Chapter 3: Ingestion and assimilation of ^{14}C labeled algae by the freshwater mussel, *Villosa iris* (Lea, 1829) at three cell concentrations.

Abstract: While some information exists on particle selection, filtration rates, and gut contents of unionids, no published information currently exists on the efficiency of unionid assimilation. In this study, assimilation efficiencies (AE) and carbon budgets were established for the rainbow mussel, *Villosa iris* , using radio-labeled cultures of *Neochloris oleoabundans* at three cell concentrations. At 1 x 10^5 cells·ml $^{-1}$ (3.4 mg dry weight · l $^{-1}$), 37% of the ingested carbon was defecated, 8% excreted as waste, 15% respired, and 40% incorporated into tissues. Assimilation efficiency at this cell concentration was 55%. At 1 x 10^4 cells·ml $^{-1}$ (0.34 mg dry weight · l $^{-1}$), 50% of the ingested carbon was defecated, 4% excreted as waste, 5% respired, and 41% incorporated into tissues. Assimilation efficiency at this cell concentration was 46%. At 1 x 10^3 cells·ml $^{-1}$ (0.03 mg dry weight · l $^{-1}$), 41% of the ingested carbon was defecated, 6% excreted as waste, 4% respired, and 49% incorporated into tissues. Interestingly, assimilation efficiencies for *Villosa iris* were independent of both cell concentration and total ingestion. Regardless of these similarities, assimilation efficiencies from this study indicate that *Neochloris oleoabundans* is readily assimilated (~50% AE) by *Villosa iris* . In addition, total assimilation was maximized at 1 x 10^5 cells·ml $^{-1}$ (3.4 mg dry weight · l $^{-1}$) which indicates that *Villosa iris* has the largest amount of energy available for growth, reproduction, and maintenance of condition in captivity at this cell concentration.

INTRODUCTION

In freshwater ecosystems, bivalves often comprise a significant portion of the zoobenthos (Hynes 1970, Kryger and Riisgard 1988). In the tidal Hudson River, for example, unionids comprise more than 50% of the macroinvertebrate biomass (Strayer et al. 1994). As filter feeders, unionids also contribute to overall ecosystem stability (Vannote et al. 1980). Thus, the gradual decline of freshwater mussel populations throughout North America (Bogan 1993) will likely have significant consequences for the ecosystem dynamics in some river systems.

Efforts to conserve declining populations of native freshwater mussels will require captive maintenance of adult unionids and propagation of juveniles (Gatenby et al. 1996, Gatenby et al. 1997). However, under laboratory and hatchery conditions, bivalve energy stores have been shown to decline without proper feeding (Calvin 1931, Pora et al. 1969, Bayne and Thompson 1970, Gabbott and Walker 1971, Patterson et al. 1997). Decreased energy reserves in adult bivalves negatively affect growth rates and energy reserves of developing offspring (Bayne 1972, Helm et al. 1973, Bayne et al. 1975). Thus, to maintain juvenile growth rates and adult fitness in captivity, more information is needed concerning the nutritional requirements of freshwater mussels.

While some information exists on particle selection (Paterson 1986, Miura and Yamashiro 1990, Tankersley and Dimock 1993), filtration rates (Stanczykowska et al. 1975, Walz 1978, Paterson 1984, Kryger and Riisgard 1988, Tankersley and Dimock 1993, Silverman et al. 1995, Vanderploeg et al. 1995), and gut contents (Coker et al. 1921, Gale and Lowe 1971, Bisbee 1984, Parker et al. in press) of unionids, no published information is currently available on the efficiency of unionid assimilation. Particle selection, filtration rate, and gut contents address food acquisition, but assimilation also must be measured and integrated with ingestion to determine food utilization. Ultimately, the best algal food resources for maintaining unionid condition in captivity should be digested and assimilated with high efficiency, while providing the necessary nutrients for growth and survival.

