Bottom trawling on a carbonate shelf: Do we get what we see?

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Continental Shelf Research
January 2021, Volume 213, Pages 104214 (7p.)
https://doi.org/10.1016/j.csr.2020.104314
https://archimer.ifremer.fr/doc/00661/77350/

Bottom trawling on a carbonate shelf: Can you get what you see?

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Abstract :

Bottom trawling is a common fisheries method and also a widespread scientific sampling method for benthic and demersal species. Selectivity and catchability are usually estimated using different meshes and studies with alternative methods are rare. In this study, to improve the estimation of trawl selectivity, we compare bottom trawl catches and recordings made by a camera fitted on the top of the net. Scientific trawling was conducted along the Northeast Brazilian shelf, a typical carbonate shelf with presence of sponges, coralline bottoms and a high water visibility. In total, 23 taxa (19 species and four genera) were identified in the videos and 30 taxa (25 species and five genera) in the trawls, indicating a higher potential of trawling to record species richness. Overall, no significant differences were observed in the assemblage structure identified by trawling and underwater footages. However, divergences were observed in fish catchability between trawls and videos. Net sampling was more selective for fish with low swimming capacity, while species with shelter and fast swimming capacity seemed to be underestimated. Furthermore, underwater footages allowed for an assessment of the trawling impacts on erect sponges with the observation of the damage on large branching and tubular sponges. The vulnerability of those habitats that concentrate higher fish diversity to mechanical impact raise the need for creation of preventive measures to protect these sensitive areas from commercial fishing operations. The use of less destructive methods is an important survey alternative under special conditions, particularly in conservation areas. In this study the videos could not be used as a complete alternative for the trawls, which presented higher efficiency, however, videos had shown to be a useful complement. Possibly, the speed and height determined by the trawling operation affected the performance of video sampling, being performed in suboptimal conditions. Using dedicated video sampling, such as towed video, could significantly improve its efficiency and compensate the disadvantages observed from the videos.

Highlights

► Video application in the evaluation of fish catchability by bottom trawling. ► Scientific trawling has shown higher performance in describing the fish community. ► Videos demonstrated to be useful complement for scientific trawling. ► The net impact on erect sponges raise the need for creation of preventive measures.

Keywords : Fish, Underwater footage, Fisheries, Catchability, Sponges, Northeast Brazil

1. Introduction

Scientific trawling is one of the most used methods to obtain abundance indices for fisheries management (Trenkel et al., 2004b; Hoffman et al., 2009). It provides the identification of a broad diversity of commercial and non-commercial fish and benthic organisms (Brind'Amour et al., 2014). Bottom trawl effectiveness is related to catchability and selectivity. Catchability is the relation of the relative abundance indices provided by a fishing technique and the actual population density (Trenkel et al., 2004a). In other words, it represents the interaction of the resource abundance and the effectiveness of the fishing gear (Arrenguín-Sanchez, 1996). Many factors can influence bottom trawl catchability, such as depth, season, fish behavior, fish vertical distribution, and gear selectivity (Beentjes et al., 2004; Yule et al., 2008). Selectivity is related to the probability of fish of a specific size to be captured by a fishing gear, providing measures of escapement (Arrenguín-Sanchez, 1996), which can occur both under and above the trawl net, also affecting trawl catchability (Pearcy et al., 1989).

Video recording methods are used to study the marine ecosystem for approximately 70 years (Barnes, 1952). They have demonstrated a satisfactory cost-effectiveness and they are non-destructive to the environment (Spencer et al., 2005, Mallet and Pelletier, 2014). Regarding trawling, videos have been used to compare the sampling effectiveness among fish species using bottom trawl and visual methods (Pearcy et al., 1989; Adams et al., 1995; McIntyre et al., 2015). However, the video footages were not simultaneous to the trawling. Studies that have used video records during trawling were in most cases investigating the behavior and escapement of a specific group of fish, such as flatfishes, elasmobranchs or other target species (Godo et al., 1999; Albert et al., 2003; Chosid et al., 2012; Hannah and Jones, 2012; Bryan et al., 2014; Melli et al., 2019; Noack et al., 2019; Young et al., 2019).

Here we compare the effectiveness of the two methodologies by performing them simultaneously during a scientific fish survey cruise. The study was performed over the Northeastern Brazilian continental shelf, which is dominated by carbonate sediments due to its low rate of precipitation and sedimentation (Camargo et al., 2015). This sediment-starved aspect provides clear waters and allows the preservation of topographic features, resulting in the presence of benthic habitats that harbor diverse biological communities (Rocha et al., 2000; Camargo et al., 2015; Eduardo et al., 2018). Specifically, we compared fish recorded by video with those captured by trawling, reported the noticeable fish reactions to the trawl net, and discussed on fish catchability according to their behavior and the characteristics of the habitat. We also recorded trawl impact on large size branching and tubular sponges, which in the region form conspicuous coral-sponge bottoms that present high biodiversity and represent a refuge for various commercial and sensitive fish species (Eduardo et al., 2018; Eduardo et al., 2020).

