

1 **This manuscript is contextually identical with the following published paper:**

2 Boros, G., Takács, P. and Vanni, M. J. (2015), The fate of phosphorus in decomposing fish carcasses: a
3 mesocosm experiment. *Freshw Biol*, 60: 479–489. doi:10.1111/fwb.12483

4 **The original published pdf available in this website:**

5 <http://onlinelibrary.wiley.com/doi/10.1111/fwb.12483/abstract>

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8 The fate of phosphorus in decomposing fish carcasses: A mesocosm experiment

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16 Running title: The fate of phosphorus in decomposing fish carcasses

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18 Keywords: fish, decomposition, phosphorus, algal bloom, mesocosm

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1 Summary

2

3 An outdoor mesocosm experiment was conducted to study the process of phosphorus (P) and nitrogen (N)
4 release during fish carcass decomposition and its implications for the functioning of warm, shallow temperate
5 lakes after massive fish kills. Specifically, we compared differences in the fate of P released from carcasses of
6 two fish species that differ in body P concentrations, and the ecosystem responses to these fish-derived nutrient
7 inputs.

8 Nutrients liberated from bluegill (*Lepomis macrochirus*) and gizzard shad (*Dorosoma cepedianum*) carcasses
9 induced phytoplankton blooms, and high total P and N concentrations in the water column within a week after
10 carcass addition; these effects persisted for ~2–3 weeks. Subsequently, water column P was transferred to other
11 ecosystem compartments, primarily sediments and benthic algae.

12 Fish species identity influenced the effects only slightly; decomposition of gizzard shad triggered the highest
13 maximal chlorophyll-*a* concentrations in the water column, while the highest coverage of benthic algae and
14 lowest biomass of periphytic biofilm were found in mesocosms containing bluegill carcasses.

15 Both bluegill and gizzard shad carcasses decomposed completely during the experimental period (~3 months).
16 Thus, apparently all carcass nutrients were mineralized into bioavailable forms and taken up by other ecosystem
17 compartments.

18 Fallen fish carcasses are not likely to represent long-term P sinks in warm-temperate shallow lakes.

19 Decomposition following large mortality events can induce fleeting algal blooms in these ecosystems.

20

1 Introduction

2 Fish can affect the nutrient cycles of freshwater ecosystems through several direct and indirect mechanisms,
3 including nutrient regeneration, translocation of nutrients within and among ecosystems, storage of nutrients in
4 bodies, carcass decomposition and cascading trophic interactions (Vanni, 2002; Vanni, Boros & McIntyre,
5 2013). However, the mechanisms by which fish affect nutrient dynamics, their relative importance and their net
6 effects on primary producers continue to be a topic of debate (Sereda & Hudson, 2010). Fish often constitute a
7 considerable nutrient pool (Kitchell, Koonce & Tennis, 1975; Sarvala & Jumppanen, 1988; Griffiths, 2006) that
8 is unavailable to other organisms (except piscivores and parasites) as long as it remains in living biomass. In
9 other words, nutrient sequestration by fish may reduce nutrients available to other organisms. Some authors
10 suggest that fish are important nutrient sinks (Griffiths, 2006; Sereda *et al.*, 2008), because of the relatively long
11 turnover time of the elements stored in their bodies. However, many other studies show that nutrient
12 translocation and excretion by fish can be an important source of nutrients for primary producers and microbes
13 (e.g., Vanni, 2002; Zimmer, Herwig & Laurich, 2006; McIntyre *et al.*, 2008).

14 A recent analysis suggests that the fate of fish carcasses is critical in determining the extent to which fish are
15 nutrient sinks or sources (Vanni *et al.*, 2013). Under natural conditions, massive mortality events (“fish kills”)
16 can occur for many reasons, including oxygen depletion, starvation, diseases or toxins. When fish die and
17 decompose, nutrients previously sequestered in fish biomass become available for primary producers, but the
18 rates and ratios at which these nutrients are made available are largely unknown (Parmenter & Lamarra, 1991;
19 Vanni *et al.*, 2013). Besides turnover time, the turnover rate of fish-bound elements is also significant when
20 assessing the role of fish as sinks or sources of nutrients. Fish decomposition can provide considerable amounts
21 of limiting nutrients that ultimately influence the structure and functioning of aquatic ecosystems, especially in
22 the case of mass mortality events (Parmenter & Lamarra, 1991; Premke *et al.*, 2010). Alternatively, because a
23 sizeable fraction of fish phosphorus (P) is tied up in relatively recalcitrant structures such as bones and scales,
24 mineralization of carcasses may take years and provide a small but steady source of nutrients (Kitchell *et al.*,
25 1975). In fact, natural fish mortality and consequent nutrient release has the potential to generate fluxes of
26 mineralized nutrients to the water column at levels comparable to loading by other major nutrient sources. For
27 instance, Kitchell *et al.* (1975) calculated that late spring and early summer bluegill (*Lepomis macrochirus*)
28 mortality represents a flux of mineralized P to the water column at rates higher than allochthonous inputs in a
29 Wisconsin lake. Moreover, Nakashima & Leggett (1980) pointed out that nutrient release due to fish
30 decomposition was equivalent to 20% of the allochthonous P entering the southern basin of Lake

1 Memphremagog (USA/Canada) in the spring. Durbin, Nixon & Oviatt (1979) also found that the influx and
2 mortality of alewives (*Alosa pseudoharengus*) had considerable impact on the food chain and nutrient cycles in
3 the Pausacaco Pond (RI, USA), causing 0.43 g m⁻² P and 2.7 g m⁻² N loading over a 2-month period. On the
4 other hand, Chidami & Amyot (2008) assessed that water-to-sediment deposition of white sucker (*Catostomus*
5 *commersonii*) carcasses alone corresponds to about 13.5% of the annual particulate P sedimentation in the east
6 basin of Lake Croche (Canada), representing a potential loss of nutrients from the pelagic food web but a
7 potential nutrient source to the benthic food web.

8 Despite the potential importance of fish decomposition for internal nutrient cycling, relatively little is known
9 about the fate of P released from or retained in decaying carcasses (Vanni *et al.*, 2013). Bones and scales of fish
10 contain considerable P (Rønsholdt, 1995; Hendrixson, Sterner & Kay, 2007) in the form of hydroxyapatite
11 (Schenau & De Lange, 2000). In fact, 86–88 % of body phosphorus content is stored in the skeleton of teleost
12 fish (Rønsholdt, 1995). These tissues may be recalcitrant and resist rapid degradation (Claeson *et al.*, 2006)
13 resulting in long-term P retention in sediments; indeed fish bones and scales are sometimes present as fossils
14 (Trueman & Marill, 2002). Because P is often a dominant limiting nutrient in controlling phytoplankton
15 production (Schindler, 1977; Lewis & Wurtsbaugh, 2008), the rate of P liberation from decomposing fish could
16 have important ecosystem-level implications and determines the role of fish as sinks or sources of P.

