BARD

FINAL REPORT

PROJECT No. I-385-81

Stable Isotope Ratios as Naturally Occurring Tracers in the Aquaculture Food Web

G. Schroeder, D. Buck, D. Coleman

1986

30
630.72
BAR/SCH
2ND COPY

a. COVER PAGEDate June 1986

BARD

P.O. Box 6

Bet Dagan, ISRAEL

BARD Project No. I-385-81

Title

Stable isotope ratios as naturally occurring tracers in the
aquaculture food web.

Investigators' Names

(Principal listed first)

Gerald L. Schroeder

D. Homer Buck

Dennis Coleman

Investigators' Institutions

Israel Agricultural Research
Organization. Bet Dagan, Israel

Illinois Natural History Survey.
Kinmundy, Illinois

Illinois State Geological Survey.
Champaign, Illinois

Project's starting date: December 1982

Type of Report: 1st Annual ——— 2nd Annual ——— Final ☒

Signatures:

Principal Investigator

Institution's Authorizing Official

*Gerald L. Schroeder**Yuval Eshdat*

Dr. YUVAL ESHDAT
Head, Div. for Research Projects and Budgets
Agricultural Research Organization

Fishes - Feeding and feeds
Fishes - Growth
Carbon - Isotopes
Radioactive tracers

636.085
639.31
541.389.681
545.82

630.72; 636.085; 639.31; 541.389.681; 545.82

BAR

SCH

2nd copy

7-1-88 1 1000 100
1000 1000 1000
1000 1000 1000 1000 1000
1000 1000 1000 1000 1000

b. Table of Contents

	Page
a. Cover Page	1
b. Table of Contents	2
c. Abstract	3
d. Objectives	4
e. Body of Report (Israeli contribution)	4
f. Description of Cooperation	20
g. Evaluation of Research Achievements	20
h. Benefits to Agriculture	29
i. List of Publications	29
j. American Contributions	32

c. Abstract

Ratios of the stable isotopes of carbon were found to be reliable indicators of the foods assimilated during animal growth. In a variety of tank and pond environments, the range of delta C values in the natural foods was sufficient to differentiate among several of the potential feeding niches via the delta C of these niches. The range of delta C values of foods typically used in fish feed (and in animal husbandry in general) is sufficiently wide to permit preparation of formulated feeds distinguishable by their delta C. In manure-loaded ponds yielding 30 kg fish growth/ha/day, quantitative estimates of carbon flow, based on measured rates of fish yields and associated delta C's of these fish, indicate that food webs accounting for half or more of all natural food production, autotrophic and heterotrophic, were harvested by the target fish. In standing water ponds receiving full rations of pellets, more than 1/2 of the common carp, tilapia, silver carp and prawn growth was based on natural foods of these ponds.

d. Objective of original research proposal.

The objective of this research was to determine the usefulness of the ratio of the stable isotopes of carbon, ^{13}C : ^{12}C (reported as δC) as a tool for studying food webs in fish ponds. Using δC , we hoped to quantify the contributions of natural foods within specific trophic niches of the ponds toward the growth of the target animals. The ponds were to receive either feed pellets or agricultural residues as sources of added organic matter.

e. Body of Report

THE STABLE ISOTOPES OF CARBON AS NATURALLY OCCURRING TRACERS IN THE
AQUACULTURE FOOD WEB

Gerald L. Schroeder, Fish and Aquaculture Research Station,
ARO, Dor, Hof HaCarmel 30-820

INTRODUCTION AND BACKGROUND

Much of the information presented herein has been presented in detail in the publications listed in this final report.

The aquaculture food web has been the subject of many investigations; however, the relative contributions of supplied feeds and fertilizers to growth of fish or other target organisms remain

unknown. In some cases the target organism yield may be attributable to the pond functioning, in effect, as a sunlit rumen wherein mineral and organic fractions of the feeds and fertilizers are converted by autotrophic and heterotrophic activity into a complex of algae, bacteria, protozoa and their exudates which serve as the food base for fish growth (Schroeder, 1978; Hobbie and Lee, 1980). Microscopic analyses of fish intestine contents identify only recently ingested foods and often reveal large masses of unidentifiable organic matter. Such analyses do not quantify which of the foods were metabolized during the growth of the fish. Algae or plants grown in the presence of ^{14}C or ^{32}P are adequately labeled to trace their metabolism and incorporation into the fish tissues. However, use of radioactively labeled substrates or foods is limited to confined systems. Naturally occurring tracers are able to follow the metabolic pathways of potential feed constituents. The ratio of the stable isotopes of carbon, ^{13}C : ^{12}C , which vary characteristically among the tissues of plants and algae, offers the potential of a naturally occurring tracer. Because these are naturally occurring and ubiquitous, large amounts of "labeled" substrates are readily available and affordable. They can be used with no exceptional environmental contamination in tank and small pond studies as well as in commercial-sized ponds.

STABLE ISOTOPE FRACTIONATION

Naturally occurring carbon is composed of two stable isotopes differing by one neutron mass: 99% of the C atoms have an atomic mass of 12 (^{12}C); 1% have a mass of 13 (^{13}C). This distribution is reflected in the composition of atmospheric CO_2 as $^{12}\text{CO}_2$ or $^{13}\text{CO}_2$. During photosynthesis, plants utilize these different molecules at differing rates. Two general types of plant exist (Park and Epstein, 1961; Smith and Epstein, 1971). Using the commonly accepted notation of:

$$\delta C = \delta C = \left(\frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right) \times 1000 \text{ (per mil)}$$

where the standard is PDB carbonate (Craig, 1957), C-3 type plants (e.g., temperate grasses, legumes, alfalfa, cotton) have $\delta C \sim -23$ to ~ -32 , while C-4 type plants (e.g., tropical grasses, corn, sorghum, sugar cane) have $\delta C \sim -10$ to ~ -16 .

Animals incorporate into their tissues the carbon isotopes of their food with little or no differentiation between isotopes (DeNiro and Epstein, 1978). Hence the isotopic composition of an animal body is expected to match the isotopic composition of the foods it has metabolized.

We have studied aquaculture food webs using delta C as an indigenous, natural tracer. The studies reported herein follow the progression: measure the delta C of homogeneous samples using several methods of sample preparation to learn the influence of these methods on the final delta C value; measure the delta C of several potential feeds to facilitate selection of feeds with large differences in delta C; measure the delta C of microbial growth, oxic and anoxic, on several of these feeds to determine whether sufficient isotopic fractionation occurs to distinguish, via delta C, between the substrate and the microbial growth; measure the delta C of selected tissues taken from animals fed one of several single-component, known delta C diets to learn the relation between feed and body delta C; measure, in freshwater fish ponds, the delta C of natural foods, supplied feeds and the target animals grown in these ponds in an attempt to identify the sources of growth of these animals.

METHODS AND MATERIALS

Sample preparation

The delta C of African fishmeal and local corn meal were measured using several methods of preparation. Samples were either dried at 90° C to constant weight (overnight) or lyophilized. Five to 15 mg portions of these dried samples were then flame-sealed in either previously evacuated, 5 mm dia, 1 mm wall thickness, 15 cm long, quartz or borosilicate tubes containing 100 mg of oxidized copper

powder plus 2 to 5 cm of 5 micron silver wire. The quartz tubes were heated to 850° C for 16 h. The borosilicate tubes were heated to 500° C for 4 to 16 h. After cooling to room temperature, each tube was mounted on a gas purification line. The line was evacuated. The tube was cracked (see Coleman, 1981, for a description of the tube cracker). The gaseous combustion products passed into the line, through a spiral cooled to -70° C in alcohol-liquid nitrogen mixture (to freeze the water vapor) and continued to a borosilicate tube cooled in liquid nitrogen (-270° C). After a 0.5 to 2 minutes collection period, the line was re-evacuated. At the low pressure now established only carbon dioxide remained frozen in the tube at liquid nitrogen temperature. This tube was flame-sealed, warmed to room temperature and stored for future delta C analysis.

Alternatively, a 5 to 15 mg, dried sample was placed in a ceramic boat over which a stream of oxygen slowly passed. the boat was heated to 850° C. The combustion products, carried by the oxygen stream, passed through a -70° C spiral (to freeze out the water vapor) and then to a spiral at liquid nitrogen temperature to freeze the carbon dioxide of combustion. After combustion was complete, the spiral containing the frozen carbon dioxide was isolated by valves, evacuated, warmed to -70° C and the liberated carbon dioxide frozen into an adjacent borosilicate tube at -270° C. This tube was then flame sealed and the sample stored for delta C analysis.

Delta C analyses for samples prepared in Israel were performed on isotope separation mass spectrometers located at the Hebrew University

in Jerusalem with occasional replicates analyzed at the Illinois State Geological Survey and at the Petroleum Institute in Wuxi.

Controlled feeds and feeding experiments

The delta C values of several feed ingredients used in aquaculture and animal husbandry, locally and abroad, were measured. To learn, for a given feed, the distribution of delta C values between the soluble and insoluble portion, samples were separated into neutral detergent fiber, NDF (Goering & Van Soest 1970), and soluble extract. Delta C was measured for each component.

Ingredients representing the range of delta C values usually observed in natural foods, -11 to -32, were selected for controlled feeding experiments. Tilapia hybrids (*Nilotica* x *Aurea*), common carp or prawns (*Macrobrachium rosenbergii*) were individually stocked in aerated, 10 to 40 liter, indoor aquariums. A single feed ingredient (e.g., corn meal) was fed to each aquarium at a daily rate of 2% to 10% of the animal weight, 4 days/week. Each feeding regime was in triplicate. Subdued light restricted algal growth. Aquarium floors were cleaned once per 10 days. After a 7-fold weight gain, samples of flesh were cut from the dorsal area of fish and tail flesh of the prawns. Occasionally scales and fins were sampled. Molts were collected during the prawn growth tests.

To learn the distribution of delta C values within the body of a large animal, a male sheep was raised on C-3 type grass for 1 year. Upon slaughter, portions of muscle, hair, plasma and heart were analyzed for delta C.

Outdoor-pond feeding experiments.

Earthen, standing water, freshwater ponds (400 m², 0.75-1.5 m deep) were stocked with tilapia nilotica x aurea hybrids (TH), common carp (CC), silver carp (SC), grass carp (GC) and the prawn (P), Macrobrachium rosenbergii. Stocking densities ranged from 10,000 to 20,000 fish/ha. Prawns, when present, were stocked at 5,000 to 20,000/ha. The banks of all the ponds were covered with fist-sized to melon-sized rocks to reduce bank erosion. Water temperature during the 90-day growth periods from fingerling to market-size varied from 25-32°C. Make up water, about 1 cm/day, was added to replace seepage and evaporation. Water alkalinity was approximately 260 mg CaCO₃/l. Water chlorinity was approximately 300 ppm.

Two rates of organic input to the ponds were used. The intensely manured, lightly fed (IM) system consisted of sorghum pellets supplied at one third of the rate of pellet application in the intensely fed ponds plus stored chicken manure initially supplied at a daily rate (based on manure dry weight content) of 50 kg/ha. This was increased over the season to a maximum of 200 kg/ha per day. The intensely fed, lightly manured (IF) system consisted of 25% protein content pellets (15% fish meal, 15% soy meal and 70% sorghum or wheat) supplied at full ration (nominally a daily rate of 2% of the TH biomass plus 6% of the CC biomass reducing to 4% of the CC biomass when the individual CC weight exceeded 150 g/m) plus stored chicken manure once per week at one day's dose of the IM pond treatment. In 3 ponds, fluid goose

manure was added at a rate such that dry weight input was similar to that of the intensely manured ponds receiving chicken manure.

Feeding rates were adjusted every 14 days by netting and weighing samples of the fish. The feed was supplied via a demand feeder. Manure was cast from the pond bank over 5-10% of the pond area. The feed/manure inputs were provided 6 days per week.

Three earthen 1000 m² ponds were stocked at densities of 2,000 to 6,000 fish/ha. These ponds received no organic inputs. Once in 2 weeks, 30 kg/ha each of agricultural grade super phosphate and ammonium sulfate were added (Hepher, Milstein and Leventer, work in progress).

