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Research Article

The behavior of the Argentine shortfin squid (*Illex argentinus*) exposed to bottom trawl gear off southern Brazil

José Angel Alvarez Perez[1], Bruna Firmino de Oliveira Yasunaka[1] & Maurício Rocha Visintin[1]
[1]Universidade do Vale do Itajaí, Escola do Mar, Ciência e Tecnologia
Universidade do Vale do Itajaí, Itajaí, SC, Brazil
Corresponding author: Jose Ángel Alvarez Perez (angel.perez@univali.br)

ABSTRACT. The behavior of the Argentine shortfin squid (*Illex argentinus*) in reaction to a trawl net is reported from images obtained during one fishing tow operated by a commercial trawler in May 2015 in slope grounds off southern Brazil (depth 370-361 m; 33°37'S, 50°49'W). Video analysis utilized criteria to classify the horizontal and vertical distribution of squid in the field of view of the camera, posture (pitch and yaw), squid color patterns, escape reaction, swimming time and use of the tail fin. The monitored bottom trawl operation intercepted dense and patchily distributed schools of *I. argentinus* that could be observed for brief periods of time swimming ahead of the mouth of the trawl. Individuals were evenly distributed in the water column but concentrated on the center and right sectors of the trawl mouth. Most squids in the path of a trawl tended to swim by jet-propulsion for 10-20 s, tail-first and with regularly spaced fin flaps. Estimated speed (1.24 m s^{-1}) was higher than cruise speed recorded for other ommastrephid squids in nature (0.3 m s^{-1}), possibly reflecting an emergency escape reaction. After a period of sustained swimming ahead of the trawl, squid reacted in different ways including: a) slowing down and swimming into the net tunnel, b) shifting directions to escape through the trawl mouth sides, and c) swimming up, trying to escape over the top trawl panel, sometimes turning around pointing the tail towards the trawl mouth.

Keywords: trawl fishery, Argentine shortfin squid, trawl camera, behavior, swimming, southern Brazil.

INTRODUCTION

Bottom trawl fishing has been historically responsible for the bulk of demersal catches off southeastern and southern Brazil (Valentini & Pezzuto, 2006). The development of this activity dates back to the 1960's when trawlers started to operate widely on the continental shelf (<100 m depth) fishing for coastal shrimps and groundfish. From 1980's onwards this fleet exhibited a continuous process of latitudinal and bathymetric expansion, which produced an increase in the diversity of resources retained for commercial purposes (Perez *et al*., 2001). Since the late 1990's, such expansion included slope areas (150-1,000 m depth), which established an unprecedented fishing regime over deep demersal resources (Perez *et al*., 2009a; Dias & Perez, 2016).

The development of slope trawling in the country produced economic and nutricional benefits (Pezzuto & Mastella-Benincá, 2015; Dias & Perez, 2016; Martínez-Musoles *et al*., 2016), but also environmental impacts, most notably, overfishing of the main target-species (Haimovici *et al*., 2006). However, the expanding 'spatial footprint' of this fishery has potentially included other effects to the marine ecosystems such as a) substantial non-target species mortality, b) alterations of demersal and benthic communities structure (Pauly *et al*., 2001), and c) physical disturbance of previously untouched marine habitats (Coleman & Williams, 2002; Kaiser *et al*., 2006). Recent studies, focused on the trawl fleet that operates off the fishing harbors of Santa Catarina State, assessed some of these impacts with the aim of producing science-based elements useful for improving ecosystem-based management measures (Perez *et al*., 2013; Visintim, 2015; Port *et al*., 2016; Martínez-Musoles, 2016).

In Brazil, the multispecies trawl fishing has been subject to a conflicted management process, principally regarding the large non-intentional mortality produced by the trawl nets. Particularly critical has been the frequent mortality of a variety of fishes considered

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threatened of extinction, whose illegal catch has restricted the commercial activity and produced severe conflicts among stakeholders (*e.g.*, Dias-Neto, 2011; Visintim, 2015). Solutions for this adverse effect of trawl fishing are complex, and one promising way involves modifications of fishing gear and operations patterns, which could minimize contact with non-target species while preserving the catch efficiency of the targeted ones (Broadhurst, 2000; Vianna & D'Incao, 2006; He & Winger, 2010). Designing and implementing such modifications, however, require a significant improvement on the understanding of the operating patterns of the bottom trawl nets and how different megafauna's components behave in reaction to these patterns (Rose, 1995; Bayse & He, 2017).

