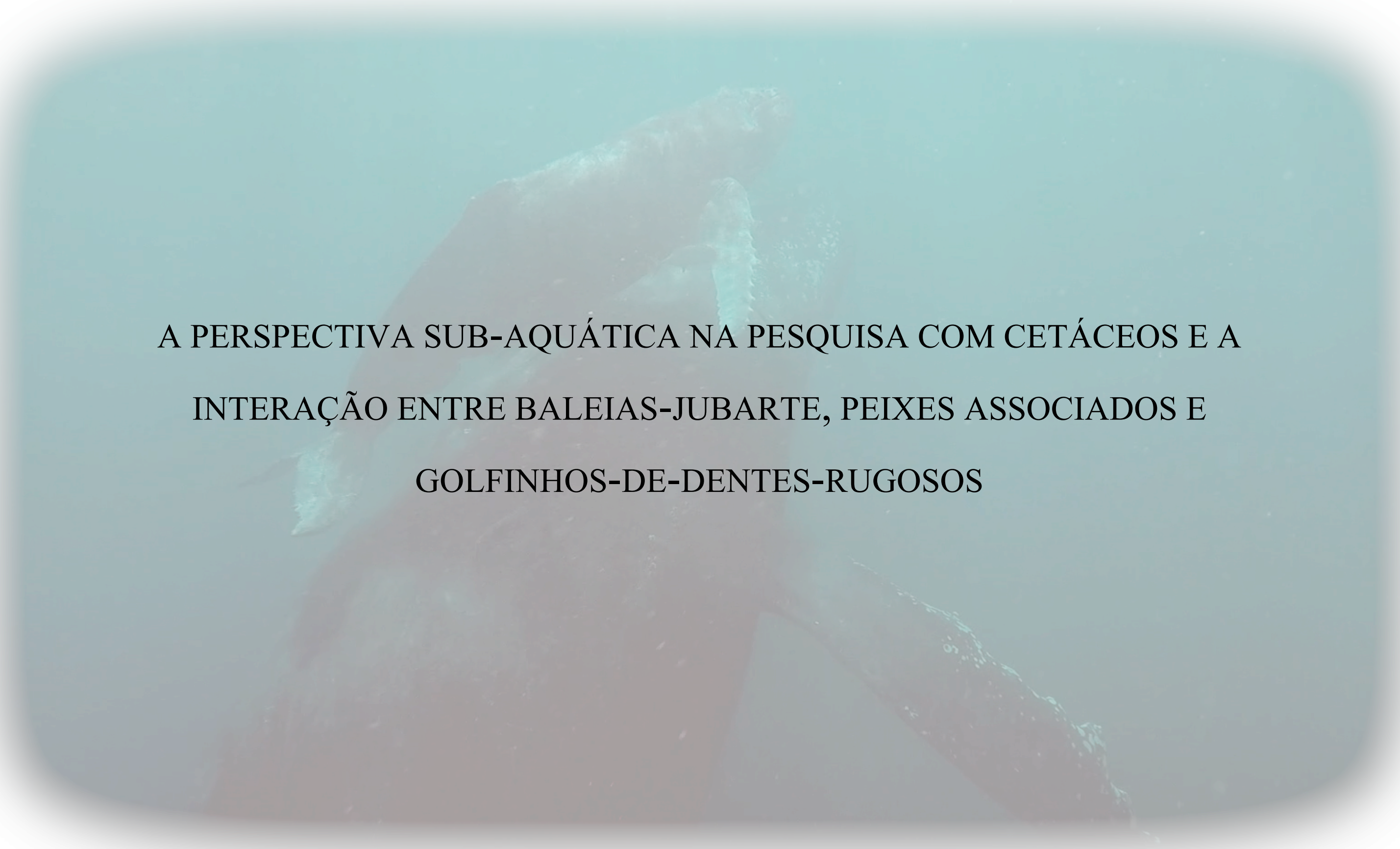
UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS

DANIEL GUSTAVO VON SPERLING DE VASCONCELLOS VENTURINI



A PERSPECTIVA SUB-AQUÁTICA NA PESQUISA COM CETÁCEOS E A INTERAÇÃO ENTRE BALEIAS-JUBARTE, PEIXES ASSOCIADOS E GOLFINHOS-DE-DENTES-RUGOSOS

BELO HORIZONTE
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DISSERTAÇÃO APRESENTADA AO DEPARTAMENTO DE ECOLOGIA, CONSERVAÇÃO E MANEJO DA VIDA SILVESTRE - INSTITUTO DE CIÊNCIAS BIOLÓGICAS, DA UNIVERSIDADE FEDERAL DE MINAS GERAIS COMO REQUISITO À OBTENÇÃO DO TÍTULO DE MESTRE EM ECOLOGIA.

ORIENTAÇÃO: FLÁVIO HENRIQUE GUIMARÃES RODRIGUES

BELO HORIZONTE

2017

"Por toda a parte, a natureza fala ao homem
com voz familiar à sua alma"

Alexander von Humboldt

APOIO INSTITUCIONAL

APOIO FINANCEIRO

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"Aqueles que passam por nós, não vão sós, não nos deixam sós. Deixam um pouco de si, levam um pouco de nós."

Antoine de Saint-Exupéry

SUMÁRIO

RESUMO

Apresentamos os resultados obtidos pela metodologia desenvolvida para a captação de imagens sub-aquáticas de cetáceos durante incursões a campo para pesquisa. A estrutura de câmeras acopladas ao casco de uma embarcação de pesquisa foi empregada ao longo de duas temporadas reprodutivas (2016-17) de baleias-jubarte no Banco dos Abrolhos. Essa técnica permitiu apontar os dados complementares que a perspectiva sub-aquática fornece acerca da detecção das reações das baleias ao disparo de um dardo para obtenção de biópsia, identificação individual, interações de natação na proa com delfinídeos, diganóstico de indivíduos emalhados e identificação dos peixes que se associam as baleias. Este último tópico é tratado de forma aprofundada, onde discutimos a interação aparentemente predatória de golfinhos-de-dentes-rugosos em relação aos peixes associados as baleias-jubarte no Banco dos Abrolhos. Ao final do trabalho discutimos as limitações, desafios e potenciais da metodologia proposta, ressaltando os melhores cenários para sua aplicação, ou de abordagens similares que nos permitam acessar uma pequena janela no mundo sub-aquático dos cetáceos.

INTRODUÇÃO GERAL

A presente dissertação é produto de uma parceria com o Instituto Baleia Jubarte (IBJ), na qual buscou-se obter maior compreensão acerca do mundo sub-aquático onde grande parte das atividades dos cetáceos de fato ocorrem. Dessa forma, propomos a utilização de uma estrutura acoplada ao casco de uma embarcação de pesquisa, portando uma câmera submersa a fim de registrar a reação sub-aquática das baleias-jubarte aos disparos de dardos para obtenção de biópsia.

Projetamos, construímos e testamos a metodologia proposta antes do início do estudo. Adaptamos às necessidades logísticas e funcionais para o período oficial de coleta de dados e, eventualmente, expandimos os objetivos para além das reações dos animais ao disparo. Durante duas temporadas reprodutivas das baleias-jubarte (*Megaptera novaeangliae*) no banco dos Abrolhos, foi possível empregar com sucesso a abordagem proposta em praticamente todos os ce
ruzeiros de pesquisa realizados – saídas a campo de normalmente três dias, mediante condições climáticas favoráveis, para amostragem sistemática dos grupos de baleias. O

produto de aproximadamente 40 dias de amostragem, durante a realização deste projeto, é descrito por mim nos dois capítulos que se seguem nessa dissertação.

O primeiro capítulo, entitulado 'Um rápido olhar abaixo da superfície: aprimorando a pesquisa de cetáceos através de uma abordagem sub-aquática', expõe os resultados complementares obtidos por meio desta metodologia. Estes são geralmente intangíveis se utilizados apenas os métodos convencionais de amostragem, isto é, observação à bordo. Foi observado o potencial das câmeras sub-aquáticas para foto-identificação individual, descrição do repertório de reações comportamentais ao disparo para obtenção de biópsia e melhorias na detecção das mesmas, além do reconhecimento dos peixes associados às baleias, interações de natação na proa com delfinídeos e diagnóstico de cetáceos emalhados. Finalmente, são discutidas as limitações e os potenciais da abordagem proposta, de forma a fortalecer a aplicação da metodologia em casos em que essa ferramenta se mostre capaz de responder as perguntas de interesse ou ainda de ampliar a obtenção de dados durante esforços de campo.

O segundo capítulo, entitulado 'De carona com os gigantes: baleias-jubarte e seus peixes e golfinhos associados', aborda de forma mais profunda um dos dados revelados pelas imagens das câmeras: as espécies de peixes que se associam com as baleias durante o período em que as mesmas permanecem por essas águas. Neste, descrevemos quali e quantitativamente essa interação e levantamos uma discussão entorno do papel das baleias migratórias nas cadeias tróficas tropicais. Este último envolve a relação entre baleias, os peixes e golfinhos associados na região do Arquipélago de Abrolhos.

Finalmente, esperamos transmitir com clareza os beneficios encontrados ao buscar uma janela de observação no mundo sub-aquático dos cetáceos, um universo tão inexplorado por restrições logísticas, mas que resguarda importantes revelações sobre o modo de vida desses animais fascinantes. Pequenos ensaios como este nos permitem avançar além das barreiras encontradas nos estudos do ecossistema marinho e estimulam inovações similares.