2. Material and Methods

2.1 Study Area

The study area (Fig.1) is situated in the Northeast Brazilian continental shelf, from 5ºS to 9ºS. The average of shelf width is 40 km, the shelf break is approximately at 60 m deep and biogenic carbonate sediments predominantly cover the area, constituting carbonate bottoms, typical of the region (Manso et al., 2003; Vital et al., 2010; Camargo et al., 2015). This region contains high biodiversity and some Marine Protected Areas, such as “APA dos Corais” and “APA Costa dos Corais”, which are in the north and south part of the study area respectively (Ferreira and Maida, 2007, Prates et al., 2007). APA is an area of sustainable use, corresponding to category V of the IUCN Protected Areas Categories (Lausche and Burhenne-Guilmin, 2011).

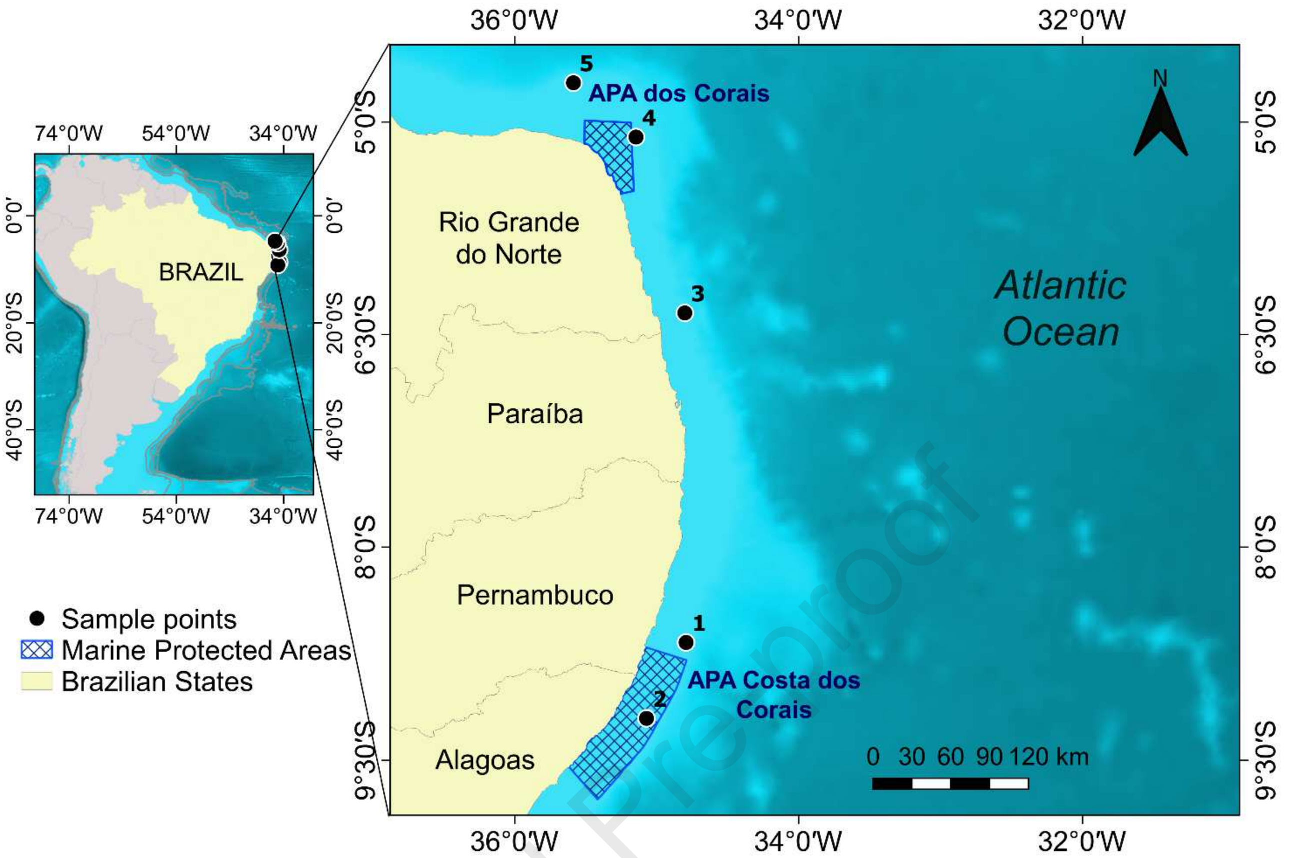

Fig.1. Study area with the five stations (sampling points) where we could compare video and trawling. Cartographic Base: IBGE, 2017. GCS_SIRGAS2000.

The study area (Fig.1) is part of the Northeastern Brazil Shelf-Edge Zone EBSA (Ecologically and Biologically Significant Area) (Secretariat of the Convention on Biological Diversity, 2014), that harbours coraline-sponge formations and may act as fish natural corridors between habitats in mesophotic zones (Feitoza et al., 2005; Olavo et al., 2011).

