Review of HYDR-D-21-00317: Foraging traits predict stage-structured feeding by freshwater fishes

I am certain that there is likely a paper that can be published on the diet data collected by the authors, however, I have major reservations about the study put forth in this manuscript. My major concerns are:

1. The authors do not clearly explain in the abstract, introduction, and most of the methods that they are distinguishing adult and pupal life stages from larval based on whether or not they feed in aquatic habitats. Overall, I found the authors’ classification of life stages into “consumer” and “non-consumer” categories to be very misleading and not well supported. Furthermore, it was unclear if and how vertebrate and zooplankton taxa were included in the analyses as these taxa do not fit easily into the conceptual framework of the authors’ objectives, but were nonetheless important fractions of some fish species’ diets (Figure S5).
2. The authors have not correctly categorized several of the fishes in this study into their proper foraging domains. Some of these improper categorizations involve species that constitute a large fraction of their sample size, and therefore, call into question the analytical results.
3. If benthic fishes are not expected to consume “non-consumer” life stages, why would this dynamic be expected to explain weak predatory effects of fish on aquatic invertebrate communities when benthic fishes are often abundant and widely distributed in these types of ecosystems?
4. The authors invoke niche partitioning and propose alternative measures of diet overlap but offer no analyses of diet overlap in their own study.

More detailed comments and editorial suggestions are offered below:

Abstract

Lines 30 – 32: The first sentence is awkwardly worded.- divided sentence in two and reworded to make it less “awkward.”

Line 33: It is not clear what is meant by “how fish consume”. Be more specific.- Sentence reworked for clarification. Now reads, “These fish traits (e.g., propensity for surface feeding), may also alter fish consumption of the remaining…”

Line 34: What is referred to by “this is potentially ecologically important”?- Restructured to, “Since aquatic insects alter their feeding at later developmental stages (pupae and adults), their consumption at differing life stages may have ecological relevance.”

Lines 34 – 35: Not all aquatic insects cease feeding at the adult life stage (e.g., dragonflies, damselflies, mosquitos, black flies, predaceous diving beetles, etc.). Are the authors referring to taxa that do not forage in aquatic environments as adults? If so, this detail needs to be clearer because it is patently incorrect to state that aquatic insects do not feed during their adult life stage. It is still not correct to assume that all aquatic insects do not forage in aquatic habitats during their adult life stage (e.g., predaceous diving beetles).- This comment has been addressed by changing the text to, “Since aquatic insects may alter their feeding at later developmental stages (i.e. pupal and adult chironomids), their consumption at differing life stages may have ecological relevance.”

Lines 36 – 37: Move “non-consumer life stages” so that this portion of the sentence reads “fraction of non-consumer life stages of terrestrial and aquatic prey that are consumed”.- Sentence formatted in accordance with this suggestion.

Lines 38 – 39: Instead of “repeatedly”, report the actual frequency of sampling and report if this study took place during a single summer.- text now reads, “…were collected from two backwater and two stream sites weekly over two months during one summer.”

Line 40: Why just report the maximum percentage? It would be more informative to report the entire range.- Total range is now included to provide reader with a clearer understanding of the data.

Line 42: I think the parenthetical should come after “life stages” and the “an” after “at ~8-10” should be removed.- we agree that this is an improvement on the sentence structure and have made the changes.

Line 43: Do the authors have the basis to conclude that these patterns are common?- text now states only that it occurs, not that it is common.

Introduction

Lines 47 – 55: This study is not about extinction risk or the effects of species loss or community disassembly on food webs or ecosystem function; therefore, it is off topic to start this paper on these issues. This information is more relevant for the discussion section as a potential implication or application of this study’s results. My recommendation is to use lines 53 – 71 as the first paragraph (just drop “however” from the first sentence) because this paragraph gets right into the issues this research addresses.- these lines have been move into the discussion while the lines that were previously lines 53-71 have been shifted to the beginning of the intro.

Line 64: Replace “such feeding” with “preferential consumption of insect pupae and adults may be ecologically important because”.- This change has been made for a clearer meaning.

Lines 64 – 67: I am going to repeat my comment from the abstract: Not all aquatic insects cease feeding at the adult life stage (e.g., dragonflies, damselflies, mosquitos, black flies, predaceous diving beetles, etc.). Are the authors referring to taxa that do not forage in aquatic environments as adults? If so, this detail needs to be clearer because it is patently incorrect to state that aquatic insects do not feed during their adult life stage. It is also not correct to assume that all aquatic insects do not forage in aquatic habitats during their adult life stage (e.g., predaceous diving beetles).- the text has been changed to clarify which insects we are referring to and to not over-generalize our statements about aquatic insect development.

