Cyprinid Nutrition (and ideas on standardized diets)

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Outline of the talk

- Common carp
- Digestive tract
- Protein
- Lipids
- Carbohydrates
- Vitamins
- Minerals
- Reference diets
- Conclusions

Common carp (*Cyprinus carpio*)

- Cultured for centuries in Asia (mainly China) and in Eastern Europe
- Raised in ponds, often with other carp species in integrated systems
- Most-studied cyprinid for nutritional data
- Is a "stomachless" fish

Digestive tract in fish

- Pharynx – swallowing; sometimes toothed
- Esophagus – transport to stomach
- Stomach – Storage and physical breakdown of food; begin chemical breakdown with pepsin and HCl
- Intestine – Finish chemical breakdown (bile and digestive enzymes); absorption of nutrients
- Associated organs like liver and pancreas

Fish digestive systems

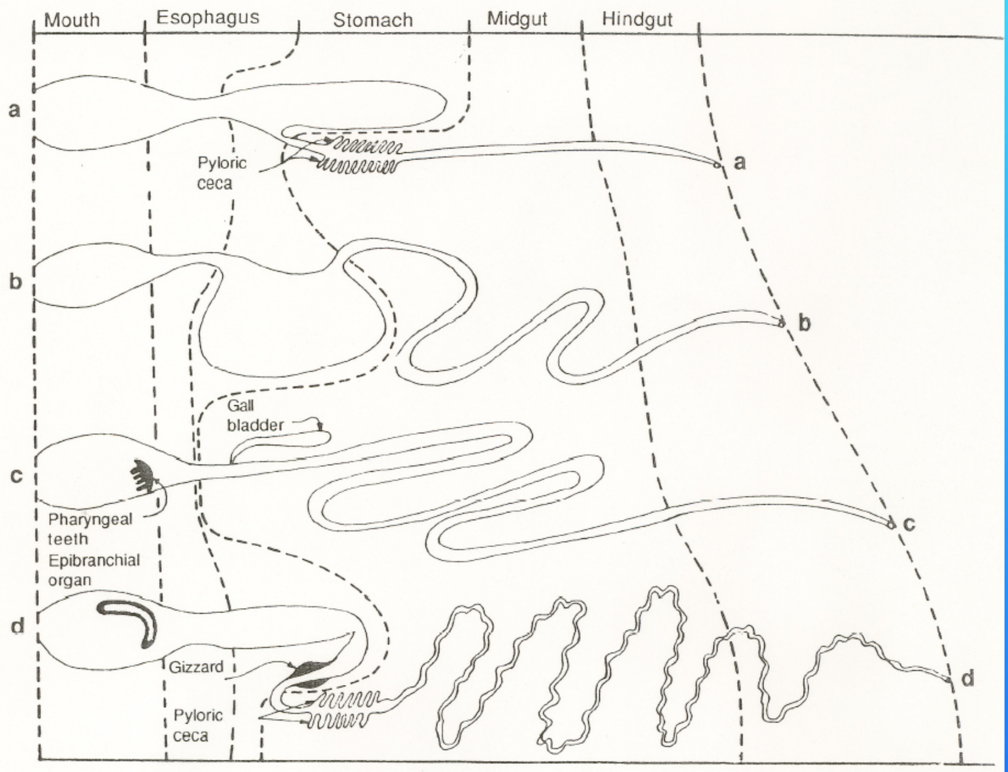

FIG. 7.5

Diagrammatic representation of typical digestive configurations. (a) Rainbow trout (carnivore, Y-shaped stomach); (b) catfish (omnivore emphasizing animal sources of food, pouched stomach); (c) carp (omnivore emphasizing plant sources of food, stomach absent); (d) milkfish (microphagous planktivore, tubular stomach with muscular gizzard). (From Smith, 1980.)

