Lindsey McDonald

ECOPHYSIOLOGICAL ASSESSMENT OF THE LITTLE FISH OF SÃO TOMÉ ISLAND USING RNA:DNA RATIOS

UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologia

2024

Lindsey McDonald

ECOPHYSIOLOGICAL ASSESSMENT OF THE LITTLE FISH OF SÃO TOMÉ ISLAND USING RNA:DNA RATIOS

Master in Marine Biodiversity, Conservation and Fisheries

Supervisor:
Vânia Baptista, PhD
Co-supervisor:
Joana Cruz, PhD

UNIVERSIDADE DO ALGARVE
Faculdade de Ciências e Tecnologia

2024

Declaration of authorship of the work

I declare to be the author of this work, which is original and unprecedented. Consulted authors and works are rightfully cited in the text and are included in the list of references.

Faro, September 2024

_____

Lindsey McDonald

Acknowledgements

I would like to thank the University of the Algarve and Algarve Centre of Marine Sciences (CCMAR) for the support they provided and for the opportunity to contribute to this research. Thank you to the ECOREACH lab and my supervisors, Vânia Baptista, PhD for making me a part of the "LittleFish-STP" project's research goals, guiding me through lab techniques and supporting me throughout the process. My thanks to Joana Cruz, PhD for her help with planning out my analysis, guidance with statistics in R and her overall cheerful support. Alizée Jamont gave me an idea of where to start with statistics for which I'm very grateful. Finally, heartfelt thanks to my mother Wendy McDonald for frequently checking in on my progress and her unwavering encouragement. This work was supported by Aga Khan, Network for Development and Portuguese Foundation for Science and Technology (FCT), through the project "LittleFish-STP: São Tomé and Príncipe little fish threatened - a big opportunity to unravel this fishery resource in tropical islands" (ref. 541718158).

List of Figures

List of Tables

List of Abbreviations

IUCN – International Union for Conservation of Nature
sRD – Standardized RNA:DNA ratio
TL – Total length
DW – Dry weight

Abstract

In the many rivers of São Tomé Island lives a group of amphidromous fish known collectively as "peixinho" or "little fish" when at the post-larval stage. The species that make up this group include *Awaous lateristriga*, *Sicydium brevifile* and *Sicydium bustamantei*. Although these species are locally very important both as a source of income and protein, there is still little knowledge available about them. To obtain information about their ecophysiological condition, this study looked at the standardized RNA:DNA ratio (sRD), DNA concentrations and RNA concentrations of these species, comparing them between different rivers, the Iô-Grande, Lembá, Martim Mendes, and Paga Fogo in the wet and dry seasons. During the dry season when food availability can be expected to be lower, sRD were significantly lower for *Sicydium* species, indicating a poorer physiological condition than in the wet season. DNA and RNA concentrations were found to be higher for all species during the dry season. Looking at these concentrations relative to one another, this could also indicate poorer physiological condition. Generally, results were consistent between species and rivers but due to sampling limitations, a robust comparison could not be made between the three species and the four rivers. This study provides insight into the ecophysiological status of these three fish species in São Tomé Island rivers, which can help to develop conservation and management strategies.

Keywords: *Sicydium* spp., *Awaous lateristriga*, sRD, nucleic acids, post-larvae, amphidromous

Resumo

Nos diversos rios da Ilha de São Tomé vive um grupo de peixes anfídromos conhecidos coletivamente como “peixinho” quando estão na fase pós-larvar. As espécies que compõem este grupo incluem *Awaous lateristriga*, *Sicydium brevifile* e *Sicydium bustamantei*. Embora estas espécies sejam localmente muito importantes, tanto como fonte de rendimento como de proteína, ainda existe pouco conhecimento disponível sobre elas. Para obter informações sobre a sua condição ecofisiológica, este estudo analisou a razão padronizada de RNA (sRD), as concentrações de DNA e de RNA destas espécies, comparando-as entre diferentes rios — o Iô-Grande, Lembá, Martim Mendes e Paga Fogo — nas estações húmida e seca. Durante a estação seca, quando a disponibilidade de alimentos pode ser considerada menor, sRD foram significativamente mais baixos para as espécies de *Sicydium*, indicando uma condição fisiológica inferior à da estação húmida. As concentrações de DNA e RNA foram mais elevadas para todas as espécies durante a estação seca. Ao analisar estas concentrações entre si, pode também indicar uma condição fisiológica inferior. De forma geral, os resultados foram consistentes entre as espécies e os rios, mas, devido a limitações na amostragem, não foi possível fazer uma comparação robusta entre as três espécies e os quatro rios. Este estudo fornece uma visão sobre o estado ecofisiológico destas três espécies de peixes nos rios da Ilha de São Tomé, o que pode ajudar a desenvolver estratégias de conservação e gestão.

Palavras-Chave: *Sicydium* spp., *Awaous lateristriga*, sRD, ácidos nucleicos, pós-larvas, anfídromo

Table of Contents

1. Introduction

1.1 The Little Fish

The little fish from São Tomé and Principe, known as “peixinho”, are post-larvae of several fish from the family Gobiidae, *Sicydium bustamantei* Greeff, 1884, *Sicydium brevifile* Ogilvie-Grant, 1884, and *Awaous lateristriga* Duméril, 1861 and Floeter, 2007. *Sicydium bustamantei* and *Sicydium brevifile* are found in middle to lower ranges of streams of the islands of São Tomé, Principe and Annobón in the Gulf of Guinea (Pezold & Harrison, 2006; Floeter, 2007). *Sicydium bustamantei* was last evaluated by the International Union for Conservation of Nature (IUCN) in 2009 and categorized as data deficient (Moelants, 2015). In 2013, *S. brevifile* was listed as least concern by the IUCN due to distribution and lack of major threats (Diouf, 2020). *Awaous lateristriga* has the largest geographical extent of the three species, and is found in rivers and streams from Senegal, and south to Angola, including the islands in the Gulf of Guinea (Floeter, 2007). The IUCN evaluated *A. lateristriga* as least concern in 2019 due to the extent of its range and relative lack of threat throughout the area (Lalèyè, 2020). All of these fish have an amphidromous life cycle with adults spawning in freshwater, fish larvae drifting down to ocean, a planktonic phase at sea, and finally after one to several months, post-larvae migrating upstream in rivers, climbing waterfalls to reach the freshwater location at which they grow and mature (McDowall, 2007; Keith et al., 2011; Baptista et al., 2020; Al-Jufaili et al., 2021; Pouil & Colsoul, 2021). Work needs to be done to assess the status of *S. bustamantei* in general, as well as the other two species in São Tomé and Principe specifically, in order to understand and maintain healthy populations.

Goby fisheries, especially those from Sicyniidae species are important all over the world (Bell, 1999). The little fish are likewise of great importance to the people of São Tomé and Principe both economically, and as a source of nutrition (Baptista et al., 2020; Pouil & Colsoul, 2021). Although, as is the case in São Tomé and Principe, they are mostly exploited on a local scale, there is evidence that fishing for post-larvae in these Goby fisheries can lead to high extraction and that many of these fisheries are declining (Castellanos-Galindo et al., 2011; Pouil & Colsoul, 2021). Anecdotal evidence from older local fishers also indicates that catches are not as great as they

once were (Castellanos-Galindo et al., 2011; dos Santos, 2023). Due to the sporadic nature of many of these fisheries and the catch being locally consumed or sold informally within the community, they are also often not well tracked and details on their impact have not been ascertained (Bell, 1999; Pouil & Colsoul, 2021). On São Tomé Island, while caught year-round, post-larvae of the little fish are caught mainly during the dry season on new and full moons (Baptista et al., 2020). Potentially due to higher run off bringing more nutrients to rivers and streams during the wet season (Gallegos et al., 1992), post-larvae of *S. bustamantei* have been found to be larger and have a higher feeding incidence rate during this time (Baptista et al., 2020).

1.2 Ecophysiological Condition – Standardized RNA:DNA Ratio

For the purpose of assessing ecophysiological conditions, standardized RNA:DNA ratio (sRD) comparisons are a relatively easy, cost-effective way to measure the condition of a fish over a short period of time (Bulow, 1970; Bhat et al., 2020). Analysis of these ratios is helpful particularly for fish larvae, but also in a number of other organisms, to help estimate growth rates (Bulow, 1970; Buckley, 1984; Rosa & Nunes, 2004). The premise behind this is that DNA levels are generally consistent, but RNA levels linked to protein synthesis vary depending on ontogeny, health and environmental factors (Bulow, 1970; Chícharo & Chícharo, 2008). These ratios have been used in many cases to measure the nutritional condition of larvae, (Buckley, 1980; Clemmesen, 1994; Mathers et al., 1994; Westerman & Holt, 1994; Morioka et al., 1996) as well as fish at older life stages (Müller et al., 2020) and crustaceans (Goncalves et al., 2021). They have also been investigated as potential measures in large marine predators (Alves et al., 2020) and deep-water sharks (Graça Aranha et al., 2023). Changes in availability of food items can be reflected in sRD as in both whelks and mussels, higher ratios have been found in response to higher food concentrations (Menge et al., 2002) and likewise, higher ratios have been observed in fed versus starved fish larvae (Buckley, 1980, 1984; Richard et al., 1991; Caldarone et al., 2003). In conjunction with lower feeding rates and growth of little fish post-larvae during the dry season, it may be expected that protein synthesis is reduced and that therefore the sRD is also lower at this time of year.

1.3 Study Objectives and Hypothesis

Since the three species, *A. lateristriga*, *S. brevifile* and *S. bustamantei* are crucial to the islands of São Tomé and Principe, and information remains limited, assessing populations and measuring environmental conditions and quality are essential steps in developing plans for conservation and population maintenance of the little fish so important to the region. The aim of the present study is to determine the ecophysiological condition of the little fish in São Tomé Island. For that, standardized RNA:DNA ratio will be calculated for samples collected in fours rivers around the island in both the wet and dry seasons of 2023. This will provide more information on the physiological condition of the post-larvae of the three relevant species in São Tomé and help to contribute to the overall goal of better monitoring and conserving these fish while improving local quality of life. As post-larvae of *S. bustamantei* have been found to be larger and have a higher feeding incidence rate during the wet season (Baptista et al., 2020) this study hypothesizes a poorer physiological condition of the little fish during the dry season relative to the wet season, potentially due to lower feeding rates.

2. Materials and Methods

2.1 Study Area and Collection of Samples

The Democratic Republic of São Tomé and Principe is located in the Gulf of Guinea, about 250 km off the west coast of continental Africa (Afonso et al., 1999; Chou et al., 2020). This island nation, along with the islands of Annobón and Bioko, form an archipelago near the equator (Afonso et al., 1999). Due to equatorial influence, the weather in São Tomé and Principe is divided into two seasons, a wet season (October to May) and a dry season (June to September) (Baptista et al., 2020; Chou et al., 2020).

Post-larvae were collected near the mouth of four rivers on São Tomé Island, the Martim Mendes River (0.098821, 6.620422), Iô-Grande River (0.110996, 6.634199), Paga Fogo River (0.28344166, 6.48399734), and further up the Lembá River (0.247246, 6.462904). Iô-Grande, the largest river on São Tomé Island, and the Martim Mendes are in the south and both drain into the Atlantic Ocean on the eastern side of the island. The Lembá and Paga Fogo rivers are in the north of the

island and drain on the western side. Post-larvae collection took place during January (the wet season) and June/July (the dry season) of 2023 during daylight hours. All collected post-larvae were measured, photographed and then stored in RNA Lock Reagent.

Figure 1 Location of São Tomé and Principe (upper map) and sampling locations in each of the four rivers on São Tomé Island (lower map). (Upper map created in QGIS version 3.34.0 with Google Maps data, April 11, 2024, lower map created in Google Earth 10.55.0.1, June 5, 2024.)

2.2 Samples

Ecophysiological condition was calculated for a total of 188 post-larvae, 98 of which were collected in January, during the wet season of 2023, and 90 of which were collected in June/July, during the dry season of 2023. The breakdown by species and river from which each sample was collected can be found in Table 1.

Table 1 Samples of little fish collected in São Tomé and Príncipe and analysed in this study, categorized by species, season (wet – January 2023; dry – June/July 2023) during which, and river from which, each was taken. Numbers are indicative of numbers of individuals.

	Dry Season				Total Dry	Wet Season				Total Wet
Species	Iô-Grande	Lembá	Paga Fogo	Martim Mendes		Iô-Grande	Lembá	Paga Fogo	Martim Mendes	
Awaous lateristriga	10	0	0	0	10	10	0	26	0	36
Sicydium brevifile	10	20	20	10	60	0	20	6	5	31
Sicydium bustamantei	0	10	10	0	20	0	20	6	5	31
Total	20	30	30	10	90	10	40	38	10	98

2.3 Ecophysiological Condition

The ecophysiological condition of little fish was inferred by determining the concentration of RNA and DNA, and standardized RNA:DNA ratio using a microplate fluorescent assay (Wagner et al., 1998; Caldarone et al., 2001) conducted at Algarve Centre of Marine Sciences (CCMAR), University of Algarve (Portugal). In preparation for analysis, all samples were washed with distilled water, stored at -80 °C, then freeze-dried for 24 hours. Approximately 1 mg was taken from the body of each sample above the tail, then mechanically and chemically homogenized via sonication of 3 pulses at 50A over a period of one minute in cold sarcosyl Tris-EDTA extraction buffer. A microplate fluorescent assay was used to quantify RNA and DNA present in each sample with Gel Red, a nucleic acid fluorochrome dye. Fluorescence was measured with an excitation wavelength of 365 nm and an emission wavelength of 590 nm on a microplate reader (Biotek Synergy HT model SIAFRTD - BioTek® Instruments, Inc., Vermont, USA). After an initial scan, microplates were incubated for 30 minutes at 37 °C to activate an added ribonuclease A solution then scanned again. Following the second scan, DNA concentrations were calculated directly from the standard curve. The DNA fluorescence of the second scan was subtracted from the total

fluorescence of the first scan in order to calculate the RNA fluorescence. Concentrations were determined by running standard curves of DNA-GR and RNA-FT with known concentrations of λ-phagus DNA (0.25 µg µL^{-1}) and 16S-23S *E. coli* RNA (4 µg µL^{-1}) (Roche). From average concentration values, RNA:DNA ratios were calculated. These RNA:DNA ratios for post-larvae were standardized using RNA and DNA slope ratios and the reference slope ratio of 2.4 as outlined by Caldarone et al. (2006). Effects of size on the concentrations of DNA, RNA and sRD (standardized RNA:DNA) were tested using a linear model (7. Appendix).

