

Communication

# Feeding Ecology of *Sicydium bustamantei* (Greeff 1884, Gobiidae) Post-Larvae: The “Little Fish” of São Tomé Island

Vânia Baptista <sup>1,\*</sup>, Ester Dias <sup>2</sup>, Joana Cruz <sup>1</sup>, Maria Branco <sup>3</sup>, Sara Vieira <sup>3</sup> and Maria Alexandra Teodósio <sup>1</sup>

<sup>1</sup> Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; jmcruz@ualg.pt (J.C.); mchichar@ualg.pt (M.A.T.)

<sup>2</sup> Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Terminal de Cruzeiros do Porto de Leixões, 4450-208 Matosinhos, Portugal; esterdias@ciimar.up.pt

<sup>3</sup> Associação Programa Tatô—Sítio da Pedragosa, 8600-013 Barão de São João, Portugal; mariaacbr@hotmail.com (M.B.); saralexvieira@gmail.com (S.V.)

\* Correspondence: vcbaptista@ualg.pt

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**Abstract:** The rivers of São Tomé Island are colonized by *Sicydium bustamantei* (Greeff 1882), an amphidromous fish that spawns in those areas. After hatching, larvae drift to the ocean with the river flow. In the marine realm, the planktonic larvae develop and migrate to freshwater as post-larvae. The migrations of post-larvae support important local fisheries at the mouth of rivers in tropical volcanic islands. Amphidromous post-larvae rely on plankton as their main source of organic matter. However, the biology and ecology of *S. bustamantei* in the West African islands are understudied, despite its importance for local fisheries. Thus, this study aimed to start bridging this gap by studying its feeding ecology. Our objectives were to identify the main prey of *S. bustamantei* post-larvae, combining gut content with stable isotope analyses. The gut contents included zooplankton (Chaetognatha, Ostracoda, and unidentified crustaceans), debris from plant and/or macroalgae-derived material, and microplastics (including microfibers). The stable isotopes analysis indicated that zooplankton and macroalgae detritus were the main sources of organic matter assimilated by this species. We also demonstrated that *S. bustamantei* post-larvae are omnivorous and secondary consumers. These data provide pioneering information that can be used in management plans that still need to be developed.

**Keywords:** Eastern Atlantic; isotopic analysis; gut contents; zooplankton; microplastics

## 1. Introduction

Amphidromy is a type of diadromy that requires freshwater-marine connectivity in the early stages of a species life cycle [1,2]. Amphidromous species such as gastropods, decapods, and fish are adapted to tropical and subtropical insular environments [2–5].

The Sicydiinae subfamily (Gobiidae) is typically associated with small oceanic islands with geologically recent volcanic origin [6–8]. They inhabit swift, high-gradient rainforest streams and rivers with large waterfalls and elevation changes [3] which are generally oligotrophic and exposed to extreme hydrological and climatic seasonal variability [3,8]. Sicydiinae fish are found in the Indo-Pacific, West Africa, Central America, and Caribbean regions [3,6–9] and have relatively high levels of endemism [6]. Adults spawn in freshwater and larvae drift seawards upon hatching (downstream migration), where they undergo a planktonic phase [3,6,7]. After several months at sea (one to nine

months, depending on the species) [3,5,10,11], post-larvae recruit and migrate to rivers (upstream migration or return migration) where they settle, grow, and reproduce [3,6,7].

Post-larvae support significant artisanal fisheries during the return migration (goby-fry fisheries), with significant nutritional, cultural, and socio-economical value in developing tropical and sub-tropical countries [3,5,7,8,11]. Returning post-larvae can be caught using beach seine nets made from mosquito nets or traps (baskets) made from vegetable fibers [12,13]. Globally, goby-fry fisheries are declining due to the degradation and loss of suitable habitat and river–ocean connectivity due to instream barriers (e.g., channelization, riverine and coastal zone development), and overfishing [7,12,14,15]. Goby-fry fisheries are largely unmanaged, with insufficient biological and fishery data [7,15], albeit some species are listed as endangered [12].

