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THE UTILIZATION OF CLADOPHORA GLOMERATA
AND EPIPHYTIC DIATOMS AS A FOOD RESOURCE
BY RAINBOW TROUT IN THE COLORADO RIVER
BELOW GLEN CANYON DAM, ARIZONA

by

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ABSTRACT

THE UTILIZATION OF CLADOPHORA GLOMERATA AND EPIPHYTIC DIATOMS AS A FOOD RESOURCE BY RAINBOW TROUT IN THE COLORADO RIVER BELOW GLEN CANYON DAM, ARIZONA

WILLIAM CHARLES LEIBFRIED

Rainbow trout in the Colorado River below Glen Canyon Dam, Arizona are known to ingest large quantities of the filamentous green alga, Cladophora glomerata. This study and others indicate C. glomerata constitutes well over 50% of trout stomach contents (dry weight). Due to the high energy available in the lipid rich diatoms epiphytic on Cladophora, I hypothesized that rainbow trout could derive nutritional benefit through digestion of diatom lipids. Feeding experiments using field collected Cladophora with epiphytic diatoms resulted in an average growth of trout by 0.15 % of body weight (BW) per day in three feeding trials. Fish fed Cladophora sonicated to remove diatoms (sonication removed +/- 60% of epiphytes) lost approximately 0.3% BW per day. Diatoms ingested by trout during feeding

experiments lost approximately 80% of their lipid, as determined by microscopic examination of stained oil droplets within frustules. Contents of diatom cells from trout collected in the field showed a 78% increase in empty diatom frustules after passage through the intestine. In experiments using unmanipulated Cladophora total nutrient assimilation efficiencies (TAE) ranged from 5.6 to 16.3%. Sonicated algae experiments resulted in mean TAE of 31.0%.

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CHAPTER 1

Introduction

Since Glen Canyon Dam, on the Colorado River near Page Arizona, was completed in 1963, a unique aquatic ecosystem has developed in the tailwaters that has been beneficial to some organisms and detrimental to others (Carothers and Minckley 1981; Carothers and Johnson 1983). The most important changes within the river environment brought about by the closure of Glen Canyon Dam are; sediment load and turbidity, seasonal discharge patterns and daily range of flows, and variation in water temperature. The release of clear and constant cold (9-11° C, centigrade) hypolimnetic water through the dam's turbines provide for a blue ribbon trout fishery. Where spring floods once scoured the river bottom and limited algal growth, post-dam regulated discharges allow the filamentous green alga, Cladophora glomerata, to proliferate in the upper reaches of the Colorado River.

Cladophora serves as a substrate for epiphytic diatoms (Diatoma, Rhiocosphenia, and Cocconeis are dominant) which

are grazed heavily by invertebrates, especially the amphipod Gammarus lacustris (Blinn et al. 1986, Usher et al. 1987). These diatoms reach densities of 7×10^6 cells per square centimeter and at times cause Cladophora to appear brown in color (Usher et al. 1987).

Cladophora filaments, which may exceed one meter in length (personal observation), also provide invertebrates with a refugium (Gosse 1981, Leibfried and Blinn 1987). Introductions of invertebrates by Arizona Game and Fish Department (AGFD) overcame low invertebrate standing crops observed in early years after closure of Glen Canyon Dam (Stone 1967). Invertebrate densities in 1985 were high and now provide an abundant food base for the fishery (Leibfried and Blinn 1987). Aquatic invertebrates found in the dam's tailwaters are dominated by chironomid larvae, Gammarus lacustris, oligochaetes and gastropods (Carothers and Minckley 1981; Leibfried and Blinn 1987). In 1985, mean benthic macroinvertebrate densities from the Colorado River below Glen Canyon Dam decreased significantly from steady to fluctuating discharges (Leibfried and Blinn 1987).

The extirpation of three of the seven native fish species (Colorado River squawfish, razorback sucker, and bonytail chub) once found in the Colorado River through Grand Canyon was a direct result of Glen Canyon Dam (Carothers and Minckley 1981); the four remaining species (humpback chub, bluehead sucker, flannelmouth sucker and

speckled dace) maintain viable populations (Maddux et al. 1987). The present fish fauna is dominated by introduced species, such as rainbow trout, carp, brook trout, and brown trout.

The fish of greatest sport and economic importance is the rainbow trout (Salmo gairdneri), typically a carnivore (Minckley 1973, Carlander 1969). Their diets usually include aquatic invertebrates, drifting terrestrial insects and other fish (Chapman 1966, Elliot 1973, Carlander 1969). In tributary streams of the Colorado River in Grand Canyon, rainbow trout exhibit this typical feeding preference (Carothers and Minckley 1981)

It is thus striking to find that stomachs of rainbow trout in the mainstream Colorado River below Glen Canyon Dam contain large quantities of Cladophora (Bancroft and Sylvester 1978, Carothers and Minckley 1981, Persons et al. 1985 and Maddux et al. 1987; Table 1). The preponderance of algae in trout guts and the concomitant low frequency of protein-rich invertebrates seems inconsistent with high growth rates reported by Carothers and Minckley (1981) and Persons et al. (1985). Although Bancroft and Sylvester (1978) felt that algae were incidentally ingested by trout as they foraged for invertebrates hiding in Cladophora filaments, the possible nutritional importance of Cladophora and its epiphytes has thus far been ignored.

