Terrestrial support of detritivorous fish populations decreases with watershed size

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Abstract. Consumers in aquatic food webs can be supported by terrestrial and aquatic primary production. However, we know little about how allochthony, i.e., the proportion of consumer biomass derived from terrestrial organic matter, varies along environmental gradients. Using hydrogen isotopes (deuterium), we quantified allochthony of an ecologically dominant detritivorous fish species (gizzard shad, Dorosoma cepedianum) in reservoir ecosystems, along a gradient of watershed land use (agriculture vs. forest). We predicted that allochthony would decline with an increase in the proportion of watershed land composed of agriculture (% agriculture). This is based on the supposition that as % agriculture increases, so does the export of dissolved inorganic nutrients to lakes, stimulating algal production and reducing the importance of terrestrial organic carbon subsidies. Allochthony accounted for ~34% of gizzard shad production (mean of 11 lakes), although this fraction varied greatly (0–68%) among lakes and isotope mixing model assumptions. Contrary to our hypothesis, we found no relationship between allochthony and % agriculture. However, allochthony was inversely related to total watershed area, as well as the absolute area of the watershed (rather than the percentage) composed of agriculture. We speculate that watershed area and allochthony are negatively correlated because watershed area exerts a strong control on the relative subsidies of dissolved inorganic nutrients vs. particulate organic carbon. Gizzard shad biomass was positively related to phytoplankton primary production but negatively related to allochthony, suggesting that phytodetritus is a higher quality resource than terrestrial detritus. Overall, our results show that both autochthonous and allochthonous carbon fuel the production of this ecologically important detritivore, the relative importance of allochthony decreases with increasing watershed size, and variation in gizzard shad production is closely tied to variation in autochthonous primary production.

Key words: agriculture; algae; allochthonous; autochthonous; carbon; deuterium; Dorosoma cepedianum; gizzard shad; isotopes; lakes; Ohio; reservoirs.

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INTRODUCTION

Historically, it was assumed that consumers in lakes are supported by in-lake (autochthonous) carbon fixation, usually by algae. However, organic carbon subsidies from terrestrial ecosystems can be an important energy source for aquatic food webs (Wetzel 1995, Polis et al. 2004, Reynolds 2008). Several studies show that terrestrial (allochthonous) organic carbon subsidies...
directly or indirectly fuel the production of a variety of lentic consumers, including bacteria (Kritzberg et al. 2004, Karlsson 2007, Berggren et al. 2010), protists (Berggren et al. 2010), metazoan zooplankton (Grey et al. 2001, Matthews and Mazumder 2006, Karlsson et al. 2003, Pace et al. 2007, Cole et al. 2011), benthic invertebrates (Solomon et al. 2008, 2011) and fish (Carpenter et al. 2005, Weidel et al. 2008, Solomon et al. 2011). In contrast, other studies suggest that in general terrestrial organic matter contributes little to the nutrition of aquatic consumers (in particular crustacean zooplankton; Brett et al. 2009, Mohamed and Taylor 2009). Thus, a major challenge is to ascertain the factors regulating “allochthony” (Cole et al. 2006), i.e., the proportion of consumer biomass derived from terrestrial organic matter.

Detritivores are often an important component of aquatic food webs, but there is uncertainty surrounding their carbon sources (e.g., Hall and Meyer 1998, Moore et al. 2004). Sediment-feeding “detritivores” often consume a relatively amorphous mix of algal detritus, terrestrial detritus, and living heterotrophic and autotrophic microbes (e.g., Smoot and Findlay 2010a, b). Because it is difficult to distinguish and quantify these energy resources, little is known about the prevalence of allochthony in aquatic detritivores. Allochthony may vary along environmental gradients. For example, allochthony may decrease with lake size and in-lake primary production, because the ratio of algal production to allochthonous organic C inputs increases along these gradients (Jones 1992, Pace et al. 2007). Reservoirs have relatively large watersheds, and may therefore receive large subsidies of both terrestrial organic carbon and inorganic nutrients (Thornton 1990, Wetzel 1990). Reservoirs in agricultural watersheds receive particularly large nutrient subsidies and generally have high phytoplankton production (Carpenter et al. 1998, Knoll et al. 2003, Vanni et al. 2011). Elevated subsidies of particulate organic carbon may increase allochthony by providing a food resource for detritivores, while dissolved inorganic nutrient subsidies stimulate phytoplankton production and may thus favor autochthony. Thus, allochthony may depend on watershed size, watershed land use, lake size, and algal productivity.

In reservoirs of the midwestern and southern USA (hereafter, “midwestern reservoirs”), fish biomass is often dominated by gizzard shad (Dorosoma cepedianum), an omnivore that exerts strong effects on food webs and nutrient cycling (Stein et al. 1995, Vanni et al. 2005, 2006b). As larvae, gizzard shad are zooplanktivores, but when they reach ~30 mm in length (~2–3 months of age) they develop morphological adaptations for sediment feeding. Individuals above this size feed primarily on sediment detritus (Mundahl and Wissing 1987, Gido 2002, Schaus et al. 2002, Higgins et al. 2006, Zeug and Winemiller 2008). Evidence based on energetics and stable isotopes shows that gizzard shad can assimilate both autochthonous and allochthonous carbon (Pilát 2007, Zeug and Winemiller 2008, Smoot and Findlay 2010a, b). However, little is known about the relative contributions of autochthonous vs. allochthonous detritus to gizzard shad production, and how these contributions vary along environmental gradients. Information on resource utilization by gizzard shad populations is critical, so that ecologists and fisheries managers can better quantify the factors limiting this ecologically dominant species.

In reservoirs, gizzard shad biomass and relative abundance increase disproportionately as watershed agriculture and reservoir productivity increase, and the ecosystem effects of gizzard shad are particularly strong in productive systems (Vanni et al. 2005, 2006b, Hale et al. 2008, Schaus et al. 2010). As mentioned, agriculture increases subsidies of both inorganic nutrients and allochthonous detritus to reservoirs. Thus, it is not clear how gizzard shad allochthony varies along gradients of watershed land use and algal productivity, or how important allochthony may be in driving the high gizzard shad biomass observed in agriculturally-impacted, high productivity systems. More broadly, we know of no studies that have examined the importance of allochthonous carbon subsidies for a lake detritivore along environmental gradients such as productivity or land use.

We quantified gizzard shad allochthony in 11 midwestern reservoirs, spanning a wide gradient in watershed land use and phytoplankton primary productivity. We hypothesized that, due to increases in algal primary production caused by
agricultural nutrient subsidies, allochthony by gizzard shad decreases with an increase in the percentage of watershed area composed of agriculture. An alternative hypothesis is that allochthony is unrelated to watershed land use but rather is driven more by in-lake variable such as lake size or shape; this could occur if increased subsidies of sediment detritus increase with agriculture in proportion to nutrient subsidies and primary productivity. We tested these hypotheses using naturally occurring hydrogen isotopes to quantify allochthony.

**METHODS**

**General approach and study sites**

We used naturally occurring stable hydrogen isotopes to quantify allochthony of gizzard shad. δD, the ratio of deuterium (2H) to hydrogen (1H) expressed relative to a known standard, has emerged recently as an effective means of separating organic matter produced via aquatic vs. terrestrial photosynthesis (Doucett et al. 2007, Solomon et al. 2009, 2011, Caraco et al. 2010, Finlay et al. 2010, Cole et al. 2011). These studies show that δD is more effective than other naturally occurring stable isotopes, such as δ13C, in distinguishing terrestrial and autochthonous energy sources.

