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ORIGINAL ARTICLE

Proposal of a non-lethal visual census method to estimate freshwater stingray abundance

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Abstract

There is not a current method to estimate South American freshwater stingray's abundance. Therefore we designed a census method and tested it in the field. It consists of making nocturnal surveys by boat in large rivers (>25m width) and floodplain lagoons of varying sizes, along transects of 100m x 10m (1000 m^2). We applied this method in the Tomo River, a tributary of the Orinoco River in Colombia. 110 transects were surveyed in 200 runs. A total of 149 rays of four species were recorded: *Potamotrygon motoro*, *Potamotrygon orbignyi*, *Potamotrygon schroederi* and *Paratrygon aiereba*. *P. motoro* was the most abundant with a density of 0.31 individuals /1000 m^2 (SD=0.5). This method gave optimal results when applied to habitats with high transparency and shallow depth (< 1m) since it permitted the identification of both adults and juveniles present.

Keywords: density; Potamotrygonidae; Orinoco river basin; Colombia.

Introduction

The family Potamotrygonidae is a complex taxonomic group of four genera: one monotypic genus (*Paratrygon* Dumeril 1865), two genera with two species respectively (*Plesiotrygon* Rosa, Castello and Thorson 1987 and *Heliotrygon* Carvalho and Lovejoy 2011), and one multispecies genus (*Potamotrygon* Garman 1877) that currently has roughly 20 nominal species (Carvalho et al. 2003, 2011, Fontenelle et al. 2014, Lasso et al. 2013a, Loboda & Carvalho 2013, Rosa 1985, Rosa et al. 2010, Silva & Carvalho 2011a-b). In Colombia, Peru, and Brazil; the family is of great importance as an ornamental fishery resource. This resource is in great demand in the international markets, and provides an important economic input to local communities. Due to the biological features of these fish (e.g. low fecundity, slow growth, partial parental care, late maturation, and annual or inter-annual reproduction), they are extremely vulnerable to human impacts and overharvesting (Lasso et al. 2013a). Thus, four of the eleven species of freshwater stingrays found in Colombia are classified as vulnerable: *Paratrygon aiereba*, *Potamotrygon motoro*, *Potamotrygon schroederi* and *Potamotrygon yepezi* (Mojica et al. 2012). The first three are found in the Amazon and Orinoco River Basin respectively, and the fourth is endemic to the Lake Maracaibo Basin (Lasso et al. 2013a).

There are five species of importance in the Orinoco River Basin: the Motoro Ray (*Potamotrygon motoro*), the Common/Tiger Ray (*Potamotrygon orbignyi*), the Macaw Ray (*Potamotrygon schroederi*), the Manta River Ray (*Paratrygon aiereba*) and the Diamond Ray (*Potamotrygon scobina*) (Lasso et al. 2013a). These species are subjected to intense fishing by ornamental collectors throughout the basin. The fishery authorities annually establish an export quota for each species at a national level to regulate fishing. A difficulty confronted by the fishery authorities is the lack of information about adequate methods to estimate stingray abundance in the exploited areas. Therefore, it is fundamental to obtain baseline information about minimum population estimates to adequately inform authorities (i.e. who set the annual quotas). Methods to census fish by direct or subaquatic observation are widely used throughout the world in fresh waters (e.g. Helfman 1992, O´Neal 2007), in Neotropical freshwater ecosystems (e.g. Cassatti & Castro 1998, Lasso et al. 2002, Nakamura et al. 2004, Sabino 1999, Sazima 1986, Teresa et al. 2011), and especially for the study of freshwater stingray behavior (Garrone Neto 2013, Garrone Neto & Sazima 2009a-b, Garrone Neto & Uieda 2009, 2012, Silva & Uieda 2007), but no method exists to permit abundance estimation of these freshwater stingrays. With this goal, we designed and field tested one method to estimate stingray abundance. It was tested in the lower stretch of the Tomo River (Orinoco River basin, Colombia) during the dry season of 2014, with the objective to validate its utility and potential use in other rivers where environmental conditions are similar. Thusly, the objective of this study is to propose and describe a non-lethal visual method to estimate freshwater stingray abundance in clear waters.

Materials and methods

Study area

The Tomo River is located in the southeast region of Colombia, in Vichada Department. It originates in the Carimagua Lagoon located in Meta; flows from west to east through Vichada; and empties into the Orinoco River in the Tuparro National Natural Park (IGAC 1999) (**Figure 1a**). It has an approximate length of 606 km and drains the high plains that discharge clear waters in the sense of Sioli (1975). It receives several clear water tributary streams and floodplain lagoons. The physicochemical characteristics vary among aquatic habitats. The floodplain lagoon has alkaline waters with a pH of 8.7, a conductivity of 83 μS, and a low measurement dissolved solids of 81 ppm; contrasting with the waters coming from the Tomo River that are acidic in pH (6.6), very low in conductivity (3 μS) and dissolved solids (1 ppm). The rainfall regime is unimodal with a dry season from December to March, and rains from April to November.

