

## Research Article

# Young Allis Shad *Alosa alosa* (Clupeidae) Would Not Be Euryphagous: An Assessment of Juvenile Diet and Prey Selectivity under Riverine-Type Conditions

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Received 21 December 2022; Revised 22 June 2023; Accepted 18 July 2023; Published 8 August 2023

Academic Editor: A. E. Naiel Mohammed

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Allis shad *Alosa alosa* hatches and develops in freshwater before migrating to the sea during its first months of life. Some field research works dedicated to the diet of the species in freshwater put forward that young stages of allis shad feed mainly on zooplankton, being opportunistic and euryphagous; their diet would diversify through ontogeny in relation to growth. However, these assumptions have never been confronted to the availability of prey, and the exact age of fish was unknown. In this work, we reared young allis shad under seminatural conditions, from 6 to 10 days posthatching until nearly 3 months old, in a trophic environment representative of a natural river. The rearing structures were designed as artificial rivers, supplied continuously by natural riverine water and zooplankton prey. Additional zooplankton was produced in separate basins and added daily in the structures. One artificial river was used to rear allis shad in 2018, and the experiment was conducted on two allis shad batches in 2019 (one batch per river). We described the nekton communities of potential prey available for allis shad in the rearing structures by sampling the water every week, and we compared these data with the stomach contents of fish captured at one and two months old posthatching to describe their diet and prey selectivity. Our results suggest that young allis shad should not be considered as opportunistic in their feeding, since clear selectivity was observed at both ages. One-month-old allis shad highly selected cladocerans, probably because they are easy to catch for fish at this age, when their swimming abilities are limited. Feeding selection at two months old drifted towards other prey taxa (dipteran larvae, ostracods, and/or copepods), whereas cladocerans were no longer preferred. Our results suggest that fish density and competition may entice two-month-old allis shad to enlarge their diet to other taxa, including benthic organisms, while one-month-old fish do not seem able to shift their diet and would fast in the absence of cladocerans. Thus, this highlights that the feeding of young allis shad may be highly challenging during their first month of growth.

## 1. Introduction

Information on the diet of a species is important to understand its ecology, since it interacts with its habitat use and selection, and therefore with its migration behavior [1–3]. In young fishes, feeding can be highly challenging because they have limited energy reserves, swimming capacities, and mouth aperture; this pressure would be especially important if their diet is highly selective ([4] and references therein).

Consequently, the availability of prey, in relation to fish size and selectivity, can strongly influence the recruitment of fish populations depending on whether the abundance of preferred prey taxa matches the first feeding and growing periods [5–7].

The allis shad *Alosa alosa* is an anadromous species that hatches and first develops in freshwater during the larval and juvenile stages, before migrating to the sea [8]. In the Gironde-Garonne-Dordogne estuary (SW France), allis shad

is likely to enter the saline estuary from 58 to 123 days old (median age 88 days old) [9]. Allis shad has undergone an important decline during the last decades, but the causes are not clearly identified [10]. In particular, the early stages of life of the species are poorly documented [8, 11]; extensive knowledge of their ecology would improve the understanding of population dynamics and recruitment, and favour adapted management practices. The diet of young allis shad in freshwater has mainly been investigated by net sampling [12–14]. Authors generally agree in describing the diet of young allis shad as composed of insect larvae and zooplanktonic crustaceans. In the Garonne River (SW France), Cassou-Leins et al. [13] put forward that allis shad of 20–50 mm in size (supposedly corresponding to one- to two-month-old individuals) would feed mainly on cladocerans, copepods and dipteran larvae. Through ontogeny, the diet of allis shad would diversify and include ephemeropterans, hymenopterans, or trichopterans [13, 15]. It is generally assumed that young allis shad are opportunistic for prey and euryphagous [14, 16], and that their diet would highly depend on their direct trophic environment. However, the selectivity of allis shad for prey remains uncertain, since the trophic environment was not described in these studies.

A high selectivity for prey may increase the risk of starvation for allis shad juveniles if a mismatch occurs between their abundance and the peak abundance of their selected prey. Conversely, matches between these abundances may favour the efficiency of population recruitment (e.g., [7, 17]). Hence, describing the feeding selectivity of young allis shad is a prerequisite to discuss the match/mismatch hypothesis. We conducted an *ex situ* experiment that allowed to characterise the trophic environment of young allis shad to provide new insights on their diet and prey selectivity. Allis shad from two cohorts were reared during their first months of life under riverine-type conditions (in terms of water and zooplankton communities). The diet of one- and two-month-old allis shad, as well as the prey availability in the water column, was assessed to test the opportunistic character of juvenile allis shad for food and its evolution through ontogeny.