Bottom 'otter' trawl nets have been designed and improved to produce a "herding" effect over some demersal fish species. Upon tiring, fishes end up overtaken by the trawl, enter the net tunnel and are finally retained in the codend. Such behavior, however, is not homogeneous among demersal and benthic megafauna, as shown for a variety of fish species, crustaceans and squids that exhibit distinctive reaction patterns (*e.g.*, Main & Sangster, 1981; Main & Sangster, 1983; Rose, 1995; Queirolo *et al*., 2010, 2012; Bayse *et al*., 2016a, 2016b). Combined with the diversity, abundance, and distribution of megafauna's species in the path of the trawl, these behavioral patterns may determine the final catch composition of fishing trawls and therefore can be partially inferred from catch analysis. Their full comprehension, however, requires direct observation, which has only been achieved through the use of video cameras attached to the trawl gear. These cameras have not been applied in the assessment of Brazilian trawl fisheries, yet should be a promising step towards exploring future practical solutions to reduce bycatch, particularly of the biologically sensitive slope benthic and demersal communities (Visintim, 2015).

The Argentine shortfin squid, *Illex argentinus* (Castellanos, 1960), has become one of the catch components of the trawl fisheries, as it expanded to the slope grounds off southeastern and southern Brazil (Perez & Pezzuto, 2006). In some years, the species was regarded a 'massive target' of this fishery (*sensu* Biseau, 1988), especially during winter months (June-September), when population groups, originated from the Uruguayan and northern Patagonian shelves, migrate to lower latitudes to spawn in Brazilian waters (Perez *et al*., 2009b). In 2015, these migrating groups were captured in video, as they interacted with a commercial trawl off southern Brazil. Despite its opportunistic nature, results obtained in this study allowed the exploration of hypotheses about how these animals react to bottom trawls and what elements of the gear and operation procedures should either enhance catch or promote escapement. These results were compared to those obtained by more robust experimental studies conducted with coastal squids (Glass *et al*., 1999; Bayse *et al*., 2016a, 2016b).

MATERIALS AND METHODS

This study analyzed images obtained during one monitored commercial trawl operation conducted by a double rig trawler in May 6, 2015, off southern Brazil slope (370-361 m depth) (initial position: 33°37'00"S, 50°49'00"W - final position: 33°27'38"S, 50°36'30"W). Four hours and 46 min-long video recording were produced by a Trawl Camera Lowlux (JT Electric®) capable of generating high resolution black & white images. This camera was sewed to the top panel, on the center of one of the paired trawls, close to the head rope, and directed slightly backward in the direction of the ground rope (angle of 30º). A light unit was positioned at the right side of the camera, which was set to low (10%) intensity (Fig. 1).

The video was initially analyzed to assess visibility levels as well as opportunities for adequately measuring/observing a) the frequency of occurrence of squids in the video, and b) different aspects of squid behavior. In the first case, patterns of squid distribution along the trawl path were assessed by conducting a second analysis of the video, when the total number of squid visualized was recorded within one-min observation intervals. The resulting time series data was analyzed using Morisita's concentration index (Morisita, 1959):

$$I_{\delta} = \frac{\sum_{i=1}^{N} n_i(n_{i-1})}{n(n-1)} N \qquad (1)$$

where n_i is the number of squids counted within the i-eth time interval; n is the total number of squids counted along the entire video; N is the number of time intervals. In this analysis, a random occurrence of squids along the video would produce $I_{\delta}{\sim}1$; an occurrence of squids at regular time intervals would produce $I_{\delta} < 1$; and clustered occurrences, $I_{\delta} > 1$. The result was tested (H_0: temporal/spatial pattern of squids along the video/ trawl path is not clustered) through the calculation of the F (Fisher) statistics ($\alpha = 0.05$) where:

$$F = \frac{I_{\delta}(n-1)+N+n}{N-1} \qquad (2)$$

Squid behavior was assessed using seven visual traits, namely: 1) position of squid around the visual field of the camera, 2) vertical distance from the camera (altitude), 3) posture-pitch, 4) posture-yaw, 5) color patterns, 6) escape reaction, and 7) swimming mode.

