Standardized Protocol for RUV Data Extraction

[version 1.0 - 05/Jun/26]

1. OBJECTIVE

1.1 This protocol aims to standardize procedures for data extraction and analysis from Remote Underwater Videos (RUVs or “videoplots”), ensuring that the resulting datasets are understandable, reproducible, and usable by any member of the ReefRUV Global Network.

2. PROCEDURE

2.1 Videoplot file organization

2.1.1 Since the ReefRUV global network aims to standardize data sampling and analysis procedures, as well as to enable data sharing with collaborators at a global scale, it is essential that, after field sampling, videoplots are organized in a standardized manner. This ensures that the data will be well understood and used by any collaborator.

2.1.2 Videoplots must be stored on external hard drives or cloud drives, and at least one additional backup copy should be maintained on another device.

2.1.3 The complete collection of videoplots generated by the ReefRUV global network will be centralized and stored on external hard drives under the responsibility of the Marine Macroecology and Biogeography Laboratory at the Federal University of Santa Catarina (Florianópolis, SC, Brazil). This ensures that the entire metadata remains updated and available for sharing among collaborators. The most recent version of the metadata will be available on the official project webpage under the file name “ReefRUV metadata”. Any video files can be requested by partners contacting: Dr Sergio R. Floeter (PI): sergiofloeter@gmail.com

2.1.4 It is recommended that videoplot files are organized in a hierarchical folder structure to ensure standardization and facilitate data sharing and interpretation among ReefRUV collaborators.

2.1.5 The suggested file structure is as follows:

- **Level 1 – Location + sampling year:** Example: “Santa_Catarina_2026”.

● **Level 2 – Site + sampling date:** Within the main folder, create subfolders for each sampled site, including location name and date in DD_MM_YY format:

Examples: “Arvoredo_10_03_26” and “Xavier_15_03_26”.

● **Level 3 – Camera (optional):** When more than one camera is used on field sampling, it is recommended to create subfolders for each device in order to facilitate organization and traceability:

Example: “Gopro_01”, “Gopro_02”, “Gopro_03”.

● **Level 4 – Videoplots (sequential order):** Within each camera folder (or directly within the site folder if only one camera is used), create sequentially numbered folders corresponding to each videoplot:

Example: “1”, “2”, “3”.

● **Level 5 – Videoplot content:** Within each numbered folder, include:

- The corresponding videoplot file;
- A subfolder named “Photoquadrat” containing the benthic photoquadrats associated with that videoplot.

● **Example of the complete recommended structure:**

Santa_Catarina_2026 > Arvoredo_20_03_26 > Gopro_1 > 1 > [videoplot + “Photoquadrat” folder]

2.2 *Video* analysis

2.2.1 Before starting videoplot analysis, the researcher must begin filling out the standard ReefRUV spreadsheet (available on the ReefRUV Global Network homepage) (Figure 1) and save a copy using the sampling location and year (e.g., “Videoplots_Santa_Catarina_2026”).

2.2.2 Then, register the videoplot file name in the previously saved spreadsheet (as in “Level 5” in item 2.1.5) in the “**video_code**” field, as well as the sampling location, site, depth, and water temperature (see Figure 1), obtained from the dive computer (Figure 2), each variable in its respective column.

2.2.3 Verify that all information is correctly filled and also matches the data recorded in the "**ReefRUV metadata**" spreadsheet (see item 2.1.3).