## 2. Methods

**2.1. Rearing Structures.** One (R1 in 2018) and two (R1 and R2 in 2019) concrete basins (25 m length, 4 m wide, ca. 100 m<sup>3</sup> in volume) located at the INRAE experimentation station (Saint-Seurin sur l'Isle, SW France) were used for rearing allis shad. The basins were enriched with substrate (sand and gravel) and two hand-made artificial habitats (one riffle and one steep slope bank) to create several habitat types mimicking a river (Figure 1). A propeller (Hydro Wizard® ECM75; Pantarhei, Wedemark, Germany) placed close to the water inlet created a circular flow within each artificial river. Various current velocities (ranging from 0 to 40 cm·s<sup>-1</sup>) were then proposed to the juveniles according to the microhabitats within the artificial rivers. Care was given to build and enrich R1 and R2 similarly, and only small differences were detected between the two artificial rivers in terms of current velocities.

The water supply came from the nearby Isle River at a semicontrolled flow rate, varying between 14.0 and 18.61 min<sup>-1</sup> during the rearing period. The riverine water was filtered at the inlet using nets of 500 μm-mesh to prevent other fish species from entering the structures, but to allow natural zooplankton prey to enter. Drilled water (temperature: 17–18°C) was added to the system to prevent the rearing temperature from exceeding 26°C (flow: 9.01·min<sup>-1</sup> from June to mid-July and 121 min<sup>-1</sup> after mid-July). Water quality was monitored every 15 min using two oxygen-temperature probes (at the water inlet and outlet), one conductivity probe, and one turbidity probe (respectively, WTW FDO 700IQ SW, WTW TetraCon 700IQ SW, and WTW VisioTurb 700IQ SW; WTW, Weilheim, Germany). Temperature and oxygen saturation were suitable for allis shad throughout the rearing period and similar between years (means ± S.D.: temperature, 2018: 21.62°C ± 1.35°C; 2019, R1: 21.12 ± 1.98°C, R2: 21.80 ± 1.57°C and oxygen saturation, 2018: 104.99 ± 17.41%; 2019, R1: 105.84 ± 15.94%, R2: 108.96 ± 18.16). Turbidity was monitored only in 2019; it was usually <10 Formazin Nephelometric Units (FNU) during the rearing period.

Since it can be assumed that allis shad juveniles in the wild enter the saline portion of watersheds between two and four months old [9], the rearing structures were maintained under riverine-type salinity conditions during the whole rearing period.

**2.2. Biological Material.** Allis shad larvae were obtained from artificial reproductions managed by MIGADO (Association for the Restoration and Management of Migratory Fishes in Garonne, Dordogne); they were conditioned in oxygen-filled plastic bags (protocol from [18] and transported to the experimentation station). On 11 June 2018, ca. 2 000 larvae (10 days old posthatching (DPH)) were released into the artificial river R1. In 2019, five times more fishes were released: ca. 10,000 larvae (7 DPH) in R1 on 10 June, ca. 10,000 larvae (6 DPH) in R2 on 17 June.

In order not to stress such fragile fish, we chose never to capture the entire rearing population to carry out counts of individuals in the artificial rivers. Consequently, we were not able to precisely monitor the mortality of allis shad during their rearing. However, mortality kinetics were estimated in another experiment based on behavioral observations (see [19]).

**2.3. Zooplankton Production and Monitoring.** Two other concrete basins of the same dimensions were used for zooplankton production in 2018 and 2019, in order to complement the zooplankton self-produced by each artificial river. The production basins were supplied with non-filtered water from the Isle River. To enhance zooplankton production, these basins were first seeded with marine guano in April (5 g·m<sup>-3</sup>) and a complement was then added every week (0.45 g·m<sup>-3</sup>). Each night, the cultivated zooplankton was concentrated (using a light trap) and sampled by filtering ca. 20 m<sup>3</sup> of water over 7 hours (from 23:00 to 06:00) using a net of 100 μm-mesh. It was collected every

