

1. INTRODUCTION

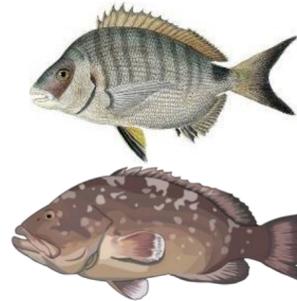
Spearfishing is a **highly selective fishing method** that allows to capture fish individually. Although it can sometimes have negative effects, such as reducing the density and size of target species and impacting marine biodiversity and ecosystem stability, it offers a valuable scientific opportunity by enabling the collection of samples from specific species or sizes that might be difficult to obtain through other methods.

The aim study is to create and validate a protocol for sample collecting and analysing **to turn spearfishing into a useful source of scientific data.**

2. MATERIALS AND METHODS

Common sea bream (*Diplodus sargus*) was used as fish species example to implement and validate the protocol. Ten fish samples were initially collected, with an additional six fresh muscle tissue samples obtained later. Two difficult-to-access target specimens dusky groupers (*Epinephelus marginatus*) were also analyzed.

- **Measures and weights:** Biological parameters and index (TL, SL, and TW, HSI, GSI, K) were calculated
- **Sample collection:**
 - Muscle (for parasitological (par), histopathological (hist) analysis)
 - Gills (par)
 - Internal organs (carried out using an isolated device similar to the one proposed by Torre et al. (2016)):
 - Digestive tract (study of microplastic and par)
 - Other organs (lives, spleen, gonads, kidney, heart) (par and hist)
 - Otoliths
- **Analyses:**
 - Organs were observed under stereomicroscope at 10x to 45x magnification to look for **microplastic** and **metazoan parasites**. The recovered parasites were stained with ferric acetocarmine, dehydrated through a graded ethanol series, cleared in eugenol, and mounted in Canada balsam. Meanwhile, the AFs were collected and mounted between glass slides with filtered distilled water for later identification under a microscope.
 - A piece of muscle (**frozen and fresh**) were fixed in 10% buffered formalin and processed using routine histology, and stained with hematoxylin-eosin for subsequent microscopic observation.



	Number of individuals affected	Intensity (range)	Location
Monogeneans	1	14	G
Gnathia	1	1	G
Copepods	1	1	G
Didymozoidae	1	1	G
Anthropogenic Fibers (AFs)	NE	NE	S, I

Table 2:. Parasitological results observed in two *E. marginatus* Abbreviations: G = Gills, S = Stomach, I= Intestine, IBW = Internal body water, NA: Not Evaluable.

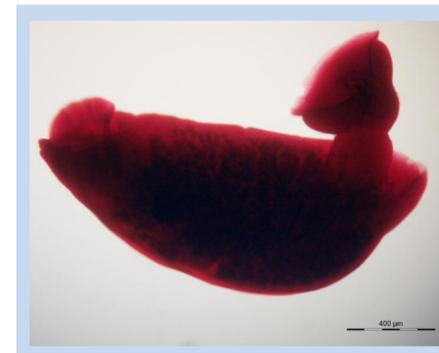


Figure 1: Monogenean parasite (*Encotyllabe vallei*) isolated from *D. sargus* gill tissue (50x magnification).



Figure 2: Microscopic visualization of anthropogenic fibers (AFs) isolated from *D. sargus* intestinal samples (400x magnification)

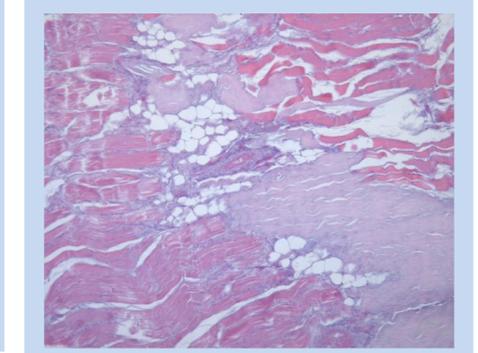


Figure 3: Microscopic image of *D. sargus* muscle fibers (100X) with increased connective tissue potentially associated to Abnormal Tough Syndrome (ATS).