2.4 Statistical Analysis

Data analysis was conducted using Microsoft Office 2021 MSO (Version 2404) and RStudio (R Core Team, 2018). Data were tested for normality of distribution (Shapiro-Wilk normality test and Q-Q plots for visualization) and homogeneity of variance (Levene's test). As the homogeneity of variance and/or normality of distribution were violated, even with the transformed data (Log x + 1), the non-parametric statistical test Kruskal-Wallis was used, to test the effects of the factorial parameters (season and rivers) on the concentrations of DNA, RNA and the sRD. When significant differences were found, a Dunn's post hoc test was used for pair-wise comparisons of treatment means. All statistical analyses were conducted to a level of significance $\alpha = 0.05$.

3. Results

From the sRD of all samples, average RNA and DNA concentrations and sRD ratios for each species by river between the two seasons were calculated. These are shown in Table 2 as means plus or minus the standard deviation. For *A. lateristriga,* the highest average total length was 24.99 ± 7.71 mm in the wet season in the Paga Fogo River. The highest sRD was in the same river and season with a value of 0.52 ± 0.21. The highest average total length of 26.19 ± 2.19 mm for *S. brevifile* was in the Lembá River during the wet season, while the highest sRD for this species of 0.53 ± 0.30 was found in the Paga Fogo River in the same season. Finally, for *S. bustamantei*, the

highest average total length of 25.54 ± 1.82 mm was in the Paga Fogo in the dry season and the highest average sRD of 0.50 ± 0.08 was in the same river but in the dry season.

Table 2 Average and standard deviation of total length (TL), RNA concentration, DNA concentration and standardized RNA:DNA ratio (sRD) of little fish collected in São Tomé and Príncipe categorized by species, seasons (wet – January 2023; dry – June/July 2023) during which, and rivers from which, each was taken.

Season	Species	River	N	TL (mm)	RNA (µg mg−1 DW)	DNA (µg mg−1 DW)	sRD
Dry	*Awaous lateristriga*	Iô-Grande	10	19.01 ± 0.88	3.02 ± 0.93	4.93 ± 0.52	0.28 ± 0.09
	Sicydium brevifile	Iô-Grande	10	26.13 ± 1.17	2.32 ± 0.51	3.49 ± 0.22	0.28 ± 0.07
	Sicydium brevifile	Lembá	20	24.91 ± 1.94	4.00 ± 1.27	3.78 ± 0.16	0.37 ± 0.12
	Sicydium brevifile	Paga Fogo	20	22.35 ± 4.82	3.04 ± 1.17	4.45 ± 0.28	0.25 ± 0.09
	Sicydium brevifile	Martim Mendes	10	24.16 ± 1.87	4.45 ± 1.55	6.81 ± 0.48	0.28 ± 0.09
	Sicydium bustamantei	Lembá	10	24.62 ± 1.26	1.75 ± 0.57	2.32 ± 0.12	0.26 ± 0.09
	Sicydium bustamantei	Paga Fogo	10	25.54 ± 1.82	4.00 ± 1.24	4.40 ± 0.47	0.31 ± 0.09
Wet	*Awaous lateristriga*	Iô-Grande	10	23.35 ± 1.52	1.68 ± 0.29	1.94 ± 0.07	0.35 ± 0.08
	Awaous lateristriga	Paga Fogo	26	24.99 ± 7.71	1.17 ± 0.35	2.41 ± 0.21	0.52 ± 0.21
	Sicydium brevifile	Lembá	20	26.19 ± 2.19	1.00 ± 0.31	1.15 ± 0.08	0.48 ± 0.18
	Sicydium brevifile	Paga Fogo	6	18.14 ± 0.82	0.54 ± 0.33	0.73 ± 0.09	0.53 ± 0.30
	Sicydium brevifile	Martim Mendes	5	23.55 ± 1.44	1.01 ± 0.28	1.34 ± 0.03	0.35 ± 0.09
	Sicydium bustamantei	Lembá	20	25.30 ± 0.85	0.78 ± 0.35	1.11 ± 0.07	0.42 ± 0.19
	Sicydium bustamantei	Paga Fogo	6	18.02 ± 0.90	0.72 ± 0.21	1.31 ± 0.27	0.50 ± 0.08
	Sicydium bustamantei	Martim Mendes	5	21.95 ± 1.67	0.75 ± 0.21	1.17 ± 0.11	0.28 ± 0.08

3.1 Awaous lateristriga

Awaous lateristriga samples were collected from the Iô-Grande River in the dry season and in the Iô-Grande and Paga Fogo rivers in the wet season (Table 1). As this species was not present in the majority of rivers sampled, it was, for the most part, analysed separately from the two *Sicydium* species. Figure 2A shows the sRD from the Iô-Grande in the dry and wet seasons and Figure 2B compares the Iô-Grande (IG) and Paga Fogo (PF) rivers in the wet season. Despite slight tendencies for higher sRD in the Iô-Grande River in the wet season over the dry season, and in the Paga Fogo River over the Iô-Grande River in the wet season, no statistically significant differences were found in sRD in either case (between wet and dry seasons in the Iô-Grande: Kruskal-Wallis test, p-value = 0.3643, between Iô-Grande and Paga Fogo in the wet season: Kruskal-Wallis test, p-value = 0.2298).

The average RNA concentrations from the Iô-Grande in the dry and wet seasons are shown in Figure 2C. The seasonal differences in average RNA concentrations were found to be statistically significant (Kruskal-Wallis test, p-value = 0.01261), with higher values found during the dry season. Figure 2D compares the Iô-Grande and Paga Fogo rivers in the wet season, showing similar values in both rivers, which is reflected in the lack of statistically significant differences found between average RNA concentrations in the two rivers (Kruskal-Wallis test, p-value = 0.2298).

Figure 2E shows the average DNA concentrations from the Iô-Grande in the dry and wet seasons. As well as being visibly different, the seasonal differences in average DNA concentrations were also found to be statistically significant (Kruskal-Wallis test, p-value < 0.001), showing higher values during the dry season when compared to the wet season. Figure 2F compares DNA concentrations for this species in the two rivers in the wet season, where no statistically significant differences were found between average DNA concentrations in the two rivers (Kruskal-Wallis test, p-value = 0.3585).

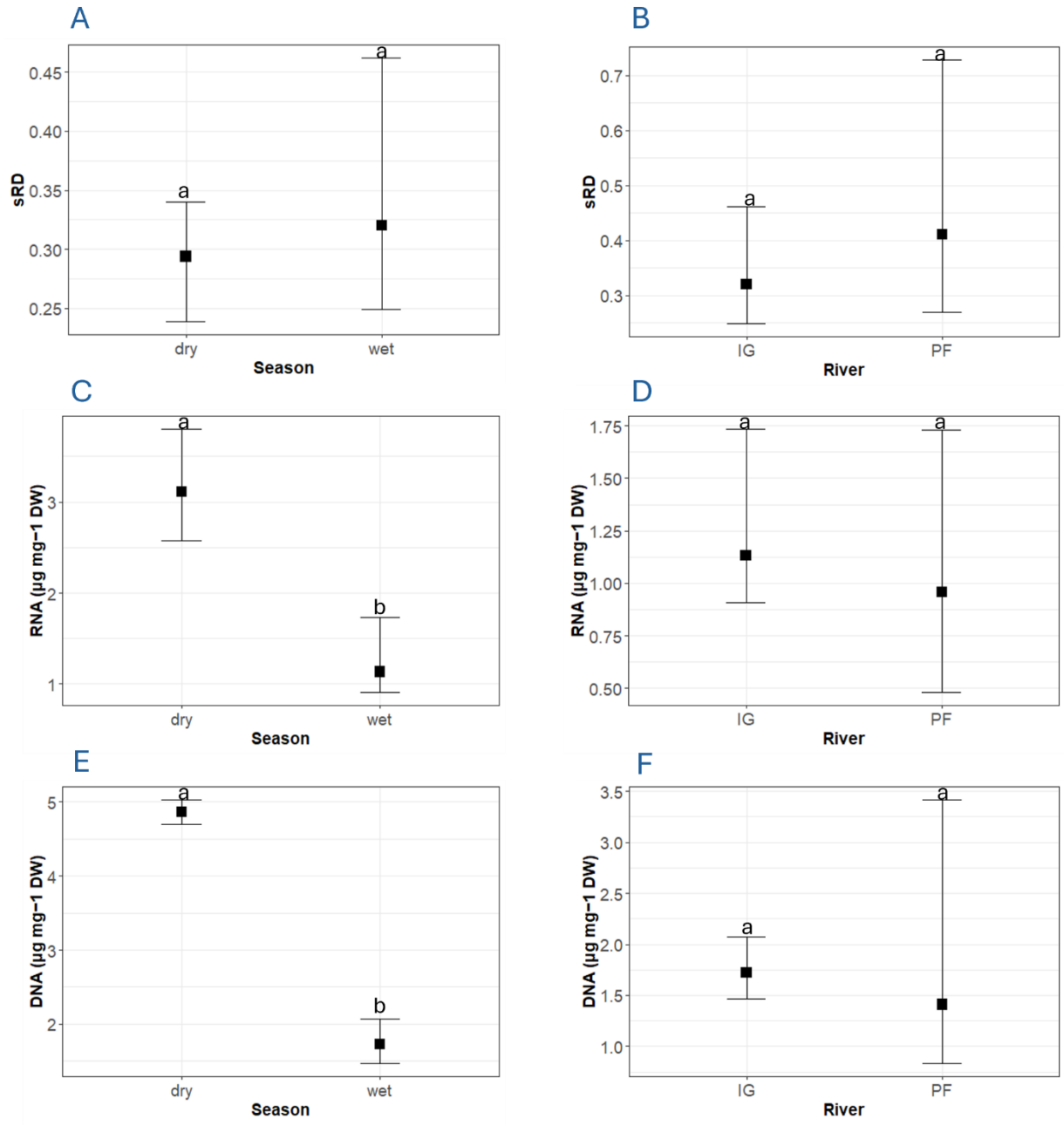

Figure 2 Ecophysiological condition indices of Awaous lateristriga collected in São Tomé and Príncipe: standardized RNA:DNA ratio (A), average RNA concentration (C) and average DNA concentration (E) in the Iô-Grande River in dry (January 2023; n = 10) and wet (June/July 2023; n = 10) seasons; standardized RNA:DNA ratio (B), average RNA concentration (D) and average DNA concentration (F) during the wet season in the Iô-Grande (IG; n = 10) and Paga Fogo (PF; n = 26) rivers. Letters denote significant differences, i.e. those presenting the same letter are not significantly different while different letters indicate significant differences.

3.2 Sicydium species

Sicydium brevifile samples were collected during the dry season in the Iô-Grande, Lembá, Paga Fogo and Martim Mendes rivers, as well as during the wet season in the Lembá, Paga Fogo and Martim Mendes rivers (Table 1). *Sicydium bustamantei* samples were collected from the Lembá and Paga Fogo rivers in the dry season, and Lembá, Paga Fogo and Martim Mendes rivers in the wet season (Table 1). For each species, sRD, average RNA concentrations and average DNA concentrations were compared across all rivers sampled in both seasons.

3.2.1 Sicydium brevifile

The sRD values for *S. brevifile*, regardless of river, are higher in the wet season than in the dry season (Figure 3A), presenting significant differences between both seasons (Kruskal-Wallis test, p-value < 0.001). Values of the average RNA concentrations for *S. brevifile* are higher in the dry season than in the wet season (Figure 3B), with statistically significant differences between both seasons (Kruskal-Wallis test, p-value < 0.001). Similarly, the DNA concentrations of *S. brevifile*, showed higher values in the dry season when compared to the wet season (Figure 3C), also with significant differences between the seasons (Kruskal-Wallis test, p-value < 0.001). When comparing the sRD in all the rivers sampled during the dry season (Figure 4A), there were no significant differences between the rivers (Kruskal-Wallis test, p-value = 0.08141). This was the same during the wet season (Figure 4B), with no significant differences for sRD between all rivers (Kruskal-Wallis test, p-value = 0.09555). Concerning the RNA concentration during the dry season, there were significant differences between the rivers (Kruskal-Wallis test, p-value = 0.01247), with higher values for the Lembá and Martim Mendes rivers in group b when compared to Iô-Grande and Paga Fogo rivers in group a (Figure 4C). During the wet season, the RNA concentrations presented no significant differences between all the rivers (Figure 4D; Kruskal-Wallis test, p-value = 0.1371). Regarding the DNA concentration in the dry season (Figure 4E), while most of the rivers are included in group a, indicating no significant difference in average DNA concentrations amongst them, the Martim Mendes is an exception. The average DNA concentration values in the dry season for the Martim Mendes River are the only values in group b, indicating a significant difference between the Martim Mendes and all other rivers (Kruskal-Wallis test, p-value < 0.001).

In Figure 4F, all rivers in the wet season are included in group a, indicating no significant differences between them (Kruskal-Wallis test, p-value = 0.08936).