2.2 Sampling

Samples were collected on board the French R/V ANTEA, during the 'Acoustics along the BRAzilian COaSt 2' (ABRACOS 2; Bertrand, 2017) survey performed on 9 April – 9 May 2017. Sampling was conducted using a bottom trawl (body mesh: 40 mm, cod-end mesh: 25 mm, entrance dimensions horizontal x vertical: 28×10 m) and video footages by an underwater camera (GoPro Hero 3+) attached in the upper part of the mouth of the net. Videos were recorded from 14 trawls, however, only five trawls presenting videos with adequate field of view (recording the entire net mouth) were considered here. Sampling was thus restricted to depths between 36 and 64 meters, which correspond to mesophotic depths (Rocha et al., 2018), and trawling was performed during approximately 6 min at 3.0 knots. Tow duration was based on video observations, from the moment of the arrival of the net on the ocean floor to the lift-off time. To ensure the net was fishing correctly we utilized SCANMAR sensors to monitor the net geometry, to give headline height, depth, and distance of wings and doors. In addition, bobbins were added to the ground rope (rockhopper) to reduce net damage and impacts on benthic habitat.

For each haul, fish were identified, counted, and preserved with a solution of 4% formalin in seawater or by freezing until processing. For video identification and counting, trained people performed visual census based on fish identification guides (Lessa and Nóbrega, 2000; Humann and Deloach, 2002). Fish that could not be identified to species level were grouped to genus level. Fish species and families were classified according to Craig and Hastings (2007) and Nelson (2016).

2.3 Data analyses

Data consisted of fish counts (N) identified at the lowest taxonomic possible level obtained from videos observations and trawling catches. The relative index of density (catch per unit of area – CPUA) was calculated considering the number of individuals per trawled area (ind.km^{-2}). To estimate the trawled area, we multiplied the distance covered by the net through the bottom (in meters) with the average mouth opening (13 m) (Table 1).

Species composition was estimated by number (relative abundance - %N). To describe species dominance patterns we used the frequency of occurrence (FO) and the relative numerical abundance in percentage (NP) (Garcia et al., 2006). Species that presented NP greater/lower than the average were considered Abundant/Scarce. Similarly, species that presented FO greater/lower than the average were considered Frequent/Rare. Based on these criteria, the degree of species dominance was classified in four categories of relative importance (relative importance index): (1) Abundant and Frequent, (2) Abundant and Rare, (3) Scarce and Frequent and (4) Scarce and Rare (Garcia et al., 2006).

Table 1

Time duration and area covered by bottom trawls and video footages in total and in all five stations (sample points). Trawls were performed along the Northeast Brazilian continental shelf (4°–9°S) and video footages were recorded simultaneously by a camera installed on top of the anterior part of the trawl net.

Stations	**Time**	**Area (m^2)**
1	8min 25sec	10110
2	6min 9sec	7387
3	5min 50sec	7007
4	5min 17sec	6346
5	4min 38sec	5566
Total	30min 19sec	36416

We investigated patterns of catchability based on the differences between the number of individuals sampled by videos and by trawls (net change). Furthermore, we used the CPUA to investigate the structure of fish assemblages from videos and trawls. To reduce the effect of shoals in the analyses, the CPUA data was log-transformed (Log(x+1)).

We built a resemblance matrix, based on Bray-Curtis similarity index. The non-metric multidimensional scaling method (nMDS) was used to represent the samples graphically in a two-dimensional plane and the non-parametric permutation procedure ANOSIM (Analysis of Similarity) was applied to test for differences between the structure of fish assemblages from videos and trawls (Clarke, 1993). These analyses were performed using the software PRIMER6 + Permanova (Anderson et al., 2008) with a significance level of 0.05. Finally, we also counted all erect sponges that appeared in the videos.

3. Results

The five hauls analyzed in this study corresponded to a total effort of 30 min and 36,416 m^2 of trawled area (Table 1). From bottom trawls, 490 fishes distributed in 23 families and identified in 30 taxa, at the level of 25 species and 5 genera were captured. Whereas 370 fishes distributed in 19 families were identified through the videos, classified in 23 taxa at the level of 19 species and 4 genera (Table 2).

Approximately 70% of fish assemblages were represented by *Holocentrus adscensionis* (26% in videos and 29% in bottom trawls), *Pseudupeneus maculatus* (20% in videos and 15% in bottom trawls), *Diodon holocanthus* (0% in videos, 15% in bottom trawls), *Fistularia tabacaria* (13% in videos and 4% in bottom trawls) and *Acanthurus* spp. (9% in videos and 4% in bottom trawls) (Fig. 2).

Of the total 34 taxa composing the fish community detected by the sum of both methods, 88.2% were captured by bottom trawls and 67.6% were identified in video observations. Four species were only observed in videos: *Caranx crysos, Halichoeres dimidiatus, Malacanthus plumieri* and *Calamus pennatula,* while 11 were only observed in trawl catches, the most abundant being *Diodon holocanthus* (Table 2).

