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The physiology of digestion in fish larvae

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Synopsis

The acquisition, digestion, and assimilation of food is critical for the growth and survival of fish larvae; a fish larva either grows or it perishes. Fish larvae are characterized by digestive systems and diets that differ from adults. Larvae undergo a pattern of trophic ontogeny, changing diet with increasing size, and these changes result in differences in digestive requirements. At first feeding, the larval alimentary canal is functional, but is structurally and functionally less complex than that of adults. The larval alimentary canal remains unchanged histologically during the larval period before transformation. During transformation, major changes that result in the development of the adult alimentary canal occur. The ontogeny of the alimentary canal differs in different taxa, and experimental evidence suggests that functional differences exist as well. Assimilation efficiency may be lower in larvae than it is in adult fishes, due to a lack of a morphological and functional stomach in larvae, but the question of improving assimilation efficiencies during larval development before transformation remains unresolved.

Introduction

The alimentary canal of fish larvae is morphologically, histologically, and physiologically less elaborate than the alimentary canal of adult fishes. Unlike the gradual development of some other organ systems, e.g., the integumentary, visual, musculature, and acoustic-lateralis systems (see review in O'Connell 1981), the development of the alimentary canal from the simple, undifferentiated, straight incipient gut of the yolk-sac larva to the complex, segmented alimentary canal of the adult proceeds by periodically rapid changes rather than continuous gradation. The incipient gut remains unchanged during yolk and oil-globule absorption (several days or weeks), then changes rapidly just before first feeding (1 to 3 d). In the main, the larval alimentary canal remains unchanged during the long larval period (several months to a year), then changes rapidly into the adult alimentary canal during transformation of the larval to the juvenile fish (weeks or months). [We use the terminology of Kendall et al. (1984) in describing early life history stages].

Concomitant with growth and the changing complexity of the alimentary canal are marked differences in diets. Most fish larvae are visual, raptorial planktivores (Hunter 1981) regardless, of whether their adult counterparts are indiscriminant filter-feeders, pelagic carnivores, or benthic pickers. Larvae begin feeding on large phyto- and small zooplankters and follow by feeding on increasingly larger zooplankters (Hunter 1981). For some species,changes in diets are required. For example,

northern anchovy, *Engraulis mordax*, larvae survive on the dinoflagellate *Gymnodinium splendens*, but do not grow well beyond 6 mm in length unless larger food organisms are included in their diets (Hunter 1977). The diets of fish larvae change again during transformation.

The alimentary canal of fish larvae also shows considerable diversity among taxa and several general dichotomies are valid. The alimentary canal of precocial larvae that generally hatch from large, negatively buoyant eggs, is more developmentally advanced than the canal of altricial larvae that hatch from small, positively buoyant eggs (terminology from Balon 1979, 1981). The larvae of most 'lower' fishes (e.g., clupeoids, salmonids, and cyprinoids) have a straight alimentary canal, whereas the larvae of 'higher' fishes (e.g., paracanthopterygians and percomorphs have a looped canal). Notable exceptions to the latter generality are some ostariophysans and salmonids that have looped guts (Iwai 1969) and some stomiatoids that have unusual, trailing, exterilial guts (Moser 1981). Other peculiarities include the occluded guts of elopomorph leptocephali (Hulet 1978, Smith 1984).

The varied structural adaptations of the fish larvae alimentary canal and the changes of these adaptations with development are characteristic of differing functional adaptations to diets and prey concentrations. There is some evidence that ingestion and digestion rates as well as assimilation efficiencies are adapted to maximize larval growth and that these adaptations differ among taxa (Houde & Schekter 1980, 1983). Within taxa, digestion rates and assimilation efficiencies may change with prey availability and ration size (Werner & Blaxter 1980, Boehlert & Yoklavich 1984a) as well as with development (Laurence 1977, Buckley & Dillmann 1982). Here we consider the morphological and histological development of the alimentary canal, review histochemical and enzymological assays of larval alimentary canal function, and describe physiological studies of larval digestion and assimilation. While this paper is a review, we also include original observations and offer recommendations for future research.

Methods

Morphology and histology

Standard histological techniques, light microscopy, transmission electron microscopy (TEM), and scanning electron microscopy (SEM), have contributed to the understanding of the structure and function of the alimentary canal of fish larvae. Standard paraffin procedures have been used with some success for light microscopy (O'Connell 1981), but techniques that use glycol methacrylate as an embedding medium (Govoni 1984) are preferred. Procedures used for TEM of larval tissues are standard (e.g., see Iwai 1969). Boehlert (1984) offers techniques for the preparation of soft larval tissues for examination with SEM.

Histochemistry

The use of horseradish peroxidase (HRP) as a histochemical tracer has resolved the morphological sites and cytological mechanisms of protein absorption in fish larvae (Stroband et al. 1979, Stroband & Kroon 1981, Watanabe 1981, 1982a, 1984a). An advantage of the HRP technique is that in addition to allowing the observation of protein absorption, the technique affords an assessment of enzymatic hydrolysis. For the present observations, 0.5% HRP solution in physiological saline was injected into the larval alimentary canal through a glass capillary inserted into either the foregut or anus. The injection volume was adjusted to fill the entire lumen. Following incubation in vivo, tissues were fixed for 4 h in cold 5% glutaraldehyde in 0.1 molar phosphate buffer (pH 7.6) and then were washed at least overnight in the same cold buffer containing 5% sucrose. For light microscope observations, the tissues were frozen and cut into 10 μm thick sections. The sections were mounted on glass slides and incubated for 5–15 min at room temperature in 0.05% 3,3-diaminobenzidine tetrahydrochloride (DAB) in 0.05 M tris-HCl buffer (pH 7.6) containing 0.01% H_2O_2 as a substrate of HRP. They then were washed in 70% alcohol and observed. Peroxidase activity was detected with light microscopy as dark brown deposits. For TEM, the tissues were

cut into small pieces, incubated in DAB, washed in distilled water, and postfixed for an hour in cold 1% OsO_4 solution in phosphate buffer (pH 7.3) containing 0.54% sucrose. This was followed by standard TEM preparations, but sections were observed without staining; peroxidase activity can be detected as electron-opaque deposits. Controls for both light and TEM were designed to show that there was no reaction product when either HRP or its substrate was absent. Groups of larvae or tissues were administered only physiological saline and observed, while another group was administered HRP solution, but without H_2O_2 substrate.

Ikeda (1959) and Prakash (1961) examined the histochemistry of alkaline phosphatase in the alimentary canal of fish larvae. Both used standard paraffin histochemical methods, although improved techniques with glycol methacrylate sections are now developed (Higuchi et al. 1979).

Enzymology

The digestive enzymes of fish larvae have been studied by testing enzyme activities of tissue homogenates against dissolved substrates (Tanaka et al. 1972, Kawai & Ikeda 1973a, 1973b, Dąbrowski 1982), whereby reaction products or the disappearance of substrates are measured photometrically; by testing the enzyme activities of tissue sections against substrate films, whereby the disappearance of substrate is assessed with light microscopy after histochemical staining (Szlaminska 1980, Vu 1983); and, by radio-immunoassay (Hjelmeland et al. 1983). The activities of amylase, maltase, pepsin, trypsin, chymotrypsin, and aminopeptidase have been assayed with the tissue homogenate method; the activities of amylase, pepsin, trypsin and chymotrypsin, and lipase with the substrate-film method. Trypsin and trypsinogen have been radio-immunoassayed.