TABLE 1. Stomach content analysis of rainbow trout from the Colorado River below Glen Canyon Dam, 1977 - 1986.
 Other = Gastropoda, Simuliidae, misc. terrestrial insects.

	C.				G.			
	glomerata	lacustris	Chironomidae	Other	Method			
1977,78 Bancroft & Sylvester 1978	34%	44%	15%	7%	(volume)			
1977,78 Carothers & Minckley 1981	40%	21%	<1%	38%	(weight)			
1980,81 Persons et al. 1985	58%	22%	17%	3%	(volume)			
1985,86 Maddux et al. 1987	75%	9%	---	16%	(volume)			

Previous work on algae in the diets of freshwater fishes has been limited to cyprinids, centrarcids, cichlids and ictalurids (Kitchell and Windell 1970, Griffith and Voorhees 1960, Moriarty 1973; Gunn et al. 1977). Ingestion of algae by salmonids is rare and thought to have no nutritional benefit (Tippets and Moyle 1978).

Carothers and Minckley (1981) suggested that the lipids contained in diatoms may provide trout with a nutritional supplement. I further postulated that diatom lipids may be used as an energy source and thereby allow trout to spare protein for growth. Elsewhere, increased dietary lipid allowed rainbow trout to grow well despite decreased protein (Watanabe 1979, Hilton and Slinger 1981). The large quantities of diatoms attached to Cladophora in stomachs of trout in the Colorado River, together with good growth rates observed for these fish indicate that this phenomenon may occur in nature.

In this work I provide evidence that ingested algae (Cladophora glomerata and epiphytic diatoms) provide nutrients to rainbow trout. I supplement available data to show the prevalence of C. glomerata in trout stomachs, describe the loss of lipid and cell contents from diatoms as they pass through the gut, demonstrate digestion of algal nutrients through analysis of foods and feces, and test the abilities of trout to grow on algal diets. These

results indicate that algae contribute to the nutrition of a typically carnivorous fish.

CHAPTER 2

Methods and Materials

Trout Stomach Content Analysis

Stomachs from 11 rainbow trout (Salmo gairdneri) collected in July 1985 from the Colorado River below Glen Canyon Dam, Arizona were examined. Invertebrates were removed from Cladophora and counted. Cladophora and the invertebrates were then dried at 60° C. for 24 hours. Comparisons were made as percent relative abundance by dry weight.

Diatom Analysis

Diatom density in cells/cm² was determined by taking a 2x2 cm sample from Cladophora covered rocks. Samples were initially preserved in AFA (Alcohol:Formalin:Acetic acid, 60:35:5, v:v:v) and latter digested with 30% hydrogen peroxide-potassium dichromate after (Patrick and Reimer 1966). The remaining diatom frustules were suspended in a known volume of distilled water and a subsample was mounted

for density determinations under a Nikon microscope at 400x magnification.

Diatom lipids were stained with Oil Red O in DMSO (dimethyl sulfoxide), which stains fat bodies red and allows counting of frustules containing oil droplets (modified from Gallager and Mann 1981). These counts were used to compare lipid content of diatoms on fresh Cladophora, dried Cladophora feed and fecal material.

Nutrient Analysis

Ash content was determined by ashing ground material in a Thermolyne muffle furnace at 550°C for 4 hours. Weight of initial samples and ash were determined on a Mettler analytical balance.

Protein determinations were performed using a modified Bio-Rad assay (Bio Rad Laboratories 1981). Samples were initially digested in 0.1 N NaOH for 2 hours and centrifuged for 5 minutes before an aliquot was withdrawn for the assay. Unknown samples were compared colorometrically with a standard of curve for bovine serum albumin.

Lipids were extracted with chloroform/methanol (2:1 v:v), filtered and washed in 0.7% NaCl according to Folch et al. (1957). Two ml concentrated sulfuric acid was added to dried lipid and heated for 15 minutes at 200°C. Samples were cooled, diluted with distilled water, and then read at

375 nm. Lipid was determined by comparison with a standard curve for cholesterol (Christie 1982).

Feeding Experiments

Rainbow trout, Salmo gairdneri, were obtained from the Page Springs Fish Cultural Station, Arizona Game and Fish Department. Fish were transported to Northern Arizona University, Flagstaff, in coolers fitted with portable aerators to assure adequate dissolved oxygen. Trout were held in Frigid Units Living Stream tanks (Model LSW-700) at 10.5°C ($\pm 1.0^{\circ}\text{C}$) and on a 12:12 (light:dark) hour photoperiod. Trout were held without feeding for two or three days, then were fed once daily with Sterling Hatchery pellets until satiated. Fish were allowed to acclimate to laboratory conditions for one week before treatments were begun.

Prior to any treatment all fish were marked to assure individual identification. Fish were anesthetized with MS-222 (tricaine methane sulfonate; Crescent Research Chemicals) and Liquitex acrylic paints were injected subcutaneously along the lower jaw (modified from Kelly 1967 and Lotrich and Meredith 1974).