We sampled 11 reservoirs in Ohio, USA, covering a gradient of watershed land use (forest vs. agriculture) and algal productivity (Table 1; Knoll et al. 2003, Vanni et al. 2006b). These ecosystems are representative of midwestern reservoirs in that they have high watershed area:lake surface area ratios and are warm and shallow, compared to natural north-temperate lakes. Gizzard shad biomass is often quite high, especially in productive reservoirs with agricultural watersheds (Table 1; Vanni et al. 2006b, Hale et al. 2008). To estimate gizzard shad allochthony, we quantified δD of gizzard shad tissue, autochthonous and allochthonous food sources (phytoplankton and terrestrial vegetation, respectively), and lake water (necessary to correct for direct uptake of water, and hence H, by fish). Because these lakes are turbid with high phytoplankton biomass, macrophytes and benthic algae contribute very little to autochthonous production; therefore, we did not sample these groups.

**Fish**

Gizzard shad were collected via electrofishing in shallow (<2 m), well oxygenated, “uplake” areas where they tend to thrive. We sampled age-1 and older gizzard shad (mean length = 206 mm, range 133–365), because these individuals have been detritivorous the majority of their lives. Except for Acton, each reservoir was sampled for gizzard shad on one date between 4 May and 12 June of 2008. Acton gizzard shad were sampled on 6 dates, in June 2007, July 2007, May 2008 (2 dates), June 2008 and October 2008. All fish were immediately frozen, returned to the lab and kept
at ~80°C until they were processed for isotope analyses, at which time they were thawed in a water bath for ~40 minutes. A small sample of white muscle (~2 g wet mass) was then collected from the dorsal region between the operculum and the front of the dorsal fin, and rinsed in deionized water prior to isotope analysis, described below.

Phytoplankton and water
We collected a 20 L water sample from the euphotic zone of each lake to characterize δD of water and seston (phytoplankton and similarly-sized particles that cannot be easily separated). For all reservoirs except Deer Creek, Pleasant Hill, and Tappan, seston and water were sampled on the same day as fish; for these 3 lakes water and seston were sampled within 2 weeks of fish sampling. To estimate δD of water, two 4 mL aliquots were filtered through 0.2 μm membrane filters and stored in brown HDPE Nalgene bottles with no air space at 4°C until analysis. Water samples from Berlin were lost in processing, so we used the mean water δD value of nearby Pleasant Hill.

Seston was collected to estimate the isotopic composition of autochthonous organic material, i.e., organic matter derived from phytoplankton photosynthesis. To obtain seston samples, we filtered water (<10 L) through a 142 mm diameter GF/F filter (nominal pore size ~0.7 μm) in a high volume filtration apparatus until the filter became clogged. The seston was then gently scraped into a metal weighing dish for drying. A subset of samples examined under 40× magnification verified that filter particles did not contaminate seston samples. Two replicate samples were collected from each 20 L container.

Seston represents a mix of phytoplankton, phytodetritus, allochthonous detritus, and bacteria nourished by autochthonous and allochthonous sources. It is difficult or impossible to physically separate phytoplankton from other sestonic particles, which presents a challenge when trying to estimate the isotopic signature of phytoplankton (Caraco et al. 2010, Cole et al. 2011, Solomon et al. 2011). Because reservoirs have large watersheds, “contamination” with terrestrial particles may be significant, and is likely most pronounced soon after storm events that deliver large quantities of allochthonous sediments. We corrected for allochthonous contamination of seston using concentrations of non-volatile suspended solids (NVSS) in lake water. During storms that cause runoff of allochthonous sediments, NVSS concentrations and loads increase greatly in inflow streams (Vanni et al. 2001), and this leads to a large increase in reservoir NVSS concentration just after storm events (e.g., Vanni et al. 2006a). Although NVSS is composed of inorganic particles, we hypothesized that NVSS concentrations and loads are correlated with those of allochthonous particulate organic carbon (POC). To test this hypothesis, we measured concentrations of both NVSS and POC, on the same stream water sample, on 56 samples collected from Acton Lake’s two largest inflow streams during 2006–08 (NVSS methods are given below; POC was measured with a Perkin Elmer CHN analyzer). Stream NVSS and POC concentrations were highly correlated ($r^2 = 0.818, P < 0.0001, n = 56$ samples pooled from the two streams; Appendix: Fig. A1). NVSS and POC loads (stream discharge × concentration) were even more strongly correlated ($r^2 = 0.921$; Appendix: Fig. A1). Although these data are from only one lake/watershed (Acton), they clearly show that watershed POC inputs are strongly associated with NVSS inputs. Although we do not have such data for all watersheds, we have observed similar increases in NVSS (in inflow streams as well as recipient lakes) in Burr Oak and Pleasant Hill, where we also have monitored watershed inputs (Vanni et al. 2011). Therefore, the relative contribution of NVSS to total suspended solids (%NVSS) in the lakes can serve as a valid measure of terrestrial detritus “contamination” of seston samples.

Concentrations of suspended solids (SS) and non-volatile suspended solids (NVSS) were measured (in addition to seston δD) in 7 reservoirs on multiple dates (except that Berlin was sampled only once) in 2007, 2008, and/or 2009 ($n = 30$ lake-dates). We used %NVSS, i.e., $100 \times (\text{NVSS}/\text{SS})$, as an index of terrestrial influence. We then created regressions of %NVSS vs. seston δD, and used the y-intercepts of these regressions as the estimated δD values of phytoplankton. This approach assumes that when %NVSS is zero, phytoplankton dominate seston, and therefore seston δD = phytoplankton δD. Using this general approach, we explored...
several scenarios, as described in the Allochthony scenarios section below. To measure SS and NVSS, water samples were filtered onto pre-weighed glass fiber filters previously combusted at 475°C. The filters were then dried at 60°C for ≥24 hours and weighed to obtain SS. Then, NVSS was determined by combusting the organic material on the filter in a muffle furnace at 550°C for 4 hours and re-weighing the filter (Vanni et al. 2006a).

**Terrestrial vegetation**

Terrestrial vegetation was sampled from the Acton, Pleasant Hill, and Burr Oak watersheds, which span our land use gradient and are geographically distributed across our study area (Vanni et al. 2005, 2011). Thus, they should represent the range of hydrologic and geologic conditions in the area. We sampled the two plant species that account for the vast majority of cropland, corn (maize, *Zea mays*) and soybean (*Glycine max*), and the most abundant tree species, maple (*Acer* spp.) and oak (*Quercus* spp.) Corn and maple were sampled in all 3 watersheds, while maple was not sampled in Pleasant Hill and soy was not sampled in Burr Oak. Tree leaves were senescent and were often collected from the ground. Leaves of corn and soy were collected from crop waste remaining on the field after autumn harvest. Within watersheds, sampling sites were selected by stratifying a watershed into three sections of equal length along its longest dimension and then randomly selecting 3 accessible sites within each stratum. If a species was present at more than two sites within a watershed, the samples from the two most geographically distant sites were used. Acton watershed vegetation was also sampled near the lake, and means for Acton include these samples.

A mean terrestrial value (δD_all) was calculated for all 11 reservoirs using species-specific δD values from the nearest sampled watershed, weighted by land use proportions. To obtain δD for forested land, we used a simple mean of maple and oak δD; this approach is similar to other studies that have characterized δD of terrestrial vegetation for aquatic food web studies (e.g., Doucett et al. 2007, Cole et al. 2011). An overall δD for agricultural land was obtained by weighting by the proportion of cropland in corn, estimated from the USDA 2007 census of agriculture (USDA 2009); soy was assumed to constitute the remaining cropland area. Finally, an overall watershed-scale terrestrial δD was obtained using the proportion of land composed of agriculture vs. forest (Table 1).

To assess whether incoming allochthonous sediments exhibited δD similar to terrestrial vegetation, we sampled sediments delivered to Acton Lake via its inflow streams. Seven samples were collected using automated samplers at Four Mile and Little Four Mile Creeks (Vanni et al. 2001, Renwick et al. 2008) during storm events in May 2008. Together these two streams drain 81% of the Acton watershed. We assume that most of this material is terrestrial in origin.