CAPÍTULO I

A quick glance below the surface: improving cetacean research through an underwater approach

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ABSTRACT

A customized set of remote underwater cameras attached to the hull of a research vessel is tested here as a tool to complement the data obtained during cetacean field surveys. We assessed the potential of this approach to gather information that is otherwise not available from conventional observation methods. The methodology was applied throughout two reproductive seasons (2016-17) of humpback whales in the Abrolhos Bank off Brazil, which yielded 76 registers of interest out of over 280 hours of sampling effort. Among the results we include:

- Significantly better detection of whale reactions to biopsy shots from the underwater perspective in comparison with surface observers;
- 28 underwater photo-identification records;
- High efficiency on the detection of body marks and individual traits during bow-riding interactions with dolphin species;
- One register of an entangled whale that was missed from the surface.

We highlight the potential of camera imagery on complementing cetacean research and provide an underwater view of their activities. Our approach is better suitable when applied under clear water conditions and associated with research procedures that require relatively close vessel approaches from the animals.

Key Words: underwater perspective, camera imagery, biopsy sampling, humpback whale, methodology.

INTRODUCTION

Studying the underwater activities of cetaceans is a rather challenging task. Visual contact with the submerged world is hampered by factors related to their fully aquatic life, high mobility and long dives, as well as environmental restrictions such as bad weather or poor water transparency. From above the surface, researchers make use of a variety of non-invasive techniques to gather relevant data for conservation efforts, such as behavioral sampling through observation methods, photograph for individual identification, biopsy sampling for molecular analysis and others (*e.g.*, Katona and Whitehead, 1981; Mann, 1999; Lambertsen, 1987). However, given the revealing questions regarding the ecology of marine animals that can be successfully addressed through the underwater perspective, researchers have also relied on technological methods to gain some observational window underneath the surface (see Mallet and Pelletier, 2014). Camera imagery in particular has been an efficient tool to study marine ecosystems since the 1950's (Barnes, 1952), though in the last few decades novel methods are being developed as practical improvements allow a wider use of video equipment (Bicknell *et al.* 2016).

Innovative and sophisticated approaches involving video technology are capable of providing substantial information on animal activities underwater, although these are often quite costly and therefore have their usage restricted to address very specific questions. An example includes animal-borne multi-sensor devices, which proved to be effective for a wide variety of applications (see Marshall, 1990; Moll *et al.* 2007).

Further, several studies have demonstrated the applicability of camera imagery as a tool to optimize data collection, frequently evidencing significant advantages in comparison with conventional sampling methods. It has been reported, for example, the benefits of using video devices for fine-scale ethological studies on bottlenose dolphins (Lopez-Marulanda *et al.* 2017). Insights into competitive behaviours of humpback whales were gained through the deployment of underwater cameras (*i.e.,* CritterCam) on fighting individuals (Herman *et al.* 2007), and customized camera systems have provided valuable information about rough areas beyond our reach, such as deep-water environments (Favaro *et al.* 2012). Additionally, a range of cases where underwater sampling *in situ* is skewed by the presence of a diver report the usage of passive image recording as an alternative to overcome this bias (*e.g.*, Harvey *et al.* 2004; Longo and Floeter, 2012).

Sampling design among the aforementioned instances varies in complexity and costs. Still, regardless of the chosen method, limitations faced by any approach employing cameras underwater are commonly similar: reduced water visibility, low light conditions and restrictions in field of view, storage capacity and batery life. These challenges often lead to small sample sizes, underutilization of data collected, time-consuming analysis process and imperfect detection of species, individuals or phenomena of interest (Bicknell *et al.* 2016), thus requiring an extra caution when planning the sampling. Generally, once a method proves itself effective, the simpler and cheaper it is, the more attractive it becomes to be broadly employed on marine research.

In the present study, we test a customized set of underwater cameras attached to the hull of a research vessel to raise complementary data during systematic surveys of humpback whales (*Megaptera novaeanglieae*) in the Abrolhos Bank, off the northeast coast of Brazil. Our initial focus was to record the underwater behavioral repertoire of whales in response to biopsy shots following close vessel approaches. Sampling procedures that require proximity from the animals are invasive and often cause behavioral changes in cetaceans (Isojunno and Miller, 2015; Williamsom *et al.* 2016). Methods for biopsy collection consist of firing a dart with a modified tip through a crossbow or rifle (Lambertsen, 1987), aiming the flanks of the whale. Despite the large number of studies assessing the reaction of cetaceans to biopsy or tagging shots (*e.g.*, Watkins, 1981; Weinrich *et al.* 1992; Brown *et al.* 1994; Gauthier and Sears, 1999; Best *et al.* 2005; Alves *et al.* 2010; Cantor *et al.* 2010; Reisinger *et al.* 2014), the majority of them have described the observed reactions solely through the perception of onboard observers, *ad libitum*, a generally biased method given the uneven attention sparked by certain behaviours (Altmann, 1974) and particularly dubious for this purpose since most of the whale's body is underneath the surface when it is hit. In spite of the convergent outcomes of these studies indicating minor impacts of this technique on whales, we hypothesize that the underwater perspective will improve detection and complement the observation of behavioral responses displayed by whales.

The short proximity from the whales required to collect biopsy samples represents a suitable scenario to capture further underwater registers of interest for research. Thereby, here we attempt to use underwater video recording to gather information that is otherwise not available from conventional observation methods, concerning:

(1) Underwater photo-identification of individuals as a tool for increasing the number of photos acquired per field effort (as first attempted by Glockner-Ferrari and Ferrari, 1990);

(2) Records of bow-riding interactions with dolphin species in order to photo-identify the individuals and detect body marks;

(3) Opportunistic registers of entangled cetaceans, seeking a better understanding of the situation through the underwater perspective.

Finally, we evaluate the effectiveness of the proposed methodology as a tool to complement data collected during field survey efforts, and discuss the limitations and potential improvements for this approach.

MATERIALS AND METHODS

Study area and sampling

The area studied comprises the Abrolhos Bank (16º 40'S to 19º 30'S, 37º 250'W to 39º 45'W), an extension of the continental shelf on the eastern coast of Brazil, where the highest densities of humpback whales from breeding stock A are found (Pavanato *et al.* 2017). Data were obtained during research cruises conducted throughout two breeding seasons of the humpbacks, from July to November 2016-17. Planned routes covered primarily the surroundings of the Abrolhos Archipelago (Figure 1), where maximum depth rarely exceeds 30 meters. Vessel used was a wooden trawler with 14.8 meters in length and 3.8 meters wide, powered by a 230hp Scania DS 112 propeller. Biopsy procedures were conducted as part of a long-term monitoring program developed by the Humpback Whale Institute, where two to four day cruises were carried along the season according to favorable weather conditions. Sighted whales were approached in attempt to obtain photo-identification of individuals and behavioral data, as well as tissue samples (see Wedekin *et al*. 2010 for a complete description of the research cruises' methodology). Whales were biopsied using a regular Kantas crossbow with 150lbs draw weight as described in detail by Cantor *et al*. (2010).

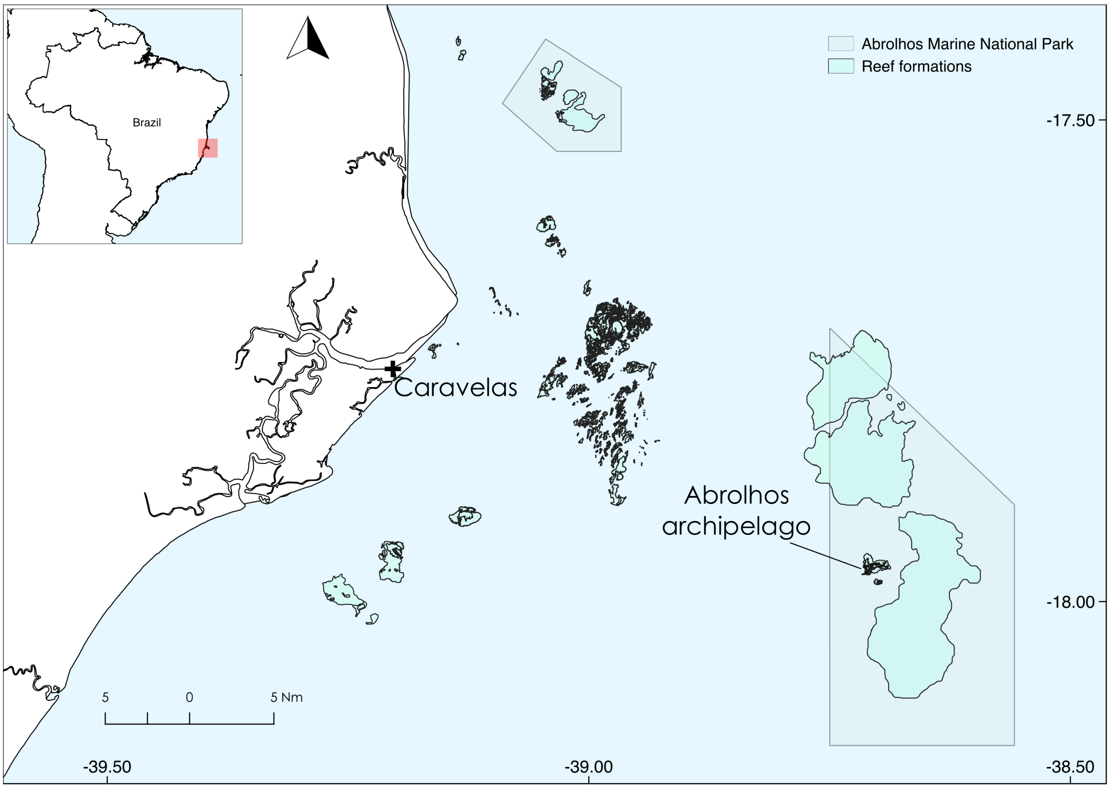

Figure 1. Study Area. Research cruises departed from Caravelas and covered primarily the surrounds of the Abrolhos Marine National Park, northeast of Brazil.

Structure design

The underwater cameras were placed 0.9 meters deep, oriented forward of the bow and angled slightly upwards. The supporting structure was a hollow, 4 meters long, stainless steel cylinder screwed along the central beam of the bow with a vertical cut, through which two waterproof cameras slid freely attached to an intern tube (Figure 2). The latter had free mobility and therefore the cameras covered an angle of 180º, allowing adjustments in the field of view according to the relative position of the whales.