In São Tomé island (São Tomé and Príncipe archipelago), *Sicydium bustamantei* (Greeff 1882), called “peixinho” (little fish), is caught as post-larvae in several rivers (e.g., the Io-Grande, Manuel Jorge, Malanza, and Ouro Rivers) and sold in local fish markets. This species is one of the main sources of income and protein for these communities. It has been found in several islands across the Gulf of Guinea (West Africa)—namely, Bioko, São Tomé, Príncipe, and Annobón [9,16–18]. Little is known about the biology and ecology of this species, particularly during the return migrations of early life-cycle stages. However, according to local knowledge in São Tomé, *S. bustamantei* forms shoals at the mouth of rivers. Here, fish are caught with baskets, mosquito nets, or even with cloths. The post-larvae are caught throughout the year, but mainly in the dry season during the full and new moon periods. These descriptions coincide with the scientific information available elsewhere for other Sicydiinae species [5,8,11,13,19].

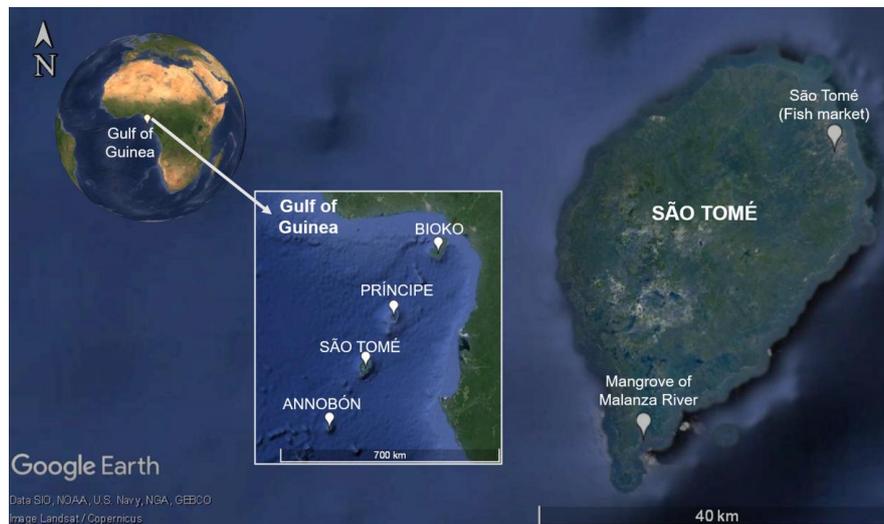
The IUCN (International Union for Conservation of Nature) has not yet attributed a conservation status to *S. bustamantei* due to insufficient scientific data [20] and, given its importance to many human populations across the species distribution range, it is necessary to obtain scientific data to start implementing sound management plans. We opted to start studying the food web ecology of this species, as well as the prevalence of microplastics in their diet. There are two main reasons for this decision. First, food web ecology discloses the relationship patterns between species on the multidimensional mosaic of habitats where they live. This is especially true for migratory species that move across ecosystems and serve as links and conduits of energy between the land and the ocean. Second, the prevalence of microplastics off São Tomé and Príncipe is unknown but likely high when considering the high levels of plastic pollution in beaches. Studying microplastic pollution is relevant because the contaminants sorbed into it or that are incorporated in the microplastics may dysregulate the physiological processes of the animals that accumulate it in their organisms [21]. These contaminants may be transferred and accumulated throughout the food web, impacting the health of multiple species, including humans [21]. Thus, our specific objective was to identify the main food sources consumed and assimilated by *S. bustamantei* post-larvae in the Gulf of Guinea, using the population of São Tomé island (São Tomé and Príncipe) as a model population. For that, we combined gut content analysis with carbon ( $\delta^{13}\text{C}$ :  $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ :  $^{15}\text{N}/^{14}\text{N}$ ) stable isotope analysis. The gut content analysis also provided the first assessment of the seriousness of microplastic pollution in São Tomé and Príncipe and its prevalence in the guts of such an important species for the people of this country.