Fish were weighed after being starved for at least 36 hours. Fish were placed in a tared container and weighed to the nearest gram on a Circuits and Systems electronic

scale. Trout were weighed at least once a week during all experiments.

Trout were fed to satiation in all feeding trials with total daily ration calculated by subtracting dried uneaten food from total dry food fed. All food not consumed after 15 minutes was removed by siphoning. All fecal material from the previous day was siphoned prior to feeding and saved for nutrient analysis and diatom quantification.

A preliminary experiment was performed to determine if trout could grow in the experimental stream tank. Fourteen rainbow trout were fed a diet of standard hatchery pellets for 24 days and exhibited excellent growth.

For experiments with algal foods, rocks covered with Cladophora were collected from the Colorado River, transported to the lab, and stored in another Frigid Units Living Stream tank at 10.0°C. Filaments of algae were cut from rocks, dried in strands overnight, and then cut into pellets roughly the size of hatchery feed. Trout were trained to eat the floating Cladophora pellets by phasing them from a diet of pure hatchery pellets, to a combination of hatchery and algal pellets, and finally to pure algal pellets. This process took approximately 5 days. An additional 7 to 10 days were required before trout would actively take Cladophora and eat until satiated. Only at this time did the actual experiments begin.

Four experimental treatments were performed to determine trout growth on algal food (Table 2). Experiment A was a separate experiment with one group of fish and experiments B, C and D utilized the same fish for all treatments.

Calculations and Analysis

Assimilation efficiencies were determined by comparing a nutrient in the food and feces against a component assumed not to be digested or absorbed. Ash was used because it is a major component in algae and assumed to be nondigestible (Montgomery 1977 and Montgomery and Gerking 1980). Equations used in determining assimilation efficiencies are as follows:

$$\text{Corrected Fecal Nutrient (\%)} = \frac{\% \text{ ash in food}}{\% \text{ ash in feces}} \times \% \text{ fecal nutrients};$$

$$\text{Nutrient Assimilation Efficiency (\%)} = \left[1.0 - \frac{\% \text{ corr. fecal nutr.}}{\% \text{ nutr. in food}} \right] \times 100\%;$$

$$\text{Total Assimilation Efficiency (\%)} = \left[1.0 - \frac{\% \text{ ash in food}}{\% \text{ ash in feces}} \right] \times 100\%.$$

A paired Student's t-test was used to compare differences between lipid content in fresh Cladophora and dried Cladophora fed to trout and between dried Cladophora feed and feces. Paired t-tests were also used to compare

TABLE 2. Summary of feeding experiments performed on rainbow trout from April through November 1986.

EXPERIMENT	DATE	N	FOOD
A	April/May (33 days)	14	<u>Cladophora glomerata</u> with diatoms
B	Sept/Oct (27 days)	13	<u>Cladophora glomerata</u> with diatoms
C	Oct/Nov (24 days)	13	<u>Cladophora glomerata</u> diatoms removed
D	Nov/Dec (27 days)	12	<u>Cladophora glomerata</u> with diatoms

loss of diatom cell contents from stomach to intestines in wild trout. One sample t-tests were run on each of the four experimental treatments to determine if weight change was significant (Zar 1984).

CHAPTER 3

RESULTS

Stomach Contents

On a dry weight basis, Cladophora glomerata averaged 89.0% of gut contents of rainbow trout surveyed in this study (Table 3). Sand and other inorganic components adhering to the tightly packed strands of C. glomerata may have elevated the algal fraction of the diet slightly, but this would not affect the magnitude of values significantly. The prevalence of C. glomerata in the diet is reflected by two other values as well. The lowest recorded percent of C. glomerata (55.0%) was more than double the highest recorded value for any other component (24.0% for dipterans of the family Chironomidae). Further, Cladophora occurred in all stomachs examined, while other individual taxa were recorded from only 4-9 (36.4 - 81.8%) of the 11 stomachs.

TABLE 3. Summary of stomach contents of 11 rainbow trout collected from the Colorado River, July 1985. Values are percentages of total dry weight of contents. Percent occurrence reflects the percent of fish sampled which contained some material. Key : OTHER = misc. terrestrial insects.

	<u>C.</u> <u>glomerata</u>	<u>G.</u> <u>lacustris</u>	Chironomidae	Simuliidae	Gastropoda	OTHER
Mean	89.0%	3.2%	4.8%	2.6%	0.3%	0.05%
S.D.	12.9	6.3	7.4	5.0	0.5	0.15
Range	55-99	0-20	0-24	0-14	0-2	----
Occurrence	100.0%	63.6%	81.8%	63.6%	36.4%	----

In contrast to plant materials, invertebrates comprised a relatively small fraction of trout diets (Table 3). No animal taxon averaged more than 4.3% of the diet, while the sums of all invertebrate taxa averaged only 11.0%. Even on an individual fish basis, animal material never constituted more than 45.0% of a given fish's gut contents. All fish had, however, taken some animal prey.