Peristalsis and digestion rates

Peristalsis and evacuation rates of the alimentary canal have been studied by gross observation of live larvae (Blaxter & Hempel 1961, Chitty 1981, Pedersen 1984) and by collecting feces after larvae were fed. Enhanced resolution of evacuation rates can be obtained by feeding larvae alternately with stained and unstained food organisms (Laurence 1971) or with radioactively labeled food and unlabeled food. The latter technique also affords continuous as well as pulsed feeding experiments.

Assimilation

The use of the physiological energetic approach (Brett & Groves 1979) and of radiotracer techniques (Sorokin 1966) have provided assessments of assimilation efficiency of the larval alimentary canal. Assimilation is normally a difficult physiological parameter to measure (Johannes & Satomi 1967, Conover 1978). This problem is particularly difficult for fish larvae because of their small size, planktonic habitat, and lack of discrete feces production. The alimentary canal of fish larvae discharges both liquid and solid feces, and it is often impossible to separate excreted products of catabolism from defecated dissolved feces. As a result, assimilation efficiency is often estimated by difference, i.e., by the subtraction of other measurable energetic parameters from the matter or energy of ingested food. Assimilation efficiency as calculated by the differences in energy budget parameters is subject to the cumulative biases of these measurable parameters. Radiotracer methods offer a direct, short-term measure of assimilation, but are also subject to error. Based on the retention of metabolically active tracers (e.g., ^{14}C, ^{3}H, or ^{32}P), estimates of assimilation can be biased if the specific activity of the tracer in the food changes during the experiment, or if there are metabolic pools of unknown boundaries and rapid turnover rates that result in short-term loss of tracer from the animal (Conover & Francis 1973). Applications of radiotracer methods to fish larvae are discussed in Govoni et al. (1982) and Boehlert & Yoklavich (1984a).

Results and discussion

The development of the alimentary canal

The development of the alimentary canal of fishes encompasses morphological, histological, and functional changes that are aligned with major changes in gross morphology (Fig. 1). Here we describe the development of the alimentary canal of a generalized altricial larva with exceptions noted where marked variations occur. Embryonically, the alimentary canal develops from the involution of columnar endodermal cells that lie above the yolk (Devillers 1961). At hatching, the alimentary canal is a straight tube lying dorsal to the yolksac, is closed at the mouth and anus in some species, and is histologically undifferentiated along its length (Engen 1968, Tanaka 1969a, Fukusho 1972, Umeda & Ochiai 1973, Vu 1976, Govoni 1980, O'Connell 1981). The incipient gut remains unchanged until the completion of yolk- and oil-globule absorption when the undifferentiated tube becomes segmented by muscular valves into a buccopharynx, fore-, mid-, and hindgut (Kostomarova 1962, Engen 1968, Tanaka 1969a, 1969b, Fukusho 1972, Umeda & Ochiai 1973, Govoni 1980, O'Connell 1981). With the exception of some precocial young that develop from negatively buoyant eggs, most notably the salmonids, gasterosteids, and mouth brooding cichlids, fish larvae lack both a morphological and functional (secretory) stomach; the posterior region of the foregut (Watanabe & Sawada 1985) and the midgut (Govoni 1980) can expand and function to store food in some larvae. The larval alimentary canal remains largely unchanged until the onset of transformation. The development of a stomach and pyloric caeca from the posterior foregut accompanies transformation and constitutes the last major morphological change of the alimentary canal. The liver and pancreas (along with their ducts) are formed at hatching and are functional by the end of yolk absorption (Tanaka 1969a, 1969b, Vu 1976, Govoni 1980, O'Connell 1981, Watanabe & Sawada 1985).

An interesting exception to the above pattern of early gut development occurs in rockfish, *Sebastes*, embryos. This group is characterized by small eggs and high fecundity and has typically been referred to as being a primitive ovoviviparous genus (Wourms & Cohen 1975). Boehlert & Yoklavich (1984b), however, demonstrated that embryos receive additional nutrition during gestation. The uptake of nutritional substances by *S. melanops* embryos does not occur across epidermal surfaces, as in many other viviparous species (Veith 1980). Epidermal microridges on *Sebastes* embryos, characteristic of fish larvae epidermis (Yamada 1968, Roberts et al. 1973), are present, but microvilli, characteristic of epidermal absorptive surfaces on embryos of viviparous fishes (Veith 1980), are absent (Fig. 2a). Instead, uptake occurs across the alimentary canal mucosa, which develops while the embryo is still within the egg envelope and a significant yolk mass remains (Boehlert & Yoklavich 1984b). Histological observations on the alimentary canal of *S. melanops* embryos demonstrate that in early embryos the foregut is not open,

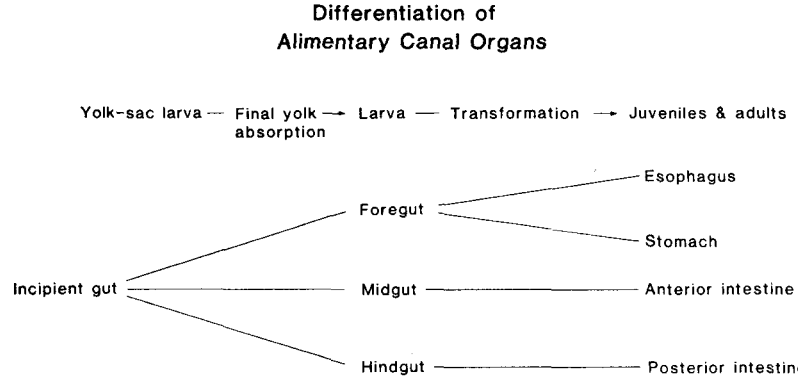

Fig. 1. The derivation, sequence, and timing of alimentary canal organs in typical larval fishes.