Figure 1: The category of “prey consumer prey taxon” is redundant and confusing. The study reported in this paper does not quantify top-down effects on food webs or primary productivity and so this figure is not relevant. Furthermore, the conceptual food webs are not needed to support the ideas described in the text.- We agree that “prey consumer prey taxon” is confusing and have simplified the text. While our study does not report the effects described here, we believe that this conceptual model can help readers to understand the relevance of stage-structured feeding and how it could have a cascading impact throughout a food web (lending more relevance to our study). This figure also provides a visual demonstration of stage-structured feeding for readers to follow. Finally, this figure demonstrates an area for future research into this topic (quantification of these top-down effects). We appreciate the suggestion for removal of this figure, but would prefer to keep this figure in the text to assist readers with a more visual understanding of concepts and less in-depth knowledge of this kind of research.

Lines 84 – 86: If benthic fishes are not preferentially consuming “non-consumer” life stages, why would stage-structured predation explain weak top-down effects of fish on aquatic invertebrates when benthic fishes are often quite abundant and widely distributed in these types of ecosystems?- We have moved away from the feeding guild approach and decided to address stage-structured feeding at a species level. As such, we have removed this statement.

Overall, there is almost no coverage in the introduction of what is known about fish traits and associated trophic roles even though the authors highlight fish traits in their predictions (lines 84 – 87). I recommend the authors provide more context for these predictions by covering past studies of fish ecomorphology, habitat use, and foraging behavior (particularly invertivory).- We have changed the manuscript to focus on individual species rather than feeding guild. We believe that explaining our findings at the species level keeps our data relevant and useable by others while allowing us to simplify our overall message.

Methods

Lines 96 – 97: I assume that the authors meant that the stream sites were downstream and upstream relative to each other. Please rewrite this sentence for clarity.- Sentence rewritten to clarify site placement.

Line 99: “Gut” sampling is more commonly referred to as “diet” sampling.- text has been changed to reflect this.

Lines 99 – 100: If sampling began 6/6/19, when did it end?- The end date of July 25, 2019 has been added.

Lines 100 – 103: More detail is needed on the sampling effort used to collect these fishes. For example, how long of a stream reach was sampled at each site? How large are the backwater sites and what fraction of these environments were sampled? Otherwise, it is not clear how representative the data is of these habitats and the fish assemblage. What time of day or night were the samples collected? Field studies of fish diets are often greatly affected by diel activity patterns of species within the assemblage.- Time of day for sampling has been added.

Line 104: Report the MS-222 dilution level used.

Line 112: Table 1 not Figure 1.- text corrected

Lines 120 – 121: Were the incomplete specimens size-matched to the whole specimens used to estimate their mass? There is no Wesner et al. (2020) in the literature cited section.- No, estimates of dry mass were generated from random draws of a distribution. These distributions were informed by literature that documented the dry mass of aquatic insects in the area. We have modified to sentence to better represent this process. Literature added to citations.

Lines 124 – 127: The study flips from being about aquatic insects in general to specifically about chironomids. What about other pupal and adult insects? The focus on chironomids would be a less jarring transition if some justification was provided.- These lines have been reworked to refer to aquatic insects in general. The justification for a later focus on chironomids is given in the results section. This allows us to provide justification for the future focus on chironomids in parts of the discussion: “Among the 59 prey taxa in fish diets, chironomids made up 64% of all diet items by abundance, 45% by biomass, and occurred in 438/617 fish stomachs (71%).”

Lines 124 – 131: Emergence was measured, but densities (and therefore availability) of larval aquatic invertebrates were not quantified.

Lines 129 – 130: Emergence traps were deployed for 3 – 4 nights and then sampled twice per week? Wouldn’t the second sample have less sampling effort (hours deployed) than the first or was each sample after 3 – 4 nights of continuous sampling by the traps? Please clarify.-Added a sentence to the end of the paragraph to improve clarity: “Emergence estimates from these traps were averaged over the number of days they were deployed (3 or 4 days).”