Two cameras (GoPro Hero 4) were hold still in the submerged end of the structure, inside a waterproof housing. To control the cameras remotely, a modified backdoor (CamDo Solutions, 2017) was attached to each camera, in order to capture their wi-fi signal. A coaxial cable RG-174 safely placed inside the hollow structure sent the signal to a dry end on the deck of the vessel, where it paired with a remote control. The exact moment of the shot was registered by a third camera, also paired, and placed in the head of the one in charge of biopsying. Consequently, all three cameras operated in synchrony and were activated simultaneously by the researcher, allowing videos to be promptly recorded whenever there was a shot, a group of whales or any subjects of interest within the field of view of the underwater cameras. Cameras held 32gb memory cards and 1160mAh lithium-ion batteries. We had three extra batteries for replacement. Total cost for implementing this methodology on ongoing field researches, including 3 cameras, material and labor services, memory cards, wi-fi cable and exta batteries was ~US$ 2.570,00, following values valid for the study period (2016-17).

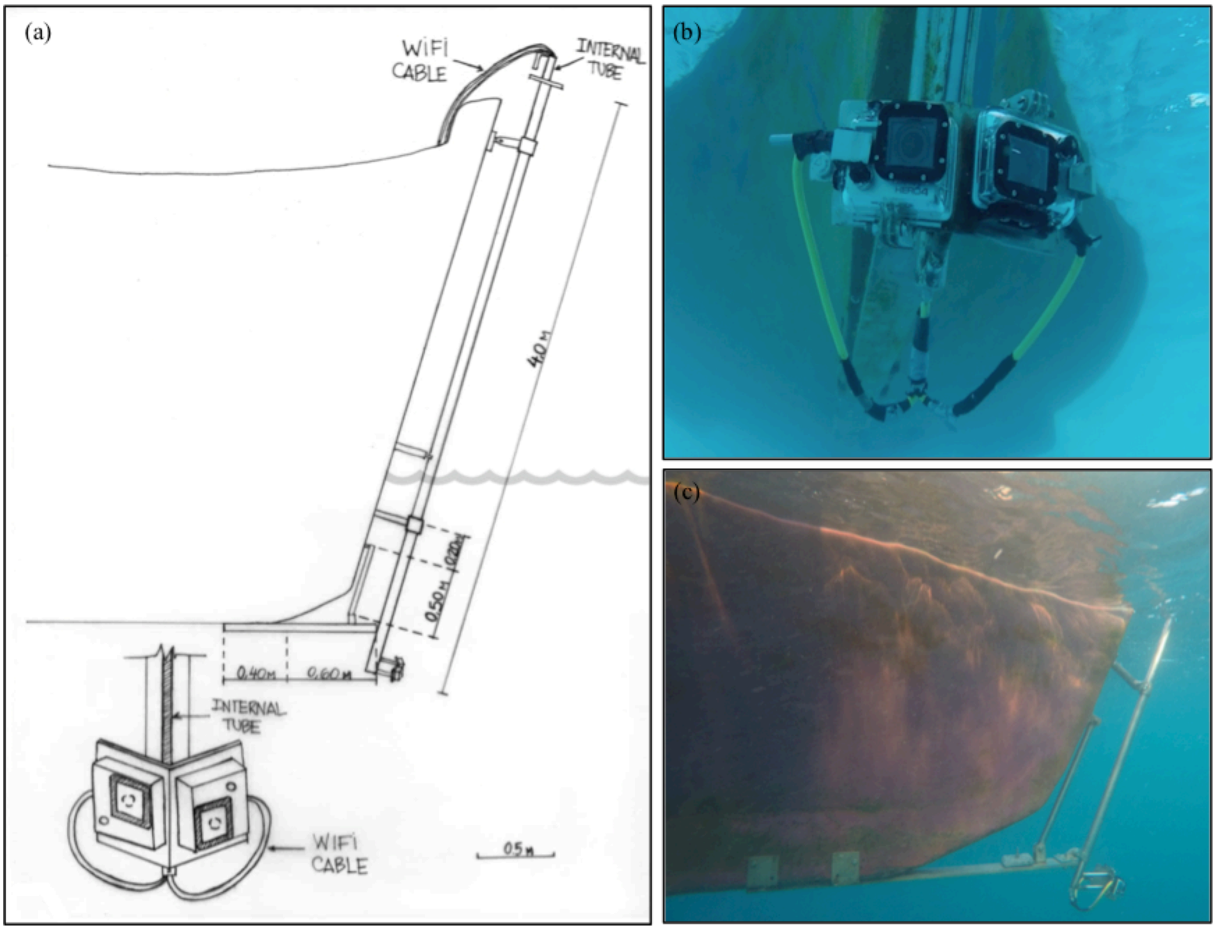

Figure 2. Customized structure designed to hold the underwater cameras. (a) Illustration and measures (Thais Peixoto Macedo); (b) Front view in detail; (c) Lateral view.

Underwater Sampling and video processing

Underwater cameras were positioned in the water when the vessel reached the sampling area. Once in position, the remote control operator started recording whenever he assumed whales were within the camera's sight. At the end of each day, cameras were taken out of the water, data downloaded and batteries recharged. Water transparency was measured with Secchi disk every two hours during sampling time, of after major, sudden weather changes. These were used to verify the water conditions under which our methodology was more effective (*i.e.*, recorded good-quality images).

Video files resulted from each camera were classified chronologically in the end of each sampling day. Both perspectives (first person shot and underwater) were spliced together in one single file, using the software Final Cut Pro 10.3 (Apple Inc, 2016) for image editing. Whenever needed, images were manipulated in order to color balance and enhance the contrast of the object within the water column, making it easier to distinguish and identify individuals for posterior analysis. Thereafter, videos were sorted and the final product was composed of three groups of images: (i) whales submitted to biopsy shot, (ii) whales not submitted to biopsy shot, (iii) opportunistic registers addressing any of our goals aforementioned. Each of the sorted videos were then linked to their corresponding data sheets filled in field, which logged time of sight for each group, group composition, number of individuals, behaviours observed *ad libitum*, geographic coordinates and depth.

Data analysis and definitions

According to our criteria, underwater reactions were defined as an immediate behavioral change, caused by a responsive movement, in front of a 'noxious stimulos that would not otherwise have occurred' (adapted from the concept of disturbed behavior by Weinrich *et al.* 1992). Herein the external stimuli are represented by two scenarios: (i) the moment of maximum proximity between the vessel and the whales, usually <10 meters, or (ii) the exact moment of the shot impact, within similar vessel proximity and accurately indicated in each video by the shooter's perspective (Figure 3). The absence of reaction was assigned to individuals that showed no detectable behavioral change in either of these scenarios. Given our focus on describing any underwater behaviors that exceeded the individual tolerance threshold to a stressor, enough to be potentially detected by onboard observers, binominal data (presence or absence) for the variable of interest 'reaction' was considered.

Figure 3. Shooter's perspective (left) in synchrony with the underwater cameras (right).

Underwater recordings were exhibited to three experienced researchers (*i.e.*, > 2 years working with biopsy sampling) to examine the overall reliability of the data collected and validate the perceived reactions (or the absence thereof). Only registers with unanimous agreement were considered in the following analysis. In order to test whether the underwater videos improved the detection of noticeable reactions, these were fit into a comparative table against the naked eye observations. A chi-square test was used to verify the assumption that reactions are observed more frequently in animals hit by the biopsy shot. Then we filled the frequencies of perceived reactions in chi-square contingency tables to compare whether or not the reaction *per se* was noted by both methods.

We attributed the status of *false-negatives* for events where the biopsy sampling yielded an observable reaction caught on cameras' view but were assigned as 'no reaction' by observers, or vice-versa. To assess whether these misleading assignments had an influence of a measurable external factor, we conducted a generalized linear model in R (R Core Team 2016) with a binomial distribution for the variable of interest *'false negatives'* as a function of three variables related to potential distractors for the observers onboard: (i) the number of whale individuals in the sampled group, (ii) group behavior (either surface active or not) and (iii) hours elapsed since the beginning of the daily sampling. The function 'hnp' from the hnp package (Moral *et al.* 2017) was used to test whether the residuals fitted the model properly, and an analysis of variance (ANOVA) of the model tested the significance of each factor. Best model was selected according to parsimony, i.e the simplest one including significant variables ($p < 0,05$) only. Finally, the descriptions of both video analysis and field data sheets were inspected for qualitative differences or similarities, in order to check whether significant information was added by the video images recorded.

Photo-identification of individuals were taken opportunistically from video images and were consisted of the ventral side of the flukes, which is the most common tool for individual discrimination of humpbacks (Katona and Whitehead, 1981). Fluke photos were checked for duplicity with photos acquired from the surface to highlight the ones obtained exclusively from the underwater cameras. Finally, further information obtained from the cameras during bow riding interactions with dolphins and from encounters with entangled animals were described in detail.

RESULTS

The underwater approach was employed on a total of 35 days during 12 field campaigns throughout two years of study. That yielded over 280 hours of sampling effort, which resulted in 76 registers of interest from which our data was extracted. These accounted for 39 minutes of sorted video footages (mean 31.3 ± 30 seconds per video). Mean water transparency in areas where usable images were recorded was 9,4 ± 2,6 meters, though this measure for the whole covered area was 7,9 ± 2,4 meters. Maximum water transparency was 14 meters. No images were obtained in areas with visibility inferior to 6,5 meters or sea state that exceeded 3 in the Beaufort scale.