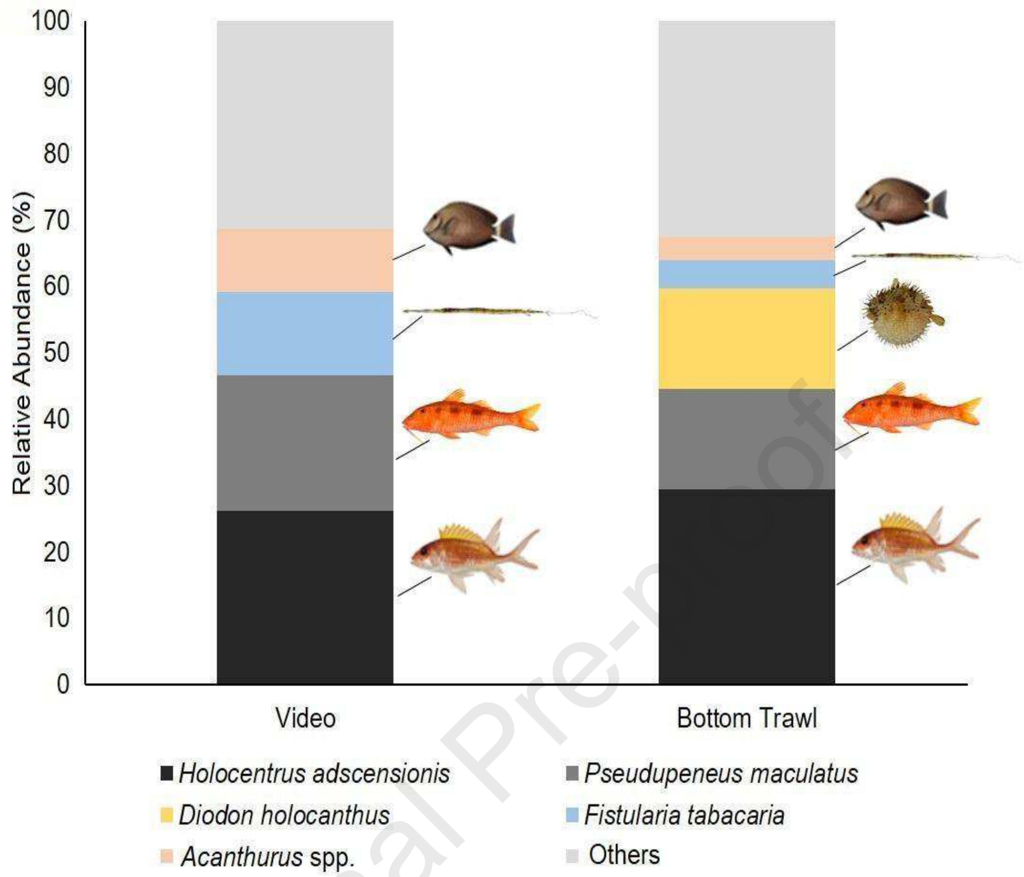

Fig. 2. Relative abundance of fishes (%) sampled by two different methods along the Northeast Brazilian continental shelf (4°–9°S): video and bottom trawl.

Table 2

List of species, number of individuals (N), relative importance index (1: Abundant and frequent; 2: Abundant and rare; 3: Scarce and frequent; 4: Scarce and rare) for fishes sampled in all five stations by videos and bottom trawls along the Northeast Brazilian continental shelf (4°–9°S).

Family	Genera / Species	N		Relative Importance Index	
		Video	Bottom Trawl	Video	Bottom Trawl
Acanthuridae	*Acanthurus* spp.	35	18	1	1
Aulostomidae	*Aulostomus maculatus*	0	5		4

Balistidae	*Balistes* sp.	1	2	4	3
Carangidae	*Caranx crysos*	1	0	4	
Chaetodontidae	*Chaetodon ocellatus*	9	19	3	1
	Chaetodon striatus	2	3	3	3
Dactylopteridae	*Dactylopterus volitans*	1	1	4	4
Dasyatidae	*Hypanus guttatus*	0	1		4
	Hypanus marianae	7	7	3	3
Diodontidae	*Diodon holocanthus*	0	74		1
Fistulariidae	*Fistularia tabacaria*	47	21	1	1
Gerreidae	*Eucinostomus* sp.	0	3		4
Haemulidae	*Anisotremus virginicus*	2	2	4	4
	Haemulon aurolineatum	9	13	4	4
	Haemulon plumierii	10	15	3	1
Holocentridae	*Holocentrus adscensionis*	97	144	1	1
Labridae	*Halichoeres dimidiatus*	1	0	4	
Lutjanidae	*Lutjanus synagris*	0	4		3
	Ocyurus chrysurus	1	2	4	4
Malacanthidae	*Malacanthus plumieri*	6	0	3	
Microdesmidae	*Ptereleotris randalli*	0	1		4
Monacanthidae	*Aluterus monoceros*	0	1		4
Mullidae	*Pseudupeneus maculatus*	75	74	1	1
Ostraciidae	*Acanthostracion* spp.	6	34	3	1
	Lactophrys trigonus	16	13	1	3
Pomacanthidae	*Holacanthus ciliaris*	6	6	3	3
	Pomacanthus paru	9	8	3	3
Pomacentridae	*Stegastes pictus*	0	1		4
Rhinobatidae	*Pseudobatos percellens*	0	1		4