Contents of Diatom Cells

As they are carried on fresh C. glomerata into the stomach and posterior intestine, many diatom frustules lose cytoplasm. Although quantitative data are lacking for fresh algal material, casual surveys indicate that more than 95% of diatom frustules on fresh C. glomerata contain cytoplasm. Fourteen percent (SD = 8.5%, range = 3-31%, n=14) of the frustules in the stomach were empty, suggesting some digestion of diatom cytoplasm takes place in the stomach. In significant contrast to values for diatoms in the stomach, 67.0% (SD = 17.3%, range = 33-96%, n=14) of those from the intestine were empty ($t=10.3$, $p<0.05$, $df=26$). If data are adjusted to account for frustules already emptied in the stomach, then approximately 78.0% of intact diatoms leaving the stomach were emptied by passage through the intestine. The disparate results for stomach and intestine are accentuated

by non-overlapping ranges for values from the two sections of the gut (3-33% vs. 33-96%, respectively).

Lipids in Diatom Cells

Approximately 81.0% of the diatoms in prepared algal foods lost their oil droplets before feces were egested ($t=9.5$, $P<0.05$, $df=19$; Table 4). There was a significant loss of lipid in the process of preparing algae for experimental feeding; 62.0% of diatoms on fresh C. glomerata contained lipid droplets, while only 36.0% of those on prepared foods showed lipid droplets were present ($t=2.8$, $p<0.05$, $df=38$; Table 4). Thus, fish consuming C. glomerata in the field ingest almost twice the lipid that fish used in experiments consumed.

Assimilation of Nutrients

Total assimilation efficiencies (TAE) for two sets of experiments offer several insights into the abilities of rainbow trout to digest algal nutrients (Table 5). First, average TAE's are low. For experiments using unsonicated algae, TAE's were 5.6, 12.3 and 16.3%. These low values are consistent with expectations due to the generally low digestibility of algal biochemicals, such as cellulose and siliceous diatom frustules.

Second, the composition of algal foods may vary considerably within a season and may affect assimilation. For example, mean assimilation efficiencies were inversely

TABLE 4. Relative abundance of empty diatom frustules in stomachs and intestines of rainbow trout collected from the Colorado River at Lees Ferry, Arizona, 1986.

	<u>Mean % Empty</u>	<u>S.D.</u>	<u>Range</u>	<u>N</u>
STOMACH	14%	8.5%	3-31%	14
INTESTINE	67%	17.3%	33-96%	14

TABLE 5. Summary of total assimilation efficiencies (TAE)* for two sets of feeding experiments conducted during 1986.

	MEAN	S.D.	N
APR-MAY			
Algal Collection 1	16.2%	3.8%	3
Algal Collection 2	12.3	7.3	6
SEP-OCT			
Unsonicated	5.6%	5.3%	2
Sonicated	31.0	1.5	4

*Calculations:
$$\text{TAE (\%)} = \left[1.0 - \frac{\% \text{ ash in stomach}}{\% \text{ ash in feces}} \right] \times 100\%$$

correlated with ash levels in the first experiment (A: April-May 1986; Table 5). In that experiment, two separate collections of algae were used as foods. Mean ash contents for foods and feces, respectively, for the first collection (TAE= 16.2%) were 32.6% and 46.8%, and for the second collection (TAE=12.3%) 71.8% and 82.8%.

Third, sonication may enhance digestibility. Assimilation efficiencies for unsonicated algae in the September-October experiment (B), were very low (mean=5.6%), while those for sonicated material were very large (31.0%; Table 5). Sonication may disrupt algal cells in ways not evident under a microscope, thus allowing greater digestion.

Protein. - As expected, trout digest major fractions of available protein (Phillips 1969; Table 6). With the exception of fish fed on unsonicated material from the first algal collection in April 1986, mean protein assimilation efficiencies (PAE) ranged from 90.1% to 93.2%; PAE for the first collection was 79.8%.

Protein digestion was apparently not influenced to any consistent degree by levels of ash in the food. The highest ash levels in foods were recorded for algae from Collection 2, which correlated with a lower TAE than with Collection 1; despite the lower TAE, PAE was higher for Collection 2 than for Collection 1 and was

TABLE 6. Protein assimilation efficiencies (PAE) for rainbow trout fed on Cladophora glomerata and epiphytic diatoms.

		Available Protein (% algal dry weight)	PAE (%)	S.D.	N	RANGE
APR-MAY						
Collection	1	2.5%	79.8	9.7%	6	64-88
Collection	2	3.0	93.2	2.7	7	90-97
SEP-OCT						
Unsonicated		5.5%	90.1%	5.0	3	84-93
Sonicated		4.7	91.8	1.7	3	90-93

indistinguishable from PAE for the September-October experiment which used foods with low ash levels (Table 6).

Total available protein also did not influence protein assimilation. Protein availability roughly doubled between April - May (2.5-3.0% protein in foods) and September - October (4.7-5.5%), but no substantial changes in protein assimilation were apparent (Table 6). Sonication appeared to reduce available protein slightly from 5.5% to 4.7%.