Underwater reactions

Underwater behavioral responses, or the absence thereof, were recorded for 65 vessel approaches in procedure of biopsy sampling. Of these, 28 (43.1%) led to successful biopsy shots, and other 37 (56.9%) resulted in close vessel approaches only, with no shooting attempt. No registers were discarded following the test with experienced researchers. We found a significant difference between the occurrences of noticeable reactions in both scenarios, where 21 (75%) of the biopsied whales exhibited an observable response, contrary to 5 (13.5%) among the no-hit whales ($X^2 = 22.61$, df = 1, $p < 0.001$). Out of the images obtained, we managed to identify eight categories of underwater behavioral responses, which were displayed alone or in combination with each other. These are described in Table 1 with their respective observation rates in each circumstance.

Table 1. Description of each underwater behavior verified in response to biopsy shots, with the respective frequency these were displayed under shot and no-shot circumstances.

Underwater Behavior	Description	Frequency (%)	
		With shot	No shot
Hard tail flick	Whale performs single or continuous (2+) vigorous vertical movements with the tail fluke, returning to previous, unchanged behavior in sequence (Weinrich *et al.* 1992; Gauthier and Sears 1999*).	25	0
Peduncle spasm	Whale displays a subtle startle movement with the fluke peduncle, apparently reflexive for its mildness.	3,6	0
Snaking	Whale curves its back laterally in a movement that extends all along the caudal peduncle (tail sweep from Gauthier and Sears, 1999*).	3,6	0
Dive	Whale rapidly arches its back in a vigorous dive straight bottomward (Gauthier and Sears, 1999*).	7,2	2,7
Spinning dive	Whale dives vigorously down, spinning on its body axis.	3,6	5,4
Sprint	Whale boosts speed quickly through a repetition of vertical propulsions in horizontal displacement (Gauthier and Sears, 1999*).	3,6	2,7

Pectoral spasm	Whale displays a subtle startle movement with the pectoral fin, apparently reflexive for its mildness.	7,2	0
Change in direction	Whale changes its traveling course in any direction other than that in which it was initially heading (Gauthier and Sears, 1999).	0	3
Unchanged	Whale shows no sudden, apparent change in behavior.	25	86,5
Hard tail flick + Dive	-	7,2	0
Hard tail flick + Pectoral spasm	-	7,2	0
Sneaking + Sprint	-	3,6	0
HTF + Pectoral spasm + Dive	-	3,6	0

* References that have previously described the assigned behaviors.

We found a significant difference in the capacity of both methods to detect whale reactions (X^2 = 8.68, df = 1, p < 0.05). In 43% of the shots, onboard observers missed a perceptible response displayed by the sampled individual (false-negatives), assuming that correct classifications of behavioral responses were assigned for the underwater videos. Most common missed reactions were *hard tail flick* (n=4), followed by *pectoral spasm* (n=2) and *hard tail flick* plus *pectoral spasm* (n=2). *Peduncle spasm*, *snaking*, *snaking* plus *sprint* and *spinning dive* were missed once each (Figure 4). It is worth noting that none of these behaviors showed clear indicatives of their occurrence through above-the-surface signs, such as white water or splash. Further, in 4 of the 9 (44%) circumstances where both methods agreed upon the presence of reaction, onboard observers missed minor details in the description, such as subtle movements in the pectoral fin or sudden dives after a vigorous surface behavior.

Our best predictive model for the occurrence of false-negatives included only the variable *number of individuals* (X^2 = 4.51, df=1, p=0.03; Figure 5), where larger groups led to a higher occurrence of missed reactions by the observers.

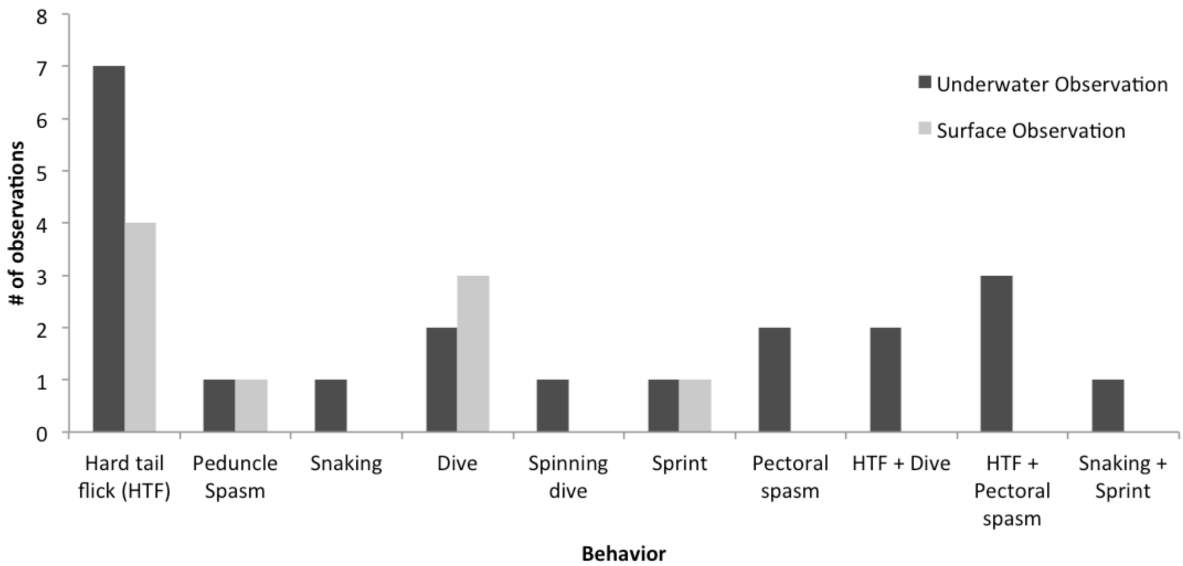

Figure 4. Frequency of observable reactions through each of the compared perspectives.

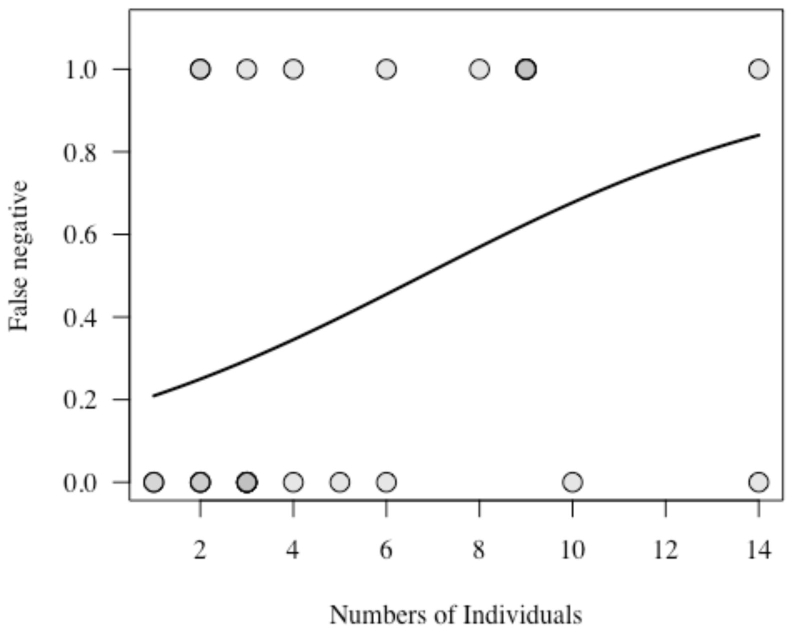

Figure 5. Occurrence of *false negatives* as a function of number of individuals in the group. Grey circles are in shaded scale for visualizing overlapping plots.

Opportunistic records

Underwater photo-identification of individuals

A total of 28 underwater photos on the ventral side of the whales' fluke were obtained through our method. However, 12 of these were also obtained from surface (*i.e.*, conventional method). The novel ones were all of proper quality to be added in the catalog of individual identification, and 13 (46,4%) of the total photos taken referred to the biopsied whale, providing a link between the fresh tissue sampled and a particular individual in the database. During the study period, 101 whales were biopsied but only 52 of these (51,4%) were identified from conventional methods, despite our constant efforts in doing so. In that sense, our underwater approach was responsible for matching up to 13% of the biopsied whales with their respective flukes.

Bow-riding interactions

We had 10 encounters with dolphin species that commonly perform bow-riding behavior. In half of these the interaction actually occurred - twice with bottlenose dolphins (*Tursiops truncatus*), involving 7 adults and two calves, and three times with rough-toothed dolphins (*Steno bredanensis*), involving 7 adults, totalling 16 interacting individuals. Underwater images were used to photo-identify the dorsal fin of 75% of these. During the longest bow-riding interaction, with bottlenose dolphins (five minutes long), images were used to review the group size perceived by the onboard observers, from five to seven individuals instead. In that same occasion individual traits could be verified - including one lactating mother indicated by her swollen mammary glands, two calves that were not swimming in synchrony with their mothers, one adult with deep marks from interaction with fishing gear and lastly, the group was identified as the off-shore ecotype of bottlenose dolphins (Simões-Lopes, *p.c.*).

Underwater images made it clear the difference in the amount of body covered by marks in each dolphin species. Bottlenose dolphins had much more intraspecific marks, while rough-toothed dolphins rarely exhibited these. Finally, by comparing the underwater photo-ID's we managed to resight one bottlenose dolphin with an year interval (Oct 2016 – Sep 2017).

Entangled cetaceans

Throughout our fieldwork campaigns we sighted one entangled whale. The circumstances are described bellow:

August 7th, 2016

Two adult humpback whales were sighted in the southern tip of the inner arch of reefs of the Abrolhos Bank, known as 'Parcel das Paredes'. One of them was photo-identified as it raised its fluke tail several times, while the other did not exhibit his fluke tail at all. After following the group for ca. 25 minutes we left the area. Thereafter we analysed the underwater images and verified a ~15 meters rope being pulled by the aforementioned whale. No steps were taken as we assessed the recording just late in that day.