Radiolabeled algal cultures have been widely used to study carbon assimilation in bivalves including *Corbicula fluminea*, Muller 1774 (Lauritsen 1986), *Crassostrea virginica*, Gmelin 1791 (Newell and Langdon 1986, Crosby et al. 1990), *Mytilus edulis*, Linnaeus 1758 (Kreeger et al. 1996, Wang and Fisher 1996), *Mytilus trossulus*, Gould 1850 (Kreeger and Langdon 1994), *Ostrea edulis*, Linnaeus 1750 (Allen 1962), *Argopecten irradians concentricus*, Lamarck 1819 (Peirson 1983) and *Geukensia demissa*, Dillwyn 1817 (Kreeger et al. 1988, Kreeger et al. 1990, Kreeger and Newell 1996). Many of these studies indicate that the efficiency of carbon assimilation is highly dependent on the concentration of food. Consequently, the objectives of this experiment were to feed the freshwater mussel, *Villosa iris* (Lea 1829), unialgal cultures of

radiolabeled *Neochloris oleoabundans* (Chantanachat and Bold 1962) at three cell concentrations and determine 1) the amount of carbon ingested at each cell concentration, 2) the proportion of the ingested carbon assimilated at each cell concentration, and 3) which cell concentration maximized total assimilation.

METHODS

Algae

Algal cultures were grown in Bold's Basal Medium (Nichols 1973) under continuous cool white fluorescent light (photon flux: 60 - 100 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 20 $\pm$ 1°C. Algal cells were counted in a hemacytometer and near-stationary phase determined. A 100 mL aliquot was filtered onto pre-ashed Whatman GF/F filters, dried (100°C, 18 h), and weighed. A media supplement and rinse solution of $(NH_4)_2HPO_4$ in autoclaved distilled water was prepared to pH 7.9, and 10 mL of this were added to 435 mL of algae prior to inoculation with ^{14}C. When cell concentrations reached 1 x 10^4 cells $\cdot mL^{-1}$, algae were inoculated with 100$\mu Ci \cdot L^{-1}$, [^{14}C]-sodium bicarbonate (57 $\mu Ci \cdot mmol^{-1}$; ICN Pharmaceuticals, Inc., Irvine, CA), stoppered without aeration, and allowed to grow under the same conditions stated above until cell concentrations reached 5 x 10^5 cells $\cdot mL^{-1}$ (> 5 generations). Cultures were then centrifuged at 7000 - 10,000 rpm for 25 min, the medium decanted, and the algae rinsed with a $(NH_4)_2HPO_4$ solution for a total of 3 washings. Algae were resuspended in autoclaved distilled water and 2 mL aliquots radio-assayed to verify that counts were over 100,000 cpm. Final specific activity of the stock algal culture was 0.25 dpm $\cdot$ cell $^{-1}$.

Mussel collection and acclimation.

On February 18, 1998, 30 male *Villosa iris* (shell lengths, 34-56 mm; dry tissue weights [DW], 0.11-0.44 g) were collected from Copper Creek in Scott County, Virginia. Mussels were scrubbed to remove epiphytes and placed in 250 mL experimental feeding chambers at 18°C for 12 h. Mussels were fed non-labeled *N. oleoabundans* and allowed to acclimate in the feeding chambers prior to use in the experiment. Feeding chambers were vigorously aerated to maintain algal cells in suspension.

Delivery of labeled algae

We changed the water in each feeding chamber, and after the mussels exhibited shell gape and aperture opening, they were fed ^{14}C labeled algae using a pulse-chase approach. Labeled algae were delivered to three groups of ten mussels at 1 x 10^5 cells $\cdot mL^{-1}$ (3.4 mg $\cdot L^{-1}$), 1 x 10^4 cells $\cdot$

mL^{-1} (0.34 mg · L^{-1}), and 1 x 10^3 cells · mL^{-1} (0.03 mg · L^{-1}). Mussels were allowed to feed on radiolabelled algae for 2 h, and upon removal from the pulse chamber, shells were scrubbed to remove any settled or attached ^{14}C labeled algae. Preliminary experiments indicated that the 2 h pulse allowed for sufficient labeled algae to be ingested, but not defecated, excreted, or respired.

Ingestion and Assimilation

During the experiment, ingestion for each mussel was estimated by adding the activities of the ^{14}C-budget components because our previous experiments showed that ingestion values obtained by measuring the loss of radioactivity from suspension were highly variable. Kreeger and Newell (1996) also found measurements of ingestion using the removal of labeled particles from suspension to be more variable than summation of budget components, and they concluded that the latter approach was more reliable. In addition, our previous experiments showed that ingestion values obtained by summation of budget components were not significantly different ($p>0.2$) from those obtained from the sacrifice of animals immediately following the pulse. This result indicated that the various components of the energy budget accounted for all of the ^{14}C activity ingested by mussels.