Cages in cement ponds.

Rigid plastic net cages, minimally 1m cubic, with 1 cm net opening, were set in 1m deep, cement-lined, 100 m² standing water, freshwater ponds. Cages were stocked with juvenile prawns, approximately 1g each, at densities of 5 to 30/m². Water temperatures ranged from 24° C to 31° C. Fish meal enriched feed pellets, containing 20% to 40% protein, were supplied to each cage once/day, 6 days/week, at daily rates of 2% to 10% of the prawn biomass. In each experiment, 1 or 2 cages received no feeds other than the growth of natural foods. In one experiment, fish were stocked outside the cages in an attempt to deplete the supply of natural foods.

RESULTS AND DISCUSSION

Delta C values vs methods of sample preparation

The delta C measured for homogeneous samples was found to be independent of the method of preparation, for those methods described above, provided that the combustion period exceeded 8 h. For a homogeneous sample analyzed on a single mass spectrometer, replicate analyses agreed to within ± 0.1 o/oo of the average of these values. With shorter combustion periods, occasional spurious values were observed. The reliability of 500° C combustion permitted use of borosilicate tubes in place of the more costly and more difficult to-work-with quartz tubes.

Agreement among the 3 mass spectrometers compared in this study was within 0.5 o/oo. Therefore, observed trends in delta C could be compared between Israeli and U.S. data, while detailed analysis of the data was best confined to results taken on a single instrument.

Delta C of feeds and feed fraction

The delta C values of several feeds and grasses used in fish farming are listed in Table 1. Considering that the delta C data are reproducible to within 0.2 o/oo, these ingredients have a sufficient spread of delta C values (total range is 21 o/oo) to permit preparation of manures and feeds readily identifiable by their delta C.

TABLE 1

Representative delta C values of organic matter used in aquaculture

<u>C-4 type plants</u>	<u>Delta C</u>	<u>C-3 type plants</u>	<u>Delta C</u>
Sugar cane	-11	Oats	-24
Corn syrup	-11	Wheat	-25
Corn silage	-11.5	Copra meal	-25.5
Corn meal	-12	Soybean oil meal	-25.5
Oil from this corn	-15	Soybean oil	-29
Sorghum halepense	-12	Banana	-25
Pineapple	-13.5	Rye	-26
Sorghum dura	-17	Barley	-27
Tropical grasses:		Polished rice	-27
Eluesine indica (yard grass)	-14	Paddy rice	-28
Pennisterum purpureum		Rice bran	-29.5
(elephant grass)	-14	Grapes	-25
Sorghum sudanese		Mulberry leaf	-27
(sudan grass)	-14	Silk worm paupa	-27
Vallisneria spiralis		Cotton seed	-27.5
(a water plant)	-16.5	Sweet potato	-28
Fish meals:		Tobacco	-28
South African	-17	Many legumes	-24 to -28
Oil from S. African	-22	Tomato	-28
Philippine	-17	Beta vulgaris (sugar beet)	-30
Costa Rican	-19	Temperate grasses:	
Anchovy meal	-16.5	Hordeum spontineus Koch.	-23
Squid meal	-18.5	" " " grains	-22
		Sporobolus virginicus	-25
		Lolium perenne (rye grass)	-26
		Arrhenatherum botchyi. Boiss	-26
		Trifolium repens L. (colver)	-26
		Eichhornia crassipes	
		(water hyacinth)	-27
		Alternanthera philoseroides	
		(water peanut)	-29.5

Corn silage and alfalfa hay were each separated into NDF and soluble fractions. For corn silage (grains removed) the solubles delta C was 0.5 to 1.0 o/oo less negative than the fiber delta C. For alfalfa hay, the solubles delta C was 0.4 to 1.5 o/oo more negative than the fiber delta C. Since the delta C of the total matter includes both fiber and solubles, and since solubles are considered to be more digestible than fiber, the animal's body might reflect this delta C difference.

In all cases for plant and animal tissues, extracted lipids were 3 to 5 o/oo more negative (i.e., containing relatively more ^{12}C) than the whole plant or animal muscle from which they were extracted. This appears to reflect a phenomenon occurring at pyruvate oxidation.

In the biokinetics of energy metabolism and lipid formation, at the stage of oxidation of pyruvate to acetyl coenzyme A, there appears to be preferential storage of molecules relatively enriched in ^{12}C and respiration of molecules relatively enriched in ^{13}C (DeNiro and Epstein, 1977). An indication of this ^{13}C respiration is the observation reported by Tyrrell et al. (1984), that the delta C of carbon dioxide exhaled by cows is 2 to 8 o/oo less negative (i.e., enriched in ^{13}C) than the feed delta C.

Delta C of microbial growth vs delta C of the growth substrate

We observed no consistent differences between the delta C of microbial slimes and the substrates on which they were grown. For both aerobic and anaerobic fermentations, the resultant slimes had delta C

values within 0.5 o/oo of the feed stock. In fermentation of corn meal and soybean oil meal (SBOM), the delta C of the liberated carbon dioxide was also within 0.5 o/oo of the substrate delta C. Therefore, it was not possible, via delta C, to distinguish between original and fermented feeds or manures. This means that it was also not possible, via delta C, to determine whether target animals assimilated feed directly or following microbial processing of that feed.

Range of delta C values within individual animals

It is useful to be able to sample the delta C of a fish without the need to kill or to homogenize the entire animal. For this reason, we performed several analyses on scales, fins and flesh taken from the same fish. The data are shown in Table 2. Consistently, we observed an order of increasingly lighter (more negative) delta C from scales to fins to flesh. The goal of this exercise was to be able to predict the delta C of the fish flesh since this represents the food of the fish. The quantitative relationship between scales and flesh does not appear to be precise. However, for an approximate indication of the flesh delta C prior to harvest, the delta C of the fins is useful. This can be taken to be approximately 1-2% heavier than that of flesh for common carp and tilapia, and 0-3% heavier for silver carp.

Carbonate in the scales and fins accounts for their delta C being different from the muscle delta C. In mature animals, bone carbonate is ~8 o/oo less negative than the diet being metabolized. This results from an 8 o/oo isotopic fractionation in the equilibrium between

TABLE 2

Examples of delta C of fish scales, fins and flesh, and prawn flesh and shell

Fish	Delta C		
	Scales	Fins	Flesh
CC	-18.8	-19.1	-19.9
		-21.5	-22.9
TH	-21.0	-22.2	-23.1
		-24.3	-26.5
		-25.5	-26.1
SC	-25.5	-29.7	
		-26.5	-29.6
		-28.7	-28.4
Prawns		Flesh	Shell
Prawns grown on two diets			
Fish meal		-14.5	-14.5
		-14.4	-14.1
		-14.2	-14.2
Soymeal (50%		-19.8	-19.3
weight gain		-19.4	-22.0
on this diet)		-18.7	-19.8
		-18.6	-19.0

dissolved CO_2 and HCO_3 in aqueous solutions at body temperature. The CO_2 is formed by oxydation of the diet (and so has the delta C of the diet and the body muscle if the diet has been constant). Bone carbonate is precipitated from the carbonate dissolved in the blood. Therefore bone carbonate reflects this 8 o/oo difference. Soaking the fish fins in HCl (to release the carbonate-C) prior to preparation for delta C analysis resulted in fin and muscle delta C being equal. With this acid treatment, mg portions of fin clipped from the live fish can be used to measure the fish delta C.

The delta C of molts or shell of prawns and Penaeid shrimp was found to be similar to the animal's flesh delta C provided that the diet had been constant. When we changed the diet from C-4 (corn) to C-3 (SBOM), or C-3 to C-4, newly shed molts had delta C values which were between the delta C's of these feeds. This implies that the molts were formed from existing body tissues plus the current foods. The molts were not effective in determining the current diet.

We studied tissues of an adult male sheep raised on C-3 grasses (delta C -23 to -26). The deposited fats, hard and soft (delta C -28.2), were 5 o/oo more negative than its protein-rich tissues (muscle -23.4; hair -23.4; intestine wall -23.4; plasma -23.5; bone marrow -24.4).

The close and quite consistent relation between feed and muscle delta C makes the muscle a reliable indicator of which feeds were assimilated.

Delta C of feed vs delta C of animal body

The relation between food delta C and muscle delta C is presented in Figure 1. The range of feeds covers most of the range normally observed in nature, from -11 o/oo for C-4 plants (in this case corn grains) to -31 o/oo for C-3 plants and micro-algae. The data for the common carp (marked CC in Figure 1) all originate from a single fish. Initially this CC was grown to 2 g on planktons (delta C -26 to -31), then to 20 g on corn (delta C -11.4), then to 170 g on soy meal (delta C -24.4). At each stage a few mg of muscle was cut from the dorsal region. The delta C of the CC flesh mimicked the changes in feed delta C. For this entire delta C range of feeds tested and for all the animals included in this study, the delta C of the muscle tissue averaged 0.8 o/oo (S.D. 0/8 o/oo) less negative than the feed. DeNiro and Epstein (1978) reported similar food-flesh delta C relations, but using multi-ingredient diets. Differences in ingredient digestibilities can effect the observed flesh delta C. The purpose of our work was to determine the food-flesh delta C relation when the animal consumed a single feed and therefore had a relatively narrow spread in the delta C of the ingested compounds.

In one study we added a poorly digestible ingredient, rice bran, to a corn meal pellet. As expected, fecal matter was enriched in the bran delta C while flesh delta C was biased toward the more digestible corn delta C.

For 3 years, growth trials were conducted with prawns in cages set in cement-lined ponds. The results of the delta C data were

1. The relation of the δC of muscle tissue of several animals to the δC of their diets.

consistent: natural foods were consistently major contributors to prawn growth. Only when efforts were made to deliberately suppress the development of the natural foods did prawn growth reflect the feed pellets. Growth of prawns in cages not receiving pellets, usually was similar to, or equalled, growth in cages receiving pellets for situations in which natural foods were allowed to develop. Delta C data for a representative prawn growth experiment are listed in Table 3. The prawn delta C differed from the pellet delta C by 3.5 o/oo, but only by 0.8 o/oo from the zooplankton (daphnia dominant) delta C. Based on these data, zooplankton appear to have been a main source of assimilated food in the prawn diet.

When the range of delta C values in the target animal's diet was small, delta C was not useful in identifying sources of growth. Such was the case described in Tables 4 and 5. The 4 feeds (Table 4) had delta C values between -18.1 and -25. The delta C range of the adult prawns was only -18.1 to -20.0, including those prawns held in cages not receiving pellets. In the presence of abundant natural foods (East Pond, Table 5), the growth in all cages, including non-fed cages, was equal. The fact that prawns receiving the "Phil" feed pellet (delta C -25) had delta C values (-19 to -20) similar to the prawns receiving the other feeds (-18.5 to -19), indicates that the highly negative delta C feed ingredients of these feeds (Table 4) were not efficiently assimilated.

TABLE 3.

Delta-C and related data for prawns grown in cages set in cement-lined ponds.

<u>Specimen</u>	<u>delta-C (o/oo)</u>
Prawn flesh	-20.2 $\pm$ 0.4
feed pellet	-23.7 $\pm$ 0.2
zooplankton	-21.0 $\pm$ 0.4
filamentous algae	-25.8 $\pm$ 0.8

Notes:

Prawns stocked at 1 g and harvested at > 30 g.

Pellets supplied 6 days per week at 2 to 4% of prawn biomass.

Pellet composition: wheat 45% (delta C ~ -24); SBOM 20% (delta-C ~ -25); fish meal 15% (delta-C ~ -19); meat meal plus oil 10%; sorghum 10% (delta-C ~ -17).

Daphnia were dominant in the zooplankton.

Data are the averages of 4 or more analyses, $\pm$ 1 std deviation.

TABLE 4.

Composition of feed used in cage feeding trials described in Table 5

	Ingredients of Feed Pellets							
	Fishmeal	SBOM	Chicken oil	Sorghum	Copra meal	Rice	Rice bran	Pellet delta-C
delta-C o/oo -----	-18	-25	-27	-17	-25	-27	-29	
Pellet composition (o/oo) -----								
G25 + oil	15	20	5	60	--	--	--	-19.0
G25 - oil	15	20	--	65	--	--	--	-18.5
G35	20	25	--	55	--	--	--	-19.0
Phil	15	15	--	--	15	45	10	-25.0

TABLE 5.