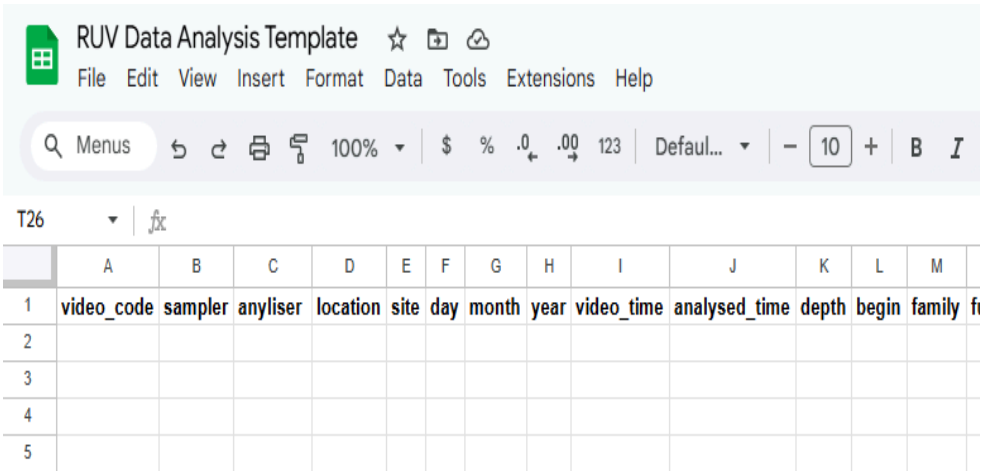

Figure 1. Standard ReefRUV spreadsheet. The complete version is available on the official project homepage. **Annex I** provides a template example.

Figure 2. Diver displaying the dive computer at the start of recording, showing depth (yellow circle) and temperature (°C) (red) (Photo: Lucas Nunes).

2.2.4 To begin analysis, open the videoplot file in a media player with speed control (e.g., VLC media player, Media Player Classic).

2.2.5 Delimit a **2 m² area** on the screen using the measurements recorded by the diver at the beginning of the video as a spatial reference. Use illustration softwares or digital ruler tools such as ***MB Ruler***. Only interactions within this area will be analyzed (Figures 3 and 4)**.**

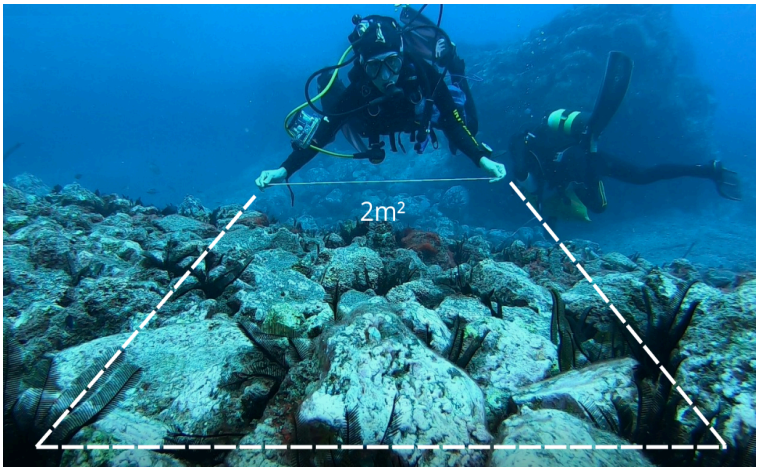

Figure 3. Delimitation of the area of analysis. All analyzed interactions must occur within the delineated polygon (Foto: Debora Ferrari).

2.2.6 After delimiting the area, define the **central 10 minutes** of the video to be analyzed. For example, in a 15-minute video, analyze from 02:30 to 12:30 min.

NOTE 1: If any video is shorter than 15 minutes due to technical issues, maintain the analysis of the central 10 minutes. However, exclude segments where the diver is still present. Start timing only after the diver leaves the field of view.

2.2.7 When estimating fish individual size (Total Length), consider perspective effects (trapezoidal distortion), where distant fish appear smaller. Use proportional scaling based on known references (e.g., measuring tape, rope, diver body parts).

NOTE 2: Always compare with the maximum size reported in the literature. If the estimated size exceeds the known maximum, adjust accordingly. Record size in centimeters (cm).