At the end of the pulse, mussels were transferred to 250 mL chase chambers containing unlabeled *N. oleoabundans* and allowed to feed for 24 h. During the 24 h chase, cells were replenished every 8 h and mussels were fed the same cell concentrations they received in the pulse to prevent changes in feeding physiology. Because the pH of the chase water was between 8.2 and 8.3, most of the respired $^{14}CO_2$ should have remained in solution. In the event that any $^{14}CO_2$ diffused from the chase water, however, chase chambers were sealed and small $^{14}CO_2$ traps containing 15 mL of a 2:1 mixture of ethylene glycol monomethyl ether and ethanolamine were attached inside (Peirson 1983). The duration of the chase (24 h) was assumed sufficient to allow mussels to purge their guts of unassimilated ^{14}C, while avoiding behavior modification due to low dissolved oxygen in the sealed chase chambers. *Geukensia demissa* (Kreeger et al. 1988) and *Mytilus trossulus* (Kreeger 1993), for example, have been shown to defecate > 95% of the total fecal ^{14}C within 18 h.

After the chase, the $^{14}CO_2$ trap was removed and sealed until analysis. Mussels were removed, and any feces adhering to shells were thoroughly rinsed back into the chase chamber. Tissues were removed from the shell and frozen (-10°C) until analysis. A buffer solution (pH=9) was added to the chase water to trap any remaining dissolved $^{14}CO_2$. Feces were collected from the buffered chase water by filtering onto pre-ashed (450°C, 24 h), Whatman GF/F filters.

Total respiration was determined using two methods, the "Difference Method" of Kreeger and Langdon (1994), and the "Trap Method" of Newell and Langdon (1986). First, a 1 mL aliquot was removed from the ethanolamine trap for radio-assay (Trap Method). The amount of respired $^{14}CO_2$ and excreted ^{14}C dissolved in the chase water was determined using the Difference

Method. Two 5 mL aliquots of the buffered chase water were collected after filtering fecal samples (Difference Method). One 5 mL aliquot was acidified (pH<4) to evolve any dissolved $^{14}CO_2$ and was placed in a dessicator containing 10 mL of concentrated HCl overnight.

Radio-Assay Procedure

Respiration and Excretion. We added 10 mL of Beckman Ready Scintillation (BRS) cocktail and 1 mL of methanol to enhance mixing of the cocktail with the ethanoloamine trap mixture (Peirson 1983). We added 15 mL of BRS cocktail to both 5 mL Difference Method aliquots. Specific activities, determined with the liquid scintillation counter (Beckman LS 6000SC; Beckman Instruments, Inc., Fullerton, CA), were then corrected for the original volumes in the traps and purge beakers to calculate total ^{14}C respired or excreted by each mussel.

Defecation. Filters were placed in scintillation vials and homogenized in 3 mL of deionized water. The homogenate was digested with 1 mL of tissue solubilizer (Scintigest) in a 55 ^0C water bath overnight. We then added 10 mL of BRS cocktail and 130 μL of glacial acetic acid to reduce chemoluminescence of the tissue solublizer prior to LSC analysis.

Ingestion and Incorporation. Mussel tissues were macerated with scissors and homogenized in 20 mL distilled water. Two, 200 μL aliquots of the tissue homogenate were then removed. One aliquot was added to a dried, pre-weighed vial, dried (100^{o}C, 24 h), and weighed. We digested the second aliquot with 2.5 mL of tissue solubilizer (Scintigest) in a 55 ^0C waterbath overnight (Lauritsen 1986). After digestion, we added 10 mL of BRS cocktail and 130 μL of glacial acetic acid for radio-assay. Analyzed ^{14}C activities were then corrected for the total dry tissue weight of mussels.

Energy Budget Calculation

A complete ^{14}C-budget was calculated for each mussel as follows:

$I = T + D + R + E,$

where ingestion (I), tissue incorporation (T), defecation (D), respiration (R), and excretion (E) were expressed in units of disintegrations per minute per gram tissue dry weight (Kreeger and Langdon 1994). Assimilation efficiencies (AE) were estimated as the proportion of ingested ^{14}C that had been either incorporated or respired, as follows (Kreeger and Langdon 1994):

$AE = [(T + R)/I] \times 100\%.$

A ratio comparing the percentage of ingested ^{14}C that was respired (R) to that incorporated into tissues (T) also was calculated for each mussel. Changes in the 'R/T ratio' will provide further insight into the relative catabolism or incorporation of carbon at different cell concentrations

(Kreeger 1993). Individual values were arcsine square root transformed and then compared with ANOVA.

