GREAT LAKES FISHERY COMMISSION

Project Completion Report[1]

Feeding ecology and habitat use by larval lampreys in Great Lakes tributaries

by:

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Great Lakes Fishery Commission
Board of Technical Experts

Task Area on

Feeding Ecology and Habitat Use by Larval Lampreys in Great Lakes Tributaries

Final Report of the Michigan Technological University Task Area Team

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INTRODUCTION AND RATIONALE

Exotic sea lamprey are prevented from destroying fish stocks in the Great Lakes only by an extensive program of chemical control. In response to both economic and environmental considerations, the Great Lakes Fishery Commission has made a commitment to reduced reliance on chemical control and increased use of alternative control technologies. All alternative technologies identified to date achieve much less that the 99% elimination of larvae that is possible with chemical lampricides. For example, preliminary work with sterile males suggests about 50% reduction in viable larvae may be expected.

The value of techniques that eliminate < 90% of the larvae in a population is in question. Both field and laboratory studies show larvae compete with each other for resources. When densities are reduced, the larvae that remain are released to some extent from the effects of competition and as a result growth faster and enjoy greater survivorship. We do not yet know enough about these density effects to predict the extent to which they will reduce or nullify the benefits of alternative controls. Over what densities does competitive release occur and what is its magnitude? For what resources are the larvae competing, and is the limiting resource always the same in every stream? Is resource limitation equally important at all times of the year? Is there some feature of the stream environment that we can measure to know its capacity to support the growth of larvae? Answers to these questions will be important for the success of reduced dependence on chemical control.

In the work reported here, we studied sea lamprey and northern brook lamprey larvae (ammocetes) in streams tributary to the Laurentian Great Lakes in an effort to begin to answer many of these questions. Because there are long stretches of streams that appear to offer suitable habitat but are not occupied, we concluded that simple physical space *per se* is not a limiting resource. Instead, we focused on the food resources. Earlier studies showed that lamprey larvae feed on seston consisting of detritus, algae and bacteria. More recent publications refer to this material as biofilm. As a food resource, biofilm has been shown to vary considerably in quality across time and space. Thus, we concentrated our research on more precise determination of the food resources used by lamprey larvae, especially their variation in quality over time and across habitats, selection and digestion of food by larvae, the effects of density on food utilization, and the consequences of these relationships for larval lamprey fitness.

Significance of Organic Detritus in the Diet of Larval Lampreys in the Great Lakes Basin

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Larval sea lamprey (*Petromyzon marinus*) and northern brook lamprey (*Ichthyomyzon fossor*) were collected monthly from three streams in the Upper Peninsula of Michigan from May 1992 through May 1993 and larval sea lampreys were collected during summer months from sites throughout the Great Lakes basin. Organic detritus made up most of the diet ash-free-dry-mass (AFDM) throughout the year, averaging 97.79%, with algae (2.12%) and bacteria (0.09%) making up the remainder of the diet AFDM. Assimilation efficiency for AFDM averaged 72% during warmer months and 53% during cooler months (annual mean = 61%). Gut fullness (amount of AFDM in the anterior one-tenth of the intestine) was low (mean = 0.10 mg diet AFDM·g^{-1} ammocoete). There were no significant differences in these measures between ammocoetes collected from the Upper Peninsula and those collected throughout the Great Lakes basin. From a laboratory-determined relationship between gut fullness and feeding rate, feeding rate in the field was estimated to be extremely slow, ranging from 4.2 to 5.5 mg diet AFDM·g^{-1} ammocoete·d^{-1}. These observations indicate that larval lampreys efficiently utilize a diet of organic detritus during warmer months when stream temperatures and food quality are more favorable for feeding, digestion, and growth.

Des larves de lamproie de mer (*Petromyzon marinus*) et de lamproie du nord (*Ichthyomyzon fossor*) ont été collectées mensuellement dans trois cours d'eau de la haute péninsule du Michigan de mai 1992 à mai 1993; des larves de lamproie de mer ont aussi été collectées durant les mois d'été dans des sites du bassin des Grands Lacs. Les détritus organiques représentatient la plus grande partie du poids sans cendre (PSC) des aliments au cours de l'année (moyenne de 97,79 %), les algues (2,12 %) et les bactéries (0,09 %) constituant le reste. L'efficacité de l'assimilation était en moyenne de 72 % pendant les mois les plus chauds et de 53 % pendant les mois les plus froids (moyenne annuelle de 61 %). La plénitude intestinale (PSC des aliments présents dans le dixième antérieur de l'intestin) était faible (moyenne = 0,10 mg de PSC des aliments par gramme d'ammocète). Il n'existait aucune différence appréciable quant à ces mesures entre les ammocètes collectés dans la haute péninsule et ceux qui provenaient du bassin des Grands Lacs. À partir d'un lien établi en laboratoire entre la plénitude intestinale et le taux d'alimentation, on a estimé que le taux d'alimentation était extrêmement lent (de 4,2 à 5,5 mg de PSC des aliments par gramme d'ammocètes par jour). Ces observations indiquent que les larves de lamproies utilisent efficacement leur alimentation en détritus organiques pendant les mois les plus chauds lorsque la température des cours d'eau et la qualité des aliments sont plus favorables à l'alimentation, à la digestion et à la croissance.

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Larval lampreys (ammocoetes) are suspension feeders that inhabit burrows in the soft sediment of streams. The larval phase may last from 3 to 13 yr. Their diet consists of a mixture of algae (primarily diatoms), organic detritus, and bacteria (Manion 1967; Moore and Beamish 1973). Although most investigators have focused their attention on the algal component (Creaser and Hann 1929; Schroll 1959; Manion 1967), algae actually comprise little of the diet. Based on indirect calculations, Moore and Potter (1976b) estimated that algae accounted for only 0.14 to 1.5% by volume of the diet of larval European brook lamprey (*Lampetra planeri*) in a eutrophic stream in England. They concluded that because algae contributed so little to the diet, ingested detritus and bacteria are probably more important to the nutrition of these animals.

Although organic detritus is frequently reported in the gut contents of all species of larval lampreys (Schroll 1959; Sterba 1962; Hardisty and Potter 1971), there are few estimates of its importance as a nutritional resource. A laboratory experiment by Moore and Potter (1976a) found that *L. planeri* ammocoetes increased in wet weight by 2.1% when fed 20–30 mg·L^{-1} detritus for 60 d at 15°C, but lost weight at this concentration over the same period at 5°C. Thus, the significance of organic detritus in ammocoete diets remains unclear.

The purpose of the present study was to directly quantify each dietary component (algae, bacteria, and detritus) and measure total diet assimilation over a thirteen month period for two species of larval lampreys to identify their principal nutritional resource. Feeding rate, estimated from gut fullness,

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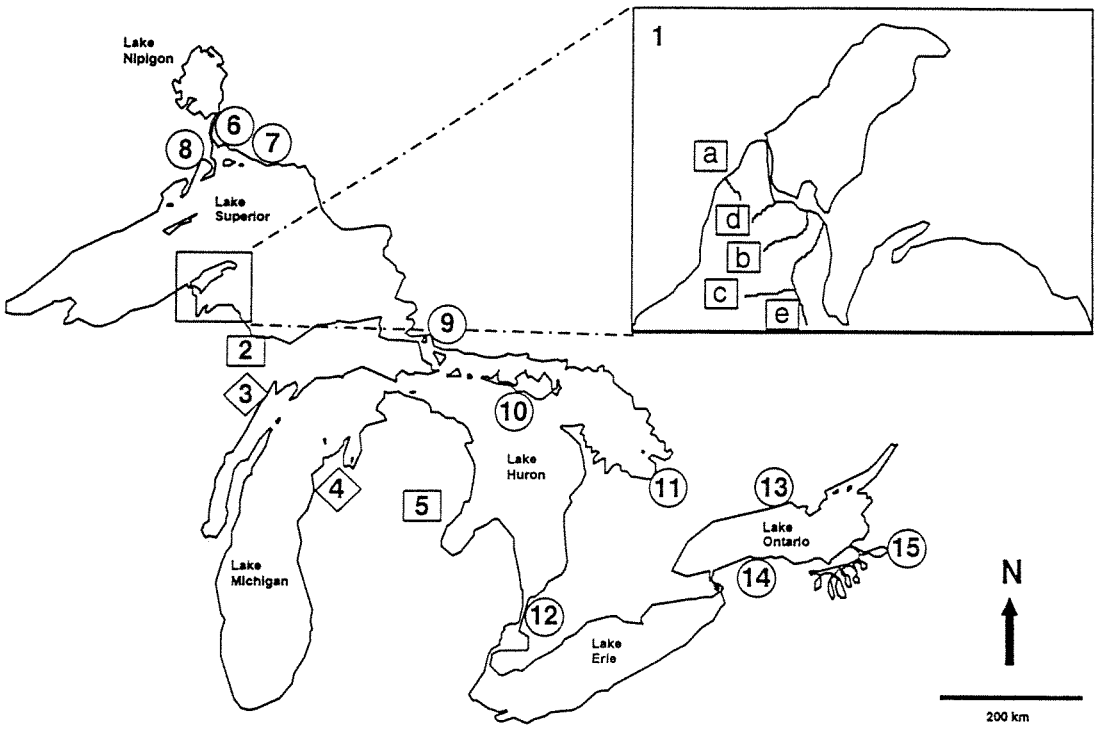

FIG. 1. River sites sampled for lamprey ammocoetes throughout the Great Lakes basin. Sites denoted with squares were sampled by Michigan Technological University, diamonds by the U.S. Fish and Wildlife Service, and circles by the Canadian Department of Fisheries and Oceans. Keweenaw sites are as follows: 1a-Misery River, 1b-Pike River, 1c-West Branch of the Sturgeon River, and 1d-Pilgrim River. For the remainder of site designations, see Table 1.

was also determined to further interpret the annual cycle of ammocoete feeding.

Methods

Sample Collection

Both sea lamprey (*Petromyzon marinus*) and northern brook lamprey (*Ichthyomyzon fossor*) ammocoetes were collected by electrofishing at monthly intervals from three streams in the Upper Peninsula of Michigan (hereafter termed Keweenaw sites) from May 1992 through May 1993 (Fig. 1; sites 1a, 1b, and 1c). Preliminary examination showed no differences in diet composition, assimilation efficiency, or gut fullness for samples collected at 4-h intervals over 24-h periods on two streams, so survey collections were made during morning hours for convenience. Due to prolonged flooding in all three streams as a result of ice and snow melt, ammocoetes were not sampled in April. At other sites throughout the Great Lakes basin, ammocoetes were obtained on single dates during the summer and fall of 1991 and 1992 by the U.S. Fish and Wildlife Service (Fig. 1; sites 3 and 4) and the Canadian Department of Fisheries and Oceans (Fig. 1; sites 1e-2 and 5-15).

Because ammocoetes are not responsive to electrofishing at temperatures <2°C (J. Weisser, U.S. Fish and Wildlife Service, Marquette, Mich., personal communication), diet samples during winter months (December–March) were obtained by placing lampreys in a cage in each stream. Cages were constructed of 1.27-cm plywood with outside dimensions: 0.3-m high × 0.6-m long × 0.45-m wide, providing a surface area of about 0.3-m^2. Openings in the front and back of the cages (0.3-m wide × 0.15-m high) were covered by 3-mm mesh netting that allowed flow through the cages but retained the lampreys. The cages were placed in areas of favorable ammocoete habitat and soft sediment was added to a depth of 0.15-m in each cage. Prior to introduction, ammocoetes were allowed to acclimate in the laboratory to ambient stream temperatures for two weeks. At monthly intervals, all ammocoetes were removed for analysis and replaced. As ammocoete density in each cage (ten per cage) was similar to densities observed in the field, no density-dependent cage effects should have occurred. This was confirmed in a field test conducted in May 1993, as diet composition, assimilation efficiency, and gut fullness of caged lampreys did not differ from those collected from streams by electrofishing (t-test; all $p > 0.25$).

For each sampling period and stream, ten freshly caught lampreys were held on ice until quiescent and anaesthetized with MS-222 (Sigma Chemical Corp.). This resulted in no loss of ingested material by regurgitation (Doxtater 1963). Lampreys were then fixed in 10% buffered formalin for 24 h and placed in Carosafe post-fixation preservative (Carolina Biological Supply) pending analysis.

Gut Content Analyses

The ammocoete digestive tract is a straight, undifferentiated tube approximately 45% of total body length (n = 305; SE = 0.01). Preliminary examination using yeast as a marker for detritus-fed ammocoetes indicated that there was no mixing or differential movement of diet contents. Thus, it can be assumed that ingested food material moves along the length of the intestine as plug flow. Contents were removed from the

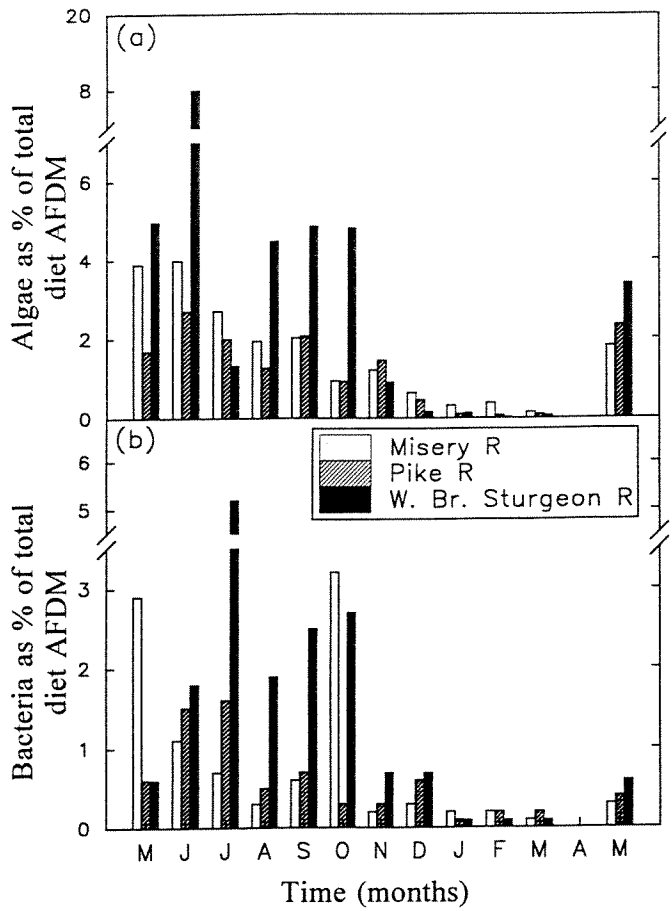

FIG. 2. Seasonal variation in the contribution of (a) algae and (b) bacteria ingested in ammocoete diets as a percentage of total diet AFDM for the Keweenaw sites from May 1992 through May 1993. Values for each month and stream are means of five individual ammocoetes.

anterior one-fourth of the intestine for five lampreys per sampling period per stream and a 50-μL subsample was analyzed by quantitative microscopy according to Ahlgren (1990a). Algal biovolume was estimated and converted to ash-free-dry-mass (AFDM) with an empirically determined weight/volume conversion factor (0.3633 mg AFDM·mm^{-3}) that is similar to ratios reported by Nalewajko (1966).

