Environ Biol Fish (2022) 105:905–916
https://doi.org/10.1007/s10641-022-01296-8

Stage-structured feeding by freshwater fish assemblages in eastern South Dakota, USA

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Received: 18 March 2022 / Accepted: 17 June 2022 / Published online: 4 July 2022

Abstract Aquatic insects undergo substantial shifts in their ecology during development, such as changing from a benthic consumer stage (e.g., larva) to a pelagic non-consumer stage (e.g., non-feeding pupae). Fish may differentially target these life-stages during predation, but the prevalence of this feeding is largely unknown. To determine how stage-specific feeding varies among fish species, we sampled fish diets from four freshwater habitats in southeastern South Dakota, USA. We measured the fraction of gut contents in fish that consisted of prey that do not feed in the aquatic food web (e.g., terrestrial insects or non-consumer stages of aquatic insects). We also compared estimates of dietary overlap using methods that either included or did not include prey life-stage information. When averaged across 22 fish species, 17% (10 to 25%) of diet dry mass consisted of prey that were not consumers in the aquatic food web. Non-consumer prey came from a mix of terrestrial subsidies (11%) and non-feeding life-stages of aquatic prey (6%). The overall fraction of non-consumer prey varied widely among fish species, from ~ 2% in a darter (*Etheostoma nigrum*) to ~ 52% in a minnow (*Notropis stramineus*). Adding prey life-stage information to estimates of dietary overlap revealed the presence of prey life-stage specialists and generalists in the fish populations, causing overlap to decline. The magnitude of this decline increased as individual fish ate more non-larval stages of aquatic insects. These results reveal the importance of considering developmental changes in prey when estimating fish diets and indicate that stage-structured prey partitioning reduces dietary overlap.

Keywords Stage-structured feeding · Food webs · Freshwater · Aquatic insects · Fish

Introduction

Despite the near ubiquity of fish-insect interactions in freshwater food webs, evidence for consistent top-down control of aquatic insects by fish is mixed (Matthews 1998; Power et al. 2008; Wesner 2019). A proposed explanation for mixed effects of fish predation is that some fishes feed more heavily on drift and/or are subsidized by terrestrial prey, thereby reducing direct consumption of benthic insects (Garman 1991; Nakano et al. 1999; Pusey and Arthington 2003;

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10641-022-01296-8.

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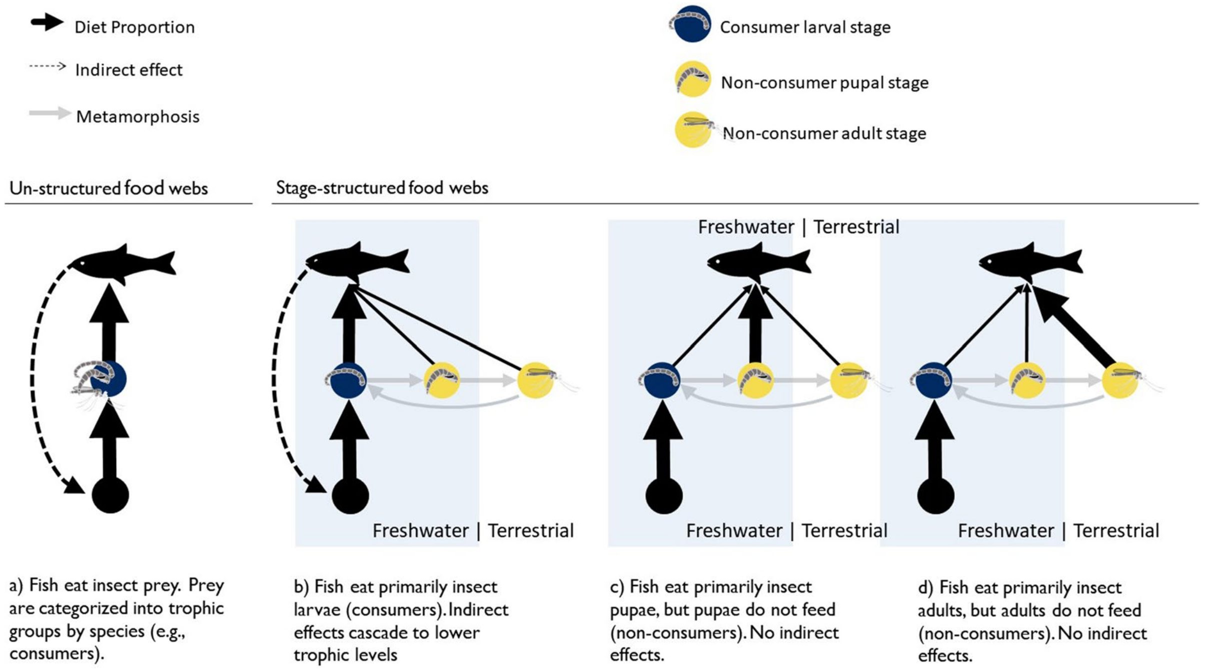
Baxter et al. 2004). In addition to terrestrial subsidies, a complementary but less well-studied explanation for the weak effects of some fishes is that they also target later-developing stages of aquatic insects, such as pupae (Wagner et al. 2012; Wesner 2019). For example, Wagner et al. (2012) found that chironomid pupae made up 20–48% of prey mass in spring diets of lake-dwelling Eurasian Perch (*Perca fluviatilis*). Similarly, Matthews et al. (1978) reported that 19–70% of stream-dwelling duskystripe shiners (*Luxilus pilsbryi*) contained chironomid pupae in their diets. Similar rates of pupal feeding by green sunfish (*Lepomis cyanellus*) were associated with weaker control of benthic insects compared to fish that fed exclusively on larvae (Warmbold and Wesner 2018). Variation in these feeding patterns among fish species is expected, in part, due to variation in foraging traits associated with benthic, midwater, or surface feeding (Dahl and Greenberg 1996; Ross 2013 p. 234).