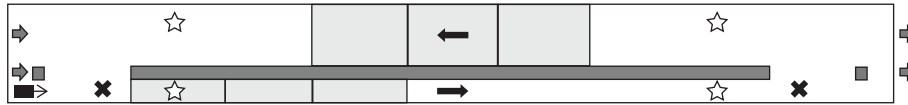


FIGURE 1: Diagram of one artificial river used for *Alosa alosa* rearing. The dark grey rectangle and squares represent concrete walls; the light grey rectangles represent the location of the artificial habitats (riffle in the narrow channel; steep slope bank in the large channel); the white and light grey areas were available for fish; the grey arrows represent the water inlet (left) and outlet (right); the black rectangle represents the propeller used to create a circular current; the black arrows represent the main direction of the water current; the black crosses represent the release points of cultivated zooplankton that were close to the probes; the white stars represent the four sampling points of zooplankton.

morning, mixed between the two zooplankton production basins, and equally distributed in each artificial river at two points (Figure 1).

To describe the zooplankton communities available to allis shad in the artificial rivers, the water was sampled every week in June and July 2018 and 2019 at noon, by twice filtering the upper 50 cm of the water column (i.e., at least 30 cm above the benthos) using a sieve of 100  $\mu\text{m}$  mesh and 20 cm diameter (corresponding to a filtered volume of 15.7 l) at four points (Figure 1, two replicates per location). The eight subsamples were concentrated, pooled in 75 ml water with 4% Lugol in plastic bottles, and conserved at 4°C. Zooplankton prey were counted and identified (at class or order level) using a stereomicroscope (Olympus SZX-12; Spach Optics, NY, USA) at  $\times 12.5$  magnification, with a Bogorov counting chamber (Hydro-Bios; Altenholz, Germany). The benthos was not sampled, as young allis shad were not supposed to feed extensively on prey from this compartment [13]. For the same reasons, biofilm and microbial production were neither monitored.

**2.4. Fish Sampling.** To describe their diet in the artificial rivers, young allis shad were captured using a trap (supporting information 1). Captures were made in 2018 at one (31 DPH) and two months old (at 59 and 63 DPH; two captures were made as the first capture was poorly efficient) at 09:00. In 2019, captures were made at similar ages, but at two schedules (in the morning and in the afternoon) (R1: 28 DPH, 09:00 and 17:00; 58 DPH, 09:00 and 15:00; R2: 27 DPH and 57 DPH, 09:00 and 17:00). All captured fishes were immediately anesthetised (bath in 25  $\text{mg}\cdot\text{l}^{-1}$  benzocaine 10%) and euthanised (250  $\text{mg}\cdot\text{l}^{-1}$  benzocaine 10%). They were measured for total length (TL) to the nearest 0.01 mm, weighted to the nearest 0.01 g (Cubis®; Sartorius, Göttingen, Germany), and rapidly conserved in 75% ethanol before dissection of their stomachs (gastric and pyloric regions); intestine contents were not analysed because the prey organisms were highly degraded. Stomach contents were analysed following the same method as for water samples. In cases of high prey degradation, only the heads were counted as prey items.

**2.5. Statistical Analysis.** Statistical analyses were made using R 3.6.1. software [20];  $p$  values  $< 0.05$  were considered significant. Differences in allis shad TL and fresh weight were assessed between years, between ages, and between rivers (in 2019) using Mann–Whitney tests or Kruskal–Wallis analysis of variance (followed by a post hoc Dunn test with

a Bonferroni correction), after rejecting parametric assumptions. The condition of allis shad during rearing was estimated for each group using the Fulton's index [21] calculated as follows:  $K = 100 \times \text{weight (in g)} / \text{TL}^3$  (in cm). Vacuity rates were calculated within each fish group as the proportion of fish with an empty stomach among the total number of individuals in the group. For the nonempty stomachs, the numbers of prey were compared between years, rivers, and ages using Kruskal–Wallis analysis of variance followed by post hoc Dunn tests with Bonferroni correction. The relative abundance (%N) of each prey taxon was calculated, in both water samples and fish stomachs, as  $\%N = n_i / n_t \times 100$  (with  $n_i$  = the number of prey of taxon  $i$  in the sample,  $n_t$  = the total number of prey in the sample). The occurrences (%O) of each prey taxon were calculated in the different allis shad groups as  $\%O = n_c / n_t \times 100$  (with  $n_c$  = the number of fish in the group that consumed the considered prey and  $n_t$  = the total number of fish in the group). Many indices can be used to assess the prey selectivity of an organism, each one having advantages and weaknesses (see [22] for a review). Here, the Ivlev's electivity index [23] was chosen because it is less sensitive to sampling bias than other indices [22]; it was calculated as  $E = (r_i - p_i) / (r_i + p_i)$ , with  $r_i$  = the abundance of the taxon  $i$  in the stomach contents and  $p_i$  = its abundance in the artificial river. The Ivlev's index ranges from  $-1$  (total avoidance of the prey) to  $1$  (exclusive preference for the prey);  $E = 0$  indicates a nonselective consumption of the prey taxon.