Pearson correlation coefficients were calculated for ingestion vs. assimilation efficiency, ingestion vs. total assimilation, ingestion vs. tissue dry weight, and assimilation efficiency vs. tissue dry weight at each cell concentration using SAS. Total ingestion, total assimilation, and assimilation efficiency at each cell concentration were compared with ANOVA. If significant differences were detected, the Scheffe F-test was used to determine the statistical significance of individual treatments.

RESULTS

During the 2 h pulse, carbon ingestion by individual mussels fed the same cell concentration was highly variable. This variability, however, could not be explained by differences in dry tissue weight ($R<0.2$, $p> 0.5$). Reduced ingestion may cause abnormal digestive functioning and warrant the removal of individual bivalves from feeding experiments. Statistical analysis, however, showed that assimilation efficiencies were not dependent on total ingestion for mussels fed 1×10^5 cells $\cdot$ mL^{-1} ($R=0.23$, $p>0.5$), 1×10^4 cells $\cdot$ mL^{-1} ($R=0.19$, $p>0.5$), or 1×10^3 cells $\cdot$ mL^{-1} ($R=0.45$, $p=0.19$). In fact, assimilation efficiencies remained relatively constant because total assimilation (T + R) was linearly related to ingestion at each cell concentration ($R>0.9$, $p<0.01$). Consequently, removal of animals was not warranted, and the data from all mussels were combined for analysis.

Total ingestion of carbon was significantly higher ($p<0.05$) for mussels fed 1×10^5 cells $\cdot$ mL^{-1} (449.7 ± 330.5 μg C $\cdot$ g dry tissue weight $^{-1}$) than mussels fed either 1×10^4 or 1×10^3 cells $\cdot$ mL^{-1} (78.4 ± 42.6 and 22.1 ± 12.7 μg C $\cdot$ g dry tissue weight $^{-1}$, respectively). Total assimilation of carbon also was significantly higher ($p<0.05$) for mussels fed 1×10^5 cells $\cdot$ mL^{-1} (259.1 ± 203.7 μg C $\cdot$ g dry tissue weight $^{-1}$) than mussels fed either 1×10^4 or 1×10^3 cells $\cdot$ mL^{-1} (34.1 ± 29.6 and 12.7 ± 10.0 μg C $\cdot$ g dry tissue weight $^{-1}$, respectively). The mean R/T ratio also was significantly higher ($p<0.05$) for mussels fed 1×10^5 cells $\cdot$ mL^{-1} (0.44 ± 0.19) than mussels fed either 1×10^4 (0.14 ± 0.12) or 1×10^3 cells $\cdot$ mL^{-1} (0.10 ± 0.05). Despite differences in total ingestion, assimilation, and R/T ratios, assimilation efficiencies at the three cell concentrations were not significantly different ($F=0.645$, $p>0.5$). Individual budget components also were similar among the different cell concentrations, as seen in the following carbon budget equations.

Mussels fed 1 x 10^5 cells · mL^{-1} (3.4 mg dry weight · L^{-1}) ingested ≈17 % [449.7 ± 330.5 μg dry weight C (g dry tissue weight)$^{-1}$)] of the available carbon (Table 1). Of the carbon ingested by mussels, roughly 37% was defecated [164.9 ± 138.8 μg dry weight C (g dry tissue weight)$^{-1}$)], 8% excreted as waste [25.7 ± 12.1 μg dry weight C (g dry tissue weight)$^{-1}$)], 15% respired [76.7 ± 68.3 μg dry weight C (g dry tissue weight)$^{-1}$)], and 40% incorporated into tissues [182.4 ± 138.7 μg dry weight C (g dry tissue weight)$^{-1}$)]. The energy budget calculated for *V. iris* at this cell concentration was:

100% I = 40% T + 37% D + 15% R + 8% E.

