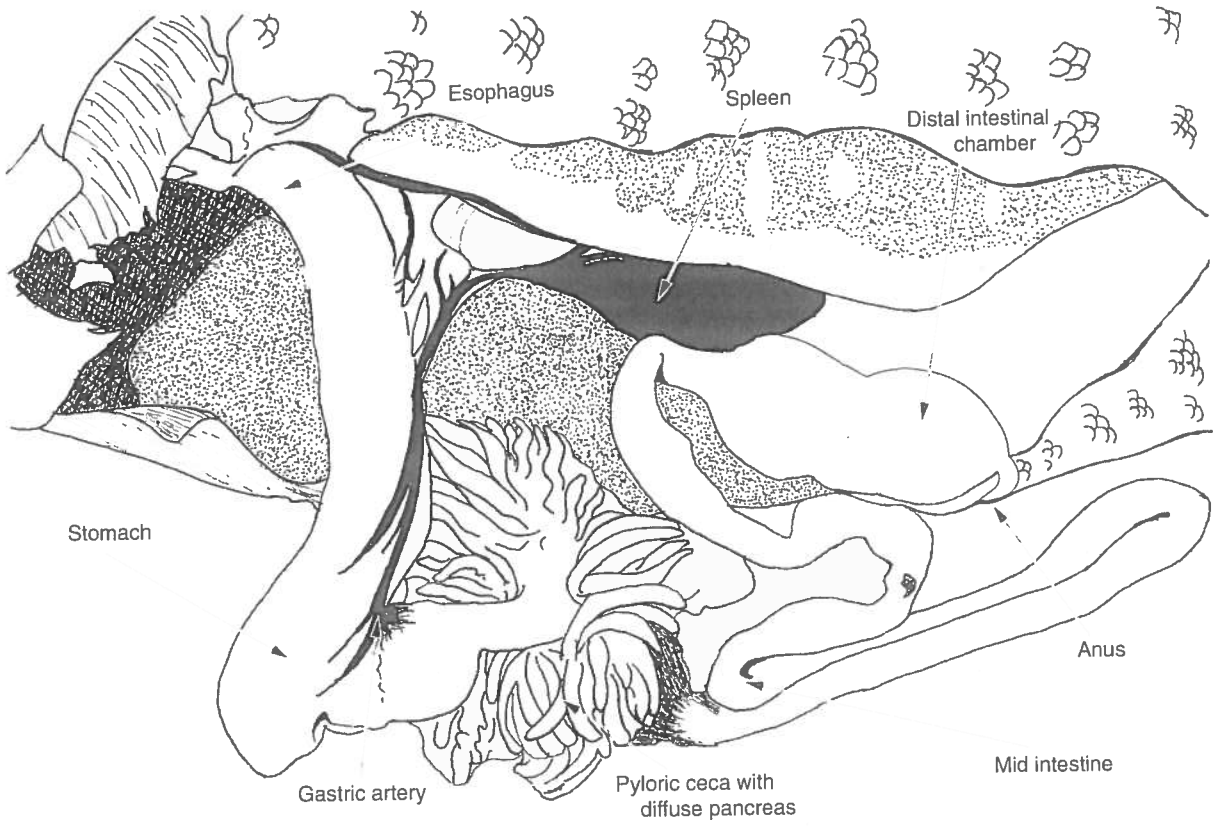

FIGURE 3-3 Drawing of stomach and pyloric ceca in Atlantic cod (*Gadus morhua*). Illustration courtesy of Victoria Blondin, University of Guelph, Ontario.

spp.), but in most species it is diffusely dispersed in mesenteric tissue along the intestines and blood vessels, as in salmonids and/or in the liver (hepatopancreas), as in breams. The structure and function of the diffuse fish pancreas is difficult to study and not well known for any species. It seems clear, however, that the acinar cells of the pancreas, found in clusters, produce and store digestive enzymes that, upon signaling from the intestine, are secreted into tubules that converge into the pancreatic duct (Kurokawa and Suzuki, 1996; Morrison et al., 2004). Water and bicarbonate are added from cells along the tubules. In some fish the tubules converge into main pancreatic ducts leading to the intestine, bile duct, or both, whereas in others the large number of fine tubules opens directly into the pyloric ceca or intestine (Einarsson and Davies, 1997).

Livers in most fish species are organized as a single organ, some with two or more lobes. In contrast to other vertebrates, fish livers are not organized into well-defined lobules of acinar units (Rust, 2002). They have a complex network of blood vessels, tubules, sinusoids, and ducts. Bile ducts drain bile into the gall bladder, and, in fish with a hepatopancreas, pancreatic ducts drain pancreatic juice to the intestinal tract. Hepatocytes comprise the main volume of the liver, storing glycogen and lipid. Great variations exist among fish species regarding liver lipid levels. Some fish, such as the cod, store lipid almost solely in the liver and accumulate large amounts depending on feed composition and intake (Rosenlund et al., 2004). In the liver of Atlantic cod (*Gadus morhua*) lipid may exceed 70% (Karlsen et al., 2006), whereas Atlantic salmon generally show levels below 10% (Pratoomyot et al., 2010). Also the European sea bass (*Dichentrarcus labrax*) liver has the capacity for high lipid accumulation, whereas the sea bream accumulates lipid at levels comparable to the salmon (Peres et al., 1999). In contrast to mammals, but similar to birds, fish seem to transport lipids from the intestine to the body via the portal vein and the liver. Lacteals, which are ducts that transport lipids and larger molecules and complexes, have not been observed in fish. Fish fed high-carbohydrate diets often accumulate large glycogen depots in the liver. The function of such livers may be compromised (Hilton and Dixon, 1982). The mechanism behind this high glycogen accumulation is not well understood (Enes et al., 2009).

Secretions

Mucus

All along the digestive tract, from the mouth to the anus, mucus is secreted from specialized cells. Water, ions, and mucins (i.e., highly glycated proteins with large water binding capacity) are the main components of the mucus, which also contains bicarbonate and may contain antibodies. The density of mucus-producing cells in the mucosa varies among intestinal compartments and is typically lower in the mouth region and high in the intestinal regions (Kuperman and Kuzmina, 1994; Sklan et al., 2004; Abate et al., 2006; Diaz et al., 2008a; Dezfuli et al., 2009; Manjakasy et al., 2009). The components of mucus protect the surface of the tract from mechanical damage by rough dietary components and from chemical damage by endogenous acid, alkali, and digestive enzymes. The mucus is also important for the protection of the body against microbes and chemicals that may challenge the health and well-being of the animal (Shephard, 1994). The number of mucus-producing cells and mucus flow may be affected by feeding habits and feed composition. An increased number has been observed with increasing dietary inclusion of plant ingredients (Olsen et al., 2007).