DISCUSSION

The methodology we propose demonstrated a satisfactory potential, as well as fair cost-effectiveness when employed in association with ongoing field surveys to improve data collection from the underwater perspective. Video recording was extremely punctual due to the remote control system, which resulted in a significantly optimized process of image analysis. We managed to conduct the video sampling under the same thresholds of environmental conditions required to perform the conventional research, albeit higher Beaufort Sea states (3 >) frequently implied on unsteady images that were often discarded. Not surprisingly, water transparency was our major challenge and the poor visibility during the winter period (beginning of the humpbacks' breeding season) led to several unused images.

Our results indicate that even though whales exhibit some sort of behavioral responses under close vessel approaches (as verified here in five instances and more deeply investigated by Morette *et al.* 2007 and Williamsom *et al.* 2016), most whales exhibit punctual responses when hit by biopsy shots, in accordance with previous studies (*e.g.*, Brown *et al.* 1994; Best *et al.* 2005; Cantor *et al.* 2010). Further, as predicted in our first hypothesis, we found that the detection of perceptible reactions differs substantially between the onboard observers and the post-analyzed, underwater videos. Observers missed over half of the displayed reactions, represented by six behavioral categories. As expected, these responses commonly occurred right underneath the surface and were often quite subtle – such as peduncle or pectoral spasms. The latter was never reported as a behavioral reaction to biopsy shots in previous studies and here is first described as such. Hard tail flick was the most missed response and is assigned here as the same vertical, vigorous movement which describes the named 'Fluke Slap' or 'Lobtailing', that when displayed on the surface 'throws much spray and produces white water' (as in Weinrich *et al.* 1992, Gauthier and Sears, 1999; Cantor *et al.* 2010). This result indicates that different intensities of reactions were missed by surface observation alone. Thus, the same behavioral response that would otherwise produce rather notable cues for detection may be missed for occurring below the waterline.

Our best predicting model for the occurrence of false negatives highlights the influence of group size on missed responses – bigger, and hence more surface-active groups caused more missed reactions. It is reasonable to state that these conditions bring more distractions to the observers, who in turn tend to lose attention to the subject. Although not examined here, it is expected that more experienced researchers will suffer less from lack of attention in these situations. In that sense, the circumstances here resemble what was reported by Oliveira *et al.* (2017), who verified that during dolphin counts for population estimates, observers on canoes detected much fewer individuals in comparison to counts from aerial images. Together with communication problems, the observer experience and sources of distraction are common limitations for visual observation of naturally occurring behaviors (Dawson *et al.* 2008). Thus, it is likely that the simple use of continuous recording cameras would improve this flaw, as frame by frame reviewing allows a level of detailing which is hardly achieved by observing real-time, freely occurring behaviors. Indeed, this alternative has been employed in other studies for this purpose: Barret-Lennard *et al.* (1996) reported that biopsied killer whales commonly displayed subtle, barely perceptible responses, often detected only after reviewing the recorded videos.

Minor, short-lived reactions displayed by biopsied whales are not likely to affect neither survival nor fecundity, and therefore poses no threat to population level. Moreover, the shot impact is rather negligible when compared to other disturbances related to the biopsy procedure – *e.g.*, the long-lasting pursuits required to reach minimum shot-distances (as examined by Alves *et al.* 2010). However, transpassing the individual tolerance threshold to a stressor, enough to trigger a behavioral reaction, represents an effect on the welfare of the individual and therefore counts for to the total disturbance.

Short-lived responses are also an indicator of whether certain classes of species, groups or individuals are more or less sensitive to biopsy sampling (*e.g.*, Clapham and Mattila, 1993; Brown *et al.* 1994; Cantor *et al.* 2010). By assessing the intensities of individual reaction to biopsy shots, researchers have modeled the best alternatives of dart delivery systems, vessel approaching techniques and targeted groups, in order to optimize the procedure and minimize net disturbance (see Noren *et al.* 2012, for an extensive review on this topic). Additionally, a short-lived reaction may be sufficient to disrupt certain activities cetaceans are engaged in, such as resting, nursing or foraging. Jahoda *et al.* (2003) found that fin whales (*Balaenoptera physalus*) ceased feeding and commenced traveling when hit, and Weinrich *et al.* (1992) found the same effect on resting humpbacks. We defend that, to avoid underestimated assessments and to infer long-term impacts of sampling shots on large whales, a proper detection of the most basic, momentary responses is fundamental and the underwater perspective assists on that.

The opportunities provided during close vessel approaches as a function of the biopsy sampling procedure allowed us to gather further images of research interest. These were entirely opportunistic registers but improved the sampling effort as additional data. With regard to the underwater photo-identification, the gross amount of fluke pictures represented an increase of 3,8% in the number of identified individuals when compared to conventional sampling. Glockner-Ferrari and Ferrari (1990), who first relied on underwater photography for individual identification of humpbacks, reported an increase of 35% in the number of identified adults over a period of 12 years. In that occasion, they performed snorkel dives with camera equipment according to favorable whale behavior. Diving to photograph is a more direct and effective method, despite the higher logistical demand, considerable risk and need for special permits in accordance with local legislation. Therefore, this alternative only should be considered under very specific circumstances, whilst our methodology may be employed for that purpose in a safer way under normal conditions, regardless of whale behavior.

We managed to photo-identify the biopsied individual in almost half of the shots that were successfully registered by the underwater cameras. This was likely due to the common momentary responses of tail flicking or sudden diving, when the whale exposes the ventral region of its fluke. This result is particularly relevant because, despite striving to biopsy only identified individuals, achieving this is not always possible and the sampled whale often remains unidentified. Therefore, in these cases underwater photos provide a straight link between the tissue sample and a specific individual in the database, followed by his entire history of resightings over the years. Also, in cases where the sampled whale had been identified in previous campaigns, this link avoids duplicity in the catalog caused by the presence of one tissue sample plus one fluke photo for two theoretically distinct individuals, which are in fact the same. Whether better results in photo-ID is the primarily goal, an alternative for employing our methodology would be placing the cameras slightly deeper in the water column, and angle them upwards. That would be also suitable for sexing the individuals during close vessel approaches.

The results from bow-riding interactions demonstrated that our structure was efficient in gathering detailed information on free-ranging dolphins. Two of the three dolphin species that commonly occur in the Abrolhos Bank are renowned for interacting with vessels: *T. truncatus* (Acevedo, 1991; Hawkins and Gartside, 2009) and *S. bredanensis* (Jefferson, 2009; De Boer, 2010). Sightings of both species in Brazilian waters are rather scarce and data prevails on resident populations (*e.g.*, Simões-Lopes and Fabian, 1999; Giacomo and Ott, 2016) or from stranded carcasses (*e.g.*, Lemos *et al.* 2013). Therefore, opportunities to sample data on their natural habitat should be fully exploited. Certainly, dolphins will not always interact with vessels – studies report interacting rates that range from 4,5% to 22% of the sampled groups (Bas *et al.* 2014; Hawkins and Gartside, 2009, respectively), to over 77% of the hours surveyed (Samuels and Bejder, 2004). The explanation for this remarkable variation is still discussed, as does the purpose of bow-riding behavior itself (Hawkins and Gartside, 2009).

Nevertheless, the majority of interacting dolphins had their dorsal fins identified and their body scanned for body marks. The latter can provide valuable information particularly on the dolphins' social behavior with conspecifics (Lockyer and Morris, 1985; Scott *et al.* 2005), and such a complete assessment is rarely possible from conventional, onboard sampling. Images also allowed us to identify the sex of some individuals and traits like the swollen mammary glands that indicated a lactating mother. We further managed to identify the ecotype of a *T. truncatus* group, which comes to be relevant given the current discussion about coastal and offshore bottlense dolphins (Costa *et al.* 2016). One photo-ID of the dorsal fin, obtained exclusively by the underwater cameras, allowed a successful comparison match of one resighted individual. Studies of small cetaceans in the Abrolhos region are limited to their occurrence and distribution (Rossi-Santos *et al.* 2006), and there are virtually nothing addressing site fidelity of bottlenose or rough-toothed dolphins. Thus, for this purpose and also for systematic surveys in areas with suitable water transparency and frequent encounters with interacting dolphins, the methodology we propose would be of great support.

In the single event of encounter with an entangled cetacean, reviewing the recorded images was essential to comprehend the dimension of the fishing gear attached to the individual. The underwater cameras allowed us to perceive a situation that would otherwise go through unnoticed. The dynamic under which we conducted our sampling did not allow us to see the images in real-time, therefore this register did not result in any measures taken. However, real-time transmission is possible and would, as we concluded from this event, support considerably in detecting and diagnosing entangled animals. In fact, Coughran (2004) suggests in his report on procedures for disentanglement of large whales the use of pole cameras (instead of fixed structures as in here) to safely assess the whale conditions and improve data collection.

CONCLUSION

We highlight here the potential of a simple, non-invasive and low-cost system for improving cetacean research from the underwater perspective. Camera imagery itself allows precise and detailed measurements of animal activities as the recordings can be reviewed multiple times and viewing rate can be slowed, providing frame-by-frame analysis. In addition, employing this approach into the submerged world provides us with the ability to catch occurring events that are rarely or never perceived from the surface. Naturally, our proposal has some limitations. Despite being easily replicated and adaptable for different vessel platforms, it is only suitable if the ongoing research procedure requires relatively close approaches from the sampled individuals. Long-term systematic monitoring programs are more prone to benefit from this methodology, as a continuous sampling effort increase the odds of getting opportunistic data. Poor water transparency drastically impairs the usage of this and virtually any other approaches that involve underwater images. Alternatively, areas with crystal clear waters may fully exploit this method and benefit from even greater applications than those we found here. Various modifications could be applied to our structure in order to meet specific goals, such as changes in camera depth or angle and even more sophisticated improvements like zoom and real-time transmission of the images. Anyhow, we defend that valuable data may be obtained from a quick glance below the surface through the method we suggest here.