Scaridae	*Sparisoma* spp.	9	13	3	3
Sciaenidae	*Pareques acuminatus*	0	1		4
Serranidae	*Alphestes afer*	0	2		4
	Cephalopholis fulva	15	1	1	4
Sparidae	*Calamus pennatula*	5	0	3	

Most species only registered either in videos or in trawl samples were considered scarce and rare in terms of relative importance index. Four species were considered abundant and frequent in both sampling methods (*Acanthurus* spp., *Fistularia tabacaria*, *Holocentrus adscensionis* and *Pseudupeneus maculatus*) and five were scarce and frequent (*Chaetodon striatus, Hypanus marianae, Holacanthus ciliaris, Pomacanthus paru* and *Sparisoma* spp.). We did not find any abundant and rare species (Table 2).

Considering the net change between fish sampled by the two different methods, the species that prevailed in the videos were *Malacanthus plumieri* (which was identified only by videos), *Acanthurus* spp., *F. tabacaria* and *Cephalopholis fulva,* while in the bottom trawls *Acanthostracion* spp., *H. adscensionis* and *Diodon holocanthus* (which was only identified on trawls) were predominant (Fig. 3).

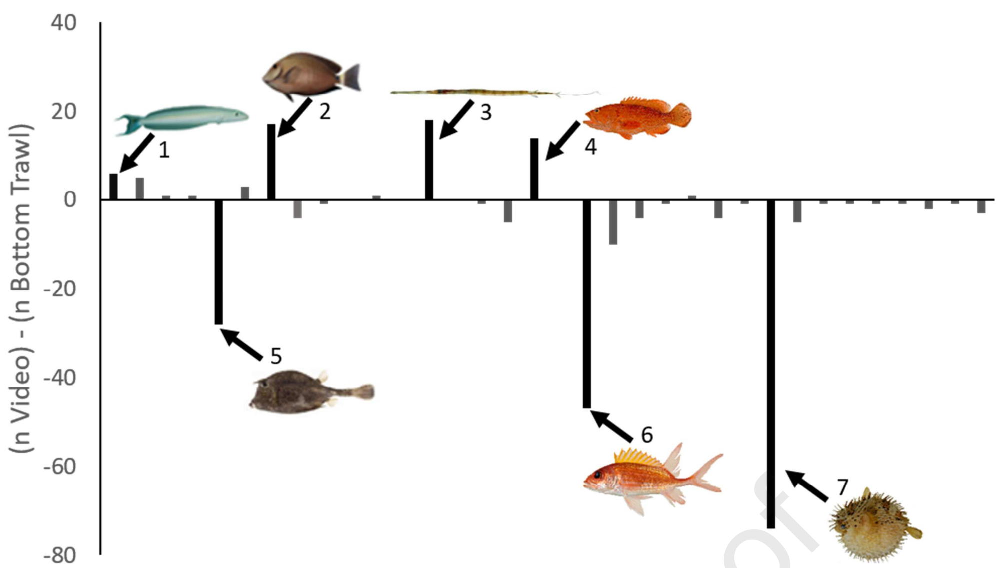

Fig. 3. Net change between fish sampled by videos and by bottom trawls along the Northeast Brazilian continental shelf (4°–9°S). The main differences are highlighted (1. *Malacanthus plumieri*; 2. *Acanthurus* spp.; 3. *Fistularia tabacaria*; 4. *Cephalopholis fulva*; 5. *Acanthostracion* spp.; 6. *Holocentrus adscensionis*; 7. *Diodon holocanthus*).

The segregation between samples is more noticeable than between methods (Fig. 4). Additionally, the ANOSIM analysis, based on the log-transformed CPUA dataset, did not reveal a significant difference between the structure of fish assemblages from video and bottom trawl ($R = -0.092$; $p = 0.72$).