## 2. Materials and Methods

### 2.1. Study Area and Collection of Samples

The Democratic Republic of São Tomé and Príncipe includes two islands, São Tomé and Príncipe, that form an archipelago with the Bioko and Annobón islands (Equatorial Guinea) in the Gulf of Guinea. São Tomé island (Figure 1) is a volcanic island with a high relief, located about 150–200 km off the west coast of Africa, and is the second-largest island (859 km<sup>2</sup>) of the archipelago [22].

*Sicydium bustamantei* post-larvae were acquired in the city of São Tomé fish market (caught mostly in the southern part of the island) and caught in the Mangrove of Malanza River (in South of São Tomé) in January 2017 and August 2017, corresponding to the wet season (October to May) and dry season (June to September), respectively (Figures 1 and 2). Samples were preserved in ethanol 96% and later identified as *S. bustamantei*, an endemic gobiid in the region of Gulf of Guinea, with the help of Dr. Peter Wirtz (independent researcher). Since samples from the fish market included multiple species, we separated and quantified the individuals by taxonomic groups. The total length of larvae (TL;  $\pm 0.01$  mm) was measured based on photographs taken under a stereomicroscope (Leica 58APO, coupled with a Leica MC170 HC camera) and using Image J (v1.50i). The standard deviation was used as a measure of data dispersion in this paper. A *t*-test was used to analyze the differences in total length between the wet and dry seasons. The analysis was carried out using the R 3.5.3 statistical software, with the level of significance set at  $p \leq 0.05$ .



**Figure 1.** Sampling locations of *Sicydium bustamantei* (Greeff 1882) post-larvae collected in São Tomé Island in 2017. Maps retrieved from Google Earth Pro.



**Figure 2.** *Sicydium bustamantei* (Greeff 1882) post-larvae collected in the island of São Tomé (São Tomé and Príncipe) in 2017.

## 2.2. Gut Content Analysis

The diet of *S. bustamantei* post-larvae was determined by analyzing the guts of 30 individuals collected in each season. The gut contents were exposed after dissecting the abdomen with fine needles and identified under a stereomicroscope (Leica 58APO) and an inverted microscope (Zeiss MB). The prey items were identified to the lowest taxonomic level possible. The presence of microplastics and microfibers was also recorded.

The incidence of food items was calculated as the percentage of post-larvae with at least one prey item in their guts. A chi-square test was used to compare the incidence of each food item between the wet and dry seasons. The analysis was carried out using the R 3.5.3 statistical software, with the level of significance set at  $p \leq 0.05$ .

The graphical method proposed by Costello [23] and modified by Amundsen et al. [24] was used to analyze the feeding strategy of *S. bustamantei* post-larvae. Individuals with no gut content were excluded from the analysis. Briefly, each point in the plot corresponds to the frequency of occurrence (i.e., the percentage of guts with a specific prey item) and prey specific abundance (i.e., the percentage of a prey taxon in relation to all prey items in the guts in which this prey was present). The importance of prey and feeding strategy were inferred by examining the points' distribution along the axes in the plot.

## 2.3. Stable Isotope Analyses

The main sources of organic matter assimilated by post-larvae were identified and quantified using carbon ( $\delta^{13}\text{C}$ :  $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ :  $^{15}\text{N}/^{14}\text{N}$ ) stable isotopes. We analyzed five individuals collected during the wet season. Samples were also collected during the dry season, but due to visible signs of deterioration after collection they were not included in the analysis. The potential prey were collected near the mouth of the Malanza River on the south coast of São Tomé island also during the wet season (January 2017) and included zooplankton (Chaetognata *Pterosagitta draco* (Krohn 1853), Copepoda Calanoida *Candacia* spp. (Dana 1846), and Siphonophora *Muggiaea kochii* (Will 1844)), macroalgae (*Caulerpa* sp. (Lamouroux 1809) and *Dictyota* sp. (Lamouroux 1809)), tree leaves (Cocoseae (Linnaeus 1753), *Terminalia catappa* (Linnaeus 1767), *Pandanus thomensis* (Henriques 1887), and *Rhizophora mangle* (Linnaeus 1753)), and seagrass (*Halodule wright* (Asch 1868)). Zooplankton were collected using a plankton net with a mesh size of 500  $\mu\text{m}$ . Macroalgae and seagrasses were collected in the intertidal and subtidal areas through freediving. Tree leaves were hand collected on the beaches near the mouth of the Malanza River.