Cyprinid digestive tracts

- Many (all?) are stomachless – ca. 1500 species, not all investigated
- True stomach has *gastric glands*, which produce pepsinogen and HCl
- Zebrafish appear to lack a true stomach, but do have a stomach-like structure as part of the intestine
- Herbivorous species have longer intestines than do carnivorous species

Protein

- Proteins are made of amino acids
- Ten of the 20 amino acids are *essential*, which means that they cannot be synthesized by vertebrates and have to be supplied in the diet
- We determine the quantitative requirement for a given amino acid by preparing diets with graded levels of the AA, feeding the diets to fish, and measuring their growth

"Dose-response" results of nutritional experiments

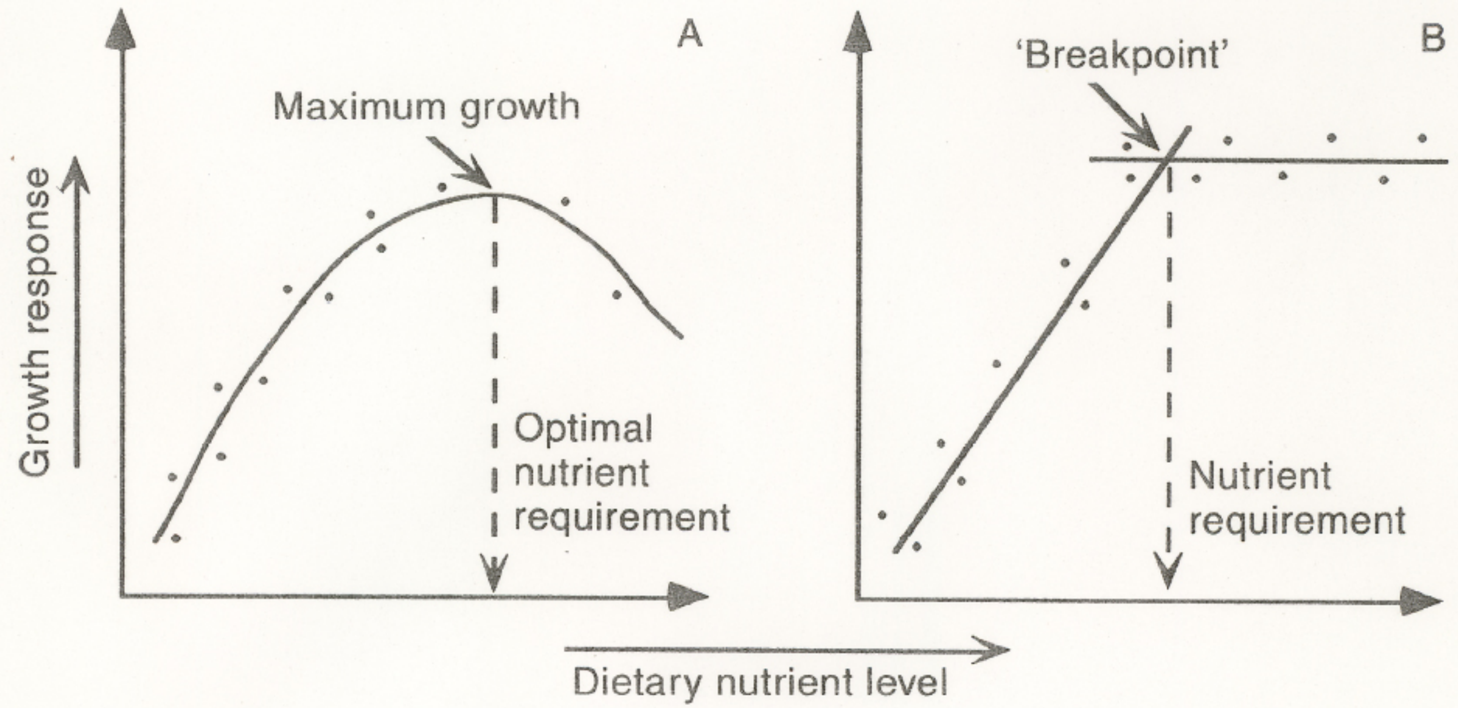

Figure 12.1 The two types of growth responses that are generally observed in nutritional requirement experiments.