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Capítulo II

Hitchhike with the giants: humpback whales and their associated fish and dolphins

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ABSTRACT

In this article we describe registers of fish in association with humpback whales in a shallow reef area off Brazil. We discuss the motivations for this co-occurrence and correlate its predictability with the potential development of a foraging technique by rough-toothed dolphins (*Steno bredanensis*). We analyzed 24 records of whale groups with accompanying fish obtained throughout two breeding seasons of humpback whales *Megaptera novaeanglieae* (2016-17) in the Abrolhos Bank. Two fish species were recorded in association: *Echeneis naucrates* and the *Caranx* spp. Generalized linear models indicated a higher probability of fish to associate with whale groups composed of lactating females with calves, nearby reef formations. We also reviewed the encounters with rough-toothed dolphins during field surveys from 1998 to 2017 over the whale's season, when 41 out of the 67 (61,2%) dolphin groups encountered were interacting with the whales. Whale groups under these interactions displayed aggressive behaviors significantly more often then non-interacting ones. The area where interacting dolphins were sighted spatially overlapped with the occurrence of fish in association with the whales. The evidence gathered here indicates that large whales may play a further role in tropical trophic chains, where they act as fish attractors, which in turn are foraged by rough-toothed dolphins.

Key Words: abrolhos bank, associated fish, diet, foraging behavior, interspecific interactions, Megaptera novaeanglieae, Steno bredanensis.

INTRODUCTION

A wide variety of interspecific interactions among cetaceans are reported in the literature. In most cases, these account to closely related species (e.g. Frantzis & Herzing, 2002; Rossi-Santos *et al.,* 2009; Koper & Plön, 2016), but also encompasses other marine animals such as sea turtles (Fertl & Fulling, 2007), pinnipeds (Pitman & Durban, 2009) and fish (Alling, 1985; Lucena *et al.,* 2015). Some cases are easily interpreted given the nature of these encounters, which may span from extremes of positive (such as foraging cooperation) to negative interactions (such as predation and aggression). However, in others this relationship is still poorly understood, as these often demand more complex and subtle interpretations (e.g. Deakos *et al.,* 2010; Pitmann *et al.,* 2017).

With regard to the interactions between Mysticeti and Odontoceti, it has been noted for instance that large whales frequently display agonistic, avoidance behaviours in the presence of dolphins (e.g. Ciano & Jorgensen, 2000; Rossi-Santos *et al.,* 2009; Koper & Plön, 2016), suggesting that the latter somehow disturb the whales. Some authors propose that for small cetaceans such as bottlenose dolphins (*Tursiops truncatus*), the motivations for this interaction are either riding the waves created by the whales as an analog to bow-riding (Würsig, 2008), or playful behaviors (Deakos *et al.,* 2010). However, Wedekin *et al.* (2004) presented evidence where rough-toothed dolphins (*Steno bredanensis*) were preying on sharksuckers (*Echeneis naucrates*) while interacting with humpback whales off Brazil, which demonstrates a predatory interest.

Indeed, several fish species are commonly observed in association with cetaceans (Alling, 1985; Silva Jr. & Sazima, 2008; Lucena *et al.,* 2015), though records of large whales and associated fish are rather scarce or receive little scientific attention, particularly in regard to humpback whales (*Megaptera novaeanglieae*). While describing four fish species in association with humpbacks in the oceanic island of Trindade, Lucena *et al.* (2015) discuss the suggest the forage interest of fish in this association and highligh the unknown importance of these whales on trophic chains of tropical latitudes. In summary, the benefits of this interaction for both fish and whales are not well elucidated, even though evidence indicate that this relationship is at least commensal (Katona & Whitehead, 1988), where fish take advantage of some benefits without interfering their host.

Remoras or diskfishes (Echeneidae) are perhaps the most acknowledged species from such interactions, as these fish truly attach themselves to their host through a suction disk on top of their heads. Two remora species have been registered in association with cetaceans: the whalesucker (*Remora australis*), and sharksucker. The former is rather host-specific, exhibits offshore pelagic habits and has been recorded associated exclusively to cetaceans, whilst the latter is the most generalist among diskfishes, often seen in warm shallow waters and nearby coral reefs (Lachner, 1986; Fertl & Landry, 2009). Suspected advantages of this association for diskfishes include expanded feeding opportunities, increased mating odds, energy saving rides, enhanced gill ventilation and potential protection against predators (Alling, 1985; Silva-Jr. & Sazima, 2008). Similar benefits apply to other reef-dwelling fish that aggregate around cetaceans, with the addition of an apparent interest of predaceous fish in preying on the same target of their hosts (Gudger, 1930; Sazima *et al*., 2006), and offal-feeding behaviour by plankton-eating fishes as an alternative form of foraging (Sazima *et al*., 2003). The latter strategy was described for reef fishes that associate with spinner dolphins (*Stenella longirostris*) in a shallow bay in Fernando de Noronha archipelago, where hundreds of dolphins congregate regularly to rest (Silva-Jr. *et al*., 2005). Similarly, humpback whales perform annual migrations to low latitude breeding grounds. Off Brazilian coast, the whales migrate to the Abrolhos Bank, their main birthplace in the southwestern Atlantic Ocean (Andriolo *et al.,* 2010). Abrolhos is an area of shallow reefs where different fish species are known to aggregate around whales, such as sharksuckers, the Serra Spanish mackerel (*Scomberomorus brasiliensis*) and horse-eye jack (*Caranx lattus*) (LW, personal observation), and where rough-toothed dolphins and other species of small cetaceans also occur (Rossi-Santos *et al.,* 2006).

In this context, the present study objectives to:

(i) Describe fish species in association with humpback whales at the Abrolhos Bank. We intend to test the foraging interest of fish for this interaction, and verify whether environmental variables drive the fish occurrence, namely depth and distance to nearest reef formation;

(ii) Review previously recorded interactions between rough-toothed dolphins and humpback whales within the same area, in order to better interpret the motivations for this association.

We hypothesize that fish prefer to associate with whale groups that provide offal resources, and that rough-toothed dolphins seek for whale groups in order to forage on their companion fauna.

MATERIAL AND METHODS

The Abrolhos Bank (between 16º40' S - 19º30' S, to 37º25' - 39º45' W) is a large extension of the Brazilian continental shelf with mean water depths of about 30 meters. It is the major breeding ground for the breeding stock A of humpback whales (Andriolo *et al.,* 2010; Wedekin *et al.,* 2010), and encompasses the largest reef complex of the South Atlantic Ocean (Castro & Pires, 2001; Francini-Filho *et al*., 2013).

Data for associated fish were collected during systematic research boat cruises conducted by the Humpback Whale Institute throughout two years of whale's breeding season

(2016-17) (see Wedekin *et al.* 2010 for a description of the methodology employed in the cruises). Sampling routes covered the surroundings of the Abrolhos Marine National Park. During monitoring activities, groups of whales and other cetaceans were located and approached for data collection. Underwater records of fish in association with whales were obtained through a system of underwater cameras attached to the hull of the research vessel. One extra record was obtained from an aerial image above the whale. Underwater images were analyzed and records containing fish in association were used for identification to species level according to Carvalho-filho (1999) and Humann (1994). Sighting position, water depth and whale group composition were taken from the datasheet filled in the field. Fish abundance and position in relation to the body of the host was obtained from video recordings. Distance from nearest emerged reef formation was estimated with the software QGIS.

For a period of 20 years of this same monitoring program (1998 – 2017), datasheets were reviewed for information regarding whale-dolphin interactions. In this period, other cetaceans were approached regardless of whether they were interacting with humpbacks. Behavior was recorded *ad libitum* (Mann, 1999) and the team registered group characteristics, location and photographs for species and individual identification. Identified individuals during interaction events were compared for resightings over the period of 2005 to 2017. Geographical location where interactions occurred were plotted in the software QGIS (QGIS Development Team, 2015) and compared with the area where we recorded associated fish with the whales.

Generalized linear models with binomial family were used to test the influence of environmental variables on the occurrence of associated fish, namely 'distance from nearest reef formation', 'depth' and 'resource', defined by the presence of either lactating females or calf as hosts. We assumed that the latters are the main source of offal residues, given that adult humpbacks rarely defecate in breeding grounds. The function 'hnp' from the hnp package (Moral *et al.,* 2017) was used to test whether the residuals fitted the model properly, and an analysis of variance (ANOVA) of the model tested the significance of each factor. The best model was selected parsimoniously, i.e the simplest one including significant variables ($p < 0,05$) only. To test the avoidance of whale groups in the presence of dolphins, we chose five movements that characterize disturbed behaviors: trumpet blows, bubble exhalation, pectoral and tail slaps and fluke strikes, all extracted from datasheets filled in the field. Disturbed state was considered when the group exhibited at least two of those. We then compared the variable 'disturbed behavior' between 27 whale groups under interaction with dolphins, against 70 randomly chosen groups that were not interacting. Given that the chosen behaviors are also commonly observed in competitive groups (Clapham *et al.,* 1992), only non-competitive groups were used in this analysis. For comparative purposes, data from monitoring activities at Praia do Forte (800~km north of Abrolhos) was also considered here.

RESULTS

Records of associated fish

In 24 out of 76 underwater registers we verified fish in interaction with humpback whales (Figure 1). Two fish species were reliably identified in association: sharksuckers, and *Caranx* spp., most commonly the blue runner (*Caranx crysos*). Table 1 summarizes the mean abundance, depth and distance from reef formations found for each species. Sharksuckers and blue runners were found inhabitting the same host in four registers and at least two individuals per host were found in 80% of the sights. Our best predictive model indicated a positive relationship between fish occurrence and the interacting variables 'nearest reef' and 'resource' (X^2 = 7.09, df=35, p=0.007).