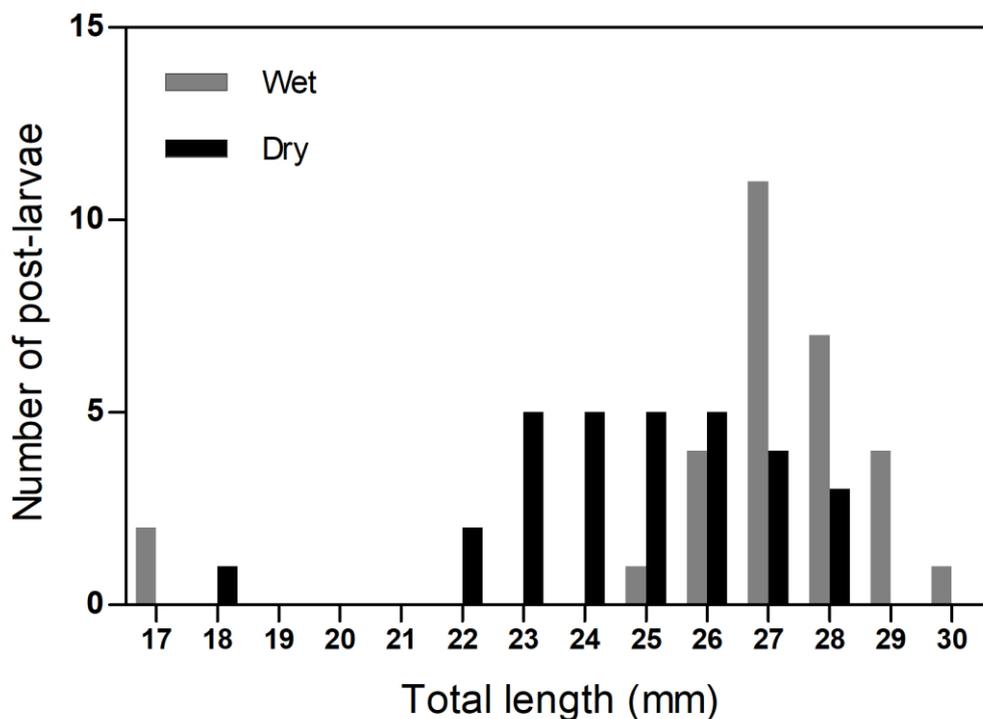
Samples were cleaned with deionized water, oven-dried at 60 °C for at least 48 h, and ground to a fine and homogenous powder using a mortar and pestle (animals) or a mixer mill (plants and macroalgae). Stable isotope ratios were measured using a Thermo Scientific Delta V Advantage IRMS via Conflo IV interface (Marinova, University of Porto). The raw data were normalized by three-point calibration using international reference materials, such as IAEA-N-1 ( $\delta^{15}\text{N} = +0.4\text{‰}$ ), IAEA-NO-3 ( $\delta^{15}\text{N} = +4.7\text{‰}$ ), and IAEA-N-2 ( $\delta^{15}\text{N} = +20.3\text{‰}$ ) for the nitrogen isotopic composition, and two-point calibration using USGS-40 ( $\delta^{13}\text{C} = -26.39\text{‰}$ ) and USGS-24 ( $\delta^{13}\text{C} = -16.05\text{‰}$ ) for the carbon isotopic composition. Stable isotope ratios were reported in  $\delta$  notation,  $\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$ , where X is the C or N stable isotope, and R is the ratio of heavy/light stable isotopes. Vienna Pee Dee Belemnite and air are standards for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. The analytical error, the mean standard deviation of the replicate reference material, was  $\pm 0.1\%$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . The zooplankton and *S. bustamantei* post-larvae  $\delta^{13}\text{C}$  values were corrected for lipid content [25], and the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were corrected for ethanol preservation [26].