Amino acid requirements of common carp (as % of protein)

- Arginine – 3.8-4.3
- Histidine – 1.4-2.1
- Isoleucine – 2.3-2.5
- Leucine – 3.3-4.1
- Lysine – 5.3-5.7
- Methionine – 1.6-3.1
- Phenylalanine – 4.9-6.5
- Threonine – 3.3-3.9
- Tryptophan – 0.3-0.8
- Valine – 2.9-3.6

What we know about the amino acid requirements of zebrafish

Protein odds and ends

- Younger fish usually have higher protein requirements than older fish of the same species
- Larval fish often require more digestible protein than juvenile fish (e.g., live feed)
- Broodstock fish may have different requirements from juvenile fish
- Amino acids in excess of levels required are metabolized for energy, amine portion becomes ammonia that fish excrete and foul the water with (so it's best to have AA's in exact amounts)

Lipids

- Made of fatty acids (and other things)
- Certain fatty acids that cannot be synthesized are considered *essential* and must be provided in the diet
- We (and fish) synthesize saturated fatty acids (16-C and 18-C chains with no double bonds) and can modify them into monounsaturated fatty acids by incorporating one double bond

Lipids (cont.)

- Polyunsaturated fatty acids (two or more double bonds) have to be provided in the diet – these are referred to as the n-3 and n-6 series of fatty acids, based on the position of the first double bond along the carbon chain
- The quantitative requirements for n-3 and n-6 fatty acids vary by species

Common carp lipid requirements

- Linoleic acid (18:2n-6) requirement is 1.0% of dry diet – from this, carp can chain elongate and desaturate the molecule to arachidonic acid (20:4n-6)
- Linolenic acid (18:3n-3) requirement is 0.5-1.0% of dry diet – from this, carp can chain elongate and desaturate to 20:5n-3 and 22:6n-3 (EPA and DHA, resp.)
- These molecules are important in membrane structure and also serve as precursors to eicosanoids (prostaglandins, etc.)

What we know about zebrafish lipid requirements

Carbohydrates

- Carbohydrates serve only as an energy source in fish – there are no *essential* carbohydrates, but fish do have an overall dietary energy requirement
- *Structural* carbohydrates (e.g., cellulose, chitin) have β linkages that are difficult to break
- *Storage* carbohydrates (e.g., glycogen, amylose) have α linkages that are easy to break
- Cyprinids have long intestines that allow the breakdown of both structural and storage forms of carbohydrates

Vitamins

- Vitamins are compounds required, usually in very small quantities, for a wide variety of normal physiological/biochemical functions in the body
- Water-soluble vitamins (most of them)
- Lipid-soluble vitamins (A, D, E, K)
- Qualitative requirements can be determined by leaving a vitamin out of a diet
- Quantitative requirements are determined by providing a graded series of diets and measuring growth, assessing pathology, etc.

Vitamin requirements of common carp (units/kg diet)

• Vitamin A –	4,000 – 20,000 IU
• Vitamin E –	100 mg
• Thiamin –	0.5 mg
• Riboflavin –	4 – 7 mg
• Vitamin B_6 –	5 – 6 mg
• Pantothenic acid –	30 – 50 mg
• Niacin –	28 mg
• Biotin –	1 mg
• Choline –	1,500 mg
• Myoinositol –	440 mg

What we know about vitamin requirements of zebrafish

Minerals

- Of the 90 naturally occurring elements of the periodic table, 29 are known to be essential for animal life
- Living matter is mainly C, H, N, O, S
- Fish require some macroelements in g/kg quantities (Ca, Mg, P, Na, K, Cl)
- The remaining “trace” elements are required in mg or μg per kg quantities (As, Br, Cd, Cr, Co, Cu, F, Fe, I, Mb, Mn, Ni, Pb, Se, Si, Sn, Vn, Zn)

Minerals (cont.)

