Habitat distinctness doesn't influence on the abundance and relative growth of the juveniles *Atherinella brasiliensis* (Quoy & Gaimard, 1824) in a subtropical estuarine ecosystem

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ABSTRACT

Shallow coastal habitats with and without vegetation may perform nursery function from distinct way for many species, raising questions about the role of vegetation on structuring nursery ground. Unfortunately, degradation and inappropriate human use of these areas associated with the lack of ecological data about fish populations can limit appropriate strategies to protect both species and habitats. In this paper we examine the nursery value of different estuarine habitats by comparing the abundance and relative growth of juveniles *Atherinella brasiliensis*. Fish were sampled once a month from November 2018 to October 2019 in five habitats: no vegetation close to the ocean, no vegetation close to the river, no vegetation and distant from other sampling points (control), mangrove and saltmarsh. Juveniles are present and abundant in all habitats and specimens had similar relative growth in the salt marsh, mangrove habitats and non vegetated habitats. The results indicate that estuarine habitats offers suitable conditions to *A. brasiliensis* in early stages of life, indicating that these sites have nursery value for juveniles of this species. Conservation and/or preservation of estuarine shallows habitats along the coast is important for *A. brasiliensis* and probably for other species, and the identification of priority areas could help to provide subsidies to management policies on conservation of coastal marine environments.

1. Introduction

Coastal ecosystems (CE herein, e.g., estuaries and coastal lagoons) provide services for human well-being, such as the protection of the coastal environment, nutrient cycling (Barbier, 2019), recreation (McLusky and Elliott, 2004) and fisheries production (Blaber et al., 2000; Constanza et al., 1997; Jordan and Peterson, 2012). CE are comprised by mosaic of habitats (Able, 2005; Nagelkerken et al., 2015), generating a high spatial heterogeneity in seascape. For example, in intertidal zones, CE is usually composed of shallow habitats (< 5 m;

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Blaber et al., 1995; Nagelkerken, 2009), functioning as nursery sites for vertebrates and invertebrate's species (Beck et al., 2001; Lefcheck et al., 2019).

The shallow estuarine habitats may have vegetation in their intertidal plains, generating physical conditions and environmental constraints that shape local fish assemblages (Bloomfield and Gillanders, 2005). For instance, mangroves and salt marshes (Dorenbosch et al., 2005; Kimirei et al., 2011; Minello et al., 2003) provides sites for food, growth, and shelter for several marine nekton species (Beck et al., 2001; Heck et al., 2003; Kimirei et al., 2013). Also, non-vegetated shallow habitats may also act as a nursery for marine species (Nagelkerken et al., 2015). Therefore, it is broadly recognized that shallows estuarine habitats are imperative for maintaining marine populations and, consequently, the world's fishing activity (Baker et al., 2020).

Nursery habitats provide, on average, greater growth, density, survival and/or movement than other habitats where fish juveniles occur (Beck et al., 2001). Therefore, fish population parameters may be essential indicators for determining whether a habitat acts as a nursery. For example, the relative abundance (Beck et al., 2001; Bloomfield and Gillanders, 2005) and the body condition extracted from relative growth rate of organisms (e.g. weight-length relationship; see Lowe and Peterson, 2015) can be used to highlight the most suitable habitats for organisms occurrence (Lowe and Peterson, 2015; Al Nahdi et al., 2016; Ma et al., 2017).

Understanding the nursery function of habitats has great scientifical value, both for theoretical ecology (Beck et al., 2001; Able, 2005) and conservation strategies (Sheaves et al., 2015). Heterogenous estuarine ecosystems where mangroves, salt marshes and non-vegetated habitats co-occurs are suitable for testing hypotheses in scientific studies (McKee and Rooth, 2008). This is the case of the Laguna Estuarine System (LES) that support salt marshes and mangroves habitats (Soares et al., 2012; Schaeffer-Novelli, 1995), both encompassed in the territory of a the Right Whale Environmental Protection Area (APA-BF), a wide protected area that comprises along 130 km of Santa Catarina state coast (De Freitas et al., 2022) and covers 154,867 ha (120,718 marine and 34,149 terrestrial).

Besides habitat structure, water quality shapes species distribution in estuarine ecosystems (McLusky and Elliott, 2004), influencing fish abundance and body condition (Blackwell et al., 2000; Cavraro et al., 2019; Lugendo et al., 2017; Molina et al., 2020; Duque et al., 2020). Among the variables, the role of salinity on the abundance of fish stands out (Barletta et al., 2005), which respond by moving within the estuary (Sheaves et al., 1999; Barletta et al., 2003), generating specific assemblage composition along ecocline variation (Barletta and Lima, 2019). Thus, is essential to relate the fish patterns in the estuarine ecosystems with both habitat and water quality spatial constraints.