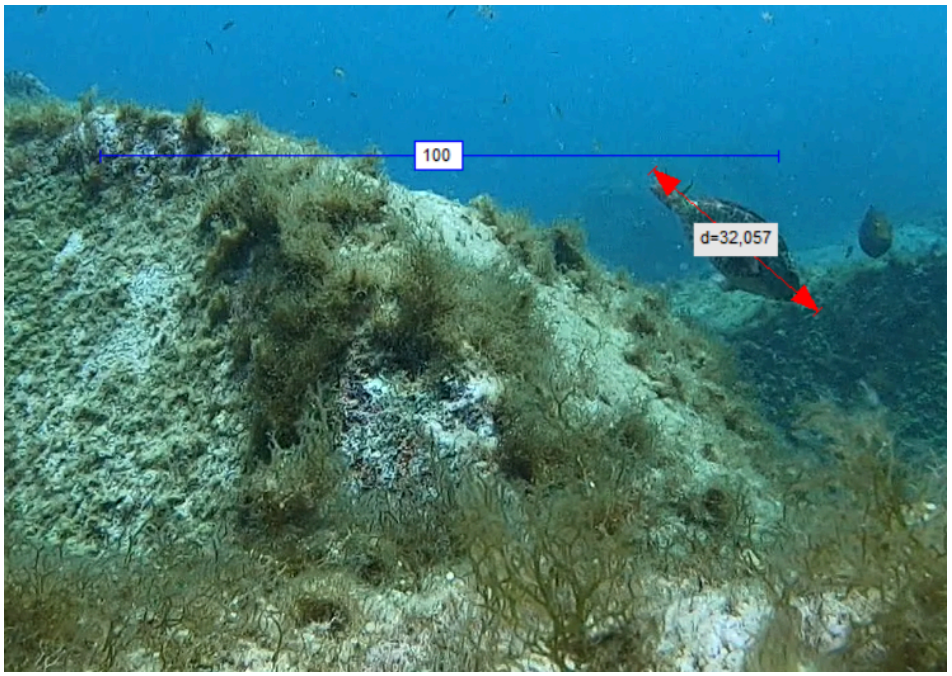

Figura 4. Example of size estimation using scale references. The blue line represents a one-meter-wide scale, previously delineated by the diver using a rope; the red line represents the estimated size of a fish measured based on the blue scale (Photo: Caroline Leão).

2.2.8 Very small individuals (< 5 cm) far from the camera (near the 2 m limit) should be excluded from the analysis. In such cases, the camera's distance does not allow for precise identification of the individual's size or behavior (e.g., chasing, cleaning, defecating, scratching, feeding).

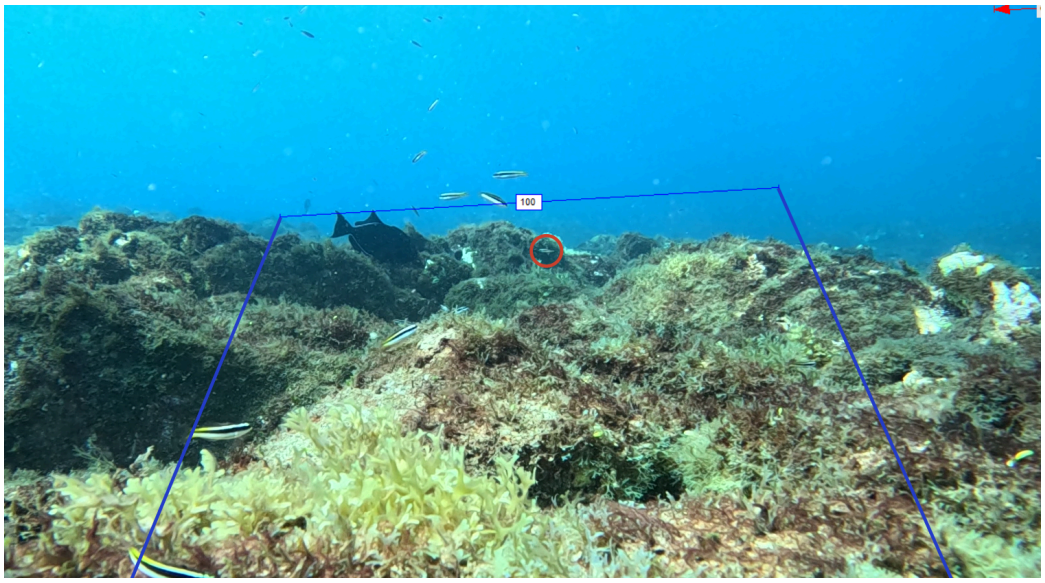

Figure 5. Example of a very small individual (< 5 cm), highlighted by the red circle, in which it is not possible to accurately identify the species, size, or interaction. Although it is within the area to be analyzed (delimited by the *MB Ruler*), it is excluded from the analysis. (Foto: Caroline Leão).

2.2.9 Record all observed behaviors (e.g., chase, cleaning, defecation, rubbing, feeding), including entry time ("**begin**"), exit time ("**end**"), and duration ("**obs_time**"), as specified in the spreadsheet. Record the kind of behavior under "**activity**".