**Isotope analysis**

All samples for δD were dried to constant mass at 60°C and ground to a fine powder using a Retsch centrifugal mill (terrestrial vegetation) or a mortar and pestle (all other samples). Approximately 350 μg of the powdered material was packaged in silver capsules, and analyzed at the Colorado Plateau Stable Isotope Laboratory (Doucett et al. 2007, Cole et al. 2011). Samples were pyrolyzed at 1400°C and isotope concentrations were measured using gas isotope-ratio spectrometry. Water was measured using headspace equilibration with H₂ gas and a Pt catalyst. Samples were normalized for exchangeable hydrogen using 3 standards as described in Doucett et al. (2007) and Solomon et al. (2009). All data are presented as delta units (δD) in per mil (%o) notation.

**Allochthony calculations**

We estimated allochthony of gizzard shad in each lake using a two-source mixing model, with phytoplankton and terrestrial vegetation as possible sources. However, because fish can obtain H from water they drink ("dietary water") as well as their food, we first corrected for dietary water using equations adapted from Solomon et al. (2009):

\[
\delta D_{\text{fish}} = \frac{\delta D_{\text{fish}} - 1000 \delta D_{\text{water}}}{1 - 1000}
\]

where \(\delta D_{\text{fish}}\) and \(\delta D_{\text{fish}}\) represent δD of fish tissue before and after correction for dietary water,
respectively; \( \omega \) is the proportion of tissue H obtained from dietary water; and \( \delta D_{\text{water}} \) is \( \delta D \) of lake water. This assumes no fractionation of H by gizzard shad, which is reasonable given that Solomon et al. (2009) found negligible H fractionation by aquatic consumers.

We attempted to measure the contribution of dietary water (\( \omega \)) for gizzard shad using controlled lab experiments, following methods of Solomon et al. (2009). However, we were unsuccessful because of high mortality, which unfortunately is typical for gizzard shad in lab settings. Thus, we explored several scenarios in which we varied \( \omega \), as described in the Allochthony scenarios section below.

Allochthony, defined as the proportion of gizzard shad biomass derived from terrestrial organic matter (\( p_{\text{alloc}} \)), was estimated as:

\[
p_{\text{alloc}} = \frac{\delta D_{\text{fish}} - \delta D_{\text{autoc}}}{\delta D_{\text{alloc}} - \delta D_{\text{autoc}}} \tag{2}
\]

where \( \delta D_{\text{autoc}} \) and \( \delta D_{\text{alloc}} \) are values for phytoplankton and terrestrial vegetation, respectively.

**Allochthony scenarios**

Because we could not directly measure the contribution of dietary water (\( \omega \)) or the isotopic signal of phytoplankton (\( \delta D_{\text{autoc}} \)), we had to estimate these parameters through other means. We recognize that this increases the uncertainty of our allochthony estimates. To address this uncertainty, we explored several scenarios in which we varied \( \omega \) and \( \delta D_{\text{autoc}} \) and examined the sensitivity of \( p_{\text{alloc}} \) to this variation. Our approach to estimating uncertainty in \( p_{\text{alloc}} \) is thus similar to that of other recent papers using deuterium to study aquatic food webs (Caraco et al. 2010, Cole et al. 2011, Solomon et al. 2011).

We explored 24 scenarios for each lake, in which \( \omega \) and \( \delta D_{\text{autoc}} \) were varied over reasonable ranges (Fig. 1). Each scenario yielded an allochthony (\( p_{\text{alloc}} \)) estimate for each lake. We then evaluated the mean and distribution of \( p_{\text{alloc}} \) produced by these scenarios. Our scenarios (Fig. 1) represent a fully factorial design in which we crossed 3 values of \( \omega \), \( \delta D_{\text{autoc}} \) values generated by 4 different NVSS-seston \( \delta D \) regressions, and 2 corrections to \( \delta D_{\text{autoc}} \) based on partial algal decomposition (no correction or correction).

Our moderate value of \( \omega \) (which we consider most realistic) was 0.17 (Fig. 1). This is the mean \( \omega \) estimated by Solomon et al. (2009) from a...
variety of aquatic consumers, including invertebrates and fish. We also explored a high $a >$ estimate (0.23), which is the maximum $a$ observed from several fish species (all salmonids) as reported in Solomon et al. (2009), and a low estimate (0.08). The latter value is based on data from six gizzard shad individuals collected from Acton and Burr Oak Lakes, from which we estimated $\delta D$ of both muscle tissue and gut contents. For these six fish, we calculated an estimate (0.08). The latter value is based on data as reported in Solomon et al. (2009), and a low estimate (0.23), which is the maximum $\delta D$, yielded one $\delta D_{autoc}$ value for Acton, and another for all other lakes pooled. We then used the respective intercepts as estimates of $\delta D_{autoc}$, yielding one $\delta D_{autoc}$ value for Acton, and another for all other lakes. This removes potential bias due to frequent sampling of Acton, but does not allow for variation among the other lakes. We accounted for variation among lakes using two more regression approaches, each using regression residuals to derive lake-specific $\delta D_{autoc}$ values (Fig. 1). In the “Common Regression w/ Residuals” approach, we used the residuals of the all-lakes regression of %NVSS vs. seston $\delta D$. Thus, for each of the 7 lakes for which he had %NVSS data, we obtained a mean residual (from residuals on individual sampling dates) and added the residual to the intercept to get $\delta D_{autoc}$ for that lake. For the 4 lakes for which we lacked NVSS data, we used the intercept to estimate $\delta D_{autoc}$ as in the Common Regression scenario. Finally, in the “Separate Regressions w/ Residuals” scenarios, we employed the same approach except that for lakes other than Acton we used residuals of the regression specific to this set of lakes. In this scenario, we could not obtain a residual for Acton because it contributed all the points to its own regression; therefore we used the Acton-specific intercept in this scenario.

As described above, we regressed %NVSS vs. seston $\delta D$ to correct for terrestrial contamination and obtain $\delta D_{autoc}$; however, within this general scheme we used four different regression approaches (Fig. 1). For our simplest approach, we obtained a single regression of %NVSS vs seston $\delta D$ using data pooled from all lakes ($n = 30$ lake-dates). Then we used the intercept of this regression, i.e., the $\delta D_{autoc}$ value corresponding to $%\text{NVSS} = 0$. A potential advantage of this approach, which we refer to as the “Common Regression” approach, is that it utilizes all the data. However, it produces a single $\delta D_{autoc}$ value for all lakes, and therefore does not allow for any variation among lakes. In addition, most of the points in this regression were derived from Acton Lake, where we have an intensive long-term sampling program, and the regression could be biased by this one lake. Therefore, our three other regression approaches addressed these issues. In the “Separate Regressions” scenarios, we used two separate regressions of %NVSS vs. seston $\delta D$, one for Acton and another for all other lakes pooled. We then used the respective intercepts as estimates of $\delta D_{autoc}$, yielding one $\delta D_{autoc}$ value for Acton, and another for all other lakes. This removes potential bias due to frequent sampling of Acton, but does not allow for variation among the other lakes. We accounted for variation among lakes using two more regression approaches, each using regression residuals to derive lake-specific $\delta D_{autoc}$ values (Fig. 1). In the “Common Regression w/ Residuals” approach, we used the residuals of the all-lakes regression of %NVSS vs. seston $\delta D$. Thus, for each of the 7 lakes for which he had %NVSS data, we obtained a mean residual (from residuals on individual sampling dates) and added the residual to the intercept to get $\delta D_{autoc}$ for that lake. For the 4 lakes for which we lacked NVSS data, we used the intercept to estimate $\delta D_{autoc}$ as in the Common Regression scenario. Finally, in the “Separate Regressions w/ Residuals” scenarios, we employed the same approach except that for lakes other than Acton we used residuals of the regression specific to this set of lakes. In this scenario, we could not obtain a residual for Acton because it contributed all the points to its own regression; therefore we used the Acton-specific intercept in this scenario.