Lines 142 – 143: Were all possible interactions included in the model? Please clarify.- Sentence clarified: “To determine whether the proportion of each prey stage in diets varied across fish species, we fit a generalized linear mixed model with prey mass per stomach (mg dry mass) as the response variable, date, fish species, and prey stage, and all of their interactions as the predictor variables, and site as a random intercept.”

Line 145 – 147: Instead of “consumer status”, which I do not believe is correct, use a three-level life stage variable (adult, pupa, and larva)?- We have decided to take this approach throughout the manuscript. This sentence is particular has been reworded to, “To determine what fraction of aquatic prey were eaten in each life-stage, we used a similar model as above, but with prey stage(larval, pupal, or adult) instead of prey origin.”

Lines 145 – 154: Combine these lines into a single paragraph with a relevant topic sentence.- The second paragraph has been removed as we simplified our analysis to provide a clearer message and focused on proportion of each life-stage within the diets of each fish species.

Lines 148 – 150 and Figure S1: Though these results support the lack of a relationship between fish size and diet mass, because many fish species have distinct size- and stage-based foraging behavior, there choice of prey types may vary by size. For example, the *Micropterus* and *Lepomis* species in this study have well-documented size-related shifts in prey electivity. At the very least, the authors should include mean and range of fish sizes per species in Table 1.

Lines 150 – 151: Presumably, foraging domain was not in the species-specific model. Please clarify.- This has been clarified within the paragraph.

It was very unclear how or if the authors incorporated vertebrate and zooplankton prey taxa within their analyses. If these prey are included as “aquatic prey” in their analyses, how did they include them in their stage-structured prey models? Overall, the authors have greatly over-simplified aquatic food webs by overlooking other organisms likely to be consumed by fishes in their study system (e.g., zooplankton and fish prey). If this study is specifically focused on aquatic insects to address a specific prediction about why fish may have weak consumptive effects on aquatic invertebrates, this focus needs to be clearly identified.-This study is meant to address aquatic insect prey. We have modified our goals in the introduction to make this clear to the reader: “The goal of this study was to quantify natural variation in terrestrial and stage-structured feeding by fishes. In particular, we measured the fraction of total insect prey in fish diets that was eaten as larval, pupal, or adult life-stages (Figure 1).”

Results

Table 1: I suggest reordering table entries by foraging domain rather than sample size. I disagree with the categorization of Smallmouth Buffalo and Bluegill as surface-feeding fish () because Smallmouth Buffalo are known as midwater to benthic planktivores and Bluegill flexibly feed throughout water column and benthos. This incorrect categorization is problematic because these species compose almost 50% of the surface-feeding category sample size. From my experience and what I know of the literature, I would categorize all of the *Lepomis* species as being rather flexible and similar in their foraging domain. Poff and Allan (1995) do not classify Bluegill or Smallmouth Buffalo as surface feeders, and they also did not classify several of the cyprinid species in this study as surface feeders. These mistaken categorizations add a great deal of uncertainty and error to the results. In addition, I noticed that many of the species categorized as having an intermediate foraging domain are primarily piscivores (Grass Pickerel, Northern Pike, unidentified gar, Largemouth Bass, Smallmouth Bass, and Walleye; 6 of 9 species and 13% of sample size in this foraging domain), a detail backed up by the amount of vertebrate prey in the intermediate-domain diets (Figure S5). Depending on how the diets of these piscivores were used (e.g., how were vertebrate prey treated in analyses?), this fraction of the intermediate domain species could also cloud analyses concerned primarily with questions regarding aquatic insects.- In response to this comment, we have reworked the manuscript to focus on individual species. This change has removed any problematic foraging domain classifications. Due to this, we have kept the table entries in order of sample size. However, we are open to further reordering suggestions if the reviewer believes a different configuration would improve table readability.

Lines 186 and 189 – 190: Diet items were identified to a finer scale than taxonomic class and so why report percentage of items that were Branchiopods or Insecta, which are not very useful distinctions because of the large diversity of organisms within these classes.- The percentage of Insecta within the diets of fish has been removed from the paragraph. Branchiopods were not identified to a finer scale since the focus of our study was insect development. We have included this percentage to provide the readers with this diet information though we do not analyze it further.

Because this paper is a diet study, prey taxa found in fish diets should be reported in the main body of the manuscript, not relegated to supplementary files. Reporting species-specific diets is also important, but I would recommend that these details be summarized in a supplementary table.