The effect of various feeds on prawn growth and delta-C in cages, for sparse versus abundant natural foods situations delta-C and weights of prawns at harvest, grown in cages set in cement-lined ponds.

		West Pond sparse natural foods		East pond abundant natural foods	
		Prawn delta-C (o/oo)	Prawn Avg Wt. (g)	Prawn delta-C (o/oo)	Prawn Avg Wt. (g)
<u>Diet name</u>	<u>Diet delta-C</u>				
G25 + oil	-19	-18.3	28	-18.1	29
G25 + oil	-19	-18.3	28	-17.7	30
G25 - oil ³	-18.5	-19.2	30	-19.0	25
G35	-19	-19.1	26	-18.2	26
G35	-19	-19.2	25	-18.5	27
Phil	-25	-19.8	29	-18.9	30
Phil	-25	-20.0	26	-19.2	29
no feed	--	-20.0	14	-19.5	29

Delta C indications of carbon flow in fish ponds

Table 6 presents a summary of the delta C data taken from several pond treatments. The data reveal several aspects of interest. For a given pond treatment, the delta C of animals of a given species was consistent to $\pm 1\%$. Within the confines of each pond the delta C of all organisms grown extended from -19 to -31 o/oo. This range was sufficient to distinguish clearly among the carbon compositions of the different organisms grown in the pond.

There existed within each of the ponds studied at least 4 sources of natural foods: bottom sediment with a delta C of -24 to -25; rock slime (a mixture of attached algal growth and associated micro-organisms plus mud and precipitated pelagic algae) with delta C of -24 to -28; pelagic microalgae (that seston passing through a 37 um screen and the only size fraction present in concentrations exceeding 0.1 mg dry weight/l pond water; microalgae accounted for >90% of total sestonic primary productivity, PP) with a delta C range of -28 to -33; and chironomids. On those surfaces of the rocks lining the pond banks that were sheltered from fish grazing, chironomids were present in numbers ranging from 0-30 per 20 cm² of rock surface. Chironomids were rarely found on rock surfaces exposed to fish grazing. Fifty chironomids from ponds receiving intense chicken manuring were held in distilled water for 2 - 24 hours to remove gut contents. Their delta C was then -30.8 ± 0.3 . The tube-like slime that surrounded them also had a delta C of -30.8. The adjacent mud had a delta C of -25.

Table 6. Summary of delta C data related to manured and fertilized, earthen, freshwater fish ponds.

Pond treatment:	Chicken manure	Feed pellet +	Fresh fluied	Chemical				
	+ 1/4 sorghum	1/4 chicken manure	goose manure	fertilization only	Feed pellet + 1/6 manure			
Pond area (ha)	0.04	2.6 3.5	0.04	0.1 0.1 0.1	0.04			
No. fish/ha:	12,000	20,000 20,000	10,000	3,000 5,600 2,000	12,000			
Fish yield (kg/ha/day)	40	40 40	30	8 12 7	-26.4			
Target animal	delta C of muscle tissute (or intestine lumen) as PDB 0/00							
Tilapia hybrid (TH)	-23.7(-24.4)	-16.8 -20.3(-21.3)	-20.0	-25.6 -23.1 NS	-26.4			
Common carp (CC)	-20.8	-17.3 -18.8(-19.8)	-17.8	-24.8 -22.7 NS	-22.8			
Grass carp (GC)	-20.5	NS NS	-18.8	NA NA NS	-20.8			
Silver carp (SC)	-31.5(-32.0)	NS -18.0(-23.3)	-21.7	NS -23.8 -20.9	-30.0			
Prawn	-22.4	NS NS	-19.3	NS NS NS	-22.5			
Natural foods								
Dominant phytoplankton	mixed green	100% BG 90% BG 10% scenedesmus	mixed green	mixed greensome BG	mixed green			
Seston>150u	-21M to -34Z	NA NA	-24M to -28Z	NA NA NA	-25 to -28			
150u>seston>37u	-22M to -34A,Z	-20.4A -22.6A	-23M to -30Z	NA NA NA	-25			
Seston<37u	-28 to -33A	-19.6A -23.3A	-25M to -28A	NA NA NA	-28 to -33A			
Epilithic slimes from littoral zone	-24 to -28	-19.3 -20.0	-21 to -27	-22.4 -21.2 NA	-24 to -28			
Chironomid larvae	-30 to -31	-16.7 -19.5	-18 to -23	NA NA NA	NA			
Sediment surface OM	-24.8	-15.6 -20.2	-23.7	-26.3 -25.2 -24.6	-25.0			
Allochthonous carbon added daily at 2 to 4% of the TH + CC biomass								
Manure	-16.8	-17.2 -17.2	-13.0	----	-16.8			
Feed pellet (25% protein)	--	-23.7 -23.7	----	----	-20.7			
Sorghum supplied at 1/3 to 1/4 the manure or pellet rate								
	-17.0	-17.0 -17.0	----	----	----			

Notes to Table 6:

NS = not stocked; NA = not analyzed; OM + organic matter (carbonate-carbon removed by soaking sediment in phosphoric acid prior to delta C analysis); BG = blue green; M = mixed zoo - and phytoplanktons plus detrital OM; Z = > 90% zooplankton; plus detrital OM; A = > 90% algae.

BG algae included oscillatoria, microcystis and spiroolina. No zooplankton were found in these ponds with BG algae dominant.

BG algal globs taken from the bottom of the 2.6 ha pond had average delta C = -20.4. BG algal globs taken from the bottom of the 3.5 ha pond had average delta C = -23.0. The 0.04 and 0.1 ha ponds were at the Dor Research Station on the coastal plain of Israel. The 2.6 and 3.5 ha ponds were in the Jordan valley near Bet Shan.

Single data represent averages of 3 or more individual analyses. The range of data are occasionally listed to indicate the spread of delta C values available to fish feeding in the niche. Standard deviation in muscle tissue delta C was < 1 0/00 for all cases.

Data reported here supercede previously reported data which, especially for sediment organic matter, may have included some inorganic carbon.

It is interesting to note that the chironomids and the SC had an almost identical delta C. It is possible that both of these life forms, the largest and the smallest of the animals visible in the pond environment, graze on micro-algae or micro-organisms derived from microalgae: the SC while the algae are suspended in the water column and the chironomids when the algae have precipitated to the rock surfaces.

It is important to recall that in the intensely manured ponds, manure organic matter (OM) was added at rates similar to rates at which pellets are supplied in pellet-fed ponds, i.e., 2 to 4% of the fish biomass. This averaged ~ 3 g manure organic carbon/m²/day. The average net primary productivity (PP) of these ponds was ~ 5 g carbon/m²/day.

Muscle and intestine delta C data were taken for ponds with dominantly green and dominantly blue-green algae. In the manured ponds with green algae (first column of data in Table 6), the gut contents delta C corresponded to the muscle delta C, indicating assimilation of the ingested food. In the ponds with blue-green algae, only the intestine contents of the SC showed evidence (based on delta C and microscopic examination) of blue-green ingestion. However, the delta C of the muscle was 5.3 o/oo less negative than the blue-green delta C. This indicates an inability of SC to effectively digest these algae. In these pellet-fed ponds, the delta C of the muscle and intestine contents of the TH and CC were quite different from the delta C of the supplied feed pellets although this pellet was provided daily at

the full rate of ~3% of the CC + TH biomass. Natural foods appear to have been selected and assimilated in preference to the supplied pellet.

Of the manure-loaded ponds, the most typical of Israel are those receiving chicken manure with green algae dominant. The delta C of the sediment OM (-25) indicates ~55% origin with microalgae (delta C ~ -32; microalgae accounted for > 90% of total PP) and 45% with the added manures and grains (delta C ~17). This is similar to the relative rates at which these forms of carbon entered the ponds.

For these ponds, the delta C data indicate that 60% of the TH, 25% of the CC and GC, and, 100% of the SC growth originated with food webs based on algal-fixed carbon. The remaining growth is attributable to food webs originating with added manure and/or grains. Gut analyses of fish from these ponds (Spataru, 1982; Spataru et al., 1983 consistently showed unidentifiable OM (subsequently referred to as detritus) for TH and detritus plus zooplankton and zoobenthos for CC, to be the highly dominant components of gut contents. Few if any intact algal cells were observed in the TH guts although the TH delta C indicated a >60% dependence of TH on the algal food web. Comparison of the delta C data taken from the pond environment with the gut content data reported by Spataru indicates that TH harvest upper sediment OM with little discrimination. CC appear to have selected for benthic OM associated with the manure or sorghum (delta C of CC muscle was 3 o/oo less negative than TH muscle and hence considerably more similar to the manure and sorghum delta C than was the TH delta C).

The delta C and gut data for SC (intact and lysed algal cells were the main gut SC gut components) indicate the pelagic harvesting of live algae and associated seston. Only microseston was present in abundances able to satisfy the measured growth rates of SC.

These delta C data were combined with the measured rates of fish growth to calculate the absolute amounts of carbon originating with PP and with manure OM that were metabolized by the target animals. Data for ponds receiving either dried chicken, fresh duck or goose manures are listed in Table 7. For these treatments, the total amounts of carbon fixed in fish growth plus that respired in energy producing reactions were 1.7 to 2.0 g/m²/day. Of this total, the delta C data indicate that for the chicken manured ponds 1.1 g, for the duck manured ponds 0.9 g, and for goose manured ponds 0.8 g carbon originated with algal-based food webs. In these ponds, net PP averaged 5 gm C/m²/day. Thus, approximately 20% of the net algal production was accounted for by fish metabolism. Based on gut analyses, only SC consumed the algae directly as live, intact cells. TH and CC harvested the algal food web as detritus or detritus plus zoobenthos and zooplankton. There are inherent losses in organic carbon across each trophic level in a food web. Moriarty (1985) estimated a factor of 3 for carbon loss in microbial processing of detritus. Invertebrate processing of detritus can result in 10-fold losses in carbon (Warwick, 1985). With these losses, the 0.6 g of processed algal carbon that were metabolized by the TH and CC in the chicken manured ponds originated with at least 2 g, and probably more,

of live algal carbon. This plus the 0.5 g carbon metabolized by SC accounts for at least half the net PP in these ponds. Considering the inefficiencies in harvesting, there is very little algal carbon remaining for additional target animal production.

A yield 30 kg/ha/day fish growth (Fig. 2) requires a daily consumption of 1.5 g food carbon/m²/day. PP supplied 2/3 of this in the manured ponds at Dor (Table 7). The remaining 0.5 g came from the manure based food web. Daily manure input to these ponds averaged 3 g manure carbon/m²/day. Hence at the trophic level of harvest, 15% of the added manure carbon was metabolized by the target animals. The manures used in this work were poor direct foods. The cow manure OM contained <1% pepsin-digestible protein and had >65% fiber content. The chicken manure OM contained ~10% pepsin digestible protein and >55% fiber. Approximately 85% of the OM in fluid cow manure and 80 to 90% of the OM in chicken manure settled to the pond bottom (Schroeder, 1978). As with the detrital algal-based food web, 3 to 10 fold losses can be expected in the microbial processing required to convert the manure crude matter into useful foods after the manure has entered the detritus web. This means that >50% of the manure carbon was consumed in food webs finally metabolized by the target animals.

With current management practices it appears that the attained maximum yields of 30 kg fish growth/ha/day exploits almost the entire base of the 2 main natural food webs (the webs based on PP and on added manure).

Table 7. Data related to carbon consumed by target animal metabolism in manure ponds.