We also wished to account for any change in $\delta D_{autoc}$ that may occur if phytoplankton partially decompose before being consumed by gizzard shad. Because these fish feed on surface sediments, it is possible that algae decompose to some extent before being consumed, and this could conceivably alter $\delta D_{autoc}$. Thus, all of the 12 scenarios described above were run both with no adjustment to $\delta D_{autoc}$ (i.e., using intercepts and residuals as described in the preceding paragraph), or with an adjustment that accounted for changes in $\delta D_{autoc}$ during partial decomposition of phytoplankton (Fig. 1). To estimate the change in $\delta D_{autoc}$ due to partial decomposition, we conducted an experiment in which we incubated two samples of Acton Lake seston in the lab in the dark to simulate conditions at the sediment-water interface, measured the change in $\delta D$, and used this change to adjust $\delta D_{autoc}$ relative to the scenarios without decomposition (Fig. 1). We sampled the decomposing seston after 3, 6 and 9 days incubation. However, we could not use data from any of the samples that had incubated for >3 days because they contained insufficient organic matter to obtain reliable deuterium values; most algae had already decomposed. Therefore, we used data only from the day 3 sampling. Seston $\delta D$ increased by 20% after 3 days. Thus, in the decomposition scenarios, we increased $\delta D_{autoc}$ by 20% relative to the same scenario without decomposition.

In total, we thus explored 24 scenarios: 3 $a$ values $\times$ 4 regression approaches $\times$ 2 algal decomposition states. We consider the most feasible scenario to be that in which we set $a = 0.17$, used the Separate Regressions w/ Residuals method, and assumed no algal decomposition (Fig. 1). Thus we consider this our “baseline”
scenario. We consider 0.17 to be the most realistic estimate of ω, even though it is derived from literature values and not our own comparison of gizzard shad tissue and their gut contents, for two reasons. First, our sample size is only six fish, from only two lakes. Second, although estimating ω by comparing consumer tissue vs. diet δD is valid when one cannot conduct controlled lab experiments, this method seems to yield lower ω values than controlled experiments (Solomon et al. 2009). We feel that the Separate Regressions w/ Residuals scenarios offer the most realistic means of estimating δDauto because it provides lake-specific values and avoids possible bias in the regression due to disproportionate sampling of Acton. Finally, we contend that scenarios without algal decomposition are most parsimonious given our current knowledge, for a couple of reasons. First, our decomposition experiment was based on only two replicates and one sampling period; thus we are not as confident as we would like to be in our estimate of the effects on δDauto. Furthermore, as we argue below (Discussion), it seems unlikely that all phytoplankton consumed by gizzard shad have decomposed for 3 days. However, because we observed a rather large change in δDauto (20%), it seems important to explore the consequences for allochthony.

Statistical analyses

We used IsoError (Phillips and Gregg 2001) to obtain error estimates for palloc under the baseline scenario. IsoError requires standard deviations (SDs) of δD estimates of consumers and food sources. For gizzard shad muscle tissue, we used the SD among individual fish (usually, n = 5 fish on a single date). For Acton Lake, where we sampled gizzard shad on several dates, we averaged the SDs from individual dates to obtain a single SD. To obtain an overall SD for terrestrial vegetation (δDalloc), we used error propagation methods (Ku 1966) that incorporated SDs of individual plant species δDs and their proportional contributions to watershed land area. For δDalloc, we used the SD of the intercept of the %NVSS vs. seston δD regression.

To test the hypothesis that allochthony varies with watershed land use (defined as the proportion of watershed area composed of a particular land use type) and/or lake characteristics, we regressed palloc against a variety of land use and reservoir parameters that have been previously identified as being important in determining phytoplankton primary production and gizzard shad biomass (Knoll et al. 2003, Vanni et al. 2006b, Bremigan et al. 2008, Hale et al. 2008). These included the proportion of watershed area composed of row crops, all agriculture (% agriculture, row crops plus pasture), or forest; reservoir surface area; the watershed area:reservoir area ratio; reservoir volume; reservoir mean depth; and the proportion of reservoir area consisting of littoral zone. In addition, we used the numerators of our ratios as predictor variables, i.e., total watershed area and the area of agricultural land in the watershed. Proportional data (land use proportions and palloc) were arcsin-square root transformed prior to analyses. We conducted two sets of regressions, one using palloc estimates generated from the baseline scenario and the other using the median palloc generated from the 24 scenarios (Fig. 1).

We also asked whether gizzard shad allochthony and biomass are related. If allochthonous organic matter provides a subsidy for gizzard shad, biomass and palloc should be positively correlated (Hoffman et al. 2007, Solomon et al. 2011). Alternatively, biomass and palloc may be unrelated if gizzard shad simply substitute allochthonous detritus for autochthonous detritus in systems with low primary production (Solomon et al. 2011). Still another possibility is that biomass and palloc may be negatively related; phytodetritus is probably a much better quality food resource that terrestrial detritus (Cebrian and Lartigue 2004, Brett et al. 2009) and if gizzard shad consume terrestrial detritus at the expense of phytodetritus, this could negatively impact production and hence biomass. Previous studies show that gizzard shad biomass is positively related to phytoplankton primary production (PPr) (Bachmann et al. 1996, Vanni et al. 2006b, Bremigan et al. 2008, Hale et al. 2008). To examine the potential singular and combined effects of autochthony and allochthony on gizzard shad biomass, we conducted simple and multiple regressions using PPr and palloc as independent variables and gizzard shad biomass as the dependent variable (Table 1). PPr was measured between 15 May and 15 September 2008, as part of a related project, on 9 of our
study lakes (all except Berlin and Grand Lake St. Mary’s). PP was quantified using the $^{14}$C method, details of which are provided in Knoll et al. (2003). We used gizzard shad biomass from hydroacoustic surveys (methods described in Vanni et al. 2006; Hale et al. 2008) conducted in August of 2006, 2007 and 2008 (Table 1). For each lake, we calculated mean biomass from the three yearly estimates, because most of the shad sampled during our study (2008) were probably 2–3 years old. Biomass data were not available for Grand Lake St. Mary’s. As with the regressions described above, we used $p_{alloc}$ from the baseline scenario as well median $p_{alloc}$, and $p_{alloc}$ was arcsin-square root transformed before analyses.

**RESULTS**

dD varied among terrestrial plant species and watersheds (Table 2). Soy was the most depleted species with a mean dD of −133‰, while corn was most enriched at +91‰. For the two species sampled in all three watersheds (corn and maple), there was a significant effect of both watershed and species (2-way ANOVA, F = 6.65 and 24.41, P = 0.007 and <0.0001, df = 2, 19 and 1, 19, respectively), but no interaction (F = 0.95, P = 0.400, df = 2, 19). Sediment entering Acton Lake via streams during storm events had a mean dD of −118‰, within the range of terrestrial plants (Fig. 1) and similar to the simple mean of corn and soy dD (−112‰), species that dominate Acton’s watershed. Despite the differences among species and watersheds, 95% confidence intervals for watershed-scale terrestrial dD, which take into account species-specific dD and land use proportions, overlapped for all 11 reservoirs (Fig. 2).

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Table 2. dD values (‰) for four terrestrial plant species, as well as sediment entering Acton Lake via its inflow streams. For each plant species, tissue from several individuals was pooled to provide a sample, where n = the number of samples.