Contribution of bacteria to total diet AFDM was determined by staining a 50-μL subsample of the contents from the anterior one-fourth of the intestine with acridine orange (0.1 mg·mL^{-1} in 0.1 M potassium phosphate buffer, pH 7.5) according to Hobbie et al. (1977). Bacterial biomass per sample (mg AFDM·mL^{-1}) was calculated as [cells·mL^{-1}] × [mean biovolume (1 μm^3·cell^{-1}; vanDuyl and Kop 1990)] × [2.2 × 10^{-10} mg C·μm^{-3}; carbon conversion factor of Bratback and Dundas 1984] × [0.5 AFDM·mg C^{-1}].

The remainder of the sample was dried to constant weight at 110°C and total sample AFDM was calculated as weight loss after combustion at 550°C. The AFDM of organic detritus was calculated indirectly as total AFDM minus microorganism AFDM (Bowen 1979a).

Assimilation Efficiency

Assimilation efficiency (%AE), the net amount of food assimilated through the gut wall expressed as a percentage of the amount ingested, was determined for total organic matter as described by Conover (1966). AFDM in gut contents taken separately from the first and last tenths of the digestive tract were compared, using diet ash as an unassimilated reference material. The assumption that ash is not assimilated is reasonable inasmuch as most of the ash appears to be small sand grains and diatom frustules. To the extent that this assumption is incorrect, assimilation efficiency will be underestimated. Because there was not enough food in the first and last tenth of the intestine to calculate individual assimilation efficiencies, gut contents of five lampreys per sampling period per stream were pooled for this analysis.

Feeding Rate

Gut fullness

As an index of feeding rate, fullness of the anterior one-tenth of the intestine was measured for ammocoetes caught in the field as mg diet AFDM·g^{-1} ammocoete wet weight. The same five lampreys per sampling period per stream used in determining assimilation efficiency were used to estimate gut fullness.

Laboratory feeding experiment

Sea lamprey ammocoetes were obtained from the Hammond Bay Biological Station (U.S. Fish and Wildlife Service), Millersburg, Mich., in December 1992 and were acclimated to laboratory conditions for 3 wk on a diet of stream detritus.

Detritus was collected from an area in the Pilgrim River known to support ammocoetes. The upper 2.5 cm of sediment were removed using a plastic scoop, sieved, and the fraction >45-μm allowed to settle for 10 min. Suspended detritus was poured off and fed to 36 groups of three ammocoetes (average 3.21 ± 0.64 g live weight per aquaria; 0.21–0.32 g·L^{-1}). Ammocoetes were held in aerated, glass aquaria with 12 L of conditioned water. Each aquarium contained sieved sand (<1 mm) to a depth of 7.5 cm and photoperiod was maintained on a 12 h L:12 h D cycle. To control temperature, twelve glass aquaria were held in each of three Living Stream refrigerated tanks with constant water circulation (Frigid Units Inc.). Experiments were conducted at 2, 9, and 16°C, simulating average winter, spring/fall, and summer temperatures, respectively, in Upper Peninsula streams.

To measure feeding rate, Red 40 Alum Lake (a water insoluble, particulate food dye; Warner-Jenkinson Inc.) was used as a visible tracer. Preliminary experiments showed that a concentration of 100 mg·L^{-1} of the dye was needed to allow detection in ammocoete guts. This concentration appeared not to disturb the normal feeding behavior of ammocoetes. Because ammocoetes normally feed on particles at much lower concentrations (<40 mg·L^{-1}), it appears that their feeding mechanism has a low capture efficiency for these dye particles. Detritus was added to the aquaria at a concentration of 30 mg·L^{-1}, as this is comparable to that found suspended in depositional areas in many natural waterways (Moore and Potter 1976a).

The detritus/dye mixture was added to each aquarium and ammocoetes were allowed to feed for several hours before being removed and sacrificed. Each digestive tract was removed and preserved in 10% buffered formalin and the distance the detritus/dye mixture traveled was measured to the nearest millimetre. Contents were removed, dried to

TABLE 1. Means (SE) for percent AFDM in the diet, percent contribution of algae and detritus to diet AFDM, assimilation efficiency of AFDM, and gut fullness of AFDM for sea lamprey ammocoetes sampled throughout the Great Lakes basin.

Site No. (Fig. 1)	Stream	Date	AFDM	Algae	Detritus	Assimilation efficiency	Gut fullness
8	Black Sturgeon R	08-13-92	54.42 (3.72)	0.64 (0.16)	99.36 (0.16)	86.44	0.31 (0.06)
2	Chocolay R	09-06-91	61.76 (5.19)	2.15 (0.38)	97.85 (0.38)	—	—
2	Chocolay R	09-12-91	44.20 (9.96)	7.83 (1.07)	92.24 (1.05)	—	—
15	Fish Cr	09-17-92	65.32 (6.93)	0.81 (0.10)	99.19 (0.10)	63.57	0.44 (0.08)
7	Gravel R–Mtn Bay	08-15-92	58.94 (7.38)	0.44 (0.22)	99.56 (0.10)	78.63	0.18 (0.05)
6	Nipigon R–Lake Helen	08-28-92	63.83 (10.63)	0.91 (0.31)	99.09 (0.31)	69.05	0.19 (0.04)
11	Nottawasaga R	09-09-92	68.04 (1.29)	1.90 (0.57)	98.10 (0.57)	49.06	0.34 (0.06)
4	Platte R	03-23-92	85.20 (4.91)	0.82 (0.07)	99.18 (0.07)	66.44	—
4	Platte R	04-29-92	62.35 (7.61)	2.87 (0.34)	97.13 (0.34)	77.92	—
4	Platte R	06-23-92	62.23 (3.40)	1.49 (0.23)	98.51 (0.23)	76.26	0.14 (0.01)
3	Rapid R	10-24-91	73.83 (7.34)	1.66 (0.59)	98.34 (0.59)	—	—
5	Rifle R	07-21-92	57.66 (6.59)	4.23 (1.27)	95.77 (1.27)	79.11	—
13	Salem Cr	09-19-92	74.43 (5.57)	1.80 (0.45)	98.20 (0.45)	62.33	0.27 (0.03)
14	Salmon Cr	09-11-92	59.93 (6.00)	1.97 (0.72)	98.03 (0.72)	70.69	0.41 (0.12)
12	St. Clair R	07-27-92	61.39 (7.29)	1.13 (0.46)	98.79 (0.54)	81.36	0.37 (0.10)
9	St. Marys R	08-27-92	76.61 (3.61)	0.20 (0.07)	99.80 (0.07)	85.58	0.21 (0.14)
1e	Sturgeon R	08-20-91	46.72 (9.44)	2.49 (0.55)	97.52 (0.54)	—	—
1e	Sturgeon R	08-31-91	44.35 (5.16)	5.53 (1.74)	94.50 (1.75)	—	—
10	Timber Bay Cr	07-07-92	39.77 (7.04)	0.53 (0.12)	99.47 (0.12)	56.93	0.10 (0.01)
	Mean		61.10 (6.26)	1.98 (0.43)	98.03 (0.43)	71.67 (2.95)	0.25 (0.04)

constant weight at 110°C, combusted at 550°C, and reweighed. Gut fullness and feeding rate were measured as mg diet AFDM in the anterior one-tenth of the intestine·g^{-1} ammocoete wet weight, and mg diet AFDM ingested·g^{-1} ammocoete wet weight·h^{-1}, respectively.

Results

Diet Composition

Throughout the Great Lakes basin, for all sites and months sampled, organic detritus made up most of the diet, averaging 97.79% of total diet AFDM. Algae contributed 2.12%, with diatoms alone comprising 1.79%. Bacteria were limited to 0.09% (data collected for Keweenaw sites only) of the total diet AFDM. Diets of sea lamprey and northern brook lamprey ammocoetes were made up of algae, bacteria, and detritus in proportions that were not significantly different (ANOVA; all $p > 0.25$).

Algal and bacterial abundance in streams sampled at monthly intervals at the Keweenaw sites from May 1992 through May 1993 followed distinct seasonal patterns. As a percentage of diet AFDM, algal abundance peaked in May/June and September for all three sites (two-factor ANOVA; both $p < 0.001$; Fig. 2a). The cycle of bacterial abundance lagged about one month behind algal abundance, with peaks in July and October (two-factor ANOVA; both $p > 0.25$; Fig. 2b). Abundance of algae and bacteria in the diet declined in all three streams from November through March (Fig. 2). Winter samples (November–March) contained a significantly lower algal and bacterial biomass than samples collected during warmer periods (May–October) in all three streams (*t*-test; all $p < 0.001$). Algal and bacterial abundance in the diet, as a percentage of total AFDM, increased in May following the spring thaw as streams resumed more typical flows, temperatures warmed, and light levels increased (Fig. 2).

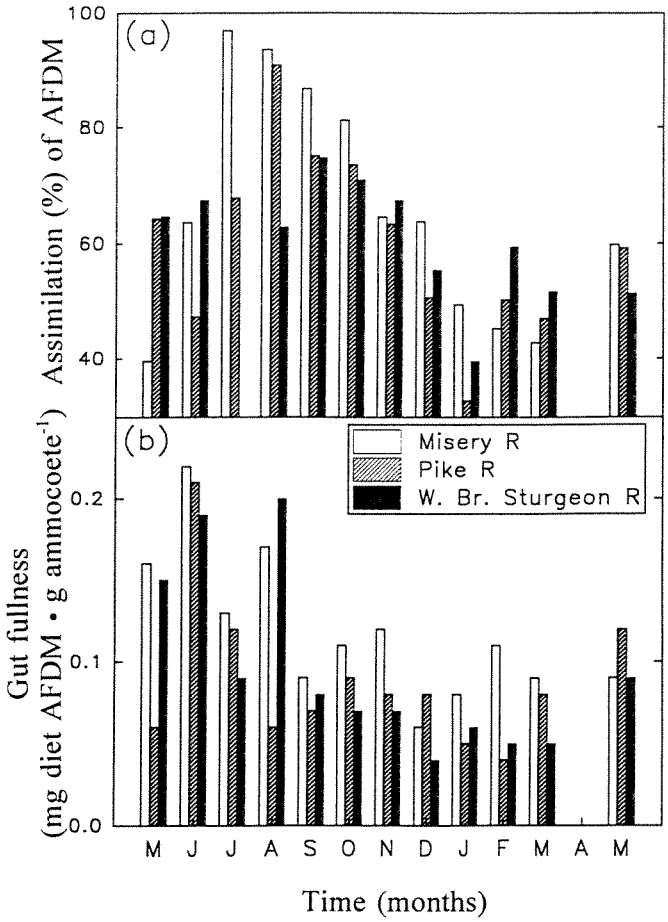

FIG. 3. Seasonal variation in the (a) assimilation and (b) gut fullness of organic matter ingested by ammocoetes for the Keweenaw sites from May 1992 through May 1993. Estimates for each month and stream consist of five pooled ammocoetes for assimilation efficiency and means of five individual ammocoetes for gut fullness.

From May through October 1992, the amount of algal AFDM in the diet was higher, on average, in the West Branch of the Sturgeon River (6.46%), which has a canopy that remains relatively open, than in either the Misery (2.57%) or Pike (1.77%) rivers, which have closed canopies (ANOVA; $p < 0.001$). An unusually large spate in the Sturgeon River in July appears to have swept away much of the algae, resulting in a lower algal abundance in the diet for that month relative to all other summer months in that stream (ANOVA; $p < 0.01$; Fig. 2a).

Ammocoetes sampled from the remainder of the Great Lakes basin showed similar diet composition to ammocoetes sampled at Keweenaw sites (ANOVA; $0.15 < p < 0.20$; Table 1). Riparian canopy was categorized as open or closed by field personnel of the Canadian Department of Fisheries and Oceans Sea Lamprey Control Centre. The amount of algal AFDM in the diet was typically higher for streams with open canopies (Fig. 1; sites 1d, 2, 3, 4, 5; Table 1). Algal abundance in open canopy streams was, on average, 3.98% of total diet AFDM, whereas closed canopy streams had an algal abundance averaging only 1.27% (t-test; $p < 0.02$). Four deep-water sites were unshaded by riparian vegetation but ammocoetes collected from these contained little algae. Two were lentic environments (Fig. 1; sites 6 and 7) and

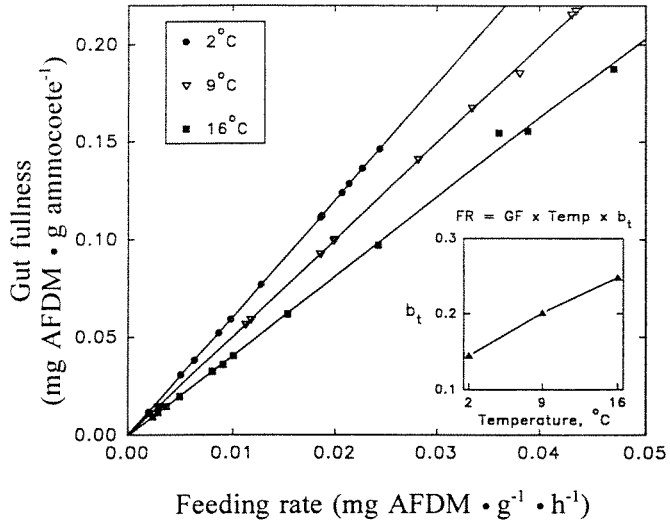

FIG. 4. Relationship between feeding rate and gut fullness at 2, 9, and 16°C as determined from the laboratory feeding experiment.

two were in large rivers (Fig. 1; sites 9 and 12). Both habitat types have low light penetration to bottom substrate which impedes the development of algae. However, a suitable environment exists for ammocoetes (Wagner and Stauffer 1962).

Assimilation Efficiency

In July 1992, we determined assimilation efficiencies for nine large individual northern brook lamprey ammocoetes (range 120–155 mm TL) from the Pike River. Mean %AE was 78.08% with a relatively low level of variation (range 71.01–84.38%; CV = 5.92%). More typically, ammocoetes collected were too small (60–90 mm TL) to provide adequate gut samples to determine individual %AE. As there was little variation between ammocoetes collected at the same time within a stream, we pooled samples from five individuals for monthly measures of %AE.