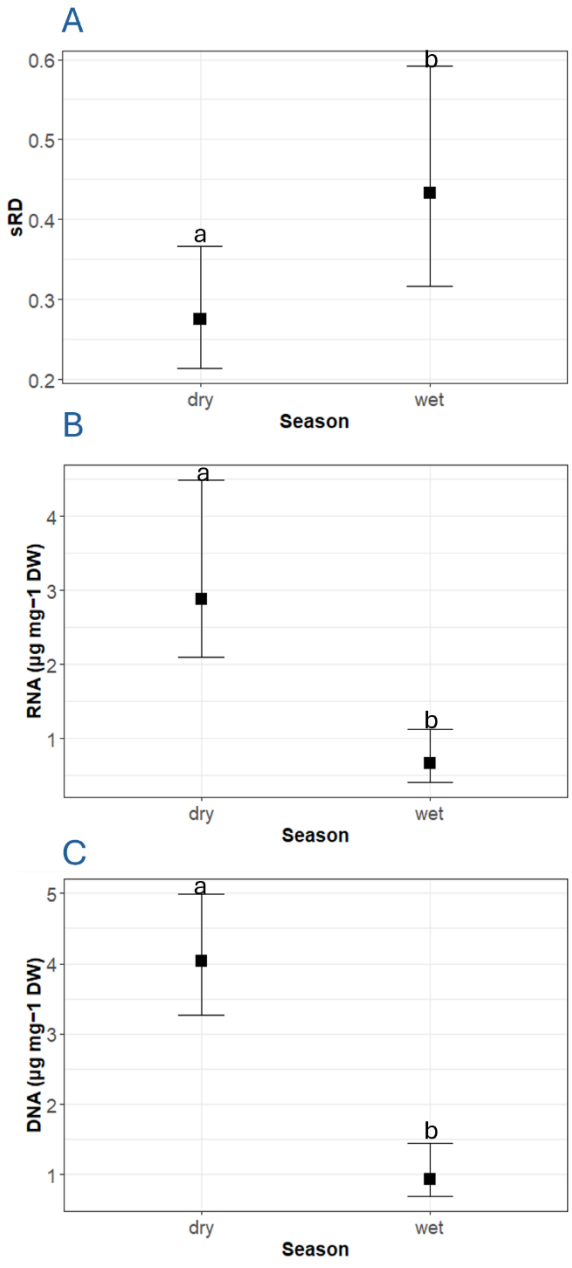

Figure 3 Ecophysiological condition indices of Sicydium brevifile collected in São Tomé and Príncipe: standardized RNA:DNA ratio (A), average RNA concentration (B) and average DNA concentration (C) of Sicydium brevifile in the dry (January 2023; n = 60) and wet (June/July 2023; n = 31) seasons for all rivers. Letters denote significant differences, i.e. those presenting the same letter are not significantly different while different letters indicate significant differences.

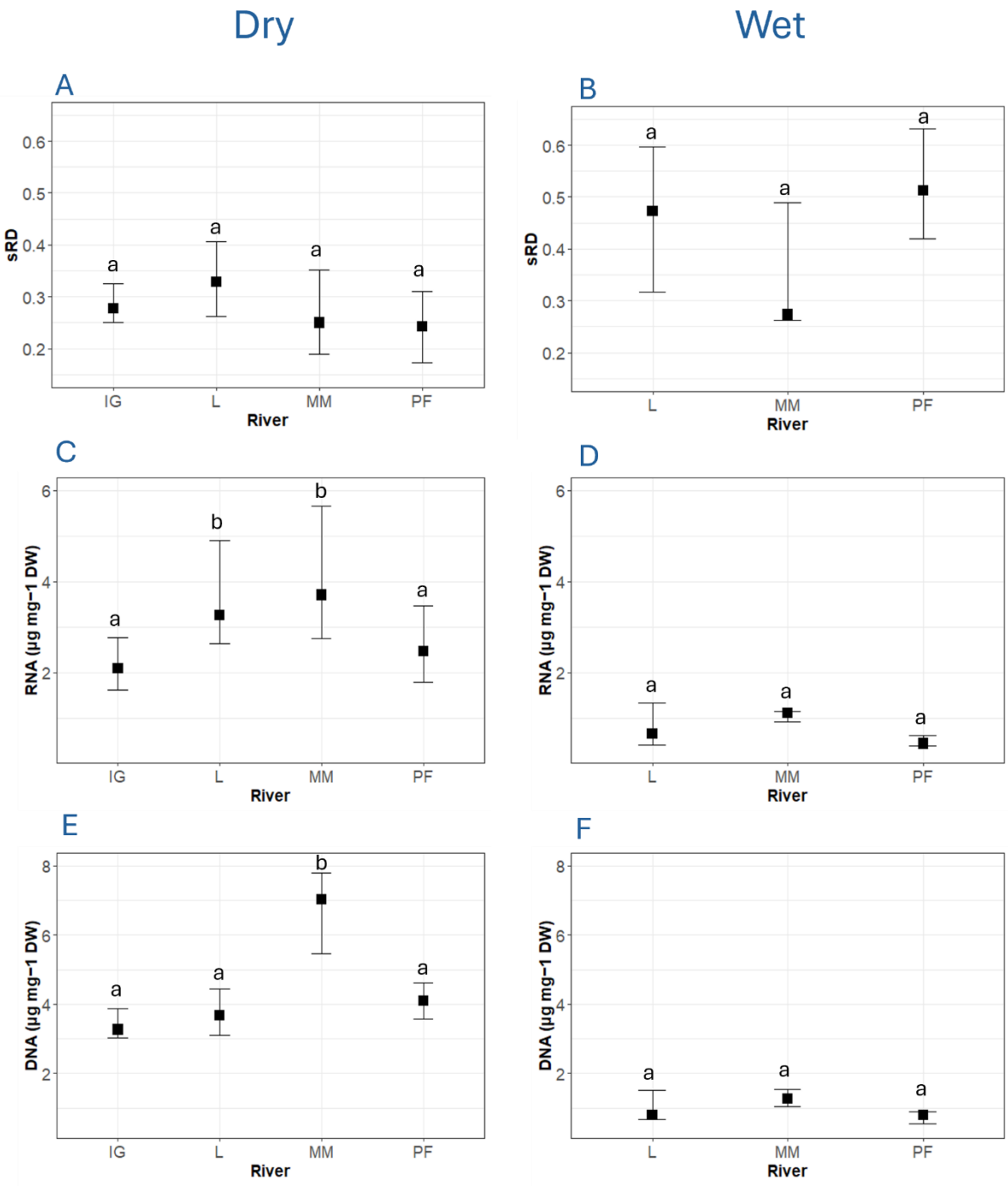

Figure 4 Ecophysiological condition indices of Sicydium brevifile collected in São Tomé and Príncipe: standardized RNA:DNA ratio (A), average RNA concentration (C) and average DNA concentration (E) of Sicydium brevifile in the dry season (January 2023) for the Iô-Grande (IG; n = 10), Lembá (L; n = 20), Martim Mendes (MM; n = 10) and Paga Fogo (PF; n = 20) rivers; standardized RNA:DNA ratio (B), average RNA concentration (D) and average DNA concentration (F) of Sicydium brevifile in the wet season (June/July 2023) for the Lembá (L; n = 20), Martim Mendes (MM; n = 5) and Paga Fogo (PF; n = 6) rivers. Letters denote significant differences, i.e. those presenting the same letter are not significantly different while different letters indicate significant differences.

3.2.2 Sicydium bustamantei

Regarding the sRD values for *S. bustamantei*, there were significant differences between the seasons (Kruskal-Wallis test, p-value = 0.04085), presenting higher values during the wet season (Figure 5A). For the RNA and DNA concentrations, there were also significant differences between seasons (RNA: Kruskal-Wallis test, p-value < 0.001; DNA: Kruskal-Wallis test, p-value < 0.001), but with higher values for the dry season (Figure 5B). Looking at the comparison of sRD average values between the rivers (Figure 6A and B), there were no significant differences in both seasons (dry: Kruskal-Wallis test, p-value = 0.1509; wet: Kruskal-Wallis test, p-value = 0.4125). For both RNA and DNA concentrations (Figure 6C and E), there were significant differences between the rivers during the dry season (RNA: Kruskal-Wallis test, p-value = 0.006502; DNA: Kruskal-Wallis test, p-value < 0.001), with higher values found in the Paga Fogo over the Lembá River. On the other hand, there were no significant differences in nucleic acid concentrations between the rivers during the wet season (Figure 6D and F; RNA: Kruskal-Wallis test, p-value = 0.911; DNA: Kruskal-Wallis test, p-value = 0.4344).

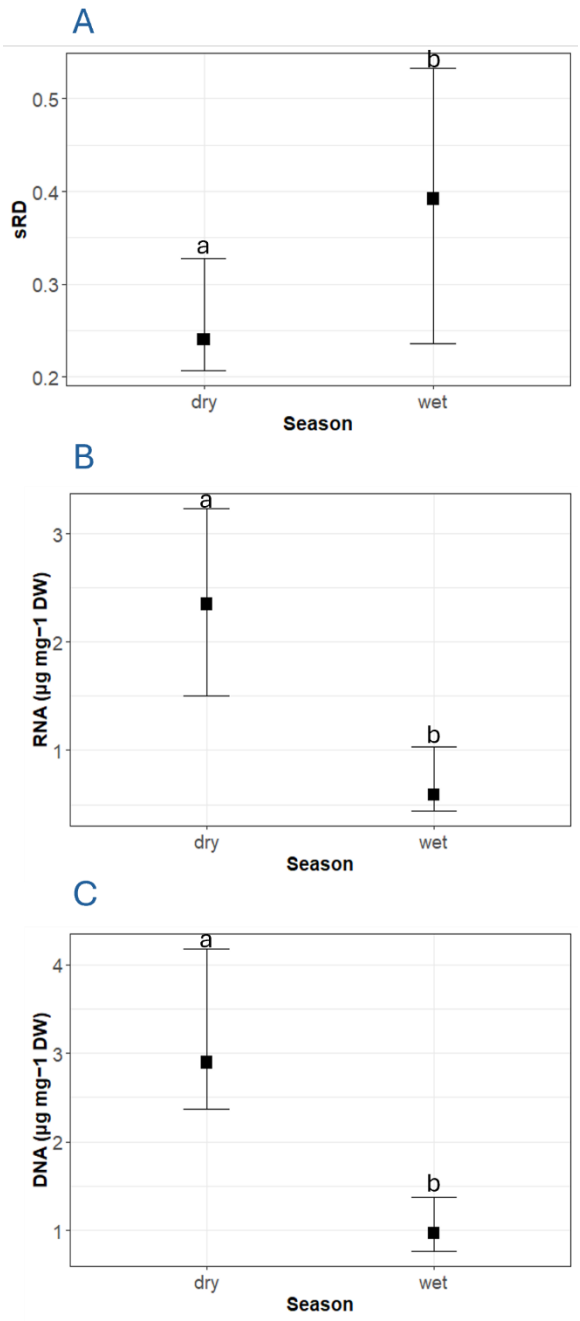

Figure 5 Ecophysiological condition indices of Sicydium bustamantei collected in São Tomé and Príncipe: standardized RNA:DNA ratio (A), average RNA concentration (B) and average DNA concentration (C) in dry (January 2023; n = 20) and wet (June/July 2023; n = 31) seasons for all rivers. Letters denote significant differences, i.e. those presenting the same letter are not significantly different while different letters indicate significant differences.