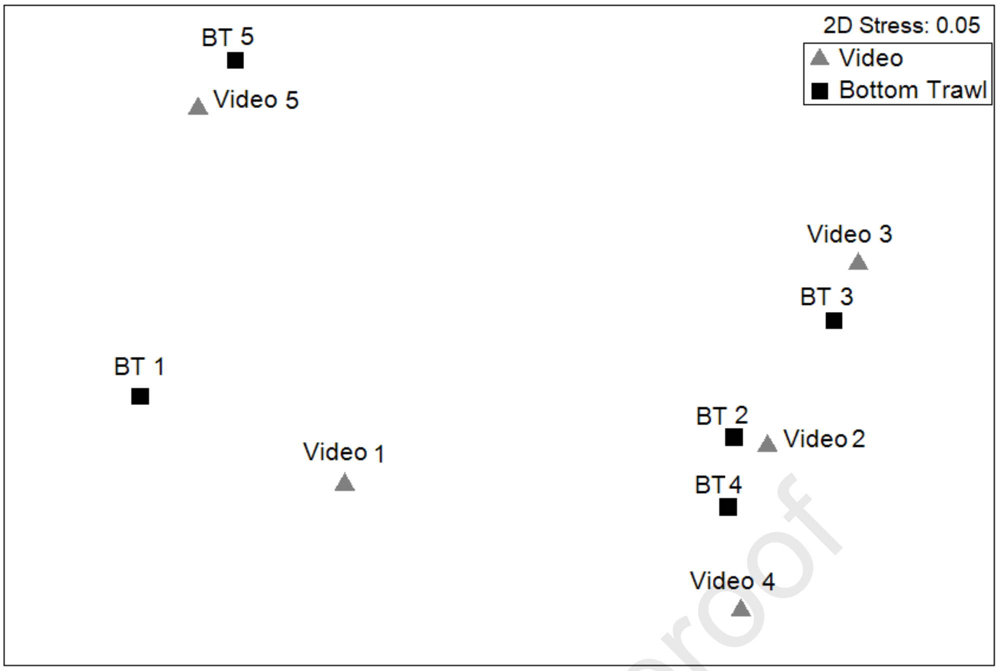

Fig. 4. Non-metric multidimensional scaling method (nMDS) of the CPUA data (ind.km^{-2}) of the fish assemblages sampled by videos and by bottom trawls along the Northeast Brazilian continental shelf (4°–9°S). The numbers (1; 2; 3; 4; 5) represent the stations (sample points); BT: Bottom trawl.

In total, 126 individual sponges were observed in videos, where 119 were classified as belonging to family Aplysinidae (94%). Of those, most resemble genera *Aplysina* and a few could be *Aiolochroia.* Remaining, seven organisms were classified as class Demospongiae, possibly belonging to the genera *Aplysina, Aiolochroia, Oceanapia* or *Geodia* All sponges hit by the trawl were damaged (Fig. 5).

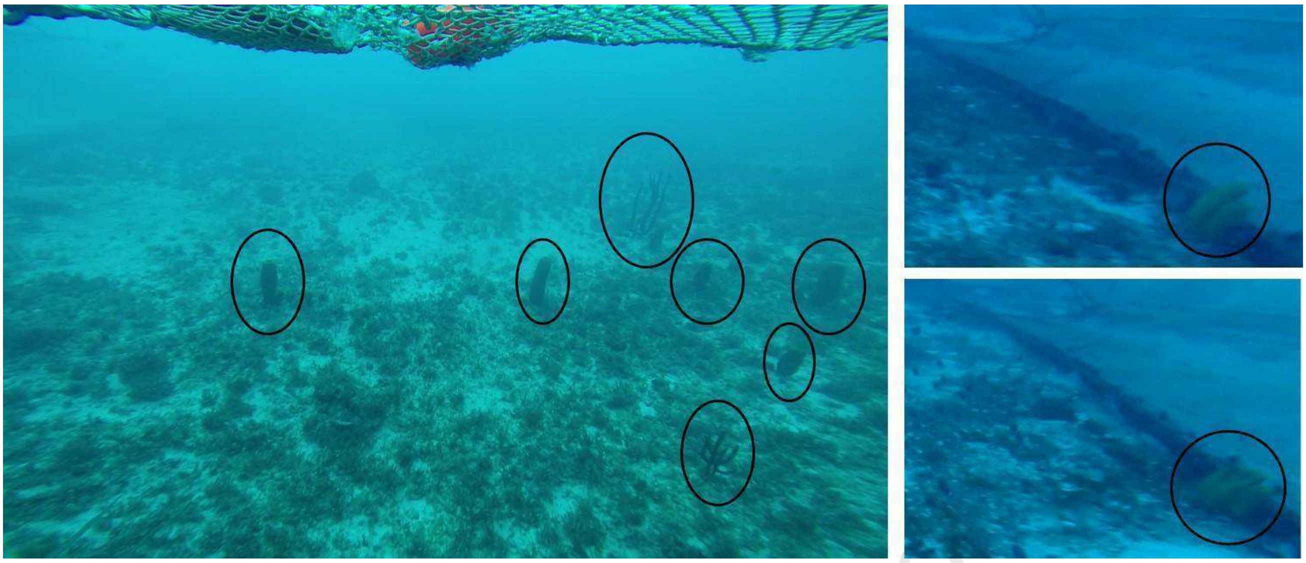

Fig. 5. Images examples of the sponges dragged by the trawl net along the Northeast Brazilian continental shelf (4°–9°S).

4. Discussion

This study is the first analyzing and comparing the fish community structure as observed from videos and bottom trawls carried out simultaneously. In total, 29 species were recorded by our subset of 5 hauls and video sampling, corresponding to 24% of the total number of species (120) recorded during the 35 hauls of the entire survey (Eduardo et al., 2018), which included trawling at depths as shallow as 10 meters.