Gastric Juice

The principal digestive components secreted in the stomach are pepsinogen and hydrochloric acid (HCl), both secreted from cells embedded in the stomach wall with the gastric juice. In most fish, pepsinogen and acid are secreted from the same cell type, the oxynticopeptic cells. In some species, however, such as in some elasmobranchs, cells of the gastric mucosa that seem specialized to secrete either pepsinogen or HCl are found among oxynticopeptic cells (Holmgren and Olsson, 2009). In winter flounder (*Pleuronectes americanus*), mucosal mucus-producing cells expressing a proton pump have been observed, indicating that these cells also secrete HCl (Gawlicka et al., 2001). Feed intake stimulates the secretion of both pepsinogen and acid. However, at least partly independent regulation seems to occur as some stimuli with strong effects on pepsinogen secretion weakly affect acid secretion and vice versa (Holmgren and Olsson, 2009). Depending on the species, feeding rate, diet composition, and time after a meal, stomach pH varies between 1 and 6 (Pérez-Jiménez et al., 2009). The pH in stomach chyme is the result of acid secreted from the stomach wall and buffering capacity of the feed components and elements in the drinking water. Proteins, carbohydrates, and minerals are among the buffering components (Thompson and Weber, 1979; Lawlor et al., 2005). A study of effects on chyme pH after ingestion of a single meal in rainbow trout showed a pH just below 3 in chyme from the stomach before the meal, rising to a pH between 5 and 6 immediately after feed intake (Bucking and Wood, 2006). The pH was stable until about 6 hours after the meal and then gradually decreased to pH 4 at 24 hours. Similar changes in pH have been observed with gilthead sea bream (*Sparus aurata*) (Deguara et al., 2003). Stomach pH in fish seems to be quite regulated and most likely, as in other species, the regulation involves stimulants such as gastrin, acetylcholine, ghrelin, and orexin and inhibitors such as somatostatin, nitric oxide, and dopamine (Schubert, 2009). Whether histamine, a potent stimulator of acid secretion in humans and other nonruminant land animals, is also present in fish is not clear. Histamine has not been observed in fish. The integration of mechanisms that regulate pH is not well understood, either in fish or in other animal species (Schubert, 2009).

The proenzyme pepsinogen is activated to pepsin in the stomach catalyzed by the HCl. Fish seem to secrete more than one form of pepsinogen, and the different forms show different activation rates, pH optima, specific activities, and specificities regarding which peptide bonds they hydrolyze most efficiently (Wu et al., 2009). Most fish pepsins show more than one pH optimum between pH 1 and 5, and some show appreciable activity even at higher pH. Pepsins are endopeptidases (i.e., an enzyme that hydrolyzes peptide bonds at some distance from the terminal amino acids) with specificity for peptide bonds adjacent to aromatic amino acids (i.e., phenylalanine and tyrosine). Secretion of pepsinogen from the gastric mucosa seems to be regulated according to dietary protein level in Atlantic cod (Krogdahl et al., 2009) and accumulates in the mucosa during fasting (Einarsson and Davies, 1996).

There are yet no clear indications that gastric juice of fish contains lipase and amylase. Reports of high specific activity of lipase and amylase in homogenates of stomach tissue from the Brazilian catfish "pintado" (*Pseudoplanty stoma corruscans*) and tambaqui (*Colossoma macropomum*) are suggestive that the stomachs of these fish are a source of the enzymes (Lundstedt et al., 2004; De Almeida et al., 2006). However, the enzyme activities were observed in extracts of tissue samples from fasted fish and not tested in contents of the stomach. A possible explanation for the high activity is the presence of pancreatic tissue in or on the stomach wall.

Whether fish have an endogenous mechanism for the digestion of the exoskeleton of crustaceans is unclear. Chitinolytic activity has been measured in the stomach of many fish species (Divakaran et al., 1999; Gutowska et al., 2004; Ikeda et al., 2009; Fange et al., 2010). The enzyme seems to be associated with fish that consume chitinous prey but do not have mechanical structures breaking down the crustacean exoskeleton. Genomic studies of a species in the pufferfish family, *Takifugu rubripes* (Altschul et al., 1997), show the presence of gene sequences with high similarity to sequences coding for chitinase. However, whether these genes code for proteins to be secreted into the digestive tract is unclear. Chitinase activity in the digestive tract may originate from the prey and/or microbiota. Several species of microbes common to fish intestine have the potential of producing chitinases (Sugita and Ito, 2006).

Bile

The important digestive components of bile are bile acids, phospholipids, and bicarbonate. Bile also contains cholesterol, fatty acids, bile pigments from catabolism of heme, and other inorganic salts. Bile acids, produced from cholesterol or taken up from the blood by the hepatocytes, and phospholipids are excreted continuously from the cells into the bile canaliculi and transported to the gallbladder. From the gallbladder the bile is emptied into the proximal midgut upon stimuli from the intestine triggered by the entering chyme. Cholecystokinin seems to be an important mediator of the contraction of the gallbladder in fish (Aldman et al., 1992; Aldman and Holmgren, 1995; Einarsson et al., 1997). However, knowledge on regulation of bile output in fish is very limited. Between meals, bile accumulates and is concentrated in the gallbladder, which becomes distended and darkly colored. In the intestine the bile acids stabilize lipid droplets and form micelles for the dispersion of lipid components produced in the chyme by lipolytic activities. The bile acids are also essential cofactors for the action of the main lipases acting in the intestine and possibly for the stability of other digestive enzymes and mucosal integrity (Ogata et al., 2003). Taurocholic, taurolithocholic, and taurochenodeoxy cholic acids seem to be the major forms of the bile acids in fish, but glycocholic acid has also been reported (Haslewood, 1967; Une et al., 1991; Bogevik et al., 2009; Velez et al., 2009). Accordingly, taurine, either supplied with the feed or produced from cysteine, is essential for efficient nutrient digestion. Bile can also be classified as an excretory secretion because it serves as the major excretory route for many components that have no known physiological function in the biliary or intestinal tract, including cholesterol; bilirubin; conjugates or hormonal steroids; lipophilic xenobiotics in conjugated or unconjugated form; polyvalent cations, such as iron and copper; and cobalamins. In humans, bile also carries immunoglobulins. Whether this is the case for fish is not known (Hofmann, 1994). An investigation with Atlantic salmon (*Salmo salar*) fed diets with wax esters replacing fish oil showed that wax esters increased bile volume and concentrations of bile acid and phospholipids (Bogevik et al., 2009). However, there seemed to be a limit to the compensatory mechanism. Limitations in compensatory responses were also observed in rainbow trout (*Oncorhynchus mykiss*) in a study of effects of dietary soybean meal inclusion on bile salt concentration in intestinal segments. The study showed a rapid decrease in the bile salt concentration up to 40 days of feeding (Romarheim et al., 2008).

Pancreatic Juice

Pancreatic secretions carry water and bicarbonate, adding to the solubilizing and buffering capacity of the intestine. The digestive enzymes are considered the most important components in the pancreatic juice. The diffuse nature of the exocrine pancreas in most fish makes investigation particularly difficult, and present knowledge needs to be strengthened to understand its function, capacity, and limitation in different feeding situations. The fish pancreas seems to produce most of the same digestive enzymes as the pancreas in mammals and birds, some in proenzyme forms. They comprise the proenzymes of the endopeptidases trypsin, chymotrypsin, and elastase I and II; the exopeptidases carboxypeptidase A and B; the active forms of lipase; phospholipase; α-amylase; and DNase and RNase (Kurtovic et al., 2009). Very little information is available on fish colipase. It seems that digestive lipases in most fish species are independent of a colipase, although colipase is present in some species (Kurtovic et al., 2009). The exocrine pancreatic cells store the digestive enzymes in granules and excrete them with the pancreatic juice upon signals from the intestine (Einarsson et al., 1996; Einarsson et al., 1997).