17 The elemental composition of fish varies considerably among taxa (e.g., Czamanski *et al.* 2011) due to the
18 differences in their anatomy (Hendrixson *et al.* 2007). Thus, the possible interspecific differences in the relative
19 proportions of hard structures in fish bodies, such as bones or integuments (rich in P), may yield varied rates of
20 decomposition among fish species (Hendrixson *et al.* 2007). Most studies on fish decomposition have been
21 restricted to salmonids (e.g. Parmenter & Lamarra, 1991; Schuldt & Hershey 1995; Cederholm *et al.*, 1999;
22 Claeson *et al.*, 2006; Bretherton *et al.*, 2011), which represent only a small fraction of fish diversity. In addition,
23 the vast majority of fish decomposition studies have focused on either the loss of total body mass during
24 decomposition or the fate of visible carcasses (e.g. Schneider, 1998; Premke *et al.*, 2010), rather than explicitly
25 examining nutrient mineralization during fish decomposition. Yet, decomposing fish can mineralize much of
26 their nutrient content quickly. For instance, Parmenter & Lamarra (1991) found that rainbow trout
27 (*Oncorhynchus mykiss*) carcasses decomposing in a Wyoming freshwater marsh lost 80% of their original
28 nitrogen (N) but only 50% of the original P content after 15 to 30 days, and 95% of their original N and 60% of
29 their original P after 10 months. They highlighted the fact that 40% of the total fish P remained immobilized in
30 bones and scales, and suggested fish carcasses can function as source of N but as a partial sink for P. Notably,

1 the long-term fate of bone and scale P is not known, and the P- mineralization rates in their study may have been
2 low due to cold water temperatures (4 – 14 °C in bottom waters where fish decomposed). Chidami & Amyot
3 (2008) also quantified decomposition rates of rainbow trout in a boreal lake (temperature 6 – 22 °C) and found
4 that decomposition rates were rapid in shallow and warm regions where carcasses were exposed to scavenging,
5 but slower in deep waters where decomposition was driven primarily by bacteria. The scarcity of data and the
6 variability among the results of previous studies on fish decomposition renders it difficult to draw overall
7 conclusions about the role of fish as nutrient sources or sinks.

8 Recent models exploring whether fish are P sources or sinks in lakes show that the net role of fish in nutrient
9 cycles depends on the rate at which fish carcass P is mineralized, the spatial scale of interest (i.e., whether fish
10 are sources or sinks for the benthos, water column, or entire ecosystem) and the fate of mineralized P (Vanni *et*
11 *al.*, 2013). To elucidate the fate of P during fish decomposition in freshwater ecosystems, we conducted an
12 outdoor mesocosm experiment that simulated massive summer fish kills in a warm-temperate shallow lake.
13 Moreover, we explored interspecific differences in P release rates and fates from decomposing carcasses using
14 carcasses of two fish species that differ in body P content (as % dry mass). We had two main hypotheses. First,
15 we hypothesized that a majority of fish carcass nutrients (from both species) would rapidly become available to
16 primary producers, and be quickly integrated into the food web, in warm, oxygenated water. Second, we
17 hypothesized that a greater fraction of body P would be mineralized from the low-P species (gizzard shad:
18 *Dorosoma cepedianum*) than carcasses of high-P species (bluegill: *Lepomis macrochirus*) and result in more
19 intense phytoplankton blooms. This prediction was based on the assumption that low-P species contain a greater
20 fraction of body P that is labile (e.g., as RNA or phospholipids) and a smaller fraction that is recalcitrant (i.e.,
21 bones and scales).

22

23 Methods

24 Experimental design

25 We conducted an outdoor, mesocosm experiment between 15 June and 11 September 2012 at the Ecology
26 Research Center of Miami University (Oxford, OH, USA). Each of the nine mesocosms (Fig. 1a) had a volume
27 of 5 m³, a bottom area of 4 m², and a water depth of 1.2 m. We added a 5 cm layer of dried sediment that was
28 collected from eutrophic Acton Lake (OH, USA) to the bottom of each tank, and filled them with 4.8 m³ of water
29 from a nearby mesotrophic pond. Water was circulated in the tanks with an air-lift mixing system (González,
30 Knoll & Vanni, 2010). This system prevented stratification and circulated the upper layer of the water column by

1 inducing slow currents, but did not pump air directly into the mesocosms. We applied three different treatments,
2 each replicated in three randomly selected mesocosms: bluegill carcasses (BG treatment), gizzard shad carcasses
3 (GS treatment) and control (no fish). To realistically simulate a massive fish kill, we assumed 500 kg ha⁻¹ fish
4 biomass and 80% mortality; thus we added ~400 kg ha⁻¹ (wet mass) of fish to the BG and GS treatments
5 (160–170 g fish per mesocosm). While this biomass is relatively high, it is within the range observed in nearby
6 eutrophic reservoirs (Hale *et al.*, 2008). We added 15–20 carcasses to BG tanks (wet mass of individual
7 carcasses: 8.9±4.4 g; total length: 75±13 mm) and two carcasses per GS tank (wet mass: 84.7±9.4 g; total length:
8 200±9 mm). Fish size was determined by availability in Acton Lake, where we collected fish for this experiment.
9 Carcass inputs were equal to 1.2 g P loading per mesocosm in the BG and GS treatments (Table 1), or 250 µg P
10 L⁻¹. To determine the amount of P we added in fish carcasses, we analyzed the body P contents of a sample of
11 fish collected at the same time and in the same manner as fish added to mesocosms (Table 1).
12 In the BG treatment, three bluegill carcasses per tank were placed in polystyrene weighing dishes (10 cm in
13 diameter and 3 cm depth), covered with mosquito netting (Fig. 1c), and embedded into the sediments. This
14 method was applied to help find and identify visible fish fragments at the end of the experiment. However, we
15 used these trays only on a subset of carcasses, because we were unsure if the fate of carcasses would be the same
16 inside and outside the trays. Gizzard shad carcasses were tagged with cable ties threaded through the opercular
17 opening (Fig. 1b) to facilitate finding carcass remains in sediments at the end of the experiment. Because we
18 added only two carcasses per tank in the GS treatment, we chose not to use the trays in this treatment.
19 One of our primary goals was to assess the availability and fates of fish carcass P in the mesocosms. Therefore,
20 we traced the flow of fish carcass P into the water column, the benthic organisms growing on the sides and
21 bottoms of the mesocosms and in the sediments.

22

23 Sampling

24 Water column N, P and chlorophyll-*a* were sampled weekly with a plastic tube sampler (5.5 cm diameter)
25 lowered into the water column. Water column chlorophyll-*a* was quantified as a measure of the bioavailability of
26 carcass nutrients and to gauge the response of phytoplankton to carcass decomposition. Temperature and
27 dissolved oxygen were also monitored weekly 30–40 cm above the sediment surface, using a YSI Pro20 oxygen
28 meter (YSI Inc., OH, USA), to determine if temperature was similar among treatments and to see if fish
29 decomposition occurred in an oxic or anoxic environment.

1 To estimate the flux of P from carcasses to other ecosystem compartments, we quantified total P in the water
2 column, on mesocosm walls, in benthic algae and in the sediments. To assess P flux to mesocosm walls, plastic
3 strips (5.5 × 8.5 cm) were fixed on the walls at three depths and in all four cardinal directions (12 strips per
4 mesocosm) to assess P uptake via biofilm growth during the course of the experiment. At the final sampling in
5 September, the plastic strips were collected, biofilm was scraped off and the total biomass of biofilm on strips
6 was calculated and extrapolated to the entire inner wall surface (8.48 m²) of mesocosms. Subsamples of biofilm
7 taken at the final sampling were used to estimate P content. To assess P flux to benthic algae, three samples were
8 collected from random locations in each tank with a plastic tube sampler (15 cm diameter); the measured
9 biomasses were averaged and converted to the bottom area of the mesocosms (4 m²) by extrapolating coverage
10 (%) in samples to total biomasses of benthic algae per mesocosm. To quantify P flux to sediments, three samples
11 of the entire sediment depth (excluding benthic algae on the sediment surface) were collected with the same tube
12 sampler mentioned above, prior to the experiment's start in June and at the final sampling to quantify initial and
13 final sediment P pools, to explore changes in chemical composition and to search for visible fish remnants or
14 scavengers. In addition, in GS treatments, we collected sediment samples from locations where fish decomposed
15 (identified by the presence of cable ties; Fig. 1b) and those not near carcasses, so we could compare P
16 concentrations in sediments in those locations. At the end of the experiment, polystyrene weighing dishes
17 containing bluegill carcasses (Fig. 1c) were collected from the bottoms of BG mesocosms to inspect if they
18 contained any visible fish remnants or if all fish-derived material decomposed during the experimental period.