Sampling

This pilot project was carried out between February 23rd and March 2nd of 2014 during the low water period. The method was tested with nocturnal runs; using a boat in large rivers (>25m width) and in floodplain lagoons (variable area). 110 transects were delimited during 200 runs (**Table 1**). During this time, the following three minimum conditions were met to allow the application of the census method: 1) the emergence of beaches in large rivers; 2) greater water transparency; and 3) lower water

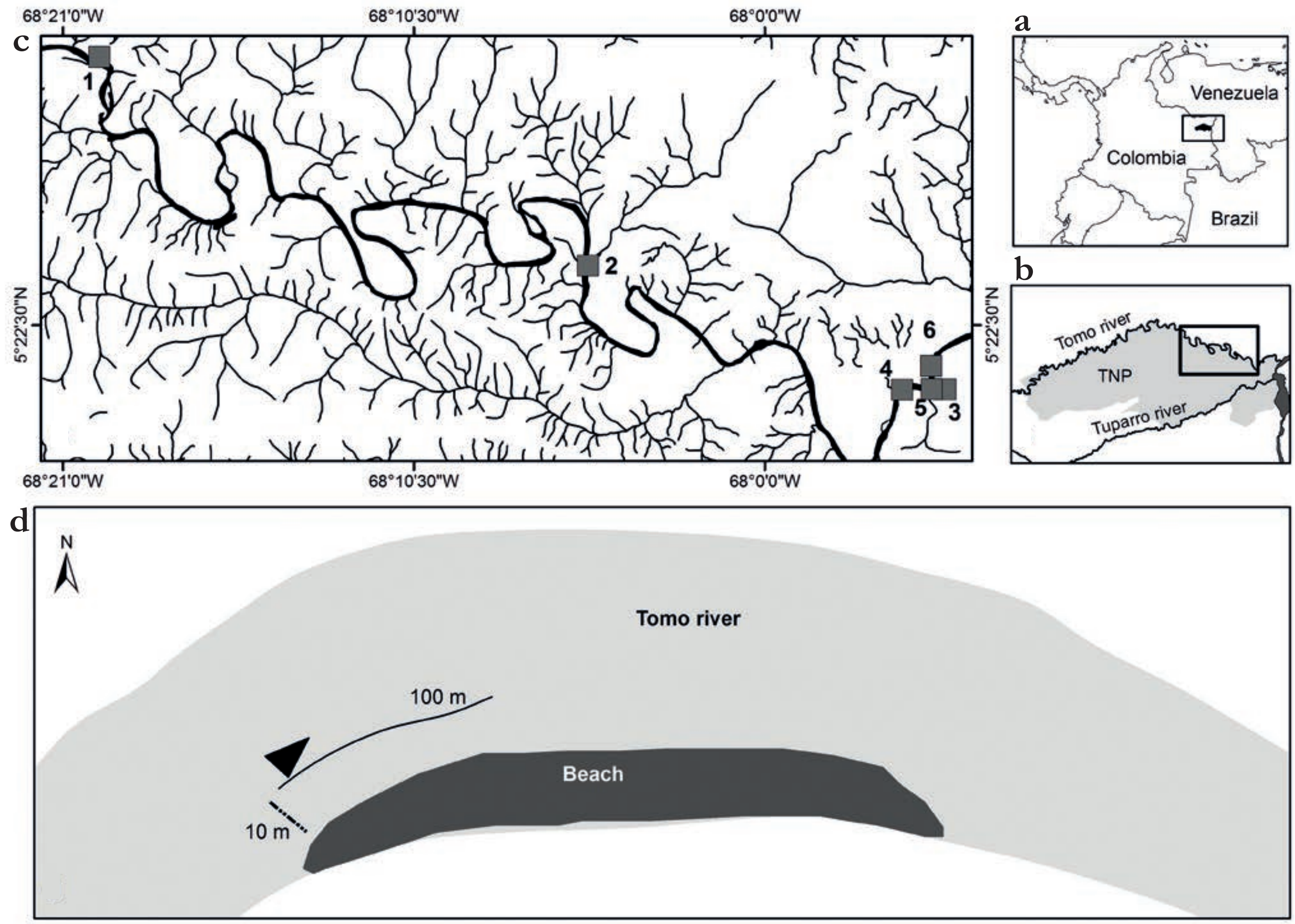

Fig. 1. Study area and transect model location. **a)** Location of Tuparro National Park (TNP); **b)** detail of sampling area; **c)** sampling stations: 1) floodplain lake I, 2) floodplain lake II, 3) floodplain lake III, 4) large river (beach I), 5) large river (beach II), 6) large river (beach III); and **d)** location of transect model in river channel.