In this study we analyzed the abundance and relative growth of the Brazilian silverside *Atherinella brasiliensis* (Quoy & Gaimard, 1824) in saltmarsh, mangrove, and non-vegetated habitats in the Laguna Estuarine System (LES). The *A. brasiliensis* is a resident estuarine species (Sczepanski et al., 2007; Contente et al., 2010) abundant in shallow water environments in southern and southeastern Brazil (Pessanha and Araújo, 2001). Although not of high commercial value (Bervian and Fontoura, 2007), *A. brasiliensis* can have local fishery importance as a food supplement for fishing communities as well as bait for fishing activities. In the LES *A. brasiliensis* is a conspicuous species, being also an important resource to distinct artisanal fisheries gears (Wahrlich et al., 2023).

Due the ecological aspects and fishery importance of *A. brasiliensis* and the presence of different estuarine habitats in the LES, we aimed to evaluate how estuarine habitat types influences relative growth and the abundance of juvenile *A. brasiliensis*. Specifically, we aimed to assessed if (1) vegetated habitats provide better nursery habitats for juveniles of *A. brasiliensis*; and (2) *A. brasiliensis* juveniles are more abundant in vegetated habitats and has better relative growth than non-vegetated habitats.

2. Materials and methods

2.1. Study area

The Lagoon Complex South of Santa Catarina comprises the largest lagoon formation in Santa Catarina state, Brazil (Giannini, 1993; Giannini et al., 2010), in which the LES (Fig. 1) is inserted. The LES is considered a typical strangulated lagoon (Kjerfve, 1994) formed by different cells, named Imaruí, Mirim and Santo Antônio dos Anjos (LSA), being the last one where the present study was carried out. The LSA receives direct input from the Tubarão River, the main contributor to LES (D'Aquino et al., 2011). The bar channel is permanently open, where the exchange of water masses with the ocean takes place. On its southern margin of LES there is the co-occurrences of mangroves and salt marshes in its intertidal plains.

2.2. Estuarine habitats

In the LSA, we selected five sampling areas to assess the influence of vegetation (presence x absence) associated with different types of habitats, as well as to analyze the role of water quality on the juvenile population of *A. brasiliensis*. Mangroves and salt marshes are located within the Right Whale Environmental Protection Area (APA-BF) limits. This Conservation Unit has a large territory (154,381 ha) on the south coast of Brazil, being its main objectives to conserve biodiversity and promote more sustainable practices in using natural resources.

The sampling habitats have the follows aspects: (i) habitat without vegetation near ocean (NV_Oce): small beach without vegetation, limited on its sides by a cluster of rocks and with the direct influence of the tides, due to its proximity to the ocean; (ii) habitat without vegetation located near the Tubarão River (NV_Riv): site without vegetation and strongly influenced by freshwater discharge; (iii) habitat with salt marsh vegetation (Veg_Sal): site with intertidal vegetation composed predominantly of *Spartina alterniflora* and *S. densiflora* (Fonseca and Netto, 2006); (iv) habitat with mangrove vegetation (Veg_Man): area with small mangrove forest, formed by species of white mangrove *(Laguncularia racemosa)*, black mangrove *(Avicennia schaueriana)* (Netto, 2018); (v) control area without vegetation (NV_Con): non-vegetated habitat, located far from the Tubarão river and the ocean. We considered this our control site due to the absence of intertidal vegetation and distance from our study site and ocean (Fig. 1).

2.3. Sampling

Three trawls of 220 m^2 were carried out for each habitat type (n=5), from twelve months (November 2018 to October 2019), totaling 180 trawls. We used a picket net with a bagger (210/206 multifilament mesh with 0.5 cm internodes), with an opening of 6 m. We pulled the nets using cables (23 m of length and 1 m of height). Before carrying out the trawls, temperature (°C) and salinity (PSU) were measured using a Hanna InstrumentsR Multiparameter Probe (HI9829).

We kept collected individuals in coolers with ice until brought to the Laboratory of Applied Ecology and Conservation of the State University of Santa Catarina (UDESC). We weighed (Wt) and measured (Lt) all individuals in the laboratory to calculate their body condition (see more below). We stored them in a freezer below 0°C and identified *A. brasiliensis* following the protocol described by Figueiredo and Menezes (1978).