19

20 Sample processing and analytical methods

21 To estimate initial fish body (carcass) P content and P input to the mesocosms, whole carcasses were dried to a
22 constant weight at 60°C, then coarsely ground with a coffee mill and finally ground to a fine powder with a
23 Retsch ZM100 centrifugal mill (Retsch GmbH, Germany). Sediment, benthic algae and biofilm samples used to
24 estimate P pools were ground with a mortar and pestle prior to P-content analyses. P concentrations were
25 measured with a Lachat QC 8000 FIA autoanalyzer (Lachat Instruments, CO, USA) following the digestion of
26 samples at 550 °C and 1N HCl solution (e.g. Vanni *et al.*, 2002; 2011). Water column total N and P was also
27 measured with the auto-analyzer, following potassium persulphate digestion. Chlorophyll-*a* was measured with a
28 fluorometer following extraction in ethanol.

29

30 Statistical methods

1 Differences in temperature, dissolved oxygen, total P and chlorophyll-*a* content of the water column within and
2 among treatments were analyzed by using one-way ANOVA tests combined with Tukey's pairwise comparisons.
3 These comparisons were done on each sampling date because we wished to know if and when carcass-derived
4 nutrients could be detected in the water column. Kruskal-Wallis tests and one-way ANOVA tests were used to
5 reveal the differences between treatments in biomass and coverage of benthic algae, biomass of biofilm and total
6 P content of sediment samples. Statistical analyses were performed using PAST 2.17 (PAleontological
7 STatistics, Norway), and decisions about the statistical significances were set at $P=0.05$ level.

8

9 Results

10 Both water temperature and dissolved oxygen exhibited similar dynamics in all treatments during the course of
11 the experiment and significant differences were not observed among treatments (ANOVA, Tukey's post-hoc
12 test; $P > 0.05$ on all dates). Water temperature varied between 18.7 and 29.3 °C (avg. \pm SD; 24.5 ± 2.9 °C), while
13 dissolved oxygen ranged from 7.5 to 10.2 mg L⁻¹ (Table 2).

14 Total phosphorus (TP) in the water column did not differ significantly ($P > 0.05$) among treatments on the first
15 sampling date (44.7 ± 7.2 µg L⁻¹ in the mesocosms) prior to fish carcasses being placed in the mesocosms.
16 However, after one week, TP was significantly higher in treatments with fish carcasses, increasing by ~50 µg L⁻¹
17 during this period in each mesocosm; this corresponds to 20% of the P added as carcasses. Peak values of TP
18 were observed one week after carcass addition: 95.8 ± 7.6 µg P L⁻¹ in BG treatment and 98.6 ± 5 in GS treatment,
19 more than 2.5-times greater than the control (38.3 ± 8.0) on the same sample date. Total P levels were
20 significantly greater in the mesocosms containing fish carcasses for two more weeks, but by week 5 there were
21 no further differences among treatments (Fig. 2a). There was no significant difference in TP between the two
22 fish species treatments throughout the experiment (Fig. 2a). Total nitrogen (TN) in the water column exhibited a
23 similar pattern, peaking one week after carcass addition (939.0 ± 70.3 and 865.4 ± 55.9 µg L⁻¹, respectively) in
24 both the BG and GS treatments. The high-TN period persisted for three weeks, and after week 5, there were no
25 significant differences between treatments except for the last sampling date when TN increased markedly in the
26 GS treatment (Fig. 2b).

27 Chlorophyll-*a* dynamics in the water column were similar to those of TP and TN; chlorophyll concentration was
28 the greatest one week after carcass additions in treatments with fish (41.9 ± 12.04 µg L⁻¹ and 50.3 ± 10.4 µg L⁻¹ in
29 BG and GS mesocosms, respectively). These values were 5.5–6.5-times higher than those of the controls at the
30 same time (Fig. 2c). The highest mean chlorophyll-*a* levels were detected in the GS treatment, but the difference

1 was significantly greater than the BG treatment only in week 2. From week 4 on, there were no significant
2 differences in chlorophyll-*a* among any treatments (Fig. 2c).

3 By the end of the experiment, the bottoms of all mesocosms were covered with benthic algae to various extents
4 (Fig. 1d; Fig. 3a,b). Submerged macrophytes did not develop in the mesocosms, except in one of the control
5 tanks where a shoot of *Myriophyllum* sp. was found (this was included in the pool of benthic algae). Biomass of
6 benthic algae (dry mass) varied between 108 and 507 g m⁻², with coverage between 50–100 % (Fig. 3a,b).
7 Biofilm growth occurred on the walls of all tanks (Fig. 1 d; Fig. 3c). It consisted mainly of ball-shaped “jelly”
8 bubbles formed by cyanobacteria (*Rivularia* sp.) embedded in a gelatinous matrix. The wet mass of this
9 periphytic biofilm was relatively high (between 0.18–1.8 kg m⁻²), but due to its very low P content and high
10 water content (98.1±0.9 %), it did not constitute a large P pool (Tables 1 & 3). The BG treatment had the lowest
11 biofilm mass (Fig. 3c), which differed significantly from the other two treatments ($P < 0.05$ in both cases).
12 Biofilm dry mass did not differ significantly between GS and control treatments ($P=0.44$).

13 P content of sediments at the end of the experiment did not differ significantly among treatments ($P > 0.05$,
14 Kruskal-Wallis tests; Fig. 4a). Furthermore, P content of sediments taken from locations where fish decomposed
15 and from other, randomly selected locations in the GS tanks did not differ ($P > 0.05$; Fig. 4b). All sediment
16 samples taken at the end of the experiment were visually analyzed for carcass remnants and scavengers. We
17 could not detect any fish remnants in sediments; thus virtually all carcasses decomposed completely during the
18 experimental period. Notably, we did not find any potential scavengers in the sediment samples. Contents of
19 polystyrene weighing dishes from the BG treatment were visually analyzed and fish remnants were not found in
20 them; the weighing dishes contained only settled detritus.

21 To better evaluate the fate of carcass P, we constructed P budgets for the mesocosms (Table 3). Sediments were
22 by far the largest P pool in the mesocosms (> 98% of total P mass in all mesocosms). We observed a slight
23 increase in the total mass of P in the mesocosms (mostly in sediments); however, the difference between the
24 initial and final P pools was less than 2.3% in all cases, and initial and final total mesocosm P mass did not differ
25 significantly among treatments ($P > 0.05$ in all cases).