Whale mother and calf pairs were present in 19 records (79,1%), adults in competitive groups were the hosts in 4 cases (16,6%) and a singleton in 1 instance (4,1%). Number of whales per group ranged from 1 to 14 (mean = 3,8 ± 2,7). Fish were observed either swimming freely around the host or attached to different regions of the whales' body. The blue runner was most commonly found nearby the genital region of its host (68,7% of the cases, n = 11). In one instance we observed a *Caranx* spp. in the surroundings of a calf's mouth. Diskfishes on the other hand were found more frequently attached to the flanks of their host (75%, n = 6) (Figure 2).

Figure 2. Registers of fish associated with humpback whales. *C. crysos* and *E. naucrates* in association with a mother and calf pair (left) and *C. crysos* in association with a mother and calf pair (right).

Table 1. Summary of mean abundance, distance from nearest reef formation and depth of the registers containing fish species in association with humpback whales.

Species	*n*	Mean Abundance	Distance (Nm)	Depth (m)
Caranx spp.	15	7,1 ± 6,1	3,3 ± 2,9	19,7 ± 6,6
E. naucrates	8	3,6 ± 2,3	4,8 ± 3,4	25 ± 3,6
Unidentified	5	4 ± 2,5	2,8 ± 0,5	21,3 ± 2,9
Total	28	5,6 ± 5,1	3,7 ± 2,9	21,1 ± 5,9

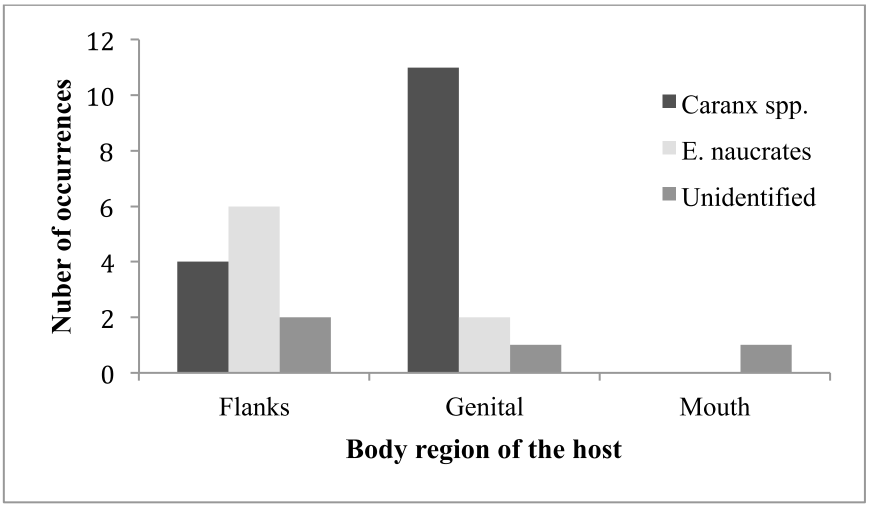

Figure 2. Graph illustrating the regions of the targeted host fishes were found when interacting with humpback whales.

Review of interactions with rough-toothed dolphins

From 1997 to 2017, 67 groups of rough-toothed dolphins were observed at Abrolhos Bank, 41 of which (61,2%) were interacting with humpback whales. These accounted for 312 individuals, sighted in mean group sizes of 4,8 ± 3,85 individuals. Encounters with interacting dolphins were spatially overlapped with the occurrence of associated fish when considering the area sampled for whales with accompanying fauna (Figure 3). Groups of whales targeted by dolphins were composed of singletons, pairs or trios of adult humpbacks in 14 (34,1%) encounters. Four or more adults were targeted in other 12 (29,2%) occasions, and mother and calf pairs were present in only five (12,1%) interactions, 3 of which in companion of 1-3 male escorts. For the other 10 cases (24,4%) we had no reliable data on the group composition of whales (Figure 4a). We also reviewed 63 sightings of bottlenose dolphins (*Tursiops truncatus*) over the same period. These were interacting with whales only in 11 (17,4%) of the encounters. For Praia do Forte, north of the Abrolhos Bank but also within the distribution area of the humpbacks, data obtained from research cruises and whale watching operations indicate a similar proportion. From 2002 to 2016, 38 groups of rough-toothed dolphins were sighted, 21 of which (55.2%) were interacting with humpback whales. For bottlenose dolphins, 33% of the 165 groups encountered were interacting with whales (Figure 4b).

From 1997 to 2017 we reviewed 30 data sheets with reliable behavior descriptions. In these, whales were observed reacting in an aggressive, disturbed manner during 21 out of 30 encounters (70%) with the dolphins. Therefore, our analysis demonstrated that whale groups were found to exhibit a 'disturbed behavior' significantly more often during interaction events with rough-toothed dolphins ($F = 21{,}61_{1,95}$; $p < 0{,}001$) than without interaction.

Good-quality photo-identifications of rough-toothed dolphins were obtained for 48 individuals over the period of 2006 to 2017. Of these, 28 were from groups in interaction with humpback whales, from which we found two matches of individuals that were first seen foraging together during an interaction with a group of whales, and resighted within the Abrolhos Region four and eight years later, respectively.

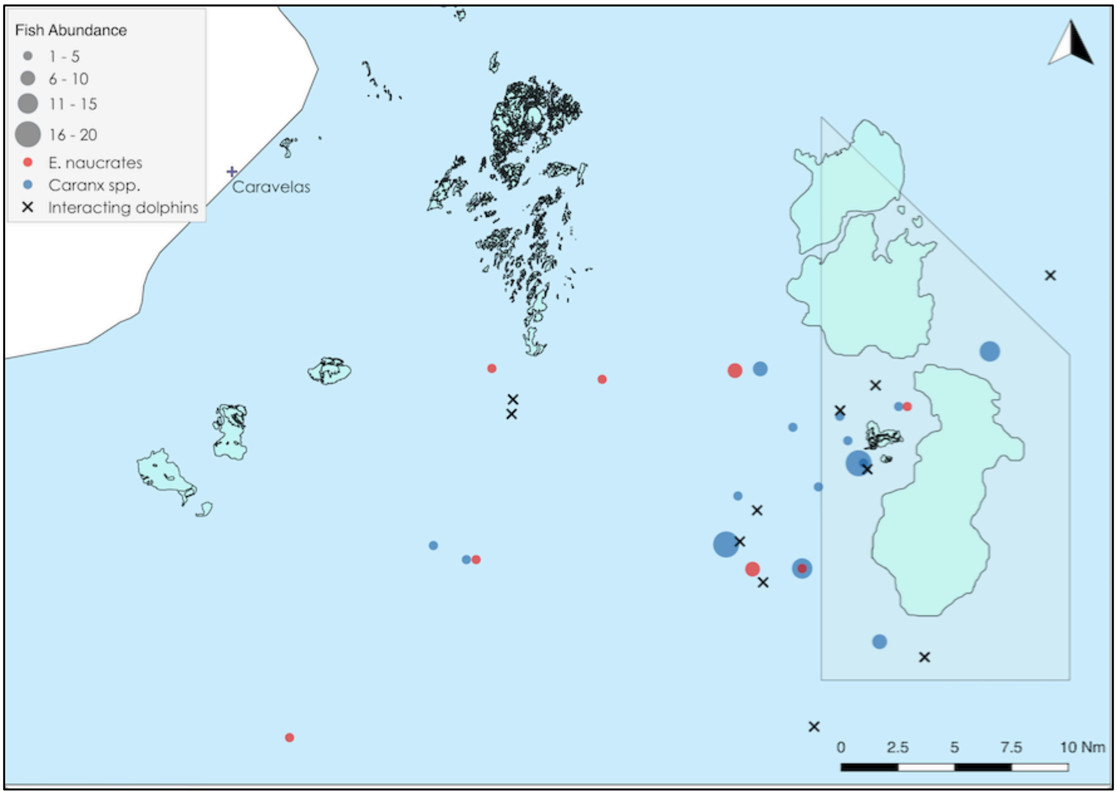

Figure 3. Locations within the study site where fish were found in association with humpback whales, and points where rough-toothed dolphins were sighted interacting with humpback whales.

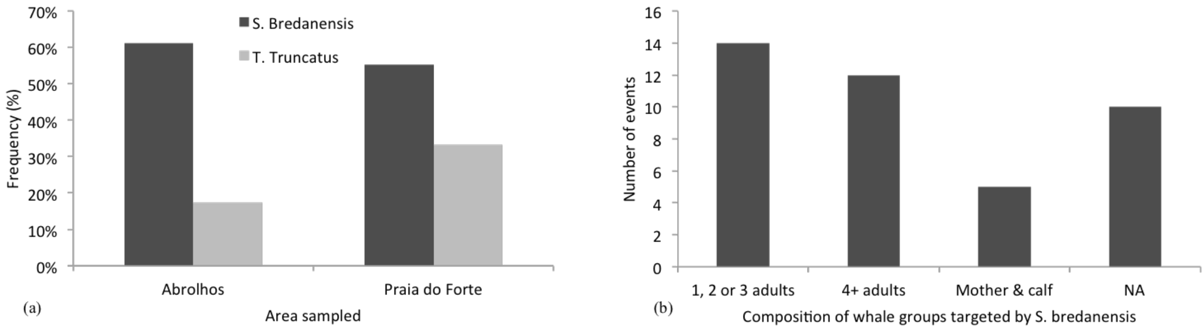

Figure 4. (a) Frequency of encounters with *T. truncatus* and *S. bredanensis* in interaction with humpback whales from Abrolhos and Praia do Forte. (b) Group composition from whales targeted by interacting *S. bredanensis*.

Finally, we reviewed three events of direct predation on associated fish during dolphin-whale interactions. In those, circumstances were roughly the same: the sighted whale group behaved in a disturbed manner as the dolphins swam in a foraging behavior. Suddenly at some point a rough-toothed dolphin surfaced with a fish in its mouth. In one case the fish species could be identified as a Serra Spanish mackarel (*Scomberomorus brasiliensis,* Scombridae) (Figure 5).