2.4. Data analysis

Considering that water quality variables, as salinity and temperature, and habitat structure may shape fish abundance and their relative growth (McLusky and Elliott, 2004; Barletta et al., 2005) and that *A. brasiliensis* populations is influenced by these abiotic variables in shallow estuarine habitats (Fávaro et al., 2007), we adopted the follow
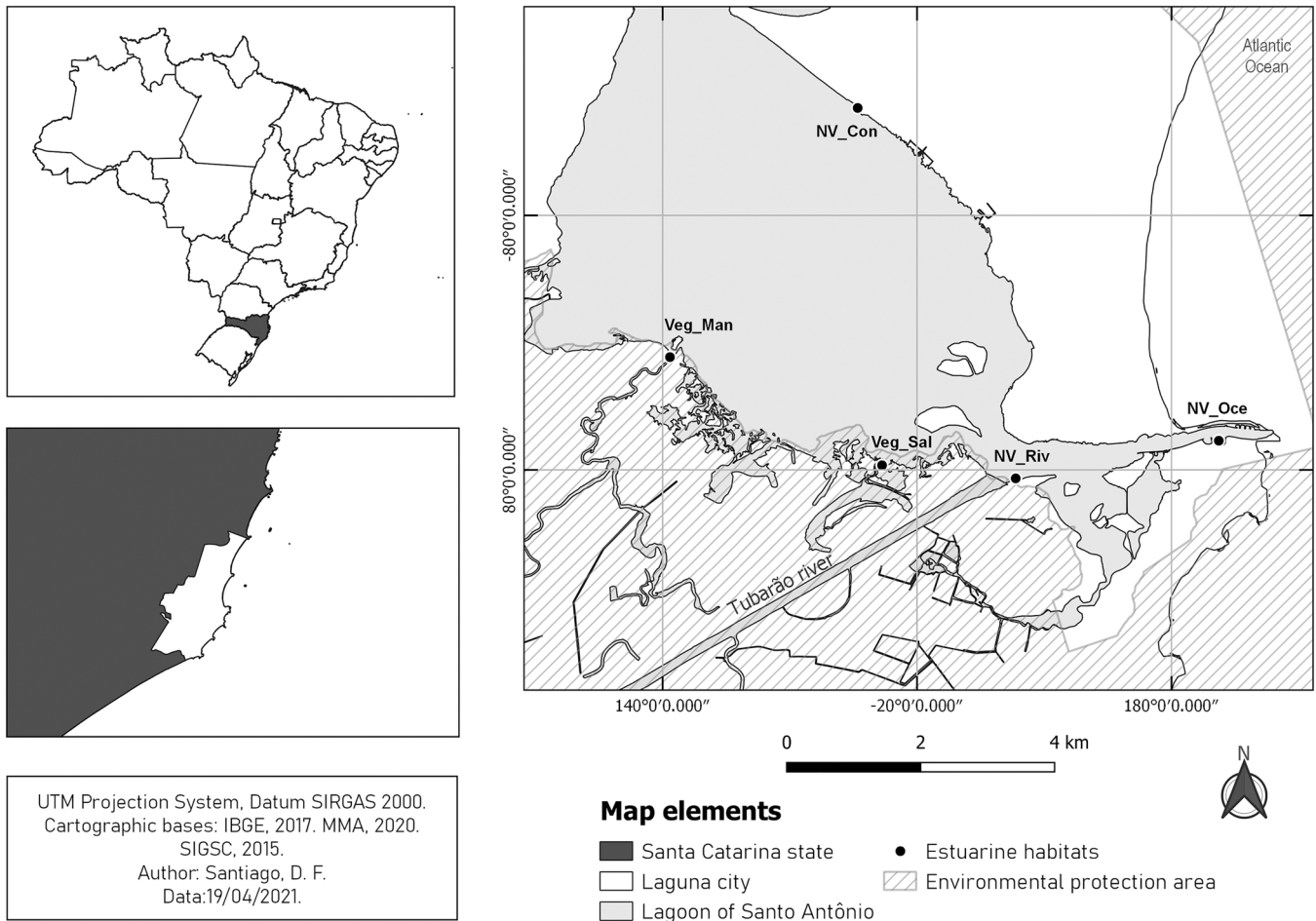
Fig. 1. Study site map in the municipality of Laguna, Santa Catarina, southern Brazil. Black dots reffer to the data collection sites in Santo Antônio dos Anjos Lagoon, being as follows: NV_Oce (non-vegetated area near the ocean), NV_Riv (non-vegetated area near Tubarão River), Veg_Sal (vegetated area with saltmarsh-like species), Veg_Man (vegetated area with mangroove-like species) and NV_Con (non-vegetated area - control site).

approach to avoid biasing analysis between both mentioned factors.