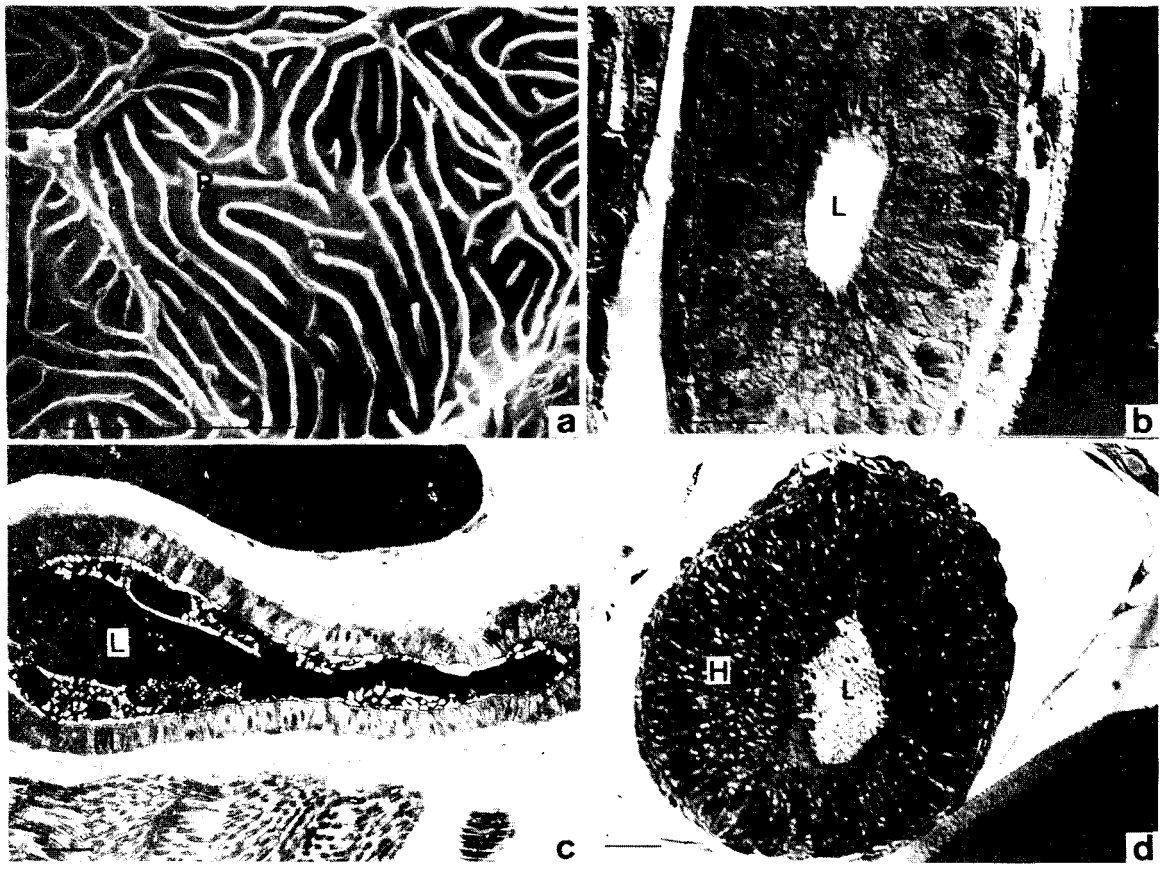

Fig. 2. Micrographs of the epidermis and gut tissue of developing embryonic states from the viviparous species *Sebastes melanops* showing the mechanism of nutrition of pre-parturition embryos. Scanning electron micrograph (*a*) of a dorsal epidermal cell just posterior of the cranium at Oppenheimer (1937) stage 31 (29 d past-fertilization, approximately 7 d before parturition). Note the well developed microridges (R) which characterize the embryonic epidermis throughout development and the absence of absorptive microvilli (scale bar is 10 μm). Photomicrograph of cross section (*b*) taken just dorsal to the yolksac (Y) of a stage 21 embryo (13 d post-fertilization). Although the midgut epithelium (M) is well developed, the lumen (L) is devoid of material (scale bar is 20 μm). Photomicrograph of a longitudinal section (*c*) of the mid- (M) and hindgut (H) of a stage 28 (11 d later than *b*). At this stage, the gut is complete and the mouth open. Note the densely stained, acidophilic substance through lumen of the gut (scale bar is 50 μm). Photomicrograph of a cross-section of the hindgut of a stage 31 embryo (*d*). Note that the lumen contains an amorphous, granular substance, and that the epithelial cells have supranuclear granules (scale bar is 20 μm).

and the gut epithelium is relatively narrow (Fig. 2b). With development past stage 27 (of Oppenheimer 1937), however, observation of whole embryos and serial sections show that the gut is open and functional. The lumen now contains acidophilic, amorphous material (Fig. 2c), and the hindgut epithelium becomes markedly deeper. Additionally, there are now large, supranuclear cellular inclusions and some vacuolation (Fig. 2d). These inclusions are discussed below and are generally indicative of a functional alimentary canal. Additional physiological energetic evidence (Boehlert & Yoklavich 1984b) indicates that the embryos ingest and assimilate ovarian fluid.

The larval fore-, mid-, and hindgut are histologically and functionally distinct (Richards & Dove 1971, Tanaka 1971, Theilacker 1978, Umeda & Ochiai 1973, O'Connell 1976, 1981, Vu 1976,

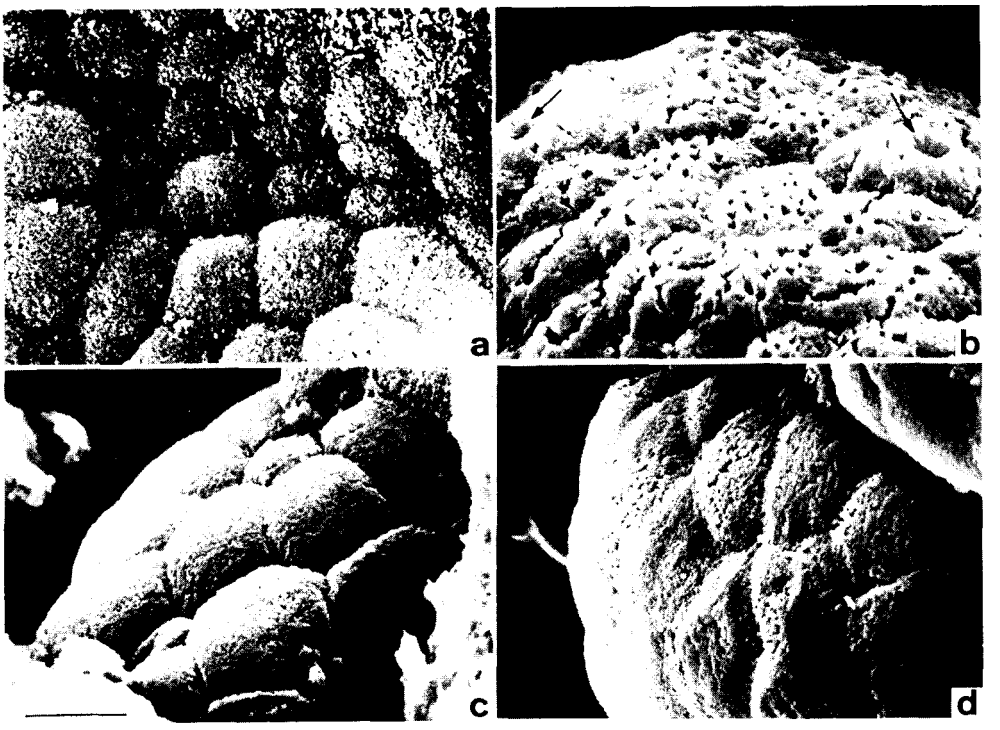

Fig. 3. Scanning electron micrographs of the apical epithelial surface of the mid- (*a*) and hindgut (*b*) of *Brevoortia patronus* larva and the mid- (*c*) and hindgut (*d*) of *Leiostomus xanthurus* larva. Note the striated border of microvilli and discharging mucous cells (arrows). (Scale bar is 5 μm; electromicrographs courtesy of J.T. Turner).

Govoni 1980, Watanabe 1981). A single layer of cuboidal epithelial cells interspersed with mucous cells lines the larval foregut. Mucous cells are more densely distributed in the anterior than in the posterior foregut of some species (Watanabe & Sawada 1985). A single layer of columnar epithelial cells with a well defined striated border of microvilli (Fig. 3) subtended by a terminal web (Fig. 4) lines the larval midgut (Iwai 1968a, 1968b, Iwai & Tanaka 1968a, 1968b, Tanaka 1971, Umeda & Ochiai 1973, Vu 1976, Stroband & Dąbrowski 1979, Stroband & Kroon 1981, Govoni 1980, O'Connell 1981, Watanabe 1982b). A single layer of columnar epithelia with microvilli (Fig. 3) and no subtending terminal web (Fig. 4) lines the larval hindgut. Mucous cells are seen in the hindgut of some species, for example gulf menhaden, *Brevoortia patronus,* while they are absent in others, for example spot, *Leiostomus xanthurus* (Fig. 3). Similarly the striated border of the hindgut in gulf menhaden is deeply furrowed, whereas it is without furrows in spot (Fig. 3).