	Harvest	Stocking	Harvest biomass	Daily growth	Carbon respired	% of total nutrition from algal food webs	Algal carbon assimi- lated	respired
	(no./ha)	(g/animal)	(g/m ²)	(g/animal)	(mgC/m ²)	(mg/m ² /d)	(mg C/m ² /d)	
Pond treatment: field-dried chicken manure plus 1/4 ration sorghum								
TH	7275	20	142	2.0	142	568	60	85 340
CC	2630	25	107	4.2	111	444	25	28 112
GC	450	200	43	8.2	37	148	25	9 36
SC	970	225	105	9.4	91	364	100	91 364
P	8450	1	25	0.3	25	100	50	13 52
Sum	11325 (fish only)		422		406	1624		226 904
Pond treatment: fresh duck manure plus dropped duck feed								
TH	10630	10	163	1.5	159	636	~ 60	95 380
CC	5220	40	195	3.5	183	732	~ 25	46 174
SC	950	115	53	4.7	45	180	~ 70	32 128
Sum	16800		411		387	1548		173 682
Pond treatment: fresh goose manure								
TH	8000	20	170	1.6	131	524	50	66 264
CC	2800	30	180	5.2	146	584	35	51 204
GC	380	200	22	3.2	12	48	45	5 20
SC	950	250	70	4.9	47	188	65	31 124
Sum	12130		442		336	1344		153 612

Notes to Table 7:

TH = tilapia hybrid; CC = common carp; GC = Grass carp; SC = silver carp; P= prawn.
Carbon content of fish is estimated to 10% of their biomass.

Carbon respired is calculated to be 4 times the carbon assimilated in growth.

For the fish populations in these ponds this value agrees to within 15% of the values calculated using the Winberg (1971) equation based on individual fish weights.

The fraction of fish growth attributed to algal food webs is based on delta C data presented in Table 6.

CONCLUSIONS

- 1) Measured delta C values for a given organic sample are similar for a variety of sample preparation techniques, including combustion at 500° C or 850° C, provided that the combustion time exceeds 8 h.
- 2) The range in delta C values for feeds used in aquaculture is sufficient to formulate diets with components easily distinguished by their delta C.
- 3) Microbial processing of the wide variety of feeds and manures tested in this work resulted in delta C values in the residual substrate plus microbial slime that equalled to within 0.5 o/oo the delta C of the original substrate. This similarity in delta C between fresh and fermented organic matter means that delta C values can not be used to determine whether supplied feeds were consumed prior to, or following, microbial processing within the pond.
- 4) The delta C of a fish's fin is similar to, and usually 0 to 1 o/oo less negative than the delta C of that fish's flesh. If soaked in acid to remove carbonate-C, the delta C of the residual matter in the fin equalled the delta C of that fish's flesh.
- 5) When fed a simple, constant, digestible diet, fish muscle averaged 0.8 o/oo (S.D. 0.8 o/oo) less negative than the delta C of the feed on which the fish grew. This relationship was for feeds with delta C values ranging from -11 to -30. Muscle delta C is therefore a good indicator of the feed delta C.

6) Delta C data can identify and quantify which natural foods were dominant in the feeding regimes of fish and prawns grown in standing water ponds.

7) In polyculture, standing water, earthen ponds natural foods accounted for the following per centages of growth of the target animals even when feed pellets containing 25% protein were supplied daily, 6 days/week, at 2 to 4% of fish biomass: carp > 50%, tilapia hybrid >60%, silver carp >95%. In cement-lined ponds, with prawn growth in cages, delta C data and weight gain data indicated that natural foods accounted for half or more of the prawn growth even in the presence of a full ration of feed pellets containing 35% protein. Considering this information, it would seem advisable for pond managers to invest as significant an effort in development and maintenance of these natural foods as is invested in the development and purchase of supplied feeds.

ACKNOWLEDGEMENTS

Thanks are given to the staff of the Fish and Aquaculture Research Station at Dor, and in particular to Dr. G. Wohlfarth who supervised the portions of this research related to fish production. Aquaculture Production Technology (ISRAEL) Ltd., kindly allowed the use of their facilities for work related to prawn culture.

REFERENCES

- Coleman, D. 1981. Tube cracker for opening samples sealed in glass tubing. Anal. Chem. 53: 1962-1963.
- Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of CO. Geochim. Cosmochim. Acta 12, 133-149.
- DeNiro, M. & Epstein, S. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197, 261-263.
- DeNiro, M. & Epstein, S. 1978. Influences of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta 42, 495-506.
- Goering, H. & Van Soest, P. 1970. Forage Fiber Analysis (apparatus, reagents, procedures and some applications). Agric. Handb. No. 379, U.S. Dept. of Agric., U.S. Govt. Printing Off., Washington, D.C.
- Hobbie, J. & Lee, C., 1980. Microbial production of extracellular material: importance in benthic ecology. In: K. Tenore and B.

Coull (Editor), Marine Benthic Dynamics. Belle Brauch Symposium on Benthic Ecology. Univ. South Carolina Press, Columbia, S.C.

Moriarity, D., 1985. Methodology for determining biomass and productivity of microorganisms in detrital systems. ICLARM Conf. on Detrital Systems for Aquaculture. Bellagio, Italy.

Park, R. & Epstein, S. 1961. Metabolic fractionation of ^{13}C and ^{12}C in plants. Plant Physiol. 36, 133-138.

Schroeder, G., 1978. Autotrophic and heterotrophic production of microorganisms in intensely mantured fish ponds and related fish yields. Aquaculture, 14: 303-325.

Smith, B. & Epstein, S., 1971. Two categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. Plant Physiol., 47: 380-384.

Spataru, P., 1982. A contribution to the study of natural foods of Sarotherodon hybrids grown under conditions of polyculture, supplemental feeding and intensive fertilization. Bamidgeh, 34: 144-157.

Spataru, P., Wohlfarth, G. & Hulata, G., 1983. Studies on the natural foods of different fish species in intensively manured polyculture ponds. Aquaculture, 35: 283-298.

Tyrrell, H., Pelletier, G., Chevalier, R., Hillaire-Marcel, C. & Ganon, M. 1984. Use of carbon 13 as tracer in metabolism studies. Can. J. Anim. Sci. 64 (Suppl.), 127-129.

Warwick, R., 1985. Meiofauna: their role in marine detrital systems. ICLARM Conf. on Detrital Systems for Aquaculture. Bellagio, Italy.

Winberg, G., 1971. Methods for Estimation of Production of Aquatic Animals. Academic Press. New York, N.Y. 175 pp.

f. Description of cooperation

Throughout the project the principal investigations were in contact, via post and telephone. In addition, there were visits by Buck to Israel and Schroeder to Illinois. We exchanged samples for replicate analyses on our respective mass spectrometers to verify the ability to compare results from our instruments. We designed our experiments together in order to that our efforts be complementary. At the conclusion of the experiments we exchanged data for joint analysis.

g. Evaluation of research achievements

The main goal of this research, to demonstrate the usefulness of delta C as a tool for studying the flow of macronutrients in fish ponds, was achieved. Because the delta C of fermentation products is very similar to the delta C of original feed stock, delta C could not distinguish between assimilation by the fish of a food in its original state or after that food had been microbially processed within the pond detrital system. To gain that information, we compared delta C data of feeds and detritus with delta C and microscopic evaluation of fish gut contents. We are currently attempting to learn whether delta N ($^{15}\text{-N}$: $^{14}\text{-N}$) or delta D ($^2\text{-H}$: $^1\text{-H}$) change in a systematic way during microbial processing of a feed stock. If they do, then there is the possibility of their being useful as indigenous food web tracers.

h. Benefits to agriculture

Understanding the flow of foods within fish ponds, from input to the pond to assimilation by the target animal, has eluded researchers. This has hampered development of cost-effective pond management strategies. The usefulness of delta C as a tool for studying paths of macronutrients within fish ponds was demonstrated in this research. Delta C permitted us to measure, quantitatively, the contributions which specific natural foods made to target animal growth. This enabled estimates of competition among various fish species growing in polyculture. We were able to calculate the fraction of the total amount of carbon (originating either with primary production or added manure) that was consumed in the food web ultimately harvested by the target animals. This has given us the opportunity to plan pond management strategies that may in the future increase yields of harvestable natural foods and therefore increase target fish yields.

As a spin-off, we worked with persons in ruminant nutrition, using delta C to follow feed ingredients through the digestive tracts of sheep and to incorporation in cow milk.

i. List of publications

Schroeder, G., 1983. Stable isotope ratios as naturally occurring tracers in the aquaculture food web. *Aquaculture*, 30: 203-210.

Schroeder, G., 1983. Sources of fish and prawn growth in polyculture ponds as indicated by delta C analyses. *Aquaculture*, 35: 29-42.

Schroeder, G., 1983. Natural food web contributions to fish growth in manured ponds as indicated by stable carbon isotope ratios. J. World Mariculture Soc. 14: 505-509. (Best paper award).

Schroeder, G. & Plavnik, I. 1984. The use of stable carbon isotopes in measuring the transfer of macronutrients in poultry. Nutr. Rpts. International 30, 359-365.

Bruckental, I., Halevy, A., Amir, S., Neumark, H., Kennit, H. & Schroeder, G. 1985. The ratio of naturally occurring ^{13}C and ^{12}C isotopes in sheep diet hay and maize grain digestibilities in mixed diets. J. Agric. Sci., Camb. 104, 271-274.

Shan, J., Chang, L., Gua, X., Fang, Y., Zhu, Y., Chou, X., Zhou, F. & Schroeder, G., 1985. Observations on feeding habits of fish in ponds receiving green and animal manures in Wuxi, People's Republic of China. Aquaculture, 46: 111-117.

Schroeder, G. & Ben-Ghedalia, D., 1986. The fate of dietary components in sheep digesta as indicated by stable carbon isotopes. Nutr. Rpts. Internatl., in press.

Cohen, D., Schroeder, G., Ra'anan, Z., & Stern, S. 1986. Contributions of natural foods as a main source of prawn growth: observations

based on stable carbon isotope analyses. J. World Mariculture Soc., in press.

Bruckental, I., Schroeder, G., & Halevy, A. 1986. Delta C in studies of milk fat, casein and lactose metabolism in milking cows. Conf. on Nutr., Cornell U., in press.

STABLE ISOTOPE RATIOS AS NATURALLY OCCURRING TRACERS IN THE AQUACULTURE FOOD WEB

Final report on Segments of the project conducted in the USA.

The USA segment of the investigations involved two cooperating investigators:

David Homer Buck, Aquatic Biologist for the Illinois Natural History Survey, was leader for (1) studies conducted in ponds, tanks and aquaria at the Sam A. Parr Fisheries Research Center, a field station of the Illinois Natural History Survey near Kinmundy, IL, and (2) for analytical and bacteriological studies conducted in laboratories of the Illinois Natural History Survey at Champaign, IL. He was assisted in this work by Dr. Michael Henebry, Microbiologist, who was partially supported by project funds. Dr. Henebry has prepared reports on those sections of the study for which he had primary responsibility.

Dennis D. Coleman, Chief of the Isotope Laboratory of the Illinois State Geological Survey, was leader for those sections of the investigations conducted in laboratories of the Illinois State Geological Survey at Champaign, IL, including the processing of samples through the mass spectrometer to determine ratios of the natural carbon isotopes (^{13}C : ^{12}C). Dr. Coleman was assisted by J. Liu and Tom Morin. Mr. Morin obtained partial support from project funds and took responsibility for major parts of the work conducted at the Illinois State Geological Survey.

The U.S. portion of the report has been prepared in three parts:

1. That prepared by Dr. Buck
2. That prepared by Dr. Henebry
3. That prepared by Dr. Coleman and Mr. Morin.

Although the project was not officially funded or initiated until January 1, 1983, preliminary investigations were conducted in both 1981 and 1982. At Kinmundy investigations were conducted in indoor aquaria (40 l) and tanks (450 l), as well as in outdoor tanks (2-m diameter), pools (3-m diameter), and 0.04-ha earthen ponds.

Work Completed:

Over the period 1981-1985 a total of 30 individual experiments were established at Kinmundy, of which 17 produced data of sufficient substance to be reported here. Portions of the data have been organized into manuscripts which, following further refinement, will be submitted for publication. A paper entitled "The Use of Stable Carbon Isotope Ratios as Naturally Occurring Tracers in the Aquaculture Food Web" was presented by Dr. Buck at Aquaculture 86 (Reno, Nevada, USA, January 19-22, 1986) in joint meetings of the World Mariculture Society, Catfish Farmers of America, U.S. Trout Farmers Association, and the Fish Culture Section of the American Fisheries Society. It will be refined for publication in the Journal of the World Mariculture Society.