26

27 Discussion

28 Our results demonstrated that nutrients liberated from the decomposing carcasses of fish can stimulate
29 phytoplankton blooms and high TP and TN concentrations in the water column. Total P increased considerably
30 in the water column one week after fish addition and corresponded to the remineralization of 20% of the P added

1 as carcasses, suggesting that a large fraction of carcass P was released directly into the water column. The
2 intensive algal bloom that occurred in both of the fish treatments lasted only 2–3 weeks, and correlated with high
3 water-column P. Similar results were obtained by Durbin *et al.* (1979) who also reported a large, but short-lived,
4 phytoplankton bloom after carcass addition in a mesocosm experiment. These dynamics suggest that under these
5 conditions, a significant fraction of the P stored in fish carcasses becomes available to primary producers within
6 a relatively short time.

7 TN exhibited a trend similar to TP, with a remarkable increase one week after carcass addition, and with
8 significantly higher TN values in the BG and GS mesocosms for two more weeks. Parmenter & Lamarra (1991)
9 reported that 80% of the original N content of fish carcasses was released within one month, presumably because
10 the vast majority of body-N content is stored in soft tissues such as muscle (Pangle & Sutton 2005; Vrede *et al.*
11 2011) that decompose and release nutrients relatively rapidly. Interpreting N dynamics using budgets is more
12 problematic than for P because of processes such as N fixation, nitrification and denitrification. Nevertheless, we
13 can presume that decomposition-derived nutrients elevated N levels after carcass addition.

14 The decrease in water column P and associated increase in benthic algal P show that P taken up in the initial
15 phytoplankton bloom became unavailable to water-column organisms in a relatively limited period of time.
16 Dissolved oxygen levels were consistently high ($> 7.5 \text{ mg L}^{-1}$) above the sediment surface in all mesocosms
17 throughout the experiment (Table 2), and we presume that these conditions in the overlying water might have
18 kept redox potentials high enough in sediments to retain P permanently (i.e., anoxia and low redox potentials in
19 sediments are not likely to occur when the overlying water is saturated with oxygen) (Boström, Jansson &
20 Forsberg, 1982). Thus, redox-dependent P retention in sediments might have played an important role in
21 reducing P and consequently chlorophyll levels in the water column. There was no significant difference in
22 sediment P content across treatments, but this is probably because the amount of P added as fish carcasses was
23 very small compared to the size of the sediment pool (Table 3). In addition, a considerable mass of benthic algae
24 had started to develop in all mesocosms 2–3 weeks after the experiment's start, which sequestered P and
25 facilitated P retention in sediments by oxygenating the upper sediment layers and consequently by raising redox
26 potential (Scheffer, 1998; Dodds, 2003; Zhang *et al.*, 2013).

27 We recognize that the distribution of habitats (water column, sediments, vertical benthic structures) in our
28 mesocosms differs from that in a lake and that decomposition in our mesocosms occurred in absence of
29 scavengers. Thus, in an actual lake ecosystem, the distribution of carcass-derived nutrients into various
30 ecosystem compartments may differ from what we observed, and will be strongly influenced by factors such as

1 ecosystem size and productivity (e.g., the relative areas and productivity of littoral vs. pelagic regions). However,
2 we can infer that any P transferred from carcasses to the pelagic zone or to mesocosms walls must have first
3 been converted to a bioavailable (presumably dissolved inorganic) form. Thus, in an actual lake ecosystem, with
4 similar conditions, these nutrients would be available to benthic and/or pelagic primary producers and carcasses
5 would not represent nutrient sinks. Thus, it is potentially instructive to discuss the factors that may have
6 influenced P fluxes among compartments.

7 Both average biomass and coverage of benthic algae tended to be higher in treatments containing fish carcasses,
8 although the only significant difference occurred in coverage between BG and the control treatments (Fig. 3b).
9 Biofilm growth on the inner walls of mesocosms was similar in GS and control treatments, and was considerably
10 lower in BG treatment. However, because the biofilm was a relatively small P pool (Table 3), we assume that it
11 had negligible effect on phytoplankton via nutrient competition. In lakes, competition for P between benthic
12 algae and phytoplankton influence the fate of carcass P. For example, if benthic primary producers account for a
13 large fraction of total primary production (as might occur in small shallow lakes, or in clear lakes; Vadeboncoeur
14 *et al.*, 2008), a large fraction of carcass P may be redistributed to benthic producers, particularly if they are good
15 P competitors compared to phytoplankton. Alternatively, in deep lakes, profundal scavengers could sequester
16 much of the carcass P; the subsequent fate of this P may depend on the extent to which these scavengers move P
17 to other habitats and/or are consumed by mobile consumers such as fish.

18 Using two fish species enabled us to study interspecific differences in decomposition rates and associated P
19 fluxes. Even though bluegill and gizzard shad differ in P content (Torres & Vanni, 2007; Table 1), probably due
20 to their differing anatomical features (Hendrixson *et al.*, 2007), we did not observe large differences in TP levels
21 in the water column, sediments or other pools between treatments containing the two different fish species.
22 Treatments differed significantly in peak phytoplankton (chlorophyll-*a*) concentrations (highest in GS
23 mesocosms) (Fig. 2) and in the coverage of benthic algae (higher in BG mesocosms than in the controls) (Fig. 3).
24 The amount of carcass P input was nearly equal in both fish treatments, and we expected that gizzard shad
25 carcasses may trigger higher P levels in the water column as they are less “bony” than bluegills, and
26 consequently a higher proportion of body P of gizzard shad could be liberated more effectively and rapidly. This
27 assumption was partially supported by the chlorophyll results, although a difference in water column TP levels
28 was not detectable between BG and GS treatments (Fig. 2). On the other hand, decomposition of bluegill
29 carcasses promoted the development of the highest coverage of benthic algae, indicating some variance in
30 ecosystem responses to the decomposition of different species. While the difference in body P between our two

1 species (3.1 vs. 2.6% of dry mass) may seem relatively small to elicit different ecosystem responses, it could
2 easily have resulted in large differences in P dynamics. For example, if we assume that non-bone P comprises
3 0.9% of dry mass (Hendrixson *et al.*, 2007), the amount of non-bone P added to mesocosms in the GS and BG
4 treatments would have been 87 and 73 $\mu\text{g P L}^{-1}$ (the mass of bone-P would have been 163 and 177 $\mu\text{g P L}^{-1}$,
5 respectively). Thus, substantially more non-bone P was added in the GS treatment than in the BG treatment,
6 expressed per volume of mesocosm water, yet water column TP dynamics were indistinguishable in the two
7 treatments. These results, in conjunction with the apparently complete decomposition of carcasses, suggests that,
8 under the conditions of our experiment, fish species identity as determined by body P content does not matter in
9 terms of the fate of carcass P.

10 One of our primary goals was to assess whether fish carcasses decompose completely and release all sequestered
11 P, or if a fraction of body P remains immobilized and buried in sediments permanently. We find contradictory
12 results in the literature concerning fish decomposition; for example, Claeson *et al.* (2006) found that inner body
13 tissues of salmon decomposed completely after two months in the Wind River (WA, USA), (mean water
14 temperature between 11.4 – 12.8°C; minimum and maximum 8.9°C and 15.4°C), but some bone and skin
15 remained intact in the long term (they did not measure nutrients in carcasses). Similarly, Premke *et al.* (2010)
16 reported total decomposition of fish carcasses after 2–3 months in the pre-alpine Lake Constance, Germany
17 (water temperature ranged between 4 – 25 °C). In contrast, Kitchell *et al.* (1975) and Parmenter & Lamarra
18 (1991) showed that 40–50% of fish carcass P remained immobilized in recalcitrant materials and was stored in
19 sediments, excluded from the active P pool of the ecosystem, at least in the short term. However, Kitchell *et al.*
20 (1975) recognized that this portion of carcass P can be remineralized slowly over longer periods. Based on these
21 studies, we hypothesized that fish decomposition would be relatively fast in our mesocosms due to the elevated
22 water temperature (24.5 °C on average, and much higher earlier in the experiment), but that some recalcitrant,
23 fish-derived material would act as a "permanent" P sink.