Pupal feeding by fishes is important for several reasons related to the ecology of pupae compared to other insect life-stages. First, pupae do not feed. As a result, consumption of pupae by fish does not remove feeding links from the aquatic food web in the same way that consumption of larval stages would (Fig. 1) (Wesner 2019). Second, pupae are pre-emergent. When fish eat them, the effect is transferred to the terrestrial ecosystem by reducing adult insect emergence, similar to of the effect of fish eating larval stages. However, unlike consumption of larval insects, the effects of pupal consumption may or may not be reflected in benthic densities (depending on the location of the pupae in the benthos versus water column (Wesner 2016)). Feeding on larval stages is known to influence the growth of other benthic species and impact benthic community structure (Gilinsky 1984; Rasmussen 1985). Pupal feeding is not known to share these effects. Third, pupal feeding is likely to vary among fish species due to variation in benthic versus pelagic feeding traits, for example (Wagner et al. 2012; Warmbold and Wesner 2018), reducing dietary overlap between fish and allowing



a) Fish eat insect prey. Prey are categorized into trophic groups by species (e.g., consumers).

b) Fish eat primarily insect larvae (consumers). Indirect effects cascade to lower trophic levels

c) Fish eat primarily insect pupae, but pupae do not feed (non-consumers). No indirect effects.

d) Fish eat primarily insect adults, but adults do not feed (non-consumers). No indirect effects.

Fig. 1 Conceptual figure showing different predictions for food webs without and with information on prey life-stages with chironomids used as prey example. Each of the food webs (a, b, c, d) has the same number and identity of prey species. a) Fish feed on a single prey taxon, generating indirect effects to lower trophic levels. b) Fish target larval life-stages, generating indirect effects because only larvae are consumers in aquatic food webs. c–d) Two of the life-stages (pupae and adults) do not feed. Fish that target those life-stages have no indirect effects on lower trophic levels. The prevalence of this type of stage-structured feeding in aquatic food webs is unknown coexistence of species that otherwise compete for the same resource (De Roos et al. 2008; Miller and Rudolf 2011).

The ecological importance of pupal feeding is a specific case of the broader concept of stage-structured food webs, in which predator–prey interactions are described by adding life-stage information into the food web (Miller and Rudolf 2011; Nilsson et al. 2017). For example, a single taxonomic prey species may represent multiple trophic species throughout its development, providing an additional way for fish to partition prey by feeding more heavily on some life-stages (e.g., stage-specific predation; De Roos et al. 2008; Nonaka and Kuparinen 2021). For fish-insect interactions specifically, stage structure extends beyond pupal chironomids to include any prey taxon that contains distinct life-stages, whether pupal or not. For example, mayflies and other hemimetabolous insects do not undergo pupation, but do stop feeding prior to emergence during the wingpad stage (i.e., during the onset of metamorphosis). In this paper, we refer to these stages as "consumer" stages (e.g., larvae) or "non-consumer" stages (e.g., pupae, wingpad, adult) to account for intraspecific variation in prey within aquatic food webs (Fig. 1).

While studies of fish diets routinely include life-stage information for some prey taxa, such as chironomids (Cable 1928; Matthews et al. 1978; Wagner et al. 2012), we are unaware of studies that quantify this feeding as a proportion of overall fish diets or consider its importance to prey partitioning. To address this, we conducted a field study to (1) quantify natural variation in fish predation of consumer versus non-consumer prey life-stages (Fig. 1), (2) compare that to variation in more commonly studied terrestrial feeding, and (3) use that information to compare species dietary overlap when life-stages are included versus not included.

Sampling methods

Four sites, two lentic backwaters and two lotic stream sites, were sampled during summer 2019 for this study. Sites were chosen to obtain a breadth of fish species across various shallow freshwater habitats. The backwater sites were connected to the main channel of the Missouri River, the longest river in North America. The two backwater sites were Burbank beach (latitude: 42° 40′ 11.88″ N, longitude: 96° 47′ 22.82″ W) and Gunderson backwater (latitude: 42° 44′ 56.56″ N, longitude: 96° 57′ 12.08″ W). Stream sites were at different locations along the same ephemeral stream system, a first order tributary of the Vermillion River called Spirit Mound Creek, with a width less than 3 m and a depth of ~ 1 m (fluctuating with precipitation). The stream sites were upstream (latitude: 42° 52′ 4.29″ N, longitude: 96° 57′ 16.25″ W) and downstream (latitude: 42° 51′ 7.39″ N, longitude: 96° 56′ 39.28″ W) within the same stream system. All sites are in SE South Dakota, USA.