<table>
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<tr>
<th>Watershed</th>
<th>Corn (Zea mays)</th>
<th>Soy (Glycine max)</th>
<th>Maple (Acer spp.)</th>
<th>Oak (Quercus spp.)</th>
<th>Incoming sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SE n</td>
<td>Mean SE n</td>
<td>Mean SE n</td>
<td>Mean SE n</td>
<td>Mean SE n</td>
</tr>
<tr>
<td>Burr Oak</td>
<td>−93.0 10.0 2</td>
<td>−120.9 2.5 2</td>
<td>−106.2 12.0 2</td>
<td>−118.0 11.5 7</td>
<td></td>
</tr>
<tr>
<td>Pleasant Hill</td>
<td>−101.8 4.0 2</td>
<td>−121.5 0.1 2</td>
<td>... ... ...</td>
<td>... ... ...</td>
<td></td>
</tr>
<tr>
<td>Acton</td>
<td>−89.1 7.5 6</td>
<td>−103.7 10.7 10</td>
<td>−97.9 7.4 10</td>
<td>−118.0 11.5 7</td>
<td></td>
</tr>
</tbody>
</table>

Note: “...” refers to a lack of data.

Seston dD varied among reservoirs (ANOVA, $F = 78.288, P < 0.0001, df = 10, 25$), ranging −133‰ among lakes (Fig. 2). Variation in seston dD among reservoirs did not correspond to variation in mean water dD, which also varied among lakes but by less than 25‰ considering all...
replicates from all lakes. Seston δD, uncorrected for terrestrial contamination, was always more depleted than terrestrial vegetation, but by a variable amount (mean difference = 68‰, range 2–140; Fig. 2).

Seston δD was significantly and positively correlated with percent non-volatile suspended solids (%NVSS), our indicator of terrestrial contamination of seston (Fig. 3). The y-intercept of the Common (all-lakes) Regression was $-225.9$‰ (SD = 55.1) and was used as $\delta D_{\text{autoc}}$ in these scenarios (Fig. 1). However, regression fits were better (i.e., $r^2$s were higher) when we used separate regressions for Acton (which contributed more than half the points in the single regression) and other lakes (Fig. 3). For the Separate Regressions scenario, the intercept, i.e., estimated $\delta D_{\text{autoc}}$ was $-213.7$‰ for Acton and $-243.7$‰ for the other lakes (Fig. 3). For the two scenarios in which we used residuals, we added the mean lake-specific residual to the appropriate intercept to obtain $\delta D_{\text{autoc}}$ for each lake. Within each residuals scenario, $\delta D_{\text{autoc}}$ increased in some lakes and decreased in others, relative to scenarios in which residuals were not used (Figs. 1, 3).

In our experiment in which Acton Lake seston was incubated in the dark to simulate algal decomposition at the sediment surface, seston δD increased by 20‰. Therefore, for all Algal Decomposition scenarios, we added 20‰ to the corresponding $\delta D_{\text{autoc}}$ value without decomposition to obtain the new $\delta D_{\text{autoc}}$. For example, in the Common Regression scenarios, $\delta D_{\text{autoc}}$ was set equal to $-205.9$‰ (Fig. 1).

Variation among lakes in gizzard shad muscle δD was small relative to seston, ranging from $-206.6$‰ in Dillon to $-167.6$‰ in Burr Oak (Fig. 2). These values are corrected for dietary water ($\omega = 0.17$, as in the Baseline and other moderate $\omega$ scenarios), which decreased gizzard shad δD (made it more negative) by $\sim 24$%. Fish δD was not significantly related to water δD (linear regression, $r^2 = 0.199$, $P = 0.197$, $n = 10$ lakes; see also Fig. 2). In Acton Lake, gizzard shad δD did not show significant seasonal variation in tissue δD (Babler 2009), so we present here the simple mean of all dates. For all six gizzard shad for which diets (gut contents) were analyzed, muscle δD values were enriched relative to gut contents, with a mean difference of 10.0‰ (SD = 6.0), yielding a mean estimate of $\omega$ of 0.076 (SD = 0.040; Table 3).

Allochthony ($p_{\text{allo}}$) ranged from 0 to >0.6 across all scenarios and lakes (Fig. 4, Appendix: Fig. A2). Averaged across all lakes and scenarios, mean $p_{\text{allo}}$ was 0.343 (median = 0.364). Median within-lake $p_{\text{allo}}$ ranged from 0.168 in Dillon to 0.471 in Burr Oak, and the baseline scenario produced $p_{\text{allo}}$ estimates that ranged from 0.209 to 0.549 (Fig. 4). For most lakes, the baseline scenario yielded a $p_{\text{allo}}$ estimate that was higher than median $p_{\text{allo}}$ (mean difference = 0.079), although in two lakes (Burr Oak and Acton) baseline and median estimates were very close, and in another lake (Delaware) the baseline estimate was slightly lower than the median (Fig. 4). The baseline and median estimates were highly correlated with each other ($r^2 = 0.75$).

As expected based on other recent studies (Caraco et al. 2010, Cole et al. 2011, Solomon et al. 2011), $p_{\text{allo}}$ estimates were sensitive to variation in $\omega$ (Appendix: Fig. A2). Increasing $\omega$ from 0.17 (baseline) to 0.23 decreased $p_{\text{allo}}$ by...
0.095, while decreasing $\omega$ to 0.08 increased $p_{\text{alloc}}$ by 0.127, averaged across all scenarios (Appendix: Fig. A2). $p_{\text{alloc}}$ was also very sensitive to the increase in $\delta D_{\text{aloc}}$ attributed to partial algal decomposition (Appendix: Fig. A2). Averaged over all scenarios, decomposition decreased $p_{\text{alloc}}$ estimates by 0.117, and this effect was greater at high $\omega$ (0.127) than at low $\omega$ (0.103). The four regression approaches yielded variable $p_{\text{alloc}}$ estimates; the Separate Regressions scenarios (with or without residuals) produced higher mean $p_{\text{alloc}}$ estimates than the Common Regression scenarios (Appendix: Fig. A2). Using the regression residuals, rather than only the intercepts, had little effect on mean $p_{\text{alloc}}$ but increased variation among lakes, as expected (Appendix: Fig. A2).

In 7 of the 264 $p_{\text{alloc}}$ estimates (<3% of all scenarios), a solution for $p_{\text{alloc}}$ was not feasible because gizzard shad tissue (corrected for $\omega$) was more depleted in $\delta D$ than either end member (phytoplankton or terrestrial vegetation). These seven non-feasible estimates were observed in three different lakes (Fig. 4); six of seven cases occurred in high $\omega$ (0.23) scenarios, and the other instance occurred with $\omega = 0.17$ and with algal decomposition. We calculated median $p_{\text{alloc}}$ two ways: setting $p_{\text{alloc}} = 0$ for the seven non-feasible observations, and by omitting these scenarios. Median $p_{\text{alloc}}$ estimates were affected very little by omission of these points (<0.02) except in Delaware, where the median $p_{\text{alloc}}$ was 0.067 higher with the non-feasible points omitted (Fig. 4).

Overall, $p_{\text{alloc}}$ estimates were lowest under the High $\omega$, Common Regression (with or without residuals), and algal decomposition scenario (Appendix: Fig. A2). In contrast, $p_{\text{alloc}}$ was highest, on average under the Low $\omega$, Separate Regressions (with or without residuals), and no algal decomposition scenario (Appendix: Fig. A2).

Allocruthy ($p_{\text{alloc}}$) was not related to land use per se, i.e., the percentage of watershed area...
comprised of agricultural land or forest (Fig. 5; Table 4). However, \( p_{\text{alloc}} \) was negatively correlated with all ratios relating watershed area to lake size, including watershed area:lake area, agricultural area:lake area ratio, row crop area:lake area and watershed area:lake volume (Table 4).