Mussels fed 1 x 10^4 cells · mL^{-1} (0.34 mg dry weight · L^{-1}) ingested ≈23% [78.4 ± 42.6 μg dry weight C (g dry tissue weight)$^{-1}$)] of the available carbon (Table 2). Of the carbon ingested by mussels, roughly 50% was defecated [43.2 ± 30.0 μg dry weight C (g dry tissue weight)$^{-1}$)], 4% excreted as waste [3.2 ± 1.4 μg dry weight C (g dry tissue weight)$^{-1}$)], 5% respired [3.6 ± 2.9 μg dry weight C (g dry tissue weight)$^{-1}$)], and 41% incorporated into tissues [30.5 ± 28.8 μg dry weight C (g dry tissue weight)$^{-1}$)]. The energy budget calculated for *V. iris* at this cell concentration was:

100% I = 41% T + 50% D + 5% R + 4% E.

Mussels fed 1 x 10^3 cells · mL^{-1} (0.03 mg dry weight · L^{-1}) ingested ≈60% [22.1 ± 12.7 μg dry weight C (g dry tissue weight)$^{-1}$)] of the available carbon (Table 3). Of the carbon ingested by mussels, roughly 41% was defecated [8.3 ± 4.4 μg dry weight C (g dry tissue weight)$^{-1}$)], 6% excreted as waste [1.2 ± 0.9 μg dry weight C (g dry tissue weight)$^{-1}$)], 4% respired [0.9 ± 0.8 μg dry weight C (g dry tissue weight)$^{-1}$)], and 49% incorporated into tissues [11.8 ± 9.4 μg dry weight C (g dry tissue weight)$^{-1}$)]. The energy budget calculated for *V. iris* at this cell concentration was:

100% I = 49% T + 41% D + 4% R + 6% E.

DISCUSSION

Assimilation efficiencies for *Villosa iris* are similar to those obtained for several marine and freshwater bivalves. Assimilation efficiencies for *Corbicula fluminea* ranged from 47 to 57% depending on the algal species (Lauritsen 1986), and typical assimilation efficiencies for *Mytilus*

edulis have been reported between 52 and 85% (Thompson and Bayne 1972, Thompson and Bayne 1974, Kiorboe et al. 1980). Assimilation efficiencies in marine bivalves, however, are known to be affected by many factors including the physiological condition of the animals and the quality and quantity of their food (Peirson 1983, Kreeger 1993, Kreeger et al. 1995, Wang and Fisher 1996).

Marine bivalves often exhibit seasonal changes in physiological condition in association with the reproductive cycle that may affect feeding physiology (Kreeger 1993, Kreeger et al. 1995). Kreeger et al. (1995), for example, showed that the efficiency of protein assimilation in *Mytilus trossulus* changed four fold throughout the year despite little change in the efficiency of carbohydrate assimilation. In fact, protein assimilation increased during periods of high protein synthesis, indicating that bivalves can increase protein utilization in response to increases in metabolic protein demands (Kreeger et al. 1995). Consequently, these authors concluded that physiological condition may affect assimilation efficiencies as much as shifts in food quality. Additional studies on the efficiency of unionid assimilation, therefore, should examine the effect of these seasonal changes in physiological condition.

The biochemical composition or quality of the algal food also may affect assimilation efficiency. Wang and Fisher (1996) found that the chlorophytes, *Chlorella autotrophica* and *Nannochloris atomus*, were not readily assimilated (8-21%) by *Mytilus edulis* . Inefficient assimilation of chlorophytes was reported in clams, oysters and bay scallops (Floyd 1953, Peirson 1983, Bass et al. 1990). The presence of a resistant substance resembling sporopollenin in the outer cell wall may make some chlorophytes resistant to bivalve digestion (Atkinson et al. 1972, Bricelj et al. 1984, Wang and Fisher 1996). If the algal contents are indigestible, bivalves may obtain little or no energy despite high rates of filtration and ingestion. For example, Peirson (1983) showed that bay scallops, *Argopecten irradians concentricus*, ingested nearly equal portions of the available *Chlorella autotrophica* and *Chroomonas salina* (77% and 81%, respectively). However, the scallops assimilated 84% of the ingested *C. salina* and only 17% of the ingested *C. autotrophica.* Thus, assimilation efficiencies obtained in the laboratory provide valuable information on the digestibility of a particular algal species and its usefulness in aquaculture. In my experiment, the chlorophyte, *Neochloris oleoabundans*, was assimilated by *Villosa iris* with relatively high efficiency (~50%). The presence of a thin cell wall (Prescott 1978) and naked zoospores during asexual reproduction (Bourelly 1966) likely enhanced the digestibility of *N. oleoabundans* in the bivalve gut. Gut content analyses of juvenile *V. iris* reared on *N. oleoabundans* showed large masses of chlorophyll and few intact cells, indicating juveniles had ingested and digested this alga (Gatenby et al. 1997).