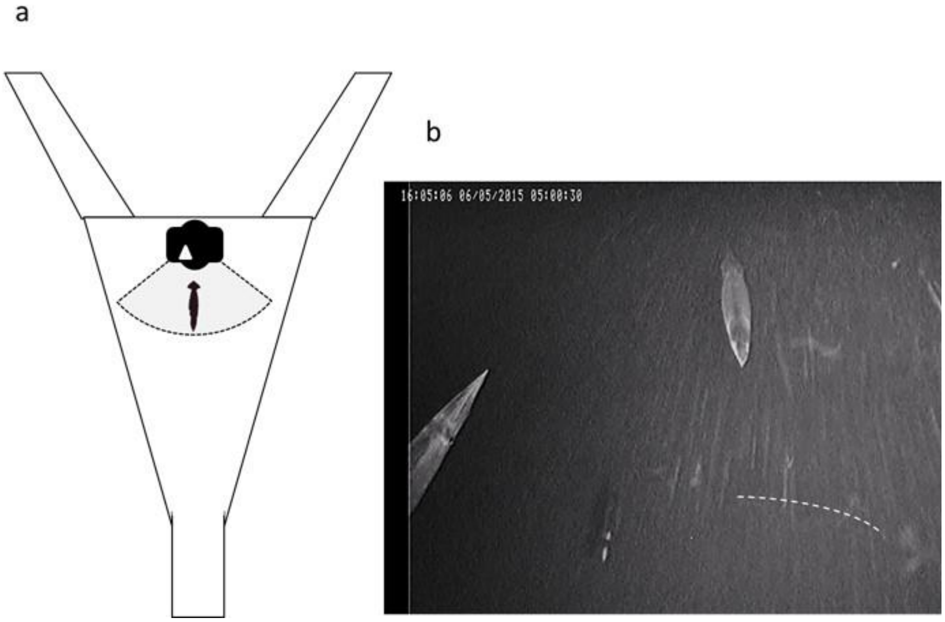

Figure 1. Argentine shortfin squid *Illex argentinus* behavior recorded by a trawl net camera system. a) Schematic view of the bottom trawl net showing position and field of view of the trawl camera (triangle = light source), b) three squids swimming in front of the camera system in three altitude levels (dashed line = ground rope).

These traits were initially described and defined categories into which all squid encounters could be classified. Once the categories were defined, video segments containing squid encounters were extracted from the main video and, from these intervals, still images (frames) were extracted and cataloged. These digital images were overlaid by a six-quadrant grid (quadrants identified clockwise by numbers 1 to 6) and each squid visualized was assigned an identi-fication code (Fig. 2). A data matrix was constructed where individual squids and describing traits were displayed in lines and columns, respectively. Each squid was then classified according to the position around the visual field (code 1 to 6) and the vertical distance between the camera and the seafloor according to the scale: 1) squid very close to the camera, 2) mid-distance from the camera to the seafloor, and 3) squid swimming near the seafloor. Posture components, pitch, and yaw, of visualized squid, were then recorded following two specific scales with eight levels (Fig. 2). Lastly, from the digital images observation, color patterns of squids were described, and their frequency of occurrence in selected still images was recorded and analyzed (see results).

The last two attributes, escape reaction and swimming mode, were analyzed directly in the extracted video segments, which were repeatedly observed to a) classify the type of escape reaction (see results), b) record swimming time in front of the camera (in seconds), and c) record the frequency of flaps of the tail fin.

The frequency of traits 1 and 2 occurrences was analyzed using Chi-square tests (H_0: the classifying categories occurred homogeneously along all observed images; H_1: the classifying categories did not occur homogeneously along all observed images; *i.e.*, behavior patterns emerged) (Zar, 2009). Pitch and yaw levels (Fig. 2) were transformed into directions around the circle (0 = 0º; 1 = 45º; 2 = 90º; 3 = 135º; 4 = 180º; 5 = 225º; 6 = 270º; 7 = 315º) and their distribution analyzed using 'circular distribution' statistical procedures (Zar, 2009). These included the angular dispersion index (r), angular deviation (s), and standard circular deviation (s_0), defined by the equations:

$$r = \sqrt{\left(\frac{\sum_{i=1}^{n} \cos a_i}{n}\right)^2 + \left(\frac{\sum_{i=1}^{n} sen a_i}{n}\right)^2} \qquad (3)$$

$$s = \frac{180^o}{\pi}\sqrt{2(1 - r)} \qquad (4)$$

$$s_0 = \frac{180^o}{\pi}\sqrt{-2 \times \ln r} \qquad (5)$$

where n is the number of pitch and yaw levels measured in the analyzed images, and a is each of the i-eth transformed angles (directions). r is a measure of angle concentration that can vary from 0 (when squid are widely dispersed around the circle) and 1.0 (when squid