Digestive enzymes secreted from the pancreas are produced in several isoforms that show variation in molecular weights, molecular structure, pH optima, efficiency, and stability, both within and between species (Krogdahl et al., 2005; Asgeirsson and Cekan, 2006; Ogiwara and Takahashi, 2007). Table 3-1 summarizes some of the general biochemical aspects. Molecular characteristics of digestive enzymes from fish are under investigation, and new nucleotide and amino acid sequences are being published with increasing frequency (Froystad et al., 2006; Psochiou et al., 2007; Manchado et al., 2008; Kurtovic et al., 2009). Pancreatic enzymes mainly act freely mixed in the chyme. However, these enzymes also seem to be associated with the brush border membrane of the enterocytes, exerting their action in close vicinity to the nutrient transporters of these cells (see review by Kuz'mina, 2008).

Species differences exist regarding enzyme output and activity, particular for α-amylase (Krogdahl et al., 2005). In general, herbivorous species seem to produce higher levels of amylase than do omnivores. The lowest activities are observed in carnivores such as eel (*Anguilla anguilla*), which have been shown to have amylase activities less than 1/100th of the activity observed in carp (*Cyprinus carpio*) (Hidalgo et al., 1999). In a comparison of capacity for starch hydrolysis in intestinal contents among Atlantic salmon, Atlantic cod, and rainbow trout, Atlantic salmon ranked the lowest (Froystad et al., 2006). The cod had an intermediate level. The low activity of the salmon amylase was suggested to be due to a defect in a substrate-anchoring structure of the molecule. Species differences have also been described for the proteases in terms of their molecular structure, pH optima, and thermal stability (Glass et al., 1989).

In general, fish seem to be able to adjust their secretion of the pancreatic digestive enzymes according to dietary level and quality of the corresponding nutrient (Buddington et al., 1997). Increasing lipase activity with increasing lipid level has been shown for both rainbow trout and yellowtail (*Seriola quinqueradiata*) (Morais et al., 2004; Ducasse-

TABLE 3-1 Digestive Enzymes of the Digestive Tract[a,b]

Source	Enzyme	Substrate	Specificity or Products
Stomach	Pepsins (pepsinogens)	Proteins and polypeptides	Peptide bonds adjacent to aromatic amino acids
Exocrine pancreas	Trypsins (trypsinogens)	Proteins and polypeptides	Peptide bonds adjacent to arginine or lysine
	Chymotrypsins (chymotrypsinogens)	Proteins and polypeptides	Peptide bonds adjacent to aromatic amino acids
	Elastase I (proelastase I)	Elastin, some other proteins	Peptide bonds adjacent to aliphatic and neutral amino acids
	Elastase II (proelastase II)	Elastin, some other proteins	Peptide bonds adjacent to aliphatic and neutral amino acids
	Carboxypeptidase A (procarboxypeptidase A)	Proteins and polypeptides	Carboxy terminal amino acids that have aromatic or branched aliphatic side chains
	Carboxypeptidase B (procarboxypeptidase B)	Proteins and polypeptides	Carboxy terminal amino acids that have basic side chains
	Colipase (procolipase)	Fat droplets	Binds to bile salt-triglyceride-water interface, making anchor for lipase
	Pancreatic lipase	Triglycerides	Monoglycerides and fatty acids
	Cholesteryl ester hydrolase	Cholesteryl esters	Cholesterol and fatty acids
	Pancreatic α-amylase	Starch	1,4,α-linkages, producing α-limit dextrins, maltotriose, and maltose
	Ribonuclease	RNA	Nucleotides
	Deoxyribonuclease	DNA	Nucleotides
	Phospholipase A (prophospholipase A)	Phospholipids	Fatty acids, lysophospholipids
	Enteropeptidase	Trypsinogen	Trypsin
	Aminopeptidases	Polypeptides	N-terminal amino acid from peptide
Intestinal mucosa	Dipeptidases	Dipeptides	Two amino acids
	Glucoamylase	Maltose, maltotriose	Glucose
	Sucrase	Sucrose	Fructose and glucose
	Nuclease and related enzymes	Nucleic acids	Pentoses and purine and pyrimidine bases
Cytoplasm of mucosal cells	Various peptidases	Di-, tri-, and tetrapeptides	Amino acids

[a]Adapted from Ganong (2009).
[b]The corresponding proenzymes are shown in parentheses.

Cabanot et al., 2007; Murashita et al., 2007). Replacing fish oil with wax esters from *Calanus finmarchicus* increased specific activity of lipolytic enzymes in the intestinal contents (Bogevik et al., 2009). However, at the highest inclusion level of wax ester (25% of the diet), reduced lipid digestibility was observed. Similarly, dietary protein and amino acids stimulate pancreatic secretion of proteolytic enzymes (Cahu et al., 2004). Protease secretion seems to respond to substrate level in the diet up to a limit. The mechanism behind the response may be related to the level of free proteases in the chyme. Protease inhibitors mixed at increasing levels into diets of rainbow trout have been found to cause a curvilinear increase in total trypsin protein concentration in the intestinal content although trypsin activity decreased (Berg-Lea et al., 1989). However, at an inclusion level of about 5 g/kg diet, the capacity for trypsin synthesis seemed to be exceeded. Strain differences in responses to dietary protein level have been described for the winter flounder (*Pseudopleuronectes americanus*) (Gawlicka et al., 2001).

The regulatory mechanisms behind exocrine pancreatic secretion are not well known. Cholecystokinin is involved in the endocrine regulation of pancreatic secretion (Koven et al., 2002), but other peptide hormones as well as neurological signals play roles in the regulation (Volkoff, 2006; Koji et al., 2008; Holmgren and Olsson, 2009).

Bicarbonate Secretion and pH of the Intestine

In fish with a functional stomach the acid chyme entering the proximal intestine seems to be quickly neutralized supposedly by HCO_3^- in bile and pancreatic juice. Secretion from epithelial cells may also add to the pH adjustment of the chyme (Cooper et al., 2010). Only limited information has been published on variation in intestinal pH and the effects of diet composition. Reduction in luminal pH in European flounder intestine has been found to stimulate HCO_3^- secretion (Wilson and Grosell, 2003; Cooper et al., 2010). Buffering capacity in the intestine seems well adjusted in light of the constant liberation of amino and fatty acids. The pH is observed to be above 7 all along the intestinal tract, for example in rainbow trout, with an increasing trend toward the distal sections (Bucking and Wood, 2006). In the distal most compartments, in which the microbial activity is higher than in the more proximal sections, pH would be expected to be lower, such as in Atlantic cod (Seppola et al., 2005). Secretion of HCO_3^- from epithelial cells appears to play an important role also in preventing excessive uptake of Ca^+ ingested by marine fish via drinking water and prey fish. Bicarbonate precipitates Ca^+ as $CaCO_3$, which is unavailable for absorption. This process seems to be an important element in intestinal water absorption (Whittamore et al., 2010).

Membrane Bound Digestive Enzymes

The brush border of the absorptive cells is equipped with membrane bound peptidases that complete the hydrolysis of peptides before transport into the cells. The peptidases act on bonds at the amino terminal end of the peptides. They are numerous, with different specificities. Peptidases from different fish species show different characteristics in terms of pH optima, thermostability, and distribution along the intestinal tract (Kuz'mina, 2008). Dietary protein level affects brush border aminopeptidase activity in herbivorous, omnivorous, and carnivorous fish (Buddington et al., 1997), with moderate differences between the groups of fish (Cahu and Infante, 1995).