Although not directly involved with the digestion and absorption of food, other structures, mainly the buccopharynx and the tunicae that envelop the alimentary canal, effect the processing and transport of food. At yolk absorption, the buccopharynx is lined with squamous epithelium along with scattered mucous cells and taste buds (Tanaka 1971, Govoni 1980). In relation to changes in the diets of fish larvae, taste buds become more numerous and functional as larvae grow (Twongo & MacCrimmon 1977, Appealbaum et al. 1983). Teeth develop in the areolar connective tissue underlying the buccopharyngeal epithelium, subsequently erupting during the larva period (Twongo & MacCrimmon 1977, Govoni 1980).

The dentition of fish larvae is often different

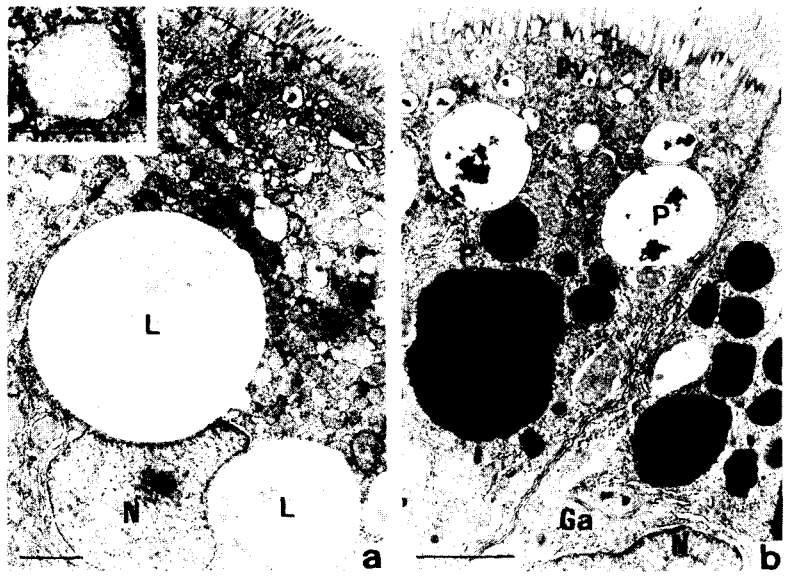

Fig. 4. Transmission electron micrographs of the mid- (*a*) and hindgut (*b*) epithelium of *Chaenogobius annularis* larva showing protein inclusion bodies (P), microvilli (MV), and terminal web (TW). Note the well defined terminal web, small, electron-lucent lipid particles (arrows), and large electron-lucent, supranuclear lipid droplets (L) in the midgut; inset is a magnification of a lipid particle. The lack of a terminal web, the presence of pinocytotic invaginations (PI) and vesicles (PV), and electron-opaque, supranuclear protein inclusion bodies are apparent in the hindgut. The lumenal, mucosal surface is in the upper right corner of each micrograph. Other abbreviations: GA, Golgi apparatus; N, nucleus. (Scale bars are 2 μm for *a* and *b*; 1 μm for inset; after Watanabe & Sawada 1985).

from that of adults, reflecting differences in feeding. For example, spot larva have retrorse conical teeth on the upper (premaxillary) and lower (dentary) jaws, but dentary teeth are lost in adults. Larval teeth are used for grasping rather than masticating food as prey are typically ingested whole.

Only three of the four enveloping tunicae typical of the adult fish alimentary canal (Kapoor et al. 1975, Reifel & Travill 1977, 1978, 1979) are present in larvae. A mucosa of absorptive epithelium, a muscularis consisting of a single layer of circular smooth muscle, and a serosa of fibrous connective tissue is present, but a muscularis mucosa or submucosa is absent until transformation. Longitudinally oriented smooth muscle in the muscularis is absent in larvae and does not develop until transformation. Whereas the longitudinal folds of the larval foregut compare well with the convolutions of the esophagus of adult fishes, the mid- and hindgut mucosa lack the distinct villi and crypt-like indentations of adults (Reifel & Travill 1979). Instead, the mucosa forms shallow rugae.

Digestive mechanisms

The function of the larval mid- and hindgut has received considerable attention inasmuch as their functions are in some ways analogous to the function of the anterior and posterior intestines of adult stomachless fishes. Moreover, there has been some controversy over the mechanisms of digestion and absorption in the mid- and hindgut (O'Connell 1976, Govoni 1980, O'Connell 1981). Cytological evidence suggests that the large supranuclear, vacuolar, electron-lucent structures of the midgut mucosal epithelial cells are the result of lipid absorption after lumenal hydrolysis to fatty acids and monoglycerides and intracellular resynthesis to lipids (Iwai 1968a, 1968b, 1969, Iwai & Tanaka 1968a, 1968b, Tanaka 1972a, 1972b, Umeda & Ochiai 1975, Stroband & Kroon 1981, Watanabe & Sawada 1985). In contrast, the acidophilic, granular, electron-opaque, supranuclear inclusion bodies of the hindgut epithelial cells are the result of pinocytotic absorption of macromolecules from

the gut lumen. In addition, the terminal web underlying the striated border of the midgut shows no signs of interruption during digestion, whereas the apical plasma membrane of the hindgut shows numerous invaginations especially in recently fed larvae (Iwai 1969). Tanaka (1972b) showed that inclusion bodies become indistinct with the development of a stomach with gastric glands during transformation, but remain present in adult stomachless fishes. Govoni (1980), however, found similar supranuclear acidophilic inclusion bodies in juvenile spot that possess a functional stomach as well as in larvae that did not. Furthermore, O'Connell (1981) did not report lipid deposits in the midgut epithelial cells and found that the granular, acidophilic inclusion bodies of the hindgut epithelium stained heavily with OsO_4, a reagent that stains lipids black (O'Connell 1976).

Recently, Watanabe (1981, 1982a, 1984a), using HRP as a histochemical tracer of protein, observed pinocytotic absorption and intracellular digestion of macromolecular protein in the hindgut mucosal epithelium and revised Iwai's (1969) proposed mechanism of protein absorption. When larvae were injected with the HRP solution and their hindgut tissues incubated with H_2O_2, the epithelial cells showed HRP reaction products within inclusion bodies (Fig. 5). Control larvae showed no reaction products. It was clear that HRP was absorbed into the cells without losing enzymatic activity, i.e., without hydrolysis into peptides or amino acids.