EXPERIMENT 1 - 1981

Purpose: To identify sources of carbon to tilapia when supplied corn stillage and algae, or corn stillage only.

Procedures: We measured initial and final δC 's of growing tilapia under three feeding regimes involving two principal sources of carbon (algae and corn stillage):

1. Indoor tanks fed fermented corn stillage in absence of sunlight/photosynthesis.
2. Sunlit tanks supplied inorganic nitrogen and phosphorus to stimulate algal growth.
3. Sunlit tanks supplied both stillage and inorganic fertilization. Corn stillage was prepared for fermentation (1) by drying (7-12 hours at 70-90° C) to obtain initial dry weight; (2) by hydrolysis in 0.1 N NaOH at pH 8.5; (3) by assuming stillage to be 50% carbon and adding sufficient N and P to establish C:N:P ratios of about 20:1:0.2; (4) by addition of trace minerals Mg, K, Ca and SO₄. The material was fermented at room temperature in pre-weighed, covered petri dishes.

Results: After tenfold gains in weight δC 's of tilapia fed corn stillage (δC -11.8) changed from an initial δC of -21.7 to final δC 's of -14.8 and -16.7; tilapia supplied only algae (δC -21.6, -22.5) had final δC of -20.1; tilapia fed both algae and corn stillage had final δC 's of -18.9 and -21.2. Thus, the tilapia derived all or most of their carbon from algae when available, from corn stillage only in the absence of algae. A pellet of bacteria separated from the fermenting stillage had a δC of -11.6.

EXPERIMENT 1 - 1982

In this preliminary experiment we determined $\delta^{13}C$'s of samples from various sections of a tilapia and prepared in various ways, as follows:

<u>Treatment of sample</u>	<u>Sample 1</u>	<u>Sample 2</u>	<u>Average</u>
Homogenized, freeze dried	-19.2	-21.3	-20.3 ± 1.0
Boiled, homogenized with "soup"	-20.3	-19.5	-19.9 ± 0.4
Boiled, homogenized without "soup"	-20.7		-20.7
"Soup" from above	-19.8	-18.3	-19.1 ± 0.8
Homogenized, heat dried	-19.0		-19.0
Gills, freeze dried	-20.4		-20.4
Guts, freeze dried	-23.0	-22.5	-22.8 ± 0.3
Filet, freeze dried	-18.6	-18.3	-18.45 ± 0.15
Fin, caudal, freeze dried	-18.4	-18.0	-18.2 ± 0.2
Fin, pectoral, freeze dried	-18.8	-18.3	-18.5 ± 0.3
Fin, dorsal, freeze dried	-17.0	-18.6	-17.8 ± 0.8

The means ranged from -17.8 ± 0.8 for freeze-dried dorsal fin to -22.8 ± 0.3 for freeze-dried guts. These data emphasize the importance of a standardized sampling procedure. In subsequent experiments we attempted to use muscle tissue when possible.

EXPERIMENT 2 - 1982

Purpose: To monitor growth and sources of carbon used by tilapia and prawns under three feeding regimes in sunlit tanks.

Procedures: Juvenile prawns and tilapia were stocked in six 2-m diameter aluminum tanks with soil substrates under the feeding regimes listed below:

Results:

<u>δC's of Feeds</u>	<u>Final δC's of Consumers</u>
Swine manure: -13.6	Prawn flesh: -16.9, -17.1 Tilapia flesh: -16.7, -17.7
Corn stillage: -11.8	Prawn flesh: -16.9, -16.9 Tilapia flesh: -15.5, -15.5
Poultry feed: -15.8	Prawn flesh: -17.8, -18.1 Prawn shell: -17.0 Tilapia flesh: -18.5, -19.0

Both tilapia and prawns under all treatments obtained carbon from sources other than those supplied. We expect that the primary alternate source was seston, composed primarily of pelagic algae. If we were to assume that the algae in these systems had δC's in the range of -20 to -24, as common in similar pools in subsequent years, both prawns and tilapia were obtaining substantial amounts of carbon from both supplied and natural foods. The δC's for tilapia and prawns supplied manure were similar (-16.7 to -17.7 for tilapia, -16.9 to -17.1 for prawns). In ponds supplied corn stillage mean δC for tilapia was less negative (heavier) than that for prawns (-15.5 vs. -16.9), indicating a heavier use of corn stillage by tilapia than by prawns. In ponds supplied poultry feed (δC -15.8), prawn δC's were heavier than those for tilapia (means -17.9 vs. -18.75), indicating heavier use of poultry feed by prawns than tilapia. The shell of a prawn fed poultry feed was found to be heavier (more ¹²C than ¹³C) than prawn flesh (-17.0 vs. -17.8 and -18.1).

EXPERIMENT 1 - 1983

This and subsequent "missing" experiments will not be reported because they produced no useful information.

EXPERIMENT 2 - 1983

Purpose: To trace the path of carbon from the atmosphere, as fixed by algae, through zooplankton to fish.

Procedures: A 3-m diameter outdoor, sunlit pool was supplied inorganic fertilizer to sustain healthy populations of pelagic microalgae and of a water flea (*Daphnia* sp) which fed on the microalgae. The daphnia were collected and fed to tilapia fry maintained indoors with no food (no light, no algae) other than the supplied daphnia.

Results:

✓C of seston (primarily microalgae) from sunlit pool	-20.0 to -22.0
✓C of zooplankton: Freshly removed from sunlit pool	-21.0
After 24 hours in distilled water	-20.7
✓C of tilapia after tenfold gain	-20.6, -20.8

EXPERIMENT 3 - 1983

Purposes:

1. To test hypothesis that accumulating food and fish wastes in uncleaned aquaria (vs. aquaria from which excess food and fish wastes were removed daily) would benefit fish and/or prawn growth through an increased production of foods associated with the detritus-rich sediments.
2. To evaluate corn stillage (by product from alcohol production) as fish food.
3. To compare fresh vs. fermented stillage as fish food.
4. To evaluate contributions of silt/sand substrate to food production and/or fish growth.

Procedures: Eight aquaria were each filled with 45 l of aged tap water and held in subdued light to eliminate production of algae. Four were supplied a silt/sand substrate to a thickness of 6-7 mm, four received no substrate. For each of the sets of four, two were fed a slight excess of fresh stillage and two were fed an excess of fermented stillage. Corn stillage was fermented in covered petri dishes as described in Experiment 1 1981. Successful fermentation yielded a light-colored bacterial slime over the surface of the stillage. Fresh and fermented stillage were supplied in equal amounts in terms of dry organic matter. Finally, one aquarium in each pair was cleaned daily by siphoning off excess food and wastes just prior to distribution of new daily rations, and one in each pair was left uncleaned for the 24-day length of the experiment. All aquaria received initial inoculations of a mixture of water and sediments from tilapia holding tanks and a pond fertilized with swine manure. All were stocked with 5 tilapia having mean weight of 0.63 g.

Results:

Substrate	Treatments		Gains by fish in grams
	Food	Cleaned	
No	Fresh	No	3.4851
No	Fresh	Yes	1.4512
No	Fermented	No	2.2289
No	Fermented	Yes	1.6098
Yes	Fresh	No	3.5086
Yes	Fresh	Yes	1.4483
Yes	Fermented	No	2.3486
Yes	Fermented	Yes	2.4680

1. Tilapia in aquaria not cleaned made significantly greater gains than in those in which wastes and excess food were removed daily.
2. Tilapia fed fresh stillage made significantly greater gains than those fed fermented stillage.
3. No significant differences due to presence or absence of sand/silt substrate.

The data were weakened by absence of replication in treatments. The evidence is strong, however, that the fermentation of accumulating foods and wastes in the sediments was more beneficial than efforts to ferment the stillage prior to distribution, and that fish gains were not influenced by presence or absence of silt/sand substrates. While differences in gains by treatment were significant, gains, per se, were not impressive. The corn stillage was judged to be a poor quality food whose value was greatly enhanced by fermentation in the tank bottom, with increased production of heterotrophs (bacteria, protozoa, etc.).

EXPERIMENT 4 - 1983.

Purposes:

1. To compare production in earthen ponds of prawns in monoculture fed Purina MR 25 with production of prawns in polyculture with tilapia and Chinese carps fed either swine manure or corn stillage.
2. To use carbon isotope analyses ($\delta^{13}C$) to indicate the foods under each regime which contributed to prawn and/or fish growth.

Procedures: We used six 0.04-ha earthen ponds to establish each of three treatments in two ponds. Four ponds were stocked with prawns (0.26 g; 32,500/ha) in polyculture with tilapia (18 g; 825/ha) and fingerling Chinese carps (silver, bighead, common and grass carps in ratios of 73:14.7:10:1) for a total fish density of 8225/ha. Swine were maintained so that fresh manure continuously entered the ponds at daily rates (dry weight basis) of from 2.5 to 4% of the biomass of fishes and prawns. Corn stillage was fed at rate of 15% of fish/prawn biomass, and feeding rate of pellets to prawns was initiated at 15% of initial weight of prawns and declined over the season to 5% of biomass of the consumers. Fish and prawns were recovered by pond drainage after 150 growing days for fish and 90 growing days for prawns.

Results: Production figures (Table 1) show that net gains by both fishes and prawns fed corn stillage were less than 50% as great as for those supplied swine manure. Deficiencies in the stillage-fed ponds were traceable to lower densities of both algae and bacteria. Prawn production in monocultures fed Marine Ration 25, a commercial prawn ration, was 245% larger than in ponds fed stillage, but only 32% larger than in ponds which received manure. It should be noted that prawns in the manured ponds were in direct competition for food with at least two (tilapia and common carp) of the five fish species, and that the mean gain of fishes in the manured ponds was on the order of 10X the gains made by prawns. The primary sources of nutrition for both fishes and prawns in the manured ponds were the natural foods produced by recycling of the nutrients present in the swine and fish manures, a great majority of which was utilized by fish. These data demonstrate the abundance of natural foods that can be available in a manured pond.

Table 2 lists the consumers and mean $\delta^{13}C$'s, with standard deviations for various numbers of samples analyzed (N) under each treatment (swine manure -13.6, corn stillage -11.8, or pellets -20.9). We believe the seston (>95% <38 microns) was the primary source of natural food in these ponds. In ponds receiving corn stillage the mean $\delta^{13}C$ of the seston (-22.7) was within the normal range of plankton algae in our ponds and experimental pools, but the $\delta^{13}C$ of seston in ponds receiving manure (-12.9), was unusually heavy and very close to that of the manure (-13.6). The seston may have contained large numbers of bacterial aggregates, or of organisms subsisting on bacteria, or may have incorporated large amounts of CO_2 respired by bacteria. The $\delta^{13}C$ of the manure was strongly reflected in the tissues of all fishes and prawns in the manured ponds. It was strongest in tilapia, common carp and grass carp, all having $\delta^{13}C$'s heavier than that of the manure, indicating their entire food was manure based. Contributions of the manure to the prawn (-14.2), silver carp (-14.5) and bighead carp (-13.1) appeared only slightly less strong. It is notable that the $\delta^{13}C$ of silver carp (-14.5) reflected the $\delta^{13}C$ of the manure (-13.6) in the manured pond and closely matched that of the seston (-22.6 vs. -22.7) in the ponds supplied corn stillage (Table 2). In stillage-fed ponds the $\delta^{13}C$'s indicated that common carp and grass carp derived substantial amounts of carbon from the stillage, the tilapia and bigheads were more dependent upon algae, and the silver carp appeared totally dependent upon algae. The much lighter $\delta^{13}C$ of prawns in pellet-fed ponds indicated the pellets to be their major source of carbon.

EXPERIMENT 6 - 1983

Purpose: To compare growth and sources of carbon for tilapia and prawns under two feeding regimes: (1) in pools supplied a commercial ration (MR 25) and (2) in pools also stocked with silver carp and fertilized with inorganic N and P to stimulate algal growth. We hypothesized that prawns, and perhaps tilapia as well, could meet their nutritional requirements on natural foods supplemented by silver carp fecal matter, and associated microfauna, as well as in pools provided a formulated ration.