2.2.10 Use playback controls to review segments if needed, including slow motion.

2.2.11 For behavioral interaction analyses, individuals entering the videoplot (2 m²) and only swimming—even if only for a short period (e.g., 1 second)—should be recorded only once per species, regardless of the number of individuals observed throughout the video. The entry time of the first individual of each species into the sampled area should be recorded; subsequent entries of the same species without interactions do not require additional records. In order to estimate species diversity per videoplot, the goal is to record all species, even if no behavioral interaction is observed.

NOTE 3: For other objectives, such as abundance estimates, different approaches can be employed in a complementary way.

2.2.12 In the spreadsheet "**activity**" column, enter "NA" for any cases like those mentioned in item 2.2.11.

2.2.13 Below, there is a brief description of how to fill in each column of the project's standard spreadsheet:

- **video_code:** code of the videoplot to be analyzed;
- **sampler:** name of the person responsible for collecting the videoplot in the field (preferably full name);
- **analyser:** name of the person responsible for analyzing the videoplot in the laboratory (preferably full name);
- **location:** location where the videoplot was collected;
- **site:** site within the location where the videoplot was collected. For example, a video may have been collected in the Arvoredo Marine Biological Reserve (Florianópolis, SC), but within the reserve, there are different sites such as Rancho Norte, Saco do Capim, Baía das Tartarugas, etc.;
- **day:** day on which the videoplot was collected;
- **month:** month of videoplot collection, to be recorded in numeric format (01–12);
- **year:** year of videoplot collection, to be recorded using four digits (e.g., 2026);
- **video_time:** total duration of the videoplot, to be recorded in **hh:mm:ss** format (e.g., a video lasting 15 minutes and 30 seconds should be recorded as 00:15:30). This format must be used to facilitate subsequent analyses in software such as R and RStudio;
- **analysed_time:** actual duration of the videoplot analysis (see item 2.2.6), also recorded in **hh:mm:ss** format;
- **depth:** depth at which the videoplot was collected, as recorded by the dive computer;
- **begin:** start time of the individual's interaction within the analyzed area, recorded in **hh:mm:ss** format;
- **end:** end time of the individual's interaction within the analyzed area, recorded in **hh:mm:ss** format;
- **obs_time:** total duration of the individual's interaction within the analyzed area, obtained by subtracting the start time from the end time;
- **individual:** sequential numeric identifier assigned to each new individual observed. Each individual must receive a unique and sequential number (e.g., 1, 2, 3…).

NOTE 4: If the same individual leaves the delimited area and later returns, a new row must be created in the spreadsheet. However, the same number should be maintained in the "**individual**" column, although that should happen only if it is possible to ensure that it is the same individual.

- **family:** family to which the identified fish individual belongs;
- **functional_group:** functional group to which the species belongs (e.g., macrocarnivore, macroalgal browser, herbivore, omnivore, detritivore, etc.; Ferreira et al., 2004; Siqueira et al., 2019; Nunes et al., 2023);
- **species:** genus and species identified in the analyzed videoplot (e.g., *stegastes_rocasensis*, *abudefduf_saxatilis*, *thalassoma_noronhanum*). Entries

must be written in lowercase only, using "_" (underscore) instead of spaces between genus and species in order to ensure standardization and compatibility with analysis software such as R and RStudio;
● **spp_code:** species code, composed of the first three letters of the genus and the first three letters of the species (e.g., for *abudefduf_saxatilis*, the code should be "abu_sax");
● **size:** estimated size of the individual in centimeters (cm);
● **activity:** observed behavior of the individual (e.g., chase, cleaning, defecation, rubbing, feeding);
● **bites:** number of bites performed by the individual when the observed behavior is feeding, recorded in numeric format (e.g., 1, 2, 3…).

NOTE 5: Although the behavior is classified as feeding, this method does not allow to ensure actual ingestion. Thus, a "bite" is defined as any strike toward the substrate or water column indicating a feeding-related movement.

2.2.14 ***Additional notes on spreadsheet completion*:**

2.2.14.1 Do not use blank spaces, as they hinder data analysis in software such as R and RStudio. Instead, use "_" (underscore);

2.2.14.2 Empty cells must always be recorded as "NA" to facilitate data analysis in software such as R and RStudio;

2.2.14.3 The spreadsheet must be completed entirely in English to facilitate data sharing, access, and analysis by collaborators from different nationalities;

2.2.14.4 Each row in the spreadsheet must correspond to a single individual observed within the videoplot sampling area. See **NOTE 4** for further details;

2.2.14.5 The 1 m height measurement relative to the reef substrate may be used when it is necessary to determine the fish position in the water column. Although it is possible to consider a three-dimensional volume (i.e., a 2 m^3 cube), this approach should not be used in comparative studies over time, in order to maintain compatibility with historical datasets that did not include this dimension. This ensures methodological standardization and spatial and temporal comparability of the data.