Absorbed HRP molecules were digested in the epithelial cells through 5 successive stages: pinocytosis, transport, accumulation, digestion, and extinction (Fig. 6). Pinocytosis of HRP molecules occurred along the intermicrovillous plasma membrane. Within the cell, membrane-bounded, pinocytotic vesicles moved toward the nucleus. HRP was then accumulated in supranuclear inclusion bodies by the coalescence of vesicles. Lysosomes, presumably derived from Golgi, became associated with these inclusion bodies soon after their formation. HRP molecules in the supranuclear inclusion bodies finally lost their enzymatic activity as indicated by a waning of reaction products, probably as a result of lysosomal hydrolysis, and

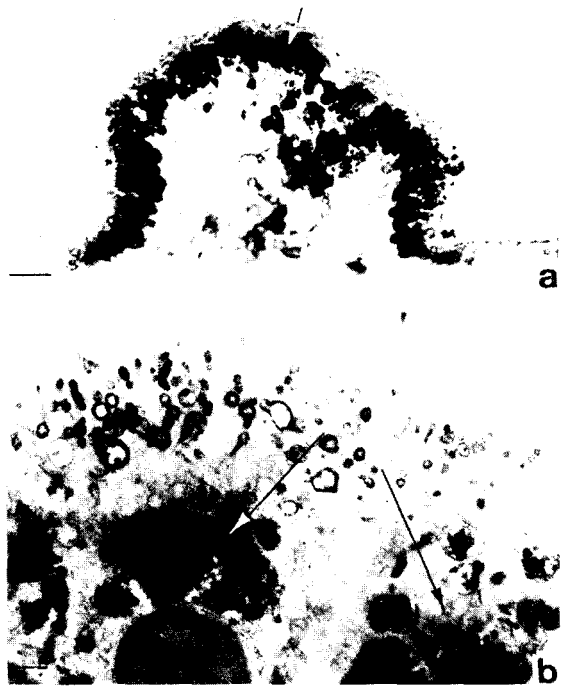

Fig. 5. Photomicrograph (*a*) and transmission electron micrograph (*b*) of the horseradish peroxidase (HRP) histochemistry of the hindgut epithelium of larval *Cottus nozawae*. Note granular and electron-opaque reaction products of HRP in pinocytotic vesicles and supranuclear inclusion bodies (arrows). (Scale bars are 30 μm for a; 1 μm for b).

eventually became extinct.

The time required for complete digestion of HRP differed with species from 10 to 24 h in pond smelt, *Hypomesus transpacificus nipponensis*, to 1 to 2 weeks in cherry salmon, *Oncorhynchus masou*. The intracellular digestion time also depends on the developmental interval of fish, e.g., it was shorter in larvae than in juveniles.

Most fish larvae lack a stomach with functional gastric glands until the completion of transformation, while the larvae of some taxa develop a functional stomach before yolk absorption and first oral feeding. Secretions of gastric glands, pepsin and HCl, effect preliminary protein digestion, thereby facilitating the complete hydrolysis of proteins to peptides and amino acids through the action of trypsin, chymotrypsin, and aminopeptidase in the mid- and hindgut. One might expect greater pi-

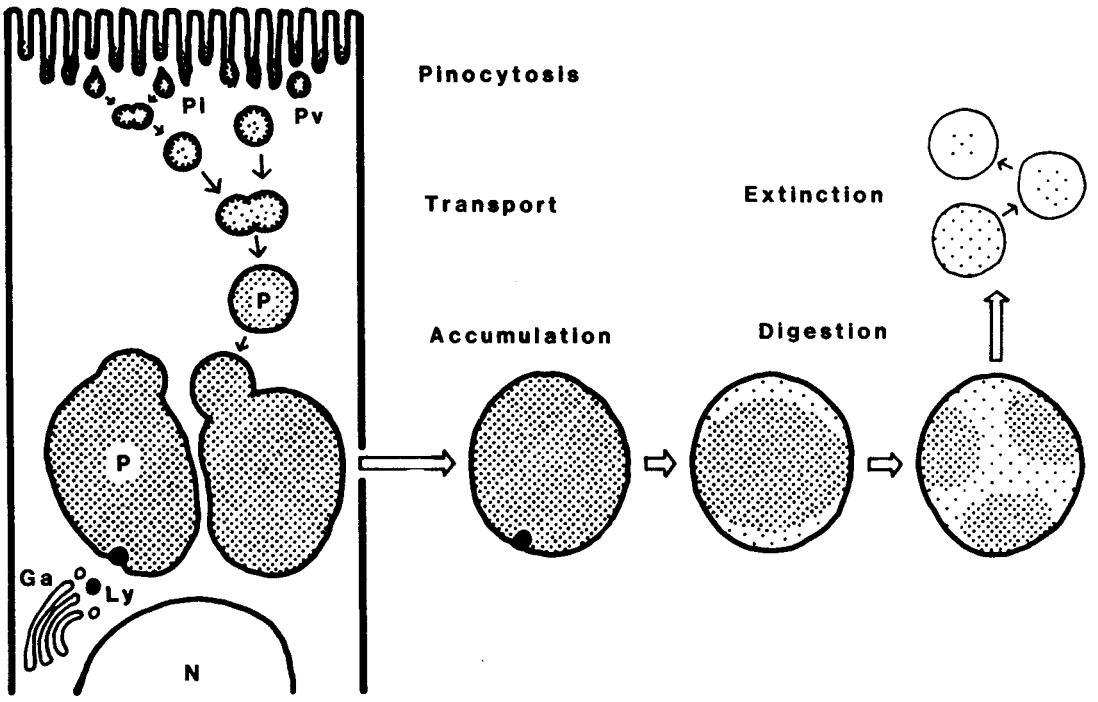

Fig. 6. Successive, 5 stages of protein absorption and intracellular digestion by the hindgut epithelial cells of fish larvae. Organelles include pinocytotic invaginations (Pi), pinocytotic vesicles (Pv), protein inclusion bodies (P), Golgi apparatus (Ga), lysosomes (Ly), and the nucleus (N). (After Watanabe 1984a).

nocytotic absorption of partially digested macromolecular proteins by the hindgut epithelial cells in fish larvae and adults that lack a stomach and preliminary protein digestion. Cherry salmon may serve as an example of a fish larvae with a functional stomach (Watanabe 1984c); pond smelt may serve as an example of a more typical fish larva without a stomach (Watanabe 1984b). Based on the absorption of HRP, the capacity to pinocytotically absorb proteins appeared before yolk absorption and first feeding in both species and persisted long after the development of gastric glands (Fig. 7). The amount of protein absorbed from food by pinocytosis, as indicated by the number of electron-opaque granules in epithelial inclusion bodies, was much greater in pond smelt larvae before gastric development than in cherry salmon larvae (Fig. 7). Pinocytotic absorption diminished in pond smelt after transformation. Pond smelt illustrated the typical pattern of fish larvae, the mechanism of protein digestion and absorption changes from pinocytosis and intracellular digestion to extracellular digestion and membrane transport with the development of gastric glands, whereas in cherry salmon, the digestive mechanism of larvae is similar to that of adults. Pinocytotic

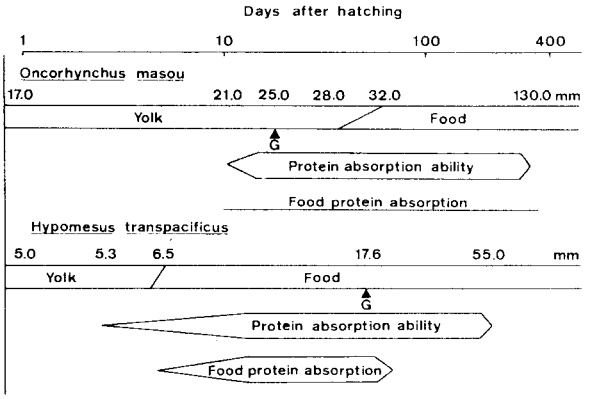

Fig. 7. Post-hatching changes in digestive mechanisms of *Oncorhynchus masou* and *Hypomesus transpacificus nipponensis*. (After Watanabe 1984d).