### 3. Results

**3.1. Fish Sizes and Condition.** For all allis shad groups, sampled individuals were significantly bigger and in better condition at two months old than at one month old (Mann–Whitney  $U$  tests,  $p < 0.001$ ; see Table 1). At one month old, allis shad reared in R1 were slightly heavier but similar in size in 2018 than in 2019, resulting in a better condition in 2018 (Kruskal–Wallis tests,  $p < 0.001$ ; post hoc Dunn tests,  $df = 2$ , weight:  $p = 0.0086$ ; TL:  $p > 0.05$ ; Fulton's index:  $p < 0.001$ ). At two months old, allis shad from R1 were heavier, larger, and in better condition in 2018 than in 2019 (Kruskal–Wallis tests,  $p < 0.001$ ; post hoc Dunn tests,  $df = 2$ , weight:  $p = 0.0086$ ; TL:  $p < 0.001$ ; Fulton's index:  $p < 0.001$ ). The individuals reared in R2 in 2019 were much smaller and in poorer condition than the two groups reared in R1 at one month old in both years (Kruskal–Wallis tests,  $p < 0.001$ ; post hoc Dunn tests,  $df = 2$ , weight:  $p < 0.001$ ; TL:  $p < 0.001$ ; Fulton's index:  $p < 0.05$ ). They were still significantly smaller at two months old (Kruskal–Wallis tests,  $p < 0.001$ ; post hoc

TABLE 1: Biometrics (TL and fresh weight), Fulton's K condition index (mean  $\pm$  SD), and vacuity rate of *Alosa alosa* captured in the rearing artificial rivers R1 and R2 at one and two months old in 2018 and 2019, with the corresponding number of individuals captured.

Age	One month old			Two months old		
	2018		2019	2018		2019
	R1	R1	R2	R1	R1	R2
Number of individuals	25	30	30	25	31	30
Total length TL (mm)	23.49 $\pm$ 1.53	22.76 $\pm$ 1.73	16.83 $\pm$ 2.37	44.25 $\pm$ 2.41	34.04 $\pm$ 3.78	22.33 $\pm$ 4.15
Weight (g)	0.06 $\pm$ 0.02	0.04 $\pm$ 0.02	0.01 $\pm$ 0.01	0.52 $\pm$ 0.09	0.21 $\pm$ 0.08	0.08 $\pm$ 0.08
Fulton's K	0.47 $\pm$ 0.09	0.34 $\pm$ 0.06	0.29 $\pm$ 0.08	0.59 $\pm$ 0.05	0.50 $\pm$ 0.05	0.62 $\pm$ 0.14
Vacuity rate (%)	12	56.7	73.3	0	0	73.3

Note that the data from R2 will be excluded from the further analysis of prey selectivity because of the very low growth of the fish and the high vacuity rate of the stomachs.

Dunn tests,  $df = 2$ , weight:  $p < 0.001$ ; TL:  $p < 0.001$ ), despite a similar or better condition (post hoc Dunn tests,  $df = 2$ , vs. year 2018, river R1: Fulton's index:  $p > 0.05$ ; vs. year 2019, river R1: Fulton's index:  $p < 0.001$ ). However, their biometrics was close to those of one-month-old fish. Although the number of fish in the artificial rivers could not be monitored, it was estimated to be 10 times lower in R2 than in R1 in 2019 when the fish were 1 to 2 months old.

**3.2. Vacuity Rates.** Vacuity rate was very variable among one-month-old allis shad (Table 1). It was low in 2018 (12.0%,  $n = 3/25$ ) but quite high in 2019: it reached 56.7% in R1 with more empty stomachs sampled in the morning (73.3%,  $n = 11/15$ ) than in the afternoon (40.0%,  $n = 6/15$ ); in R2, vacuity rate was high at both schedules (73.3%,  $n = 11/15$ ). Among the two-month-old fish, no empty stomachs were found in R1, while the vacuity rate was high in R2 (73.3%,  $n = 22/30$ ). As an indication, the few nonempty individuals sampled in the batch reared in R2 contained only 1 or 2 prey items each (cladocerans or dipteran larvae). Given this result and especially because their growth was not representative of a good development, this batch was excluded from the analysis of prey selectivity.