Throughout the year, lamprey ammocoetes assimilated, on average, 61% of organic detritus ingested. For the Keweenaw sites, %AE followed a seasonal cycle, peaking in July or August (Fig. 3a). Percent AE was higher during warmer months (May–October; mean = 72%) than during colder sampling periods (November–March; mean = 53%) (t-test; $p < 0.05$). Flooding in the West Branch of the Sturgeon River in July caused a change in diet composition resulting in no assimilation of ingested material (Fig. 3a). Assimilation efficiency estimates from the remainder of Great Lakes basin sites (Table 1) fall within the range of values for ammocoetes sampled during late summer at the Keweenaw sites (Fig. 3a).

Feeding Rate

For the Keweenaw sites, gut fullness followed a seasonal cycle, peaking in June and decreasing through the remainder of the year (Fig. 3b). During warmer months (May–October), gut fullness was higher (0.13 mg diet AFDM·g^{-1} ammocoete wet weight) than in colder sampling periods (November–March; 0.07 mg diet AFDM·g^{-1} ammocoete wet weight) (t-test; $p < 0.02$; Fig. 3b). Ammocoetes sampled from the remainder of Great Lakes basin sites typically had gut fullness values (Table 1) within the range found for ammocoetes sampled at the Keweenaw sites during late summer (Fig. 3b).

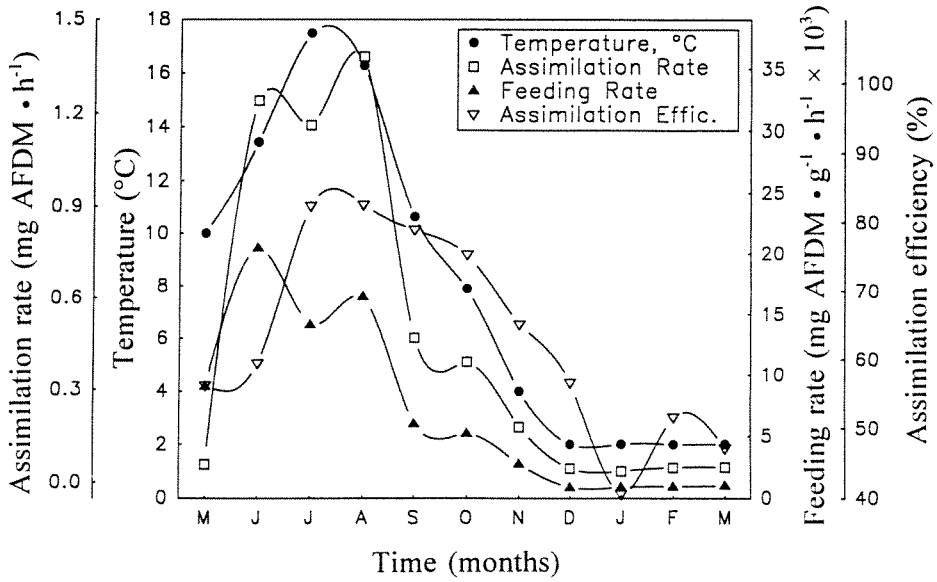

FIG. 5. Seasonal variation in stream temperature, assimilation rate, feeding rate, and %AE as estimated for the Keweenaw sites.

In the laboratory feeding experiment, mean feeding rates were 1.43×10^{-2}, 3.67×10^{-2}, and 3.21×10^{-2} mg diet AFDM·g^{-1} ammocoete wet weight·h^{-1} at 2, 9, and 16°C, respectively. Gut fullness values at these temperatures were 0.08, 0.11, and 0.07 mg diet AFDM·g^{-1} ammocoete wet weight. Although within-treatment variance was relatively high (CV = 58.51%), regression slopes of feeding rate and gut fullness between temperature treatments were significantly different (ANCOVA; both $p < 0.001$).

From field gut fullness estimates and the experimentally determined slope of the regression relationship between gut fullness and feeding rate (b_t; Fig. 4), we calculated feeding and assimilation rates for wild populations of lamprey ammocoetes from the Keweenaw sites for the period May 1992 through March 1993. Feeding rate (FR) was estimated as the product of gut fullness (GF), stream temperature (Temp), and the slope (b_t) (Fig. 4). Assimilation rate (AR) was estimated as the product of feeding rate (FR) and field determined %AE.

Discussion

Seasonal Variation in the Abundance of Algae and Bacteria

Variation in the abundance of algae in ammocoete diets follows the general annual cycle described for north temperate streams (Hynes 1970). Algae in diets peaked in late spring and early autumn when light availability and stream temperatures support high levels of diatom production (Chapman and Demory 1963; Hynes 1970). A similar cycle of algal abundance in ammocoete diets has also been reported in other studies (Moore 1972; Moore and Beamish 1973; Moore and Mallatt 1980). Bacterial abundance in the diet also followed a seasonal cycle, lagging about a month behind peaks in algal abundance. This may reflect the time required for bacterial population growth that is fueled by organic substrates from physiologically mature or senescent algae. Thus, variation in the abundance of algae and bacteria in ammocoete diets is consistent with the expected pattern of their availability in the annual stream cycle.

Sources of Nutrients Supporting Lamprey Larvae

Organic detritus is the principal trophic resource supporting the metabolism and growth of both sea lamprey and northern brook lamprey larvae in the populations studied. Microorganisms averaged only 2.2% of diet AFDM. Even if these are digested with 100% efficiency, their low concentration means they could account for no more than 4% of the organic matter assimilated. Because detritus makes up an average of 98% of diet AFDM and diet AFDM is assimilated with a mean efficiency of 61%, it is necessarily the case that most organic matter assimilated is detritus. The same conclusion was reported for two teleosts, *Oreochromis* (*Sarotherodon*) *mossambicus* (Bowen 1981) and *Prochilodus lineatus* (*platensis*) (Bowen et al. 1984), that feed on similar mixtures of aggregated detritus and microorganisms.

Although microorganisms provide a small fraction of the digesta, they may be important as sources of specific nutrients, especially vitamins, or as supplementary sources of digestive enzymes. Of much greater significance, they may play a critical role in production of the detrital fraction of the diet. There is a strong correlation between microorganism abundance and the digestibility of detritus, with assimilation efficiency lagging about one month behind microorganism abundance. Algal and bacterial abundances in ammocoete diets peaked in June and July, respectively, whereas peak %AE occurred in July and August. After October, %AE and microorganism abundance declined as stream temperatures cooled. Percent AE reached a low in January, increasing slightly in February before declining again in March (Fig. 3a). This February increase coincided with a period of warmer air temperatures from mid-January to early February 1993 that caused some stream ice melt, allowing light penetration to the stream bottom in some reaches for approximately two weeks.

The correlation in the annual cycles of microorganism abundance and assimilation efficiency points to algae and bacteria as agents responsible for the production of more digestible detrital aggregate. At the Keweenaw sites, the four- to six-week-long period of high discharge following spring snow melt either buries or scours from the system

much of the algae, bacteria, and organic detritus. This is followed in May and June by a spring diatom bloom during which cells begin to lay down layer upon layer of extracellular polymer matrix. Production of extracellular matrix is greater after the stationary growth phase as diatom cells become more physiologically mature (Decho 1990). This material entraps existing particles, adsorbs dissolved organic matter (DOM) and gradually builds up a "biofilm" layer (Decho 1990) comprised of "detrital aggregate" (Bowen 1979b). We speculate that the lag between peaks in diatom abundance and detritus digestibility (%AE) reflects the time required for detrital aggregate to accumulate, be dislodged by physical erosion, sloughing (Decho 1990), and invertebrate activity (Allanson 1973), and become a significant component of suspended particulate organic matter (POM) in streams. These newly produced particles are likely to be less degraded and refractory, and to have a higher food value than older particulate matter, especially that derived from vascular plant debris (Bowen 1979b). Not only do biofilms potentially represent a highly labile carbon source because they are more abundant in easily hydrolyzable polysaccharides, but the DOM adsorbed to their surface provides a reservoir of nutrients and organic compounds that are otherwise not available to particulate feeding organisms (Decho and Lopez 1993). Thus, we believe that the peak in %AE is likely due to increased availability of recently produced, more digestible detrital aggregate relative to other sources of detrital particles in streams. The correlation between bacterial abundance and the digestibility of detrital aggregate for lamprey larvae suggests that both heterotrophs derive their nutrition from labile detrital aggregate.

This interpretation of the annual cycle is consistent with the effects of the July flood in the West Branch of the Sturgeon River. Flood waters appear to have swept away both algae and detritus, leaving only indigestible sediment in suspension for ammocoete feeding. This heavy bedload movement resulted in ammmocoete diets containing an extremely high percentage of mineral matter compared with other months of the year. Typically, ammocoete diets contained, on average, 35% mineral matter (range 14.80–61.25%; SE = 2.06). However, in July, ammocoete diets contained 75% mineral matter. After stream discharge returned to normal, algal abundance increased in August and diet digestibility peaked later in September. Thereafter, the pattern for the Sturgeon River was comparable to the other two streams.

Seasonal Variation in Gut Fullness and Feeding Rate

Gut fullness and feeding rate appear more closely associated with stream temperature than with diet digestibility. An increase in stream temperature up to 15°C in early summer was associated with an increase in gut fullness, feeding rate, %AE, and assimilation rate (Fig. 5). Peak stream temperatures in July coincided with a decrease in feeding and assimilation rate, but a peak in %AE. Reduced feeding at this time may have been a response to the increased availability of digestible detrital aggregate, allowing the larvae to meet their nutritional needs while ingesting a smaller quantity of food. As stream temperature declined after the peak, feeding and assimilation rates increased again before declining through the remainder of the year (Fig. 5). This pattern points to the annual temperature cycle as a major influence on ammocoete feeding in north temperate streams (R^2 = 0.60 and 0.57 for %AE and gut fullness, respectively; both $p < 0.001$). Malmqvist and Brönmark (1981) reported a similar response, in that stream water temperature has a significant effect on the rate of particle filtration in *L. planeri* ammocoetes. However, the availability of a higher quality food source in biofilm detritus during the summer makes it difficult to separate the relative contribution of both temperature and food quality on ammocoete feeding.

Ammocoete Adaptations to Detritivory

Compared with other detritivorous fishes, the digestive tract of lamprey ammocoetes is unspecialized (Hardisty and Potter 1971). It consists of little more than a simple tube about half as long as the animal's body, with some differentiation into esophagus and intestine. As no mucosal folds or lateral caeca are evident, the surface area for absorption appears to be small. Also, the pH in the intestine of ammocoetes is circumneutral, typically around 7.5 (Barrington 1972). Other detritivorous fishes studied to date show morphological adaptations for digestion of detritus (Bowen 1980, 1984; Mundahl and Wissing 1988). Yet lamprey ammocoetes assimilate an average of 61% of the organic matter in their diets, a percentage similar to that reported for other detritivorous fishes (Bowen 1981; Bowen et al. 1984; Mundahl and Wissing 1988; Ahlgren 1990b). The question remains, how can lamprey ammocoetes so efficiently utilize a low quality food resource such as detritus while having no apparent morphological adaptations of their digestive tract?

Ammocoetes feed very slowly. We estimate from gut fullness data and the experimentally determined gut fullness/feeding rate relationship that at between 9 and 15°C, ammocoetes feed at rates ranging from 4.2 to 5.5 mg diet AFDM·g^{-1} ammocoete·d^{-1}. This is far below the 60 mg diet AFDM·g^{-1} fish·d^{-1} typical of other juvenile fishes fed highly digestible diets *ad libitum* (Brett and Groves 1979, *inter alia*). Slow feeding is consistent with observations that ammocoetes' respiratory pumping rate and metabolic rate are both slower than those observed for other fishes (Mallatt 1982; Sterba 1962; Hill and Potter 1970) and with the long time period required for gut clearance in ammocoetes (Moore and Mallatt 1980). In the absence of discernable morphological adaptations for digestion of detritus, it appears that ammocoetes achieve a high assimilation efficiency with an extraordinarily long period of digestion. This suggests that natural selection may have favored a low metabolic rate as an adjustment to the limited nutrient assimilation rate associated with this digestive strategy. In turn, low metabolism is linked to slow growth and the extended length of the detritivorous larval phase (3–13 yr; Applegate 1950; Lowe et al. 1973). Thus, many of the life history characteristics of lamprey larvae can be interpreted as adaptations to organic detritus as a food resource.

The destructive impact of sea lamprey predation on Great Lakes fishes is currently partially controlled by chemical treatment of streams to kill larvae before most metamorphose into the parasitic form. Due to both cost and limited effectiveness, the level of control in many parts of the basin is not adequate to support management objectives. There is a growing need to supplement or replace the current control technology with less expensive, more effective, and more environmentally benign technologies. Identification of the link between the detritus food resource and larval

lamprey production may provide a step toward identifying opportunities for new control strategies.

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Part II

Yap, M. R. and S. H. Bowen. 1998. Habitat Selection Affects Diet Quality for the Biofilm-Feeding Northern Brook Lamprey (*Ichthyomyzon fossor*). (In preparation)

ABSTRACT

We studied the relationships between habitat, feeding and fitness in larval northern brook lamprey (*Ichthyomyzon fossor*) in three low order streams in Michigan's Upper Peninsula. Larvae were less abundant in sand (site means = 0.2 - 0.5 larvae / 0.5 m^2) than in organic sediment (0.5 - 2.85 / 0.5 m^2). Within patches of organic sediment, larvae aggregated at sites where obstacles to flow caused localized accumulation of flocculent material ($\leq$ 26 larvae / 0.5 m^2). Both field and laboratory experiments showed that food particles were taken from the seston rather than from sediment. Mucus from the buccal cavity used to trap and ingest seston made up 8% of foregut organic matter, by mass. Ingestion was selective, with both organic matter and the amino acid concentration in organic matter higher in lamprey foreguts than in seston. Habitat type had a small effect on levels of organic matter and amino acids in the diet, but had a major effect on assimilation efficiency for both organic matter and amino acids with highest values in depositional areas where larvae aggregated. Condition factors (weight per length) were similarly higher in these depositional areas. Throughout the warm season (May through October), monthly variations in diet composition suggest a cycle of seston production resulting from biofilm sloughing from solid surfaces as controlled by stream discharge and the availability of light.

INTRODUCTION

Larval northern brook lamprey (*Ichthyomyzon fossor*) build burrows in stream sediments. The burrow is connected to the overlying water by a small tube through which they draw a respiratory current. Their diet consists of an aggregated mixture of nonliving organic matter, algae and bacteria that appears to be identical to material widely described in the literature as biofilm (Sutton and Bowen 1994). Many of the factors that affect lamprey use of this dietremain unknown. Food particles are trapped on a buccal mucus network, and food and mucus are ingested together (Mallatt 1981). We do not know if particle capture is selective, or if all particles have an equal probability of capture. Interpretation of the diet is difficult since we do not know how much of the gut content is mucus and how much is food. Various authors have suggested that lamprey larvae may collect their food as sediment inside or outside the burrow, or as free particles of seston in the respiratory current (Moore and Beamish 1973, Mallett 1983). It is likely that the source will have significance for both the quantity and the quality of food available to larvae (Bowen 1987). Food characteristics may also be affected by turbulent stream currents as they differentially suspend,

transport and redeposit particles in various habitats. Seasonal variation in stream discharge, temperature and light are other factors likely to affect the production, distribution and quality of biofilm particles (Sutton and Bowen 1994). Our purpose in the research presented here was to examine factors that influence of use and value of biofilm as the food resource used by larval lampreys so that we better understand the effect of diet on their growth, transformation and survivorship.