- We don't have nearly the database on dietary mineral requirements of fish as we do on vitamin requirements, because
  - Fish can take up these elements from water across their gill surfaces
  - Marine fish drink seawater for osmoregulation and take up these elements in intestine
  - It's difficult to formulate a test diet that has none of what you're studying

Minerals (cont.)

- Because there are so many minerals in sea water, we only have to worry about mineral deficiencies in freshwater fish
- Even so, it’s very unusual to see mineral deficiencies

Common carp mineral requirements

- Phosphorus – 0.6-0.7% of diet
- Magnesium – 0.05% of diet
- Iron – 150 mg/kg of diet
- Copper – 3 mg/kg of diet
- Manganese – 13 mg/kg of diet
- Zinc – 15-30 mg/kg of diet

What we know about mineral requirements of zebrafish

So if we don't know anything about zebrafish nutritional requirements…

What do we do?

There is hope

- Not to worry…we don't know the nutritional requirements of most of the species we raise in aquaculture
- We can make some best guesses
- We're not trying to provide diets at minimal cost
- Carp requirements might be a good approximation of zebrafish needs

Diet standardization

- There have been a few attempts to develop standard or reference diets for fish (and other aquatic organisms) used in research
- Key principle: The diet has to be standard, not necessarily optimal or ideal, so that the organisms will be in comparable physiological condition.

Aquatic toxicity research

- About 30 years ago, need for standardization of aquatic toxicity testing practices in government, industry, and academic labs
- American Society for Testing and Materials provided a vehicle for writing consensus standard methods, including rearing of test organisms
- EPA lab in Narragansett, RI was interested in effects of nutrition on toxicity test results

EPA - Narragansett

- Research on formulated diets for juvenile fish, but toxicity testing was beginning to focus on larval stages
- Research on nutritional variation in brine shrimp nauplii from different geographical sources (AA's, FA's, organochlorine pesticides, heavy metals, naupliar size) and impacts on survival and growth of larvae of several marine species
- Bottom line: Fatty acid composition of nauplii was the key factor – dependent on the algae that the female brine shrimp were eating when they produced the cysts