Lipid. - Lipid levels in foods are characteristically low (\pm 5.0% of total weight), and are reduced by sonication which removes diatoms. Unsonicated foods contained approximately 9.0% lipid, on an ash-free dry weight basis, while lipid levels in sonicated material dropped to 6.4%; this represents a decline of 29%.

Growth Experiments

The initial experiment (Figure 1; Table 7) demonstrated that rainbow trout obtained from the hatchery would live and grow well under laboratory conditions. These fish, fed on commercially available pelleted foods, exhibited excellent growth. On average, trout gained 58 g (41.7% of initial body weight [IBW]) during the 24 day experiment; their average daily increase in weight was 2.4 g (1.7% IBW).

Similar experiments were subsequently run with C. glomerata (and attached epiphytes) as the sole food source.

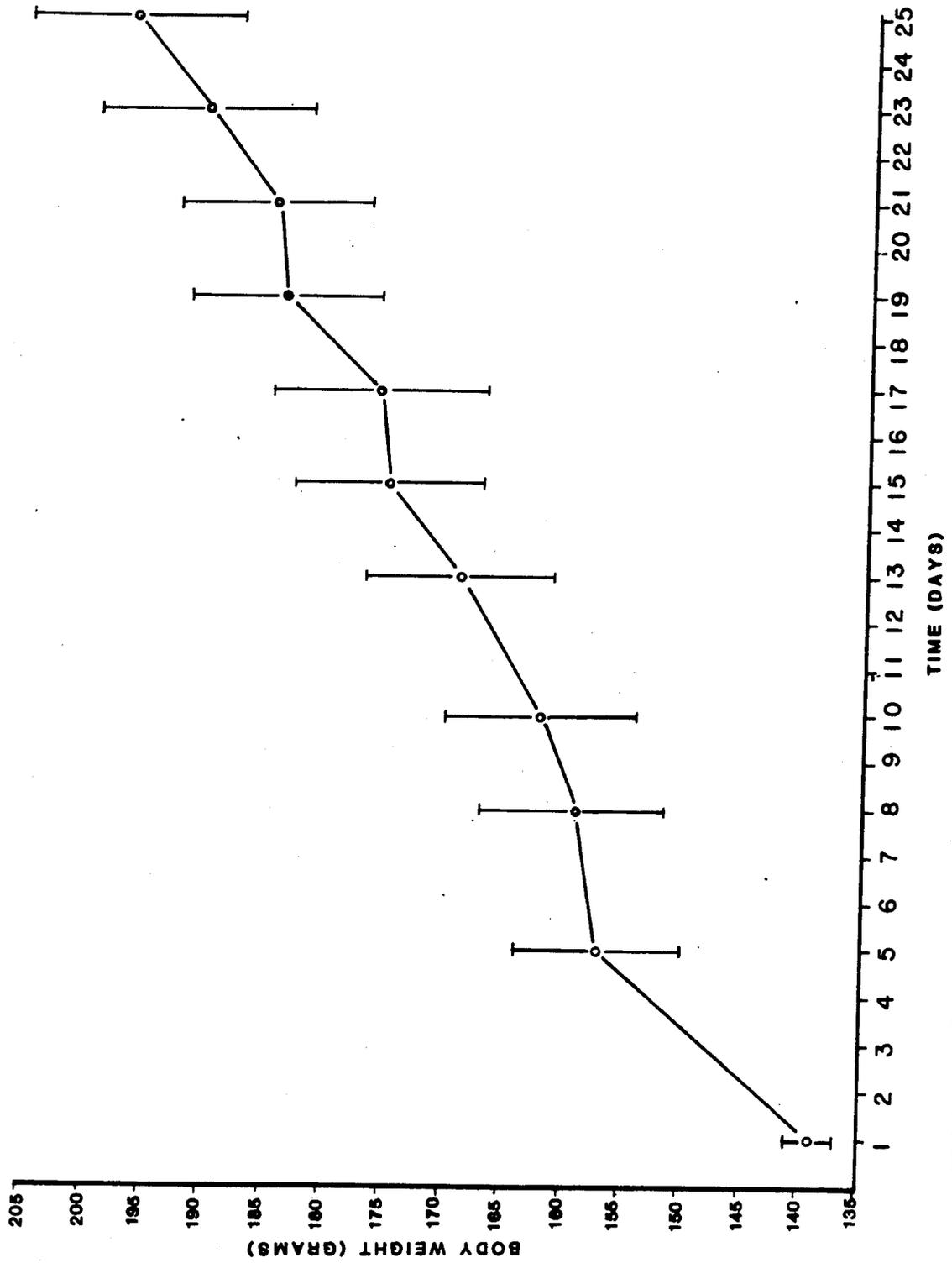


FIGURE 1. Growth of rainbow trout fed commercial hatchery pellets (n=12). Plotted values represent mean body weight \pm one standard error.

TABLE 7. Summary of results from rainbow trout growth experiments. High epiphytes = unsonicated; low epiphytes = sonicated; IBW = initial body weight. "Algal Collections 1 and 2" from Tables 6 and 7 provided foods for experiment A (High Epiphytes 1); "Unsonicated" served as food for experiment B (High Epiphytes 2); "Sonicated" was the food for experiment C (Low Epiphytes). Experiment D utilized unsonicated foods, but foods were not analyzed for nutrient content.