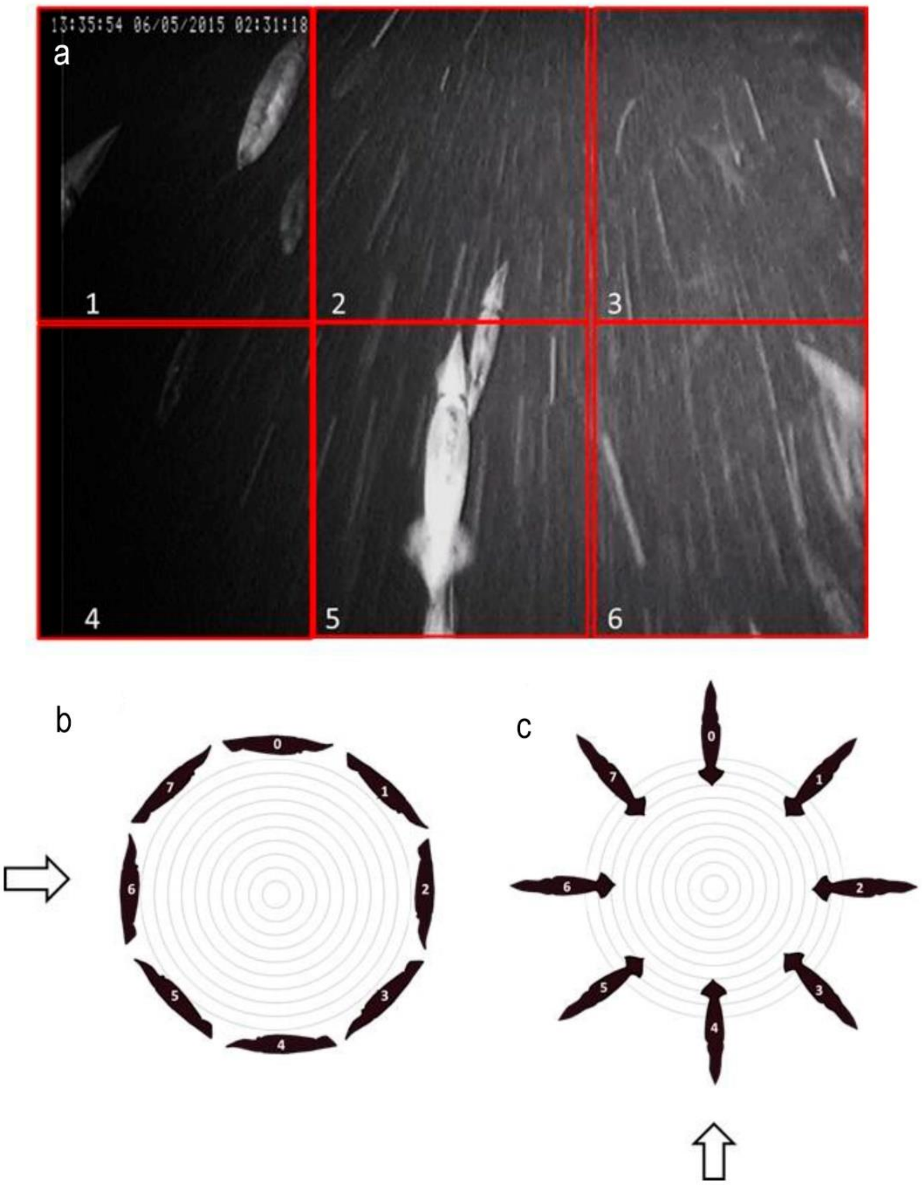

Figure 2. Argentine shortfin squid *Illex argentinus* behavior recorded by a trawl net camera system. a) Grid overlaid still images that allow recording squid position around the field of view of the trawl camera, b) classification levels for pitch (side view), and c) yaw (upper view). Arrow indicates the position of the camera.

were all concentrated in the same direction). Angular deviation (s) varies from 0 to 81.03º, and standard circular deviation (s_0) varies from 0 to ∞. The smaller the difference between s and s_0 the more concentrated the squid were around a direction; *e.g.*, when indices differ from each other by less than 2º then $r > 0.8$ (Zar, 2009). A mean direction value describes the probabilities for pitch and yaw directions (H_0), were calculated using Z statistics and the Raleigh test (Zar, 2009), where

$$Z = n \times r^2 \tag{6}$$

RESULTS

Spatial distribution patterns

A total of 832 squids were observed in the field of view of the camera during the entire video observation. This procedure was affected by the eventual recounting of individuals that may have moved in and out of the field of view more than once. Still, tens to hundreds of individuals were counted during short time intervals (4 to 7 min) interspersed by longer periods when few or

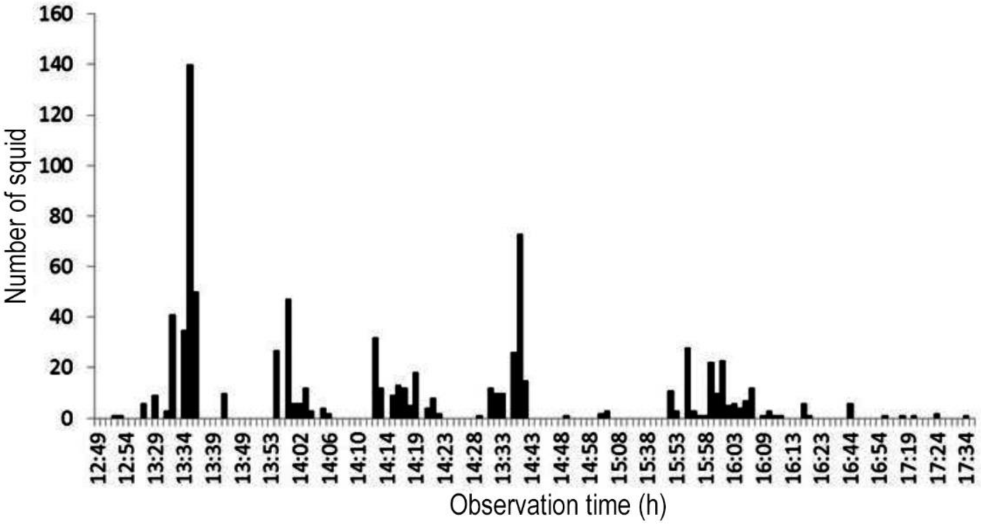

Figure 3. The occurrence frequency of the Argentine shortfin squid (*Illex argentinus*) along a 4 h and 46 min-long trawl on the slope of southern Brazil.

no squids were visible (Fig. 3). The visual evidence suggested that the trawl encountered dense schools of squid, extremely concentrated in time/space as confirmed by Morisita's index (16.1; F = 44.7; $P < 0.001$).