Formation of functional gastric glands are indicated by (G).

absorption and intracellular digestion of proteins lends a possible adaptive advantage. This mechanism of digestion may compensate for incomplete digestion by allowing for the assimilation of macromolecular proteins and is apparent in the undeveloped alimentary canals of some other chordates, including some fetal and infant mammals (see reviews in Yamamoto 1982, Gauthier & Landis 1972) as well as in larvae and adult stomachless fishes (Yamamoto 1966, Kapoor et al. 1975, Noaillac-Depeyre & Gas 1976, Weinberg 1976, Stroband 1977, Stroband & Van Der Veen 1981).

Lipids are apparently digested to fatty acids and monoglycerides in the midgut lumen, absorbed into the midgut epithelium, resynthesized in the agranular endoplasmic reticulum, and deposited in large lipid droplets (sensu Watanabe & Sawada 1985) of the mucosal epithelial cells (Iwai 1969), but the exact mechanism of lipid absorption is not known. Similarly, lipids are digested and absorbed in the anterior intestine of adult fishes (Barrington 1957, Kapoor et al. 1975, Fange & Grove 1979). Lipids observed in the midgut of fish larvae and the anterior intestine of adult stomachless fishes (Noaillac-Depeyre & Gas 1974, 1976, Stroband & Debets 1978) are thought to be temporary storage (Tanaka 1972a, Stroband & Dąbrowski 1979, Watanabe & Sawada 1985).

The morphological position and mechanism of carbohydrate absorption is unexplored in fish larvae. The high densities of mitochondria in the midgut epithelial cells, some of which are associated with lipid droplets (Iwai & Tanaka 1968a, Iwai 1968b, 1969), suggest that these cells are energetically active and capable of active transport. Whether effected by non-saturable influx or by

Table I. Digestive enzyme activities reported for fish larvae. Positive signs (+) indicate presence of enzyme activities; negative signs (−) absence; NA indicates that the enzyme was not assayed.

Species	Age range	Pepsin	Trypsin	Chymo-trypsin	Amino pepti-dase	Lipase	Amylase	Maltase	Authors
Salmo gairdneri	first feeding to 110 d	NA	+	+	+	NA	NA	NA	Lauff & Hofer (1984)
S. gairdneri	first feeding to 66 d	+	+	NA	NA	NA	NA	NA	Dąbrowski (1982)
S. gairdneri	hatching to 60 d	+	+	NA	NA	NA	+	+	Kawai & Ikeda (1973a)
Coregonus hybrids	first feeding to 130 d	NA	+	+	+	NA	NA	NA	Lauff & Hofer (1984)
Coregonus pollan	first feeding to 42 d	+	+	NA	NA	NA	NA	NA	Dąbrowski (1982)
Plecoglossus altivelis	yolksac to transformation	+	+	NA	NA	NA	+	NA	Tanaka, Kawai & Yamamoto (1972)
Esox lucius	18 d	+	+	NA	NA	−	−	NA	Szlaminska (1980)
Cyprinus carpio	hatching to 125 d	+	+	NA	NA	NA	+	+	Kawai & Ikeda (1973b)
Gadus morhua	hatching to 30 d	NA	+	NA	NA	NA	NA	NA	Hjelmeland et al. (1983)
Acanthopagrus schlegellii	hatching to 35 d	+	+	NA	NA	NA	+	NA	Kawai & Ikeda (1973b)
Dicentrarchus labrax	hatching to 30 d	+	+	+	NA	NA	NA	NA	Alliot, Pastoureaud & Treller (1977)
D. labrax	2 to 60 d	+	+	+	NA	NA	NA	NA	Vu (1983)

sodium-dependent active transport (Crane 1975, Ferraris & Ahearn 1984), the mechanism of carbohydrate absorption is unknown.

Enzymology

Enzyme assays of the alimentary canal in fish larvae indicate that pepsin, trypsin, chymotrypsin, and amylase activities are apparent in several freshwater and marine fish larvae (Table 1). Maltase and aminopeptidase activities are present in the two species so far examined. Surprisingly, lipase activities have not been reported in the larval alimentary canal, although lipase has been assayed in only one species. The liver and the mucosal epithelium are likely the sources of lipase secretion (Kapoor et al. 1975), but zymogen granules, the histological evidence of secretory enzyme precursors, have not been reported in hepatocytes or mucosal epithelial cells of fish larvae. The pancreas is well developed at hatching in northern anchovy and spot, and its acinar cells are invested with conspicuous, acidophilic, zymogen granules, presumably the precursors of trypsin and chymotrypsin (O'Connell 1976, Govoni 1980). Aminopeptidase, another secretion of the mucosal epithelium has not been observed as zymogen. The origin of maltase is unknown in fishes (Kapoor et al. 1975). Pepsin, a secretion of the gastric mucosa, is not apparent (in those fish larvae that lack a stomach) until gastric glands in the developing stomach become functional during transformation (Tanaka et al. 1972, Kawai & Ikeda 1973b, Alliot et al. 1977, Vu 1983).

There is some indication that the activities of digestive enzymes are low at first feeding and increase during the larva period before transformation. Kawai & Ikeda (1973b) observed that activities of amylase, maltase, and trypsin increased in bulk assays of larvae of increasing age after yolk absorption. In contrast, Hjelmeland et al. (1983), using the more sensitive and specific radio-immunoassay technique, observed that after an initial increase betweeen hatching and yolk absorption, trypsin and trypsinogen activities decreased to low levels for 14 d, then increased.

There are two possible explanations for the possible increase in carbohydrolytic and proteolytic enzyme activities in fish larvae after yolk absorption and first feeding. First, the enzymes that are inherent in the food of fish larvae may increase as a result of increasing ration size. Second, enzyme production by the larval liver, pancreas, and mucosal epithelium may be stimulated in response to initial food consumption and increasing ration size. Lauff & Hofer (1984) found that exogenous trypsin activated by the high pH (9) of the hindgut of whitefish larvae, *Coregonus*, accounted for a high percentage of the total tryptic activity. Enzyme stimulation has not been observed, but the deterioration of pancreatic zymogen granules (O'Connell 1976, Theilacker 1978) and of proteolytic enzyme activities in the alimentary canal (Dąbrowski 1982, Hjelmeland et al. 1983) of starved fish larvae implies that enzyme production is variable.

Histochemistry

Beyond the histochemical application of HRP to define the mechanism of protein digestion and absorption, few studies have examined the histochemistry of the developing alimentary canal. Prakash (1961) found increasing activities of alkaline phosphatase in the striated border of the hindgut of rainbow trout, *Salmo gairdneri*, larvae with the greatest changes in the intensity of activity during transformation. Ikeda (1959) related the activities of alkaline phosphatase to the development of a functional stomach in Japanese killifish, *Oryzias latipes*. Inasmuch as alkaline phosphatase activity is associated with sodium-mediated active transport in absorptive tissues (Ugolev 1965), changes in the intensity of alkaline phosphatase reactions may indicate increased absorption through active transport.

Peristalsis and digestion rates

Constriction of the single layer of smooth circular muscle in the muscularis that progresses posteriad in a wave-like manner effects the transport of food toward the anus of fish larvae. Peristalsis is apparent in young Atlantic menhaden (*B. tyrannus*), spot, and rockfish embryos. Blaxter (1969) and

Rosenthal & Hempel (1970) reported that *Artemia* nauplii move rapidly and undigested through the midgut to the hindgut of Atlantic herring, *Clupea harengus harengus* larvae. Pedersen (1984), in contrast, observed that ingested copepods passed through the foregut of herring within seconds, passed through the anterior midgut within minutes, and stopped in the posterior midgut at the ileocaecal valve. Copepods remained in the posterior midgut and hindgut for several hours. Constrictions of the ileocaecal valve also check the transport of food from the mid- to the hindgut in Atlantic menhaden and spot larvae.