Weekly diet sampling of fish was conducted in the mornings (between 6 am and 12 pm) at each of the four study sites starting on June 6, 2019, and ending on July 25, 2019. First, fish were collected via seine net and put into a 5-gallon bucket containing fresh water where other team members quickly began the next stage in this process to reduce stress and handling time for the fish. Water in the buckets was regularly refreshed. To ensure that we sampled a variety of fishes, various seining techniques were used at each site, including kick-seining for benthic fishes, seining around vegetation, short seine-hauls, and long seine-hauls. Seining was conducted for ~ 1 h at sites along ~ 100 m of shoreline. The seine net had a mesh size of ¼ inch. Fish were then identified and moved from the holding tank to the anesthetizing tank (a 5-gallon bucket containing water and a dilute [25–100 mg/l] MS-222 solution). Once immobilized, fish were measured for standard length and gut contents were extracted with gastric lavage (Kamler and Pope 2001; Kraus et al. 2016). To do this, a garden sprayer (acting as a pump for this procedure, Figure S1) with an appropriately sized tube for the mouth of the fish (≥ 3.2-mm inner diameter) was fed into the gut. Water was then pumped through the gut of the fish and any dislodged stomach contents were collected in a 250-µm sieve and preserved in 95% ethanol. The fish was then placed into a recovery tank (a 5-gallon bucket containing fresh water), monitored for recovery, and released. In a previous study with similar fishes in this area, we found no difference between diet results from gastric lavage compared to gut removal (Wesner and Seidel 2020). Fish species and the number of individuals sampled are summarized in Table 1.

Prey items from the diet samples were identified to family or order and life-stage using Merritt et al. 2008. Prey were further classified as aquatic or terrestrial

Table 1 Number of diet samples collected per species

Fish species	*n*
Semotilus atromaculatus	95
Etheostoma nigrum	74
Ictiobus cyprinellus	73
Ameiurus melas	62
Lepomis macrochirus	59
Cyprinus carpio	49
Cyprinella spiloptera	47
Pimephales promelas	30
Luxilus cornutus	27
Lepomis cyanellus	24
Ictiobus bubalus	14
Notropis stramineus	11
Cyprinella lutrensis	10
Notropis blennius	8
Esox americanus	6
Esox lucius	5
Lepomis gibbosus	4
Lepisosteidae	3
Micropterus salmoides	2
Culaea inconstans	1
Micropterus dolomieu	1
Sander vitreus	1

(based on their larval habitat) and as either a consumer or non-consumer, indicating their trophic status in aquatic food webs. Pupal, wingpad, and adult stages of aquatic insects along with all terrestrial insects were classified as non-consumers, while aquatic larvae were classified as consumers. All prey in a sample were enumerated and a random subset (up to 10 individuals per sample per stage) was measured for dry mass (mg) using length-mass regressions (Benke et al. 1999). For taxa that did not contain whole specimens ($n = 2/58$ taxa), we were unable to measure lengths. In those cases, we used mass estimates obtained from different studies in the same region (Warmbold 2016; Wesner et al. 2020). We multiplied the mean individual dry mass of each prey taxon by the number of individuals in a sample to generate an estimate of sample dry mass.

Analysis

To compare feeding among fish species, we fit a generalized linear mixed model (GLMM) with prey mass per stomach (mg dry mass) as the response variable, and consumer status (consumer vs non-consumer), larval origin (terrestrial vs. aquatic), and their interaction as predictor variables. Site, date, and fish species were included as random intercepts. Fish species was also included as random slopes to allow comparisons of feeding among fish species. Due to zero inflation, we fit this model as a hurdle model to account for the excess zeros (Huang et al. 2019). Doing so allowed us to fit two submodels simultaneously. The first model estimates the probability of containing a prey item using a binomial GLMM. We fit this as an intercept-only model with random intercepts and slopes for each species. The second part of the model is for the non-zero data, which we fit using a gamma likelihood with a log-link, since the data are continuous and positive. We used prey mass per fish, rather than prey mass per fish length (e.g., corrected for fish size), because there was no evidence that larger fish had a higher mass of prey (Figure S2). We also analyzed prey mass, rather than prey abundance, to capture the potential energetic contributions of prey to fish. However, to determine whether results differed between mass and abundance, we re-ran the above model with abundance as the response variable (number of prey items per fish) (Figure S3).

After fitting, we corrected the gamma-based estimates by multiplying each iteration of the posterior by the probability of containing a prey item (Huang et al. 2019). We used the resulting posterior distribution to estimate derived quantities of total prey mass per fish, the proportion of prey that were eaten at as terrestrials, the proportion of aquatic prey eaten as non-consumers, and the overall proportion of all prey eaten as non-consumers. In each case, "non-consumer" means a prey taxon or stage that does not feed in the aquatic food web, even if it may feed in terrestrial food webs.