In addition to the effects of food quality, the quantity of food also has been found to have a dramatic effect on the assimilation efficiency of marine bivalves. Assimilation efficiencies in the

blue mussel, *Mytilus edulis*, fed uni-algal cultures of *Tetraselmis suecica* were 85% at 1 x 10^3 cells · mL^{-1} (0.66 mg · L^{-1}) and 0% at 2.5 x 10^3 cells · mL^{-1} (1.65 mg · L^{-1}) (Thompson and Bayne 1972). Similarly, when fed *Dunaliella primolecta* at cell suspensions between 5 x 10^2 (0.06 mg · L^{-1}) and 8 x 10^3 cells · mL^{-1} (0.90 mg · L^{-1}), assimilation efficiencies of the black mussel, *Choromytilus meridionalis* (Krauss 1848) reached 80% and then dropped to 0% between 3 x 10^4 (3.36 mg · L^{-1}) and 4 x 10^4 cells · mL^{-1} (4.48 mg · L^{-1}) (Griffiths 1980). In contrast, at much higher algal concentrations of *Phaedactylum tricornutum* (1 x 10^5 cells · mL^{-1}; 4.4 mg · L^{-1}), the assimilation efficiency of *Crassostrea virginica* remained around 70% (Langefoss and Maurer 1975). Because of this discrepancy, some authors have concluded that assimilation efficiency is dependent on the amount of algae ingested rather than the concentration of suspension directly (Foster-Smith 1975, Navarro and Winter 1982). Interestingly, assimilation efficiencies for *Villosa iris* in this experiment were independent of both cell concentration and total ingestion.

These findings are interesting because high cell concentrations and increased ingestion may cause food particles to pass through the gut at a faster rate, not allowing sufficient time for complete digestion and assimilation (Bricelj et al. 1984). One potential explanation for the consistency of assimilation efficiencies at different cell concentrations is that *Neochloris oleoabundans* may have particular cellular components that are indigestible in the bivalve gut. Consequently, even if gut passage time is reduced, no additional carbon can be extracted by the digestive enzymes. Studies on gut residence time in unionids, as well as additional ^{14}C tracer studies with algae that differ in their relative lability or refractoriness are needed to confirm this hypothesis. A second possible explanation is that *V. iris* has not developed the ability to alter gut passage time. This evolutionary trait would likely be an adaptation to environments that experience seasonal fluctuations in food availability. The rainbow mussel, *V. iris* , usually inhabits small streams that may experience only 2-3 fold changes in background FPOM (Webster 1983). Significant increases in FPOM of 9 or 10 fold may occur during storm events, but these fluctuations are typically short in duration (Webster 1983). Thus, *V. iris* may have adapted to an environment that lacks 100 fold differences in food concentration like the ones used in this experiment. Additional experiments on the gut residence time of unionids are needed in which animals are acclimated to low cell concentrations for an extended period.

Regardless of the effect of cell concentration, assimilation efficiencies obtained in this experiment indicate that *N. oleoabundans* is readily digested by *V. iris* at moderately high cell concentrations (3.4 mg dry weight · L^{-1}). This algal species, therefore, may serve as a good food resource for freshwater mussels held in captivity. The recommended concentration to feed in captivity should be no less than 1 x 10^5 cells · mL^{-1} (3 mg dry weight · L^{-1}), because this amount lead to the highest total assimilation of carbon. By maximizing total carbon assimilation, *V. iris* had the largest amount of energy available for growth, reproduction, and maintenance of condition

in captivity. Significant reductions in the R/T ratio at 1 x 10^4 and 1 x 10^3 cells · mL^{-1} also indicate that carbon, because it was being conserved from catabolism, was limiting at these lower cell concentrations. Extrapolation of assimilation data obtained in the laboratory to determine the effects of filter-feeders on riverine systems, however, is problematic because normal physiological functions can be altered under artificial testing conditions (Bayne and Newell 1983, Lauritsen 1986). In addition, natural seston contains a complex mixture of phytoplankton species, cell sizes and shapes, as well as suspended benthic algae, detrital materials, microheterotrophic organisms and inorganic particles. Hence, the relative food value of these particles varies considerably, and results from laboratory studies under defined conditions with a nutritious algal food are probably not indicative of food utilization in nature. Our studies clearly indicate that future studies on the culture and propagation of freshwater mussels can use ^{14}C tracer experiments to determine which algal species are digestible by freshwater mussels and thus, suitable as food resources for the maintenance of adult fitness in captive propagation.