METHODS

Study Areas and Habitats

We studied the food and feeding of northern brook lamprey (*Ichthyomyzon fossor*) in three streams in Michigan's Upper Peninsula: Pilgrim and Pike Rivers (Houghton County), and the West Branch of the Sturgeon River (Baraga County). These streams do not contain sea lamprey, and thus are not treated with lampricide. This was essential to our study because, only in untreated streams were we able to collect sufficient numbers of larger (< 7mm total length) animals needed to obtain enough gut content per animal ($\geq$ 0.5 mg dry mass) for chemical analyses. Pilgrim (47°02'N 88°50'E) and Pike (47°52'N 88°50'E) are second order streams with widths that range from 4-9m in the study reaches. West Branch of the Sturgeon (46°30'N 88°50'E) is third order and ranges from 10-14m in width. During the summer, the canopy of the Pike is closed, that of the Pilgrim is partly closed and that of the West Branch of the Sturgeon is open.

We adopted the system of habitat distinctions developed by sea lamprey control agents which is based on sediments types (Klar and Weise 1994). Type I is soft, organic sediment; Type II is firm, sandy sediment with a relatively low organic content; Type III is dominated by gravel or larger particles.

Field Collections

Larvae were obtained from stream sediments using a backpack electroshocker specially designed for collection of these animals (ABP-2, Instrumentation Systems Center, University of Wisconsin, Madison, WI.) Immediately after capture, the larvae were anaesthetized with 50 mg / L MS-222 (Sigma Chemical) to prevent stress-induced regurgitation or defecation, and then fixed in 4% formalin. Later they were rinsed with water and stored in Carosafe preservative (Carolina Biological Supply). Weight and length were measured from preserved animals.

Condition factor (K_n), a ratio of weight to length that reflects the nutritional status of individual animals, was calculated according to LeCren (1951) as

$$K_n = W_o / W_e \, , \, W_e = a \, L^{\,b}$$

where, W_o is the observed mass of the larvae, W_e is the expected mass of the larvae, L is the total length of the larvae, and a and b are fitted constants for a reference population of larvae. The reference population used here was made up of pooled samples from monthly collections from the Pilgrim, Pike and West Branch of the Sturgeon Rivers from May through October, 1994.

The way in which the stream bottom was sampled depended on the purpose of the collection. For studies of the relationship between habitat and diet, we collected five animals from each of two patches of Types Ia (defined below), I and II habitat within each stream. Collections were made monthly from May through October 1994. Only during the May - October period do we expect significant variation in diet, and feeding rate is much higher during this period (Sutton and Bowen, 1994). The June sample for the Sturgeon River was missed as a result of a persistent flood that prevented our finding the animals.

For study of larval density, a square aluminum frame was laid on the bottom to define a 0.5 m^2 sample area which was shocked for a fixed length of time. The frame was then systematically turned on a lateral or upstream edge to define the next square in the sampling grid. In Pilgrim and Sturgeon Rivers, we sampled 60 0.5 m^2 areas in each of two type I habitat patches and 30 0.5 m^2 areas in each of two type II and two type III habitat patches. In the Pike River, only type I habitat is common so our sampling there was limited to 60 0.5 m^2 areas in each of two type I habitat patches.

Samples of particulate matter were collected in May, 1993 from Pilgrim River for comparison to lamprey gut contents. Seston was collected by emersion of a clean, 7.5L plastic container at the mean depth for each sample site. The sample was passed through a 250μm sieve to remove large particles normally rejected by the lamprey feeding apparatus, and the remaining particles were collected by centrifugation. Sedimenting particles were collected in shell vials pressed into the sediment with about 2 mm of the vial above the sediment, and surficial sediment was collected with shell vials pressed all the way into the sediment so that the tops were flush with the sediment surface. After three days, the vials were recovered and the content transferred to a 1L bottle with 500 ml of filtered tap water. The bottle was shaken vigorously, the contents were allowed to stand for 60 seconds so that sand in the sample could settle, and the remaining suspended particles were decanted and allowed to settle for several hours. Seston and settled benthic particles were collected in pre-weighed aluminum pans, dried at 50°C, and stored in vials pending analyses.

Diet Analyses

The digestive tract of lamprey larvae is a simple tube, undifferentiated except for a single longitudinal dorsal fold. Initial experiments with larvae in aquaria fed yeast as a diet marker indicated the food moves through the digestive tract in plug flow with little

mixing of diet components ingested at different times. Thus, samples from the anterior and poster gut were taken as representative of food and feces, respectively. We collected the contents or the anterior and poster 10% of the digestive tract for individual animals. Gut contents were dried to constant mass at 50°C, homogenized by grinding, and stored in airtight vials pending analysis.

Composition of the diet was determined in terms of ash-free-dry-mass (AFDM), and total amino acids. AFDM was determined as weight loss on ignition at 555°C. Five minutes at this temperature was sufficient to reach constant mass for samples of < 10 mg. Amino acids were liberated by alkaline hydrolysis (Hirs, 1967) and quantified by the ninhydrin assay (Moore and Stein 1948, James 1978, James 1984) with minor modifications as described by Lemke (1992). Although different amino acids give different color intensities with ninhydrin, the amino acid composition of organic detritus varies little and is expected to have a minor effect on either accuracy or precision (Bowen 1987). The coefficient of variation (standard deviation divided by mean) for five replicate samples was 7.2%.

Assimilation efficiency (AE%) is the mass of a nutrient that passes through the gut wall expressed as a percentage of the amount of nutrient ingested. In this study, we calculated assimilation efficiency using the ash ratio method (Conover 1966) which is based on the assumption that materials that contribute to the ash component of the gut content are not assimilated. This assumption is appropriate for study of lamprey larvae since most of the ash in their diets is indigestible sand particles and diatom frustules. For some collections made up of mostly smaller animals, it was necessary to pool gut contents from several animals to obtain the quantity of material required.

Laboratory Feeding Experiments

Polystyrene beads similar to biofilm particles in density (1.05 g/cm^3) and diameter (48-50 μm) were used to trace sources of particles ingested by lamprey larvae in aquaria. To each of five 600 ml aquaria (beakers) filled to a depth of 5 cm with sand, we added one large larva (10 cm total length). The aquaria were kept in a incubator at 12°C with a 12 h light / 12 h dark photoperiod, and the larvae were allowed to build burrows and acclimate for two days. The relatively large surface area to volume ratio and cool temperature obviated the need for aeration. Biofilm containing algae, mostly diatoms, was scraped from an aquarium wall and added to the water to a concentration of 1 mg dry mass / L. White colored beads were added to the aquaria (1 mg / L water) and allowed to settle for 24 hrs, and then a suspension of green colored beads was added to produce the same concentration. Experiments were terminated 6 h after addition of green beads. With care not to disturb the sediment, water was siphoned into a collection bottle to within 2 mm of the sand surface. The last 2 mm of water was swirled in the aquarium and siphoned into a second collection bottle together with the sediment. Larvae were anesthetized with MS222 and the contents of the anterior one tenth of the gut was collected. White and green beads in each of the three samples

(suspended particles, sedimented particles, gut contents) were counted using light microscopy.

Selective ingestion of suspended particles was assessed in 10 L aquaria. Larvae were put in glass tubes to simulate burrow conditions (Mallatt 1982) an four larvae were suspended in the center of each aquarium. Temperature and photoperiod were as described above. Flocculent, organic sediment from Pilgrim River was kept suspended by constant aeration. Two aquaria contained sediment that passed through a 125 μm sieve, and two contained sediment that passed through a 64 μm sieve. Each day, a sample of the suspended material was collected from in front of each tube. The animals were allowed to feed for 5 to 7 days, anesthetized with MS222 and the contents of the anterior one tenth of the gut was collected and dried for determination of AFDM and amino acids.

We assessed the extent to which mucus contributes to gut contents using three replicate aquarium experiments (Bowen 1981) Eight larvae were individually placed in glass tubes and suspended in the aquarium with a precisely measured quantity of unfiltered Pilgrim River water containing seston at 5.56 mg/L. A magnetic stirrer kept particles in suspension. A second aquarium not containing larvae was used as a control. Aquaria were held under controlled temperature and photoperiod as described above. Larvae were allowed to feed for twelve hours after which they were anesthetized and the gut contents were collected and pooled. Seston remaining in the aquaria was collected by centrifugation. All AFDM samples were dried pending analysis. The quantity of AFDM ingested by lamprey larvae was calculated as the quantity in the control minus the quantity in the test aquarium. The amount of mucus in gut contents was estimated as the AFDM in gut contents, minus the AFDM ingested.

RESULTS

Distribution

Larvae were most abundant in type I habitats, with mean densities per habitat patch ranging from 0.5 - 2.85 larvae / 0.5 m^2. Much lower densities were found in type II habitats (0.2 - 0.5 / 0.5 m^2), and larvae were rarely found in type III habitats. Within habitat patches, larvae showed a high degree of aggregation that was significantly different from the expected Poisson distribution (Fig. 1; $\chi^2 = 129.6$, $p < .001$, Statgraphics 1987). Specifically, we found a higher than expected proportion of 0.5 m^2 areas with no larvae, and a few areas had very much higher numbers of animals than expected had they been distributed at random. In one 0.5 m^2 area, we collected 26 larvae and others no doubt escaped before we could catch them. Within type I habitats, we observed that sites where larvae aggregated where often downstream of obstacles to flow such as stones or woody debris that favored localized deposition of flocculent, organic sediment. We termed these areas "type Ia" habitat.

Contribution of Mucus to Gut Contents

Mucus made up about 8% of the organic matter in gut contents. Results from the three replicates are similar, due in part to the relatively large number (8) of larvae in each test aquarium.

Table 1. AFDM (mg) in test and control aquaria, and lamprey gut contents used to calculate the contribution of mucus to gut contents.

Replicate	1	2	3
Control Aquarium (A)	3.301	15.560	14.116
Test Aquarium (B)	2.329	14.604	12.619
Gut Contents (C)	1.013	1.039	1.596
Ingested by Lampreys (A-B = D)	0.909	0.956	1.497
AFDM as Mucus (C-D = E)	0.104	0.083	0.099
Mucus as % of Gut AFDM ((E/C) * 100)	10.30%	7.98%	6.20%

Selective Ingestion

Data from field studies showed mean AFDM values were higher in gut contents than in any of the three particle categories collected from the Pilgrim River (Fig. 2). Two-factor ANOVA showed mean AFDM values were significantly different across sources ($p<0.001$) and habitats ($p=0.002$), and an *a posteriori* multiple comparisons test (SNK) revealed two statistically distinct groups: gut contents and seston, and sedimenting particles and sediment.

In aquarium experiments using polystyrene beads, the beads were readily ingested by larvae. From 4 to 16% of the beads recovered from the aquaria were in lamprey gut contents (Table 2). Green beads intended to simulate seston mostly stayed in suspension, while white beads intended to simulate sediment mostly stayed on the bottom. Most beads ingested by lamprey larvae were green, although a small percentage (mean = 13.4%) was white.

Table 2. Distribution of 48-50 μm diameter green polystyrene beads relative to white beads in food choice experiments.

Replicate	Percentage of Green Beads (percentage of all beads in replicate)		
	Seston	Sediment	Foreguts
1	92 (26)	7 (68)	83 (6)
2	98 (25)	3 (65)	96 (10)
3	87 (30)	6 (54)	88 (16)
4	96 (25)	1 (68)	86 (7)
5	99 (61)	4 (35)	80 (4)
Means	94.3 (33.4)	3.9 (58.0)	86.6 (8.6)

Table 3. Comparisons of AFDM and amino acids in lamprey foreguts and seston collected from Pilgrim River during the summer of 1994.

Month	Foreguts (95% C.I.)	Seston (95% C.I.)	Ratio
Ash-Free-Dry-Mass (mg / mg dry mass)			
May	0.860 (0.038)	0.335 (0.019)	2.57
June	0.596 (0.074)	0.321 (0.019)	1.86
July	0.660 (0.059)	0.387 (0.055)	1.71
August	0.408 (0.010)	0.336 (0.023)	1.21
Amino Acids (mg / mg AFDM)			
May	0.151 (2.020)	0.049 (.008)	3.08
June	0.101 (0.018)	0.051 (0.008)	1.98
July	0.105 (0.021)	0.060 (0.023)	1.75
August	0.052 (0.010)	0.057 (0.014)	0.91

Larvae held in glass tubes in aquaria and exposed only to suspended natural particles collected from Pilgrim River selectively ingested particles higher in AFDM and amino acids (Fig. 3). *In situ* in the Pilgrim River, larvae in glass tubes and those in the sediment both concentrated AFDM relative to the ash component, but in this case did not concentrate amino acids. Similarly, comparison of foregut contents and seston from a type Ia habitat in the Pilgrim River in four different months showed foregut contents to be higher in AFDM and amino acids than seston collected from above the site (Table 3). For both nutrients, the degree to which the nutrient was concentrated relative to other diet components declined over the course of the summer. This resulted from a change in the selective ingestion process itself and not from any quantitative change in the seston for which AFDM and amino acid values remained relatively constant.

Differences Among Habitats

Averaged across the six month sampling period, habitat type appeared to have a significant effect on AFDM and amino acid concentrations in foregut contents in the Pike River, but not in Pilgrim or Sturgeon Rivers. In contrast, habitat type had a very highly significant effect on the assimilation of both AFDM and amino acids, and on the subsequent condition factor of lamprey larvae in all three systems studied (Fig. 4). In all cases with statistically significant differences, nutrient concentration, nutrient assimilation and condition factor were highest in type Ia and lowest in type II.

Differences Across Months

Within the limitation imposed by the missing sample for Sturgeon River in June, patterns of change were the same for all three streams. We found a trend of decreasing AFDM in foregut contents from May through August, a substantial increase in September, followed by a decrease in October (Fig. 5). Foregut amino acid values show a clearer trend of continuous decline from May through October (Fig. 6). Assimilation efficiency values for both AFDM (Fig. 7) and amino acids (Fig. 8) follow a pattern very similar to that found for AFDM , with a gradual decline from May through August, an increase in September, and a decline in October.