To identify and quantify the contribution of the most likely food sources to the *S. bustamantei* post-larvae biomass, we combined biplot analysis (post-larvae  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were adjusted for trophic fractionation [27]), with the results from the dual-stable isotope mixing model produced by SIAR (Stable Isotope Analysis in R) [28,29]. This mixing model uses Bayesian inference to solve the indeterminate equations (more than  $n + 1$  sources relative to  $n$  stable isotopes) and produces a

probability distribution that represents the likelihood a given source contributes to the consumer biomass [28]. The model also allows each of the sources and the trophic fractionation (TEF; or trophic enrichment factor) to be assigned as a normal distribution [28]. SIAR produces a range of feasible solutions to the mixing problem to which are assigned credibility intervals (CIs) (in this study, 95% CI) [28]. SIAR also includes a residual error term. For the SIAR mixing model, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were adjusted for one trophic level using the trophic fractionation estimates from Vander Zanden and Rasmussen [27] ( $+0.47 \pm 1.23\text{‰}$   $\delta^{13}\text{C}$ ,  $+3.40 \pm 0.41\text{‰}$   $\delta^{15}\text{N}$ ).

### 3. Results and Discussion

Post-larvae collected during the wet season were larger than those collected during the dry season ( $t(29) = 67.08$ ,  $p < 0.001$ ). The total length of the *S. bustamantei* post-larvae varied between 17 and 30 mm ( $26.7 \pm 2.9$  mm) in the wet season and between 18 and 28 mm ( $24.8 \pm 2.2$  mm) in the dry season (Figure 3). These values are within the range described for the total length of post-larvae of other species of Sicydiinae during their return migrations (recruitment) [3,7].



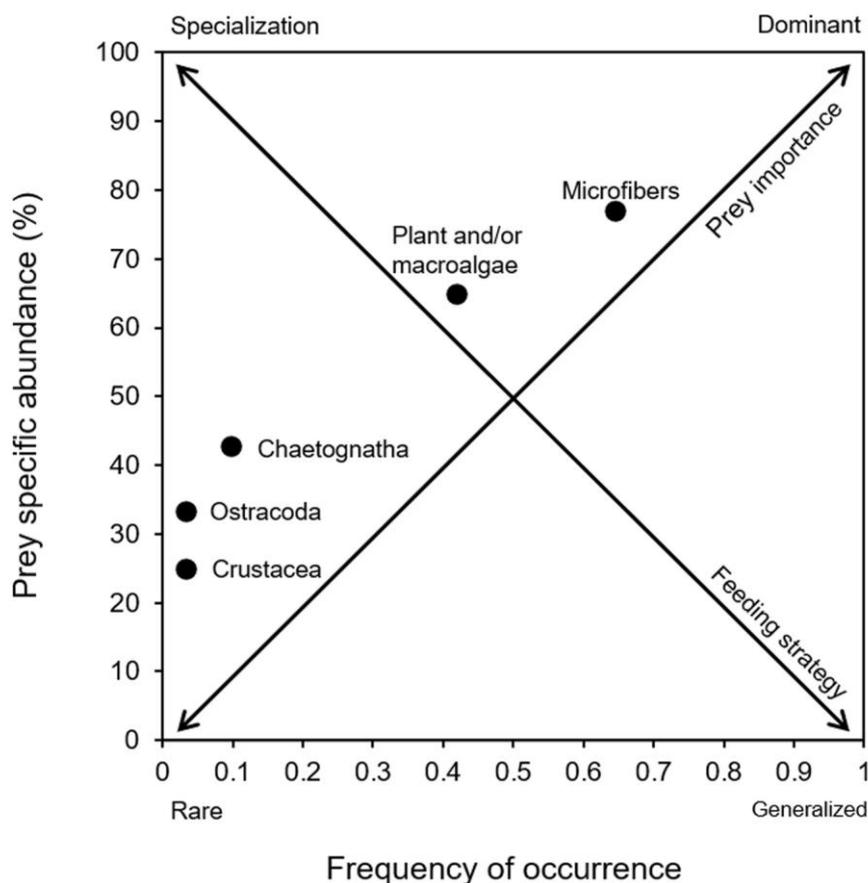
**Figure 3.** Histogram of the total length (mm) of *Sicydium bustamantei* (Greeff 1882) post-larvae collected during the wet ( $n = 30$ ) and dry ( $n = 30$ ) seasons of 2017 in the island of São Tomé (São Tomé and Príncipe).