Further inspection revealed that these relationships were driven by the numerators of these ratios, i.e., correlations were similar when we used watershed area, agricultural area, and row crop area (Table 4; Fig. 5). None of the lake morphometry variables were significantly related to \( p_{\text{alloc}} \) (Table 4). Correlations were similar whether we used \( p_{\text{alloc}} \) from the baseline scenario or median \( p_{\text{alloc}} \) (Table 4; Fig. 5). We also assessed whether we could better predict \( p_{\text{alloc}} \) using multiple regressions with two independent variables. Specifically, we used either watershed area (WA) or watershed area:lake area (WA:LA) as the first independent variable, and either percent agriculture, percent row crop or percent forest as the second independent variable (i.e., 6 different regressions). We tried this for both \( p_{\text{alloc}} \) estimates (baseline and median). In some cases \( r^2 \) increased slightly, compared to simple regressions with either WA or WA:LA alone. However, Akaike’s Information Criterion (AIC) from the multiple regressions were always within 2 of the simple regressions, suggesting that the more complex models were not an improvement over the simple regressions. We did not explore multiple

Table 4. \( r^2 \) values from linear regressions of various watershed and lake predictor variables against the proportion of gizzard shad biomass derived from terrestrial organic matter (\( p_{\text{alloc}} \)). Separate regressions were conducted using \( p_{\text{alloc}} \) values from the baseline scenario, and the median \( p_{\text{alloc}} \) across all 24 scenarios. Proportional data were transformed (arc-sin-square root) prior to analyses.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Baseline scenario</th>
<th>Median of all scenarios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion agricultural land in watershed</td>
<td>0.222</td>
<td>0.248</td>
</tr>
<tr>
<td>Proportion row crop land in watershed</td>
<td>0.212</td>
<td>0.192</td>
</tr>
<tr>
<td>Proportion forested land in watershed</td>
<td>0.094</td>
<td>0.111</td>
</tr>
<tr>
<td>Lake area</td>
<td>0.193</td>
<td>0.166</td>
</tr>
<tr>
<td>Lake volume</td>
<td>0.180</td>
<td>0.146</td>
</tr>
<tr>
<td>Mean lake depth</td>
<td>0.054</td>
<td>0.026</td>
</tr>
<tr>
<td>Proportion of lake area composed of littoral area</td>
<td>0.000</td>
<td>0.009</td>
</tr>
<tr>
<td>Watershed area:Lake area</td>
<td>0.471*</td>
<td>0.699**</td>
</tr>
<tr>
<td>Ag area:Lake area</td>
<td>0.693**</td>
<td>0.809***</td>
</tr>
<tr>
<td>RowCrop area:Lake area</td>
<td>0.782***</td>
<td>0.739***</td>
</tr>
<tr>
<td>Watershed area:lake volume</td>
<td>0.508*</td>
<td>0.664**</td>
</tr>
<tr>
<td>Total watershed area</td>
<td>0.425*</td>
<td>0.764**</td>
</tr>
<tr>
<td>Agricultural area in watershed (Ag area)</td>
<td>0.596**</td>
<td>0.828***</td>
</tr>
<tr>
<td>Row crop area in watershed (RowCrop area)</td>
<td>0.666**</td>
<td>0.735***</td>
</tr>
</tbody>
</table>

Notes: Values in boldface indicate \( P < 0.05 \), \* \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \).
regressions with more than two independent variables, given that we have 11 observations (lakes).

Gizzard shad biomass was positively related to phytoplankton primary production (PPr) (Fig. 6A) and negatively related to palloc (Fig. 6B). Results of multiple regression analyses differed somewhat depending on whether baseline or median palloc was used as the independent variable. Using baseline palloc as the independent variable, the multiple regression did not improve the model fit beyond that with only palloc as the independent variable; although $r^2$ was marginally increased when both variables were included (from 0.860 to 0.876), AIC was lower when only palloc was included (75.274) compared to the model with both variables (76.199). In contrast, when median palloc was used, the model with both PPr and palloc performed better than either single variable model. Specifically, $r^2$ was 0.921 using both variables, considerably higher than simple regressions with either PPr or palloc (Fig. 6), and AIC was much lower with the multiple regression model (72.140) than with either single-variable model (86.708 and 87.432, using PPr or palloc respectively). In addition, a model incorporating an interaction term (PPr $\times$ palloc) yielded even higher $r^2$ (0.975) and lower AIC (63.751).

**DISCUSSION**

Our two most important findings are that (1) autochthonous energy supports the majority of gizzard shad production, although allochthony is also important; and (2) the reliance on allochthonous energy sources (palloc) is negatively related to watershed size and gizzard shad biomass. We discuss the implications of these findings, as well as the implications of uncertainty in our estimates.

**Carbon sources supporting gizzard shad production**

Autochthonous energy, i.e., phytoplankton production, supported the majority of gizzard shad production. Averaged across all scenarios, autochthonous production supported >50% of gizzard shad production in all lakes (Fig. 4). However, gizzard shad production is supported to some extent by terrestrial organic carbon; even in the scenarios with high $\omega$ and with algal decomposition, which yielded the lowest allochthonous energy sources, on average ~15% of production was supported by terrestrial sources.

The ability of gizzard shad to utilize both autochthonous and terrestrial energy sources is supported by studies on diets, digestive physiology and energetics, isotope labeling experiments, and experimental subsidy additions. Gizzard shad feed preferentially on detritus with higher concentrations of carbon and nutrients than ambient sediments (Mundahl and Wissing 1987, Higgins et al. 2006), apparently by selectively
feeding on low-density sediment particles that are relatively enriched in organic matter. Ingested particles include terrestrial plant detritus, algae, and heterotrophic bacteria (Smoot and Findlay 2010a). Using lipid biomarkers, Smoot and Findlay (2010a) showed that sediment-feeding gizzard shad in Acton Lake assimilate carbon from algae and heterotrophic bacteria in approximately equal amounts, and that assimilation of C from these sources is sufficient to meet gizzard shad energetic demands. Thus, terrestrial organic C may contribute indirectly to gizzard shad production, because sediment bacteria utilize terrestrial detritus (Smoot and Findlay 2010a, b), a situation also shown for invertebrates in forested streams (Hall and Meyer 1998). In a mesocosm experiment in which gizzard shad were provided with reservoir sediments mixed with corn (Zea mays) that was previously labeled with $^{13}$C and $^{15}$N, gizzard shad incorporated corn C and N into their tissues (Pilati 2007). This shows that shad can assimilate terrestrial C (and N), although it is not clear if they assimilate it directly or from microbes that utilize corn detritus. Population-level experiments also support the notion that gizzard shad can utilize either energy source. In an experimental pond study, food resources for gizzard shad were enhanced either by fertilization with inorganic nutrients, which stimulated phytoplankton production, and/or by direct addition of allochthonous sediments. Both subsidies (allochthonous and autochthonous) greatly increased gizzard shad production, and there was no difference in their effects (Pilati et al. 2009).

Recently, Zeug and Winemiller (2008) showed, using $^{13}$C, that terrestrial detritus supported >80% of gizzard shad production in the main channel of the Brazos River and one of its oxbow lakes; in contrast, terrestrial and autochthonous matter contributed equally in another oxbow lake in this floodplain system. The overall lower reliance on terrestrial C in our study reservoirs compared to that in the Brazos system may be related to hydrology. Specifically, the ratio of terrestrial inputs to autochthonous production should be higher in rivers than in lakes, with reservoirs intermediate (Thornton 1990, Wetzel 1990). Thus, allochthony should be most pronounced in ecosystems with large terrestrial subsidies, and during periods of high subsidy.

For example, coastal ecosystems sometimes receive large terrestrial subsidies, and consumers in these ecosystems may depend more on terrestrial matter during wet periods when high stream discharge delivers large allochthonous subsidies (Hoffman et al. 2007, 2008, Wai et al. 2008).