Experiment	GROWTH (%IBW)	DURATION (days)	DAILY GROWTH (%IBW/day)
Pellets	+ 42%	24	+ 1.7%
High Epiphytes A	+ 10	32	+ 0.3*
High Epiphytes B	0	26	0
Low Epiphytes C	- 6	23	- 0.3*
High Epiphytes D	+ 4	27	+ 0.14*

* = significant weight gain or loss ($P < 0.05$)

Three experiments (Figure 2, A, B and D; Table 7) used dried, but otherwise unmanipulated, algae; a fourth (C) used sonicated and dried material. As noted above, sonication removed approximately 60% of the diatoms.

The initial experiment (Figure 2, A; Table 7) yielded growth averaging 0.3% IBW per day ($t=6.8$, $P<0.05$, $df=13$), approximately one-ninth the rate obtained on hatchery pellets. In the second experiment (B) fish showed no weight gain or loss. Fish fed sonicated algae in the third experiment (C) lost, on the average, approximately 0.3% IBW per day ($t=2.8$, $P<0.05$, $df=13$). This latter experiment was particularly instructive; although the experiment is plotted as a separate line on Figure 2, it was in fact a continuation of experiment B. The significant loss in weight was not, therefore, an artifact due to alteration in holding conditions or food preparation. Experiment D continued with the same fish as in C, except that unsonicated algae was again fed. These fish showed significant ($t=2.11$, $P<0.05$, $df=11$) growth after 27 days of feeding with C. glomerata and epiphytic diatoms (Table 7; Figure 2, D)

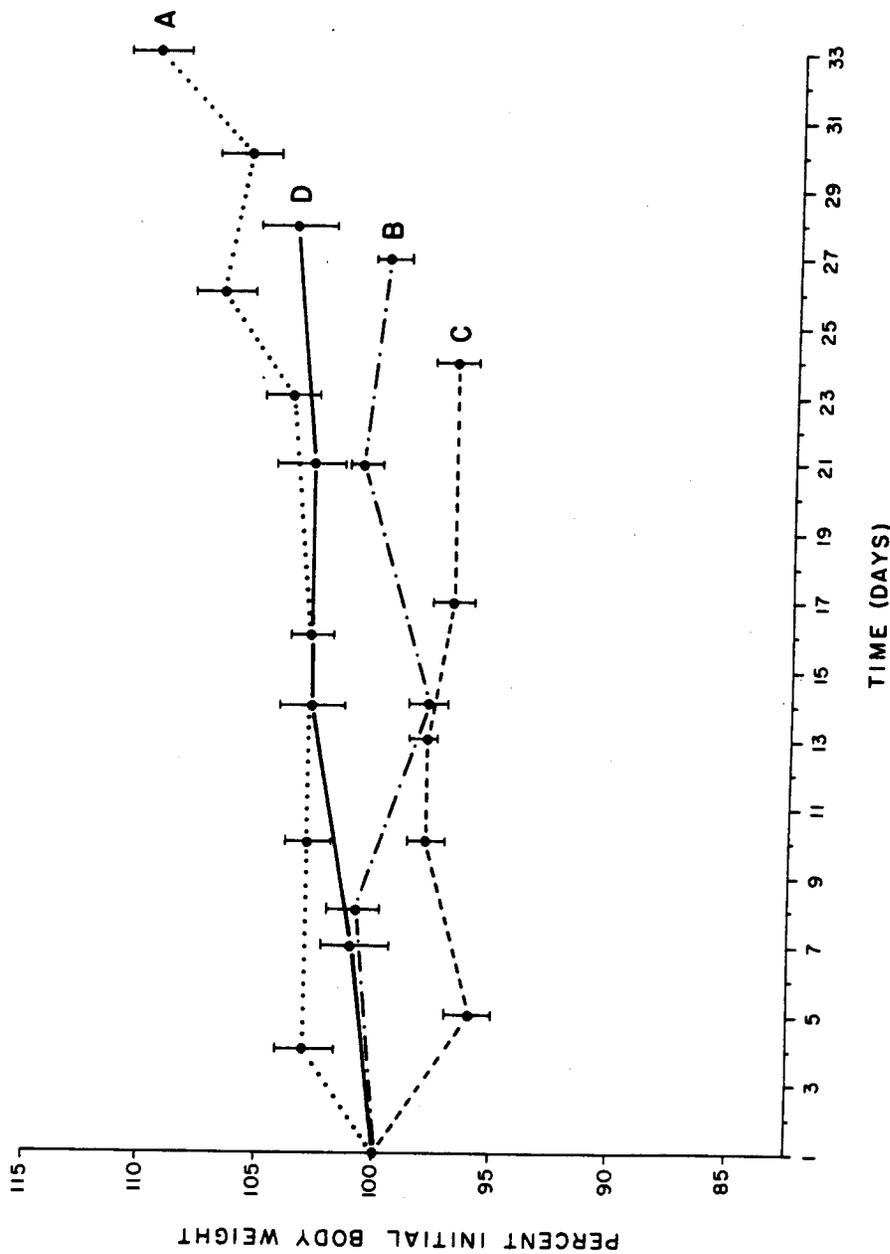


FIGURE 2. Mean percent change from initial weight for rainbow trout used in feeding experiments. Experiment A, B and D: trout fed *Cladophora glomerata* with epiphytic diatoms (n=14,13,12). Experiment C: *C. glomerata* sonicated to remove diatoms (n=13). Bars represent \pm one standard error.