Differences between the structure of fish assemblages sampled by videos and bottom trawls were not significant, probably due to small sample size and differences between samples sets. Previous studies have demonstrated differences among species when comparing visual surveys and bottom trawl sampling efficiency (Adams et al.,1995; McIntyre et al., 2015). In this study, despite the non-significant difference between the overall fish assemblages sampled by the two methods, it was clear that the bottom trawls were more efficient to describe the fish community, as indicated by the higher number of species caught by this method. It is noteworthy, however, that in the present study the results from videos were determined by trawls' height and speed, possibly limiting the efficiency of the method per se. Species captured only by trawls were mostly those considered scarce and rare. However, their detection is important, as rarity is a common feature in reef fish communities and they might be highly important in the ecosystem despite they are not the most abundant or frequent (Grande et al, 2019).

Furthermore, species-specific patterns were observed. For instance, *Cephalopholis fulva, Malacanthus plumieri, Calamus pennatula,* and *Halichoeres dimidiatus* were present in videos but escaped the trawl net passage due to their ability to hide in caves, to take refuge underneath coral rubble or to dive into the sand (Clark et al., 1988; Fiorentini et al., 1999; Claydon and Kroetz, 2007; Guidetti et al., 2008). Noteworthy, the rockhopper prevented the net of digging into the bottom. The Surgeonfishes *Acanthurus* spp. were also able to evade capture possibly because of their shelter attempts (Benevides et al., 2016).

Although squirrelfish *Holocentrus adscensionis* frequently hide in coral crevices at daytime (Greenfield, 1981), and thus potentially had the ability to escape, they did not escape and were more abundant in the trawl catches than in video. This is related to the occurrence of a large aggregation event in one of the stations, probably a spawning aggregation since most individuals were spawn ready (BPF, pers. obs.), which leaves them more vulnerable (Chollett et al., 2020). In a shoal, the behaviour of an individual fish can be influenced by the movement of the surrounding fishes, resulting in different reactions of the fish in front of the trawl and consequently enabling a tendency to have high catchability at high densities and low catchability at low densities (Godo et al., 1999). In most cases, *Holocentrus adscensionis* appeared in videos trying to hide into the corals and sponge's structures. However, aggregating individuals behaved swimming away from the net with other fish. The aggregation may also have induced a bias in fish counting, causing underestimated abundance in the video census (Tessier et al., 2005; Lowry et al., 2012).

The blue runner *Caranx crysos* (Mitchill, 1815) was not captured in the trawls. Probably, its fast-swimming capacity allowed the fish to keep its position ahead of the net, eventually escaping the net during or at the end of the haul (Beentjes et al., 2004). Similarly, the cornetfishes *Fistularia tabacaria* (Fritzsche, 1976) has high swimming endurance and this possibly contributed to their escapement from the trawl net (Beentjes et al., 2004).

On the other hand, the porcupine puffer fish *Diodon holocanthus* was not identified in videos but was the most abundant fish in the bottom trawls catches. This species was likely not detected in videos since the upper vision of the camera make difficult to identify the fish due to its camouflage with the substrate and immobility. Also, they react to threats inflating or expanding their body with air or water (Shipp, 2003). Thus, we believe that when the porcupine puffer fish felt threatened by the trawl net they were not in the camera's field of view anymore, and as a consequence of their immobility and behavior they were immediately dragged to the end of the net. The boxfishes *Acanthostracion* spp. are also slow swimming benthic-dwelling (Matsuura, 2003) and present a good camouflage with the substrate, which similarly contribute to their high catchability and complicate their identification in video census (Beentjes et al., 2004).

Catchability differences were noted as discussed above and were possibly associated with fish behavior, swimming ability, density and distribution (He, 1993; Godo et al., 1999; Beentjes et al., 2004; Trenkel et al., 2004a; Yule et al., 2008; McIntyre et al., 2015). Escaping was also associated to habitat complexity, which was offered by the rugose carbonate bottom that forms crevices and by coral-sponge formations, typical of the carbonate shelf.

Videos illustrated strong bottom-trawl impact with the destruction of erect sponges. Indeed, all observed sponges touched by trawling gear were destroyed or damaged. Trawl nets remove rocks and corals and smooth out natural topography, consequently reducing the structural heterogeneity, which is an important factor to the recruitment and survival of countless fish (Auster et al., 1996; Pilskaln et al., 1998; Kumar and Deepthi, 2006). Sponges together with corals and other benthic organisms play an important role in the formation of mesophotic ecosystems and are vulnerable to different fishing activities (Portela et al., 2015; Chimienti et al., 2019). In some areas of the Northeast Brazilian continental shelf, the sponge community represents the only shelter for various fish species and functions as an habitat 'oases' (Rocha et al., 2000). The direct damage to benthic organisms may affect a variety of demersal fishes because of the depletion of food supply and shelters, which exposes the fishes to predation (Kumar and Deepthi, 2006).

Along the Northeastern Brazilian coast, artisanal and commercial bottom trawling operations target mostly shrimp, being thus restricted to muddy or sandy-muddy bottoms and generally in shallow and coastal waters (Dias-Neto, 2011; Bandini, 2014; Port et al., 2016; Bomfim et al., 2019; Silva-Júnior et al., 2019). However, bottom trawling operations on carbonate bottoms and in mesophotic depths (under 30 meters deep) is reported in other parts of the world (Colman et al., 2005; Eigaard et al., 2016; Chimienti et al., 2019). In Brazil, experimental fishing operated by international vessels has already been conducted on northeastern Oceanic banks (MMA, 2006), so there is reason for concern that this kind of fishing may be eventually implemented over carbonate bottoms of continental shelves.