Squid position and posture

73 high quality still images were extracted, in which 139 visible squids were identified and had their behavior traits analyzed. Swimming squid concentrated in the left (51%) and central quadrants (36%) of the field of view, with only 11% occurring in the right quadrants ($\chi^2 = 40.0$; $P < 0.01$) (Fig. 4). Squids were also observed swimming close to the camera (30.9%), close to the seafloor (33.8%) and at half distance (35.2%) ($\chi^2 = 0.40$; $P = 0.99$). Pitch categories 0 (horizontal swimming) and 7 (swimming slightly upwards) were characterized jointly in 98% of the observed squid (Fig. 4). Yaw categories 0, 1 and 7 (swimming straight ahead or slightly yawing to the left and to the right) were, altogether, characterized in 98% of observed squid (Fig. 4). Both descriptors of squid posture transformed into directions around the circle were shown to be highly concentrated, with *r*-values ranging between 0.87 and 0.91, and the difference between S and S_o ranging between 0.45 and 1.79 (for yaw and pitch, respectively). The Raleigh test, applied to these distributions, confirmed that observed squid could be described by significant mean pitch and yaw directions ($P < 0.001$).

Color patterns

Color patterns of squid swimming ahead of the trawl mouth were highly homogeneous, including the typical chromatic components described for the species, *i.e.*, mantle generally pale with a dark longitudinal band along the mid-dorsal surface, extending anteriorly to the dorsal head and arms (Fig. 5).

However, a few remarkable chromatic components were consistently observed, namely, an unpigmented area at the left dorsal end of the mantle, and two pairs of white spots behind the eyeballs and on the mantle mid-dorsal surface, on both sides of the dark longitudinal band (Fig. 5). Also remarkable, was the regular contrast formed between the first (dark) and the second (pale) pair of dorsal arms, forming a V-shaped line outlining the arms (joint together during the swimming posture) (Fig. 5). Four progressive stages of the dorsal mantle, head, and arms pigmentation were defined, varying from the dark with very few contrasting components, to bright pale with important dark/light contrasts (Fig. 5); 72% (100) of individuals displayed the darker patterns, whereas 28% (39) displayed the lighter patterns, a result found to be statistically significant ($\chi^2 = 3.85$; $P < 0.01$).

Squid swimming and reactive movements

The visualization of complete movement sequences of squid, intercepted by the camera field of view during the trawl operation, was only considered sufficiently clear in 68 encounters along the entire video. All other encounters were either exceedingly fast or with insufficient resolution/illumination, which increased levels of uncertainty. In 21 encounters, squid sustained regular swimming in front of the camera allowing for reliable measurements of swimming times at the trawl/camera speed (4 knots), and the frequency of utilization

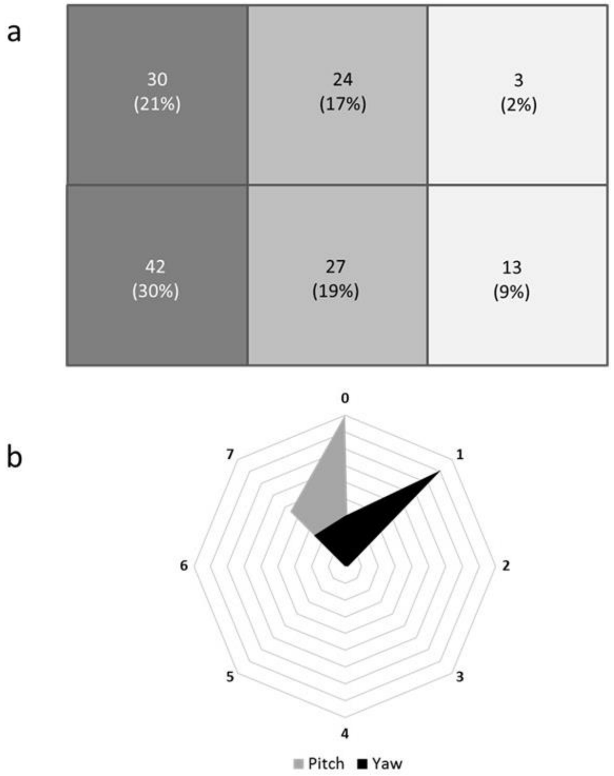

Figure 4. Reaction of the Argentine shortfin squid (*Illex argentinus*) to a trawl net operation off southern Brazil. a) squid distribution around the field of view of the trawl camera (n = 139, χ^2 = 40.0, P < 0.01); b) frequency of occurrence of pitch (n = 139, r = 0.81, P < 0.01) and yaw (n = 139, r = 0.92, P < 0.01) categories.