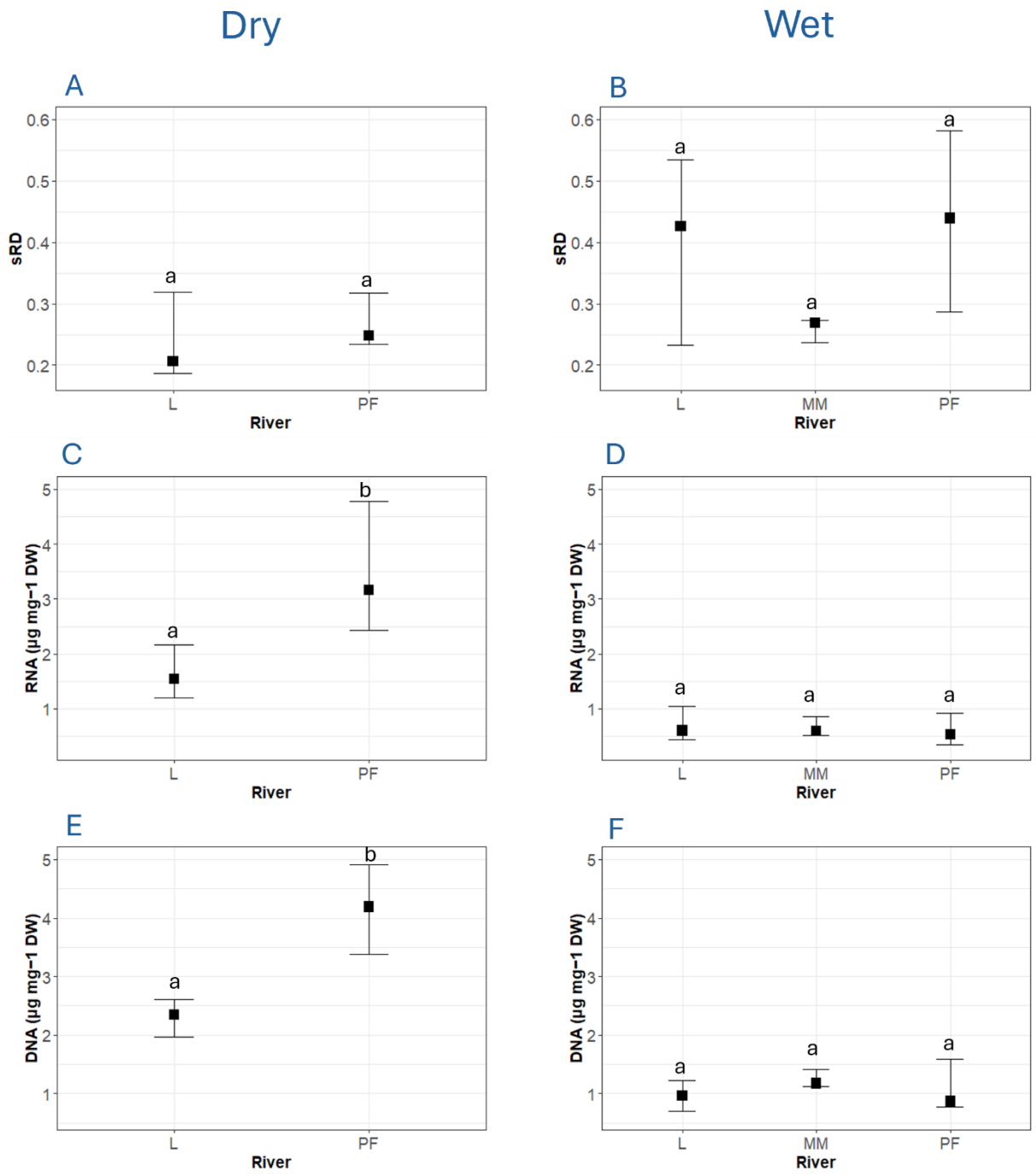

Figure 6 Ecophysiological condition indices of Sicydium bustamantei collected in São Tomé and Príncipe: standardized RNA:DNA ratio (A), average RNA concentration (C) and average DNA concentration (E) of Sicydium bustamantei in the dry season (January 2023) for the Lembá (L; n = 10) and Paga Fogo (PF; n = 10) rivers; standardized RNA:DNA ratio (B), average RNA concentration (D) and average DNA concentration (F) of Sicydium bustamantei in the wet season (June/July 2023) for the Lembá (L; n = 20), Martim Mendes (MM; n = 5) and Paga Fogo (PF; n = 6) rivers. Letters denote significant differences, i.e. those presenting the same letter are not significantly different while different letters indicate significant differences.

3.3 Sicydium Species Comparison

In order to check for significant differences between the two *Sicydium* species, comparisons were made between their respective data. As both species were present in only the Paga Fogo, Lembá and Martim Mendes rivers in both seasons, data for these three were analysed (Table 1). Data for the Iô-Grande River were not included as only *S. brevifile* post-larvae were sampled from this river, and only for one season.

In both dry and wet seasons (Figure 7A and B), there were no significant differences between the sRD of the two *Sicydium* species (dry: Kruskal-Wallis test, p-value = 0.5761; wet: Kruskal-Wallis test, p-value = 0.08716). Concerning the RNA and DNA concentrations during the dry season (Figure 7C and E), there were significant differences between both species, with higher values found for *S. brevifile* when compared to *S. bustamantei* (RNA: Kruskal-Wallis test, p-value = 0.04669; DNA: Kruskal-Wallis test, p-value = 0.002154). During the wet season, no significant differences were observed for these nucleic acid concentrations between the *Sicydium* species (Figure 7D and F; RNA: Kruskal-Wallis test, p-value = 0.4859; DNA: Kruskal-Wallis test, p-value = 0.6272).

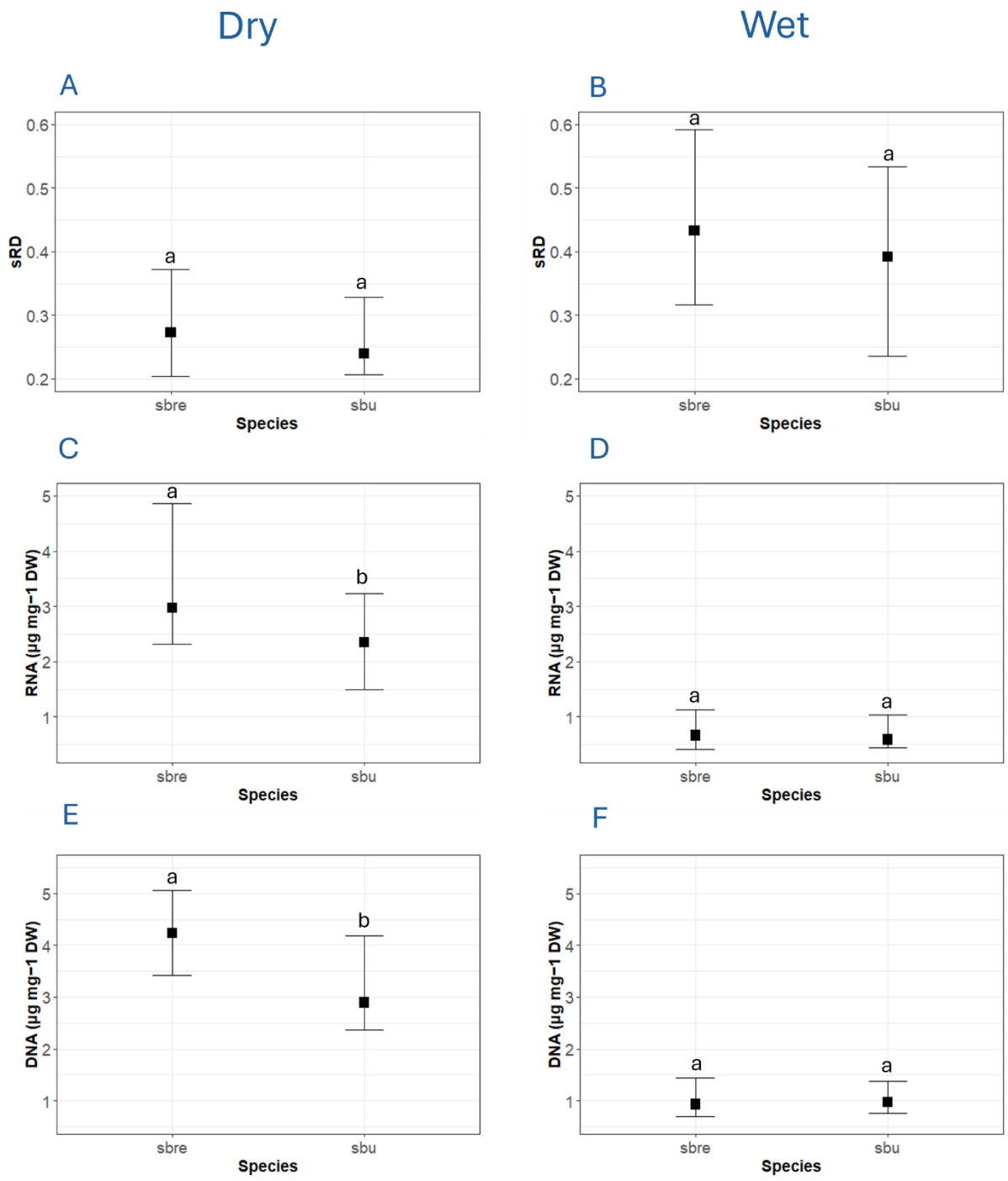

Figure 7 Ecophysiological condition indices of Sicydium brevifile and Sicydium bustamantei collected in São Tomé and Príncipe: standardized RNA:DNA ratio (A), average RNA concentration (C) and average DNA concentration (E) of Sicydium brevifile (sbre; n = 60) and Sicydium bustamantei (sbu; n = 20) in the dry season (January 2023); standardized RNA:DNA ratio (B), average RNA concentration (D) and average DNA concentration (F) of Sicydium brevifile (sbre; n = 31) and Sicydium bustamantei (sbu; n = 31) in the wet season (June/July 2023). Letters denote significant differences, i.e. those presenting the same letter are not significantly different while different letters indicate significant differences.

3.4 All Little Fish Species Comparison

In order to check for significant differences between the three Little Fish species, the two *Sicydium* species and *A. lateristriga*, comparisons were made between their respective data. As all three species were present in only the Paga Fogo River, and in only the wet season, data for these three were analysed during this season only (Table 1).

Regarding the sRD average values, although *S. brevifile* presented higher values than the other two species, there were no significant differences between all the species (Figure 8A; Kruskal-Wallis test, p-value = 0.5536). Additionally, there were no significant differences between all species for the RNA and DNA concentrations, although *S. brevifile* presented slightly lower values when compared to the other species (Figure 8B and C; RNA: Kruskal-Wallis test, p-value = 0.2022; DNA: Kruskal-Wallis test, p-value = 0.07056).

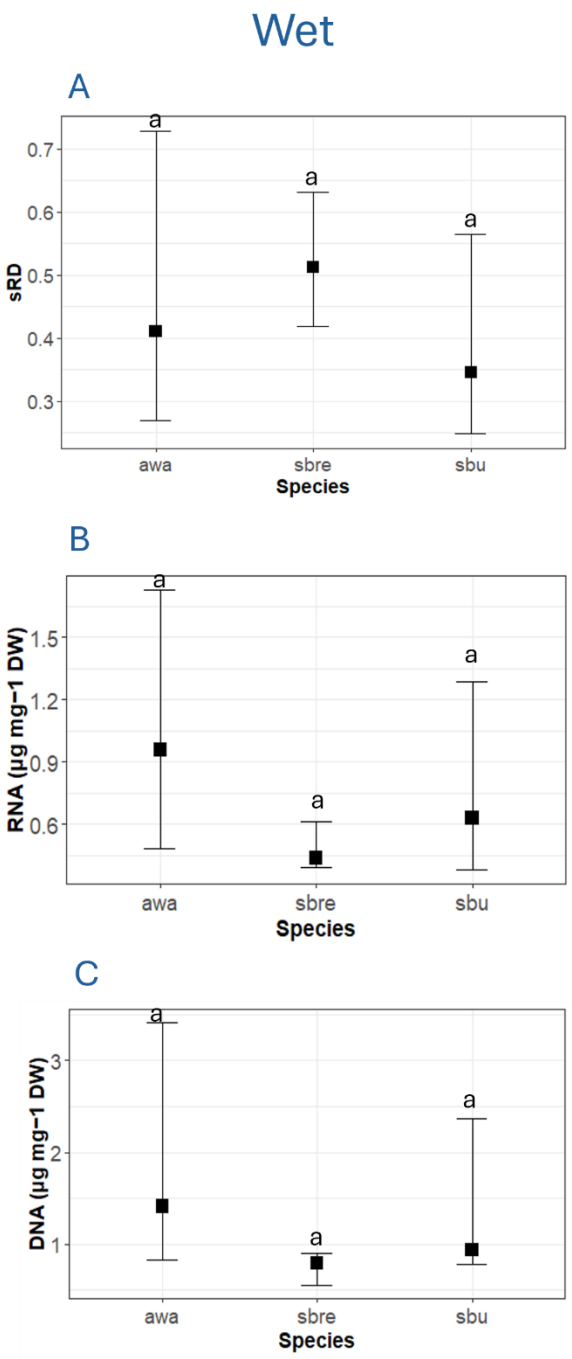

Figure 8 Ecophysiological condition indices of Awaous lateristriga, Sicydium brevifile and Sicydium bustamantei collected in São Tomé and Príncipe: standardized RNA:DNA ratio (A), average RNA concentration (B) and average DNA concentration (C) of Awaous lateristriga (awa; n = 26), Sicydium brevifile (sbre; n = 6) and Sicydium bustamantei (sbu; n = 6) in the wet season for the Paga Fogo river. Letters denote significant differences, i.e. those presenting the same letter are not significantly different while different letters indicate significant differences.

4. Discussion

A large number of studies have found that increases in nutrient ingestion cause a rise in RNA:DNA ratios while ratios decrease during times of starvation (Buckley, 1980, 1984; Richard et al., 1991; Mathers et al., 1994; Westerman & Holt, 1994; Foley et al., 2016; Bhat et al., 2020). This has been found to be due to a rise in RNA activity and subsequent increase in protein synthesis in a time of higher nutrition (Chícharo & Chícharo, 2008). These changes in nutrient ingestion can be linked to differences between seasons as shown by Rosa & Nunes (2004), who found evidence of seasonally related differences in the nutritional condition of organisms, possibly due to seasonal changes in feeding activity. In the case of the little fish, statistically significant differences in sRD were found in the two *Sicydium* species between the wet and dry seasons, with higher sRD observed during the wet season. *Awaous lateristriga* also displayed higher sRD values in the wet season than the dry, but for this species, differences were not statistically significant, perhaps due to fewer rivers being sampled when compared to the two *Sicydium* species. As higher sRD has also been linked to recent growth rates (Bulow, 1970; Buckley, 1984; Bhat et al., 2020), the results of the *Sicydium* species are consistent with that of Baptista et al. (2020) which found larger post-larval size of *S. bustamantei* during the wet season. In a comparison of sRD between rivers however, for all species individually, sRD was not found to be statistically different. This may be indicative of a consistent general physiological condition across rivers from which samples were collected.

Setting comparisons between rivers aside for now and focusing on seasonal differences, while sRD was only affected significantly by seasons, and followed the pattern of seasonality expected at the beginning of this research, RNA and DNA concentrations showed results contrary to what was anticipated. For all species, despite sRD being higher in the wet season than during the dry, both RNA and DNA concentrations were found to be higher during the dry season, a difference which was found to be statistically significant for all little fish species. On the surface, this outcome seems counterintuitive, but in all cases, as the change in DNA concentration is greater than that of the RNA concentration (leading to the observed sRD), these concentrations relative to each other are indicative of poorer condition during the dry season. This increase in both DNA and RNA

concentrations with the former increasing to a greater extent, may be due to a number of reasons, such as stress response (Lieder et al., 2016; Valcarce et al., 2024), growth rate changes (Baptista et al., 2020) and smaller cellular size during times of less favourable seasonal conditions (Bergeron, 1997).