We tested the correlation between water quality variables from non-parametric Spearman's correlation coefficient (Zar, 2010). As the variable were correlated (p < 0.05), we used only salinity to test differences among habitats. Then, we checked for normality and variance homogeneity in salinity using Shapiro-Wilk and Bartlett test (Zar, 2010), respectively. Since the dataset presented non-normal distribution and heterogeneity of variances and to avoid pseudo replication regarding sampling design (Heffner et al., 1996), we used a generalized linear mixed model (GLMM) to investigate the if the salinity is equal habitats. The GLMM had identity link function (Zuur et al., 2009) and area as fix term and sampling trawl as random term in the equation. On detection of significance of habitats on salinity by GLMM analysis, the pairwise comparisons between them habitats) were performed using Tuckey-Kramer test with Bonferroni correction in the p-values. For all tests, a significance level of 5 % (p < 0.05) was adopted. This abiotic analyze allowed us to define which habitats had similarity in water quality, allowing used only them to compare abundance and relative growth restrictedly with spatial component. All statistical analysis were performed in the software R version 4.0.3 (R R Core Team, 2020). The graphical outputs were prepared with packages *ggplot* (Wickham, 2016). GLMM Modes were fitted using the 'glmmTMB' package (Magnusson et al., 2017). The pairwise comparison was performed with multicomp package (Hothorn et al., 2008)

To understand the juvenile relative growth patterns relationship (i.e weight *vs.* length) and abundance among habitats we performed several statistical analyses. For the weight *vs.* length relationships analysis, we

checked for outliers in weight and length data using the Interquartile Range (IQR) method using the *influencePlot* function of the *car* packages (Fox and Weisberg, 2019). Also, based on the scale's accuracy capability (0.03 g) and the low precision to distinguish the weight of small fish over a wide range of lengths (Ogle, 2013) we log transformed weight and length data to perform a lower cut-off analysis (Supplementary Material – Figs. 1 and 2).

After exclusion of outliers and lower values, we fitted a weight-length (W-L) relationship power equation: $Wt = aLt^b$ (Huxley, 1924), where Wt is the weight total (g), Lt is the length total (cm). We obtained linearization in W-R relationship applying the ln (natural logarithm), allowing to estimate a and b curve's coefficient from least square method: $\ln(Wt) = \ln(a) + bxln(Lt)$.

We adjust a global W-L relationship following alternative models (Zuur et al., 2009): simple linear model (LM1); generalized least squares models (GLS1); GLS with different variance link functions, namely: fixed (GLS2), identity (GLS3), power (GLS4), power constant (GLS5) and exponential (GLS6). We compared models using an ANOVA test (p<0.05), and once we detected statistical differences, the best-fitted model was selected using the lowest Corrected Akaike Information Criterion (AICc). The most constant power model (GLS5) showed the best fit (Supplementary Material – Table 1).

From GLS5 model we extracted normalized residuals and plotted then (y-axis) against a moving average with sequential interval readjustments of 0.5 cm in the total length (x-axis) using a *smooth.spline* function of the *stats* package (R R Core Team, 2020). From this graphical approach, we expected to identify possible trends in residuals
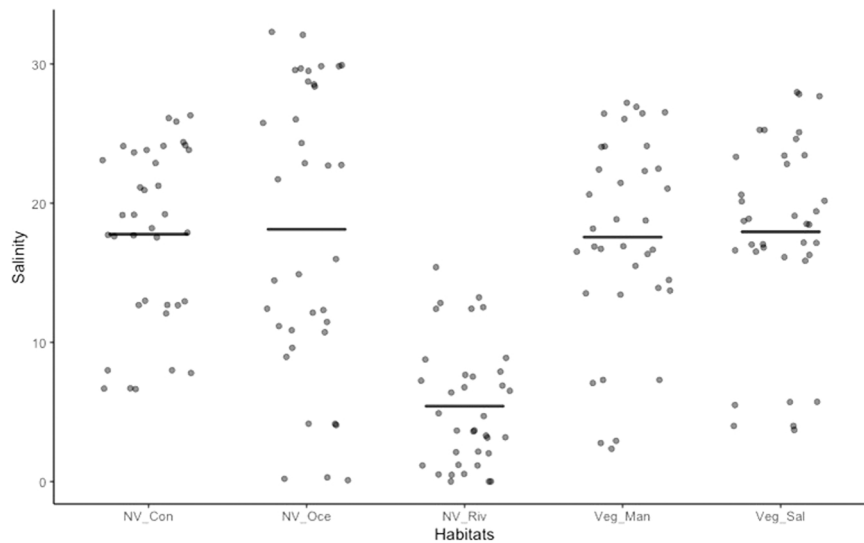
Fig. 2. GLMM`s predicted effects (bars are the medians) and observed values (points) of salinity on estuarine habitats.