Figure 5. Rough-toothed dolphin preying on a *Scomberomorus brasiliensis* (left) and fish tossing (right).

DISCUSSION

Our study assesses the fish interest in associating with humpback whales and supports the predatory interest of rough-toothed dolphins (*Steno bredanensis*) when interacting with the whales. There are several reasons for which fish might be interested in associating with humpback whales. Previous studies have suggested that large aggregations of fish within the same host may be linked to its increased opportunities to mate (Alling, 1985; Cressey & Lachner, 1970). Silva-Jr & Sazima (2003) reinforce that with cases of long-term bonds between remoras and spinner dolphins, proposing that the highly social nature of the spinner dolphins may facilitate encounters between fish mating partners.

Our best predictive model indicated a higher occurrence of fish in association with whale groups that provide resources, i.e. composed of lactating mother and calf. Alternative foraging strategies are an interest for associated fish (Alling, 1985; Silva-Jr & Sazima, 2008, Strasburg, 1962), particularly offal feeding. Coprophagy is a common foraging behaviour among different trophic levels of reef-dwelling fishes in the Pacific (Robertson, 1982). Regarding cetacean offal, both the blue runner and sharksucker were among the twelve fish species recorded feeding on feces and vomits of spinner dolphins (*Stenella longirostris*) at Fernando de Noronha archipelago, off Brazil (Sazima *et al.,* 2003). In that site dolphin feces appear to be so plentiful that it is the presumed reason for the rarity of fish-feces eating by other reef fishes (Sazima *et al.,* 2003). This particular feeding alternative would be remarkably advantageous in areas where marine mammals congregate in great numbers, which is the case of the Abrolhos Bank. Even though adult humpback whales are rarely seem eating or defecating at breeding grounds, mothers are intensely nursing their calves, which in turn defecate a nutrient-rich material.

Another potentially attractive food source for associated fishes is the fat-rich milk of a lactating mother. Calves are expected to spend substantial time suckling given their growth rates of 0.5 – 1 meter per month in length (Christiansen *et al*., 2016). Indeed, it has been estimated an average of 20% of their post-birth time spent on this activity (Videsen *et al.*, 2017). Therefore, suckling events seem to be quite predictable, thus associated fish may take advantage of nursing mothers once the milk becomes available in the water. Zoidis & Lomac-MacNair (2017) reported, in two out of five focal sessions with nursing humpback whales, the presence of free floating milk in the water column. Despite no direct observations of fish feeding on dispersed milk in the present study, we assessed a good-quality underwater footage obtained from a tourism company in Silver Bank, where several fish (*Caranx* sp.) concentrate around the tip of the mouth of a humpback whale calf during suckling activity (Conscious Breath Adventures, 2010). Moreover, the prevalence of mother and calf pairs in our records (78,2%), in addition to the common positioning of fish in the surroundings of the genital and ventral region of both females and calves in these cases, corroborate the hypothesis that the two species found in this study may feed on dispersed milk and on feces defecated by calves.

Finally, an apparently obvious advantage for associated fishes is the protection from predators (Cressey & Lachner, 1970; Alling, 1985). It is reasonable to assume that a free-swimming fish would be more vulnerable to predation than one that remains next to a large whale. Indeed, small fishes have been observed schooling underneath bigger ones for protection during predation attacks (Macieira *et al*., 2010). However, given the evidence of predation presented here, fish in association with humpback whales in the Abrolhos region may face to a trade-off situation between (i) fitness-related benefits from this association, and (ii) a risky ride on foraging ground potentially targeted by the dolphins. Rough-toothed dolphins are distributed in the vicinity of coral reefs along the Abrolhos region (Rossi-Santos *et al.,* 2006). Our predictive model indicated a positive correlation between fish occurrence and close distances from reef formations. Further, the locations where dolphins were sighted interacting with humpback whales, including the described events of direct predation on associated fish, grossly overlaps with the area where we recorded the occurrence of fish in association with the whales.

Also relevant are the aggressive behaviors displayed by the whales during interaction events with rough-toothed dolphins, especially when the latter are foraging (according to the descriptions reviewed in Wedekin *et al.,* (2004) and here). The behavioral repertoire of forceful movements that indicate annoyance is similar to what is observed during social interactions among males in competitive groups (Clapham *et al.,* 1992) and during predatory interactions between killer whales (*Orcinus orca*) and humpback whales (Florez-Gonzales *et al.,* 1994). It is probable that these vigorous responses are triggered by the rapid foraging movements performed by the dolphins in proximity to the whales. Whether this is the case, these response movements are probably intended to drive away the dolphins. In that scenario, the events reported here would resemble the interactions described by Pitman *et al.* (2016), in which humpback whales protect cetaceans, pinnipeds and fish from killer whale predation attacks. Therefore it is reasonable to state that, at least to some extent, associated fish gain an active protection from predators from this interaction. Howsoever, additional underwater observations of such events may help to elucidate the dynamics occurring during these interactions.

Little is known about the ecology of rough-toothed dolphins or their feeding habits (Jefferson, 2009), and most data on the diet of this species are obtained from scattered records, generally from stomach content (Pitman & Stinchomb, 2002). However, rough-toothed dolphins frequently associate with others delphinid species and are most likely the only cetacean that regularly associates with flotsam (Jefferson, 2009). The high rate of encounters where rough-toothed dolphins are sighted interacting with humpback whales (61,2%) in the Abrolhos Bank indicates some strong motivation for this association. The similar proportion of interacting groups found for Praia do Forte, and the reduced rate of interaction between whales and bottlenose dolphins reinforces the significance of this result. This evidence suggests that their major motivation is predatory. Records from diet items of rough-toothed dolphins are rather scarce, and only one species from our underwater records (*E. naucrates*) was previously recognized as their prey (Wedekin *et al.,* 2004). Our reviewed interactions between rough-toothed dolphins and humpback whales identified for the first time the Serra Spanish mackerel as a prey item for this species.

The comparison of interacting dolphin individuals indicated that this sort of foraging strategy might be frequent, which corroborates our hypothesis that local rough-toothed dolphins developed a foraging technique from this unique interaction with the whales. However, we found a divergence in our results concerning the composition of the whale group targeted by fish and dolphins. While mother and calf pairs were frequently present in fish interaction records, dolphins rarely targeted these. We hypothesize that this divergence may have occurred due to (i) the small sample size of fish in association with whales, or (ii) dolphins might use acoustic cues to seek and find the whales, therefore singing males may be targeted more often than mother and calf pairs.

CONCLUSION

The evidence gathered here draw attention to a neglected but important role of humpback whales for associated fish and rough-toothed dolphins. Approximately 10.000 humpbacks congregate in the Abrolhos region anually (Pavanato *et al*., 2017). Their social nature in breeding grounds is to form from small groups of male escorts with lactating females, to large competitive groups in their breeding grounds (Clapham, 1996). Abrolhos is the habitat of more than 266 reef and shore fish species (Moura & Francini-Filho, 2006), distributed over an area that coincides with the occurrence of whales and rough-toothed dolphins. These numbers build up a scenario of resource abundance for fish species that benefit with this association through the advantages inspected here. Therefore, whales act as fish attractors, and we reported here several instances where groups of dolphins search for whales to obtain food. The predictability of available fish in association with seasonal migrating whales may have facilitated the development of a unique foraging alternative for this population of rough-toothed dolphins.

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CONCLUSÕES

O principal produto dessa dissertação foi o desenvolvimento e o teste de uma metodologia com câmeras submersas simples, barata e eficiente, a ser empregada em paralelo à pesquisas de campo e monitoramentos sistemáticos de cetáceos. Demonstramos que é possível obter resultados relevantes através de um rápido olhar sob a perspectiva sub-aquática, com implicações para estudos comportamentais, identificação de indivídos e atributos, condição corporal e diagnóstico de animais encalhados. O uso de câmeras otimiza a detecção de objetos de interesse – sejam fenômenos, indivíduos, etc -, tornando as campanhas de coleta mais proveitosas, ampliando os dados coletados e otimizando os resultados. A metodologia proposta pode ainda ser explorada em seu potencial máximo se aplicada em condições ótimas de transparência da água (>20m) – maior limitante para seu sucesso.

Aprofundando a discussão acerca das relações interespecíficas entre peixes e baleias-jubarte - um dos resultados obtidos durante esse trabalho —, conseguimos juntar mais informações sobre um tema há anos observado pelos pesquisadores do Instituto Baleia Jubarte: o interesse de golfinhos-de-dentes-rugosos em forragear nos peixes associados às baleias-jubarte. Essa complexa interação não dispõe de uma interpretação simplificada, e os dados aqui obtidos foram essenciais para reunir as evidências que apontam para a hipótese levantada no capítulo II deste trabalho: a previsibilidade da migração sazonal das baleias-jubartes para um ambiente recifal fornece recursos de interesse para os peixes que ali habitam, que por sua vez se associam às baleias e tornam-se alvos móveis para o forrageamento dos golfinhos. Estes últimos, que no habitat oceânico se alimentam de forma oportunística, podem então contar com a disponibilidade de peixes no entorno das baleias, organismos consideravelmente mais fáceis de serem encontradas devido às pistas acústicas do que peixes menores e silenciosos.

Vários desafios limitam a amostragem e visualização das atividades de animais marinhos em seu hábitat natural. Similarmente, são também diversas as implicações voltadas para pesquisa e conservação aplicada dessa fauna obtidas por meio dessa abordagem. A metodologia aqui proposta pode (e deve) ser adaptada e ganhar complexidade de acordo com a demanda e com as perguntas a serem respondidas. É válido ressaltar que quaisquer avanços em direção ao estudo de animais marinhos são dignos de reconhecimento e devem ser propagados, discutidos e melhorados.