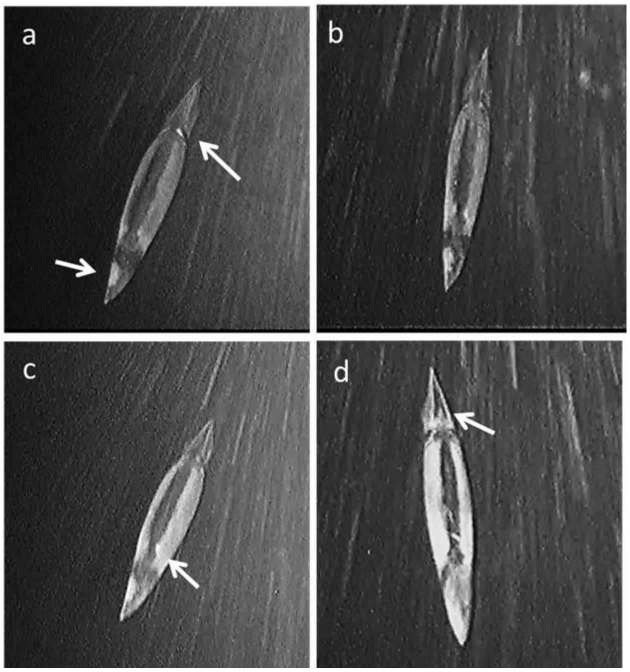

Figure 5. Color patterns exhibited by the Argentine shortfin squid (*Illex argentinus*) while swimming ahead of a trawl net off Southern Brazil. Four progressive stages were differentiated according to the dorsal pigmentation of the mantle, head, and arms ranging from generally dark with few contrasting features (a) to generally bright pale with strong contrasting features (d), and including two intermediate stages (b) and (c). The arrows indicate an unpigmented area at the mantle tip in (a), white spots behind the eyeball in (a), white spots in the middle of the dorsal mantel on either side of the dark longitudinal band in (c), and contrasting 2nd (dark) and 3rd (pale) pair of arms in (d).

of the tail fin along this movement. In these encounters, squid exhibited the general 'cruise' swimming pattern of ommastrephid squids, *i.e*., jet-propelled tail-first displacement and the use of occasional flaps of the tail fin. Stable swimming was sustained for up to 39 s, with more frequently measured time intervals (25-75% quartiles) ranging between 9 and 16 s (median = 13 s, n = 21). During these swimming intervals, up to six tail fin flaps were recorded, with values concentrating between 2 and 5 (median = 3.6 flaps, n = 21). In general, one flap was recorded every 2.5-4.2 s (quartiles 25-75%), being less spaced (<2 s) in very fast encounters (<5 s swimming intervals) and more spaced (>5.0-9.5 s) in swimming intervals longer than 20 s (Fig. 6).

Clear reactions of squids to the trawl gear were observed in 68 encounters grouped into three general patterns (Fig. 7). The first was observed ten times (15.6% of the encounters) and can be described by a swimming deceleration followed by the movement towards the tunnel of the trawl net (and potential retention in the cod-end). A second pattern, observed in half of the encounters (32 times), included a sudden shift in the swimming direction and an escape movement in the direction of the lateral panels of the trawl net mouth. The third, and more complex reaction, observed 22 times (34.4%), involved a sudden upward thrust and an escape movement towards the top panels of the net. This movemente was sometimes preceded by the full reversion of movement (180º gyre), turning the head and arms towards the camera (Fig. 7). Often such reactions also included sideways (left and right) shifts. The occurrence of observed reactions was significantly unbalanced towards escape reactions (χ^2 = 11.4; P < 0.01).

DISCUSSION

This study described encounters of a bottom trawl operation on the slope off southern Brazil with dense and patchily distributed schools of *Illex argentinus*.