Additionally, trawlers frequently operate illegally in areas where fishing is prohibited according to federal fishery laws, including ecologically sensitive zones (Brandini, 2014; Isaac and Ferrari, 2016). Therefore, although bottom trawling is not currently disseminated on carbonate bottoms in the Northeast Brazilian continental shelf, fishing operations may develop faster than regulatory agencies can respond (Berkes et al., 2006), thus preventive measures and regulations are important to this region.

We acknowledge the limits of our study including the restricted sample size. This study, however, represents a first contribution combining simultaneously video and bottom trawling and thus provides insight into the performance of two dissimilar sampling methods and an evaluation of a potential fishing impact. Thus, the video observations on sponge depletion and also on fish behavioral reactions are important to assist in taking preventative actions.

Certainly, scientific trawl surveys are much less destructive than commercial operations and are useful to collect biological samples for diet and ageing studies, allowing the estimation of population age structures, the calculation of growth and mortality rates, which are essential for the management and monitoring of fish populations (McIntyre et al., 2015). However, despite the higher efficiency of bottom trawls, our study has shown that videos may be an important addition to bottom trawl methods, recording elusive species and also detecting impacts, complementing previous studies showing that video is an appropriate method for studies aiming at analyzing the distribution of fish or community structure (Assis et al., 2007; Stoner et al., 2007; Schaner et al., 2009; Mérillet et al., 2018).

5. Conclusion

Here, we compared the fish community on a carbonate shelf as observed using bottom trawl and video footage simultaneously. Overall, the difference in fish assemblages structure was not significant. However, trawling has shown higher performance in relation to the number of species captured and thus in describing the fish community. Catchability differences were noticeable for some species, associated with behavioural and ecological characteristics. In addition, video footages allowed the observation of sponges' destruction by the trawls net. Although bottom trawl fishing is not under operation in the region, we recommend that preventative measures and regulations should be addressed to these vulnerable bottom type areas, as the regulatory agencies are often slow to react to the fast development of fishing activities.

The videos analyzed in this study do not represent a complete alternative for scientific bottom trawling, however, it demonstrated to be a useful complement, allowing the observation of species that were captured by the trawls and species-specific reactions to the net. Furthermore, the implementation of less destructive methods may be necessary, especially in conservation areas. Possibly, video sampling was performed in suboptimal conditions, that is, at the speed and height determined by the trawling operation. A use of dedicated video sampling (such as towed video) could significantly improve the performance and compensate the disadvantages of the videos observed in this study.

Acknowledgements

We acknowledge the French oceanographic fleet for funding the research expedition ABRACOS 2 and the crew of the R/V ANTEA for their assistance and guidance in the field operations. We also acknowledge the assistance of Dr Thaynã Cavalcanti (UFPE) in the sponges' identification, as well as the help of all participants from the LECOR and BIOIMPACT Laboratories. This study was partially financed by the project PELD-TAMS (Long Duration Ecological Program – Sustainable Tamandaré), by CAPES (Coordination for the Improvement of Higher Education Personnel) through a student grant to Maria Jacqueline Gomes de Barros, Leandro Nolé Eduardo and Alex Souza Lira, and by CNPQ (National Council for Scientific and Technological Development) through a productivity research grant to Beatrice Padovani Ferreira and Flávia Lucena Frédou. This work is a contribution of the LMI TAPIOCA and of the PELD-TAMS (grant number 441632/2016-5).

Declarations of interest

None.

Funding

This work was supported by the French oceanographic fleet, through the project ABRACOS 2 (http://dx.doi.org/10.17600/17004100), and partially financed by the project PELD-TAMS (Long Duration Ecological Program – Sustainable Tamandaré), by CAPES (Coordination for the Improvement of Higher Education Personnel) and by CNPQ (National Council for Scientific and Technological Development).

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Highlights

- Video application in the evaluation of fish catchability by bottom trawling;
- Scientific trawling has shown higher performance in describing the fish community;
- Videos demonstrated to be useful complement for scientific trawling;
- The net impact on erect sponges raise the need for creation of preventive measures.

Conflict of Interest Statement

This manuscript has not been published and is not under consideration for publication elsewhere. The authors have no conflict of interest. All authors approved the submission of the present manuscript version and have participated in the manuscript preparation: Beatrice Padovani Ferreira conceptualize this study, Arnaud Bertrand and Flávia Lucena Frédou coordinated the research project. Maria Jacqueline Gomes de Barros performed analyses and wrote the manuscript with contributions of Leandro Nolé Eduardo, Arnaud Bertrand, Flávia Lucena Frédou, Thierry Frédou, Alex Souza Lira and Beatrice Padovani Ferreira. All authors contributed substantially on biological analyses.

Sincerely.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

none