Determining the proportion of non-consumers assumes accurate categorization of prey life-stages within diets. Distinguishing life-stages is difficult for many prey taxa, particularly from partially digested diet samples. For chironomids, it is easier to categorize since the larvae, pupae, and adults have clearly distinguishable features, even with partial specimens (e.g., larval head cases vs folded pupal legs vs adult wings and antennae). Therefore, we fit a second model using only chironomid data. The chironomid model was similar to the model above but included only chironomid life-stage as a predictor. This allowed us to compare results that contained all prey types, but low resolution of prey stages to results that contained one prey type with high resolution of prey stages.

Dietary overlap

To determine how dietary overlap changed when information on prey life-stages was included, we calculated dietary overlap using two datasets: one that contained life-stage information and taxonomic information (e.g., Ceratopogonidae larva or Ceratopogonidae pupa) and one that contained only taxonomic information (e.g., Ceratopogonidae). Dietary overlap using these two datasets was calculated for individual fish using the *RInSp* package in R (Zaccarelli et al. 2013):

$$o_{ik} = \sum_{j} \min(p_{ij}, p_{kj})$$

where o_{ik} is the diet overlap between individuals i and k, ranging from 0 (individuals are specialists and share no common prey) to 1 (individuals are generalists and have identical diets), and p_{ij} and p_{kj} are the proportions of resource j for individuals i and k (Zaccarelli et al. 2013).

We used these estimates to compare dietary overlap among species and sites using a generalized linear mixed model with diet overlap (o_{ik}) as the response variable; dietary method (with or without life-stages), site, and their interaction as fixed and random slopes (within species); and date and fish id as random intercepts. The likelihood was beta, since the data are restricted between 0 and 1.

Finally, to determine how changes in overlap are affected by stage-specific feeding for individual fish, we calculated the difference in overlap estimates by subtracting the estimate without life-stage information from the estimate with life-stage information for each individual fish. We assumed that the difference in diet overlap would be larger for fish that ate a larger fraction of non-larval individuals, because those fish are essentially feeding on three different stages within a single taxon, while fish that eat only larvae are feeding on a single stage within a single taxon. To test this hypothesis, we fit a Gaussian linear regression with difference in overlap as the response variable; proportion of non-larval prey as the predictor variable (standardized via z-scores); and site, date, and fish species as random intercepts. This model also included a submodel for sigma that allowed the variance to change as a function of the predictor variable.

Models were specified in R (R Core Team 2021) using Bayesian inference with the *brms* package (Bürkner 2017). Posterior distributions were generated with a Hamiltonian Monte Carlo algorithm through *rstan* (Stan Development Team 2020). Prior distributions are described and justified (along with prior sensitivity analyses) in the Supplementary Information (Figures S2–4) following methods in Gabry et al. (2019) and Wesner and Pomeranz (2021). All code and data can be found at https://github.com/jswesner/kanz_stagestructure.

Results

Fish sampled

We collected a total of 606 diet samples from 22 fish species (Table 1). Creek chub (*Semotilus atromaculatus*) was the most commonly sampled fish ($n = 95$ samples), followed by Johnny Darter (*Etheostoma nigrum*, $n = 74$) and smallmouth buffalo (*Ictiobus cyprinellus*, $n = 73$).

Prey taxonomic composition

Fish diets contained an overall median of 10 mg dry mass (mgDM) per stomach (95% credible interval [CrI]: 5 to 26), ranging from 1 mgDM in *Ictiobus cyprinellus* to 56 mgDM in *Ameiurus melas* (Table 2). 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%). The next most common item was Branchiopods (14% by abundance, 4% by biomass, 24% by occurrence). All other prey taxa made up less than 4% of diets by abundance, biomass, or occurrence. One exception was crayfish chelipods, which made up less than 4% of abundance or occurrence, but 23% of biomass due to their large size (Table 2). They were found in 24/606 fish stomachs.

Table 2 Total abundance and dry mass of prey items collected from all fish in this study

Prey taxon	Total #	Total mgDM
Chironomidae	6844	4378
Crayfish	28	2240
Coleoptera	136	646
Branchiopoda	1583	443
Dytiscidae	57	406
Amphipoda	818	393
Trichoptera	105	281
Simuliidae	229	144
Frog	2	106
Hydrophilidae	15	93
Diptera	113	72
Corixidae	121	56
Mite	65	43
Ephemeroptera	104	33
Hemiptera	68	32
Collembola	30	23
Chaoboridae	34	22
Dolichopodidae	35	20
Formicidae	18	19
Carabidae	2	17
Gyrinidae	2	17
Arachnid	24	16
Cicadellidae	22	14
Zygoptera	9	13
Parasite	15	12
Fish	13	10
Worm	13	10
Culicidae	14	9
Copepoda	11	8
Haliplidae	1	8
Snail	13	7
Spider	11	7
Isopoda	7	5
Lepidoptera	8	5
Thysanoptera	7	5
Canacidae	7	4
Malacostraca	6	4
Elmidae	2	3
Aphid	6	3
Anisoptera	4	3
Exuviae	4	3
Stratiomyidae	4	3
Ceratopogonidae	4	3
Muscidae	4	3
Aphidoidea	3	2
Tetranathid	3	2
Syrphidae	3	2
Unknown	2	2
Empididae	3	2
Homoptera	2	1
Gerridae	2	1
Orthoptera	1	1
Ephydridae	1	1
Phoridae	1	1
Psychodidae	1	1
Brachycera	1	1
Dryomyzidae	1	1
Circulionidae	4	1
Caelifera	1	0.2
Grand total	10,647	9659