Fish larvae lack the layer of longitudinally aligned, smooth muscle in the muscularis that is characteristic of adults, but some have ancillary means of transporting food. Iwai (1964, 1967a, 1967b), Iwai & Rosenthal (1981), and Watanabe (1984b) have observed ciliated cells and cilia movement that aid in the transport of food in the alimentary canals of some plecoglossid, osmerid, clupeid, and salangid larvae. Ciliated cells have also been observed in some adult fishes with poorly developed musculari (Ferraris & Ahearn 1984).

The passage of food through the larval alimentary canal (Table 2) occurs at rates that are somewhat faster than rates observed in adult fishes (Kapoor et al. 1975, Fange & Grove 1979). Extremely

Table 2. Evacuation rates and times of fish larvae as determined in the laboratory.

Species	Age or length	Feeding protocol	Food	Temperature (°C)	Evacuation rate	Evacuation time	
Clupea harengus	12 mm		*Artemia salina*			19	Kurata (1959)
C. harengus	10 to 14 mm	Continuous feeding	*A. salina*			4 to 10	Rosenthal & Hempel (1970)
C. harengus	8 to 12 wk		*A. salina*		−0.09 to −0.84	3 to 7+	Werner & Blaxter (1980)
C. harengus	8–22 d	Single feeding	Copepod nauplii and polychaete larvae	6		12.5 to 22.5 h	Fossum (1983)
C. harengus	22–52 d	Continuous	zooplankton	9.5		40 min to 3 h	Pedersen (1984)
Coregonus clupeaformis	19–20 mm	Single feeding	Copepod nauplii	14		16	Hoagman (1974)
Cyprinus carpio						1 to 8	Chiba (1961)
Abramis brama	12–18 mm	Single feeding	*Bosmina longirostris*			5	Sorokin & Panov (1966)
Micropterus salmoides	2–8 d	Continuous feeding	zooplankton	17 to 23		2.0 to 2.8	Laurence (1971)
M. salmoides	2–8 d	Single feeding	zooplankton	17 to 23		3.8 to 5.2	Laurence (1971)
Lagodon rhomboides	15 to 18 mm	Single feeding	copepods	12	0.8		Kjelson & Johnson (1976)
Leiostomus xanthurus	16 to 20 mm	Single feeding	copepods	17	0.24		Kjelson & Johnson (1976)
L. xanthurus	7 to 47 d 1.8 to 9.4 mm	Single feeding	*Brachionus plicatilis*	20		5 h	Govoni et al. (1982)
Scomber japonicus	3–5 d	Single feeding	*B. plicatilis*	19	0.5 to 2		Hunter & Kimbrell (1980)
Pseudopleuronectes americanus		Continuous feeding	zooplankton	8		5.1 to 8.4	Laurence (1977)

rapid rates have been observed with some clupeoid larvae. In one report, bay anchovy, *Anchoa mitchilli*, defecated within minutes after eating (Chitty 1981). In Atlantic menhaden and spot larvae, however, defecation begins 1 to 2 h after eating. Larval age does not markedly affect the evacuation rate of herring larvae (Pedersen 1984). Evacuation rates are faster for larvae fed continuously than for larvae fed a single ration (Blaxter 1965, Laurence 1971) and are positively related to ration size (Werner & Blaxter 1980). Evacuation rates are also positively related to temperature (Laurence 1971). In any case, digestion and assimilation in fish larvae are rapid. By visual inspection, Fossum (1983) observed the digestion of copepod nauplii and polychaete larvae within 1.5 h following their ingestion by Atlantic herring larvae. Govoni et al. (1982) observed that larvae respired $^{14}CO_2$ within 3 h following the ingestion of ^{14}C-labeled food, which indicates that food is digested, assimil-

Table 3. Assimilation efficiencies and the coefficient of utilization of fish larvae.

Species	Age, length or weight	Food	Food concentration	Method of determination	Assimilation efficiency (%)	Coefficient of utilization (%)	Author
Anchoa mitchilli	10 to 100 μg dry weight	Copepod nauplii	1000 l^{-1}	gravimetric		24 to 41	Houde & Schekter (1983)
Clupea harengus	6 wk	*Artemia salina*	10^{-6} to 10^{-3} l^{-1}	radiotracer (^{14}C)		38 to 68	Boehlert & Yoklavich (1984a)
Cyprinus carpio	1–5 mg dry weight	zooplankton	10 to 100 mg l^{-1}	calorimetric	74 to 87		Filatov (1972)
Abramis brama	12 to 18 mm	*Bosmina longirostris*	20 to 2000 l^{-1}	radiotracer (^{14}C)	74 to 83		Sorokin & Panov (1966)
Micropterus salmoides		zooplankton	200 to 1200 l^{-1}	calorimetric		80	Laurence (1971)
Archosargus rhomboidalis	10 to 100 μg dry weight	copepod nauplii	500 l^{-1}	gravimetric	44 to 75		Houde & Schekter (1983)
Leiostomus xanthurus	7 to 47 d 1.8 to 9.4 mm 0.022 to 2300 μg dry weight	*Brachionus plicatilis*	10^5 to 10^6 l^{-1}	radiotracer (^{14}C)	67 to 99	9 to 65	Govoni et al. (1982)
Paralichthys dentatus	1 to 56 d	zooplankton	unlimited	nitrogen assay		27 to 68	Buckley & Dillman (1982)
Limanda limanda	0 to 32 d	oligochaetes unlimited calorimetric	unlimited		99		Calculated from Edwards et al. (1969)
Pleuronectes platessa	0 to 45 d	ologochaetes unlimited calorimetric	unlimited		96 to 99		Calculated from Edwards et al. (1969)
Pseudo-pleuronectes americanus	16 to 668 μg dry weight	copepod nauplii	2000 to 3000 l^{-1}	calorimetric		69 to 74	Laurence (1977)
P. americanus	16 to 23 d	zooplankton	>2000 l^{-1}	nitrogen assay		20	Cetta & Capuzzo (1982)
Achirus faciatus	10 to 100 μg dry weight	copepod	1000 l^{-1}	gravimetric		34 to 54	Houde & Scheckter (1983)

ated, and metabolized within this short period.

Assimilation

Mass and energy budgets (physiological energetics) as well as radiotracer experiments indicate that the assimilation efficiency of fish larvae (Table 3) is near or somewhat below the assimilation efficiency of adult fishes (Kapoor et al. 1975, Conover 1978, Brett & Groves 1979). Assimilation efficiency (the percentage of a food ration that is assimilated after loss to feces (Brett & Groves 1979)) ranges from 67 to 99% for fish larvae, whereas assimilation efficiency ranges from 80 to 90% for adults. Coefficients of utilization (the fraction of matter or energy retained after losses to defecation of feces as well as metabolic excretion of urine (Winberg 1956)) for fish larvae are also generally lower than they are for adults. The coefficients of utilization ranges from 9 to 80% for larvae, whereas coefficients average about 70% for adults (Ware 1975).