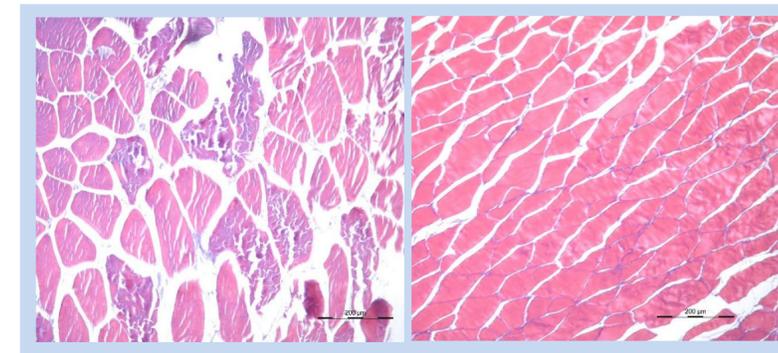


Figure 4: Histological differences between previously frozen muscle fibers (left) and non-frozen muscle fibers (right) observed under the microscope at 100x.

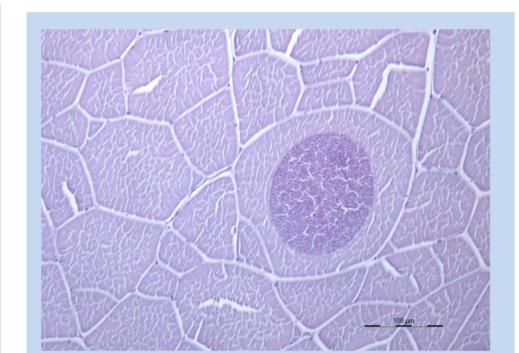


Figure 5: Muscle histology of a fish with microsporidian cysts (200X)

3. RESULTS

	Prevalence	Intensity (range)	Location
Monogeneans	30	1-2	G
<i>Encotyllabe vallei</i>	30	1-2	G
Digeneans	60	1-5	S, I, IBW
<i>Gymnophalloidea sp.</i>	50	1-5	S, I, IBW
<i>Digenea genus sp.</i>	10	1	I
Copepods	20	2	G
<i>Hatschekia sargi</i>	20	2	G
Nematodes	10	1	I
<i>Anisakidae sp.</i>	10	1	I
Anthropogenic Fibers (AFs)	70	1-13	S, I
<i>Cellulose fibers (0-1mm)</i>	10	6	S
<i>Cellulose fibers (1-2mm)</i>	60	1-4	S, I
<i>Cellulose fibers (2-3mm)</i>	40	1	S, I
<i>Cellulose fibers (3-4mm)</i>	20	1	S
<i>Cellulose fibres (>4mm)</i>	10	1	I

Table 1: Prevalence, intensity and location of parasites and microplastics found in *D. sargus*. Abbreviations: G = Gills, S = Stomach, I= Intestine, IBW = Internal body water

4. CONCLUSIONS

According to the results obtained, we can conclude that the **mentioned protocol is valid for detecting the presence of microplastics, parasites and others pathologies** in fish caught through spearfishing. This general protocol provides data from the fish species analysed in a holistic way, and could be modified depend on the aim of the study and/or other target species caught using this fishing method.

This makes spearfishing a **valuable method for the selective collection of samples and the monitoring of the marine environment** particularly for species that are difficult to capture using other methods and thus remain poorly studied in scientific research.

5. LITERATURE

Torre, M., Digka, N., Anastasopoulou, A., Tsangaris, C., & Mytilineou, C. (2016). Anthropogenic microfibrils pollution in marine biota. A new and simple methodology to minimize airborne contamination. *Marine Pollution Bulletin*, 113(1-2), 55-61. <https://doi.org/10.1016/j.marpolbul.2016.07.050>