Consumer versus non-consumer prey

A median of 17% (95% CrI: 10 to 25%) of diet dry mass contained prey that did not feed in the aquatic food web (Fig. 2a). This fraction was split between terrestrial prey, which made up 8% (CrI: 6 to 14) of total prey mass (Fig. 2c), and aquatic prey in non-consumer life-stages, which made up 5% (3 to 9%) of total prey mass (Fig. 2b). For three fish species, ~ 50% of their diets were non-consumer prey: *Notropis stramineus*, *Cyprinella spiloptera*, and *Luxilus cornutus* (Table 3).

Terrestrial feeding among fish species varied widely, ranging from 42% terrestrial in spotfin shiner (*Cyprinella spiloptera*) to < 1% in Johnny Darter (*Etheostoma nigrum*) (Fig. 2c). Similarly, variation in feeding on non-consumer aquatic prey varied from 28% (16 to 44%) in *Luxilus cornutus* to < 1% in *Etheostoma nigrum* (Fig. 2b). Fish that ate terrestrial prey also tended to eat non-consumer life-stages of aquatic prey (Fig. 2b, c), but there were exceptions to this. For example, spotfin shiner (*Cyprinella spiloptera*) ate the highest fraction of terrestrial prey (42%), but they ate only 14% of aquatic non-consumers (Table 3).

Among fish feeding on chironomids, the most common prey item, stage-specific feeding was common. All fish ate some pupal or adult chironomids, with four species eating at least 30% (Table 3).

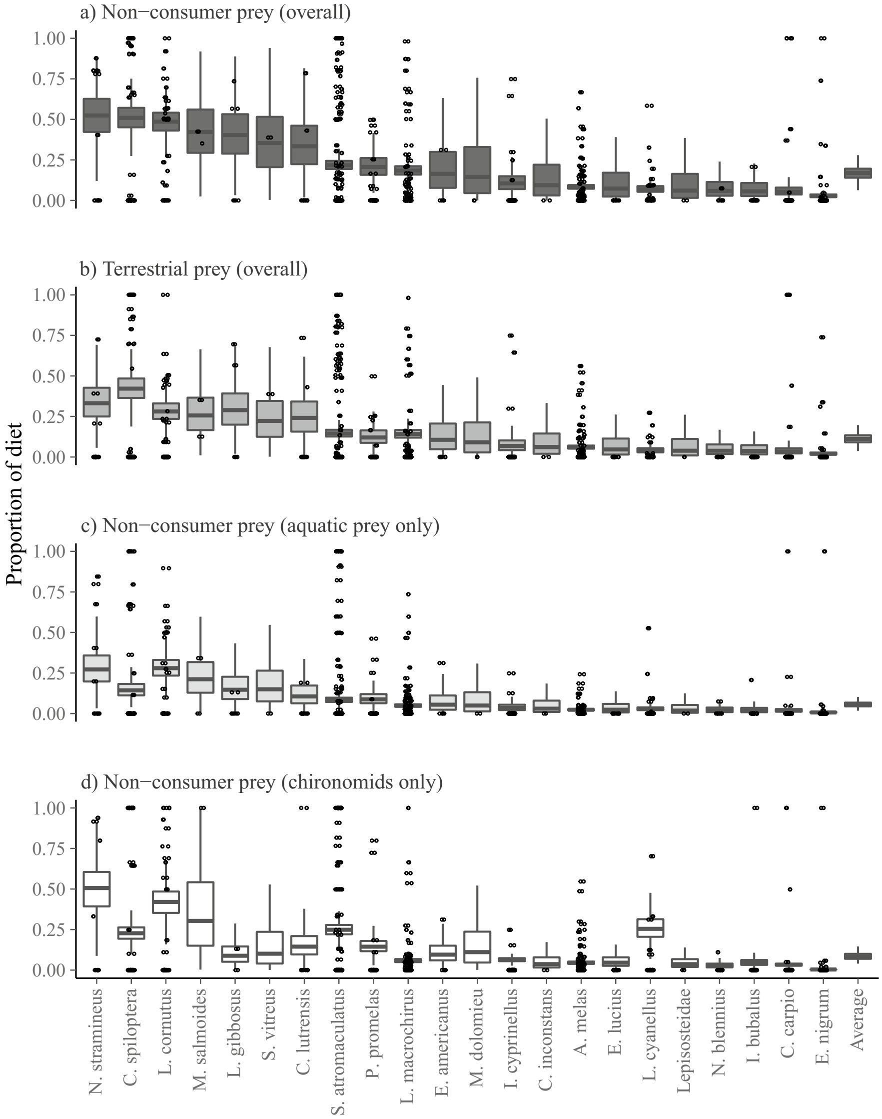


Fig. 2 Variation in stage-structured feeding by fishes. Posterior distributions, averaged across time, showing **a** the overall proportion of prey eaten as non-consumers in aquatic food webs, **b** the proportion of terrestrial prey, **c** the proportion of aquatic prey eaten in a non-consumer life-stage, and **d** the proportion of chironomids eaten as pupae or adults. Dots are raw data. All estimates are based on prey dry mass in grams

Overall, 9% (5 to 15%) of chironomids were eaten as pupae or adults (Fig. 2d).