**Allochthony in relation to environmental gradients**

Contrary to our hypothesis, allochthony was not related to watershed land use, i.e., % agriculture or forest, but was strongly and negatively related to total watershed area and agricultural area in the watershed. One possible reason for the strong effect of watershed size is that specific sediment export rate (i.e., sediment mass exported per unit watershed area per unit time) declines with watershed area, in part because in large watersheds a greater fraction of eroded sediment is re-deposited in floodplains and stream beds rather than exported completely out of the drainage area (Walling 1983). Smaller watersheds export sediment more efficiently, perhaps because of higher drainage density (length of streams per unit area), steeper slopes, and relatively fewer areas where sediment can be re-deposited (Walling 1983, Renwick and Anderson 2006). In contrast, dissolved nutrient export should be much less affected by these hydrological processes, and therefore specific export rates for dissolved nutrients should be less dependent on watershed area (Prairie and Kalff 1986). Thus, the ratio of sediment export (and presumably POC export) to dissolved nutrient export should also vary inversely with watershed area. If so, this could potentially account for our observed negative correlation between gizzard shad allochthony and watershed area. Of course, this assumes that POC behaves similarly to bulk sediment in terms of how its export is mediated by watershed area, which is probably the case in agricultural or mixed land use watersheds where eroded soil is the main contributor to POC export (Lal 2003, Saenger et al. 2008). For example, the strong correlation between NVSS and POC loads to Acton Lake (Appendix: Fig. A1) suggests that POC export and sediment export behave similarly. However, in small, forested watersheds, POC export may be dominated by leaf fall and not by eroded sediment, in which case there may not be a negative relationship between watershed...
area and specific POC export (Schlesinger and Melack 1981).

It is possible that land use and watershed size interact to influence sediment and nutrient export rates. For example, in Ohio reservoirs, specific sedimentation rate (sediment accumulation per unit watershed area) is negatively related to watershed size in agricultural, but not forested, regions (Renwick and Andereck 2006). Similarly, Prairie and Kalf (1986) found that specific export rates of total phosphorus were strongly and negatively related to watershed size in agricultural watersheds but not forested watersheds. They attributed this difference to the forms in which P is exported from different land use types; specifically, in agricultural watersheds most P is exported in particulate form (i.e., attached to sediment particles) whereas in forested watersheds a much larger proportion of P export is in dissolved form (Prairie and Kalf 1986). This supports the notion watershed size affects dissolved nutrient export less than sediment (and POC) export. These findings suggest that land use (i.e., % agriculture) and watershed size may jointly regulate the ratio at which sediments (POC) and dissolved nutrients are exported from watersheds, and both factors could interactively determine allochthony. We did not detect such an interaction effect on allochthony, but it would be difficult to do so with only 11 watersheds. Future studies employing a greater number of watersheds are needed to adequately test this hypothesis.

All else being equal, allochthony may be proportional to the ratio of terrestrial organic C subsidies to autochthonous primary production. We have data on both of these C fluxes in three of our study systems. We used annual loading rates of particulate carbon (most of which is organic C) to Burr Oak, Pleasant Hill, and Acton, measured over two years (Vanni et al. 2011) as well as phytoplankton primary production rates (PPr) from the same lakes (from Knoll et al. 2003 and Vanni et al. 2006b). We assumed that daily PPr measured in summer applies to 6 months of the year, with no production during the rest of the year, to obtain an annual PPr. We then divided annual particulate C loading rates by annual PPr (C fixation rates), both in units of mg C (m$^{-2}$ of lake area) year$^{-1}$, and obtained the following ratios for Burr Oak, Pleasant Hill and Acton: 0.26, 0.35, 0.32. Given the variation associated with both C fluxes, these ratios are probably not significantly different from each other, suggesting that terrestrial export of POC and PPr increase with agricultural land use at proportionally similar rates. Perhaps then, it is not surprising that we did not find an effect of land use on allochthony (recognizing that this is based on C flux data from only 3 watersheds/lakes). It is also worth noting that the POC inputs:PPr ratios we observed in these three reservoirs (0.26–0.35) are similar to the mean $p_{allo}$ value observed across all scenarios (0.34); this suggests that the relative assimilation of terrestrial and autochthonous C by gizzard shad may be more or less proportional to the ratio at which these two C forms are provided.

The positive correlation between PPr and gizzard shad biomass, and the negative correlation between $p_{allo}$ and gizzard shad biomass (Fig. 6), suggest that gizzard shad production is closely tied to autochthonous production. This could be because autochthonous material (phytoplankton) is a higher quality food source than terrestrial material for detritivorous age classes, i.e., juveniles and adults. Alternatively, or additionally, this relationship may arise because the recruitment of zooplanktivorous larval gizzard shad increases with autochthonous primary production (Bremigan and Stein 2001, Bremigan et al. 2008). Our results appear to contrast with research on American shad, a species in the same family as gizzard shad (Hoffman et al. 2007). Young-of-year (YOY) recruitment of American shad in an estuary was highest in a year when allochthonous subsidies were high and YOY fish showed greater allochthony. Hoffman et al. (2007) attribute this to increased food resources for YOY fish, including zooplankton and benthic invertebrates, during periods when allochthonous subsidies are pronounced. However, in our reservoir systems, gizzard shad recruitment is tied to rotifer biomass, which in turn is positively related to algal biomass (Bremigan et al. 2008). The apparently greater reliance of YOY clupeid fish on autochthonous production in reservoirs, compared to estuaries, may be due to higher rates of primary production found in reservoirs compared to estuaries. Future studies should quantify allochthony of entire food webs, along gradients of autochthonous primary production.
Limitations and sources of uncertainty

The large separation in δD of terrestrial vs. aquatic energy sources suggests that hydrogen isotopes are useful for quantifying allochthonous subsidies (Doucett et al. 2007, Solomon et al. 2009, 2011, Caraco et al. 2010, Finlay et al. 2010, Cole et al. 2011). However, there are still several sources of uncertainty associated with this new technique, including the importance of dietary water and the true δD value of phytoplankton. Allochthonous estimates are sensitive to the importance of dietary water (δo), as illustrated by our study and others (Caraco et al. 2010, Cole et al. 2011, Solomon et al. 2011). As pointed out by Solomon et al. (2009), we need much more information on δo from a variety of consumers to properly constrain allochthony across a range of ecosystems and conditions. Until such information is available, the sensitivity of allochthonous estimates to assumptions about δo must be considered. Our approach to dealing with uncertainty in δo is similar to that in other recent studies. For example, Caraco et al. (2010) and Cole et al. (2011) explored allochthony scenarios in which δo was set to a mean value and varied above and below this mean, similar to our use of a baseline value in addition to various scenarios. Furthermore, the range of δo that we explored is similar to the range investigated in these previous studies.

Another potential source of variation in fish muscle δD, for which we did not account, is variation in lipid content. Storage of H in lipids can affect fish tissue δD (Jardine et al. 2009), and because we did not extract lipids from our samples, variation in lipid content could have contributed to among-lake variation in gizzard shad δD. We do not have data on body lipid content to evaluate this possibility. However, Higgins et al. (2006) showed that whole-body gizzard shad C:N ratio varied little among three of our study lakes (Burr Oak, Pleasant Hill and Acton) that vary greatly in productivity and watershed land use. As body lipid content is correlated with body C:N (Post et al. 2007), this suggests that variation in body lipids among gizzard shad populations did not contribute greatly to variation in tissue δD. However, additional studies are needed to critically evaluate the potential role of lipids in mediating fish δD.