levels yielding shallower water depths and less extensive flooded areas. The censuses were done in the lower section of the Tomo River (**Figure 1b**) where the following aquatic environments were evaluated: 1) large river (3 sites), and 2) floodplain lagoons (3 sites) (**Figure 1c**). Species were identified and the development stages were based

Table 1. Summary of sampling effort by habitat.

Site	Number of transects (100m x 10 m)	Number of runs per transect	Total runs/site
Floodplain lake I	20	2	40
Floodplain lake II	20	2	40
Floodplain lake III	30	2	60
Large river (beach I)	10	1	10
Large river (beach II)	20	2	40
Large river (beach III)	10	1	10
Total	**110**	**10**	**200**

on the work of Charvet-Almeida et al. (2005), Lasso et al. (2013a, b, c, d, e), Loboda & Carvalho (2013), Rosa (1985), and Silva & Carvalho (2015).

A detailed description of this proposed census method is given in the Results section.

Data analysis

For our proposed method, the density was calculated for each species, as well as their respective standard deviations (SD) and percent coefficients of variance (CV).

$$\text{Density} = \text{number of individual}/\ 1000\ m^2 \qquad \textbf{(1)}$$

Results

Description of method

Three people are needed for this type of census: one observer, one recorder, and one boat operator. To begin the census, the driver must turn off the motor and proceed using a paddle or pole to avoid perturbation of the area. The boat should be, at maximum, 10 m from the shore (i.e. the maximum effective area of visibility of the observer). The observer is located at the bow with a field of observation from the boat to shore. Flashlights or headlamps are used to detect eye shine (red or yellow in stingrays) or the outline of the bodies of the stingrays sitting on the bottom. Upon observation of a stingray, the boat moves as close as necessary to permit species identification, size estimation (disc width), and maturity (adult or juvenile). Rays can be identified by the color pattern that is distinctive for each species. The recorder notes all the pertinent information at the initiation point of the run: time, date, geographic coordinates and site name and description; then records the time of each observation (meters travelled, coordinates, time, number of individuals, species, estimated size, and developmental state) and again at the end of the run the time, meters travelled and coordinates. Each transect was 100 m long * 10 m wide (1000 m^2) (**Figure 1d**).

Results of pilot test (census)

A total of 149 stingrays were recorded, of four species: *Potamotrygon motoro* (62 individuals), *Potamotrygon orbignyi* (57), *Paratrygon aiereba* (18) and *Potamotrygon schroederi* (12) (**Figure 2**). Both juveniles and adults were observed, with a predominance of juveniles in all species. Sympatric and syntopic distribution of four species of stingrays was recorded (all four species in one beach). In **Table 2**, the density of each species is shown, along with the standard deviation and coefficient of variance.

Potamotrygon motoro had the highest relative abundance values, followed by *P. orbignyi*, *Paratrygon aiereba* and *P. schroederi*. The high values and differences in SD and CV for *P. schroederi* and *P. aiereba* are probably due to spatial variation in the distribution of species since these two were only observed in the main channel of the river. The other two species, *P. motoro* and *P. orbignyi*, were observed both in rivers and lagoons, and had high density values and slightly lower coefficients of variance.