Brush border disaccharidases hydrolyze low molecular carbohydrates with 2–4 units producing free forms of their respective monosaccharides. The highest hydrolytic capacity of intestinal homogenates is found for maltose. Glucose is produced from maltose at rates several times higher than from sucrose and trehalose (Buddington and Hilton, 1987; Krogdahl et al., 1999; Kuz'mina, 2008). A homogenate of fish intestinal mucosa also shows the ability to hydrolyze lactose. The enzymes responsible for this activity seem to be cytosolic because the activity remains in the homogenate when the brush border membranes are extracted (Krogdahl et al., personal communication). Herbivorous and omnivorous fish species have several-fold higher disaccharidase activities in the intestinal brush border compared to carnivorous species (Kuz'mina, 2008). Present knowledge indicates that disaccharidases from fish living in cold waters have higher specific activities than the same enzymes from fish in warmer waters (Maffia et al., 1993). In most species the highest activities are observed in the proximal intestine with decreasing activities toward the anus. Whether disaccharidase activity of the brush border is affected by dietary carbohydrate level seems to depend on the feeding situation of the fish. A comparative study of effects of starch level in diets for rainbow trout and Atlantic salmon showed that both species increased their disaccharidase capacity with increasing starch level (Krogdahl et al., 2004). However, in other studies on salmonids, varying dietary starch level did not alter disaccharidase activity (Buddington and Hilton, 1987; Krogdahl et al., 1999; Kuz'mina, 2008). The conflicting results may be related to differences between the studies regarding dietary starch level, starch processing, technical qualities of the feed, feed intake, and/or environmental factors such as temperature and salinity. It should be kept in mind that fish can adjust intestinal brush border enzyme capacity either by increasing enzyme concentration in the tissue or by increasing brush border area or by both methods. Both possibilities should be taken into account in studies of effects on brush border enzyme capacity. No apparatus for hydrolysis of lipids has been identified in the intestinal brush border.

Intestinal Transit Time

Intestinal passage rate and transit time vary with diet composition, meal size, and feed structure in many animal species (Guilloteau, 1979; Hill, 2007). Increased flow of digestible carbohydrates, proteins, and lipids into the distal regions of the small intestine inhibits intestinal motility. Lipids elicit the strongest signals (Hasler, 2006). These observations are in accordance with the results of investigations of gastric emptying rate in fish (dos Santos and Jobling, 1988). Most investigations on intestinal passage rate in fish have focused on effects of fibers and bulking agents (Storebakken, 1985; Storebakken and Austreng, 1997; Dias et al., 1998). Soluble indigestible carbohydrates such as alginates and guar gum as well as bulking agent such as silica and zeolite in general seem to cause reduced transit rates in fish. Intestinal passage rate may be suggested to be regulated to optimize nutrient utilization and to prevent overload of nutrients in the distal intestinal compartments. However, soluble fibers and bulking agents often reduce nutrient digestibilities, particularly of dietary lipids. Insoluble fibers, such as cellulose, on the other hand, may speed up passage rate (Dohnalek, 2004). Intestinal passage rate is expected to vary among fish species. However, comparative studies are not available. The studies conducted with rainbow trout and sea bass indicate similar transit times for these species with presence in feces of markers from a meal between 5 and 35 hours after the meal (Storebakken, 1985; Dias et al., 1998).

Digestion

Stomach Digestion

A condition for efficient digestion and absorption of a nutrient is solubility in water. The concerted action of hydrochloric acid and pepsin in the stomach denatures and degrades most proteins and increases their solubility. The process also increases the solubility of other nutrients such as carbohydrates and minerals bound or trapped in the feed matrix. The low pH increases the solubility of many minerals and transforms them to their chloride forms, which often are more water-soluble than their native form in the feed. Lipids are also released. The hydrophobicity of lipids gives them a tendency to aggregate into droplets. Under normal circumstances, emulsifiers from the feed and stomach, such as phospholipids and certain proteins, will limit the size of the lipid droplets. However, if the rate of lipid release is too fast or the supply of emulsifiers is limited, accumulations of lipid will form. The result may be fat belching as seen in some farming situations (Baeverfjord et al., 2006). The condition seems to be multifactorial and is influenced by rate of pellet disintegration, rapid changes in salinity, and temperature.

The importance of enzymes present in food organisms for the digestive process of fish has been an issue discussed by several scientists (see review by Kuz'mina, 2008). Unargu-

ably, live prey animals are eaten with their own intestinal digestive apparatus providing a range of gastric, pancreatic, and membrane bound enzymes. Moreover, each cell of the prey has lysosomes that contain enzymes for the degradation of proteins, lipids, carbohydrates, nucleic acids, and other cell components at acid pH. They may be activated when stressed, for example when exposed to the host gastric juice that contains acid and enzymes. The term "induced autolysis" has been suggested for the process (Kuz'mina, 2008). Researchers have argued (Kuz'mina, 2008) that the fast degradation of whole prey animals observed in fish involves activation of digestive apparatus of the cells of the prey by H^+ ions from the gastric juice. The ions have been estimated to diffuse 1,000 times more rapidly into the prey than the digestive enzymes. In this view, gastric digestion proceeds from three starting points: the digestive tract of both the host and the prey and from within the tissue of the prey. The quantitative importance of the prey enzymes for nutrient digestion is not established, but is suggested to vary depending on the nutrient in question, the fish and its stage of development, the prey and its physiological status, environmental temperature, and oxygen level (Kuz'mina, 2008). For fish fed dry pellets, enzymes from the feed are certainly of no importance unless specifically supplemented.

Intestinal Digestion

Once the mixing and churning action of the stomach muscles and structures has processed the feed to the appropriate particle size and moisture level sufficient for further transport and processing, the partially digested feed, now called chyme, is passed on to the midgut, the intestine's pyloric or hepatopancreatic region. The product of stomach processes is a mixture of dissolved nutrients, mainly proteins and large peptides; mono-, di-, oligo-, and polysaccharides; water-soluble vitamins; emulsified lipids, including lipid-soluble vitamins; dissolved minerals and vitamins; and fine particles of any undissolved and insoluble feed material. In fish without a stomach, particle size of feed is reduced in some species by various structures such as the gizzard, and the enzymatic breakdown of nutrients starts in the midgut.

Protein and peptide hydrolysis take place in the chyme by the concerted action of the endo- and exopeptidases (for characteristics of the enzymes, see Table 3-1). Trypsin hydrolyzes internal peptide bonds adjacent to lysine and arginine, leaving them as carboxyterminal peptide ends, which are substrates for carboxypeptidase B (i.e., basic amino acids). Chymotrypsin preferentially hydrolyzes bonds to branched-chain amino acids, giving rise to carboxyterminal ends suitable for the action of carboxypeptidase A. The elastases preferentially hydrolyze peptides adjacent to aliphatic and neutral amino acids and are particularly efficient in initiating elastin hydrolysis. After the action of the pancreatic enzymes, the peptide chains are short, usually with less than five amino acids. After further hydrolysis by the aminopeptidases, a large proportion of the amino acids are absorbed as free amino acids. However, it is likely that substantial amounts are taken up as small peptides for further hydrolysis within the cell.