The effects of food type and ration size on assimilation efficiencies are poorly known for fish larvae. In stomachless pipefish larvae, *Syngnathus fuscus*, Ryer & Boehlert (1983) noted slower evacuation with smaller ration and suggested that assimilation efficiency would increase at low ration when the residence time of individual food particles increased. In Atlantic herring larvae, Werner & Blaxter (1980) observed that high rations resulted in food that was apparently less digested when defecated; indeed, at the highest ration, *Artemia* nauplii passed rapidly through the alimentary canal and were defecated alive. This observation coupled with the extremely rapid evacuation times in bay anchovy larvae (Chitty 1981) implies that large rations may result in low assimilation efficiencies, especially for clupeoid larvae with straight alimentary canals. This has been experimentally confirmed for Pacific herring larvae, *C. harengus pallasi*; the uptake and retention of ^{14}C-labeled food decreased significantly with increasing food concentration and, therefore, ration (Boehlert & Yoklavich 1984a). Herring larvae have straight guts. It would be of interest to repeat these experiments to determine if percoid larvae with coiled guts show the same change in assimilation efficiency, inasmuch as the feeding strategies of species with differing gut morphologies may differ, as demonstrated by the work of Houde & Schekter (1980, 1983). The clupeoids are adapted to utilize contagiously distributed prey in high concentrations; although assimilation efficiency and gross growth efficiency may decrease, the total energy intake increases (Fig. 8), resulting in a net energetic gain to the larva. Similarly, comparing a wide variety of fish larvae, Checkley (1984) noted that gross growth efficiency showed a peak at moderate food densities and decreased thereafter, a pattern similar to adult fishes (Brett & Groves 1979).

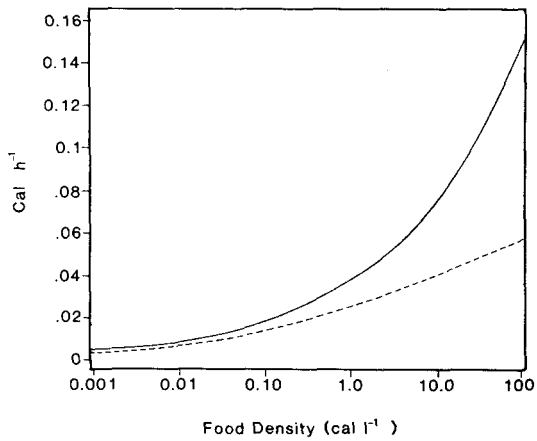

Fig. 8. The relationship of calories consumed (solid line) and calories assimilated (dashed line) as a function of food density for 6 wk-old *Clupea harengus pallasi* larva feeding on *Artemia* nauplii. Ingestion rate increases with food density for this species (Werner & Blaxter 1981). Although assimilation efficiency decreases with increasing ingestion rate, the magnitude of decrease with increasing ingestion rate is more than compensated for by increased ingestion such that the total energy assimilation continues to increase. (From Boehlert & Yoklavich 1984a).

Some work has indicated that the low assimilation efficiencies of some fish larvae improve during the larva period, while other research has failed to demonstrate improved efficiencies until transformation with its attendant elaboration of alimentary canal morphology. By subtracting the amount of energy in growth and metabolism from the energy in food, Laurence (1977) reported increasing assimilation efficiencies during the larva period before transformation. In contrast, Sorokin & Panov (1966) and Govoni et al. (1982), by using ^{14}C as a

tracer, and Houde & Schekter (1983), by subtracting the energy of growth and metabolism from the energy of food, reported no change in assimilation with development before transformation in several other species. Buckley & Dillmann (1982) measured the nitrogen excreted as ammonia in urine and defecated as primary amines in feces by summer flounder larvae, *Paralichthys dentatus*. They reported that the rate of defecation of primary amine, representing the nonassimilated fraction of ingested nitrogen, decreased with age, while the rate of ammonia, representing the assimilated and metabolized fraction, decreased until 35 h after hatching, then increased. More importantly, the coefficient of nitrogen utilization increased with age during the larva period. Cetta & Capuzzo (1982) found a similar pattern of nitrogen loss in the larvae of another flatfish, *Pseudopleuronectes americanus*. Pinocytosis and intracellular digestion of protein macromolecules is possibly a less efficient mechanism of protein digestion and assimilation than hydrolysis to amino acids and active transport across the lumenal plasma membrane (Gardner 1984). While this might account for the overall lower assimilation efficiencies of the fish larvae, what accounts for changes in assimilation during the larva period before transformation? Some of proteolytic enzymes indicate that enzyme activities increase during the larva period before transformation, and Buckley & Dillmann (1982) invoked these increases to explain increasing assimilation. This explanation is plausible, but there is no corresponding cytological evidence of an increase in the capacity of the pancreas to produce these enzymes; the pancreas has conspicuous zymogen granules at yolk absortion and these remain obvious throughout development. This lack of apparent morphological or cytological change that could cause a shift in the efficiency of digestion and assimiltion is puzzling.

Improvement of assimilation efficiency is likely with the development of a functional stomach with gastric glands and pyloric caeca at transformation due the digestive contribution of pepsin and hydrochloric acid and the increased absorptive surface area of the alimentary canal. Mironova (1974) reported increasing assimilation efficiency of *Oreochromis mosambicus* that was concomitant with transformation.

Recommendations for future research

We know that the alimentary canal of fish larvae is less complex than that of adults and that digestion and assimilation in larvae may be less efficient as a result. The activities of proteolytic enzymes are apparently lower in larvae, but may increase as larvae grow before transformation. In some flatfish larvae, nitrogen assimilation improves during the larva period, but measures of calorie and carbon assimilation with larvae other than flatfish have indicated no improved assimilation before transformation. For most fish larvae there is no apparent elaboration of alimentary canal tissues after yolk absorption and before transformation; alimentary canal development is not well studied among flatfish.

Research on the physiology of digestion in fish larvae has faced several technical difficulties. The small size of larvae, their mostly planktonic habitat, and the unavailability of natural prey sources to experimenters, among other problems, have all contributed variability to the range of results reported herein. Consequently, several questions remain to be answered. Inasmuch as it has bearing on models of larval growth and survival, the question of changing digestive and assmilative abilities with larval development before transformation warrants the most immediate attention. Several avenues of research to address this issue are possible.

The length as well as the absorptive surface area of the alimentary canal should be measured and related to larval length. The morphometric methods of Hughes (1984) could be used for this purpose. Such comparisons would indicate disproportionate increases in the absorptive surface area with development and might offer an explanation for improving assimilation.

A histochemical examination of developmental stages of various species should also contribute to the resolution of changing assimilation. Such an examination should focus on lipase and esterase because histochemical evidence of these enzymes

would indicate the capability of larvae to digest and absorb lipids. The histochemistry of alkaline phosphatase, because of its association with sodium-mediated active transport, and acid phosphatase, because of its association with supranuclear inclusion bodies and pinocytotic absorption (Ugolev 1965, Watanabe 1984a), should also be considered. We recommend the histochemical methods of Higuchi et al. (1979) with the intensity of histochemical reactions quantified with image-analysis densitometry.

The question of enzyme stimulation should be resolved by conducting carefully controlled experiments wherein developmental stages of different species are starved and then fed for various intervals, and the activities of digestive enzymes measured. Because of its sensitivity in distinguishing specific antibody and antigen reactions and its capacity to detect inhibited as well as active trypsin, we recommend radio-immunoassay (Hjelmeland 1983, Hjelmeland et al. 1983) for measurement of trypsin activities. Perhaps radio-immunoassays could be developed for other digestive enzymes.

A comparative approach to these recommendations would be clearly advantageous, for morphological and physiological differences among taxa are great. Studies on digestive physiology elucidate the feeding strategies and growth potential of fish larvae and thereby contribute to our understanding of fish larvae survival.

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