Table 1

The comparison between parameter b (regression slope - body condition) among all estuarine habitats.

Estuarine habitats	b*	CI (2.5 %)	CI (97.5 %)
"NV_Oce"	2.97[1]	2.93	3.01
"Veg_Sal"	3.07[2]	2.96	3.17
"Veg_Man"	3.04[12]	2.90	3.17
"NV_Con"	3.00[12]	2.91	3.11

* CI, confidence interval. Estuarine habitats: NV_Oce (non-vegetated habitat near the ocean), Veg_Sal (vegetated saltmarsh), Veg_Man (vegetated mangrove) and NV_Con (non-vegetated control). * 1 and 2 indicate statistical groups from ANCOVA analysis.

distributions, since residuals should distribute randomly along the y-axis zero baseline if the weight/length transformed data follow the best model fitted (Fontoura et al., 2010). Based on this approach, we might observe a break in the residual pattern around 8.10 cm (Supplementary Material – Fig. 3), inferring at this point the length of the first maturity of the individuals (Bervian et al., 2006). Thus, individuals with Lt > 8.10 cm were removed to follow analysis. It worth mentioning that we only included juveniles in our analysis due to two main reasons: (a) the body condition does not remain constant throughout the lifespan of individuals (Lovett and Felder, 1989); and (b) the nursery concept only considers the juvenile phase (Beck et al., 2001). Therefore, we picked out juveniles to exclude the gonad weight effect on the regression slope (presence of adults).

Considering the potential variance heterogeneity in W-L relationship (Ogle, 2013), the selected GLS5 model with fixed variance structure ensured a proportional variance on this model (Zuur et al., 2009). We used ANCOVA (Zar, 2010) to check whether the W-L relationships are equal among habitats. On sequence, we estimated intercept marginal means by habitat and performed a pairwise comparisons. On presence of non statistical diferences between intercepts of the habitats, we followed with slopes marginal means comparison. Intercept and slope comparisons were performed using Tuckey-Kramer's test with Bonferroni`s adjust by *emmeans()* and emtrends() function, respectively, from package *emmeans* (Lenth, 2023).

We checked the juvenile population structure among habitats by a histogram, with a bin of length class of 1.0 cm. Since juvenile abundance is discrete variable, with a wide dispersion and a great presence of zero, we adjusted a negative binomial GLMM ($p < 0.05$) with the same structure aforementioned (i.e habitat as fix term and sampling trawl as random term) to compare abundance among habitats, followed, in the

detection of differences, by Tuckey-Kramer test (Zar, 2010) with Bonferroni correction in the p-values. We performed the juvenile model validation checking deviations between observed and expected residuals using the 'DHARMa' package (Hartig, 2018; see Supplementary Material – Fig. 4).

3. Results

3.1. Environmental patterns

The salinity means showed similar values in NV_Oce (18.13 ± 10.43), Veg_Sal (17.94 ± 6.95), NV_Con (17.76 ± 6.30) and Veg_Man (17.76 ± 7.15), however in the NV_Oce habitat there was a wider broad in salinity values during the study time. The lowest mean salinity was registered in NV_Riv (5.41±4.42). GLMM showed that habitat influence on salinity ($p < 0.05$; Fig. 2), being that NV_Riv differed from all other habitats (Tuckey-Kramer with Bonferroni`s correction: $p < 0.05$). Since this distinct water condition in NV_Riv we used this habitat to highlight patterns but not to compare relative growth and abundance between habitats.

3.2. Weight vs. length relationship

A total of 1774 individuals of *A. brasiliensis* were collected during the sampling years. Of these, 1109 were juveniles with total length between higher than highlighted in cut-off analysis (4.5 cm) and up to 8.1 cm.

ANCOVA's results highlighted influence of habitats on W-L relationships (Fig. 3). The higher intercept was registered in habitat Veg_Man (a = 0.0068), follow by NV_Oce (a = 0.0067). Both NV_Con (a = 0.0059) and Veg_Sal (a = 0.0056) habitats had the lowers values, presenting statistical differences with NV_Oce. Furthermore, Veg_Man presented statistical differences with Veg_Sal. Regarding the slope comparison between of the W-L relationships of the habitats with similar intercept, the habitat Veg_Oce (b = 3.00) had higher slope than NV_Veg (b = 2.99), while Veg_Sal (b = 3.08) had slope higher than NV_Con (b = 3.05, Fig. 3). When compared statistically (ANCOVA; $p > 0.05$), there were no significant differences in slope between habitats with equal intercept.