The feeding incidence was higher during the wet season (53.3%) than during the dry season (20.0%) (Table 1). This may be due to the fact that runoff is higher during the wet season than during the dry season, which increases the downstream transport of food and nutrients from upriver to the estuaries/mangroves and adjacent coastal areas, consequently increasing food availability [30].

**Table 1.** Total number of *Sicydium bustamantei* (Greeff 1882) post-larvae guts examined and guts with food items; feeding incidence (%); and incidence of plant and/or macroalgae, zooplankton, microplastics, and microfibers (%). Samples were collected during the wet and dry seasons of 2017 in the island of São Tomé (São Tomé and Príncipe).

Seasons	Examined Guts (N)	Guts with Food (N)	Feeding Incidence (%)	Plant and/or Macroalgae Incidence (%)	Zooplankton Incidence (%)	Microplastic and Microfiber Incidence (%)
Wet	30	16	53.3	46.7	16.7	20.0
Dry	30	6	20.0	20.3	0.0	23.3

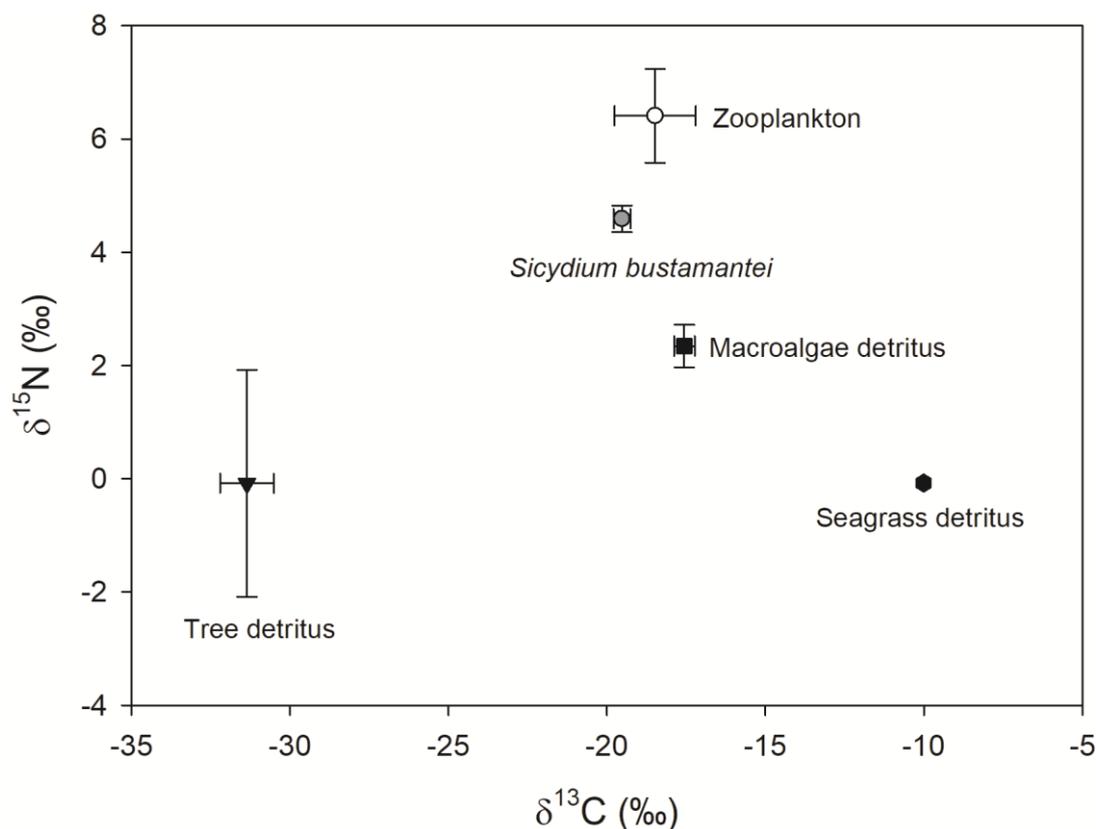
The incidence of each food was not statistically different between seasons ( $\chi^2(2) = 4.98, p = 0.082$ ). However, zooplankton (16.7%), such as Chaetognatha, Ostracoda, and unidentified crustaceans, were only observed in the guts of post-larvae during the wet season. Most of the gut contents consisted of plant and/or macroalgae detritus (46.7% in the wet season and 20.3% in the dry season) and microplastics/microfibers (20.0% and 23.3% in the wet and dry seasons, respectively) (Table 1). Thus, *S. bustamantei* post-larvae showed a specialist food strategy [22,23], feeding on a dominant prey taxon—vascular and/or macroalgae-derived material, and occasionally on small proportions of other prey types (rarest items)—zooplankton (Figure 4).