The numbers of prey identified in the stomachs of allis shad are presented in Figure 2, for R1 only. In both years, the prey was significantly more numerous in two months old than one-month-old individuals, and fish stomachs contained significantly more prey items in 2018 than in 2019 at both ages (Kruskal-Wallis and post hoc Dunn tests,  $p < 0.001$ ).

**3.3. Allis Shad Prey Selectivity.** The zooplankton organisms identified in the water samples were more diverse and much more abundant (up to  $\times 10$ ) in 2019 than in 2018, whereas fish density was 5 times higher in 2019 (supporting information 2). In 2019, prey items were often 2 times more abundant in R1 than in R2; for an undetermined reason, they were particularly scarce in R2 on 18 June, i.e., the day after the fish release. Nevertheless, the predominant taxon encountered was always cladocerans, generally followed by copepods (adults and nauplii), dipterans (adults and larvae), and ostracods, in variable proportions. The prey compositions of allis shad stomach contents and corresponding water samples are presented in Table 2. Stomach contents

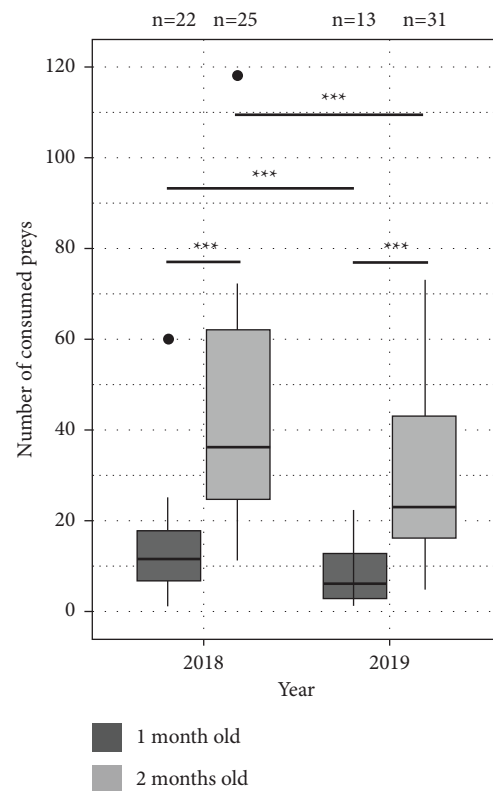


FIGURE 2: Numbers of consumed prey items counted in the stomachs of *Alosa alosa* (nonempty stomachs only) captured in the artificial river R1 in 2018 and 2019 according to their age (dark grey: one month old; light grey: two months old). The stomachs of fish from R2 were almost all empty and were thus excluded from the analysis. Corresponding numbers of fish are indicated at the top of the plot for each group. Boxplots represent median (horizontal lines), minimal, first quartile, third quartile, and maximal values; external black dots represent outliers. Significant differences between ages and years are represented at the top of the boxes (post hoc Dunn test after Kruskal-Wallis analysis of variance (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ )).

were compared with water samples taken on the closest dates (1 to 5 days apart in 2018; same dates in 2019). When allis shad were one month old, cladocerans were the predominant taxon, in the water samples (2018: %N = 66.7%; 2019: %N = 47.6%) and even more so in stomach contents (2018: %N = 73.4%; 2019: %N = 86.1%). Cladocerans were

TABLE 2: Number (N) and relative abundance (%N) of prey identified in the water samples, occurrence (%O), and mean relative abundance of prey in *Alosa alosa* stomach contents according to the age of fish (one month old or two months old) and the year (2018 or 2019).