DISCUSSION

The preference of lamprey larvae for type I habitat is consistent with patterns observed by Sea Lamprey Control agents throughout the Great Lakes basin (pers. comm. U.S. Fish and Wildlife Service Sea Lamprey Control Group, Marquette, MI, USA.). Within type I habitats, larvae tend to aggregate in areas where obstacles to flow cause localized deposition of fine, flocculent sediment. Others have reported that lamprey larvae tend to be associated with organic matter in streams (Malmqvist 1980, Potter *et al.* 1986). From the authors' descriptions, these appear to be depositional sites where vascular plant debris accumulates and thus are similar to our type Ia habitats. Not all sites that fit the type Ia description have high concentrations of larvae.

At some, very high concentrations were found ($\leq$ 26 / 0.5 m^2), but other sites that appear to be very similar had few or none. This may reflect proximity of the site to gravel beds where eggs were spawned, or it may reflect the attraction of larvae to each other.

Many fishes that feed on small particles capture them through their adhesion to mucus that lines structures in the oral and branchial cavities. Cilia then move the mucus and entrapped particles to the pharynx. This mucus could potentially affect the quality of gut contents in at least two ways that would influence our interpretation of differences between seston and gut contents. Food particles may differ in characteristics that affect their adhesion to mucus, resulting in selective ingestion. Secondly, inasmuch as mucus and food particles differ in chemical composition, the makeup of the gut contents will be affected by the proportion that is mucus. Although entrapment on a branchial mucus net is the principal means of food particle capture in larval lamprey (Moore and Mallatt 1980, Mallatt 1981) the mucus itself makes up only about 8% by mass of the organic matter in gut contents. Compared to differences between food sources and gut contents described below, this 8% contribution is small and thus not a significant factor in interpretation of the results. Thus, selective capture is the more likely mechanism responsible for differences between seston and gut contents.

Both field survey and laboratory experiment data indicate that the majority of particles ingested by larval lampreys are from the seston. Although the high values for AFDM in larval gut contents could have been achieved by selective ingestion of AFDM - rich sedimenting or sediment particles, it is more parsimonious to interpret this result as indicative of feeding on seston. Results of the bead experiments offer strong support to this conclusion. Larvae draw their food-bearing respiratory current into their burrows at the water-sediment interface. In our bead experiments, settling from seston and turbulent resuspension of sediment are likely to have mixed the green and white beads at the water - sediment interface to a greater extent than indicated by the post-experiment separation of seston and sediment. Even setting aside this likely influence, the results indicate that 90% of the ingested material was derived from seston. This finding provides experimental support for inferences made by others from comparisons of seston and gut contents (Creaser and Hann 1929, Manion 1967, Moore and Beamish 1973).

Further comparison of gut contents and seston shows that the lamprey ingestion process is selective. On average, particles higher in AFDM and amino acids are retained relative to other diet components. Concentration factors of 3 are not unusual for either nutrient. The degree of selection is variable, however, and appears to be more variable for amino acids than for AFDM. Larvae held in glass tubes concentrated amino acids in both laboratory experiments, but failed to do so in the one field experiment (Fig. 2). AFDM was concentrated in all three experiments. Early in the

summer, larvae in Pilgrim River were substantially concentrating both nutrients, but the effectiveness of selective ingestion declined over time with no apparent change in the levels of these nutrients in the seston. The proportional decline was greater for amino acids than for AFDM. In other work (Young 1995), we found no correlation between AFDM or amino acid values for seston and gut contents studied from May through November in three streams. Thus, successful concentration of nutrients appears to depend on qualitative features of the diet yet to be identified.

Based on a 13 month study of feeding by sea and northern brook lamprey larvae in the Pike, Sturgeon, and Misery Rivers, Sutton and Bowen (1994) described an annual cycle of seston generation from microbially generated biofilms that controlled the quality of larval diets in terms on AFDM and its assimilation. High discharges from spring snow melt scour much of the organic mater from the streams. Algal blooms during early summer establish layers of cells on firm substrata, but subsequent development of the riparian canopy limits algal production. As a result, attached algae and associated microbes mature and produce increasing quantities of extracellular polymer matrix that traps both dissolved organic matter and particles. This biofilm matrix becomes friable, and fragments are carried away by the current as seston. As the particles age, the more labile components are lost relative to the more refractory components, and thus the particles both lose nutrients and become less digestible over time.

The pattern of change across summer months reported here refines and extends our understanding of the annual cycle described by Sutton and Bowen (1994). Trends in the present study are clearer and more consistent across streams due, in part, to our stratified sampling by habitat. The previous authors simply collected the first ten animals encountered. From our results, it is clear that habitat differences contribute considerable variation to unstratified samples. Our more sensitive data allow us to distinguish a September peak in the abundance of diet AFDM, and in assimilation efficiency for both AFDM and amino acids. This corresponds to a late summer peak in algal biomass reported by Sutton and Bowen (1994) which they concluded was a result increased primary productivity permitted by the thinning riparian canopy. It is likely that this pattern of seston food quality applies to other seston feeders which may be similarly selective in particle ingestion.

The simultaneous declines over the summer in ability of larvae to concentrate AFDM and amino acids in their diet (Table 3) and in their ability to digest those particles they ingest (Figs. 7 and 8) is consistent with the suggestion that larvae selectively ingest recently formed biofilm particles that are both nutrient rich and labile, and select against older seston particles that have lost labile nutrients and become refractory (Sutton and Bowen 1994). If in-stream primary productivity declines due to riparian shading, the availability of recently formed particles would decline relative to the abundance of older particles. Whether or not particle age is the principal

discriminant, these observations indicate that characteristics of particles important to their digestibility are also important to the ability of larvae to select them.

Habitat selection clearly affects diet quality and condition of lamprey larvae. The majority of larvae select type I habitats, and there the animals obtain diets that are more digestible in terms of both AFDM and amino acids than the diets of larvae in type II habitats. Those that aggregate at localized depositional sites within type I habitats (type Ia habitats) benefit from even more digestible diets and achieve much higher condition factors. It is not yet clear why these differences occur. We speculate that more refractory particles may have a higher density (Bowen 1987) and thus, as water moves into less turbulent areas such as type Ia habitats, the relative proportion of more digestible suspended particles may increase. However, we have not been able to measure consistent differences in seston quality in type 1 and type 1a habitats. Since were are not able to selectively sample seston in a manner analogous to the selective ingestion of lamprey larvae, it appears that differences in seston quality occur in some fraction of the seston that we cannot discriminate based on analyses of bulk samples.

The effect of habitat on diet and condition may not be equally significant in all streams. The streams studied here are oligotrophic due to low temperatures, low nutrient levels, and riparian shading. Lampreys are also abundant in warmer, more eutrophic streams where high food quality particles are likely to be more available. Young et al. (1990) found the growth rate of lamprey larvae was correlated with factors that influence the productivity of streams. Sea lamprey control agents have observed that larvae below a sewage outfall on the Chippewa River, Isabella Co., Michigan, are consistently larger than those collected above (pers. comm., S. Morkert, US Fish and Wildlife Sea Lamprey Control Unit, Luddington, MI). Thus, there may be little benefit from selection of type 1a habitats in food rich streams or stream segments. Stratified sampling on the scale used in the present study would be required to determine if the aggregation patters found in oligotrophic streams similarly apply to more eutrophic streams.

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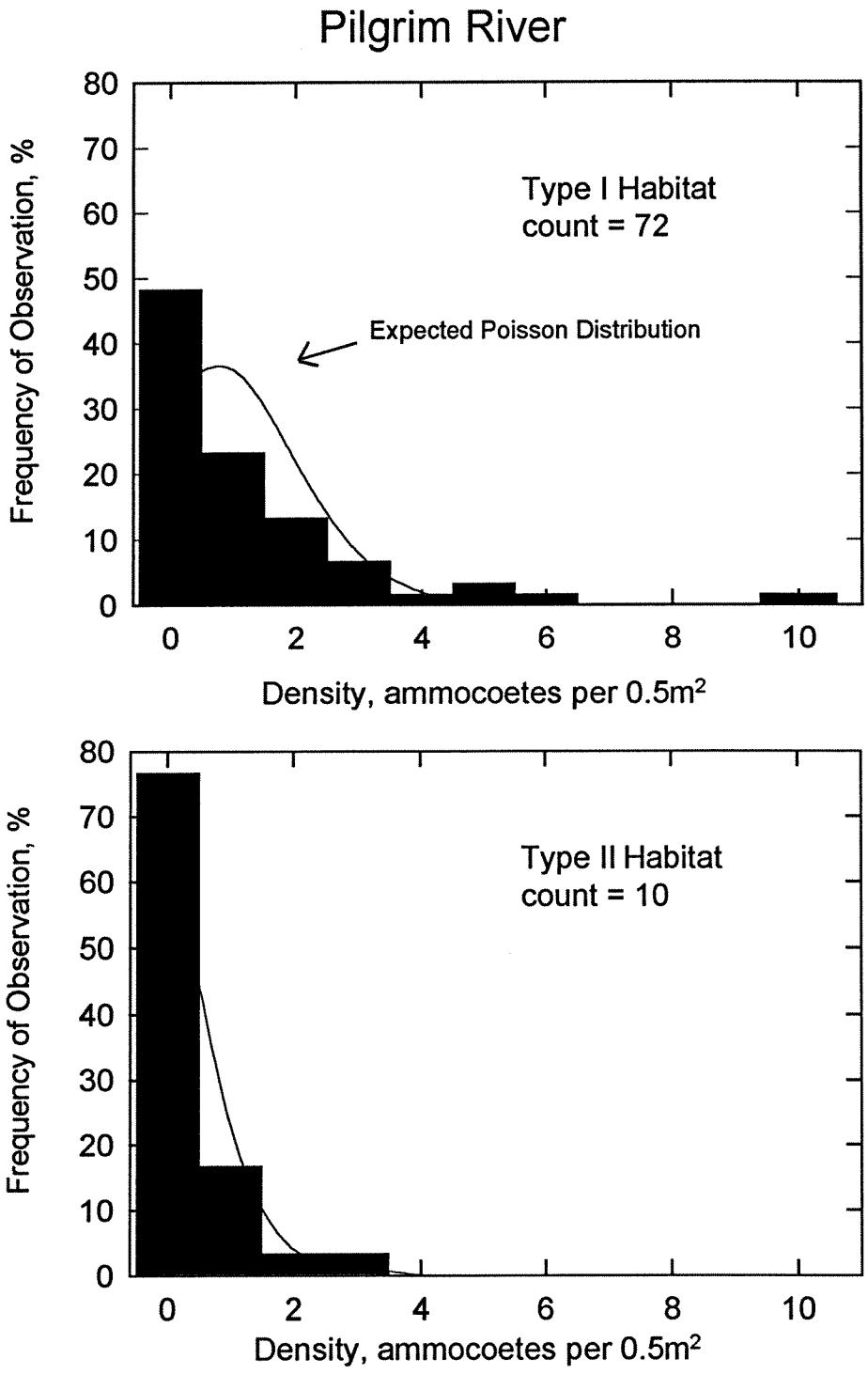

Figure 1. Frequency distributions of lamprey larvae at different densities in type I and type II habitats in the Pilgrim River, Houghton Co. MI, USA

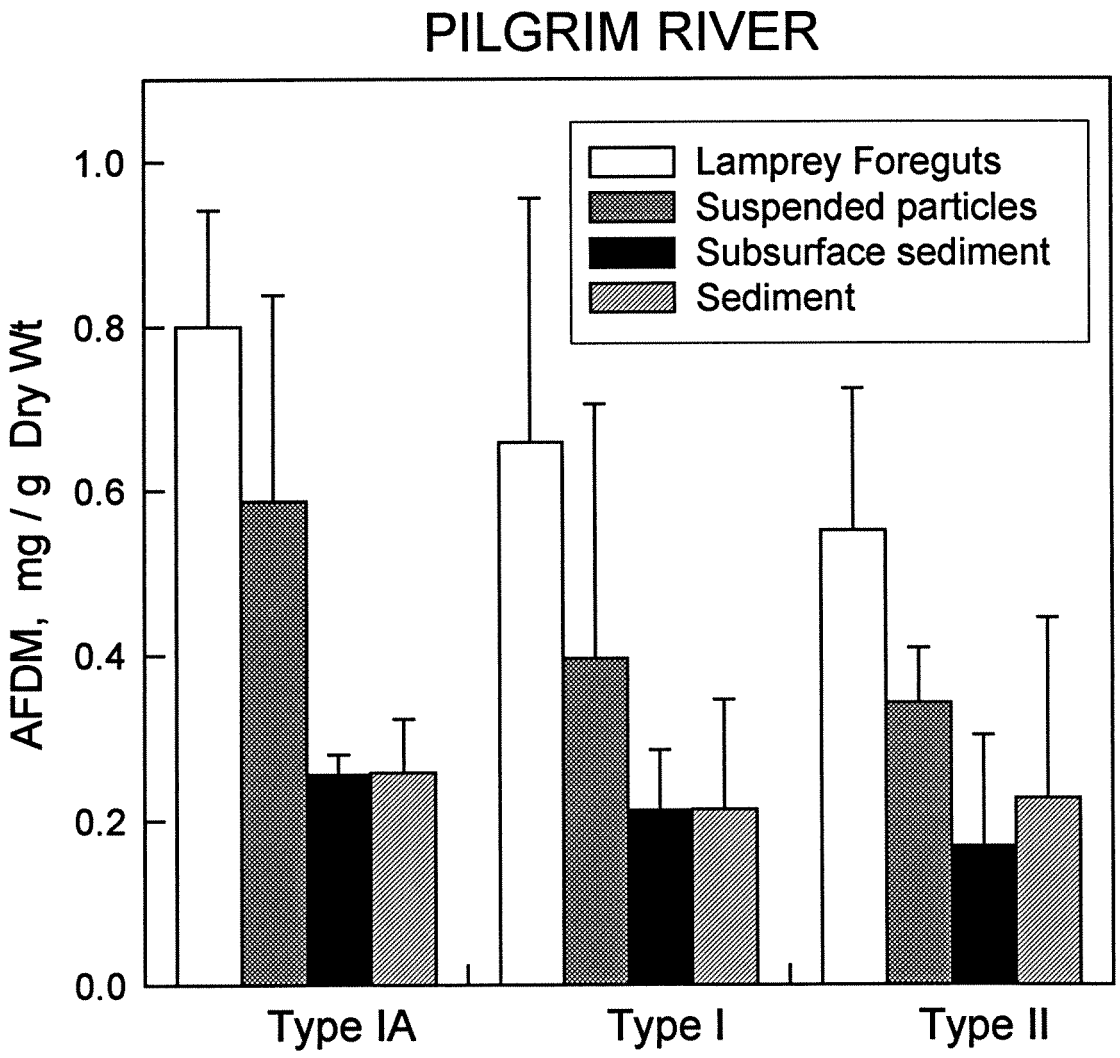

Figure 2. Comparison of lamprey foregut contents with potential food sources from the Pilgrim River, Houghton Co. MI, USA.