24 Contrary to our expectations, there was no evidence for the long-term retention of fish-derived P in sediment, as
25 the TP content of different sediment samples (locations under carcasses vs. random locations) did not differ
26 significantly. Furthermore, the sediment samples did not contain any visible fish remnants, suggesting that
27 decomposition was complete in our mesocosms. Finally, bluegills in the weighing dishes apparently decomposed
28 completely during the course of the experiment; there were no detectable remains in the dishes and we did not
29 find any scavengers that could have removed carcass fragments. Thus, our study provides evidence that even in
30 the apparent absence of scavengers, P stored in fallen fish carcasses can re-enter internal nutrient-cycling

1 pathways with high efficiency, if environmental conditions are favorable. This finding is important because
2 recent models (Vanni *et al.*, 2013) show that the rate at which fish carcasses decompose and liberate P is critical
3 in determining their net role in nutrient cycles, i.e., whether they are sources or sinks of P at the scale of the
4 ecosystem (e.g., lake) or habitat (e.g., benthic or pelagic zones). We conclude that fish are much less likely to
5 function as P sinks in shallow, warm-temperate lakes than in deeper and/or colder lakes where a significant
6 fraction of fish-derived P can be lost permanently from the available nutrient pool by incomplete decomposition
7 (Kitchell *et al.*, 1975; Parmenter & Lamarra, 1991). However, even mass mortality events occurring in winter
8 can increase water column P and stimulate phytoplankton biomass the following spring, presumably because
9 carcasses decompose rapidly when temperatures warm (Schoenebeck *et al.*, 2012).

10 Deeper insight into the process of P release from decomposing fish carcasses is essential to better understand the
11 complex role of fish in aquatic ecosystems. However, as Chidami & Amyot (2008) pointed out, the fate of fish
12 carcasses after deposition at the sediment surface is still poorly documented. Our study revealed that massive
13 fish kills can induce fleeting algal blooms in the water column of shallow lakes, and that fish species identity
14 only slightly influences the effects. Massive fish mortality may have only temporary impacts on water quality,
15 and after a few weeks of transition, the effects of such a large, pulsed nutrient input may dissipate, at least in
16 terms of water column nutrients. Some authors have concluded that fish biomass can constitute a considerable P
17 sink because fish are long-lived relative to other aquatic organisms (e.g., Griffiths, 2006; Sereda *et al.*, 2008) and
18 a significant fraction of body P can remain immobilized postmortem in heavily recalcitrant materials and buried
19 in sediments (Parmenter & Lamarra, 1991). Yet, for fish to function as true nutrient sinks, the storage of
20 nutrients in fish biomass over time must represent a permanent net reduction of the nutrients available to other
21 organisms (Vanni *et al.*, 2013). Our results cast doubt on the idea that fish carcasses act as permanent P sinks in
22 shallow lakes with higher water temperature; such lakes are abundant in both temperate and tropical regions. If
23 live fish biomass remains relatively constant, and if carcasses fully decompose and liberate all of their P (even if
24 this occurs slowly), fish are not likely to function as ecosystem-level P sinks. The variable results regarding fish
25 decomposition draw attention to the importance of context-dependent approaches when assessing the function of
26 fish as sinks or sources of nutrients, and indicate a need for additional experimental and whole-ecosystem
27 studies.

28

29 Acknowledgements

1 The Rosztochy Foundation supported Gergely Boros by providing a postdoctoral fellowship to conduct research
2 at Miami University. We acknowledge the support of NSF grant DEB 0743192. We thank E. Mette, T. Ratliff, L.
3 P. Leon, Z. Alley and A. Morgan for assistance in the field and lab, and Ecology Research Center staff for
4 logistical support.

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6 References

- 7 Boström B., Jansson M. & Forsberg C. (1982) Phosphorus release from lake sediments. *Archiv für*
8 *Hydrobiologie – Beiheft Ergebnisse der Limnologie*, **18**, 5–59.
- 9 Bretherton W. D., Kominoski J. S., Fischer D. G. & LeRoy C. J. (2011) Salmon carcasses alter leaf litter species
10 diversity effects on in-stream decomposition. *Canadian Journal of Fisheries and Aquatic Sciences*, **68**, 1495–
11 1506.
- 12 Cederholm C. J., Kunze M. D., Murota T. & Sibatani A. (1999) Pacific salmon carcasses: Essential contributions
13 of nutrients and energy for aquatic and terrestrial ecosystems. *Fisheries*, **24**(10), 6–15.
- 14 Chidami S. & Amyot M. (2008) Fish decomposition in boreal lakes and biogeochemical implications. *Limnology*
15 *and Oceanography*, **53**(5), 1988–1996.
- 16 Claeson S. M., Li J. L., Compton J. E. & Bisson P. A. (2006) Response of nutrients, biofilm, and benthic insects
17 to salmon carcass addition. *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 1230–1241.
- 18 Czamanski M., Nugraha A., Pondaven P., Lasbleiz M., Masson A., Caroff N., Bellail R. & Tréguer P. (2011)
19 Carbon, nitrogen and phosphorus elemental stoichiometry in aquacultured and wild-caught fish and
20 consequences for pelagic nutrient dynamics. *Marine Biology*, **158**, 2847–2862.
- 21 Dodds W. K. (2003) The role of periphyton in phosphorus retention in shallow freshwater aquatic systems.
22 *Journal of Phycology*, **39**, 840–849.
- 23 Durbin A. G., Nixon S. W. & Oviatt C. A. (1979) Effects of the spawning migration of the alewife, *Alosa*
24 *pseudoharengus*, on freshwater ecosystems. *Ecology*, **60**(1), 8 – 17.
- 25 González M. J., Knoll L. B. & Vanni M. J. (2010) Differential effects of elevated nutrient and sediment inputs
26 on survival, growth and biomass of a common larval fish species (*Dorosoma cepedianum*). *Freshwater Biology*,
27 **55**, 654–669.
- 28 Griffiths D. (2006) The direct contribution of fish to lake phosphorus cycles. *Ecology of Freshwater Fish*, **15**,
29 86–95.