3.3. Juvenile population structure and abundance

The number of juveniles was higher in Nv_Con (n = 305), with size class of 5 cm as predominant (Fig. 4). The habitat Nv_Sal was the second
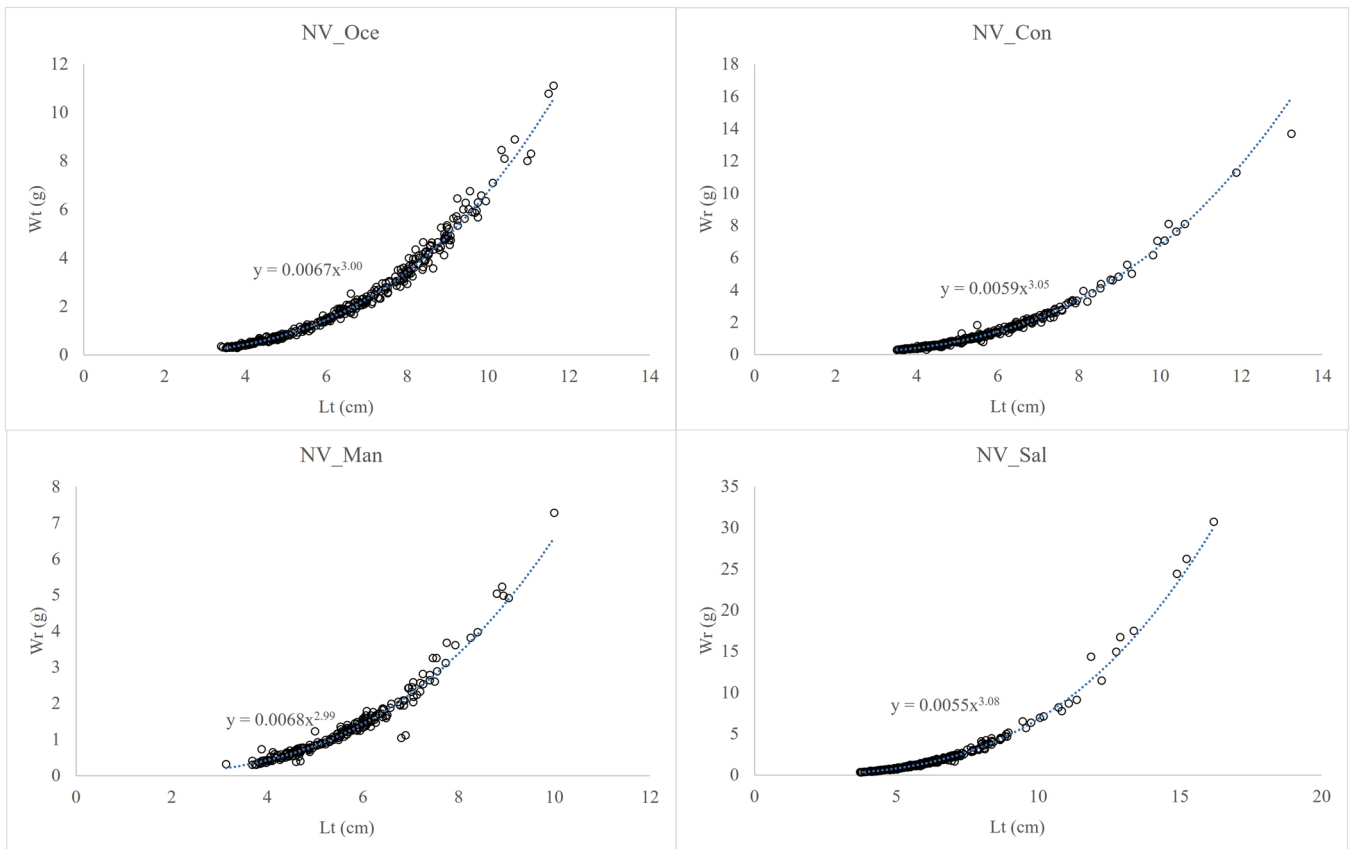
Fig. 3. Weight *vs.* length relationships for *A. brasiliensis* to the different sites in Santo Antônio dos Anjos Lagoon: NV_Oce (non-vegetated area near the ocean), Veg_Sal (vegetated area with saltmarsh-like species), Veg_Man (vegetated area with mangroove-like species) and NV_Con (non-vegetated area - control site).
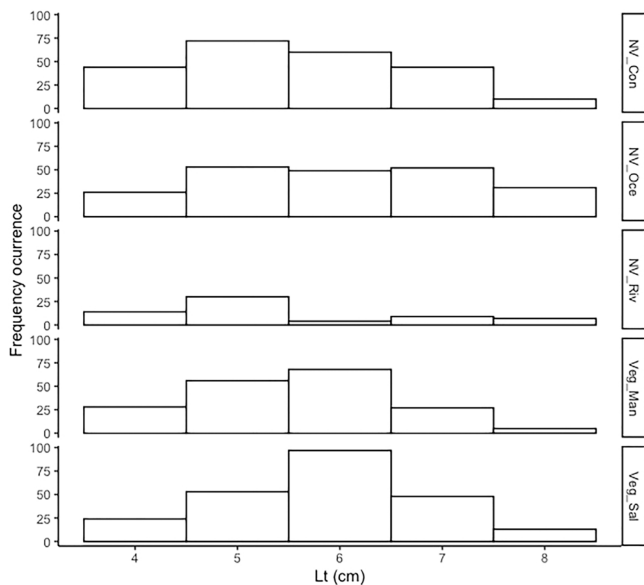