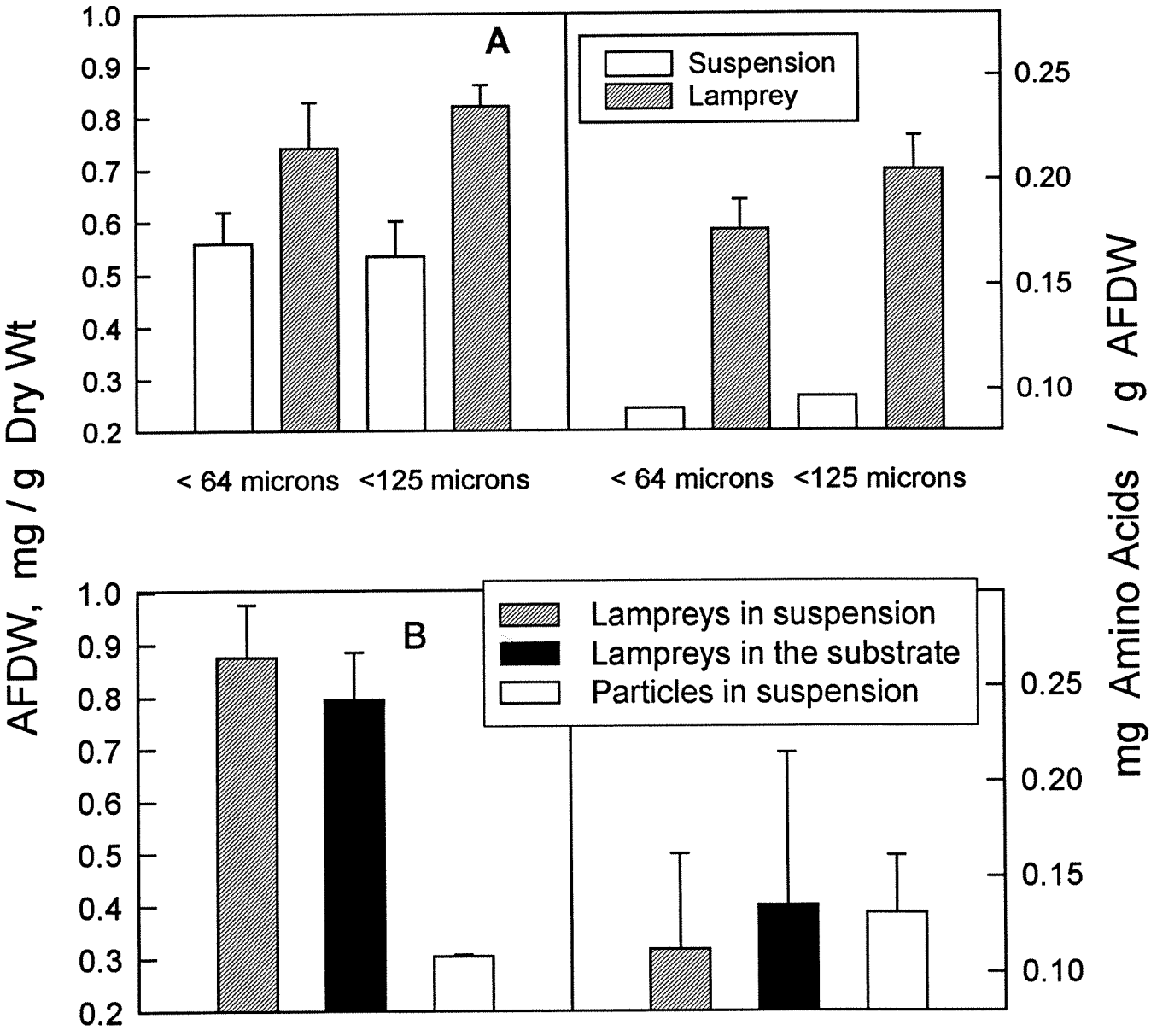

Figure 3. **A**: Laboratory experiment comparing AFDM and amino acids in foreguts and in suspension. Each bar represents the mean of 4 replicates with 95% confidence intervals. **B**: Field experiment comparing AFDM and amino acids in the foreguts of larvae held in suspension, larvae in the sediment, and particles in suspension. Error bars are 95% confidence intervals.

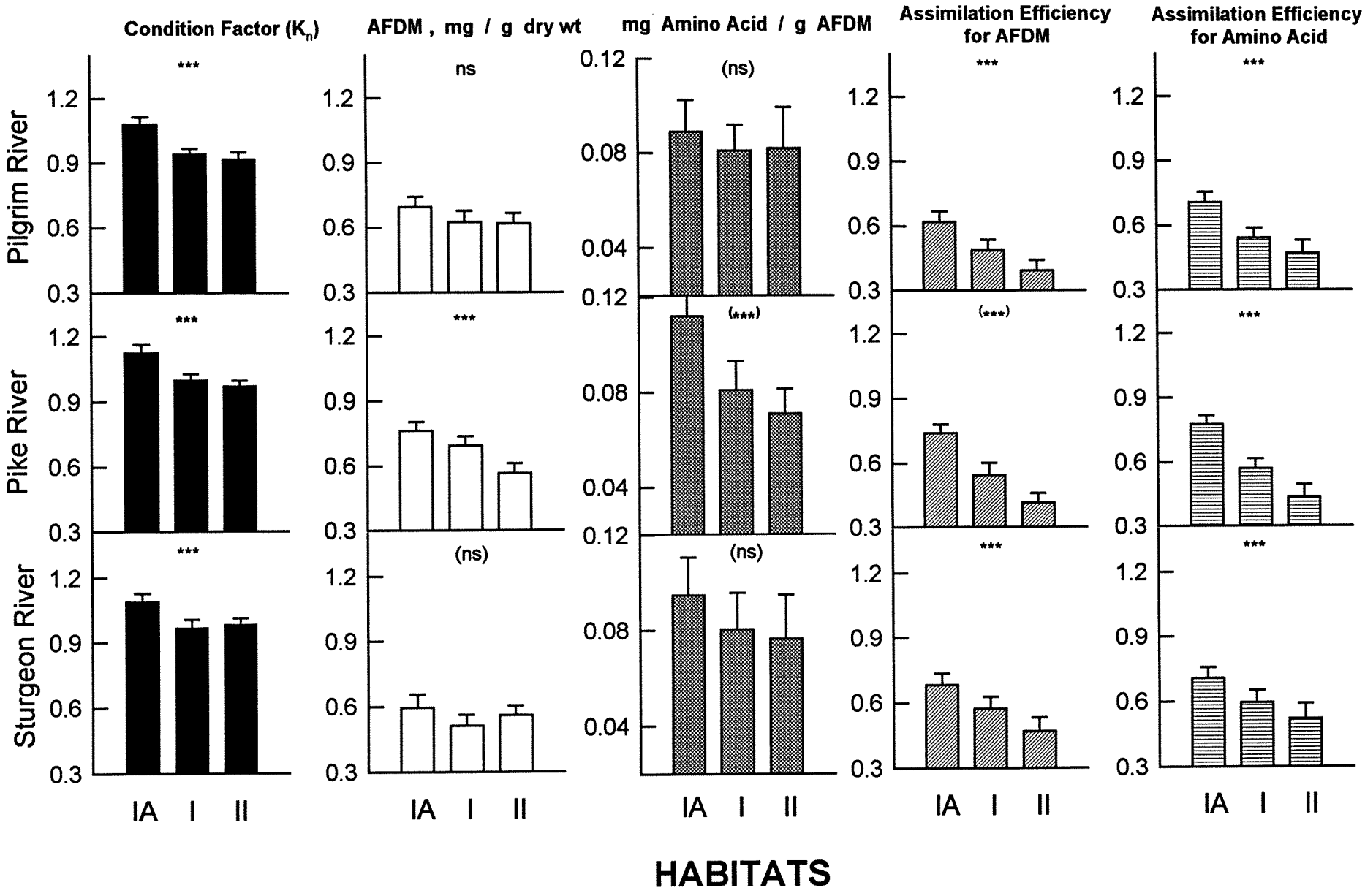

Figure 4. Differences in condition factor, diet composition and assimilation efficiency across habitats. Bars are means with 95% confidence intervals for six monthly samples (May - October). ANOVA *** = p<.0001; ns = not significant. ANOVA on Ranks (***) = p<.0001; (ns) = not significant.

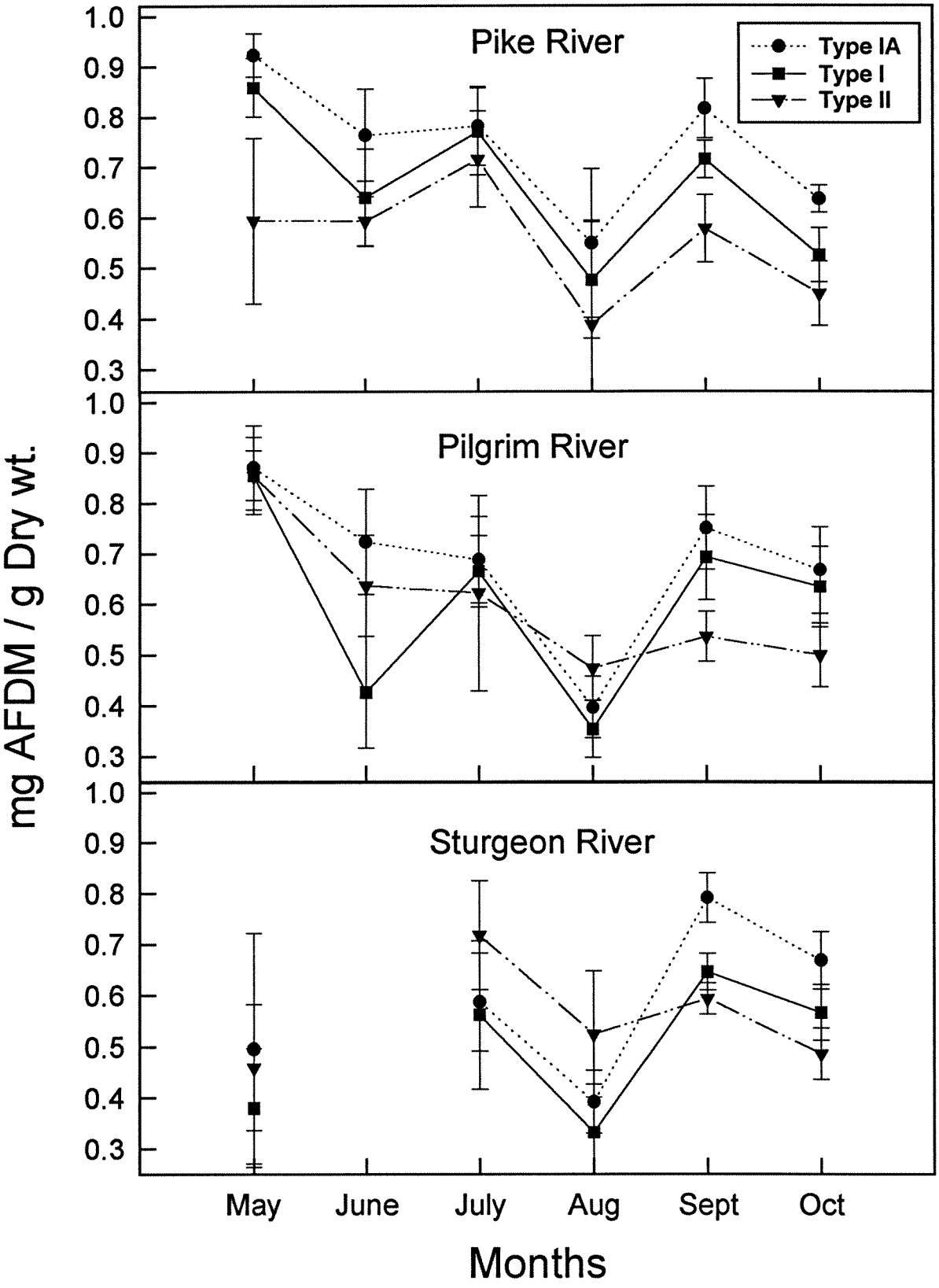

Figure 5. AFDM in the diet of lamprey larvae across summer months. Each point is the mean for ten larvae, five from each of two habitat patches, with 95% confidence intervals. Flooding prevented collection of larvae in the Sturgeon River in June.

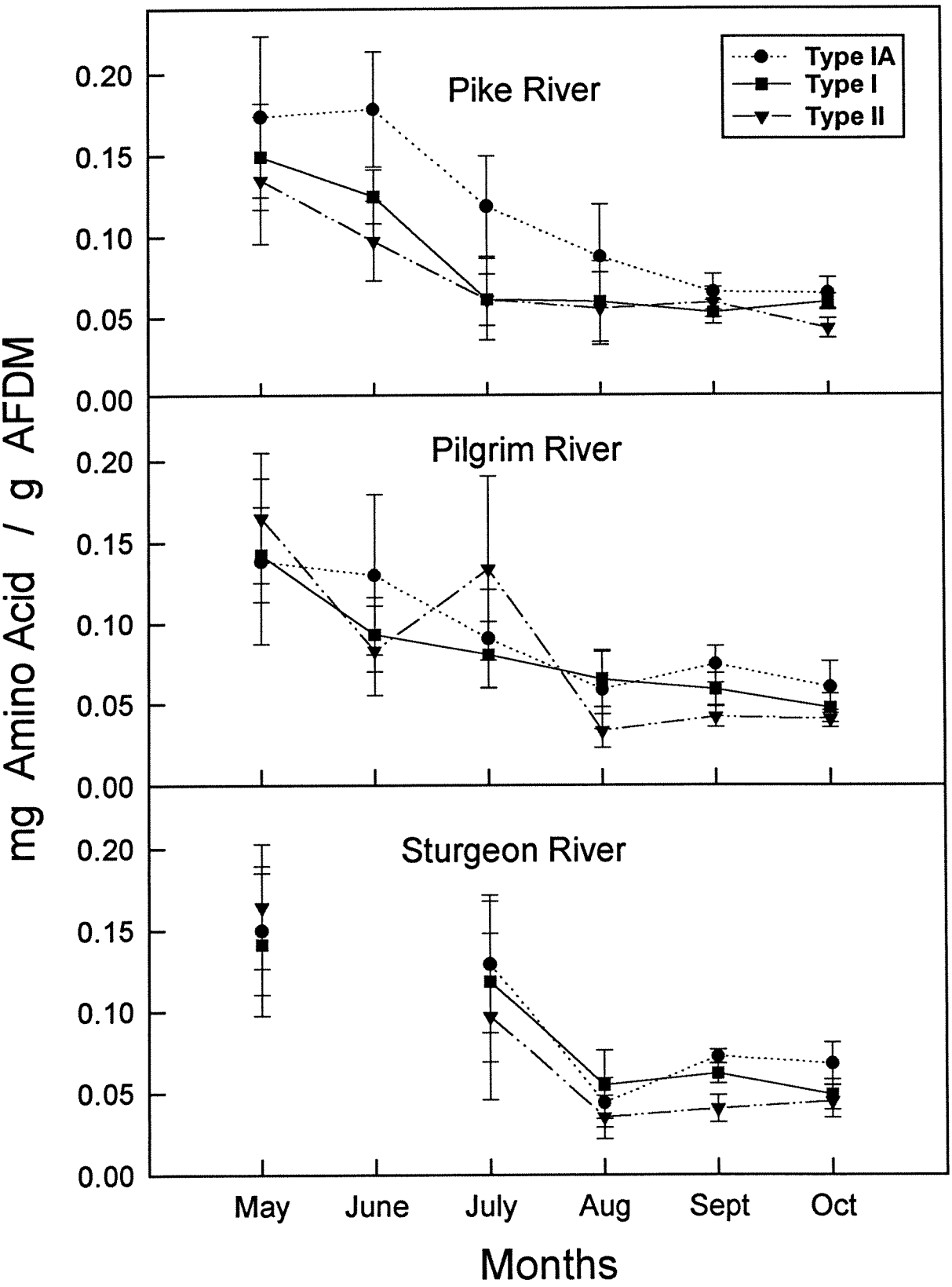

Figure 6. Amino acids in the diet of lamprey larvae across summer months. Each point is the mean for ten larvae, five from each of two habitat patches, with 95% confidence intervals. Flooding prevented collection in the Sturgeon River in June.