Lipid digestion requires emulsification of the lipids released from the feed in the stomach and intestine in the initial steps of digestion. The bile salt-dependent, carboxyl ester lipase is the dominating lipase in most fish species and the only lipase in many species. This carboxyl ester lipase seems to have broad substrate specificity, preferentially hydrolyzing bonds involving long, highly unsaturated fatty acids in the 1 and 3 positions of triacylglycerols. This lipase also has the ability to hydrolyze wax esters (Tocher and Sargent, 1984; Gjellesvik et al., 1989; Tocher, 2003; Kurtovic et al., 2009). Fish hydrolyze phospholipids quite efficiently, but a specific phospholipase has not been described (Tocher, 2003). Whether the final hydrolysis products, the results of concerted action of more than one lipolytic enzyme, are free fatty acids and monoglycerides or glycerol is not known. However, the enzymes responsible for resynthesis of triacylglycerols in the intestinal mucosa of this species seem to prefer monoglycerides before glycerol (Oxley et al., 2007), an indication that monoglycerols are important endproducts. The fatty acid products of lipolysis in the chyme are incorporated into primary micelles formed by bile acids and phospholipids. As the micelles enlarge, they are transformed into secondary micelles that have the capacity to include the more lipophilic compounds such as long-chain saturated fatty acids, cholesterol esters, and fat-soluble vitamins. The further process of absorption of lipids is not well known, but is believed to proceed as in mammals. As the secondary micelles reach the so-called unstirred water layer covering the intestinal brush border, they disintegrate because of the lower pH of this layer. The fatty acids cross the brush border membrane by diffusion or facilitated transport aided by proteins.

Low molecular weight carbohydrates, such as glucose, maltose, and sucrose, seem to be digested efficiently in all fish (Singh and Nose, 1967; Hilton et al., 1982; Hilton and Atkinson, 1982; Storebakken et al., 1998). They are all highly water-soluble and their hydrolysis is dependent only on glucosidases located in the brush border. The products of the hydrolysis are mainly glucose and fructose. Digestion of starch and chitin takes place by the action of α-amylase and chitinase, respectively. However, starch in most feedstuffs is contained in granules that are mostly insoluble and therefore not hydrolyzed by the amylase in the fish intestine unless well heat-treated in the presence of moisture (Krogdahl et al., 2005). The exception from this general pattern is starch in oats, which can be digested without heating, even in Atlantic salmon (Arnesen et al., 1990; Arnesen and Krogdahl, 1995; Krogdahl et al., 2005). Chitin seems to be quite poorly hydrolyzed even in fish species having crustaceans in their natural diet (Krogdahl et al., 2005). The reason may be low solubility

of this polysaccharide, very low (or no) chitinase production, or low uptake efficiency of N-acetyl glucosamine, the product of chitinase activity (Gutowska et al., 2004).

Microbial Digestion

The digestive tract of all animals, including fish, is inhabited by microorganisms of many kinds, aerobic, facultative aerobic, as well as anaerobic. The numbers of bacteria in fish are in general lower than in homoeothermic animals, but great differences exist among fish species. Some bacteria, the allochthonous, are transient and present in the çhyme; others, the autochthonous bacteria, are inhabitants of the mucosal surface and reproduce in situ. Until the past decade, studies of intestinal microbiota were largely hampered by methodological limitations because only live bacteria that were able to grow on the available media could be studied. The development of molecular tools and collection of data in international databases have changed the situation, and the number of studies is increasing. It is evident that the fish digestive tract harbors microbes with the ability to secrete enzymes that are able to hydrolyze and metabolize proteins, starch, cellulose, other nonstarch polysaccharides, chitin, and lignin (Kuz'mina, 2008; Ray et al., 2009). Higher concentrations of bacteria are found in the distal intestinal compartments than in the proximal. The variation is related to variation of feed sources. Also the microbiota of the environment has a great impact on intestinal microbiota. Herbivorous species normally have higher bacterial numbers than do carnivores. But also within these groups, variation is seen due to differences in intestinal anatomy. Fish such as cod, which have a chamber-like compartment that is closed by sphincters, have higher bacterial numbers than Atlantic salmon (Seppola et al., 2005). The products of bacterial fermentation of dietary nutrients are amino acids, glucose, acetate, propionate, and butyrate, all compounds that apparently can be absorbed efficiently by the distal intestinal tract of fish. However, the quantitative contribution from microbial fermentation to total nutrient supply is most likely small even in herbivorous species.

Nutrient Absorption

Products of the action of digestive enzymes can enter the organism across the brush border by diffusion or facilitated transport down a concentration gradient or by active and energy-dependent transport against a concentration gradient. Passage via paracellular pathways is also possible, but considered to be of minor importance in fish (Ferraris et al., 1990; Oxley et al., 2007). Facilitated and active transport takes place via specialized transporters unique for the nutrient or a group of nutrients with similar chemical characteristics. Both are saturable mechanisms. Fish have the apparatus for nutrient absorption all along the intestinal tract including the distal most areas (Ferraris and Ahearn, 1984; Collie, 1985; Buddington and Diamond, 1987; Bakke-McKellep et al., 2000). Distribution of the transporters along the intestinal tract differs, however, among species, although most fish show decreasing absorption rates toward the distal segments (Buddington et al., 1987; Bakke et al., 2010). Thus, the basic mechanisms of nutrient absorption seem to be similar to those found in mammals. However, for most, but perhaps not all transporters, the rate of nutrient absorption is lower in fish (Reshkin and Ahearn, 1987; Buddington et al., 1997).

The active transporters are generally dependent on ions such as Na^+, Cl^-, K^+, or H^+, and the energy for transport is needed to maintain necessary ion gradients across the cell membrane. The nutrient transporters show a high degree of conservation through evolution. However, variation among fish species has been observed in terms of traits such as substrate affinity (K_m) and maximum velocity (V_{max}). An apparent tendency for higher substrate affinity of amino acid transporters in herbivorous fish compared to carnivorous fish and an opposite trend regarding V_{max} of the glucose transporters has been suggested (Ferraris and Ahearn, 1984; Buddington and Diamond, 1987). Higher influx of nutrients per unit of tissue in freshwater than saltwater fish has also been indicated (Ferraris and Ahearn, 1984; Collie, 1985; Buddington and Diamond, 1987; Collie and Ferraris, 1995; Lionetto et al., 1996). As expected, transporter capacity tends to increase with increasing water temperature (Houpe et al., 1996).

Based mainly on studies with the European eel (*Anguilla anguilla*), it seems that fish have at least four distinct Na^+-dependent transporters for amino acids, one transporter for each of acidic, neutral, N-methylated amino acids, and proline (Storelli et al., 1989). Sodium-independent transporters seem to be present for the absorption of neutral and basic amino acids (i.e., for glycine, alanine, and lysine) as in mammals. For histidine, a highly specific transporter has been suggested because the transport seems to be independent of the presence of other amino acids (Glover and Wood, 2008). However, differences among fish species exist regarding substrate specificity of various amino acids (Collie and Ferraris, 1995).

Fish are also equipped with peptide transporters as demonstrated in herbivorous and carnivorous fish species such as the tilapia (*Oreochromis mossambicus*), European eel, rockfish (*Sebastes caurinus*), sea bass (*Dichentrarcus labrax*), rainbow trout, and Atlantic salmon (Thamotharan et al., 1996a,b; Maffia et al., 1997; Bakke-McKellep et al., 2000; Nordrum et al., 2000; Verri et al., 2000; Terova et al., 2009; Ostaszewska et al., 2010). The molecular structures of both the PepT1 and PepT2 transporters have been characterized for zebrafish (*Danio rerio*) and cod (Buddington et al., 1997; Verri et al., 2003; Romano et al., 2006). Diet composition seems to affect the expression of PepT1 as demonstrated for rainbow trout (Ostaszewska et al., 2010). A comparative study of amino acid and peptides transport has been carried out with rainbow trout and Atlantic salmon, showing species

differences in transport activity along the intestinal tract. In both species, transport decreased along the intestine. In the distal intestine, transport seemed higher in the trout than in the salmon for lysine and methionine, equal or lower for phenylalanine and proline. Soybean feeding decreased transporter-mediated uptake and increased permeability. In both species, nutrient transport was also influenced by water salinity. The results indicate that transporter-mediated uptake is of greater importance in saltwater than in freshwater (Bakke-McKellep et al., 2000; Nordrum et al., 2000).