Diet overlap

Adding life-stage information reduced the amount of diet overlap by ~ 0.06 units on average (CrI: – 0.01 to – 0.15; Fig. 3), representing a 17% reduction compared to diet overlap without life-stages. Across species, reductions were highest for fish that fed heavily on non-larval insects, like *Notropis stramineus* (33% [13 to 54%]; median [95% CrI]) and *Luxilus cornutus* (27% [11 to 44%]). These reductions were consistent across sites (Fig. 3). The effect of including prey life-stage information on dietary overlap also widened as fish ate a larger fraction of non-consumer prey stages (Fig. 4), with a > 99% probability that all slopes were negative. For example, at the Spirit Mound Creek downstream site, including life-stage information generated a 19-percentage point reduction in dietary overlap for fish that ate only non-larval aquatic insects, more than double that for fish that ate only larval insects (7 percentage point reduction; Fig. 4).

Table 3 Summaries of the posterior distribution of the proportion of non-consumer prey in fish diets (overall and for aquatic prey only), the proportion of terrestrial prey in fish diets, and the proportion of non-consumer chironomids (e.g., (pupae + adults)/total chironomids)

Fish species	Median (sd)			
	Non-consumers	Non-consumers (aquatic)	Terrestrial	Non-consumers (chironomids)
Notropis stramineus	0.52 (0.14)	0.27 (0.12)	0.33 (0.13)	0.51 (0.15)
Cyprinella spiloptera	0.51 (0.09)	0.14 (0.06)	0.42 (0.09)	0.23 (0.05)
Luxilus cornutus	0.49 (0.08)	0.28 (0.07)	0.28 (0.07)	0.42 (0.1)
Micropterus salmoides	0.42 (0.18)	0.21 (0.14)	0.26 (0.14)	0.3 (0.26)
Lepomis gibbosus	0.4 (0.17)	0.15 (0.11)	0.29 (0.14)	0.09 (0.09)
Sander vitreus	0.35 (0.21)	0.15 (0.15)	0.22 (0.16)	0.1 (0.19)
Cyprinella lutrensis	0.33 (0.17)	0.11 (0.1)	0.24 (0.14)	0.14 (0.09)
Semotilus atromaculatus	0.22 (0.04)	0.08 (0.02)	0.15 (0.03)	0.25 (0.04)
Pimephales promelas	0.21 (0.08)	0.09 (0.05)	0.12 (0.06)	0.14 (0.05)
Lepomis macrochirus	0.18 (0.04)	0.05 (0.01)	0.14 (0.04)	0.06 (0.01)
Esox americanus	0.16 (0.17)	0.05 (0.09)	0.11 (0.13)	0.1 (0.08)
Micropterus dolomieu	0.15 (0.21)	0.05 (0.13)	0.09 (0.16)	0.11 (0.19)
Ictiobus cyprinellus	0.11 (0.07)	0.03 (0.03)	0.07 (0.05)	0.06 (0.01)
Culaea inconstans	0.09 (0.16)	0.03 (0.09)	0.06 (0.12)	0.04 (0.07)
Ameiurus melas	0.08 (0.02)	0.02 (0.01)	0.06 (0.02)	0.04 (0.01)
Esox lucius	0.07 (0.14)	0.02 (0.07)	0.05 (0.1)	0.05 (0.05)
Lepomis cyanellus	0.07 (0.03)	0.03 (0.01)	0.04 (0.02)	0.25 (0.08)
Lepisosteidae	0.06 (0.15)	0.02 (0.08)	0.04 (0.11)	0.04 (0.05)
Notropis blennius	0.06 (0.09)	0.02 (0.03)	0.04 (0.07)	0.03 (0.02)
Ictiobus bubalus	0.06 (0.08)	0.02 (0.03)	0.04 (0.06)	0.05 (0.03)
Cyprinus carpio	0.05 (0.04)	0.02 (0.01)	0.04 (0.03)	0.03 (0.01)
Etheostoma nigrum	0.03 (0.01)	0.01 (0)	0.02 (0.01)	0.004 (0.002)

Discussion

We found wide variation among fish species not only in their use of terrestrial subsidies, but also in how they partitioned the remaining aquatic portion of their diet by stage-specific feeding. Terrestrial subsidies accounted for ~ 1 to 40% of prey mass in fish diets. This is similar to the range for other small-bodied stream fishes (Sullivan et al. 2012), and it approached that reported in salmonids (> 30%; Wipfli 1997). However, the most important result from our study is that a similar fraction of the remaining aquatic prey are also not consumers in aquatic food webs due to life-stage changes in prey feeding. When combined with terrestrial subsidies, this meant that 17% of fish diet mass consisted of prey that themselves no longer feed in aquatic food webs, ranging from < 1 to > 50% among species (Fig. 2c).