Estimating the actual δD value of phytoplankton is problematic because of the difficulty in separating phytoplankton from other seston. Our approach using the single regression of seston δD vs. %NVSS yielded an algal δD value of −226% in the baseline scenario, and a range among lakes of −287 to −192% in the two residuals scenarios. These values are similar to the range observed in other field studies (Doucett et al. 2007, Caraco et al. 2010, Finlay et al. 2010). One downside to our regression approach is the relatively large error term associated with our estimate of phytoplankton δD. For example, in the Common (all-lakes) Regression, the SD of the intercept was 55%. Thus, better methods for estimating algal δD are needed.

An alternative approach to estimating phytoplankton δD is to conduct laboratory dilution-regrowth experiments with phytoplankton, which yield relatively pure phytoplankton samples (Caraco et al. 2010, Cole et al. 2011). However, such experiments alter some potential-
ly important conditions that might change phytoplankton species composition, and hence δD relative to that of lake phytoplankton. For example, these experiments typically exclude grazers and do not allow for episodic nutrient inputs, which are common in our reservoir ecosystems (Vanni et al. 2006a), possibly leading to changes in algal species composition. This is potentially problematic because studies in streams show that different algal groups can vary substantially in δD within the same stream (Doucett et al. 2007, Finlay et al. 2010). On the other hand, differences among phytoplankton groups in δD may average out at the bulk seston (algal community) level. For example, the difference between water δD and algal δD seems to be restricted to a relatively narrow range among ecosystems (−150 to −170‰; Doucett et al. 2007, Finlay et al. 2010, Cole et al. 2011). Thus, it is possible to estimate phytoplankton δD simply by adding −150 to −170‰ to water δD (Finlay et al. 2010, Cole et al. 2011). If we use this approach in our reservoirs (i.e., add −160 to water δD), we obtain phytoplankton δD values that are well within the range of those in our scenarios (Fig. 2). This provides additional validation of our approach to estimating phytoplankton δD.

Allochthonous estimates were also sensitive to changes in algal δD that occur during partial decomposition. Our experiment may have yielded somewhat extreme algal δD values, compared to those of phytoplankton actually consumed by gizzard shad, because it assumes that all phytoplankton decomposes for three days before being consumed. The actual δD of algae consumed by gizzard shad will depend on whether gizzard shad eat freshly deposited or partially decomposed phytoplankton. In reservoirs, gut contents of detrivorous gizzard shad contain mostly “mud,” i.e., amorphous, brown sediment detritus, and microscopic inspection of gut contents reveals very few recognizable, intact algal cells (Mundahl and Wissing 1987, Higgins et al. 2006). This suggests that gizzard shad feed primarily on partially decomposed phytoplankton. However, lipid analyses suggest that they consume living algae in Acton Lake (Smoot and Findlay 2010a), and gut analyses in other ecosystems also show that gizzard shad consume fresh, recognizable algae (e.g., Gu et al. 1996, Roseman et al. 1996). Perhaps it is difficult to visually detect intact algae in guts of gizzard shad feeding in reservoirs because they also consume considerable amounts of inorganic matter that obscure algal cells (Mundahl and Wissing 1987, Higgins et al. 2006). Furthermore, gizzard shad feed on surface sediments, “vacuum-filtering” low-density particles; thus, freshly deposited phytoplankton are likely to be consumed relatively more than partially decomposed phytoplankton. Thus, it seems likely that gizzard shad consume algae that, on average, has decomposed for less than 3 days; if so, allochthonous estimates from our algal decay scenario are probably underestimates. Clearly, however, more research is needed on both the extent to which consumers feed on live or dead algae, and the changes in δD that arise during decomposition.

Recently, Cole et al. (2011) showed that the use of multiple stable isotopes (13C, 2H and 15N) produces more tightly constrained allochthonous estimates, i.e., narrower confidence intervals for δD. We did not design our study with the goal of using these three isotopes, but we did collect a limited amount of data on 13C in Acton Lake (but not the other lakes), which allow us to use 13C to estimate allochthonous. Specifically, we quantified δ13C of gizzard shad (two dates), seston (six dates) and terrestrial vegetation (one date, depending on species) in 2008. Cole et al. (2011) showed that phytoplankton δ13C can be derived by using deuterium to estimate the proportion of seston composed of terrestrial organic matter. We used this approach to obtain an alternative estimate of allochthonous of Acton gizzard shad. First, we used δD to estimate the proportion of seston organic matter that is terrestrial, Dg = (DpOM – Dphyto)/ (DT – Dphyto), where DpOM, DT and Dphyto represent δD of seston organic matter, terrestrial vegetation and phytoplankton (Dautoc), respectively (Cole et al. 2011). For DpOM, we used the mean seston δD for all Acton dates (−195.9‰), for DT we used Acton terrestrial δD weighted by land use and plant species, as described above (−112.4; Fig. 2), and for Dphyto we used the intercept of the Acton-specific %NVS versus seston δD regression (−213.7; Fig. 1). This produced a Dg value of 0.18. Then, the δ13C of phytoplankton was estimated as δ13Cphyto = [(δ13CpOM – Dg × δ13Cg)]/(1 – Dg), where δ13CpOM and δ13Cg are the δ13C of seston and terrestrial vegetation,
22.8) as food sources, and the Acton gizzard shad value obtained using deuterium. For \( \delta^{13}C_{phyto} = -28.6 \) and \( \delta^{13}C_{T} \) we used \(-22.8 \) (mean, weighted by land use and plant species, as of \( \delta D_{T} \)). This yielded \( \delta^{13}C_{phyto} = -28.6 \). Using the two end members (phytoplankton, \(-28.6 \), and terrestrial vegetation, \(-22.8 \)) as food sources, and the \( \delta^{13}C \) of gizzard shad tissue \((-26.7, \text{mean of 13 fish collected on two dates}) \) in a two-source mixing model yields \( p_{alloc} = 0.33 \), which is very similar to the median Acton gizzard shad value obtained using deuterium \( (p_{alloc} = 0.31) \). Similar \( p_{alloc} \) estimates obtained using \( \delta D \) and \( \delta^{13}C \) lend confidence to our allochthony estimates.

**Conclusions**

Our results show that gizzard shad rely on both terrestrial and autochthonous organic energy sources, but that autochthonous sources contribute more to production. Furthermore, the importance of autochthonous sources increases with watershed size, and we speculate that this is because subsidies of particulate organic C, per unit watershed area, decline more with watershed area than do the subsidies of dissolved inorganic nutrients that fuel algal production. However, our ability to test this hypothesis is limited because few studies have quantified both carbon and nutrient exports from the same watersheds. Future studies of allochthony should employ an eco-hydrological approach that quantifies the magnitudes of these subsidies, as well as the pathways by which they affect aquatic consumers, across gradients of watershed size and land use.

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Fig. A1. Relationship between non-volatile suspend-
ed solids (NVSS) and particulate organic carbon con-
centration (POC) in Acton Lake’s two largest
inflow streams (which together drain 81% of the lake’s
watershed). A shows the relationship between concen-
trations, while B shows the relationship for loads (load =
concentration × discharge). Each point represents a
sample (n = 56) on which both NVSS and POC was
measured.
Fig. A2. Box plots depicting the proportion of gizzard shad biomass derived from allochthonous terrestrial organic matter \( (p_{\text{alloc}}) \) in the 24 scenarios. The black bar represents median \( p_{\text{alloc}} \) from the 11 lakes, the bottom and top edges of the boxes represent the 25th and 75th percentiles, and the bars represent the minimum and maximum values excluding outliers. The open circle represents the only outlier, where an outlier is defined as a value either less than the 25th percentile minus 1.5 \( \times \) IQ, or greater than the 75th percentile plus 1.5 \( \times \) IQ, where IQ is the interquartile distance (75th percentile minus 25th percentile). \( \omega \) refers to the proportion of H obtained by gizzard shad from dietary water.