	One month old						Two months old						
	2018		2019		2018		2019		2018		2019		
	Water	Allis shad (n = 22)	Water	Allis shad (n = 13)	Water	Allis shad (n = 25)	Water	Allis shad (n = 31)	Water	Allis shad (n = 25)	Water	Allis shad (n = 31)	
N	%N	%O	%N	N	%N	%O	%N	N	%N	%O	%N	%O	
Cladocera	28	66.67	<b>95.45</b>	50	47.62	<b>100.00</b>	47	77.05	92	49.80	69.17	96.77	<b>46.36</b>
Copepoda (adults)	—	—	<b>54.55</b>	8	7.62	23.08	1	1.64	3	6.18	2.26	<b>54.84</b>	3.45
Copepoda (nauplii)	5	11.90	—	20	19.05	—	—	—	2	—	1.50	—	—
Diptera (adults)	4	9.52	4.54	—	—	15.38	—	—	1	2.52	0.75	22.58	1.54
Diptera (larvae)	—	—	<b>72.73</b>	3	2.86	<b>38.46</b>	1	1.64	4	18.43	3.01	<b>80.65</b>	17.84
Ostracoda	—	—	18.18	14	13.33	—	5	8.20	12	22.92	9.02	<b>80.65</b>	16.28
Hydrachnidia	—	—	—	2	1.90	—	—	—	4	—	3.01	25.81	1.58
Coleoptera	1	2.38	—	—	—	—	—	—	—	—	—	3.23	0.20
Terrestrial	—	—	—	1	0.95	7.69	5	8.20	1	—	0.75	3.23	0.27
Exuviae	4	9.52	—	2	1.90	—	1	1.64	2	—	1.50	—	—
Trichoptera (larvae)	—	—	—	—	—	—	1	1.64	—	0.16	—	—	—
Nematoda	—	—	—	1	0.95	—	—	—	—	—	—	—	—
Rotifera	—	—	—	4	3.81	—	—	—	12	—	9.02	12.90	2.65
Isopoda	—	—	—	—	—	—	—	—	—	—	—	<b>45.16</b>	7.30
Amphipoda	—	—	—	—	—	—	—	—	—	—	—	6.45	0.21
Gastropoda	—	—	—	—	—	—	—	—	—	—	—	3.23	2.32

Note: Terrestrial prey refers to nonaquatic prey items that fell into the artificial rivers. Results are indicated for all prey taxa sampled, whether in water or in fish. Values >33% calculated in fish stomach contents are marked in bold. The dates of water sampling and *Alosa alosa* capture are indicated for each group, along with the numbers of fish analysed (nonempty stomachs only, river R1 only).

identified in 95% of individuals on both years. The two other taxa highly consumed by one-month-old allis shad were dipteran larvae and adult copepods in both years, whereas these taxa were poorly present in the water samples in 2019 (%N=2.9% and 7.6%, respectively) and absent in 2018. Conversely, copepod nauplii and diverse exuviae were never consumed by fish at this age, even though they were relatively abundant in the water samples. No strictly benthic prey was observed in the stomach contents at this age.

When allis shad were two months old, cladocerans were still the predominant taxon in both water samples (2018: %N=77.0%; 2019: %N=69.2%) and fish (2018: %N=49.8%; 2019: %N=46.4%), occurring in almost all individuals in both years (%O>96.8%). Ostracods were present in substantial proportions in both fish (2018: %N=22.9%; 2019: %N=16.3%) and water samples (2018: %N=8.0%; 2019: %N=9.0%). Compared with ostracods, dipteran larvae represented a similar proportion of allis shad diet (2018: %N=18.4%; 2019: %N=17.8%), although they were scarcer in water samples (%N≤3.0%). Benthic prey were found in some allis shad stomachs, especially in 2019 (see Table 2).

The Ivlev's index was null (0.00) for cladocerans regarding their abundance in the environment and their consumption by one-month-old fish in 2018, but it was positive (0.27) in 2019; for all other prey taxa, negative values of Ivlev's index were obtained (see Table 3). When the fishes were two months old, in 2018, the Ivlev's index was positive for adult copepods (0.25) and ostracods (0.34), and especially for dipteran larvae (0.72), while it was negative for all other taxa, including cladocerans (-0.24). In 2019, positive values of Ivlev's index were only obtained for ostracods (0.04) and dipteran larvae (0.31). However, some variability appeared among individuals in this group of fish: adult copepods were consumed in large numbers by a notable proportion of allis shad, although the mean value of the Ivlev's index was negative (-0.22); adult dipterans, hydrachnids, and rotifers were rarely identified in stomach contents, but some fish stomachs contained many of these prey items.