During the dry season, lower levels of water entering rivers can lead to slower current flow, lower oxygen levels and higher water temperatures which can cause stress on organisms (EPA, 2024). The little fish could be facing similar stressful conditions in the dry season relative to the wet season. Stress can cause increases in DNA production due to upregulation of genes involved in DNA repair as cells work to repair themselves (Lieder et al., 2016). Additionally, stressors in fish larvae during early development have been found to lead to overexpression of some types of RNA (Valcarce et al., 2024). Although RNA production may increase in response to stress, likewise there can be RNA degradation. The interplay of factors that lead to mRNA production and degradation are complex, with different stressors and environmental factors leading to a variety of transcriptional and degradation responses and therefore, a transition to different mRNA concentrations (Pérez-Ortín et al., 2007; Shalem et al., 2008). New RNA concentrations transitioned to during the dry season may, while increasing, still increase less relative to DNA concentrations, leading to a higher sRD. While further testing is required to find specific information on little fish responses to stress conditions, stressors could be a factor leading to the higher concentrations of both DNA and RNA but a lower sRD in the dry season over the wet season.

During development, while the DNA level should stay constant within a cell, the number of cells increases which therefore leads to an overall higher DNA concentration (Ferron & Leggett, 1994). However, in response to higher stress levels and lower food availability that can be experienced during the dry season, post-larvae may compensate with altered growth rates. This has been found to be the case with *S. bustamantei* which have been observed to have smaller post-larval size and lower feeding rates in the dry relative to the wet season (Baptista et al., 2020). Percentage of DNA to dry weight has also been found to increase when low food availability leads to weight loss (Richard et al., 1991). This is likely due to there being more cells per milligram of

tissue and thus a greater concentration of DNA/mg than larvae of a similar size but in conditions of higher food availability as observed by Bergeron (1997). Along with reduced growth rates during the dry season, environmental variations experienced in this season may have a greater impact on larvae than in the wet season (Bergenius et al., 2005). However, fish and their larvae are among the vertebrate species most capable of regeneration following injury or other damage (Kawakami, 2010; Lisse et al., 2015). In the maintenance of tissues, during the dry season there may be an increase in cell production in order to replace cells that have been damaged. This, in conjunction with stress-induced degradation of RNA may contribute to the relatively low sRD in the dry season relative to the wet season despite the increase of both RNA and DNA concentrations.

Aside from seasonality, an additional factor that may have an impact on results is that RNA:DNA ratios are susceptible to differences based on the tissue from which they were extracted. Olivar et al. (2009) have observed that the head of several larval species had a lower RNA:DNA ratio than that of the muscle. During this study, as previously mentioned, a portion of approximately 1 mg of each sample was taken from the body, anterior to the tail. However, due to small dry weight of some samples, there were several cases in which the whole dry sample was used for analysis instead of a small portion. Although the head alone was not used for analysis purposes, due to the differences in RNA:DNA ratios based on the tissues from which they were extracted, there is a possibility that there could be some impact due to some samples being used in full, while for most, only a portion of the sample was used.

In this investigation into the ecophysiological condition of the little fish of São Tomé Island, in addition to comparisons between wet and dry seasons, comparisons were also made between the four rivers from which little fish were sampled. However, as none of the three species of little fish could be sampled from all rivers in both seasons, assessment of similarities and differences between the rivers were somewhat limited (Table 1). During sampling, *A. lateristriga* were only collected from the Iô-Grande and Paga Fogo rivers. When samples from the two rivers were compared, there were no significant differences in either sRD, RNA or DNA concentrations for this species between them. For the *Sicydium* species on the other hand, some differences were

observed. In the case of *S. brevifile*, while sRD values were similar between rivers in each season, RNA and DNA concentrations were found to be significantly different between the Martim Mendes and Lembá when compared with the other rivers, and Martim Mendes and the other rivers during the dry season, respectively. For *S. bustamantei*, sRD was found to be similar between rivers during each season while RNA and DNA concentrations were found to be significantly different in the Paga Fogo from those of other rivers in the dry season only. Across the island of São Tomé, while the wet and dry seasons take place at the same time, the amount of rainfall experienced is not consistent, with higher rainfall experienced in the south and longer dry seasons experienced in the north and northeast of the island (Chou et al., 2020). Differences in precipitation between the south/southeast and the north/northwest of the island can be as high as 6000 mm annually (Chou et al., 2020). As the Martim Mendes River, where higher DNA concentrations were observed in *Sicydium* species, is located in the southeast of São Tomé, it would be expected to receive much higher levels of precipitation than the Lembá and Paga Fogo rivers in the northeast. Perhaps the differences in precipitation between areas of the island leads to greater cellular proliferation during the dry season in *S. brevifile* in the Martim Mendes River relative to the rivers in the northwest. At this time however, a reason for the difference in DNA alone is unknown and further study is required to establish a firm hypothesis regarding why this difference exists between rivers. If possible, more sampling in the Iô-Grande River of the two *Sicydium* species, as well as sampling of *S. bustamantei* in the Martim Mendes in the dry season, could add important comparative data. As the Iô-Grande is in the same area and watershed as the Martim Mendes and therefore presumably faces similar climatic conditions, it would be of great interest to explore any potential similarities and differences between DNA concentrations of *Sicydium* species found in these two rivers. It could also help to highlight potential trends if *S. bustamantei* could be sampled from the Martim Mendes in the dry season, and if these samples displayed similar trends in DNA concentration to *S. brevifile*.

As the Paga Fogo River is in the same watershed as the Lembá, there isn't the same potential geographic reason for significant differences in *S. bustamantei* RNA and DNA concentrations between these rivers as there would be with the Martim Mendes. However, the Lembá is a larger river than the Paga Fogo so differences in water volume, flow and bottom surface area could be

potential factors. Additionally, as comparisons here were made based on a smaller number of samples than other groupings, and only from two rivers, there is a possibility that greater numbers and the collection of samples from other rivers could narrow the differences seen between the Paga Fogo and Lembá rivers in the dry season for this species.

On the level of species comparison, no significant difference was found between the three species for sRD, RNA, or DNA concentrations but as all species were not sampled between all rivers, this comparison is based only on data from the Paga Fogo River during the wet season which can limit the analyses. Overall, the general consistency in sRD, RNA and DNA for all three species could be indicative of similar conditions of growth and nutritional status of post-larvae between the rivers sampled (Bulow, 1970; Buckley, 1984; Bhat et al., 2020). When the two *Sicydium* species were compared, no significant differences were observed in the wet season for sRD, RNA and DNA concentrations, however, there were significant differences between the *Sicydium* species during the dry season concerning the RNA and DNA concentrations. Post-larvae in this study were collected at the mouths of the rivers, and therefore likely not long after leaving the ocean, thus differences between the two *Sicydium* species may be more attributable to factors at sea rather than those attributable to the rivers. Bell et al. (1995), found that two other *Sicydium* species, despite being from the same genus, *S. punctatum* and *S. antillarum,* had respective mean pelagic larval durations of 54-136 days and 63-139 days respectively. Thus, such differences between pelagic larval duration and/or other physiological, biological and ecological characteristics along the life cycle of the *Sicydium* species in this study may likewise differ, potentially contributing to the differences in RNA and DNA concentrations observed between the species in the dry season. Additionally, Monti et al. (2018), found selectivity of diatoms consumed by *S. punctatum* and *S. plumieri*. Should the species of the genus *Sicydium* of São Tomé Island show a similar tendency towards consumption of different food types, this could also be a contributing factor to the differences observed between the two species.

In order to make more robust comparisons in the future, it would be ideal if all three species could be collected in larger numbers from each of the four rivers in both seasons. In several cases, post-larvae could only be sampled from a particular species in a river in one season but not the other, while in three of the rivers sampled, at least one of the little fish species was not sampled

at all in either season. Numbers of post-larvae sampled from the Martim Mendes River were also low in comparison to numbers from other rivers. Greater uniformity in sampling would be ideal for more in-depth results but at this time, there is still little information available on the little fish of São Tomé so this may not be possible. Knowledge on any specific timings of migration from the ocean upriver or if they are generally panseasonal like other Goby species (Bell et al., 1995) could be helpful. Also, it would be important to find out whether waterflow during the wet season on the southeastern part of the island is too high for most post-larvae to navigate, as Iida et al. (2015) observed only low numbers of recruits of a Goby species during, or the day after, rainfall in a river in Japan. In addition, determining whether there may be greater larval preference towards specific rivers over others as observed by Engman et al. (2017) and more, could provide greater ability to conduct targeted sampling and delve deeper into the many unanswered questions about these species. Salinity measurements at the time of sampling showed a range of between 0 and 1.4, likely too low a range to have an impact on sRD, however, further environmental information would be very helpful, providing a basis for additional hypotheses. Currently, infrastructural and measurement limitations mean that little environmental data, either real-time or historical is available. For example, on the island there is only one meteorological station near the airport that has been around for decades with an additional four added in recent years (Chou et al., 2020). Due to this, available data on temperature and other environmental factors in localised regions can be limited, with little data from the past on which to base comparisons. Buckley et al. (1999) have found that RNA concentration and sRD can only be compared directly in temperature differences of less than 2°C. At the time of sampling, at the sea near the Iô-Grande and Martim Mendes rivers, water temperatures were found to range from 28.4-28.7°C during the January 2023 wet season and 25.5-26.2°C in the June/July 2023 dry season (Baptista & Cruz, personal communication). While water temperature showed little variability across the year 2023 in São Tomé and Principe, in future studies, temperature should continue to be monitored to confirm that compensation does not need to be made for temperature before comparing RNA concentration and sRD. Out of the 180 countries measured in the Environmental Performance Index, São Tomé and Principe ranks near the bottom for air pollution and in the bottom half for environmental health (Block et al., 2024). Since pollution can have negative effects on the sRD of

organisms that differ seasonally (Amaral et al., 2009), measurements linked to water and water quality such as water flow rates, oxygen levels, pH and nutrient levels could also provide helpful information on the environmental conditions the little fish face, as well as any resultant differences in habitat between rivers and seasons.

The sRD results in this study follow the pattern of seasonality anticipated from literature and while the RNA and DNA concentration results were somewhat surprising, they were in line with expected results. Further testing using other indices of health and growth of larvae and post-larvae could be beneficial for clarification of the ecophysiological condition of these species. Measurements of protein synthesis as well as protein degradation and damage could provide further elucidation, as could testing for free amino acids (Rosa & Nunes, 2004). Knowledge about the little fish of São Tomé Island is still in its infancy and measurements of environmental conditions are limited, so a wide variety of factors both about these fish and the habitat in which they live remain open for further exploration.

5. Conclusion

Standardized RNA:DNA ratios have been shown to provide a snapshot into the ecophysiological condition of a fish larvae at the time of sampling. Comparison of these snapshots among the different species of the post-larval little fish on São Tomé show that there are significant differences between their levels in the wet and dry seasons with higher values during the wet. Based on the current body of knowledge on sRD, this is indicative of a difference in the condition of the little fish between seasons; a better condition in the wet season than in the dry. Both RNA and DNA concentrations were higher during the dry season, with the higher relative increase in DNA concentration indicating poorer condition of post-larvae. In the future, more work is needed on sampling, habitat assessment and more, in order to further develop knowledge of the condition of the little fish of São Tomé Island. This study is consistent with expected results but a number of questions and other directions of inquiry remain that can be explored in subsequent research.

6. References

Afonso, P., Santos, R. S., & Barreiros, J. P. (1999). Coastal marine fishes of São Tomé Island (Gulf of Guinea). *Arquipelago Ciencias Biologicas e Marinhas*, *17A*, 65–92.

Al-Jufaili, S. M., Esmaeili, H. R., Jawad, L., & Sayyadzadeh, G. (2021). Insights into the taxonomy and mitochondrial phylogenetic affinity of the longnose goby, *Awaous jayakari* (Teleostei: Gobiidae). *Environmental Biology of Fishes*, *104*(10), 1177–1193. https://doi.org/10.1007/s10641-021-01144-1

Alves, F., Dromby, M., Baptista, V., Ferreira, R., Correia, A. M., Weyn, M., Valente, R., Froufe, E., Rosso, M., Sousa-Pinto, I., Dinis, A., Dias, E., & Teodósio, M. A. (2020). Ecophysiological traits of highly mobile large marine predators inferred from nucleic acid derived indices. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-61769-7

Amaral, V., Penha-Lopes, G., & Paula, J. (2009). RNA/DNA ratio of crabs as an indicator of mangrove habitat quality. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *19*(SPEC. ISS.). https://doi.org/10.1002/aqc.1039

Baptista, V., Dias, E., Cruz, J., Branco, M., Vieira, S., & Teodósio, M. A. (2020). Feeding ecology of *Sicydium bustamantei* (Greeff 1884, Gobiidae) post-larvae: The "Little Fish" of São Tomé Island. *Oceans*, *1*(4), 300–310. https://doi.org/10.3390/oceans1040020

Baptista, V., & Cruz, J. Personal communication (2023).

Bell, K. N. I., Brown, J. A., & Pepin, P. (1995). Seasonal, inverse cycling of length- and age-at-recruitment in the diadromous gobies *Sicydium punctatum* and *Sicydium antillarum* in Dominica, West Indies. *Canadian Journal of Fisheries and Aquatic Sciences, 52*(7), 1535-1545. https://doi.org/10.1139/f95-147

Bell, K. N. I. (1999). An overview of goby-fry fisheries. *NAGA - The ICLARM Quarterly*, *22*(4), 30–36.