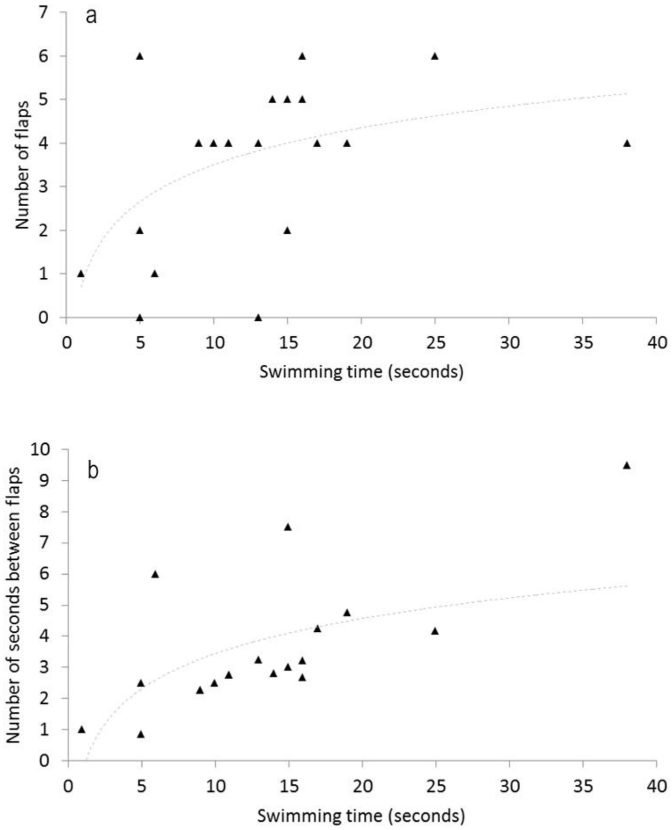

Figure 6. Swimming time of the Argentine shortfin squid (*Illex argentinus*) ahead of a trawl net off Southern Brazil and relationship with fin flaps: a) number off in flaps counted during each encounter; b) mean time interval (seconds) between consecutive fin flaps.

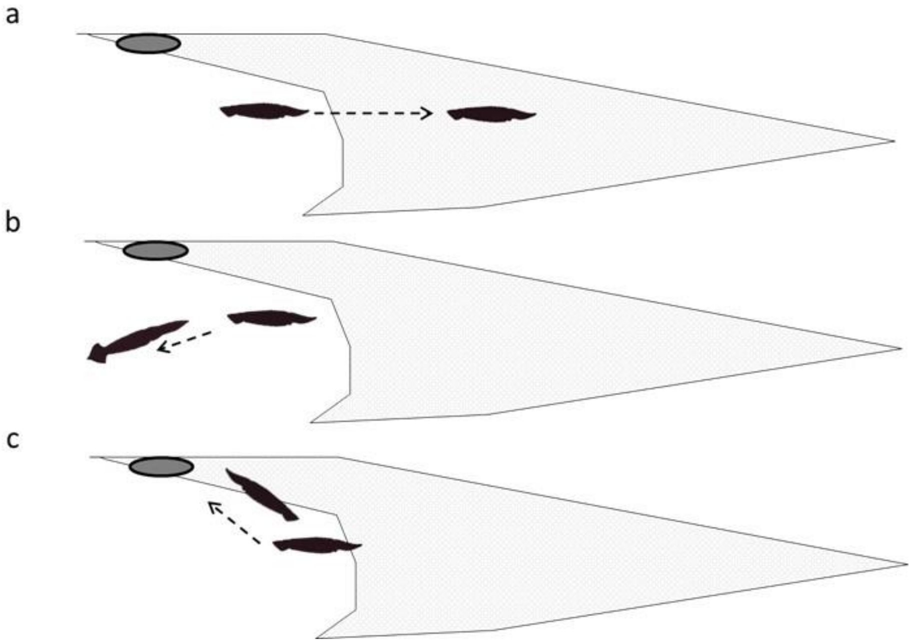

Figure 7. Types of reactions displayed by the Argentine shortfin squid (*Illex argentinus*) to trawl net operation off Southern Brazil: a) deceleration and displacement towards the net tunnel and code-end, b) shifting swimming direction for lateral escapement, and c) reversal of swimming direction and upward escapement movement.

Observed squids were evenly distributed between the camera and the seafloor but concentrated on the center and left sectors of the field of view of the camera (right sector of the trawl mouth). Jet-propelled swimming ahead of the trawl mouth was sustained for 10-20 s, tail-first and with regularly spaced fin flaps. Most individuals exhibited escape reactions, shifting the swimming direction towards the lateral or top panels of the trawl.

Similar behavior patterns were reported for the loliginid *Doryteuthis pealeii* in reaction to trawl nets in the NE Atlantic (Glass *et al*., 1999); *i.e*., patchy schools of squid swam up to “three min” tail-first in front of the trawl mouth (~3 knots) and, upon tiring, raised to the top panels and turned around, pointing the tail at the net mouth. Authors also observed that, at this point, most squid would cease swimming and were overtaken by the trawl. However, Bayse *et al*. (2016a) also for *D. pealeii* in the same area, reported squid swimming mostly individually and often ‘drifting’ motionless until overtaken by the trawl. These contrasting results obtained for the same species (Glass *et al*., 1999; Blayse *et al*., 2016a) could not be fully explained, but in the case of *Illex* the observed behavior (*i.e*., continuous swimming/escape reactions) was somewhat expected since, unlike loliginid squids, ommastrephids are known to be fast-swimming strong muscular oceanic squids, that include long migrations in their life-history.