Some dietary and endogenous proteins escape proteolytic digestion in the proximal sections of the intestine. Such proteins may be absorbed as macromolecules. Uptake of human gamma globulin, horseradish peroxidase, ferritin, prion proteins, and oral vaccines has been demonstrated in various fish species (Lavelle and Harris, 1997; Hernandez-Blazquez and da Silva, 1998; Amthauer et al., 2000a; Concha et al., 2002; Quentel et al., 2007; Uran et al., 2008; Valle et al., 2008). The distal intestine seems to be the most important site of absorption of larger peptides and proteins, and uptake of intact proteins is considered essential for development of the defense apparatus against exogenous proteins and pathogens. The nutritional importance of macromolecular uptake, however, is considered minor. Indication of an enteropancreatic circulation of proteins exists based on macromolecular uptake studies. However, despite efforts to gain information on such recirculation of proteins, data that can support the concept are scarce (Rothman et al., 2002).

Information about lipid transport across the intestinal mucosa in fish is limited, but present knowledge indicates that the processes are quite similar to those in other vertebrates. The proximal intestine seems to absorb most dietary lipids. Medium-chain and longer highly unsaturated fatty acids are absorbed in more proximal regions compared to the longer and more saturated, which are absorbed in more distal regions (Røsjø et al., 2000). It is believed that fatty acids as well as the fatty alcohols pass the brush border membrane by diffusion. However, demonstration of the presence of fatty acid binding proteins (FABP), also in the fish intestine, indicates that facilitated transport may take place (Andre et al., 2000; Concha et al., 2002; Iqbal and Hussain, 2009). Uptake of fatty alcohols from wax esters, abundantly present in some marine organisms such as copepods, is slower than uptake of fatty acids (Bogevik et al., 2008). Both fatty acids and alcohols are reesterified in the enterocytes. The triacylglycerols are produced from fatty acids and monoglycerols or glycerol-3-phosphate (Caballero et al., 2006). The monoglycerols seem to dominate as substrate for the production of triacylglycerols, whereas phospholipid synthesis utilizes glycerol-3-phosphate. The efficiency of production and partitioning between the two seems to depend on the source of lipid in the diet. Triacylglycerols produced by the mucosal cells are incorporated into lipoproteins that accumulate in lipid droplets in the cells and exit the cells via exocytosis (Hernandez-Blazquez and da Silva, 1998; Kjaer et al., 2009). The lipoproteins produced by the enterocytes should perhaps be named portomicrons rather than chylomicrons because they are not conveyed by chylus into collective lymph ducts, or so-called lacteals (Tocher and Sargent, 1984; Bogevik et al., 2008). However, the movement of lipids between the gut and the general circulation is not well known.

A dietary supply of phospholipids is essential for efficient lipid digestion, absorption, intracellular metabolism, and further transport in the body (Tocher et al., 2008). A deficiency of phospholipids has been observed to cause lipid accumulation within the intestinal absorptive cells and histological alterations in carp and salmonids (Fontagne et al., 1998; Olsen et al., 2003)

Glucose uptake has been studied in several fish species and seems qualitatively similar to that in other vertebrates. D-glucose and galactose are taken up by the same brush border transporter, SGLT1, which is electrogenic and dependent on Na^+ and energy (Krogdahl et al., 2005; Geurden et al., 2007). Fructose is also absorbed in fish. However, a putative facilitative transporter for fructose, such as GLUT5 in other vertebrates, has not been identified in fish. Other transporters may supply additional transport capacity for monosaccharides but have not yet been described in fish.

Carbohydrate absorption also takes place mainly in the proximal intestinal compartments of the fish intestine, as shown for Atlantic salmon (Krogdahl et al., 1999; Bakke-McKellep et al., 2000). This is in agreement with the observation that brush border enzymes for hydrolysis of disaccharides have the highest activities in the proximal regions. Differences exist among fish species, but most fish absorb mono- and disaccharides with high efficiency (Singh and Nose, 1967; Hilton et al., 1982; Hilton and Atkinson, 1982; Storebakken et al., 1998). No information has been found in the scientific literature on uptake mechanisms for N-acetylglucosamines, the hydrolysis products of chitinolytic activity.

Knowledge on mechanisms behind increases and decreases in nutrient transport capacities in the brush border and basolateral membranes and their regulation is very limited. Transporter concentration in the brush border membrane can change quickly, for example by the introduction of transporters stored intracellularly. The signals may be mediated by endocrine and/or neurological signals (Holmgren and Olsson, 2009).

SHRIMP

Shrimp can be filter feeders, scavengers, and predators and are classified as herbivores, carnivores, and omnivores. Investigations of stomach contents of shrimp have shown that they eat other species of crustaceans, annelids, mollusks, echinoderms, nematodes, fish tissue, insects, seeds, algae, macrophytes, vegetable matter, and detritus (Focken et al., 1998; Figueiredo and Anderson, 2009). Some species have developed more carnivorous feeding habits than oth-

ers. In extensive and semi-intensive pond-cultured shrimp, the naturally available food organisms can dominate over the exogenously supplied feed (Nunes et al., 1997; Nunes and Parsons, 2000), whereas in more intensive systems the contribution from natural feed is reduced or eliminated.

Structural and Functional Aspects of Digestive Organs

The anatomy of the digestive tract of shrimp is often divided into three major parts: foregut, midgut, and hindgut. A further division can be made of the foregut: esophagus, cardiac stomach, and pyloric stomach, a chamber where feed particles are ground and filtered (Mantel, 1983; Ceccaldi, 1997). A drawing of the structure of the tract is shown in Figure 3-4. Lamellae of various sizes, brushes and needles, and dents make the stomach structure rather complicated. Most shrimp possess a calcified structure in the stomach, known as the gastric mill. The hepatopancreas (midgut gland), the major digestive organ of shrimp, is a large multilobate structure, a diverticulum of the midgut. A blind tubule covered by a single epithelial layer with digestive characteristics is the basic unit. The tubules vary in length and fuse into larger collective ducts and end in one or two major ducts opening into the midgut. The hindgut of the intestine is straight and widens into the rectum before termination at the anus. A layer of a chitin-protein complex, which is part of the shrimp exoskeleton, covers the external surface of the foregut and hindgut. The midgut is not lined by this complex and is the only section of the intestine with characteristics of intestinal absorptive surface. Shedding and replacement of the chitin-protein layer takes place at each molt. In some shrimp species, chewing structures located in the stomach are also replaced at each molt (Ceccaldi, 1997). Shrimp larvae start out with simple intestines that develop into more complicated structures as they progress through distinct stages.