Results: Results involving growth were not complete or distinct because prawns were recovered from only three of the six pools, and silver carp in two of the three pools failed to gain weight, due apparently to deficiencies in the supply of pelagic algae as food. However, surviving prawns in the single pool containing only natural foods (algae, silver carp feces, microfauna, etc.) had both mean and total gains significantly greater than in two pools supplied commercial rations. For tilapia, however, gains in the pellet-fed pools were more than 2X those in pools containing only natural foods. In the absence of stronger algal production, competition for food probably limited growth by both tilapia and silver carp.

Results from $\delta^{13}C$ analyses were interesting, but also indecisive. Following tenfold or greater weight gains the mean $\delta^{13}C$'s under each feeding regime were almost identical for both tilapia (-17.9, -18.1) and prawns (-16.8, -16.9), and all were heavier (less negative) than either the supplied feed (-20.9), the pelagic algae (-19.2 to -22.4), or the bottom sediments (-20.2 to -21.7). It seemed clear that the primary source of carbon for both tilapia and prawns under both feeding regimes was something other than those sampled.

EXPERIMENT 10 - 1983

Purpose: To identify the sources of carbon for tilapia and prawns under the five treatments listed in Table 3.

Procedures: Two 2-m diameter pools were used for each treatment. Inputs were either nitrogen and phosphorus, inorganic fertilizers, N and P plus corn stillage (CS), swine manure (SM), or Marine Ration 25 (MR 25). Each pool was stocked on August 15 with tilapia fry and post larval prawns. All pools were drained and censused in mid-September following tenfold gains by the experimental animals.

Results: Unfortunately, prawns survived in only the indoor treatment. With no sunlight and input limited to corn stillage, the final $\delta^{13}C$'s of both tilapia and prawns were very close to that of the stillage. In sunlit, outdoor pools the final mean $\delta^{13}C$'s of tilapia were all similar (Range -16.0 to -17.1), were much lighter than in indoor pools having no sunlight or algal production (-11.9), and were substantially different from the principal food input: much lighter when the principal input was stillage (-11.8), or swine manure (-13.6), but much heavier when input was MR 25 (-20.9). We believe that the tilapia in outdoor pools derived most of their carbon from sidewall algae, which had a mean $\delta^{13}C$ in all pools of -15.6 (range in all pools: -12.2 to -19.0). Samples of seston and sediments were collected in these pools but were not analyzed because of limitations on access to the mass spectrometer,

as well as on funds.

EXPERIMENT 1 - 1984

Purposes:

1. To determine if tilapia can live and grow on a pure diet of freeze-dried bacteria.
2. To determine if substrates in form of clay colloids added to manure-enriched systems would increase availability and utilization of bacteria by fish, as indicated by fish growth.
3. To use $\delta^{13}\text{C}$ measurements to determine contributions of various available foods (manure, MR 25, algae, etc.) to fish growth.

Procedures: Three tilapia (mean weight 0.061 g) were stocked in each of fourteen aquaria (52 l), two of which were given one of the treatments listed below:

1. Fed bacteria (freeze dried) only
2. Fed MR 25 only
3. Fed algae only
4. Fed manure only (swine manure)
5. Fed manure and silt/clay
6. Fed manure and MR 25
7. Fed manure and algae

Bacteria fed to the tilapia were grown in pure culture and freeze dried. The algae was an Oedogonium sp. collected from a pond, oven dried and ground into small particles. Swine manure used had been frozen and stored the previous summer and was screened of coarse particles of corn. The silt/clay added consisted primarily of colloidal clay particles which remained in suspension in an earthen pond having clay substrate. Sufficient clay was added to impart a turbidity of 200-210 JTU's. Feeding began on February 21, 1984. All tanks were aerated and temperatures were maintained at about 28°C. Ponds were fed five days weekly and given double rations on the fifth day. Aquaria with clay were stirred daily to maintain turbid suspension. All aquaria were given initial dose of 50 ml of Taub's solution as source of nutrients.

Results: The tilapia accepted bacteria as food but made poor growth, and no advantage was gained by adding the clay colloids as a bacterial substrate. Best gains were made on diet of MR 25 only and manure only. Diet of manure and algae provided better growth than manure only.

$\delta^{13}\text{C}$ data were obtained for tilapia from only four of the treatments as shown below:

Supplied feeds and $\delta^{13}C$'s	$\delta^{13}C$'s of Tilapia	
	Initial	Final
Swine manure (-13.6)	-18.6, -18.7	-12.1, -12.2
Marine Ration 25 (-20.9)	-18.6, -18.7	-18.4, -18.6
Swine manure (-13.6) and MR 25 (-20.9)	-18.6, -18.7	-16.5, -16.6
Swine manure (-13.6) and algae (-13.2 to -15.7)	-18.6, -18.7	-12.5, -12.9

The data show that when swine manure was available the foods which made the greatest contributions to the growth of the tilapia were manure based.

EXPERIMENT 2 - 1984

Purpose: To evaluate the heterotrophic/detrital food chain in manured ponds in comparison with conventional feeds as a food base for prawns, tilapia and Chinese carps, and to use the carbon isotope technology to identify the feeds which contributed to the growth of the prawns and fishes.

Procedures: We employed three treatments in nine 0.04-ha ponds, each stocked with equal numbers of tilapia, prawns and Chinese carps (silver, bighead, common and grass) (Table 4).

1. Three ponds received fresh swine manure in quantities calculated in terms of dry organic matter at daily rates of 2.5% to 4.0% of the live weight of fishes and prawns.
2. In three ponds the prawns, tilapia, and common carp were fed a conventional ration at conventional rates - the remaining fishes do not accept formulated feeds.
3. Three ponds received similar rates of both manure and feed.

Results: Gains in kg/ha by treatment are shown in Table 5. Prawn and fish production both averaged higher in manured than in conventionally fed ponds, but highest mean rates for both occurred in those ponds which received both manure and feed. However, the highest production of both prawns and fish in an individual pond occurred in a "manure only" pond, and had it not been for special problems with turbidities, tadpoles, and crayfish in one pond in this treatment, which severely reduced fish/prawn production, mean production of fish and prawns under the "manure only" treatment would not have been significantly different from that in ponds which received both feed and manure. It should also be mentioned that on the basis of total yields (including extraneous tadpoles and crayfish), production in "manure only" ponds averaged higher than in "manure plus feed" ponds, and more than 3X higher than in ponds which received only feed. The comparison of total yields is not especially meaningful because of the uncommonly large production of tadpoles and crayfish in a single "manure only" pond; however, the total array of data clearly demonstrates that the heterotrophic/detrital

food chain in a manured system can be highly productive of prawns and of those fishes capable of exploiting bacteria, algae, and other foods produced at the lowest levels of the food chains, both autotrophic and heterotrophic.

Results of $\delta^{13}\text{C}$ analyses are shown in Table 6. Time and budget limitations restricted our sampling to the prawns, tilapia and silver carp, which species we believed could provide the most meaningful data. No less than 95% of seston in these ponds was microalgae (<38 microns), which we believe was the principal available food. It had mean $\delta^{13}\text{C}$'s within the usual range of our ponds, -25.3 in pellet-fed ponds, and -21.2 in all ponds which received manure, with or without feed. We have no understanding of why the $\delta^{13}\text{C}$ of the seston in manured ponds (-21.0) did not reflect the same strong influence as in the previous year where that for the seston (-12.9) was very close to that of the manure (-13.6). In this experiment our primary sources of carbon were from either manure or seston in the manured ponds, from seston or pellets in pellet-fed ponds, and from seston, manure and pellets in the third set of ponds. The prawns had an initial $\delta^{13}\text{C}$ of -16.0. When supplied only manure, the mean $\delta^{13}\text{C}$ followed the manure to -14.0. When supplied only pellets final mean $\delta^{13}\text{C}$ of prawns was exactly that of the pellet (-18.8), but when provided both manure and pellet, it again showed a strong influence by manure (-15.3), indicating that perhaps 75 to 80% of food metabolized by prawns was manure-based in spite of pellet availability. Tilapia showed a strong utilization of manure-based foods in manured ponds, but appeared to have derived most of their carbon from algae when manure was not present. The $\delta^{13}\text{C}$ of silver carp changed from an initial value of -27.7 to -17.0 in ponds supplied only manure, to -24.5 in ponds supplied only pellets, very close to the seston, and to -18.2 in ponds fed both manure and pellets. It seemed clear that silver carp derived a major part of their carbon from manure-based feeds where manure was present.

EXPERIMENT 4 - 1984

Purpose: To test the hypothesis that water movement (vs. static water) would increase production of sessile algae and associated fauna, and thereby increase fish production.

Procedures: Eight 3-m diameter plastic-lined pools were given a standardized silt/sand substrate, stocked with prawns, silver carp and tilapia, and were supplied fresh swine manure at rate of 3% of fish/prawn biomass either five or six times each week. In all pools a cylinder (1-m diameter) placed in the pool center excluded fish and prawns from the central "core" and created a circular raceway in which water movement could be induced. In all pools nylon netting (1-cm-square mesh) was draped on pool walls as substrate to increase production of fish foods (bacteria, protozoans, sessile algae, etc.). In four pools a current of about 0.15 m/second was induced by submerged electric pumps. In four pools one-tenth of water volume was replaced daily. In addition, four standardized squares (20 cm x 20 cm) of nylon netting were suspended in each pool, two of which were shielded by hardware cloth to prevent grazing on the nets by fish. The combination of treatments was as follows:

1. Two pools having water movement only.
2. Two pools having water exchange only

3. Two pools having water movement and water exchange.
4. Two static control pools having neither exchange nor movement.

Results: Standing biomasses of sessile algae on the nets exposed to grazing by fish were similar (no significant differences) under all treatments, but concentrations of sessile algae on protected nets in pools receiving only recirculation were significantly higher (>2X greater) than in all other pools, followed by those pools which received both recirculation and water exchange. Lowest crops of sessile algae on both grazed and ungrazed nets were in the control pools which remained static. In spite of apparent greater productions and utilization by fish of sessile algae on the net substrates in pools having water movement, gains by fish were significantly highest in the static pools. Growth rates were not improved by either reduction in fish density, or by increasing the rate of water movement. It was concluded that fish growth was inhibited by the "noise" or vibrations created by the submerged pumps. This led to a second trial in which water movement was induced by airlift (no noise or vibrations) as will be described below.

Limited growth by silver carp did not justify analysis of carbon ratios. $\delta^{13}C$'s for tilapia were surprisingly similar in all treatments, (range: -19.7 to -20.9), as were those for the seston (-23.1 to -27.7), net algae (-23.8 to -25.4), wall algae (-23.4 to -24.2), and the bottom sediments (-23.7 to -24.1). For the tilapia, the source of carbon was about evenly divided between algae and manure. The $\delta^{13}C$'s for prawns in ponds having pumped water movement were -17.9 and -17.9, those in pools without water movement were -20.0 to -21.0, indicating a stronger influence from manure in ponds having water circulation. Surprisingly, percents of organic (volatile solids) matter on net substrates in pools was highest in static pools.

EXPERIMENT 5 - 1984

Purpose: To retest the hypothesis examined in Experiment 4 - 1984 using water movement induced by other means.

Procedures: Environments and feeding rates (fresh swine manure) similar to those used in Experiment 4 - 1984 were established in four 2-m diameter aluminum pools in full sunlight.

1. Two pools with water movement induced by airlift pumps.
2. Two static pools.

Water movement was induced by passing air (and water) up through a tube (10-cm diameter) inclined at about 45° so that air and water entered the tube 5-10 cm above the sediments and were discharged at the surface. Water moved past the section of net substrate from which samples of sessile algae were taken at a rate of about 0.1 m/second.

Results: Production of sessile algae on suspended net substrate and gains by both tilapia and silver carp were significantly greater in pools having water movement than in static pools, suggesting that water movement

may indeed increase production of foods in a form useful to fish. It also suggests that negative results obtained in previous experiments were somehow related to the use of submerged electric pumps. Additional associations of observable (not necessarily significant) differences under the two treatments are listed below.