**Figure 4.** Frequency of occurrence and prey-specific abundance (%) present in the gut contents of *Sicydium bustamantei* (Greeff 1882) post-larvae collected during the wet and dry seasons of 2017 in the island of São Tomé (São Tomé and Príncipe), and plotted according to the Costello method [23] modified by Amundsen et al. [24].

The stable isotope values from *S. bustamantei* post-larvae, after being corrected for trophic fractionation, indicate that they assimilated <sup>15</sup>N- and <sup>13</sup>C-enriched sources, such as zooplankton and

macroalgae detritus, and also tree detritus (Figure 5). In fact, based on the SIAR mixing model (95% CI), zooplankton was the source with the highest relative contribution to the *S. bustamantei* post-larvae biomass during the wet season, varying between 0.42 and 0.71, followed by macroalgae detritus (0.16–0.50) and tree detritus (0.02–0.18) (Table 2).



**Figure 5.**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (‰) (mean  $\pm$  SD) of *Sicydium bustamantei* (Greeff 1882) post-larvae and potential food items collected during the wet season of 2017 in the island of São Tomé (São Tomé and Príncipe). Stable isotope values for *S. bustamantei* are corrected for trophic fractionation (0.47‰  $\delta^{13}\text{C}$ , 3.40‰  $\delta^{15}\text{N}$ ; Vander Zanden and Rasmussen [27]).

**Table 2.** Relative contribution of each food source to the biomass of *Sicydium bustamantei* (Greeff 1882) post-larvae collected during the wet season of 2017 in the island of São Tomé (São Tomé and Príncipe). The mode values represent the most likely value, and the low 95% and high 95% values represent the 95% Bayesian credibility intervals calculated by a dual-stable isotope mixing model produced by SIAR (Stable Isotope Analysis in R) [28,29].

Food Items	Low 95%	High 95%	Mode
Zooplankton	0.42	0.71	0.56
Macroalgae detritus	0.16	0.50	0.35
Tree detritus	0.02	0.18	0.10