- 1 Hale R. S., Degan D. J., Renwick W. H., Vanni M. J. & Stein R. A. (2008) Assessing fish biomass and prey
2 availability in Ohio reservoirs. *American Fisheries Society Symposium*, **62**, 517–541.
- 3 Hendrixson H. A., Sterner R. W. & Kay A. D. (2007) Elemental stoichiometry of freshwater fishes in relation to
4 phylogeny, allometry and ecology. *Journal of Fish Biology*, **70**, 121–140.
- 5 Kitchell J. F., Koonce J. F. & Tennis P. S. (1975) Phosphorus flux through fishes. *Verhandlungen des*
6 *Internationalen Verein Limnologie*, **19**, 2478–2484.
- 7 Lewis W. M. & Wurtsbaugh, W. A. (2008) Control of lacustrine phytoplankton by nutrients: Erosion of the
8 phosphorus paradigm. *International Review of Hydrobiology*, **93**, 446–465.
- 9 McIntyre P. B., Flecker A. S., Vanni M. J., Hood J. M., Taylor B. W. & Thomas S. A. (2008) Fish distributions
10 and nutrient cycling in streams: Can fish create biogeochemical hotspots? *Ecology*, **89**, 2335–2346.
- 11 Nakashima B. S. & Legett W. C. (1980) The role of fishes in the regulation of phosphorus availability in lakes.
12 *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 1540–1549.
- 13 Pangle K. L. & Sutton T. M. (2005) Temporal changes in the relationship between condition indices and
14 proximate composition of juvenile *Coregonus artedii*. *Journal of Fish Biology*, **66**, 1060–1072.
- 15 Parmenter R. R. & Lamarra V. A. (1991) Nutrient cycling in a freshwater marsh: The decomposition of fish and
16 waterfowl carrion. *Limnology and Oceanography*, **35**(5), 976–987.
- 17 Premke K., Fischer P., Hempel M. & Rothhaupt K. O. (2010) Ecological studies on the decomposition rate of
18 fish carcasses by benthic organisms in the littoral zone of Lake Constance, Germany. *Annales de Limnologie–*
19 *International Journal of Limnology*, **46**, 157–168.
- 20 Rønsholdt B. (1995) Effect of size/age and feed composition on body composition and phosphorus content of
21 rainbow trout *Oncorhynchus mykiss*. *Water Science and Technology*, **31**, 175–183.
- 22 Sarvala J. & Jumppanen K. (1988) Nutrients and planktivorous fish as regulators of productivity in Lake
23 Pyhäjärvi, SW Finland. *Aqua Fennica*, **18**, 137–155.
- 24 Scheffer, M. (1998) *Ecology of Shallow Lakes*. Chapman & Hall, UK
- 25 Schenau S. J. & De Lange G. J. (2000) A novel chemical method to quantify fish debris in marine sediments.
26 *Limnology and Oceanography*, **45**(4), 963–971.
- 27 Schindler D. W. (1977) Evolution of phosphorus limitation in lakes: natural mechanisms compensate for
28 deficiencies of nitrogen and carbon in eutrophied lakes. *Science*, **195**, 260–262.
- 29 Schneider J. C. (1998) Fate of dead fish in a small lake. *The American Midland Naturalist*, **140**(1), 192–196.

1 Schoenebeck C. W., Brown M. L., Chippss S. R. & German D. R. (2012) Nutrient and algal responses to
2 winterkilled fish-derived nutrient subsidies in eutrophic lakes. *Lake and Reservoir Management*, **28**(3), 189–
3 199.

4 Schuldt J. A. & Hershey A. E. (1995) Effect of salmon carcass decomposition on Lake Superior tributary
5 streams. *Journal of the North American Benthological Society*, **14**(2), 259–268.

6 Sereda J. M., Hudson J. J., Taylor W. D. & Demers E. (2008) Fish as sources and sinks of nutrients in lakes.
7 *Freshwater Biology*, **53**, 278–289.

8 Sereda J. M. & Hudson J. J. (2010) Comparative estimate of P fluxes in lakes: A comment on “Fish
9 decomposition in boreal lakes and biogeochemical implications” by Chidami and Amyot (2008). *Limnology and*
10 *Oceanography*, **55**(1), 463–465.

11 Torres L. E. & Vanni M. J. (2007) Stoichiometry of nutrient excretion by fish: Interspecific variation in a
12 hypereutrophic lake. *Oikos*, **116**, 259–270.

13 Trueman C. N. & Martill D. M. (2002) The long-term survival of bone: the role of bioerosion. *Archaeometry*,
14 **44**, 371–382.

15 Vadeboncoeur Y., Peterson G., Vander Zanden M. J. & Kalff, J. (2008) Benthic algal production across lake size
16 gradients: interactions among morphometry, nutrients, and light. *Ecology*, **89**, 2542–2552.

17 Vanni M. J. (2002) Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology, Evolution*
18 *and Systematics*, **33**, 341–370.

19 Vanni M. J., Flecker A. S., Hood J. M. & Headworth J. L. (2002) Stoichiometry of nutrient cycling by
20 vertebrates in a tropical stream: Linking species identity and ecosystem processes. *Ecology Letters*, **5**, 285–293.

21 Vanni M. J., Renwick W. H., Bowling A. M., Horgan M. J. & Christian A. D. (2011) Nutrient stoichiometry of
22 linked catchment–lake systems along a gradient of watershed land use. *Freshwater Biology*, **56**(5), 791–811.

23 Vanni M. J., Boros G. & McIntyre P. B. (2013) When are fish sources versus sinks of nutrients in lake
24 ecosystems? *Ecology*, **94**(10), 2195–2206.

25 Vrede T., Drakare S., Eklöv P., Hein A., Liess A., Olsson J., Persson J., Quevedo M., Stabo R. & Svenback R.
26 (2011) Ecological stoichiometry of Eurasian perch—intraspecific variation due to size, habitat and diet. *Oikos*,
27 **120**, 886–896.

28 Zhang X., Liu Z., Gulati R. D. & Jeppesen E. (2013) The effect of benthic algae on phosphorus exchange
29 between sediment and overlying water in shallow lakes: a microcosm study using ³²P as a tracer. *Hydrobiologia*,
30 **710**, 109–116.

1 Zimmer K. D., Herwig B. R. & Laurich L. M. (2006) Nutrient excretion by fish in wetland ecosystems and its
2 potential to support algal production. *Limnology and Oceanography*, **51**(1), 197–207.

3

1 Tables

2

3 Table 1: Fish-derived phosphorus loading and the phosphorus content of periphytic biofilm and benthic algae in
4 the different treatments (avg. \pm SD).

5

	Bluegill	Gizzard shad	Control
P content of stocked carcasses, dry mass %	3.1 \pm 0.6	2.6 \pm 0.8	N/A
P loading/mesocosm, g	1.2 \pm 0.02	1.2 \pm 0.02	N/A
P content of biofilm, dry mass %	8.6 $\times 10^{-4}$ $\pm 2.1 \times 10^{-4}$	8.3 $\times 10^{-4} \pm 1.3 \times 10^{-4}$	5.6 $\times 10^{-4} \pm 9.6 \times 10^{-5}$
P content of benthic algae, dry mass %	1.3 $\times 10^{-1}$ $\pm 8.1 \times 10^{-4}$	1.5 $\times 10^{-1} \pm 4.2 \times 10^{-2}$	1.5 $\times 10^{-1} \pm 4.8 \times 10^{-2}$

6

7

8 Table 2: Temporal changes in dissolved oxygen and water temperature in the mesocosms during the
9 experimental period. Measurements were taken 30–40 cm above the sediment surface.