Fish species that ate high fractions of terrestrial prey also tended to eat high fractions of non-consumer aquatic prey (Fig. 2b, c). The reason for this is unknown, but it is perhaps driven by foraging traits of the species. For example, terrestrial feeding is higher in small-bodied fishes with subterminal and terminal mouths, such as some Cyprinidae and many Fundulidae (Sullivan et al. 2012). It seems likely that similar traits also lead to pupal consumption in the water column. Wesner (2010) found that *Gambusia affinis*, a small-bodied fish with an upturned mouth, fed heavily on emerging pupal chironomids compared to benthic orangethroat darter (*Etheostoma spectabile*). In the current study, the top three feeders on non-consumer insects were all cyprinids: *Notropis stramineus*, *Luxilus cornutus*, and *Cyprinella spiloptera*. Each of these species has been described previously as a surface-oriented feeder (Sullivan and Watzin 2010; Burress et al. 2016) or as feeding heavily on terrestrial insects (Gillen and Hart 1980). We did not measure fish morphology in this study, but future studies should examine whether

Fig. 3 The effects of adding life-stage information to estimates of dietary overlap across species and sites. Boxplots summarize the posterior distributions of dietary overlap calculated with or without life-stage information

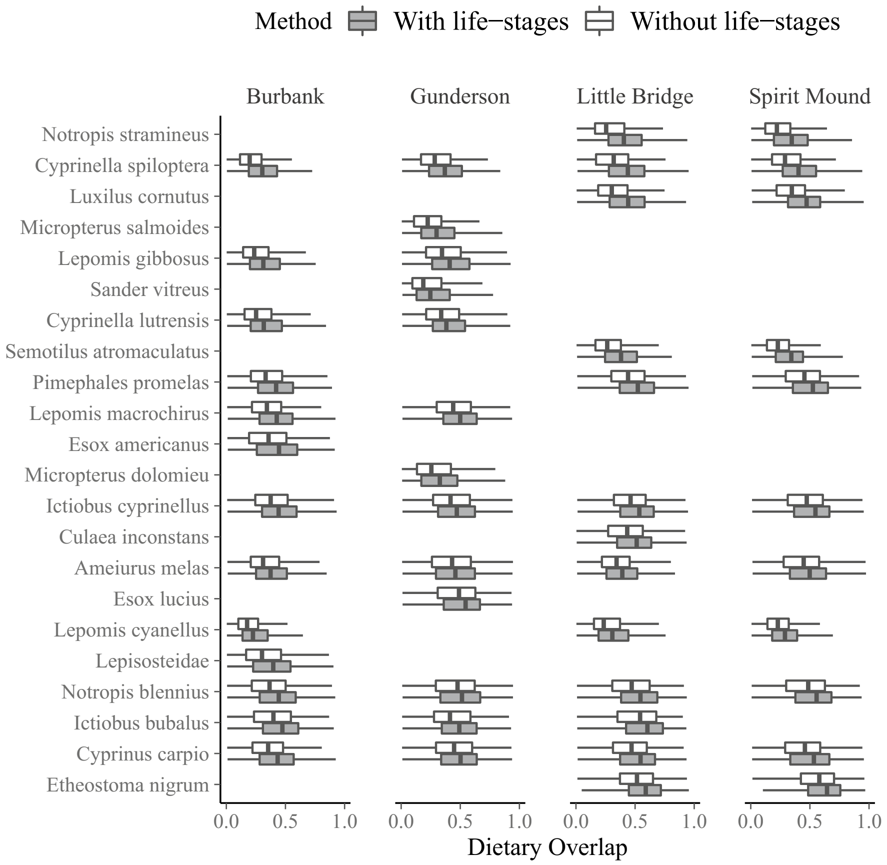
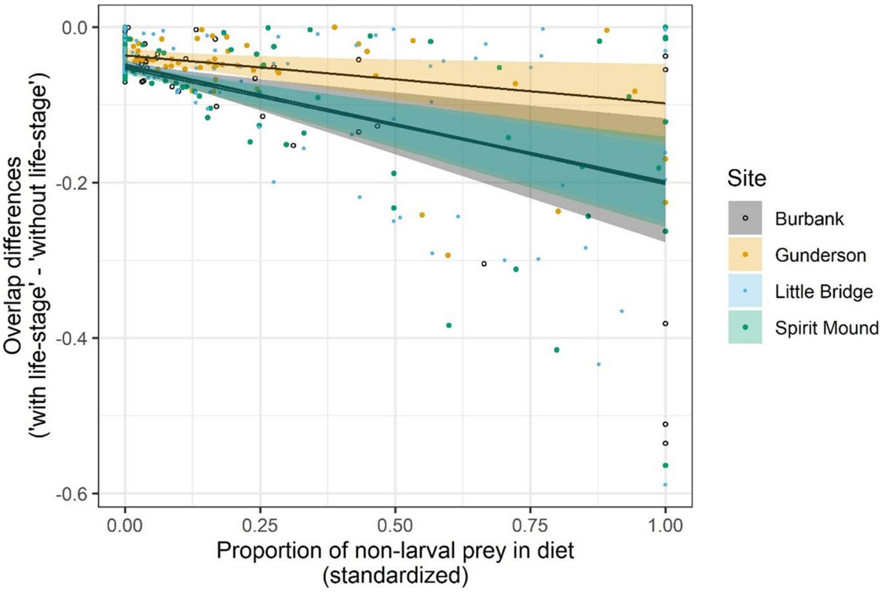