Bergenius, M. A. J., McCormick, M. I., Meekan, M. G., & Robertson, D. R. (2005). Environmental influences on larval duration, growth and magnitude of settlement of a coral reef fish. *Marine Biology*, *147*(2), 291–300. https://doi.org/10.1007/s00227-005-1575-z

Bergeron, J. P. (1997). Nucleic acids in ichthyoplankton ecology: a review, with emphasis on recent advances for new perspectives. *Journal of Fish Biology*, *51*(sA), 284–302. https://doi.org/10.1111/j.1095-8649.1997.tb06104.x

Bhat, H., Ahmad, I., Asimi, O., Shah, M., Irfan Ahmad, I., Oyas Asmi, I., Feroz Shah, I., Bilal Bhat, I., Tariq Hussain, I., Mehak Hafeez, I., Sumaira Rashid, I., Nakeer Razak, I., Raja Aadil Hussain Bhat, I., Asmi, O., Shah, F., Bhat, B., Hussain, T., Hafeez, M., Rashid, S., … Aadil Hussain Bhat, R. (2020). RNA: DNA ratio as an indicator of growth, nutritional status and condition of fish: A review. *Journal of Entomology and Zoology Studies*, *8*(6), 654–658.

Block, S., Emerson, J. W., Esty, D. C., de Sherbinin, A., & Wendling, Z. A. (2024). *2024 Environmental Performance Index*. New Haven, CT: Yale Center for Environmental Law & Policy.

Buckley, L. J. (1980). Changes In ribonucleic acid, deoxyribonucleic acid, and protein content during ontogenesis in winter flounder, *Pseudopleuronectes americanus*, and effect of starvation. *Fishery Bulletin*, *77*(3), 703–708.

Buckley, L. J. (1984). RNA-DNA ratio: an index of larval fish growth in the sea. *Marine Biology*, *80*, 291–298.

Buckley, L., Caldarone, E., & Ong, T. L. (1999). RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. *Hydrobiologia*, 401, 265–277.

Bulow, F. J. (1970). RNA–DNA ratios as indicators of recent growth rates of a fish. *Journal of the Fisheries Board of Canada*, *27*(12), 2343–2349. https://doi.org/https://doi.org/10.1139/f70-262

Caldarone, E.M.; Wagner, M.; St Onge-Burns, J.; Buckley, L.J. Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. In *Northeast Fisheries Science Center Reference Document 01–11*; Woods Hole: Falmouth, MA, USA, 2001; p. 22.

Caldarone, E. M., Clemmesen, C. M., Berdalet, E., Miller, T. J., Folkvord, A., Holt, G. J., Olivar, M. P., & Suthers, I. M. (2006). Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish. *Limnology and Oceanography: Methods*, *4*(5), 153–163. https://doi.org/10.4319/lom.2006.4.153

Caldarone, E., Stonge-Burns, J., & Buckley, L. (2003). Relationship of RNA/DNA ratio and temperature to growth in larvae of Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series*, *262*, 229–240. https://doi.org/10.3354/meps262229

Castellanos-Galindo, G. A., Carlo, G., Garcés, S., Beltrán-León, B. S., Carlo Sanchez (, G., Beltrán-León (, B. S., & Zapata, L. (2011). A goby-fry fishery in the northern Colombian Pacific Ocean. *International Journal of Ichthyology*, *35*(4), 391–395.

Chícharo, M. A., & Chícharo, L. (2008). RNA:DNA ratio and other nucleic acid derived indices in marine ecology. *International Journal of Molecular Sciences*, *9*(8), 1453–1471. https://doi.org/10.3390/ijms9081453

Chou, S. C., de Arruda Lyra, A., Gomes, J. L., Rodriguez, D. A., Alves Martins, M., Costa Resende, N., da Silva Tavares, P., Pereira Dereczynski, C., Lopes Pilotto, I., Martins, A. M., Alves de Carvalho, L. F., Lima Onofre, J. L., Major, I., Penhor, M., & Santana, A. (2020). Downscaling projections of climate change in Sao Tome and Principe Islands, Africa. *Climate Dynamics*, *54*(9–10), 4021–4042. https://doi.org/10.1007/s00382-020-05212-7

Clemmesen, C. (1994). The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. *Marine Biology*, *118*, 377–382.

Diouf, K. (2020). *Sicydium brevifile. The IUCN Red List of Threatened Species 2020*. https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T183161A58334378.en

dos Santos, E. L. P. (2023). *Captura por Esforço do Peixinho em São Tomé e espécies acessórias*. Universidade de São Tomé e Príncipe.

Engman, A. C., Kwak, T. J, & Fischer, J. R. (2017). Recruitment phenology and pelagic larval duration in Caribbean amphidromous fishes. *Freshwater Science, 36*(4), 851-865. https://doi.org/10.1086/694176

EPA. (2024, February 24). *Dissolved Oxygen*. United States Environmental Protection Agency.

Ferron, A., & Leggett, W. C. (1994). An Appraisal of Condition Measures for Marine Fish Larvae. *Advances in Marine Biology*, *30*(C), 217–303. https://doi.org/10.1016/S0065-2881(08)60064-4

Floeter, S. R. (2007). Coastal fishes of São Tomé and Príncipe Islands, Gulf of Guinea (Eastern Atlantic Ocean) - an update. *Zootaxa*, *1523*, 1–48. https://doi.org/10.5281/zenodo.177470

Foley, C. J., Bradley, D. L., & Höök, T. O. (2016). A review and assessment of the potential use of RNA:DNA ratios to assess the condition of entrained fish larvae. *Ecological Indicators*, *60*, 346–357. https://doi.org/10.1016/j.ecolind.2015.07.005

Gallegos, C. L., Jordan, T. E., & Correll, D. L. (1992). Event-scale response of phytoplankton to watershed inputs in a subestuary: Timing, magnitude, and location of blooms. *Limnology and Oceanography*, *37*(4), 813–828. https://doi.org/10.4319/lo.1992.37.4.0813

Goncalves, R., Gesto, M., Teodósio, M. A., Baptista, V., Navarro-Guillén, C., & Lund, I. (2021). Replacement of Antarctic krill (*Euphausia superba*) by extruded feeds with different proximate compositions: effects on growth, nutritional condition and digestive capacity of juvenile European lobsters (*Homarus gammarus*, L.). *Journal of Nutritional Science*, *10*(36). https://doi.org/10.1017/jns.2021.27

Graça Aranha, S., Teodósio, A., Baptista, V., Erzini, K., & Dias, E. (2023). A glimpse into the trophic ecology of deep-water sharks in an important crustacean fishing ground. *Journal of Fish Biology*, *102*(3), 655–668. https://doi.org/10.1111/jfb.15306

Iida, M., Watanabe, S., & Tsukamoto, K. (2015). Oceanic larval duration and recruitment mechanism of the amphidromous fish *Sicyopterus japonicus* (Gobioidei: Sicydiinae). *Regional Studies in Marine Science, 1*, 25-33. https://doi.org/10.1016/j.rsma.2015.03.001

Kawakami, A. (2010). Stem cell system in tissue regeneration in fish. *Development, Growth & Differentiation*, *52*(1), 77–87. https://doi.org/10.1111/j.1440-169X.2009.01138.x

Keith, P., Lord, C., Lorion, J., Watanabe, S., Tsukamoto, K., Couloux, A., & Dettai, A. (2011). Phylogeny and biogeography of Sicydiinae (Teleostei: Gobiidae) inferred from mitochondrial and nuclear genes. *Marine Biology*, *158*(2), 311–326. https://doi.org/10.1007/s00227-010-1560-z

Lalèyè, P. (2020). *Awaous lateristriga. The IUCN Red List of Threatened Species 2020*. https://doi.org/10.2305/IUCN.UK.2020-2.RLTS.T183156A21912369.en

Lieder, S., Jahn, M., Koepff, J., Müller, S., & Takors, R. (2016). Environmental stress speeds up DNA replication in *Pseudomonas putida* in chemostat cultivations. *Biotechnology Journal*, *11*(1), 155–163. https://doi.org/10.1002/biot.201500059

Lisse, T. S., Brochu, E. A., & Rieger, S. (2015). Capturing tissue repair in zebrafish larvae with time-lapse brightfield stereomicroscopy. *Journal of Visualized Experiments*, *95*. https://doi.org/10.3791/52654

Mathers, E. M., Houlihan, D. F., & Burren, L. J. (1994). RNA, DNA and protein concentrations in fed and starved herring *Clupea harengus* larvae. *Marine Ecology Progress Series*, *107*(3), 223–232. https://doi.org/10.3354/meps107223

McDowall, R. M. (2007). On amphidromy, a distinct form of diadromy in aquatic organisms. *Fish and Fisheries*, *8*(1), 1–13. https://doi.org/10.1111/j.1467-2979.2007.00232.x

Menge, B. A., Olson, A. M., & Dahlhoff, E. P. (2002). Environmental stress, bottom-up effects, and community dynamics: Integrating molecular-physiological and

ecological approaches. *Integrative and Comparative Biology*, *42*(4), 892–908. https://doi.org/10.1093/icb/42.4.892

Moelants, T. (2015). *Sicydium bustamantei. The IUCN Red List of Threatened Species 2010*. https://doi.org/10.2305/IUCN.UK.2010-3.RLTS.T182284A7849800.en

Monti, D., Lefrançois, E., Lord, C., Mortillaro, J. M., Lopez, & P. J., Keith, P. (2018). Selectivity on epilithic diatom consumption for two tropical sympatric gobies: *Sicydium punctatum* Perugia, 1986 and *Sicydium plumieri* (Bloch, 1786). *Cybium: Revue Internationale d'Ichtyologie, 42*, 365-373. https://hal.science/hal-02549067

Morioka, S., Ohno, A., Kohno, H., & Taki, Y. (1996). Nutritional condition of larval milkfish, *Chanos chanos*, occurring in the surf zone. *Ichthyological Research*, *43*(4), 367–373. https://doi.org/10.1007/BF02347636

Müller, C., Erzini, K., Teodósio, M. A., Pousão-Ferreira, P., Baptista, V., & Ekau, W. (2020). Assessing microplastic uptake and impact on omnivorous juvenile white seabream *Diplodus sargus* (Linnaeus, 1758) under laboratory conditions. *Marine Pollution Bulletin*, *157*. https://doi.org/10.1016/j.marpolbul.2020.111162

Pérez-Ortín, J. E., Alepuz, P. M., & Moreno, J. (2007). Genomics and gene transcription kinetics in yeast. *Trends in Genetics*, *23*(5), 250–257. https://doi.org/10.1016/j.tig.2007.03.006

Pezold, F. L., & Harrison, I. J. (2006). The California Academy of Sciences Gulf of Guinea Expedition (2001) V. Multivariate analysis of Sicydiines of São Tomé & Príncipe with redescription of *Sicydium brevifile*. *Proceedings of The California Academy of Sciences*, *57*(34), 965–980.

Olivar, M., Diaz, M. V., & Chícharo, M. A. (2009). Efecto del tipo de tejido sobre los cocientes ARN: ADN en larvas de peces marinos. *Scientia Marina*, *73*(SUPPL. 1), 171–182. https://doi.org/10.3989/scimar.2009.73s1171

Pouil, S., & Colsoul, B. (2021). Aquaculture as a tool to support goby-fry fishery? Current knowledge on biology and ecology of the red-tailed goby *Sicyopterus lagocephalus*. *Aquaculture, Fish and Fisheries*, *1*(1), 16–26. https://doi.org/10.1002/aff2.17

R Core Team. (2018). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. Available online at https://www.R-project.org/.

Richard, P., Bergeron, J.-P., Boulhic, M., Galois, R., & Person-Le Ruyet, J. (1991). Effect of starvation on RNA, DNA and protein content of laboratory-reared larvae and juveniles of *Solea solea*. *Marine Ecology Progress Series*, *72*, 69–77. https://doi.org/10.3354/meps072069

Rosa, R., & Nunes, M. L. (2004). RNA, DNA and protein concentrations and amino acid profiles of deep-sea decapod *Aristeus antennatus*: An indication for seasonal variations of nutrition and growth. *Aquatic Living Resources*, *17*(1), 25–30. https://doi.org/10.1051/alr:2004003

Shalem, O., Dahan, O., Levo, M., Martinez, M. R., Furman, I., Segal, E., & Pilpel, Y. (2008). Transient transcriptional responses to stress are generated by opposing effects of mRNA production and degradation. *Molecular Systems Biology*, *4*. https://doi.org/10.1038/msb.2008.59

Valcarce, D. G., Sellés-Egea, A., Riesco, M. F., De Garnica, M. G., Martínez-Fernández, B., Herráez, M. P., & Robles, V. (2024). Early stress exposure on zebrafish development: effects on survival, malformations and molecular alterations. *Fish Physiology and Biochemistry*. https://doi.org/10.1007/s10695-024-01355-0

Wagner, M.M.; Durbin, E.G.; Buckley, L.J. RNA/DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. *Mar. Ecol. Prog. Ser.* **1998**, *162*, 173–181.

Westerman, M., & Holt, G. J. (1994). RNA:DNA ratio during the critical period and early larval growth of the red drum *Sciaenops ocellatus*. *Marine Biology*, *121*, 1–9.