2.3 Photoquadrat analysis

2.3.1 To estimate the percentage of benthic cover on the reef, photoquadrats can be analyzed using the free software CoralNet (https://coralnet.ucsd.edu/). This online platform was developed by the University of California San Diego and the Scripps Institution of Oceanography and is widely used for automated analysis of coral reef photoquadrat images. It uses artificial intelligence (machine learning) to automatically identify benthic

components, allowing scientists and conservationists to rapidly quantify coral, algal, and other reef components. Another platform with a similar purpose that could be used is MERMAID (https://datamermaid.org/).

2.4 Turf analysis

2.4.1 Data entry and analysis of turf height, collected alongside the videoplots, must be linked to their respective videoplot ("**video_code**", see item 2.2.2). This standardization aims to facilitate data sharing and use among all network collaborators.

2.4.2 See Figure 6 for the standard spreadsheet template containing all the necessary information for linking turf data with videoplots.

2.4.3 Measured turf height ("**height_cm**") must be recorded in centimeters (cm).

	A	B	C	D	E	F	G	H	I	J
1	**video_code**	**location**	**site**	**sampler**	**depth**	**temperature**	**day**	**month**	**year**	**height_cm**
2	GOPRO11_20260127_001	xavier	xavier	talita	9,4	26	27	1	2026	2,4
3	GOPRO11_20260127_001	xavier	xavier	talita	9,4	26	27	1	2026	1,5
4	GOPRO11_20260127_001	xavier	xavier	talita	9,4	26	27	1	2026	1,4
5	GOPRO11_20260127_001	xavier	xavier	talita	9,4	26	27	1	2026	2
6	GOPRO11_20260127_001	xavier	xavier	talita	9,4	26	27	1	2026	2,2
7	GOPRO11_20260127_002	xavier	xavier	talita	11,2	26	27	1	2026	1,5
8	GOPRO11_20260127_002	xavier	xavier	talita	11,2	26	27	1	2026	1,7
9	GOPRO11_20260127_002	xavier	xavier	talita	11,2	26	27	1	2026	1,9
10	GOPRO11_20260127_002	xavier	xavier	talita	11,2	26	27	1	2026	1,8
11	GOPRO11_20260127_002	xavier	xavier	talita	11,2	26	27	1	2026	1,1
12	GOPRO11_20260127_003	xavier	xavier	talita	11,8	26	27	1	2026	2,1
13	GOPRO11_20260127_003	xavier	xavier	talita	11,8	26	27	1	2026	2,4
14	GOPRO11_20260127_003	xavier	xavier	talita	11,8	26	27	1	2026	1,5
15	GOPRO11_20260127_003	xavier	xavier	talita	11,8	26	27	1	2026	1,3

Figure 6. Standard spreadsheet for recording turf height data.

2.5 Reef complexity or relief - This extra and useful metric may be annotated following semiquantitative schemes, for example, Langlois (2017).

2.6 Abundance (MaxN) - We recommend all fish be identified to the lowest taxonomic level possible. The standard metric of abundance is MaxN, the maximum number of individuals of a given species present in a single video frame (see Langlois et al., 2020 and references).

2.7 Feeding pressure - Bite rates could be measured directly from the videos; however, it is recommended to use “Feeding Pressure” as the standardized measure of consumer impact on benthic resources that integrates fish biomass and feeding activity. It is calculated as the product of bite rate and consumer biomass per unit area and time, providing a proxy for the intensity of trophic interactions between reef fishes and the benthos (see Longo et al., 2019 for details).

3. REFERENCES

FERREIRA, C.E.L.; FLOETER, S.R.; GASPARINI, J.L.; JOYEUX, J.C. & FERREIRA, B.P. (2004) Trophic structure patterns of Brazilian reef fishes: a latitudinal comparison. *Journal of Biogeography*, 31: 1093–1106.