#### 4. Discussion

In the two years of this experiment, young allis shad were reared under similar conditions, with a trophic environment consistent between years, at least in terms of composition of zooplankton communities. However, the quantities of potential prey differed between years and between rivers in 2019 (supporting information 2), perhaps because of variations in luminosity or competition between zooplankton taxa. In 2019, on the first day of rearing, 7 times more zooplankton (and 23 times more cladocerans) were sampled in the river R1 than in R2, whereas fish densities were similar (ca. 10 000 individuals). Fishes from R2 were always smaller and could not be used for diet analysis since their stomachs were mostly empty. Thus, these individuals may have suffered from limiting feeding conditions, which may have led to high mortality rates in R2 during the first days of rearing and would explain the early difference in fish density between R1 and R2.

TABLE 3: Mean values of Ivlev's electivity index (E) calculated for each prey taxon in *Alosa alosa* individuals captured at one month old or two months old in the river R1, in 2018 and 2019.

	One month old		Two months old	
	2018	2019	2018	2019
Cladocera	0.00	<b>0.27</b>	-0.24	-0.24
Copepoda (adults)	*	-0.68	<b>0.25</b>	-0.22
Copepoda (nauplii)	-1.00	-1.00		-1.00
Diptera (adults)	-0.96	*	*	-0.61
Diptera (larvae)	*	-0.37	<b>0.72</b>	<b>0.31</b>
Ostracoda	*	-1.00	<b>0.34</b>	0.04
Hydrachnidia		-1.00		-0.69
Coleoptera	-1.00			*
Terrestrial		-0.85	-1.00	-0.94
Exuviae	-1.00	-1.00	-1.00	-1.00
Trichoptera (larvae)			-0.94	
Nematoda		-1.00		
Rotifera		-1.00		-0.84
Isopoda				*
Amphipoda				*
Gastropoda				*

Sometimes, prey taxa were absent from the water sample, but observed in fish: these cases are marked with a \*symbol. Ivlev's E indicates an exclusive preference when close to 1, a nonselective consumption when close to 0, and a total avoidance of prey when close to -1. Significant preferences are highlighted in bold, and significant avoidances are highlighted in italic.

In the river R1, where prey was supposed to be quite abundant, fishes with empty stomachs were sampled at one month old in both years, and especially in 2019. One-month-old fishes may be less efficient at foraging than older individuals; in addition, competition may have been higher in 2019 than in 2018 in R1, since the initial fish density was 5 times higher and zooplankton was "only" 2 to 5 times more abundant on similar dates (supporting information 2). Nevertheless, the composition of the diet of one-month-old allis shad did not seem to be influenced by fish density: globally in our structures, the selectivity of one-month-old fishes was positive or neutral for cladocerans (mainly consuming the genera *Bosmina*, *Scapholeberis*, *Eurycercus*, and *Camptocercus*) whereas they avoided all other taxa (see Table 2). At one month old, allis shad have a small mouth aperture ( $2.16 \pm 0.23$  mm in 2018;  $2.21 \pm 0.29$  mm in 2019 in average) and limited swimming capacities: thus, cladocerans might have been preferred because they are the only well-sized (0.2–6 mm), easy-to-catch organisms, whereas copepods, for example, are usually larger (2–10 mm) and can exhibit a fast flight response [24, 25].

In our experiment, two-month-old allis shad avoided cladocerans (although these were generally the dominant taxon sampled in the water column), and conversely, they selectively consumed dipteran larvae (mainly Chironomidae), adult copepods, and/or ostracods (although these taxa were not particularly more abundant in the water samples). Moreover, some individuals consumed hydrachnids, coleopterans, and rotifers, as well as benthic taxa (trichopteran larvae, isopods, amphipods (mainly Gammaridae), and gastropods) (see Tables 2 and 3). This suggests that allis shad would enlarge their diet throughout ontogeny and require other prey to maintain their energetic condition.

Unfortunately, we did not sample the benthic compartment and cannot assess whether allis shad effectively caught prey on the substratum (although attempts to catch food from the substratum or from the walls of the artificial rivers were identified during behavioral observations) (see [19]).

It is assumed that prey consumption by fishes is highly size-dependant (e.g., [26, 27]). In our experiment, the sizes of the consumed prey were uncertain because they were degraded in allis shad stomachs, but they were usually larger in the stomachs of two-month-old fishes than in those of one-month-old fishes (estimates: 200–7000  $\mu\text{m}$  length, <1400  $\mu\text{m}$  diameter vs. 160–2000  $\mu\text{m}$  length, <950  $\mu\text{m}$  diameter; data not shown). However, some large individuals fed only on small prey organisms (e.g., ostracods, rotifers, and hydrachnids), but in high numbers. Thus, the evolution of the diet of allis shad during ontogeny would not only be related to the size of prey but also to the increase in both their energy demands and their swimming capacities, enticing and allowing them to feed on more mobile but more energetic organisms (such as copepods, which are enriched in unsaturated fatty acids, which are essential for growth, see [28]).