7. Appendix

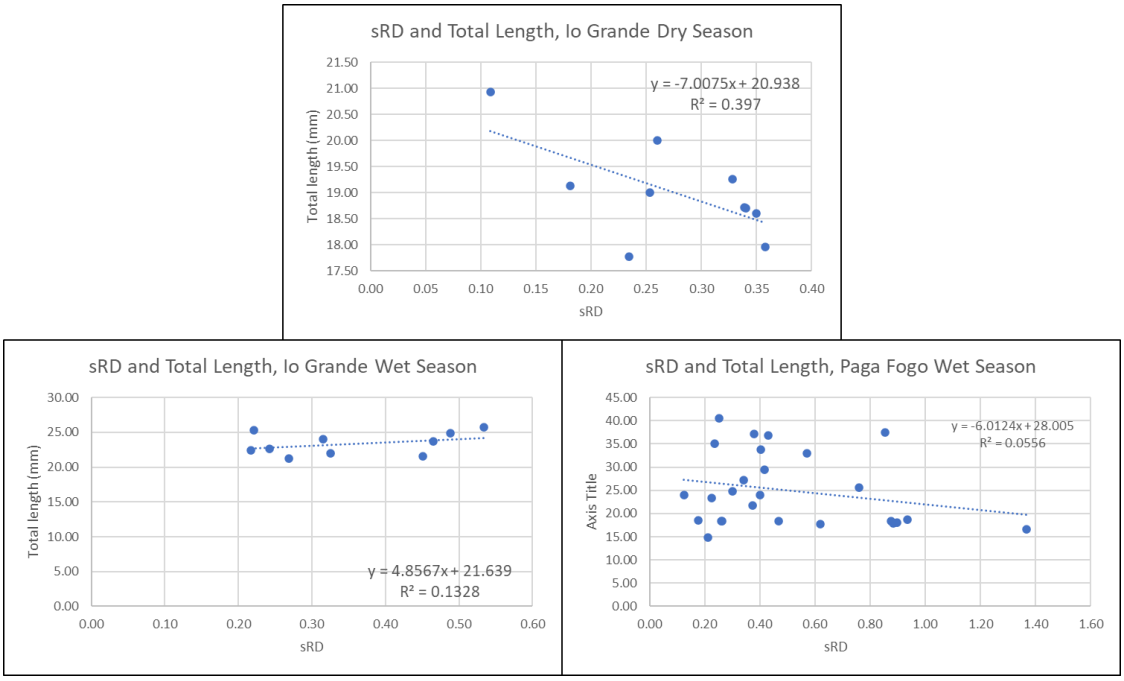

Figure A1 Linear regression of larval total length (mm) and standardized RNA:DNA ratio (sRD) of Awaous lateristriga collected in São Tomé and Príncipe during wet season (January 2023) and dry season (June/July 2023).

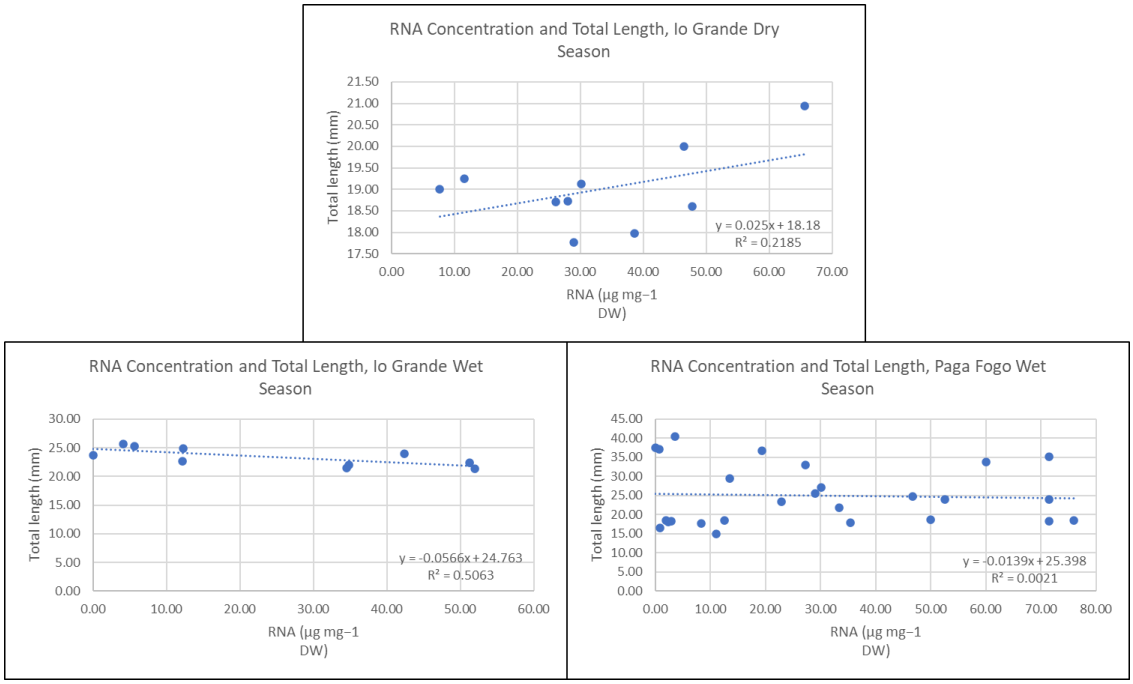

Figure A2 Linear regression of larval total length (mm) and RNA concentration of Awaous lateristriga collected in São Tomé and Príncipe during wet season (January 2023) and dry season (June/July 2023).

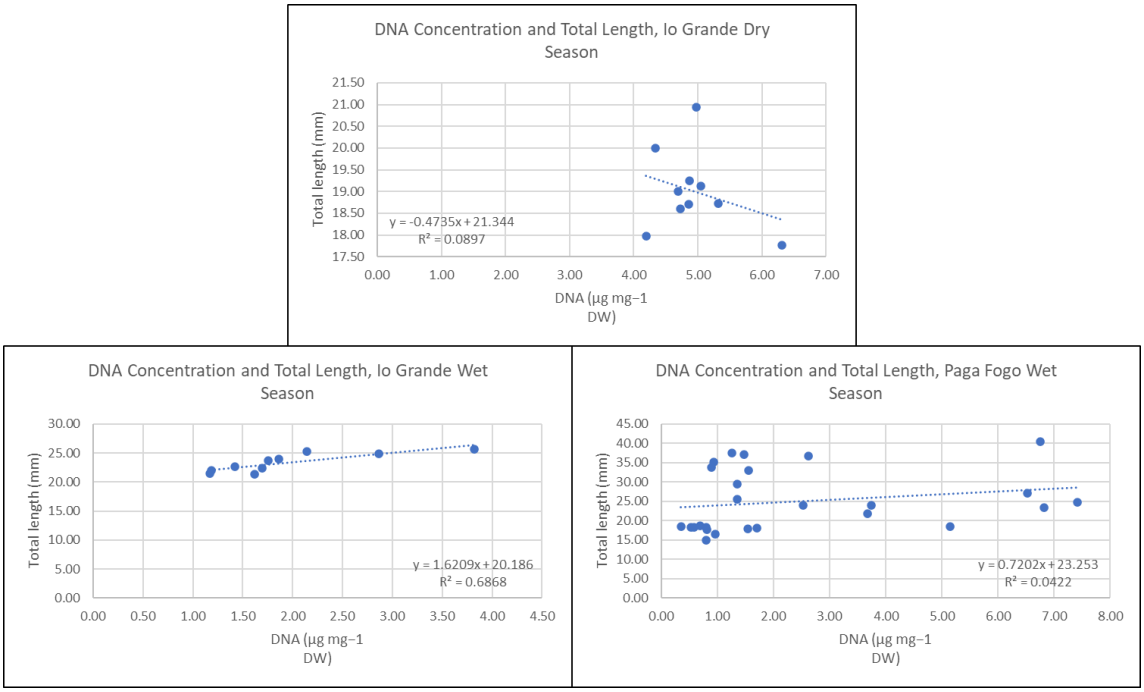

Figure A3 Linear regression of larval total length (mm) and DNA concentration of Awaous lateristriga collected in São Tomé and Príncipe during wet season (January 2023) and dry season (June/July 2023).

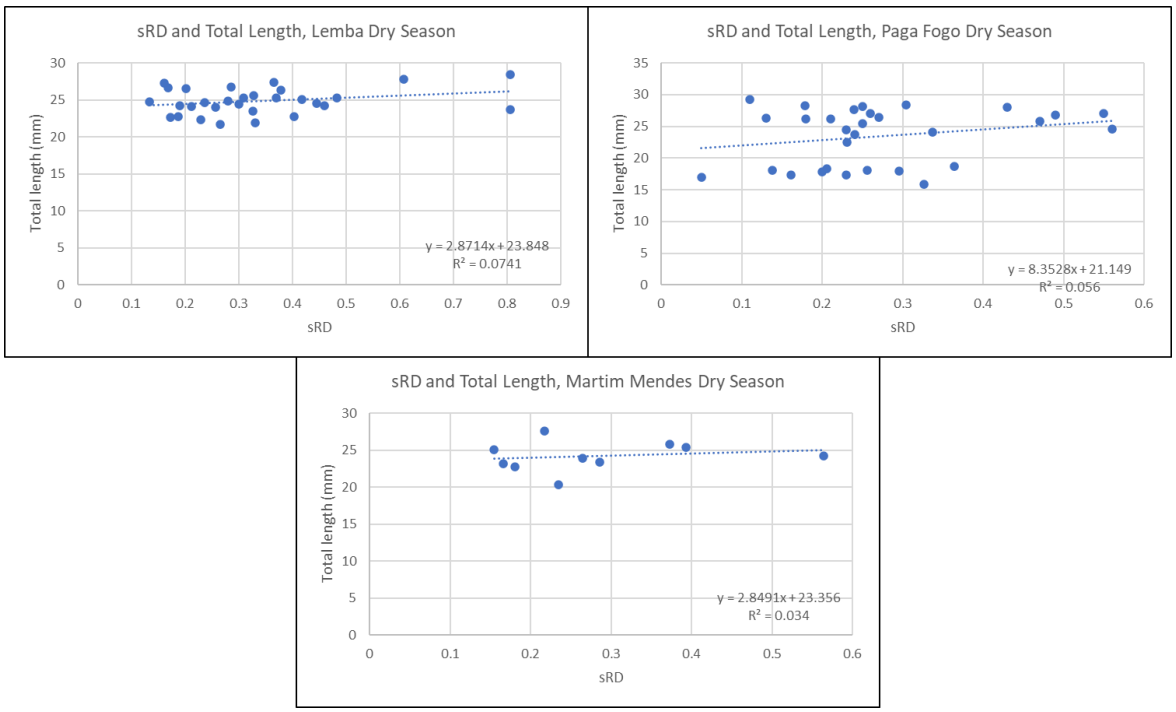

Figure A4 Linear regression of larval total length (mm) and sRD of both Sicydium species collected in São Tomé and Príncipe during dry season (June/July 2023).

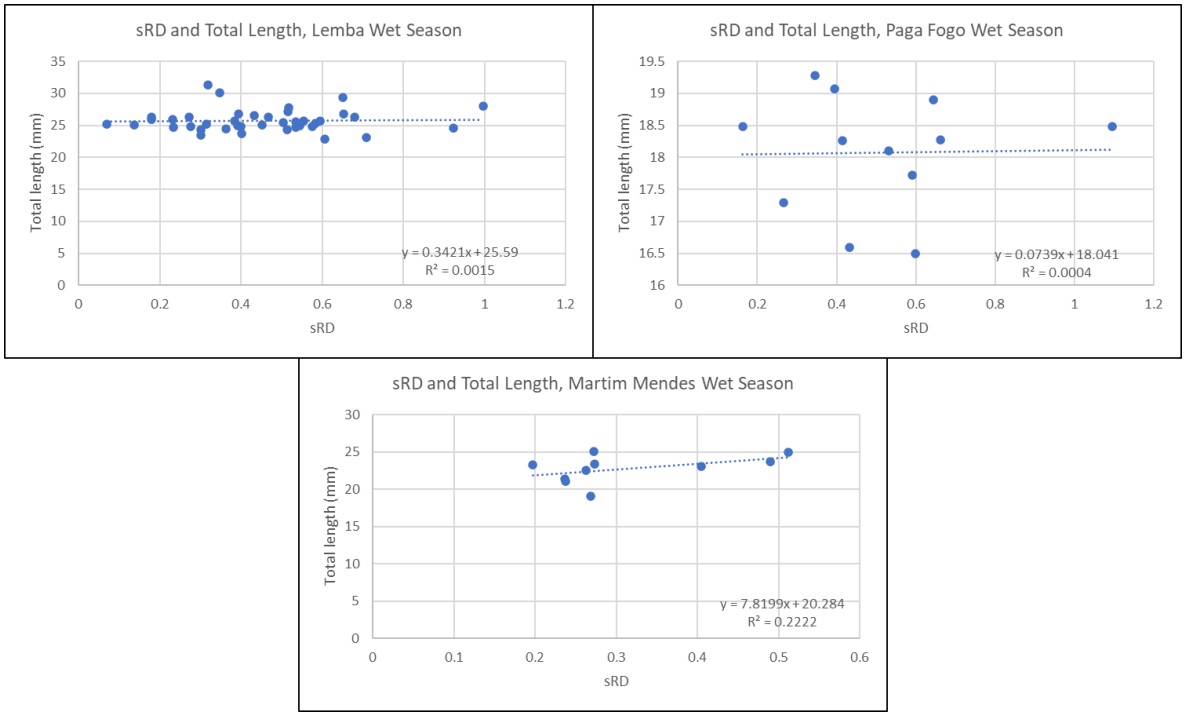

Figure A5 Linear regression of larval total length (mm) and sRD of both Sicydium species collected in São Tomé and Príncipe during wet season (January 2023).