Fig. 4 The effect of adding life-stage information to estimates of dietary overlap increases as fish eat a larger fraction of non-larval prey. Regressions are median and 95% credible intervals of the posterior distribution. Dots are the raw data of individual fish



traits that are associated with surface feeding are also associated with feeding on non-larval aquatic insects.

Whether the feeding described here affects top-down control by fish in our systems is unknown. However, similar types of stage-specific feeding have been invoked to explain top-down control of fishes on benthic insects. For example, Baxter et al. (2004) excluded *Glossosoma* pupae (a caddisfly) from their analysis of trout trophic cascades and top-down control because *Glossosoma* pupae do not feed and would have "had little influence on the food web." This implicitly recognized the fact that life-stage variation in *Glossosoma* generates different trophic species with unique roles in the food web. Similarly, insects are usually most vulnerable to fish predation during pupation as they move through the water column to emerge from the surface (Oliver 1971; Pinder 1986). They are often least vulnerable as larvae, which have physical and behavioral defenses to avoid predation (Power et al. 2008; Atlas et al. 2013). These differences in vulnerability among life-stages help to explain the general trend in which fish typically have strong control over the emergence of adult aquatic insects, but weak control over larval aquatic biomass or abundance (Wesner 2016). Pupation likely serves as a bottleneck between the larval and adult life-stages leading to different responses in adult versus larval aquatic insects to fish predation (Wesner 2019).

Regardless of their impact on top-down control, stage structure had a clear impact on dietary overlap. Including stage structure reduced average dietary overlap by 8 to 32% across fish species. This is similar to the size of overlap reduction (25%) induced by extreme morphological variation in sticklebacks (Bolnick and Paull 2009). As predicted, dietary overlap declined as individual fish ate a higher fraction of non-larval prey. This is expected, since treating prey as trophic species, rather than taxonomic species, is akin to adding additional nodes to a food web (Clegg et al. 2018). In this study, differential feeding by fish on prey life-stages allowed fish to partition the prey communities, with some fish acting as specialists on prey developmental stages (e.g., *Etheostoma nigrum* feeding on aquatic larvae) and others acting as generalists (e.g., *Cyprinella spiloptera* feeding on larvae and pupae) (Nonaka and Kuparinen 2021). This feeding has potentially important effects on ecological networks, community complexity, and community stability (De Roos et al. 2008; Nonaka and Kuparinen 2021). However, stage structure has largely been studied in freshwater ecosystems from the perspective of fish ontogeny, rather than prey ontogeny (Werner and Gilliam 1984). As our results indicate, prey life-stages alone can also reveal important patterns in how fish partition prey resources. It is also unclear how much these results are driven by fish foraging preference versus prey encounter probabilities. We did not measure the relative abundance of each prey life-stage in this study but doing so is an avenue for future research.

Freshwater fish communities have changed dramatically due to species loss and species additions. Approximately 40% of freshwater fish species are at risk of extinction or are already extinct (Jelks et al. 2008; Darwall and Freyhof 2016). Because of the importance of fishes in freshwater ecosystem functioning, this loss has the potential to alter freshwater food webs (Hargrave 2009; Vanni 2010) in addition to linked riparian food webs (Baxter et al. 2005; Sullivan and Manning 2019). Predicting how these changes affect freshwater ecosystems relies on understanding how fishes partition limited resources and control those resources. It is also clear that fishes vary in their use of terrestrial resources (Pusey and Arthington 2003), and in their vertical use of the water column (Nakano et al. 1999; Wagner et al. 2012; Ross 2013). Our study adds an additional axis of fish resource use by examining how fish partition prey across prey life-stages. The ecological importance of this partitioning has been demonstrated for the control of fishes on aquatic-terrestrial subsidies (Warmbold and Wesner 2018), but its importance on other aspects of aquatic food webs (e.g., species coexistence, trophic cascades) is unknown and deserves further study.

Acknowledgements We thank Alexis Culley, Katy McCarthy, Sarah Lane, Jacob Ridgway, and Justin Pomeranz for help in the field and the lab.

Funding This study was funded by the National Science Foundation (#1837233 and #1560048), and by the University of South Dakota Graduate Research and Creativity Grant.

Data availability All supporting data and R script for analysis can be found at: https://github.com/Abrahamkanz/stageguildms.

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Declarations

Ethics approval All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Dakota (03–03-18-21C).

Competing interests The authors declare no competing interests.

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