In terms of prey taxa identified in stomach contents, our results are consistent with those obtained by Cassou-Leins et al. [13] on wild allis shad of 20–50 mm in size in the Garonne River or with other observations made on larger allis shad in a different environment [14, 15]. Our results are also consistent with several works qualitatively describing the diet of American shad *A. sapidissima* juveniles at similar ages, although selectivity among prey has not been highlighted by all authors [29–33], and with studies devoted to the diet of the twaite shad *A. fallax*, although this species may be able to feed on larger and more active prey at earlier ages [34, 35]. Consequently, allis shad and twaite shad could possibly compete for food during their freshwater phase since they might occur in sympatry in some watersheds if allis shad spawning areas are constrained downstream by a dam [16].

The present experiment refutes the opportunistic character of juvenile allis shad feeding: it suggests a clear prey selectivity at the two ages tested and a difference in diet between these ages. This highlights the importance of abundant and adequate prey for allis shad juveniles during their development in freshwater. In fish populations, a match between adult spawning phenology and a peak in abundance of suitable zooplankton prey for juveniles improves the efficiency of recruitment [7, 17]. For allis shad in the Gironde-Garonne-Dordogne watershed, spawning can be observed from April to July [36], with a possible influence of both water temperature and river discharge [37]. Besides, in the Gironde estuary, zooplankton communities may shift in quantity and composition, notably because of thermal variability [38, 39]. Hence, a risk of mismatch may exist for allis shad, especially from their first feeding up to one month old, when their small size seems to restrict their diet to cladocerans. Moreover, they have only a few days of yolk reserve to join suitable feeding areas and start feeding on exogenous prey, which may not be sufficient if temperature and/or flow regimes are not suitable at this very moment.

This could represent an ecological bottleneck for the species and have a serious impact on the recruitment of populations, as it has been suggested for the young stages of Northern American alosine species [40].

Further analyses would be of interest in order to verify some assumptions, notably by assessing the prey selectivity of allis shad at earlier ages. More information on the composition of the zooplankton communities occurring in natural rivers during allis shad freshwater stay would be of particular interest to evaluate the suitability of the trophic environment for local populations and discuss the efficiency of their recruitment.

### Data Availability

All data used for this work are available either in the article body or in its supplementary material.

### Disclosure

An earlier version was submitted as thesis titled “Analyse de l’écologie des jeunes stades de grande alose (*Alosa alosa*, Clupeidae): Préférences écologiques, sensibilité aux pressions environnementales et restauration des populations.”

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### Authors’ Contributions

L. B. performed experimental design, was responsible for technical aspects, performed data generation, performed data analysis, and prepared the manuscript. A.A. was responsible for technical aspects, data generation, and data analysis. J. V. contributed to data generation and technical support. J. P. performed experimental design, was responsible for technical aspects and rearing routine, and performed data generation. S. M. contributed to data generation and technical support. W. B. performed production of biological material and contributed to project conception. A. B. performed project conception, contributed to experimental design, performed data analysis, prepared the manuscript, and was a PhD supervisor. M. L. A. performed project conception, contributed to experimental design, to rearing process, and to data generation, performed data analysis, prepared the manuscript, and was a PhD supervisor. Agnès Bardonnnet and Marie-Laure Acolas contributed equally to this work.

### Acknowledgments

The authors would like to thank the MIGADO Association for providing the biological material used in this work. The authors thank INRAE and the Adour-Garonne Water Agency for the grant funding of L. Baumann PhD. The authors thank J. Vega for helping in fish capture and biometrics. The authors are grateful to R. Le Barh, L. Jacobs, P. Chèvre, and F. Bouin for technical support and all the staff of the INRAE experimentation station for their contribution



to routine maintenance. This work was part of Loïc Baumann PhD and Adrien Aries MSC funded by the Adour Garonne Water Agency and INRAE. It was included in the Shad'eau project aiming at understanding the possible causes of the population decline of shads, especially in South West France, to help future restoration and conservation management.

## Supplementary Materials

A diagram of the device used to capture allis shad juveniles in the rearing artificial rivers is available in supporting information 1. Raw data for counting and identifying potential prey items in water samples are available in additional information 2. (*Supplementary Materials*)

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