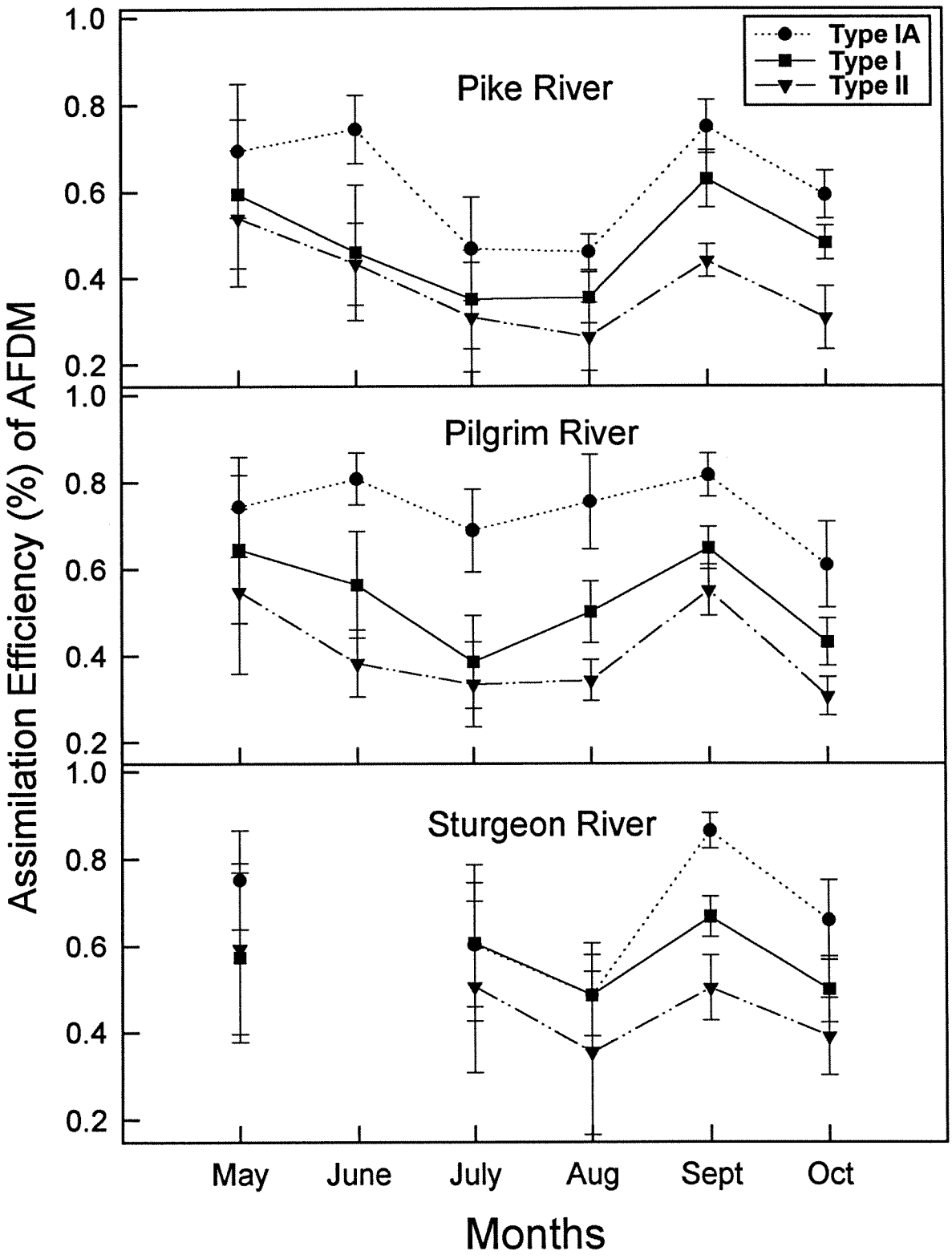

Figure 7. Assimilation efficiency for AFDM across summer months. Each point is the mean of ten larvae, five from each of two habitat patches, with 95% confidence intervals. Flooding prevented collection in the Sturgeon River in June.

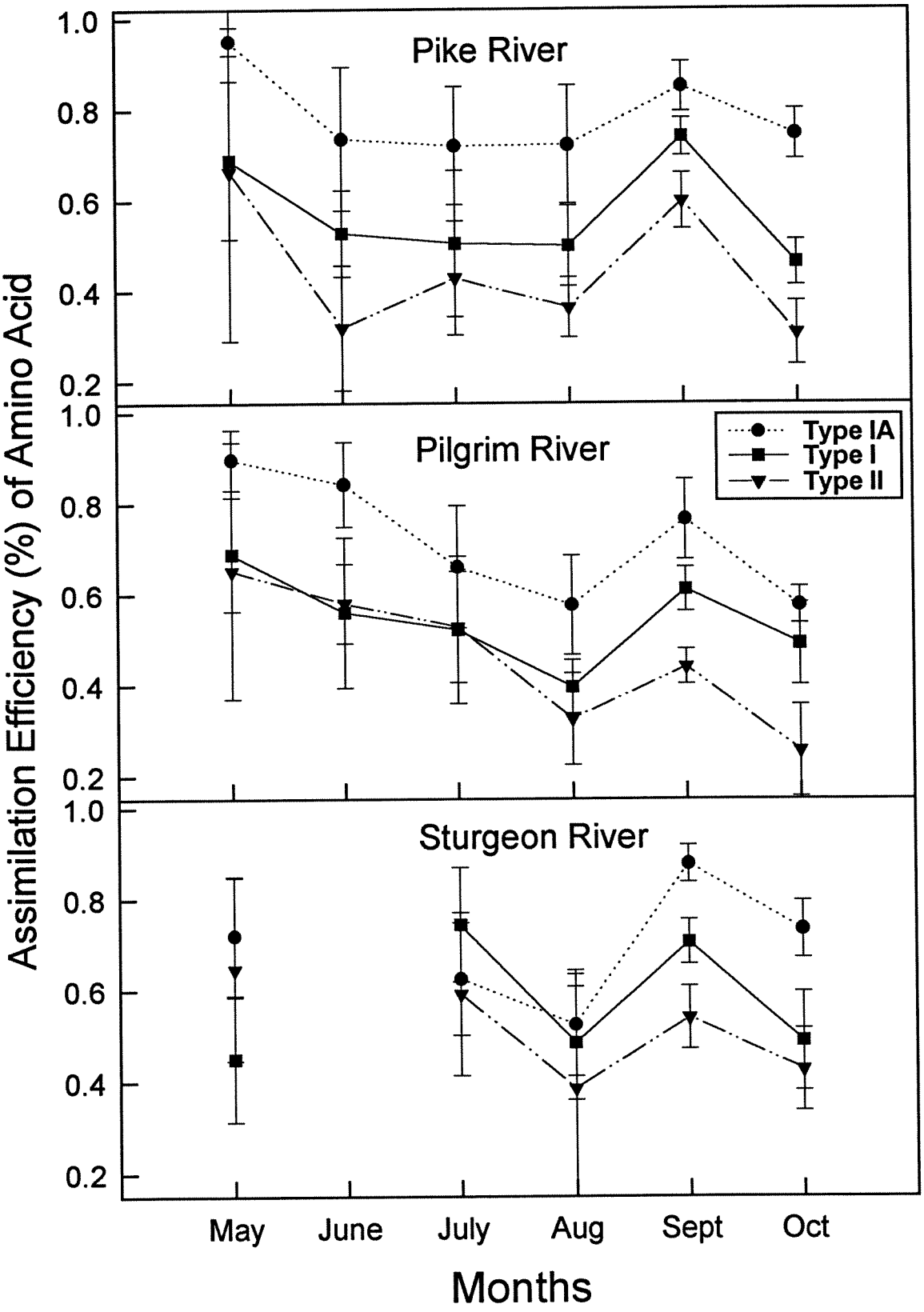

Figure 8. Assimilation efficiency for amino acids across summer months. Each point is the mean of ten larvae, five from each of two habitat patches, with 95% confidence intervals. Flooding prevented collection in the Sturgeon River in June.

Part III

Bowen, S. H., and M. R. Yap. 1998. Significance of crowding on digestive efficiency in biofilm feeding northern brook lamprey larvae (*Ichthyomyzon fossor*). (in preparation)

ABSTRACT

We studied the effect of crowding on selective feeding, feeding rate, and assimilation efficiency by larval northern brook lampreys in three Lake Superior tributaries in Michigan. Larvae held for one week in cages at three densities (5, 10, 15 individuals / 0.07 m^2) showed no effect of density on diet selection or feeding rate, but assimilation efficiency was dramatically reduced for both total organic matter and amino acids. In surveys of wild populations, we similarly found no effect of density on diet selection or feeding rate, but mean assimilation efficiency ranged from 75% at 2 larvae m^{-2} to 25% at 10 larvae m^{-2} for organic matter, and from 76% at 2 larvae m^{-2} to 32% at 10 larvae m^{-2}. These results suggest that the reduced growth and survivorship associated with increased density in populations of larval lampreys results from disturbance of digestive processes essential to use of biofilm as a food resource.

INTRODUCTION

Several studies provide evidence that increased density in populations of larval lampreys results in reduction of growth and/or survivorship. Mallatt (1983) working with *Lampetra tridentata*, and Murdoch et al. (1992) working with *Petromyzon marinus* found that for larvae held in aquaria, growth was inversely proportional to animal density (individuals per m^2). Moran (1987) held *P. marinus* larvae at a range of densities in cages in streams, and found both growth and survivorship were inversely proportional to density. Purvis (1979) and Weise (1998) found that age-specific growth rates of *P. marinus* larvae declined during successive years of re-colonization and increasing population size in streams that had been treated with lampricide. The reasons for this density effect are unclear. Larvae live in burrows in stream sediment, where they feed on suspended particles (sestonic biofilm) from the waters above through a small, tube-like connection to the sediment surface (Sutton and Bowen 1994, Yap and Bowen 1998). Even at the highest densities observed in field studies, there is ample sediment for individual animals to establish burrows (Mallatt 1983). In feeding, they remove such a minute fraction of the available particles from the seston that they are not expected to affect food availability for other larvae (Moore and Beamish 1973, Malmqvist and Bronmark 1981). Thus, it appears that lamprey larvae compete for neither space nor food, *per se*. In earlier work, we found that several aspects of food use by lamprey vary in their efficiency, and these may be affected by crowding (Yap and Bowen 1998). In the work reported here, we tested the hypothesis that crowding significantly interferes with feeding as measured by efficiency of selective ingestion, feeding rate, and

digestive efficiency in northern brook lamprey (*Ichthyomyzon fossor*) in three Upper Michigan streams.

METHODS

Northern brook lamprey (*Ichthyomyzon fossor*) larvae generally > 6 cm total length were held in plastic trays (.35 cm x .25 cm) filled with organic sediment from the stream and covered with plastic mesh that prevented their escape but allowed constant water movement through the cage. Animals were introduced at 5, 10 or 15 per cage, and two replicates of each density were placed in each of three streams: Pilgrim, Pike and Sturgeon rivers in Houghton County, Michigan, USA. Cages were located in areas of soft, organic sediment favored by wild larvae. After one week, the larvae were anaesthetized with 50 mg / L MS-222 (Sigma Chemical) to prevent stress-induced regurgitation or defecation, and then fixed in 4% formalin. Later they were rinsed with water and stored in Carosafe preservative (Carolina Biological Supply). Weight and length were measured from preserved animals. Foregut and hindgut samples were collected as the contents of the anterior quarter and the posterior quarter of the digestive tract by length, and were taken to represent the diet and feces, respectively. Gut contents were analyzed for ash-free-dry-weight, an operational measure of organic matter in the sample, and for amino acid concentration in the organic matter (Yap and Bowen in preparation). Assimilation (digestive) efficiency, the percentage of material ingested that is absorbed through the gut wall, was determined by Conover's (1966) ash ratio method. Condition factor, a ratio of weight to length that reflects an animal's nutritional status, was calculated according to Le Cren (1951). Gut fullness was calculated as mg food AFDW / g larvae weight.

We collected larvae from patches of organic sediment in three streams using a backpack electroshocker specially designed for collection of these animals (ABP-2, Instrumentation Systems Center, University of Wisconsin, Madison, WI.) A square plastic frame was placed on the bottom and the area within the frame was shocked for several seconds until no more larvae emerged. All animals collected from within the frame were anesthetized and preserved together. The frame was then systematically repositioned to construct a rectangular grid so that the entire sediment patch was sampled. In the Pilgrim River, a 1m x 1m frame was used, and in the Otter and Sturgeon Rivers we used a 0.5m x 0.5m frame. Contents of the foregut and hindgut were removed and analyzed as described above.

RESULTS

Cage Experiments

Gut fullness was not significantly different across larval densities (ANOVA $p > .05$, Fig. 1). Similarly, density had no significant effect on selective ingestion as indicated by concentrations of either organic matter or amino acids in foregut contents.

In contrast, assimilation efficiency for both organic matter and amino acids were negatively correlated with larval density (ANOVA p's < .01, Fig. 1). As densities increased, assimilation efficiency values declined and were lowest at larval density of 15. During this one week long experiment, we found no effect of density on condition factor.