CHAPTER 4

Discussion

This study and others establish that rainbow trout inhabiting the cool tailwaters below Glen Canyon Dam, near Lees Ferry, Arizona, ingest large quantities of the filamentous green alga, Cladophora glomerata. Contents of stomachs examined for this study averaged 89.0% C. glomerata on a dry weight basis. Although I did not attempt to evaluate diatom loads on C. glomerata, data collected by Usher et al. (1987) and Blinn et al. (1986) demonstrate that diatoms are common epiphytes of the green alga, and are therefore common components of trout diets at Lees Ferry.

Protein-rich invertebrates comprise a small but important fraction of trout diets, and a large fraction of dietary protein probably derives from these invertebrates. This is consistent with reports that trout grew slowly at Lees Ferry following closure of the dam (Stone 1967), but that growth rate increased after introduction of the amphipod, Gammarus lacustris. Nonetheless, the question

remains: how can trout grow on a diet containing little animal protein and large quantities of refractory plant materials?

This work contributes to answering this question in two particularly significant ways. First, I establish that trout can digest plant tissues and nutrients; in some cases their digestive abilities approach those of truly herbivorous fishes from other freshwater and marine systems (Montgomery and Gerking 1980). Second, I demonstrate that trout can grow or maintain weight when fed on plant diets in the absence of supplementary animal foods.

Total assimilation efficiencies appear low, compared to values trout achieve on animal foods (Phillips 1969), but are consistent with expectations based on the nature of plant foods, fish anatomy, and low temperatures experienced by trout at Lees Ferry. Extracellular (cell wall and middle lamella) carbohydrates of macroalgae and higher plants cannot be digested without the assistance of grinding mechanisms such as pharyngeal mills or gizzard-like stomachs or highly-specialized intestinal microflora (Lobel 1981) that prepare algae for digestion by stomach enzymes. Trout do not have grinding structures to disrupt algal cells, and there is no evidence for a consistent flora in the intestines of trout, although various microbes do occur regularly (Trust and Sparrow 1974). This highly refractory cell wall also serves as a major barrier between

the fish digestive system and digestible compounds in the cytoplasm of plant cells. Truly herbivorous fishes and other herbivorous vertebrates generally have grinding apparatus and an exceptionally long, coiled gut, compared to that of carnivorous relatives (Lobel 1981, Montgomery 1977). Trout have a short and relatively straight gut. Finally, truly herbivorous fishes are rare in cool, temperate waters and reach their greatest biomass and diversity in warm, tropical waters (Bakus 1969). Conditions at Lees Ferry are not those where one might predict the occurrence of an herbivorous fish.

Morphological specializations possessed by herbivorous fishes in order to utilize plant material include a thin walled stomach and a long intestine, a gizzard-like stomach with a long intestine, a pharyngeal mill without a stomach but retaining a long intestine, and a pharyngeal mill with a thin-walled stomach and long intestine (Lobel 1981). In addition to these physical adaptations, Lobel (1981) emphasizes the importance of low gastric pH in lysing algal cells. Although trout do not have the specialized stomachs of herbivorous fishes, they do maintain an acidic gastric pH and may be capable of utilizing algae via acid lysing (Kapoor et al. 1975, Lobel 1981, Phillips 1969).

Despite the improbability that trout can digest plant foods, total assimilation efficiencies demonstrate their ability to do so. Analyses similar to those used here

indicate that small herbivorous fishes on tropical reefs digest 20-25% of algal biomass (Montgomery and Gerking 1980); the 6-16% calculated for rainbow trout compares favorably.

One total assimilation efficiency stands out as unique. Assimilation for sonicated material (31%) jumped to a five-fold increase over unsonicated material in the same experiment (5.6%). As noted, sonication appears to disrupt some component of algal cells and allows increased access to digestible compounds.

Protein assimilation efficiencies (80-93%) met expectations based on other studies of carnivorous fishes fed on high protein foods (Barrington 1957, Phillips 1969). Despite this, the small fraction of algal biomass comprised of protein (2.5-5.5%) makes it doubtful that plant protein contributes much to trout growth. The ability of trout to digest protein is underscored by our failure to detect a substantial increase in protein assimilation with sonication, despite the accompanying increase in total assimilation. Sonication must, therefore, make compounds such as lipid and carbohydrates more available.

Counts of stained or empty diatom frustules leave no doubt that large fractions of diatoms which enter the gut lose their lipid-rich cell contents into the gut lumen. Once made available, such lipids should be readily digested and absorbed by the fish. Many studies of trout have

demonstrated their ability to digest and absorb lipids of both animal and plant origin (Cho and Slinger 1979, Sargent et al. 1979, Phillips 1969).