Shrimp catch and preprocess feed with their mouth pieces with specialized prehensile appendages. The feed is passed through the relatively short esophagus to the stomach. Feed disintegration takes place mainly in the stomach by the action of the various lamellae, appendages, and calcified parts, including the gastric mill (Ceccaldi, 1997). The feed is turned into very fine particles that are passed on to the midgut. Larger particles are conveyed by fluid streaming retrograde to the more proximal parts of the stomach for further degradation. Another sorting of feed particles takes place in the midgut by the glandular filter (ampulla). Indigestible particles are passed on to the distal compartments of the tract, whereas nutritionally valuable material enters the hepatopancreas.

The hepatopancreas combines the functions of the pancreas, intestine, and liver and is responsible for processes such as synthesis and secretion of digestive enzymes, absorption of digested material, and metabolism of lipids, carbohydrates, and minerals. It is the center for the production of materials required for the temporally distinct events of molt and vitellogenesis. The glandular tissue also serves as a detoxification organ for heavy metals and toxic organic compounds (Ceccaldi, 1997). Several shrimp species have midgut ceca of different lengths and numbers located close to the stomach, at the opening of the hepatopancreatic canal, or at the entrance to the hindgut. Cells of the ceca have microvilli indicating absorptive functions. The epithelium of the hindgut of shrimp is involved in osmoregulation (i.e., transport and metabolism of water and ions) and in condensing the material for excretion in feces (Ceccaldi, 1997).

Shrimp encase their feces in a peritrophic membrane, an acellular layer that separates ingested materials from the gut

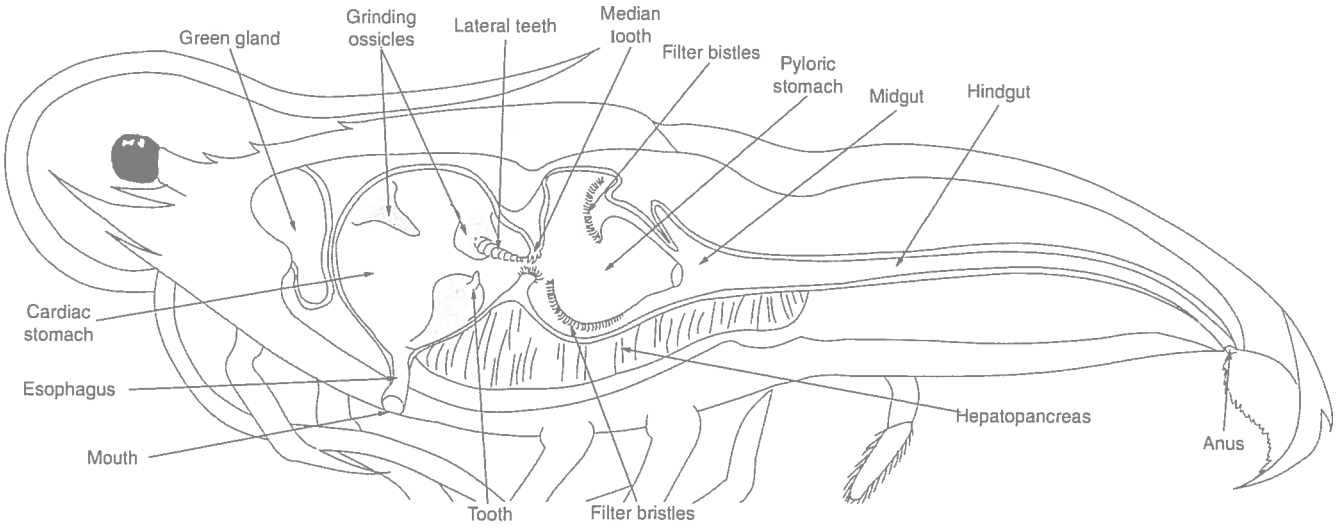

FIGURE 3-4 Anatomy of the digestive tract of shrimp.
Illustration courtesy of Victoria Blondin, University of Guelph, Ontario.

epithelium. The membrane is secreted from the midgut and contains chitin and protein (Martin et al., 2006). This has implications for the measurement of apparent digestibility. On one hand, nutrients in the membrane are lost, and on the other hand there is reduced loss of nutrients in the feces from leaching.

Digestive Enzymes

The shrimp stomach, covered with a chitin level, does not secrete acid and enzymes, but its contents often show digestive enzyme activities, some of which may originate from the hepatopancreas and some from food animals. At least in some crustaceans, digestive enzymes produced in the hepatopancreas pass from the midgut into the stomach (Vogt et al., 1989). Hence, the digestive processes can be initiated before the feed enters the midgut.

The hepatopancreas is the main secretory organ of shrimp. Digestive enzymes are synthesized in the F-cells (fibrillar cells) and accumulate in the B-cells (blister-like cells) (Vogt et al., 1989; Sousa et al., 2005). In some but not all species, the B-cells contain granules, presumably containing enzymes in active or proenzyme form (Babu and Manjulatha, 1995; Sainz et al., 2004; Ong and Johnston, 2006). The hepatopancreas secretes a wide range of digestive enzymes; proteases, including specific collagenases; lipolytic enzymes; chitinase; cellulase; laminarinase; α/β-glucosidase and/or α-amylase to be able to make use of the cellulose from plant cell walls; laminarin from brown algae; and other nonstarch polysaccharides (Xue et al., 1999; Johnston and Freeman, 2005; Carrillo-Farnes et al., 2007; Figueiredo and Anderson, 2009). Species that have high protein content in their diet show high proteinase activities. Species that feed on crustaceans synthesize chitinase. Herbivorous species tend to produce high amounts of the various carbohydrases to be able to disrupt cell walls and make use of the cellulose from plant cell walls, laminarin from brown algae, and other nonstarch polysaccharides (Xue et al., 1999; Johnston and Freeman, 2005). Omnivorous opportunistic feeders have high activities of several of the enzymes mentioned above and are able to utilize a wide range of food sources.

The main endopeptidases for most crustaceans are trypsin and chymotrypsin. Some species, however, synthesize cathepsin L as the main proteolytic enzyme (Teschke and Saborowski, 2005; Carrillo-Farnes et al., 2007; Chisty et al., 2009). Very few studies have been carried out on purified enzymes, but gene sequences are available for many (Carrillo-Farnes et al., 2007). Characteristics such as inhibition patterns, pH optima, and heat tolerance of enzymes are mainly the results of studies on crude hepatopancreatic extracts (Carrillo-Farnes et al., 2007). Shrimp trypsinogen seems to lack the enterokinase-recognizing sequence of trypsin from vertebrates. It is however activated by extracts of hepatopancreas and so is chymotrypsinogen (Babu and Manjulatha, 1995; Viader-Salvado et al., 2007). Information on exopeptidases, such as carboxypeptidase and aminopeptidases, is difficult to find in shrimp (Carrillo-Farnes et al., 2007).

Enzymes capable of hydrolyzing triglycerides and phospholipids have been observed in several shrimp species (Carrillo-Farnes et al., 2007). There is, however, some debate about whether the hydrolysis of triglycerides is catalyzed by a triglyceride lipase, a phospholipase, or both. *Litopenaeus vannamei* seem to have both. From this species, two fractions with lipolytic activity have been isolated; one with preference for triglyceride substrate, and the other for phospholipid (Carrillo-Farnes et al., 2007). A comparison of substrate specificity of lipases from *Litopenaeus schmitti* indicated a strong preference for n-3 and n-6 fatty acids. Studies of lipases from *L. vannamei*, *Farfantepenaeus californiensis*, and *Farfantepenaeus notialis* showed similar preferences. Several shrimp lipases have shown two pH optima in the range 5–11 (Carrillo-Farnes et al., 2007). The lipases and esterases are found associated with the microvilli of R-cells (resorptive cells), as well as in vacuoles of B-cells, supranuclear vacuoles of F-cells, lumen of the hepatopancreas tubule, and in intertubular connective tissue (Lopez-Lopez et al., 2003). The R-cells can take up fatty acids from the lumen and store them intracellularly.