Field Surveys

The distribution of larvae was highly aggregated. For example, in one 3m x 9m area of soft, organic sediment surveyed in the Pilgrim River, we found two 1m x 1m cells that contained relatively high densities (8 or 9 larvae), but the majority of cells contained two or fewer (Fig 2). Density showed no relationship to gut fulness or to the concentrations of organic matter or amino acids in foreguts, but density was highly significantly correlated with assimilation efficiency for both organic matter and amino acids (Fig. 3, Pearson correlation p's < .01). Very similar results were found for all three streams surveyed (Table 1).

Table 1. Slope coefficients from least squares regressions of assimilation efficiency for AFDM and amino acids on density (larvae / m^2). All regression p's < .01.

River	AFDM	Amino Acids
Otter	-0.062	-0.055
Sturgeon	-0.046	-0.055
Pilgrim	-0.077	-0.071

DISCUSSION

The larval densities in our experimental enclosures are comparable to densities in wild populations. Although densities in the field are typically reported to be in the range 1 - 13 larvae per m^2 (Morman 1987), these figures are usually based on the number of larvae collected while sampling a large area of many m^2, most of which does not contain larvae. In our own field work, we often find dense aggregations of 10 or more larvae in patches measuring approximately 25 cm by 25 cm, with a total area of 0.06 m^2. Thus, the densities of 5 - 15 individuals / 0.07 m^2 in our cage experiments are comparable to those found under field conditions, and they realistically simulate field conditions in patches where crowding is most likely to affect food use, growth, and mortality.

Feeding by lamprey larvae can be viewed as a series of three processes: selection as reflected by diet quality, ingestion as reflected by gut fulness, and digestion as measured directly in terms of assimilation efficiency. Our results indicate that crowding affects only digestion. A similar result was found for the cichlid fish

Tilapia mossambica that feeds on biofilm on littoral sands and the surfaces of macrophytes in freshwater lakes (Bowen 1979, 1981). *T. mossambica* depends on secretion of gastric acid for efficient digestion of biofilm. When disturbed by handling in aquarium experiments, these fish continue to feed but acid secretion is inhibited and assimilation declines dramatically. The mechanism whereby lamprey larvae digest biofilm has not been identified. One distinctive feature of digestion in lamprey larvae is the inordinately long time required for food to progress through the gut (54 - 70 hrs, Moore and Beamish 1973), and it is possible that extended exposure of biofilm to digestive enzymes is the key. We observed that when larvae in aquaria are disturbed with a blunt probe, a typical response is to "swim" rapidly through the sediment until they hit the aquarium wall, and then to swim up into the water in a frantic effort to escape. It seems reasonable that this behavior would disturb digestion in an animal that depends on patient and extended processing. Whatever the digestive mechanism, as in *T. mossambica* it appears to be sensitive to disturbance.

Condition factors for larvae within Type IA habitat were not related to density. Our earlier work showed that condition factors do differ when larvae from Type IA are compared to larvae from Type I and/or Type II habitats (Yap and Bowen 1998). Since condition factor is based on differences in growth of body weight relative to body length, this measurement reflects the averaged effect of density over a period of many days or weeks. Our findings indicate that, compared to movement of larvae between habitats, movement of larvae within Type IA patches is more frequent. The fact that density is strongly correlated with assimilation but not correlated with condition factor indicates that individuals move with some frequency to avoid crowding in their immediate environments.

These responses to crowding may reflect compromises in the lamprey life history strategy. Sestonic biofilm is continuously available. As animals that feed by filtering seston, lamprey larvae expend little or no additional energy in searching for, capturing or ingesting food. They avoid competition for food by feeding exclusively on an abundant food resource not generally useful to other chordates. Their digestive process allows very efficient assimilation of nutrients (Sutton and Bowen 1994), but at a considerable expense of time (Moore and Beamish 1973). Time is less critical when mortality is low. Lamprey larvae are not subjected to significant predation. Extensive studies of stream fish feeding habits rarely if ever find lamprey larvae in the diet of any fish. There are a number of stream fishes that feed by searching though sediment for invertebrate prey, but the flight response described above would allow lamprey larvae avoid a potential predator by moving laterally through the sediment. Our observations on larvae in aquaria suggest that flight is triggered in response to relatively small disturbances in the sediment. Thus, it seems likely that crowding effects observed in this study are the result of digestion being disturbed as individual larvae sense the movement in the sediment of adjacent larvae.

ACKNOWLEDGMENT

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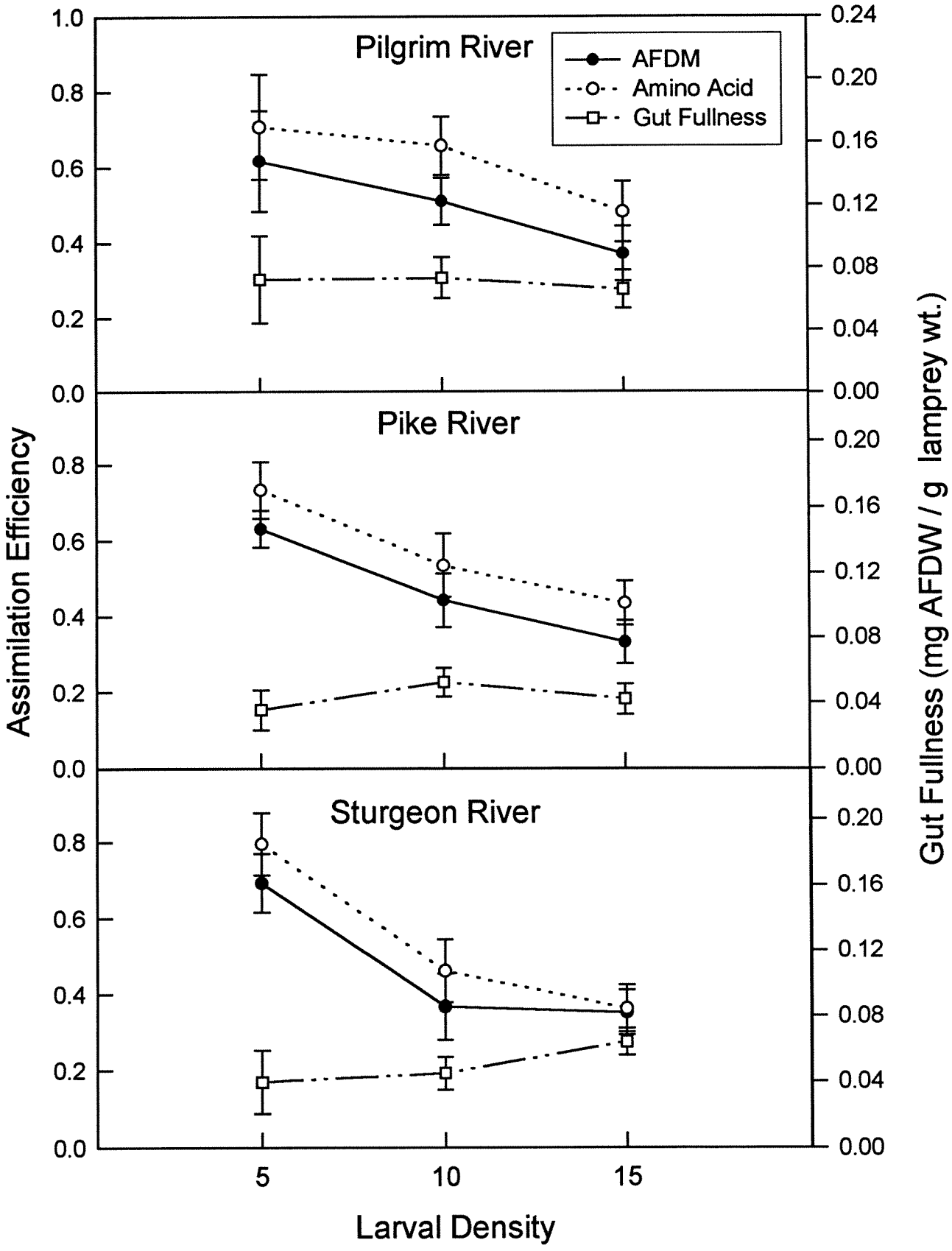

Figure 1. Effect of larval density on gut fullness and assimilation of AFDM and amino acids. Points are means with 95% confidence intervals.

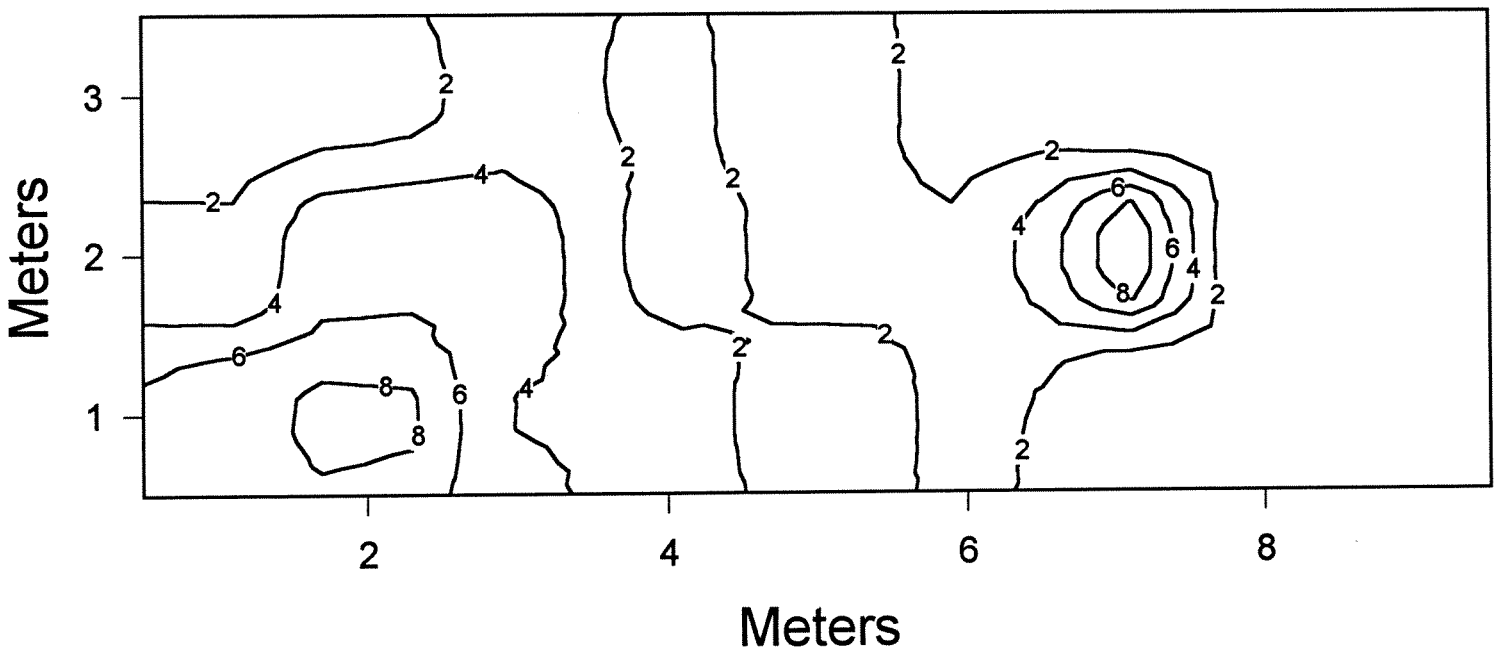

Figure 2. Contour plot from Kreiging analysis of abundances of larvae in twentyseven cells of a nine by three meter grid laid over a patch of organic sediment in the Pilgrim River. Contours are number of larvae per m^2.

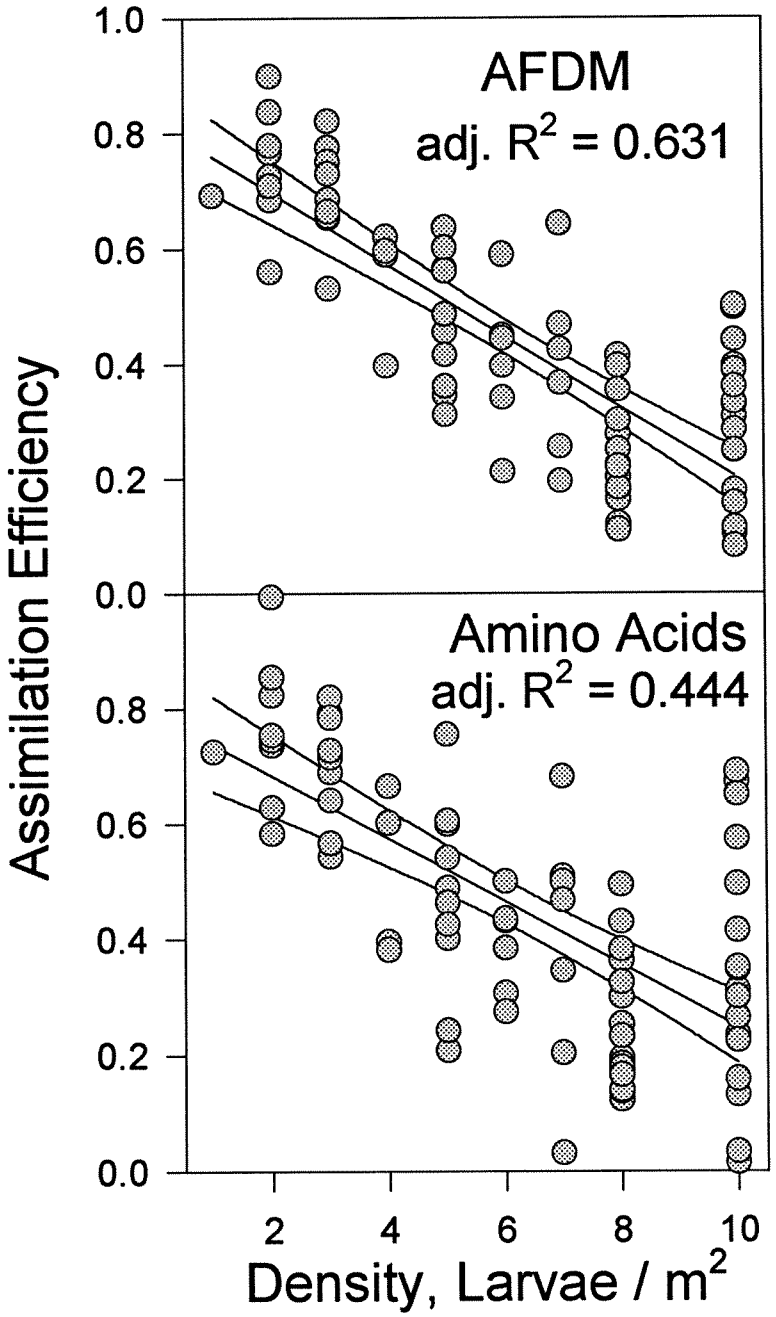

Figure 3. Effect of density on assimilation of AFDM and amino acids in larvae collected from the Otter River. Regression lines show estimated means and 95% confidence limits.

Graduate Theses Completed during this Research

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