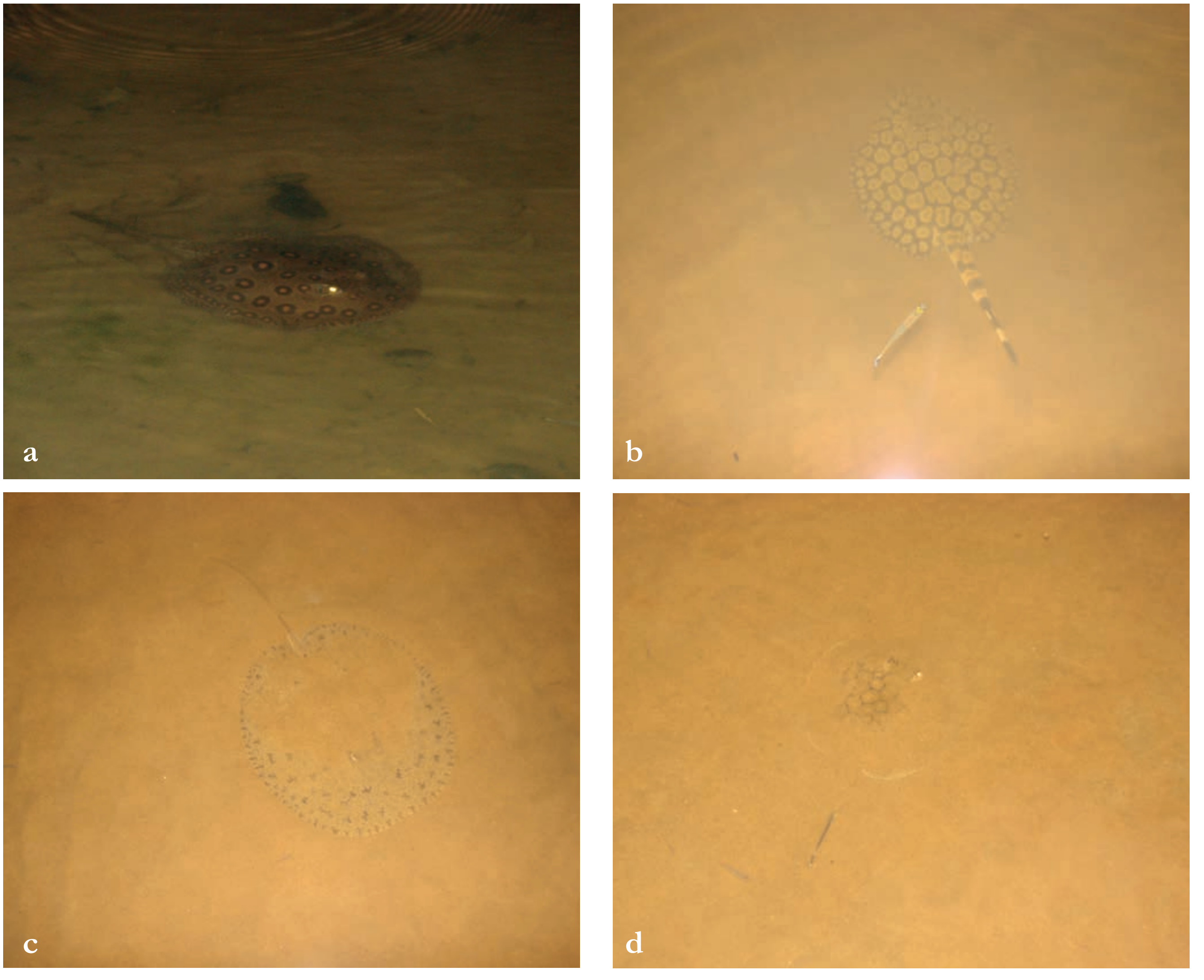

Fig. 2. Sightings of rays during the nocturnal sampling in the Tomo River: **a)** *Potamotrygon motoro*; **b)** *Potamotrygon schroederi*; **c)** *Paratrygon aiereba*; **d)** *Potamotrygon orbignyi*.

Discussion

This method is recommended for large rivers and floodplain lagoons with beaches where water depth during the dry season depth is not greater than 1 meter. This permits sampling along greater distances (longer transects) and different habitats in less time. The detection capability was good (using a maximum distance from shore

Table 2. Density (D) (ind./1000 m^2). SD: standard deviation. CV: coefficient of variance (SD/D*100). n= 149.

Species	n	D	SD	CV
Paratrygon aiereba	18	0.09	0.2	224.4
Potamotrygon motoro	62	0.31	0.5	161.3
Potamotrygon orbignyi	57	0.29	0.43	148.2
Total	**149**			

of 10 m). The information obtained by this method also allows better understanding of the habitat preference, size, and behavior of the rays observed. It is also a safe and comfortable method for the researchers doing the work. For these nocturnal surveys, the lunar cycle should be taken into account, since it will affect visibility and the ability of the observer to detect the eye shine of the rays. The observer's effectiveness diminishes based on the environment being illuminated by the moon. The lunar cycle may also affect the behavior of the rays. As would be expected, proper depth is crucial for best observation. A maximum of 1 m water depth was found optimal under field conditions encountered during this pilot test. Turbidity and wind (affecting the surface of the water) can affect the ability of observation, as well as the behavior of the stingrays; there should be caution taken to minimize their impact on the census.