Lines 195 – 196: It is not clear what the authors are referring to by “the likely trophic impact of this feeding”.- Text changed to address this comment and fit the new direction of this manuscript: “However, the proportion of chironomids in each life stage differed among fish species.”

Line 203: Delete “was”.- This has been removed.

Lines 210 – 211: What exactly is being referred to “respectively” in this sentence?

Line 224: To more explicitly address niche partitioning, the authors should also calculate an index of diet overlap. – Thank you for this suggestion. We have now included an analysis of diet overlap ~L147 and new Figure 4. The result was interesting, supporting our hypotheses that 1) including prey stage information reduces dietary overlap (a fairly obvious result), and 2) the size of the reduction is correlated with the fraction of non-larval prey in a fishes diet. This helps to support the overarching hypothesis that stage-structured feeding provides a way to reduce dietary overlap among fishes, even when they share similar prey taxa.

Lines 226 – 233: Availability of alternative prey may have been an important factor in the lack of a strong relationship with emergence.- This is a good point. We have removed the emergence analysis as both reviewers had concerns with it. The time frame of the study was likely not long enough to detect temporal trends and there are many other explanations for the lack a strong relationship.

Discussion

Lines 253 – 256: Concluding that stage-structured feeding attenuates top-down effects of fish communities on larval, benthic insects ignores all of the benthic fish species present in the fish assemblage.

Lines 266 – 267: How can the authors conclude that “in the current study, cryptic partitioning is driven by prey life-stages, rather than species” when they neither identified nor analyzed prey composition at the species level (lines 113 – 114; Figures S5, 3B, etc.)?

Lines 276 – 285: As I pointed out in the methods and results, a more parsimonious explanation for the lack of an effect of relative abundance of emerging insects is the availability of alternative prey items.

Line 288 – 293: It should be pointed out the authors did not calculate a measure of diet overlap in their study and so it is a fraught exercise to draw conclusions from their study on niche partitioning and alternative perspectives of diet overlap.- An analysis of dietary overlap is now included, as suggested above.

Reviewer #2: Overall, this manuscript is a concise and technically sound contribution to the fish diet literature that would be of general interest to freshwater ecologists. The authors provide several lines of evidence of predictable stage-structured feeding by freshwater fishes based on foraging traits and discuss the potential implications of this phenomenon at the community and whole food web scales. They demonstrate that the disaggregation of prey taxa by life stages reveals otherwise hidden diet partitioning by freshwater fishes. I concurred with the majority of the conclusions presented herein and therefore my comments will remain broader in scope.  
  
My primary concern involved the discussion of the temporal variation in stage-structured feeding on page 12. I would argue that two months of diet sampling from a single summer is simply too short of a sampling period to even begin to speculate about the drivers of this temporal variation in stage-structured feeding over the course of a growing season. The authors likely didn't detect a relationship between insect emergence and stage-structured feeding due to the small sample size for each species over just the two months, and/or the collection bias with the emergence traps addressed immediately following this discussion section. If one considers an individual macroinvertebrate species' instar lengths or complete life cycle lengths compared to fishes, sampling just two snapshots over a given year is almost guaranteed to miss capturing fishes' intra-annual feeding shifts from one stage to another if they are present. Two months is insufficient temporal coverage to assess that trend, and as such I'd caution against speculating too far into drivers of variation in the temporal component of these data.- Speculation into this topic has been removed from the manuscript. The text now reads: “However, speculation on the cause of this temporal variation would require further investigation across a time period longer than two months.”  
  
Aside from that small segment of the discussion segment, I found this manuscript to be relatively straightforward in terms of experimental design and statistical analysis. The authors provided clear rationale for their model parameterization and the discussion related stage-structured feeding to its relevance to ecosystem functioning. Therefore, I recommend it for publication with minor revisions.  
  
A handful of specific line edits and comments:  
  
36- One dash is an en dash and one is an em dash- format unified with em dash  
  
94 - capitalize "River"- correction made  
  
107 - I'd love to see a photo of this garden sprayer gastric lavage setup in the supplemental material just out of sheer curiosity  
  
149 - It's interesting that you didn't find a relationship between fish mass and prey mass  
  
215 - Did you mean to write "one exception" instead of "on exception"?- Yes, the correction has been made.  
  
224 - Cut the "per se"- text has been removed  
  
230 - Report effect size in-text as well  
  
495 - I really liked your conceptual model in Figure 1.  
  
499 - Figure 2's species labels seem a little compacted and overlapping