	Sampling							
	week	1	2	3	4	5	6	7
Dissolved oxygen, mg L ⁻¹	Average	10.2	8.2	7.6	7.5	8.0	7.7	7.9
	\pm SD	0.1	0.1	0.1	0.0	0.0	0.1	0.1
	Sampling							
	week	8	9	10	11	12	13	14
	Average	8.6	8.2	8.2	8.2	9.0	8.4	9.1
	\pm SD	0.3	0.2	0.0	0.6	0.1	0.1	0.0

	Sampling							
	week	1	2	3	4	5	6	7
Temperature, °C	Average	22.7	26.2	27.0	29.3	26.9	26.3	27.3
	± SD	0.0	0.0	0.1	0.01	0.1	0.1	0.2
	Sampling							
	week	8	9	10	11	12	13	14
	Average	26.4	23.4	22.2	21.0	24.2	23.8	18.7
	± SD	0.1	0.4	0.1	0.0	0.01	0.0	0.1

1

2

3 Table 3: Phosphorus budgets at the beginning and end of the experiment (mean ± SD).

4

	Bluegill		
	treatment	Gizzard shad treatment	Control
Initial P pool (g)			
Water column	0.21±0.02	0.21±0.02	0.21±0.02
Sediment	139.47±0.00	139.47±0.00	139.47±0.00
+ Fish	1.25±0.02	1.21±0.02	—
Sum	140.93±0.02	140.89±0.02	139.69±0.00
Final P pool (g)			
Water column	0.09±0.04	0.08±0.02	0.08±0.02
Sediment	140.28±3.14	142.40±2.85	141.35±2.93
Benthic algae	1.43±1.09	1.63±0.32	1.11±0.59
Biofilm	$2.7 \times 10^{-4} \pm 1.7 \times 10^{-4}$	$1.2 \times 10^{-3} \pm 4.1 \times 10^{-4}$	$6.2 \times 10^{-4} \pm 4.3 \times 10^{-4}$
Sum	141.80±2.46	144.11±2.57	142.54±2.51
*P surplus (g)	0.87±2.48	3.22±2.58	2.86±2.51
**P surplus (% difference)	0.62	2.28	2.00

1 * final minus initial P mass

2 ** % increase relative to P mass

3

4

5 Figure legends

6

7 Figure 1. Outdoor mesocosms at the Ecology Research Center, Miami University. (a) The array of mesocosms
8 showing the deck used for sampling. (b) Gizzard shad were marked with cable ties threaded through the
9 opercular opening, used to facilitate locating carcass remnants at the end of the experiment. (c) Bluegills in
10 polystyrene weighing dishes prior to lowering into the sediment (rocks were included to hold dishes in place).
11 (d) Considerable biomass of benthic algae and biofilm covered the mesocosms by the end of the experiment.

12

13 Figure 2. Time-courses of water column total phosphorus (TP), total nitrogen (TN) and chlorophyll-*a* during the
14 experiment. Letters above the columns denote the significance of differences between treatments: **a**, no
15 significant difference between treatments ($P > 0.05$); **b**, control differs significantly from the other two
16 treatments; bluegill and gizzard shad treatments do not differ significantly; **c**, gizzard shad treatment differs
17 significantly from the other two treatments; bluegill and control treatments do not differ significantly; **d**,
18 significant differences between all treatments.

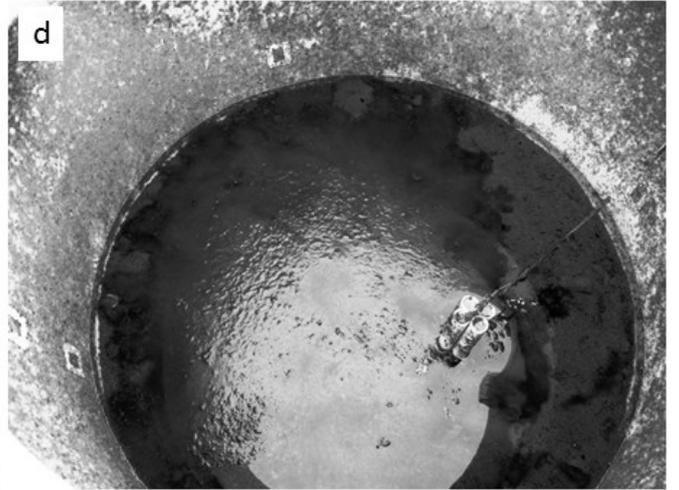
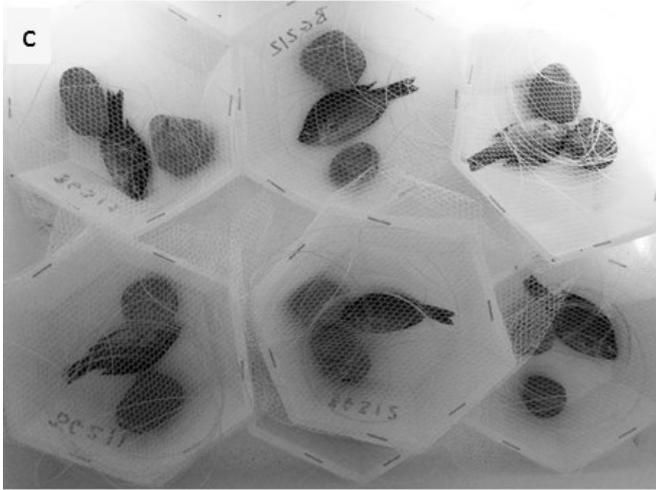
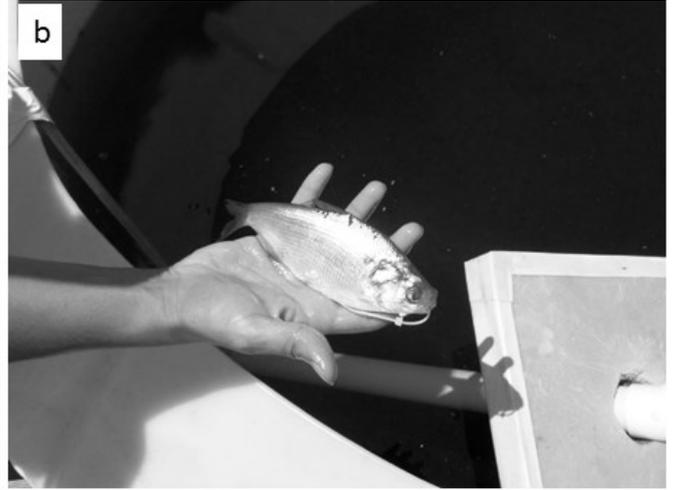
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20 Figure 3. Biomass and coverage of benthic algae, and biomass of biofilm in the three different treatments at the
21 end of the experiment (BG – bluegill treatment; GS – gizzard shad treatment; C – control). Lower case letters
22 above the columns denote the similarities and significant differences between treatments (treatments denoted
23 with the same letter do not differ significantly; $P \geq 0.05$), while whiskers denote minimum and maximum values.

24

25 Figure 4. (a) Total phosphorus (TP) content of sediments in the three different treatments at end of the
26 experiment, and (b) in the gizzard shad tanks where sediment cores from random locations were compared to
27 locations where fish carcasses decomposed. The box represents the 25 and 75 % quartiles, while the band in the
28 box denotes the mean. The whiskers represent the highest and lowest values.