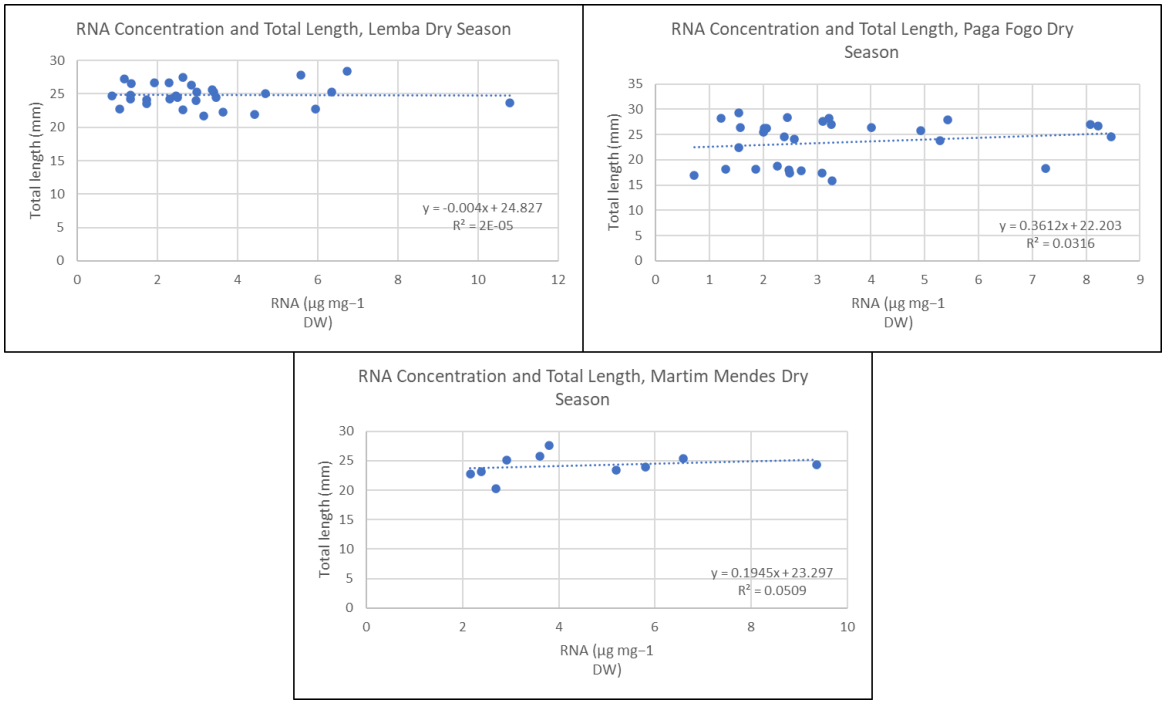

Figure A6 Linear regression of larval total length (mm) and RNA concentration of both Sicydium species collected in São Tomé and Príncipe during dry season (June/July 2023).

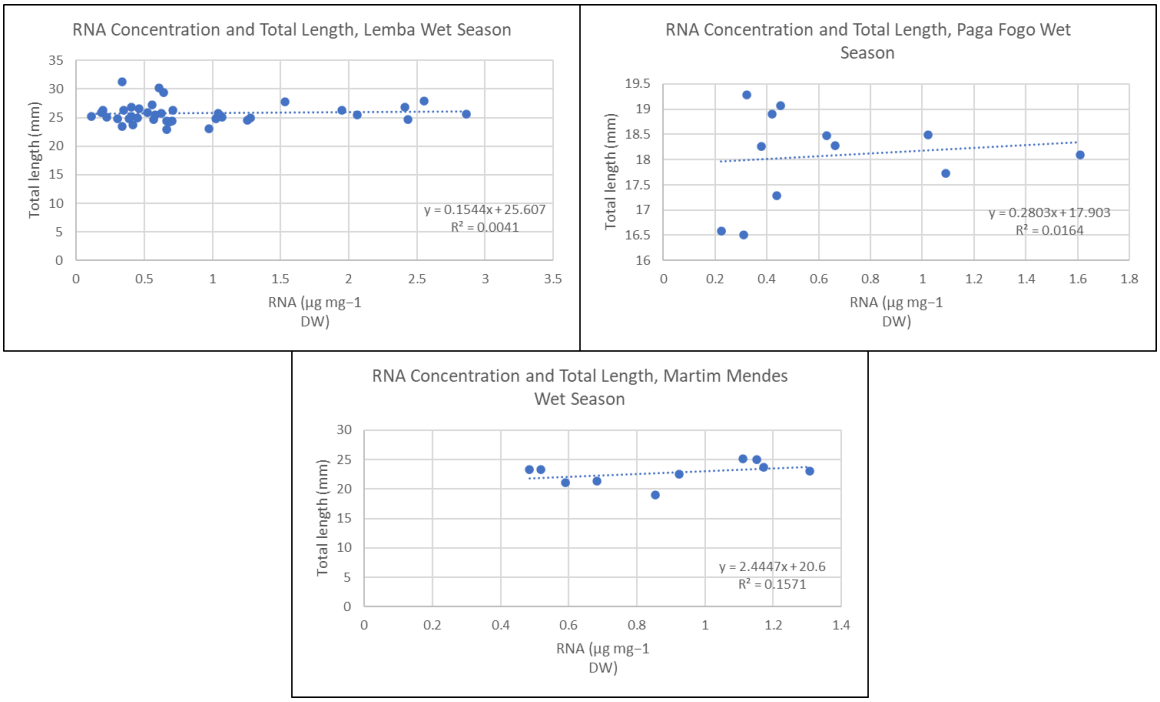

Figure A7 Linear regression of larval total length (mm) and RNA concentration of both Sicydium species collected in São Tomé and Príncipe during wet season (January 2023).

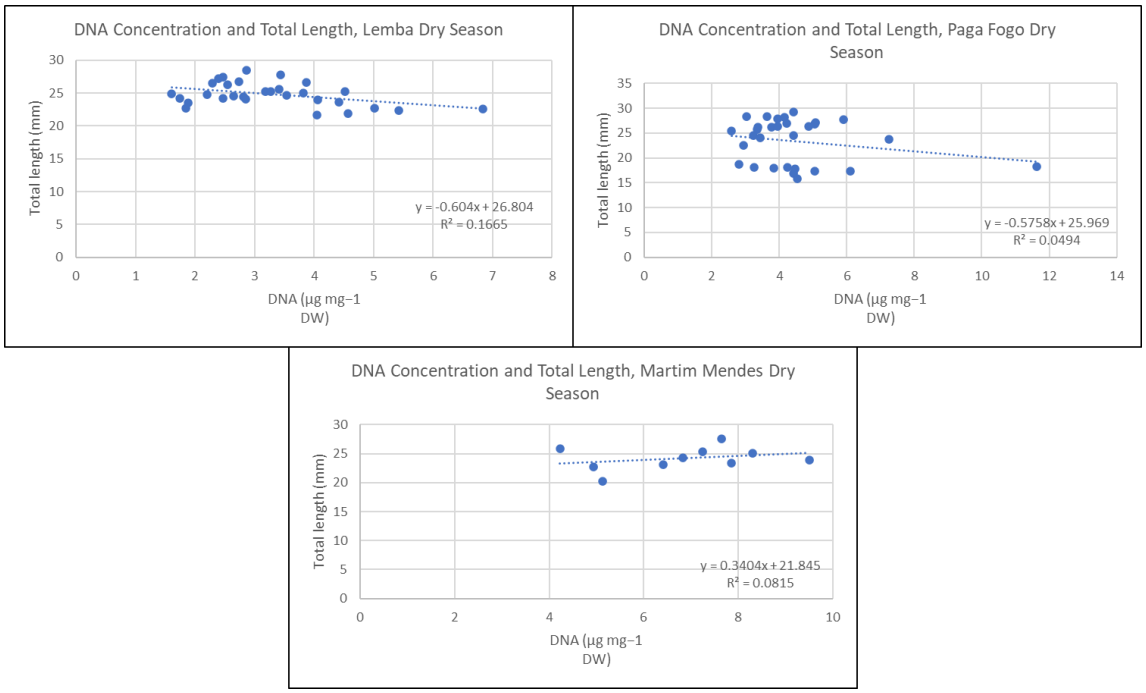

Figure A8 Linear regression of larval total length (mm) and DNA concentration of both Sicydium species collected in São Tomé and Príncipe during dry season (June/July 2023).

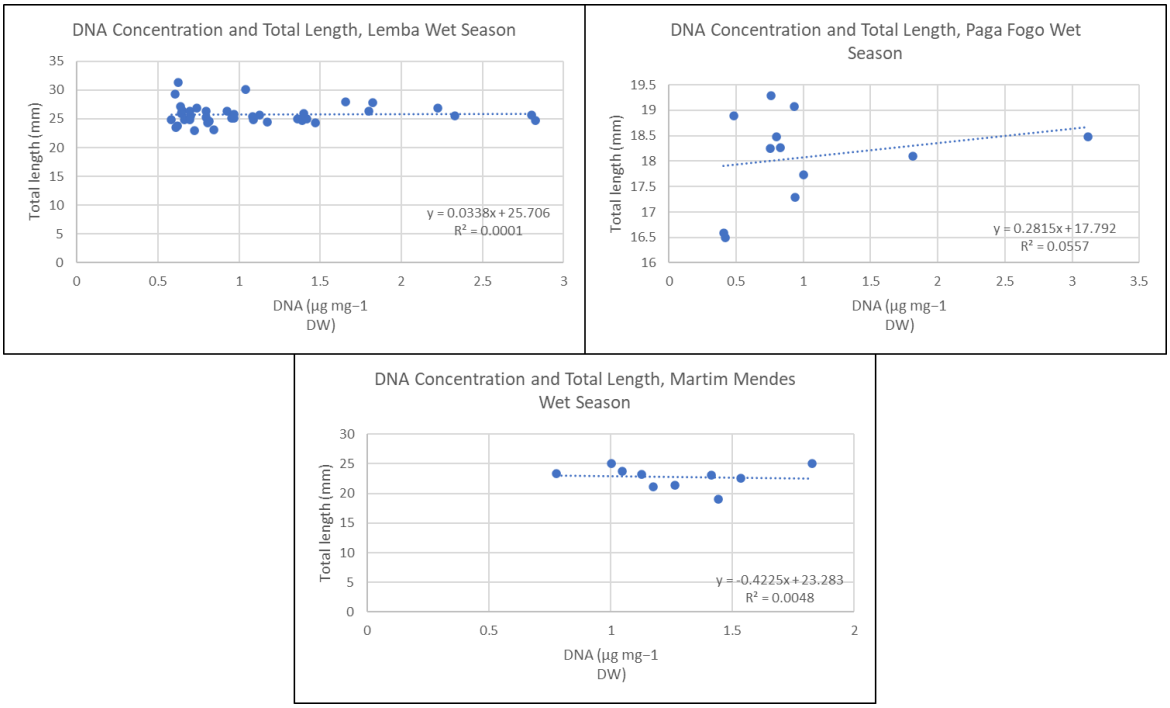

Figure A9 Linear regression of larval total length (mm) and DNA concentration of both Sicydium species collected in São Tomé and Príncipe during wet season (January 2023).

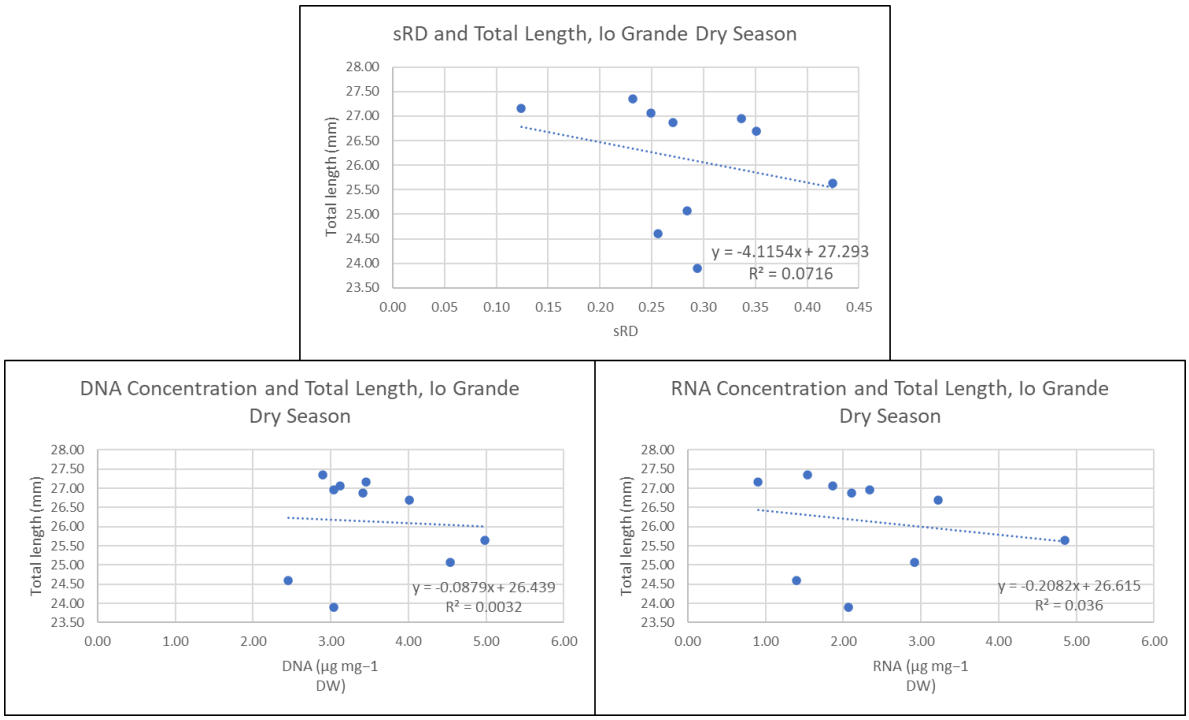

Figure A10 Linear regression of larval total length (mm) and (clockwise from top) sRD, RNA concentration and DNA concentration of Sicydium brevifile in the Iô-Grande River collected in São Tomé and Príncipe during dry season (June/July 2023).