The ability of rainbow trout to spare protein for growth by utilizing energy gained from lipids has been documented under laboratory conditions (Reinitz et al. 1978; Watanabe et al. 1979; Hilton and Slinger 1981). Laboratory trout could spare approximately 15% of their dietary protein when fed increased levels of high-quality lipid (Watanabe et al. 1979). Rainbow trout ingesting large quantities of diatoms found on Cladophora glomerata from the Colorado River are undoubtedly enhancing their diet with lipid.

If rainbow trout can, in fact, digest and assimilate substantial fractions of nutrients contained in plant cells, then that should be reflected in feeding experiments. All fish used in the experiments were obtained from a hatchery and had been grown from hatching on artificially prepared diets. They were in no way anatomically or physiologically specialized for a plant diet.

Feeding experiments demonstrate three important facts. First, fish maintained under laboratory conditions grew well. This eliminates the possibility that poor growth in subsequent feeding trials is an artifact of experimental conditions.

Second, heavily epiphytized C. glomerata can support growth or maintenance of weight in the absence of animal foods. Significant positive growth was evident in the initial experiment (A) at total assimilation efficiencies of 16.2% and 12.3% and the final experiment (D, no TAEs recorded). No growth occurred in the phase of the second experiment (B) where heavily epiphytized C. glomerata was fed. Assimilation efficiencies were inexplicably lower during this experiment (5.6%). These experiments further support our contention that algal protein contributes little to fish growth. Protein levels during the second experiment (B) were roughly double those of the first. These fish were stressed during transport from the hatchery in August and this may be responsible for their poor TAE and lack of growth during experiment B. These same fish exhibited significant growth in subsequent experiments with diatoms present (D).

Third, trout lose weight when diatoms are removed from the Cladphora host. The decline in weight (mean = 0.3% body weight per day) initially suggests that the impact of removing diatoms is relatively slight, but the true impact may be far greater than indicated.

Assimilation estimates demonstrate that assimilation of sonicated material is 5.5 times that of unsonicated material. Trout, therefore, lost weight despite a very

large increase in the efficiency with which they were digesting their available foods. If assimilation of sonicated algae were adjusted to the 5.6% value observed for unsonicated material, we would predict a decline in weight of approximately 1.5-1.8% per day ($0.3\%/day \times 5.5$ fold difference). The implication is clear: diatoms, the only component removed from C. glomerata by sonication, may be the single greatest contributor to growth or maintenance of trout on an algal diet.

Information on algae in the diets of temperate freshwater fishes is very limited (Griffith and Voorhees 1960, Gunn et al. 1977, Kitchell and Windell 1970). Kitchell and Windell (1970) discuss the potential value of algae in the diets of the bluegill sunfish, Lepomis macrochirus. Although their experiments concluded that bluegills could not digest Chara, growth of these fish was higher on diets containing both algae and animal foods than on animal or plant foods alone. Ictalurid catfishes were observed to have gut pH of 2.0 - 4.0, as in bluegills (Page et al. 1976). Gunn et al. (1977) found Ictalurus nebulosus was able to digest blue-green algae at 67% assimilation efficiency. In cichlids, Moriarty (1973) and Moriarty et al. (1973) found that stomach pH increases as the gut becomes full, thereby more thoroughly digesting stomach contents. This would allow fish to more efficiently

utilize animal material ingested along with large quantities of algae.

Rainbow trout from the Colorado River below Glen Canyon Dam ingest large quantities of algae along with a smaller percentage of aquatic invertebrates. The advantages of consuming Cladophora with its epiphytic diatoms may be twofold: the direct gain from high-energy lipids found in diatoms and the increased digestive efficiency resulting from a full stomach containing algae and animal material.

Management Considerations

Epiphytic diatoms appear to be an important food resource for rainbow trout in the Colorado River below Glen Canyon Dam. These diatom lipids may spare sparse protein, found in invertebrates for use in growth, and Cladophora in trout stomachs may provide a more efficient site for digestion of ingested animal material. Any operations of Glen Canyon Dam, i.e. changes in temperature regime or discharge fluctuations, that would alter the algal communities may have an impact on the Colorado River rainbow trout fishery.

Future management plans should address impacts to the primary producers within this system as well as higher trophic levels. Data on impacts to invertebrates and algae from operations of Glen Canyon Dam found in Blinn et al.

(1986), Leibfried and Blinn (1987), Usher et al. (1987) and my research should be used to initiate more indepth studies of this ecosystem.

CHAPTER 5

Conclusions

1. Rainbow trout showed significant growth or maintenance on a diet of Cladophora glomerata and epiphytic diatoms. Trout lost weight when fed C. glomerata from which 60% of the diatoms were removed.
2. Diatom lipid decreases significantly from food to feces, and appears to be digested by trout.
3. Wild rainbow trout have significantly fewer diatoms containing cytoplasm in their intestines than they do in their stomachs; they appear to digest diatom cell contents as do experimental trout.
4. Epiphytic diatoms may be an important food resource in the diets of rainbow trout in the Colorado River below Glen Canyon Dam. Lipids may spare sparse protein, found in invertebrates, for use in growth.

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