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Table 1. ^{14}C budget components of 12 *Villosa iris* fed ^{14}C-labelled *Neochloris oleoabundans* at 1 x 10^5 cells · ml^{-1}. The mean (± SD) ^{14}C-activity of each component is expressed as a percentage of ^{14}C ingested and ^{14}C assimilated with actual ^{14}C-activities [dpm (g dry mussel tissue weight) $^{-1}$] given in brackets and total dry weights of carbon (µg dry weight C (g dry tissue weight)$^{-1}$) given in parentheses.

Budget component	^{14}C-component as percentage of ingestion [dis · min^{-1} x 10^5 (g dry tissue weight)$^{-1}$] (µg dry weight C (g dry tissue weight)$^{-1}$)
Ingestion (I)	100 [44.3 ± 32.6] (449.7 ± 330.5)
Defecation (D)	37.5 ± 17.1 [16.2 ± 13.7] (164.9 ± 138.8)
Excretion (E)	7.8 ± 5.7 [2.5 ± 1.2] (25.7 ± 12.1)
Respiration (R)	15.2 ± 5.1 [7.6 ± 6.7] (76.6 ± 68.2)
Incorporation (T)	39.6 ± 16.7 [17.9 ± 13.7] (178.3 ± 129.4)
Total Assimilation (A)	54.8 ± 18.9 [26.9 ± 21.2] (259.1 ± 203.7)

Table 2. ^{14}C budget components of 12 *Villosa iris* fed ^{14}C-labelled *Neochloris oleoabundans* at 1 x 10^4 cells · ml^{-1}. The mean (± SD) ^{14}C-activity of each component is expressed as a percentage of ^{14}C ingested and ^{14}C assimilated with actual ^{14}C-activities [dpm (g dry mussel tissue weight) $^{-1}$] given in brackets and total dry weights of carbon (µg dry weight C (g dry tissue weight)$^{-1}$) given in parentheses.

Budget component	^{14}C-component as percentage of ingestion [dis · min^{-1} x 10^5 (g dry tissue weight)$^{-1}$] (µg dry weight C (g dry tissue weight)$^{-1}$)
Ingestion (I)	100 [7.7 ± 4.2] (78.4 ± 42.6)
Defecation (D)	49.9 ± 21.4 [4.0 ± 2.8] (43.2 ± 30.0)
Excretion (E)	4.4 ± 1.5 [0.32 ± 0.14] (3.2 ± 1.4)
Respiration (R)	6.1 ± 5.0 [0.35 ± 0.28] (3.6 ± 2.9)
Incorporation (T)	40.6 ± 19.2 [3.0 ± 2.7] (30.5 ± 28.8)
Total Assimilation (A)	46.7 ± 21.7 [26.9 ± 21.2] (34.1 ± 29.6)

Table 3. ^{14}C budget components of 12 *Villosa iris* fed ^{14}C-labelled *Neochloris oleoabundans* at 1 x 10^3 cells · ml^{-1}. The mean (± SD) ^{14}C-activity of each component is expressed as a percentage of ^{14}C ingested and ^{14}C assimilated with actual ^{14}C-activities [dpm (g dry mussel tissue weight) $^{-1}$] given in brackets and total dry weights of carbon (µg dry weight C (g dry tissue weight)$^{-1}$) given in parentheses.

Budget component	^{14}C-component as percentage of ingestion [dis · min^{-1} x 10^5 (g dry tissue weight)$^{-1}$] (µg dry weight C (g dry tissue weight)$^{-1}$)
Ingestion (I)	100 [2.2 ± 1.3] (22.1 ± 12.7)
Defecation (D)	41.1 ± 17.7 [0.81 ± 0.42] (8.3 ± 4.4)
Excretion (E)	5.7 ± 2.6 [0.11 ± 0.09] (1.2 ± 0.9)
Respiration (R)	4.2 ± 2.1 [0.09 ± 0.08] (0.9 ± 0.8)
Incorporation (T)	48.9 ± 17.6 [1.2 ± 0.9] (11.8 ± 9.4)
Total Assimilation (A)	52.1 ± 17.6 [1.3 ± 1.0] (12.7 ± 10.0)