Fig. 4. Frequency (%) of the total length of *A. brasiliensis* captured in between estuarine habitats in the Santo Antônio dos Anjos Lagoon, Santa Catarina, Brazil.

with a greater number of juveniles (n = 257), with individuals with 6 cm as dominant size class. The number of juveniles in habitat Nv_Oce was 252, with individuals with 5, 6 and 7 cm as predominant size class. In the habitat NV_Man was sampled 220 juveniles, with the individuals with 6 cm in the predominant size class. The model validation (see

results in Supplementary Material) highlighted that the negative binomial GLMM adjusted well and incorporated the presence of zeros and the high data dispersion. The GLMM results showed that abundance didn't present significant statistical differences among habitats ($p > 0.05$; Fig. 5). The NV_Riv was not compared with other habitats, however, is important to mention its low juvenile abundance values (n = 75), mainly composed by juveniles with 5 cm in the predominant size class.

4. Discussion

In this study, we checked for differences in relative growth and abundance among estuarine habitats. Among factors that are determinant in the distribution of individuals in estuarine habitats, water quality is recognized as a structuring element of the species found in these environments (McLusky and Elliott, 2004). Since coastal lagoons present a high variability of abiotic factors (Basset et al., 2013), from our approach we were able to avoid mismatch spatial comparison, using only habitats which had similar conditions in water quality parameter, here the salinity concentration. From GLMM results, we highlighted that the juveniles abundance were not influenced by habitat structure (i.e absence and presence of distinct vegetation types), while from ANCOVA we highlighted differences in W-L relationships among habitats. These ANCOVA differences were caused by intercept (a), which is a parameter with greater statistical than biological meaning, since it highlights the weight where the length is equal to zero (Le Creen, 1951; Froese, 2006). In the comparison between habitats with equal intercept, there wasn't difference in slope (b), which denotes no difference in the growth rates due habitats distinctness.

In general, resident estuarine species are well adapted to stressful environments, such as coastal lagoons (Franco et al., 2008). Corroborating with this, *A. brasiliensis* doesn't present a clear pattern in relative
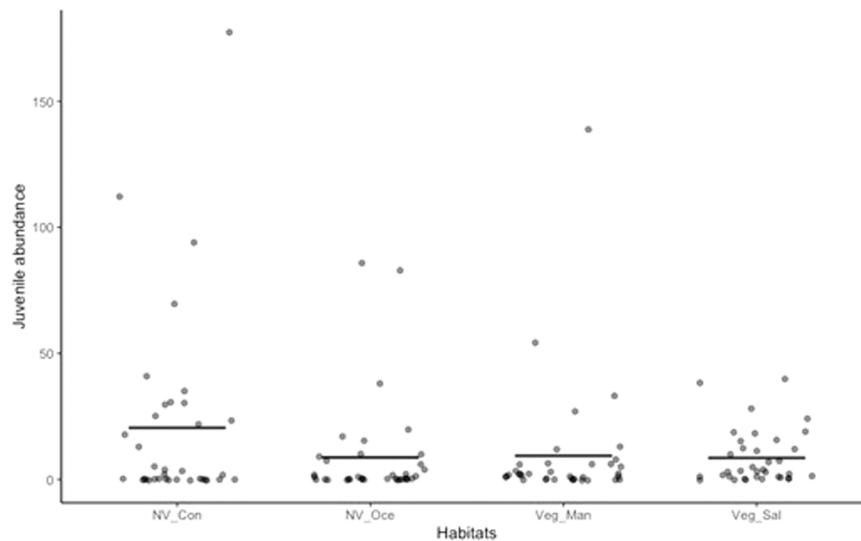
Fig. 5. GLMM`s predicted effects (bars are the medians) and observed values (points) of abundance of juveniles on estuarine habitats.