Shrimp are able to hydrolyze a great variety of oligo- and polysaccharides and seem to surpass greatly even herbivorous fish. Hepatopancreatic and/or tissues from other sections of the digestive tract of various shrimp species have shown a wide range of enzyme activities characterized as α- and β-galactosidase, α-fucosidase, laminarinase, α-mannosidase, β-glucuronidase, β-glucosaminidase, xylanase and α-xylosidase, raffinase, β-fructofuranosidase, and cellulase (reviewed by Carrillo-Farnes et al., 2007). Whether these enzymes are endogenous to the shrimp or to the food ingested by the shrimp or both is not clear. In accordance with these observations, many shrimp species seem to utilize starch and other polysaccharides very efficiently. Three α-amylases have been cloned from *L. vannamei* and show great sequence similarities with mammalian α-amylase (Van Wormhoudt and Sellos, 2003). Activities described as α- and β-galactosidases, chitobiase, α-fucosidase, laminarinase, α-mannosidases, β-glucuronidase, β-glucosaminidase, xylanase, and α-xylosidase have been observed in one or more species (Van Wormhoudt and Sellos, 2003). It may be suggested that the great ability of shrimp to hydrolyze polysaccharides is related to the fact that they all start as an herbivore or omnivore with phytoplankton as a major nutrient source (Le Vay et al., 2001; Diaz et al., 2008b).

Two of the cell types of the hepatopancreas, the R and F, are equipped with microvilli, indicating absorptive functions. Also, epithelial cells of the intestinal ceca, present in several shrimp species, have well-organized microvilli (Ceccaldi, 1997). Whether they are equipped with digestive enzymes such as aminopeptidases and disaccharidases is not clear (Ceccaldi, 1997). Homogenates of hepatopancreas show α-glucosidase activity, but the enzymes may be intracellular.

Larger changes in enzyme content of the hepatopancreas are seen during molting periods (e.g., for trypsin and chitinase) (Hernandez and Murueta, 2009). Chitinase digests the old exoskeleton so it can be resorbed and replaced by newly synthesized chitin. The production of digestive enzymes also seems to vary throughout the year and even within the species, depending on the available nutrient sources. The Caridean shrimp (*Crangon crangon*) has high trypsin activity during the summer and low activity during winter periods (Pöhlmann, 2007; Sahlmann, 2008). The latter work also indicated that shrimp may recirculate digestive enzymes. Even enzymes from ingested prey can survive the hydrolytic conditions in the intestine and be recycled via the hepatopancreas (Sahlmann, 2008).

Enzymes seem to be emptied from the hepatopancreas upon feeding (Ong and Johnston, 2006). Passage of enzymes from the midgut to the stomach has been found to induce additional synthesis and secretion of enzymes (Vogt et al., 1989). Adjustments to diet composition for proteases, lipolytic enzymes, and amylase have been shown for many species. The responses vary among species. For some species, responses are seen in proteolytic and amylase activity but not in lipolytic activity, but for other species, amylase and/or protease activity seem unresponsive (Moss et al., 2001; Gamboa-Delgado et al., 2003; Lopez-Lopez et al., 2005). A high dietary starch level was found to increase the specific activity of α-amylase and an α-glucosidase in *L. vannamei* (Le Moullac et al., 1997; Gaxiola et al., 2005). The same species has shown variation in trypsin and chymotrypsin activity with variation in protein level (Le Moullac et al., 1997; Lemos et al., 2000; Muhlia-Almazan et al., 2003, 2008). The magnitude of the stimulation seems to differ among species and to depend on the protein source of the diet.

The regulatory mechanisms behind adaptation to dietary composition are not well understood. Intestinal hormones are likely to be involved in this regulation (Santos et al., 1997). Gastrin-cholecystokinin-like peptides isolated from the stomach of the marine crustacean *Nephrops norvegicus* were found to stimulate isolated midgut gland cells (Favrel et al., 1991). Moreover, GI hormones from vertebrates, CCK-8 (desulfated form), gastrin, bombesin, secretin, and substance P were all stimulating the release of proteases and amylase from the hepatopancreas (Resch-Sedlmeier and Sedlmeier, 1999). Also, hormones from the eyestalk, such as the hyperglycaemic hormone, have been suggested to be involved in regulation of digestive functions (Carrillo-Farnes et al., 2007).

Digestion

Qualitatively, digestive processes seem quite similar in shrimp and fish. Even though shrimp do not have a secretory stomach, nutrient hydrolysis seems to be initiated in the foregut in many species by the action of enzymes delivered from the hepatopancreas or from food animals. All macronutrients may be partially hydrolyzed when they reach the midgut as the juice from the hepatopancreas contains proteases, lipases, and amylase. The breakdown of macronutrients continues in the hepatopancreatic chamber and the endproducts are supposedly small peptides and amino acids, fatty acids, and monoglycerol or possibly free glycerol. Digestion of lipids in crustaceans is similar to that of fish, and lipid digestibility is typically > 90%. The midgut gland of *L. vannamei* shows lipase activity from the very early stages of development, indicating a capacity for lipid digestion as well as the importance of lipid in development (Rivera-Pérez et al., 2010). A major difference between shrimp and fish is the fact that crustaceans do not produce bile and do not utilize bile salts in their lipid digestion and metabolism (Cherif et al., 2007). Demand for other emulsifiers may therefore be higher in shrimp than in vertebrates.

The intestinal microbiota of shrimp may play a role in some shrimp species feeding on high-carbohydrate diets. However, transit time is high and prevents extensive microbial fermentation. The microbes may supply vitamins and possibly add some digestive enzymes, but neither the proximal compartment nor the distal compartment of the intestine has a surface facilitating colonization (Ceccaldi, 1997).

Absorption

The main absorption of nutrients in crustaceans takes place in the hepatopancreas. This tubular system has a single-cell layer of epithelial cells that facilitates rapid transcellular nutrient transport to the haemolymph. However, the absorptive functions of the different cell types of the hepatopancreatic tissue have not been fully investigated. The activity of several brush-border membrane transporters in the hepatopancreas is reported to be pH-dependent (Verri et al., 2001). This has been demonstrated for the Na^+/D-glucose cotransporter, the Na^+/Cl^-/L-alanine cotransporter, the $Na^+/2Cl^-$/L-leucine cotransporter, and the Na^+/Cl^-/L-glutamate cotransporter. The low pH in the hepatopancreatic lumen facilitates nutrient influx into the epithelial cells.

CONCLUSIONS

Fish and shrimp differ greatly in the anatomical characteristics of the digestive tract, which seems to be more complicated in shrimp than in fish. However, variation in structure is greater for fish. The digestive processes, on the other hand, are less variable and generally follow the same principles as found in higher animals. Present knowledge on digestive physiology of fish far exceeds that of shrimp, but many details still require further investigation, even in fish. Better understanding is needed of the fate of the feed in the digestive tract and limitations of the digestive processes to be able to formulate and process diets optimally so that they can fulfill the nutrient requirements and secure health and wellbeing of the cultivated organisms. The processes depend