29



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2 Figure 1

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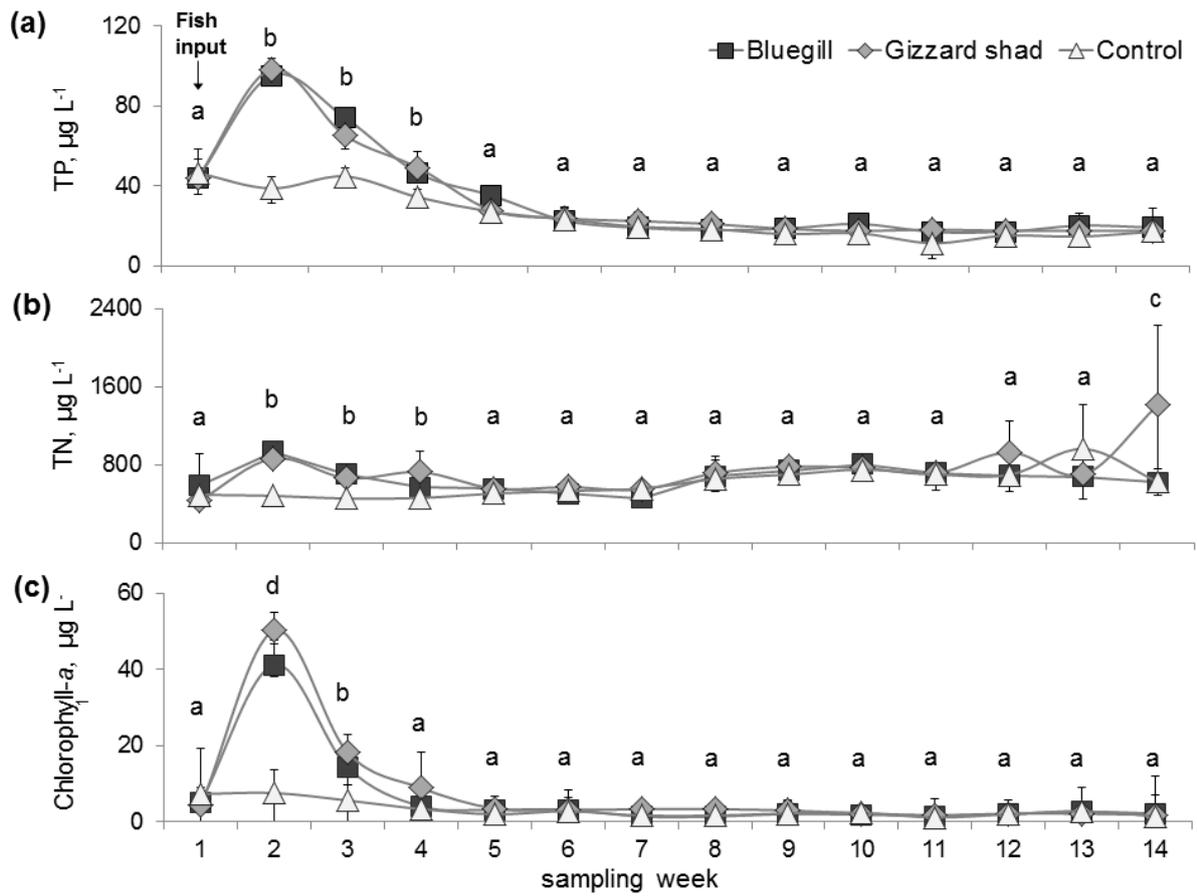
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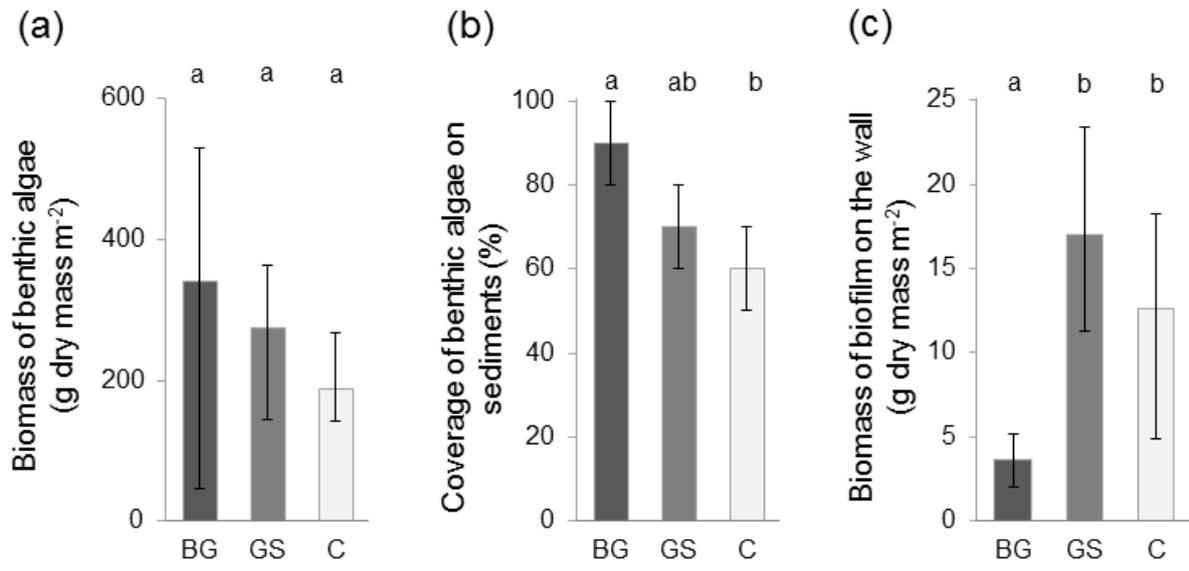
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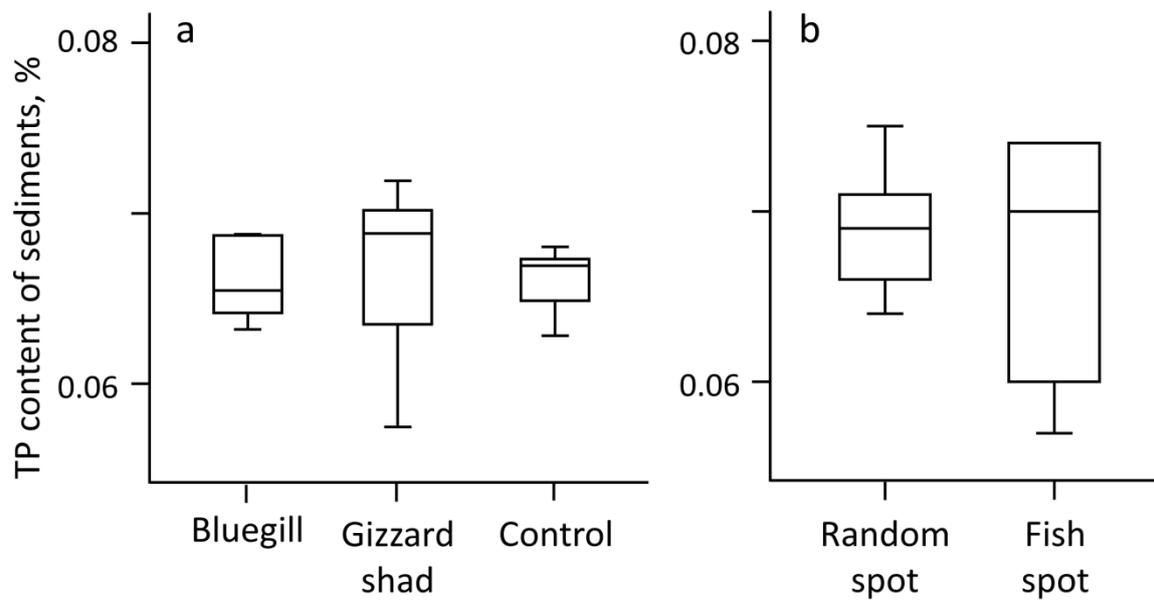


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Figure 2



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Figure 4