	<u>Airlift Tanks</u>	<u>Static Tanks</u>
Gains by tilapia	High	Low
Gains by silver carp	High	Low
Algal biomass on wall and nets	High	Low
M.O. alkalinity	High	Low
Free CO	High	Low
Transparency (Secchi)	High	Low
Algal biomass in water column	Low	High
Detrital biomass in sediments	Low	High
Dissolved oxygen	Low	High
pH	Low	High
Turbidity	Low	High

✓C data were obtained for tilapia, and various natural foods, including seston of two size fractions (<36 microns and 38-150 microns), sessile algae (on nets) and bottom sediments. The natural foods were sampled in mid-August and in mid-September, the tilapia only in mid-September.

All pools had received similar rations of manure. It is important to note that circulation by airlift increased the degree to which the manure entered the food web. This is evident in several ways (Table 7):

1. For all three components of algae, the mean ✓C's of samples collected from airlift pools in August were lighter (more negative) than for later samples collected in September.
2. On the basis of limited data, there seemed to be a slight tendency toward change in the opposite direction in the static pools.
3. From an initial mean ✓C of -19.3 ± 0.2 , final means of ✓C for tilapia were -13.7 ± 0.48 for four fish from pools circulated by airlift, and -16.8 ± 0.49 for four fish in static pools.

We suggest that conditions under which the manure decayed were

predominantly aerobic in pools circulated by airlift and predominantly anaerobic in static pools. With the greater proliferation of bacterial cells, and a consequent higher production of CO_2 under aerobic than under anaerobic conditions, the algae in the airlift pools were incorporating significant amounts of CO_2 having the same approximate $\delta^{13}\text{C}$ as the manure (about -14). This was further reflected in the heavier mean $\delta^{13}\text{C}$'s of the tilapia in the airlift pools (-13.7 vs. -16.8).

EXPERIMENT 1 - 1985

Purposes:

1. To create special feeds and manures having desired ratios of the stable carbon isotopes in effort to facilitate measurement of the relative contributions of manures and conventional rations.
2. To measure the response of the cultured animals to the special feeds.

Procedures: With the guidance of nutritionists Dr. Lori Moore of the University of Hawaii, and Dr. Robert Stickney of the University of Washington, we engineered a light feed and a heavy feed for fish (Table 8), and a light feed and a heavy feed for swine (Table 9). The results were achieved through two principal adjustments to conventional diets. For energy we exchanged wheat ($\delta^{13}\text{C}$ -26) for corn ($\delta^{13}\text{C}$ -12), and for protein we substituted meat and bone meal for soybeans ($\delta^{13}\text{C}$ -28). Light wheat and light soybeans provided light feeds (-23.4) for fishes and prawns, -26.0 for swine), and heavy corn and heavy meat and bone meal provided heavy feeds (-12.9 for fishes and prawns, -11.0 for swine). Feeding the light and heavy feeds to swine gave light (-26.2) and heavy (-14.1) manures. Responses to these engineered feeds and manures were measured in aquaria stocked with either crayfish or tilapia and prawns, two aquaria for each treatment. The aquaria were maintained indoors where controlled lighting precluded photosynthesis and algal production.

Results: The results presented in Table 10 are for animals which had made tenfold gains on the feeds or manures supplied. Tilapia with initial $\delta^{13}\text{C}$ of -17.6 became lighter (-21.6, -21.8) when fed either light feed or light manure, and heavier (-14.5, -12.8) when supplied a heavy feed or heavy manure. Crayfish and prawns responded in the same way. Consumer $\delta^{13}\text{C}$'s corresponded closely with those of feeds or manures supplied, except for tilapia and crayfish fed light manure. Here a differential of 4.4 between the consumers and the manure suggested that some component of the manure was not being assimilated. We had no data for prawns fed manure because they failed to make tenfold gains on a manure diet in the time allotted for this study.

EXPERIMENT 2 - 1985

Purposes: To measure production of fishes (Tilapia, silver carp, grass carp), prawns and crayfish fed either light and heavy feeds, light and heavy manures, or combinations of a light feed or manure with a heavy feed or manure; and (2) to employ carbon isotope analyses to attempt to identify the

foods which contributed to growth of the various consumers. We also measured autotrophic production in the water column, and heterotrophic cellulolytic activity in the water column and sediments, as reported in a separate section of this report.

Procedures: Seven 0.04-ha ponds were available for this study. All were stocked with similar numbers and sizes of tilapia, silver carp, grass carp, prawns and crayfish as shown in Table 11. Five treatments were used:

<u>Number of Ponds</u>	<u>Treatment</u>
2	Light manure (LM)
2	Heavy manure (HM)
1	Light manure with heavy feed (LM/HF)
1	Heavy manure with light feed (HM/LF)
1	Heavy feed

Manures were supplied at rates maintained within the range of 2.5 to 4% (dry weight basis) of the live (wet weight) of the fishes and crustaceans. Feeds were supplied at an initial rate of 7% of biomass of tilapia, prawns and crayfish. Samples for $\delta^{13}C$ analysis were collected at both mid- and late-season for seston and sediments, at late-season only for the consumers. All fishes and crustaceans were recovered by draining censuses in October; gains under individual treatments are shown in Table 12.

Results: The data are not strong because not all treatments could be replicated. There are, however, a number of points worth noting.

1. Seasonal gains by the principal consumers (tilapia, silver carp, prawns) were consistently higher in ponds receiving light manure than in those which received heavy manure, but differences were not significant.
2. Gains by fishes and crustaceans in ponds receiving both feeds and manures were significantly higher than in ponds receiving only manure, and gains in the latter were on the order of 2X those in the pond which received heavy feed (feed only, no manure).
3. Recoveries of prawns in this experiment were lower than usually achieved in our fish/prawn polycultures, due possibly to competition from crayfish, and probably due in part to the fact that condition of the prawns when stocked was poor due to a longer-than-usual time in transit from Hawaii.
4. Gains made by crayfish were small because recoveries in the final censuses ranged from 1 to 22%, with a mean of 8%, compared to a mean recovery of 38.5% for prawns.
5. Factors which we believe contributed to the reduction of crayfish numbers include predation by prawns and migration.

6. We can offer no logical explanation for the greater weights of tadpoles in the ponds which received only manure. It is, however, a phenomenon that we have observed before when comparing biomasses in manured and non-manured ponds.

A summary of $\delta^{13}C$ data for consumers as well as both supplied and natural feeds is presented in Table 13. They contain unique characteristics that are difficult to interpret.

1. Tilapia, prawns and crayfish seemed totally independent of influence by the manure. $\delta^{13}C$'s for each were similar whether the manure was heavy or light. Each did in fact have lighter $\delta^{13}C$'s in ponds with heavy manure than in ponds which received light manure. This suggests that something other than manure (probably seston) was a primary source of carbon for all these species in ponds which received only manure.
2. In ponds supplied both manure and feed, tilapia, prawns and crayfish all showed influence by feed, being heavier in the presence of heavy feed, lighter in the presence of the lighter feed.
3. From an initial value of -19.6, $\delta^{13}C$'s of silver carp (-20.8 to -24.1) were close to that of the seston (-23.3 to -26.5), indicating that neither the silver carp nor the seston incorporated significant amounts of carbon from the manure.

EXPERIMENT 3 - 1985

Purpose: To measure the uptake of carbon by prawns, crayfish, and tilapia presented different combinations of light and heavy manures and feeds.

Procedures: On June 17, 1985, four 3-m diameter outdoor pools with soil substrates were each stocked with 10 tilapia ($\overline{wt} = 0.032$ g), 10 crayfish ($\overline{wt} = 0.43$ g), and 12 prawns ($\overline{wt} = 0.43$ g). Two pools were supplied combined daily rations of light manure and heavy feed, two were supplied a combination of heavy manure and light feed. Samples of the principal natural foods (seston, wall algae, sediments) and all three consumers (prawn, crayfish, tilapia) were collected on August 23, 1985, by which time the cultured animals had made more than tenfold weight gains.

Results: Responses of the various consumers were similar to those exposed to the same rations in ponds (Table 14). Their $\delta^{13}C$'s did not follow either the light or heavy manure, or the light or heavy feed. Tilapia, prawns, and crayfish all appeared to have obtained major portions of their carbon from natural foods (algae, seston, sediment).

EXPERIMENT 5 - 1985

Purpose: To measure the uptake of carbon by tilapia presented either light or heavy manure, or combinations of light manure with heavy feed, and of heavy manure with light feed.

Procedures: On July 25, 1985 four outdoor 2-m diameter aluminum tanks with soil substrate were each stocked with 10 tilapia ($\overline{wt} = 0.05$ g). One pool was supplied heavy manure, one received light manure, one got a combination of heavy manure with light feed, the other a combination of light manure and heavy feed. On August 27, 1985 samples of natural foods and tilapia were taken, by which time the tilapia had made tenfold gains in weight.

Results: As in the two previous experiments, the cultured animal (tilapia) again showed a total lack of influence from the manures (Table 15). In this instance, the major source of carbon probably was seston. An unusual aspect of these data was the consistent degree of differences in $\delta^{13}C$ of the wall algae (range of means in all pools -15.1 to -16.7), and that of the seston (-21.9 to -28.2) and the sediments (-21.8 to -22.6). In a previous experiment (3-1985) mean $\delta^{13}C$ of wall algae was -14.3 in two pools which received light manure and heavy feed, and -23.3 in the two which received heavy manure and light feed. We have no understanding at this time as to the cause for these differences.

EXPERIMENT 6 - 1985

Purposes:

1. To assess uniformity in the carbon ratios of separate animals from the same environment exposed to the same food.
2. To determine the precision possible by different technicians using different machines.

Procedures: We prepared samples from skeletal muscles of three separate silver carp, and from tail muscles of three individual crayfish after the animals had made tenfold weight gains in the same environment. Each of the six samples was divided and half of each was sent to Dr. Schroeder to be analyzed in Israel. All three samples of each type were analyzed in Israel, only two of each were analyzed in the US.

Results: The results shown in Table 16 were both surprising and reassuring. We had not expected the differences to be so insignificant.

1. Standard deviations for three silver carp and three crayfish analyzed in Israel were both less than 0.2; those for two of each analyzed in the US were 0.05 or less.
2. Differences in the means of samples analyzed in Israel and the US were 0.11 for silver carp, 0.2 for crayfish.

SUMMARY

We believe that we achieved our primary objective in this study, namely, to demonstrate the usefulness of stable isotope ratios ($^{13}C:^{12}C$) as a tool

for studying, quantitatively, the aquatic food web in monoculture and polyculture ponds. The technique was particularly useful in assessing the relative contributions of supplied vs. natural foods to the growth of the cultured organisms. We were able to demonstrate that in some cultures the major source of carbon (and growth) is from natural foods which in turn derived their carbon from supplied manure, even in ponds supplied full rations of formulated feeds enriched with fish meal. We further demonstrated that the heterotrophic/detrital food chain in a manured system can be highly productive of prawns and of those fishes capable of exploiting bacteria, algae, and other foods produced at the bottom of the food chain.

We were less successful in another aspect of the study. In an attempt to facilitate and refine the identification of sources of carbon for culture animals, and to illuminate microbial pathways within the detrital system, we developed both feeds and manures having distinctive carbon ratios (either very light or very heavy). In controlled aquaria, the culture organisms (tilapia, prawns, crayfish) responded to the special feeds in a predictable way: they become "lighter" when supplied either light feed or light manure, and "heavier" when provided heavy feed or heavy manure. The response was less predictable when the same feeds and manures were used in earthen ponds and in outdoor pools and tanks having soil substrates. In three consecutive experiments the cultured animals (prawns and crayfish in two, tilapia in three) failed to respond to either the light or the heavy manure. This was a notable departure from earlier experiences wherein the manures (corn-based and heavy) invariably were reflected in the $\delta^{13}C$ of the cultured fishes and crustaceans, even in the presence of lighter feeds. It is a puzzling development that we shall continue to study. When properly understood it may provide important new insights into the aquaculture food web.

Table 1. Mean seasonal gains (kg/ha) in 0.04-ha ponds under three feeding regimes, two ponds in each treatment in 1983.