growth and abundance among estuarine habitats, potentially related to i) the high osmoregulatory capacity observed in estuarine fish (Souza--Bastos and Freire, 2011) and ii) with its generalist and opportunistic feeding habits (Brito et al., 2019; Chaves and Vendel, 2008; Contente et al., 2010). The species feeding on wide types of prey, as zooplankton, insects and diatoms (Contente et al., 2011; Alves et al., 2016) that can occur in shallows estuarine habitats. These both ecological aspects (*i.e* wide conditions tolerance and resource heterogeneity) are associated with high niche breadth (Hutchinson, 1957), which can explain the presence and abundance of *A. brasiliensis* along all vegetated and non-vegetated habitats assessed in this study.

The spatial heterogeneity associated with presence of distinct types of vegetation may provide differs growth condition to nekton species (Beck et al., 2001), generating specific nursery value on each estuarine habitat (Pihl et al., 2002; Pérez-Ruzafa et al., 2007). In general, vegetated habitats have higher nursery function values than those non-vegetated (Beck et al., 2001). Salt marsh (Minello et al., 2003) and mangrove (Nagelkerken, 2009) habitats associated with estuarine habitats are nursery areas for some species of nektons. The vegetated habitat can serve as a foraging area, with infauna being an important food resource for juveniles (Zimmerman et al., 2000; Whaley and Minello, 2002), promoting greater growth for part of the nektons (Minello, 2000).

The nursery function can depend on habitat characteristics and may vary among species (Minello et al., 2003). In our study, we highlighted similar abundance, relative growth, and length composition in estuarine habitats, indicating that non-vegetated, salt marsh, and mangrove habitats have similar nursery value for *A. brasiliensis*. These findings are consistent with previous studies (e.g., Bloomfield and Gillanders, 2005), which showed that the abundance of nekton species varies between vegetated and non-vegetated estuarine habitats, while the abundance of fishes from the Atherinidae family, the same family as *A. brasiliensis*, is not influenced by habitat structure.

Better growth rate is an attribute of nursery area (Beck et al., 2001) and this accelerated growth comprises an essential characteristic for the survival of juvenile fish, since with the increase in body size, there is a reduction in the predator's capture capacity (Sogard, 1992). Potentially, vegetated and non vegetated habitats in LES provides suitable conditions to the kingfish *A. brasiliensis* in early stage and this wide habitat utilization appears to be an ecological aspects to the species. For instance, *A. brasileinsis* is a frequent and abundant resource in fisheries activities in LES (Wahrlich et al., 2023) and, regionally (Southern Brazil), is considered one of the most representative species in estuarine fish assemblage (Falcão et al., 2006; Loebmann and Vieira, 2005; Spach et al., 2003).

Structured habitats have economic and ecological importance as they promote different types of ecosystem services (Barbier, 2019; Constanza et al., 1997). Salt marsh habitats are among the most degraded and altered in the world (Vitousek et al., 1997; Valiela et al., 2004; Lotze et al., 2006). Both mangrove and salt marsh habitats studied here are located in a Conservation Unit (APA-BF) and Brazilian legislation considers the mangroves as Areas of Permanent Preservation (APP). Considering that the objective of the environmental protection areas is to conserve biodiversity and promote the sustainable use of natural resources, the initial knowledge generated here together with future studies that incorporate more species may contribute to actions for sustainable use and protection of areas priority for ichthyofauna and other organisms that are part of the estuarine biota or that depend on this ecosystem.

5. Conclusion

Our results showed that *A. brasiliensis* juveniles are abundant in estuarine habitats and the species has similar relative growth in studied habitats, independently if the distinctness of them Water quality may shape species distribution and abundance, however in our study we only compared habitats with similar salinity condition. In general, shallow estuarine habitats harbor a high abundance of nektons species and provides suitable conditions to organisms growth in the early stages of life. Thus, it is necessary to evaluated habitats aspects and its ecological roles in the estuarine ecosystem, to subsidize better actions to environmental protection in seascape. Considering the various economic, social and environmental conflicts of interest existing in worldwide coastline, the identification of priority areas for protection and conservation can help government actions in the context of public administration.

CRediT authorship contribution statement

Jorge Luiz Rodrigues Filho: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Diogo Fernandes Santiago:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Thiago Bif Piazza:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **David Valença Dantas:** Writing – review &

editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Victor Aguiar de Souza Penha:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Joaquim Olinto Branco:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare that they have no known competing financial interests or personal relationships that influenced the work reported in this paper

Data Availability

Data will be made available on request.

Acknowledgements

Ethics declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the care and use animals were followed.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rsma.2024.103663.

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