We found that this method permits data collection, not only on the abundance of the species, but also generates information about the population structure if the development stage can be observed (adult vs. juvenile). The advantage of nocturnal observation is that it permits the observer to see and quantify the number of neonates and juveniles, that during the day, stay in the deeper parts of their habitat, and adults that migrate at night towards the beaches on shore from the deeper waters of the main channel. This has been shown for *P. motoro* and *P. falkneri* in the upper Paraná River basin by Garrone Neto & Uieda (2012).

This method requires only a basic taxonomic knowledge of the species identified when their eyes reflect light back from spotlights used to observe them and can easily be replicated by other researchers in rivers and lagoons with the physical conditions described above. We were able to observe and differentiate neonates, juveniles, and adults. The presence of young may indicate that these populations are in good condition. It is important to mention that the ornamental fishery is selective towards neonates and juveniles, and undertaken only during low water phase; the same time of year we carried out our method trials. Theoretically, little or no ornamental fishing pressure is present in this stretch of the river, since neonates and juveniles are the most sought after specimens (Lasso et al. 2013a). This method also permits observations of behavior and activity rhythms, and complements other methods based on underwater observations (Garrone Neto 2013, Garrone Neto & Sazima 2009a-b, Garrone Neto & Uieda 2009, 2012, Silva & Uieda 2007).

Our relative abundance values can not be classified as high or low since there is no comparative values available in literature and we have few replicates. This is the first data published, and as they were obtained in a protected area with no ornamental fishing, they do provide a baseline for comparison with areas where that activity does occur.

Conclusions

The method was designed to estimate freshwater stingray's abundance and field testing proved to be feasible, easily applied, and economical. Furthermore, it does not require significant technical preparation, an important factor since the objective is to provide the fishery or environmental governmental authorities with the means to implement the census using technicians with only basic training. This method was useful both on beaches and shorelines of the main river channel and floodplain lagoons; both areas where ornamental fishers catch stingrays. Successful use will depend upon the physical conditions of the areas sampled (water transparency, depth, etc.). We recommend using the method only at night because, during the day, the results may be different if we consider the activity patterns of the species. Finally, it is important to remember that the objective of this study was not to obtain estimations of the abundance of the stingrays present in the study area, but to propose and test a census method to determine its potential usefulness in other similar aquatic ecosystems. This is the first time that this method has been tested and additional testing needs to continue in the future in order to assess its validity.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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Propuesta metodológica no letal para la estimación visual de abundancia de rayas de agua dulce

Resumen. No existe actualmente un método para estimar la abundancia de las rayas suramericanas de agua dulce. Por ello se diseño un método para censarlas y se probó en campo. Consiste en hacer recorridos nocturnos en bote por ríos grandes (>25 m de ancho) y lagunas inundables de diversos tamaños, a lo largo de transectos de 100 m x 10 m (1000 m^2). Con este método se muestreó el río Tomo, un tributario del río Orinoco en Colombia. Se muestrearon 100 transectos en 200 recorridos. Se registró un total de 149 rayas pertenecientes a cuatro especies: *Potamotrygon motoro*, *Potamotrygon orbignyi*, *Potamotrygon schroederi* and *Paratrygon aiereba*. *P. motoro* fue la más abundante, con una densidad de 0.31 individuos/1000 m^2 (DE=5.06). Este método dio óptimos resultados cuando se aplicó a hábitats con alta transparencia y poca profundidad (<1m), ya que así es posible identificar tanto a los adultos como a los juveniles presentes.

Palabras clave: densidad; Potamotrygonidae; cuenca del Orinoco; Colombia.

Proposta de um método de censo visual não letal para estimar de abundancia de raias de agua doce

Resumo. Não há um método atual para estimar a abundancia de raias de água doce Sul-Americanas, assim que desenhamos um método de censo e o testamos em campo no rio Tomo, um afluente do rio Orinoco, em Colômbia. Este método consiste na realização de levantamentos noturnos por barco em grandes rios (> 25m de margem) e lagoas de várzea de tamanhos diversos, usando um transecto de 100m x 10m (1000 m^2). Em um total de 200 percursos, 110 transectos foram pesquisados. Um total de 149 raias de quatro espécies foram registradas: *Potamotrygon motoro*, *Potamotrygon orbignyi*, *Potamotrygon schroederi* e *Paratrygon aiereba*. *P. motoro* foi a mais abundante, com uma densidade de 0,31 indivíduos/1000 m^2 e um desvio padrão (DP) de 5,06. Este método forneceu os melhores resultados quando aplicado a habitats com alta transparência e baixa profundidade (< 1m) uma vez que permite a identificação de espécies tanto adultas quanto jovens.

Palavras-chave: densidade; Potamotrygonidae; bacia do Rio Orinoco; Colômbia.

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