Illex horizontal distribution on the trawl mouth, unbalanced towards one side, was equally reported for *D. pealeii* (Blayse *et al*., 2016a) and explained by a) an artifact produced by the camera orientation, b) tow direction relative to tide flow (where cross-current towing could produce such lateral concentration), and c) a turn on the vessel steaming direction. In the present study, an additional explanation could include an illumination effect, because the trawl system employed in this study had a single source of light placed on one side of the camera lens, which coincided with the side where squid was scarcely recorded. The light attraction has been frequently used in different squid fishing methods (Boyle & Rodhouse, 2005). In fishing operations, squids have been observed to concentrate in the nearby shadows observing the illuminated areas for any moving prey (*e.g*., jigs) (Martins & Perez, 2006). It is possible that *Illex* tends to avoid the bright area ahead of the trawl mouth, swimming in the less illuminated area. This hypothesis requires further investigation but could imply in the potential use of artificial illumination to direct squid either towards the net mouth or away from it (as part of an escape device).

Illex was shown to sustain a 1.24 m s^{-1} speed ahead of the trawl mouth, which is faster than the upper limits of the ‘cruise’ speed (~0.9 m s^{-1}) and much faster than average cruise speeds (~0.3 m s^{-1}) as measured for ommastrephids in nature by tracking devices (O’Dor, 2002). Because squid cruise swimming speeds are potentially kept for hours, *I. argentinus* may exhibit such high speeds as an emergency escape reaction, and therefore sustains it only for a short period. Swimming activity combined jet-propulsion with the regular flapping of the triangular tail fin. In cephalopods, jet-propulsion is based on continuous water inhaling and exhaling movements of the mantle, the latter generating thrust by displacing water through the funnel. Fin flaps may produce additional thrust, when coinciding with the exhaling movement or produce compensatory thrust when coinciding with the inhaling ‘recovery’ movement (Hoar *et al*., 1994; Bartol *et al*., 2008). Flaps occurred every 2-4 s, on average, but were more frequent during short encounters with the trawl, probably accelerating as an emergency reaction. It was not possible, however, from video observations, to assess if flaps coincided with inhaling or exhaling mantle movements.

Finally, *Illex* exhibited few chromatic variations during encounters with the trawl gear. However, dark patterns were more common than lighter ones, as usually exhibited by cephalopods under stress. Also, localized unpigmented areas on the dorsal mantle, head and arms were frequently, seen some of them outlining internal regions where female ovary and oviducts are located, as often displayed by different squid species during spawning seasons (Hanlon *et al*., 1999). Visualized squid could not have the sex determined, but Perez *et al*. (2009b) have shown that winter migrating schools, exploited by the slope trawl fishery off southern Brazil, were dominated by large females, mostly in spawning or spent maturation stages. Therefore it is possible that many of the visualized squid swimming ahead of the trawl were, in fact, large spawning females displaying a corresponding color pattern.

Unlike recent studies involving trawling of coastal loliginid squid *D. pealeii* (Bayse *et al*., 2016a, 2016b), the present study did not allow a quantitative assessment of escaped *vs* retained squid, partly due to an uneven visualization of squid behavior along the water column, more clearly discernible higher in the trawl mouth and closer to the video camera. In that sense, it was not possible to assess if squid swimming near the seafloor (over the ground rope) would be able to escape or to determine whether reacting squid escaped through the sides of the trawl mouth and over the headline or ended up entering the trawl. Video records suggested that most *D. pealeii* enter the trawl very high in the trawl mouth (Glass *et al*., 1999). When fished with a 'drop-chain' trawl, the species was shown to be caught high over the seafloor, never escaping underneath the trawl (Bayse *et al*., 2016b). The present results provided evidence that confirms that *I. argentinus* often swims high in trawl mouth, but a more precise characterization of squid behavior near the bottom, including patterns of escapement/ retention, should require different placement of the camera and illumination in the trawl gear. As concluded for *D. pealeii*, however, it seems that escaping devices (*e.g*., grids) on the top panels of the trawl net could equally allow escape of *I. argentinus* during trawl operations (Bayse *et al*., 2014). Occasional views of the top panels of the trawl allowed the distinction of squids entangled in the netting.

Underwater cameras have long provided unique opportunities to assess vulnerability and selectivity of marine megafauna's components to fishing operations. Results have helped to design and implement gear modifications in the direction of enhancing catchability of targeted species and/or decreasing catches of unwanted ones (Main & Sangster, 1981, 1983; Walsh & Hickey, 1993; Queirolo *et al*., 2010, 2012; Nguyen *et al*., 2014; Bayse *et al*., 2016a, 2016b and others). In Brazil, such studies are rare or on non-existent, which makes this analysis preliminary in nature. Obtained results were compared with more robust experimental studies conducted with coastal squids (Glass *et al*., 1999; Bayse *et al*., 2016a, 2016b) allowing for some inferences to be made about ommastrephid squid behavior when exposed to trawl fishing, as well as important caveats and potentialities of this study method to assess behavior responses of squids and other organisms vulnerable to the Brazilian trawl fishing in the future.

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