The stable isotopes and gut content analyses showed different results for the relative contribution of each food source during the wet season. While the gut contents were mainly composed of vascular and/or macroalgae-derived material, zooplankton was the source with the highest relative contribution to the post-larvae biomass. Detritus is not the main source of energy for most aquatic organisms [31], because it is less likely to be assimilated than animal-derived material [32,33]. Moreover, because we collected zooplankton using a net with 500  $\mu\text{m}$  mesh size, small-sized zooplankton such as Ostracoda were not included in the stable isotope analysis. However, this would probably not change the main

conclusions about the contribution of zooplankton to post-larvae biomass. We expect that larger zooplankton, such as the carnivore Chaetognatha, will present higher  $\delta^{15}\text{N}$  values than Ostracoda (or other small-sized zooplankton), which feed on phytoplankton and detritus. Because we do not know the origin of the basal sources that support their biomass (pelagic or benthic), we cannot speculate about the potential differences in their  $\delta^{13}\text{C}$  values. Thus, if Ostracoda had lower  $\delta^{15}\text{N}$  values than the one estimated for zooplankton, the contribution of zooplankton to the *S. bustamantei* post-larvae would likely increase.

Our data indicate that *S. bustamantei* post-larvae are secondary consumers and omnivorous during their pelagic phase. They feed on zooplankton, as reported for other Sicydiinae species [34,35], and on plant/macroalgae detritus. During recruitment, metamorphosis occurs and modifications to anatomical feeding structures lead to a change in the *S. bustamantei* diet, from being a carnivorous fish feeding on plankton to an herbivorous fish feeding on the benthos [3,5,34]. This may explain the omnivory of this species during the post-larval phase. Although the number of samples analyzed was small to draw firm conclusions about the foraging habitat of post-larvae, the fact they showed high  $\delta^{13}\text{C}$  values so close to those from marine zooplankton and macroalgae indicate that these fish spent part of their life in the marine environment before moving to freshwater streams, as described for other Sicydiinae post-larvae [36]. Still, other studies have reported that the biomass of recruiting amphidromous fishes have an inshore signature typical of environments influenced by freshwater. This suggests that *S. bustamantei* post-larvae can be retained temporarily in the freshwater plumes of rivers while waiting for the appropriate conditions to start the return migration [37,38].

Large amounts of microplastics/microfibers (20.0–23.3%) were found inside the guts of *S. bustamantei*, along with zooplankton and vascular and/or macroalgae-derived material (Table 1). Unfortunately, large amounts of plastic litter lay on the beaches of São Tomé, some of which will break into microplastics. The ingestion of microplastics by fish larvae has been associated with a decrease in growth rates, changes in feeding preferences, innate behavior, swimming behavior, response to olfactory cues, and increasing mortality [39,40]. Thus, plastic pollution may also increment the deleterious effect of overfishing upon several populations of this and other marine species across the Gulf of Guinea. It has been globally reported that amphidromous fishes suffer many anthropogenic threats beyond overfishing—namely, water abstraction, degradation and loss of suitable habitat and connectivity due to instream barriers, and pollution [1,7,12,14,15,41,42]—with consequences to their physiology, reproduction, and migration patterns between freshwater and marine coastal areas [13]. In fact, Bell [7] considered these land-use threats to be more likely to cause population declines than overfishing.

In samples collected during both wet and dry seasons, *S. bustamantei* post-larvae corresponded to 80% of the total biomass, while 20% included small crustaceans (19.4%) and other non-identified fish species (0.6%). As described for other countries, the goby-fry fishery in São Tomé and Príncipe is not selective and it is not regulated—i.e., when local people find fish schools they catch as much as they can. Small crustaceans (e.g., isopods and decapods) and post-larvae and juveniles of other fish species are commonly found performing upstream migrations together with *S. bustamantei* post-larvae. This has been observed for other Sicydiinae species, and by-catch is often discarded during goby-fry fishery [19,41].

#### 4. Conclusions

*Sicydium bustamantei* is a secondary consumer with an omnivorous diet during the post-larval phase and is not exclusively carnivorous, as described for other species of the same genus. Additionally, large amounts of microplastics/microfibers were ingested by post-larvae. Plastic pollution may cause detrimental impacts on the conservation status of this species and not only its overexploitation. Thus, the silent health risk problem that microplastic pollution may cause to humans through the consumption of *S. bustamantei*, in tandem with the ecological and economic importance of this species, represents another compelling reason to undertake a critical long-term monitoring program to assess the conservation status of the species.

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