Species	Swine Manure	Corn Stillage	MR 25
Tilapia	26.2	39.4	
Silver carp	1,237.7	323.3	
Bighead carp	243.8	40.2	
Grass carp	43.8	20.0	
Common carp	<u>280.4</u>	<u>122.2</u>	
Subtotals	1,831.9	545.1	
Prawns	<u>199.4</u>	<u>71.8</u>	<u>267.7</u>
Grand Totals	2,031.3	616.9	267.7

Table 2. σ C measurements of consumers under three treatments in 0.04-ha ponds in 1983.

Consumers	Swine Manure			Corn Stillage			Pellets		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
Prawn (-16.0)*	4	-14.2	1.0	3	-15.6	0.2	4	-18.8	0.6
Tilapia (-20.7)*	3	-12.4	0.4	3	-17.4	0.9			
Silver carp	5	-14.5	1.1	3	-22.6	0.4			
Bighad carp	3	-13.1	1.1	4	-19.6	0.4			
Common carp	3	-12.5	0.8	3	-13.1	1.1			
Grass carp	2	-12.6	0.4	4	-15.2	0.5			
<u>Natural Food</u>									
Seston	2	-12.9	0.3	3	-22.7	0.4	2	-23.4	0.4
<u>Supplied Feeds</u>									
Swine Manure		-13.6							
Corn Stillage					-11.8				
Pellets								-20.9	

* Initial σ C's

Table 3. Final δ C's for tilapia and prawns supplied corn stillage (CS), Nitrogen and Phosphorus (N and P), swine manure (SM), or Marine Ration 25 (MR 25), singly or in various combinations in 1983.

Treatment, Input (δ C)	Final Mean δ C's	
	Tilapia	Prawns
No sunlight, CS (-11.8)	-11.9	-12.1
Sunlight, N and P	-16.0	
Sunlight, N and P plus CS (-11.8)	-16.4	
Sunlight, SM (-13.6)	-16.9	
Sunlight, MR 25 (-20.9)	-17.1	

Table 4. Stocking data in 0.04-ha ponds for 1984 - all ponds stocked same.

	Numbers Stocked		$\overline{wt}$	Date	Number of
	Actual	/ha	(g)	Stocked	Growing Days
Silver carp	200	5,000	10.5	4/12	152-162
Bighead carp	20	500	1.6	4/12	152-162
Grass carp	4	100	4.4	4/12	152-162
Common carp	20	500	104.9	4/12	152-162
Tilapia	50	1,250	1.7	6/6	97-107
Prawn	2,600	65,000	0.019	6/1	103-113

Table 5. Mean gains in weight by cultured fishes and prawns and by other extraneous consumers in 0.04-ha ponds. Values presented for prawns and stocked fishes represent means of three populations under each treatment in 1984.

Species	Manure Only	Manure Plus Feed	Feed Only
Silver carp	764.34	815.29	253.02
Bighead carp	125.47	122.85	36.93
Grass carp	23.65	34.05	16.52
Common carp	247.22	271.65	161.36
Tilapia	<u>119.50</u>	<u>153.87</u>	<u>81.64</u>
Subtotals	1,280.18	1,397.71	549.47
Prawns	200.78	255.78	182.76
Extraneous*	<u>1,418.72</u>	<u>887.09</u>	<u>35.75</u>
Grand Totals	2,894.68	2,540.58	767.98

* Included young-of-year common carp in 5 of 9 ponds, mixed crayfish and tadpoles in 4 of 9 ponds, a few fathead minnows in one pond.

Table 6. $\delta^{13}C$ measurements of consumers under three treatments in 0.04-ha ponds (1984).

Consumers	Final $\delta^{13}C$'s by treatment											
	Initial $\delta^{13}C$			Manure			Pellets			Manure & Pellets		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Prawn	2	-16.0	0.1	4	-14.0	0.5	4	-18.8	0.6	4	-15.3	0.7
Tilapia	2	-13.4	0.7	3	-15.1	0.3	4	-21.2	0.5	4	-16.1	1.4
Silver carp	2	-27.7	0.2	3	-17.0	0.5	4	-24.5	0.4	4	-18.2	0.7
<u>Natural Food</u>												
Seston < 38 μ	6	-21.2	3.1	4	-25.3	1.2	4	-21.2	0.4			
<u>Supplied Feeds</u>												
Manure	4	-14.4	0.2							4	-14.4	0.2
Pellets							2	-18.8	0.6	2	-18.8	0.6

Table 7. Influence of airlift circulation on incorporation of manure into algal components of food web.

Treatment	Date of Sample	Mean σ C's of algal components			
		<38 μ (N)	38-150 μ (N)	Net Algae (N)	
Airlift	August	-19.6 (3)	-17.8 (1)	-15.1	(1)
Airlift	September	-17.1 (2)	-15.5 (2)	-12.9	(2)
Static	August	-19.5 (1)	-19.7 (2)		
Static	September	-19.5 (2)	-21.0 (2)		

Table 8. Light and heavy rations for fish and prawn diets prepared by the University of Illinois Department of Animal Science, courtesy of Bob Easter.

Ingredient	Light (✓ C -23.4)		Heavy (✓ C -12.9)	
	kg	%	kg	%
Fish meal	33.0	14.5		
Wheat	90.9	40.0		
Soybean meal	91.8	40.4		
Meat & bone meal			126.8	55.8
Corn			86.58	38.1
Cod liver oil			2.26	1.0
Dical. Phosphate	2.84	1.25	2.84	1.25
Limestone	1.93	0.85	1.93	0.85
Sod. chloride	1.136	0.5	1.14	0.5
Vitamins & minerals	<u>5.68</u>	<u>2.5</u>	<u>5.68</u>	<u>2.5</u>
Totals	227.286	99.55	227.23	99.55

Table 9. Light and heavy swine rations prepared by the University of Illinois Department of Animal Science, courtesy of Bob Easter.

Ingredient	Light (✓ C -26.0)		Heavy (✓ C -11.0)	
	kg	%	kg	%
Wheat	408.0	81.6		
Soybean	80.5	16.1		
Corn			405.0	80.9
Meat & bone meal			78.75	15.7
Dical. Phosphate	4.05	0.81	7.5	1.5
Limestone	4.5	0.9	5.75	1.15
Trace mineral salt	2.5	0.5	2.5	0.5
Vitamins	0.5	0.1	0.5	0.1
Lysine			<u>0.381</u>	<u>0.07</u>
Totals	500.05	100.01	500.381	99.92

Table 10. Final σ^c 's of consumers fed light and heavy manures and feeds in indoor aquaria (no sunlight, no algae) in 1985.

	<u>Light Feed</u>		<u>Light Manure</u>		<u>Heavy Feed</u>		<u>Heavy Manure</u>	
	σ^c -23.0	SD	σ^c -26.2	SD	σ^c -12.4	SD	σ^c -14.1	SD
Initial σ^c 's	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tilapia (-17.6)	-21.6	0.1	-21.8	0.4	-14.5	0.1	-12.8	0.2
Crayfish (-20.2)	-20.6	0.1	-21.4	0.1	-13.9	-	-13.6	0.7
Prawn (-16.9)	-20.3	-			-13.0	-		

Table 11. Stocking data in 0.04-ha ponds for 1985 -- all ponds stocked same.

	Numbers Actual	Stocked /ha	wt (g)	Date Stocked	Number of Growing Days
Tilapia	70	1,750	1.47	6/5	104-114
Silver carp	200	5,000	11.30	4/9	169-179
Grass carp	2	50	203.50	7/22	57-67
Prawns	588	14,700	0.40	6/14	95-105
Crayfish	200	5,000	0.40	6/17	92-102

Table 12. Seasonal gain (kg/ha) in 0.04-ha ponds under five treatments involving light and heavy manure (LM and HM) and light and heavy feeds (LF and HF) in 1985.

Species	Light Manure Ponds 3 & 4	Heavy Manure Ponds 5 & 6	LM/HF Pond 7	HM/LF Pond 9	HM Pond 10
Tilapia	281.2	257.8	300.6	227.4	164.0
Silver carp	567.1	511.2	873.0	803.2	231.8
Grass carp	<u>28.4</u>	<u>22.7</u>	<u>26.7</u>	<u>28.7</u>	<u>26.4</u>
Subtotals	876.7	791.7	1,200.3	1,059.3	422.2
Prawns	167.5	154.1	194.8	164.9	135.1
Crayfish	<u>26.8</u>	<u>18.4</u>	<u>1.8</u>	<u>6.5</u>	<u>12.1</u>
Subtotals	1,071.0	964.2	1,396.9	1,230.7	569.4
Tadpoles	<u>983.3</u>	<u>1,047.5</u>	<u>320.2</u>	<u>426.0</u>	<u>384.4</u>
Grand Totals	2,054.3	2,011.7	1,717.1	1,656.7	953.8

127

Table 13. Final consumer $\bar{C}$'s under five treatments involving light and heavy manure (LM and HM) and light and heavy feeds (LF and HF) in 1985.

Sample and Initial C 's	Light Manure* ($\bar{C}$ C -26.2)		Heavy Manure* ($\bar{C}$ C -14.1)		LM/HF** (-26.2/-12.4)		HM/LF** (-14.1/-23.0)		HF** (-12.4)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tilapia (-14.6)	-19.0	1.2	-22.6	0.2	-16.9	0.1	-21.6	0.4	-17.8	0.1
Silver carp (-19.6)	-24.1	0.5	-22.8	0.3	-20.8	0.4	-22.1	0.1	-23.3	0.0
Prawn (-16.9)	-20.4	0.5	-21.3	0.5	-16.2	0.2	-18.9	0.2	-18.7	0.2
Crayfish (-20.2)	-20.3	0.9	-22.1	0.3	-16.0	0.2	-22.2	0.2	-18.9	-
<u>Natural Foods</u>										
Seston	-26.2	1.1	-26.5	2.4	-23.3	1.9	-26.5	2.1	-28.5	0.5
Sediment	-22.8	1.4	-25.0	0.1	-22.4	0.4	-25.6	0.4	-23.8	0.1

* Means in this column are for four samples, two from each of two ponds.

** Means in this column are for two samples from a single pond.

Table 14. Final consumer δ C's in pools fed combinations of light manure (LM) or heavy manure (HM) with light feed (LF) or heavy feed (HF).

Consumers and initial δ C's	LM/HF -26.2/-12.4			HM/LF -14.1/-23.0		
	N	Mean	SD	N	Mean	SD
Prawn (-16.9)	4	-17.5	0.6	4	-20.2	0.5
Crayfish (-20.2)	4	-19.1	0.3	2	-21.5	0.1
Tilapia (-17.6)	4	-18.6	0.8	4	-21.3	0.2
<u>Natural foods</u>						
Seston	2	-20.2	2.3	2	-23.8	0.9
Wall algae	2	-14.3	3.1	2	-23.3	1.4
Sediment	2	-23.4	0.4	2	-23.8	0.1

Table 15. Final $\delta^{13}C$'s of tilapia fed combinations of light and heavy manures and feeds in sunlit, 2-m diameter pools (1985).

	Heavy Manure -14.1		Light Manure -26.2		HM/LF -14.1/-23.0		LM/HF -26.2/-12.4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Tilapia (-17.6)	-20.7	0.0	-19.4	0.4	-20.7	-	-18.6	0.4
<u>Natural Foods</u>								
Seston	-28.2	-	-21.9	-	-27.0	-	-22.1	-
Wall algae	-15.1	0.6	-15.6	0.6	-16.7	0.8	-16.1	0.2
Sediment	-22.6	-	-22.2	-	-21.8	-	-22.0	-

Table 16. Comparison of $\delta^{13}C$'s of same animals as determined by independent analyses in Israel and Illinois.

	Israel			Illinois		
	Samples	Mean	(SD)	Samples	Mean	(SD)
Silver carp No. 1	-19.75			-19.6		
Silver carp No. 2	-19.43	-19.66	(0.1611)	-19.5	-19.55	(0.05)
Silver carp No. 3	-19.79					
Crayfish tail No. 1	-20.12			-20.2		
Crayfish tail No. 2	-20.01	-20.00	(0.1066)	-20.2	-20.2	(0)
Crayfish tail No. 3	-19.86					