Effect of *Artemia* nauplius size on larval fish survival

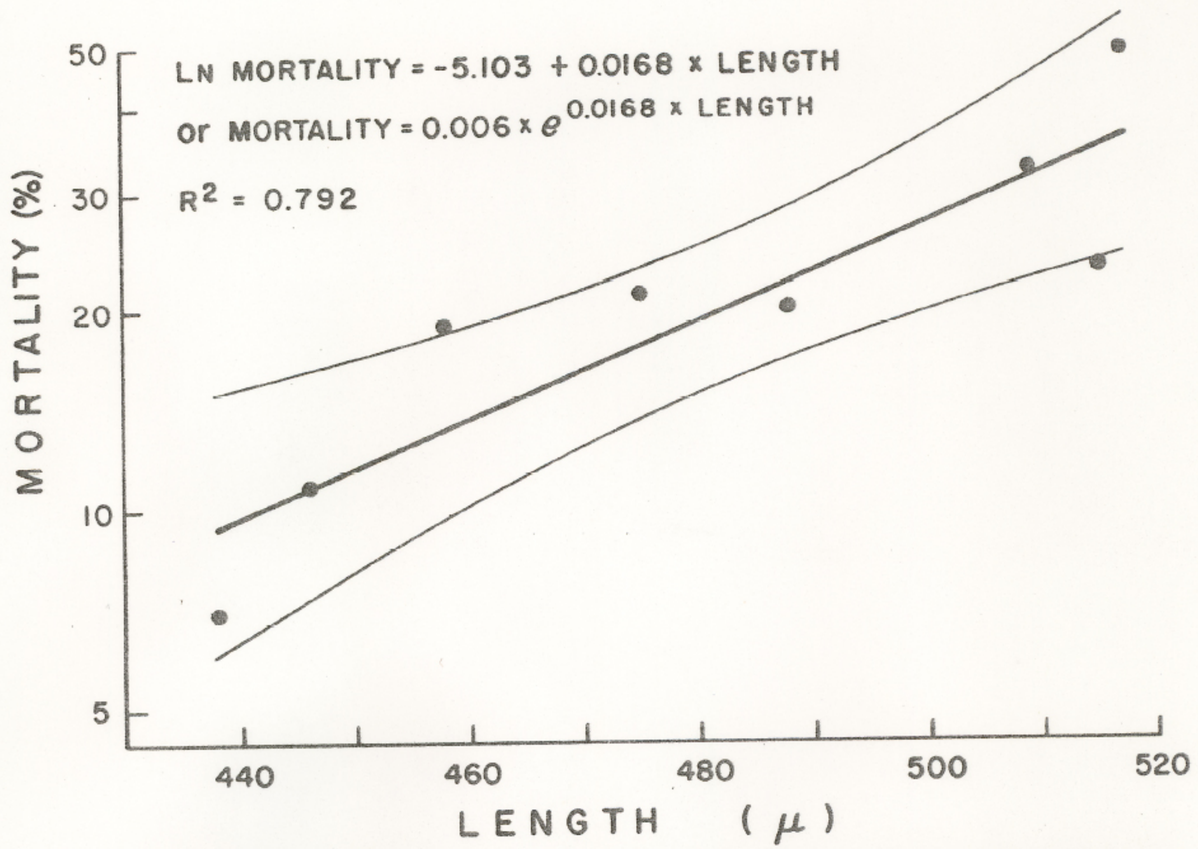

FIG. 2—*Natural logarithm of mortality of Atlantic silversides during the first 5 days of the experiment as a function of the mean length of eight strains of* Artemia *nauplii fed to the fish. The data are from the strains tested in this study and that of Beck et al* [4]. *The bold, straight line is the regression line; the thinner, curved lines represent 95 percent confidence limits.*

Nutritional impacts on toxicity test results

- *Mysidopsis bahia* 48-h LC_{50} with Cd
  - Brazil – 98.5 ppb (95% CL = 85.3 – 113.8)
  - San Pablo Bay – 64.4 ppb (55.9 – 71.9)
- *Menidia menidia* 96-h LC_{50} with Cd
  - Brazil – 821 ppb (716 – 946)
  - San Pablo Bay – 298 ppb (266 – 322)
- *Cyprinodon variegatus* 96-h LC_{50} with endosulfan
  - Brazil – 1.21 ppb (1.10 – 1.36)
  - San Pablo Bay – 1.02 ppb (0.93 – 1.15)

Results of brine shrimp research

- EPA characterized and purchased a batch of Reference *Artemia* Cysts (RAC) to be used in all their toxicity testing
- ASTM standard E1203 was published "Standard Practice for Using Brine Shrimp Nauplii as Food for Test Animals in Aquatic Toxicology"
- ICES Reference enrichment media for brine shrimp (fatty acid research)

Formulated diets

- Efforts have also been made to provide reference formulated diets for aquaculture research:
  - Through World Aquaculture Society Nutrition Task Force, purified Standard Reference Diet for Crustacean Nutrition Research
  - Through ICES Working Group on Marine Fish Culture, Standard weaning diet (for research on transition from live feed to formulated diet)

U.S. fish hatcheries

- U.S. Fish and Wildlife Service typically contracted for annual production runs of trout and salmon diets for all of its hatcheries, according to USFWS specified standards, for quality assurance of its hatchery outputs.

Zebrafish

- My understanding is that the normal feeding sequence is *Paramecium*, brine shrimp nauplii, dry diet of some sort plus brine shrimp. I believe a good strategy would be to agree on the feeding sequence, then to:
  - Standardize *Paramecium* culture practices
  - Develop Reference *Artemia* for zebrafish
  - Develop/adopt standard formulated diet

Good Luck!!