FONTOURA, L. et al. (2020) The macroecology of reef fish agonistic behaviour. *Ecography*, 43: 1278–1290, doi: 10.1111/ecog.05079

LANGLOIS, T.J. (2017) Habitat annotation of forward-facing benthic imagery. Retrieved from https://github.com/TimLanglois/Habitat-annot ation-of-forward-facing-benthic-imagery

LANGLOIS, T., GOETZE, J., BOND, T., et al. (2020) A field and video annotation guide for baited remote underwater stereo-video surveys of demersal fish assemblages. *Methods Ecol Evol.*,11: 1401–1409. https:// doi.org/10.1111/2041-210X.13470

LONGO, G.O.; HAY, M.E.; FERREIRA, C.E.L. & FLOETER, S.R. (2019) Trophic interactions across 61 degrees of latitude in the Western Atlantic. Global Ecology and Biogeography, 2018. DOI: https://doi.org/10.1111/geb.12806

NUNES, L.T.; FLOETER, S.R. & FERREIRA, C.E.L. (2023) Feeding ecology of Brazilian reef fishes (pp. 13–20). In S.R. Floeter et al. (Eds.), *Brazilian Reef Fishes* (p. 320). Editora CRV.

SIQUEIRA, A.C.; BELLWOOD, D.R. & COWMAN, P.F. (2019) The evolution of traits and functions in herbivorous coral reef fishes through space and time. *Proceedings of the Royal Society B: Biological Sciences*, v. 286, n. 1897, p. 20182672.

TEBBETT, S.B. & BELLWOOD, D.R. (2019) Algal turf sediments on coral reefs: what’s known and what’s next. *Marine Pollution Bulletin*, v. 149, p. 110542. https://doi.org/10.1016/j.marpolbul.2019.110542

[Version prepared and revised by: C.C. Leão; T.M. Beneli; S.R. Floeter]

Standardized Protocol for RUV Data Extraction

ANNEX I – STANDARD TEMPLATE FOR SPREADSHEET COMPLETION

video_code	sampler	analyser	location	site	day	month	year	habitat	video_time	analysed_t	depth	begin	family	functional_group	species	spp_code	size	bites	activity	end	ind
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	1'26"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	24	0	swimm	1'29"	1
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	1'29"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	25	0	swimm	1'49"	2
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	1'31"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	23	0	swimm	2'00"	3
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	1'33"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	25	0	swimm	1'42"	4
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	1'49"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	25	2	feed	1'51"	2
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	2'01"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	40	0	coça	2'06"	5
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	2'24"	holocentridae	mobile_invertebrate	holocentrus_adscensionis	hol_ads	22	0	swimm	2'20"	6
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	2'33"	holocentridae	mobile_invertebrate	holocentrus_adscensionis	hol_ads	22	0	swimm	2'38"	7
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	2'56"	holocentridae	mobile_invertebrate	holocentrus_adscensionis	hol_ads	23	0	swimm	3'03"	8
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	3'14"	labridae	mobile_invertebrate	thalassoma_sanctaehelenae	tha_san	7	0	swimm	3'17"	9
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	3'18"	pomacentridae	territorial_herbivore	stegastes_sanctaehelenae	ste_san	8	0	chase	3'18"	10
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	3'30"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	23	0	swimm	3'44"	11
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	3'30"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	25	0	swimm	3'45"	12
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	3'46"	holocentridae	mobile_invertebrate	holocentrus_adscensionis	hol_ads	26	0	swimm	4'20"	13
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	4'36"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	30	0	swimm	4'44"	14
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	4'45"	labridae	herbivore_detritivore	sparisoma_strigatum	spa_str	30	1	feed	4'49"	15
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	4'58"	labridae	herbivore_detritivore	sparisoma_strigatum	spa_str	30	0	swimm	5'05"	16
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	5'36"	labridae	mobile_invertebrate	bodianus_insularis	bod_ins	30	0	swimm	5'45"	17
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	6'03"	pomacentridae	territorial_herbivore	stegastes_sanctaehelenae	ste_san	6	0	chase	6'05"	18
Buttermilk_29.01.23_video_1_fundo	debora	mariana	st_helena	buttermilk	29	1	2023	interface	12'53"	10'00"	13.5	6'31"	pomacentridae	territorial_herbivore	stegastes_sanctaehelenae	ste_san	9	2	feed	6'32"	19