The following supplement accompanies the article

Resource utilization of puffer fish in a subtropical bay as revealed by stable isotope analysis and food web modeling

Mathilde Lemoine, Tom Moens, Anna-Maria Vafeiadou, Luis Artur Valões Bezerra, Paulo Lana*

*Corresponding author: lana@ufpr.br

Marine Ecology Progress 626: 161–175 (2019)

Methods

Input parameters in Ecopath with Ecosim (EwE)

Production by Biomass (P/B, in units of day^{-1}) ratios of *Sphoeroides greeleyi*, *S. testudineus*, and the "other fish" group were calculated in Fishbase (Froese and Pauly 2018) using the life history tool, while a gross production efficiency (P/Q) of 0.15 (Christensen et al. 2008) was used to estimate Consumption by Biomass (Q/B). For fish, an Ecotrophic Efficiency of 0.99 was used to estimate the Biomass (B) in $g.m^{-2}.day^{-1}$. The P/B of the virgin nerite (*Neritina virginea*) and of other infauna/ epifauna were based on Netto and Lana (1999), while a gross production efficiency of 0.25 (Christensen et al. 2008) was used to estimate Q/B for *N. virginea* and for all other invertebrates in the food web. We introduced a logical way to measure the *Spartina alterniflora* P/B, which was the difference between above-ground live and dead biomass, while B was above-ground biomass only (Lana et al. 1991). This makes sense when one conceives that P/B represents the renewal of biomass (Christensen et al. 2008), or in our case, annual mortality. The input of phytoplankton was calculated directly from results of Lana et al. (2001). Mangrove plant biomass should be the amount of leaves in the river, as renewed daily, therefore, we searched a reference providing mean daily renewals throughout the year. We found the daily variation of leaf biomass in summer (Domingos and Lana 2017) and extrapolated it to a year, while P/B was the mean renewal between flood and ebb tides. Detritus and Suspended Organic Matter (SPOM) biomasses were based on Lana and Guiss (1992). All input was based on previous studies in the Paranagua bay, except for B, P/B and Q/B of birds and mammals, that were retrieved from the Caeté estuary, northern Brazil (Wolff et al. 2000).

The initial steady state was achieved by altering the biomass of intermediate groups (*Fistulobalanus citerosum*, *Callinectes sapidus*, *Crassostrea rhizophorae, Anomalocardia flexuosa, Alitta succinea*, and zooplankton), until mass balance was achieved. In the absence of biomass information for Insecta, an EE of 0.5 was used and the Q/B retrieved from Wolff et al. (2000). For microphytobenthos (MPB), we used the mean chlorophyll a concentration in sediment obtained from Latin American estuaries and recalculated this to biomass in $g.m^{-2}$, while P/B was the variation between mean and maximum seasonal variation (Seeliger and Kjerfve 2013).

Results

Table S1. PERMANOVA on Euclidean distance matrices of δ¹³C and δ¹⁵N signatures of food sources; raw data were used for δ¹³C, while δ¹⁵N data were log transformed. P-values were obtained from a given number of permutations (perm). Significant differences are in bold.

		δ¹³C(‰)			δ¹⁵N(‰)		
	df	Pseudo-F	*P*	Perm	Pseudo-F	*P*	Perm
Mangrove sources							
Crassostrea rhizophorae	2	129.37	**0.001**	860	33.67	**< 0.001**	8912
Fistulobalanus citerosum	2	14.92	**< 0.001**	2095	7.254	**0.006**	9564
MPB	2	134.16	**0.005**	117	0.386	0.632	110
Mangrove detritus	2	5.556	0.203	6	1.763	0.467	9
Saltmarsh sources							
Neritina virginea	2	97.039	**< 0.001**	9289	95.664	**< 0.001**	9583
Alitta succinea	2	52.414	**< 0.001**	9205	381.31	**0.001**	9541
Subtidal channel							
Anomalocardia flexuosa	2	5.301	**0.03**	50	1.120	0.357	2151
Callinectes sapidus	2	3.749	**0.046**	9499	1.587	0.265	9560

Table S2. Percentage diet contribution (Bayesian 95 % c.i.) of food sources based on δ¹³C and δ¹⁵N isotope signatures of *Sphoeroides testudineus* and *S. greeleyi* per habitat as estimated with SIAR and using the TEF 1 scenario. Important food source contributions (> 10 %) are shown in bold.

Food sources	***Sphoeroides greeleyi***			***Sphoeroides testudineus***		
	mangrove	saltmarsh	subtidal channel	mangrove	saltmarsh	Subtidal channel
Spartina alterniflora	7.6(0.4-17.9)	7.8(0.4-18.9)	4.4(0.1-12.8)	**14.9(10.8-34.5)**	9.7(0.2-27.6)	7.6(0.1-25.0)
Avicennia schaueriana + Rhizophora mangle	1.0(0-2.3.7)	0.9(0-3.5)	1.0(0-3.4)	0.8(0-4)	1.2(0-6.0)	1.1(0-5.1)
Laguncularia racemosa	0.9(0-3.5)	1.0(0-3.5)	1.0(0-3.5)	0.8(0-3.5)	1.1(0-5.4)	1.0(0-4.6)
Crassostrea rizophorae	4.5(0.1-16.3)	5.5(0.2-18.5)	4.5(0.1-15.7)	4.3(0.1-21.7)	7.7(0.1-38.0)	5.8(0.1-28.0)
Fistulobalanus citerosum	6.5(0.1-22.6)	6.1(0.2-20.8)	7.9(0.3-26.5)	4.9(0-22.3)	6.6(0.1-29.5)	6.8(0.1-27.8)
Neritina virginea	**53.9(27.7-72.8)**	**51.7(26.6-70.1)**	**57.8(31.7-77.2)**	**40.8(10.8-64.6)**	**38.0(8.8-63.1)**	**46.9(16.1-70.1)**
Alitta succinea	9.2(0.3-28.5)	9.9(0.2-29.0)	6.5(0.3-20.3)	**18.1(0.1-65.6)**	**14.2(0.1-50.9)**	**12.2(0.1-45.1)**
Callinectes sapidus	7.0(0.2-25.1)	6.6(0.2-23.3)	8.5(0.3-28.7)	5.5(0.1-25.6)	7.3(0.1-31.4)	7.4(0.1-32.1)
Anomalocardia flexuosa	3.8(0.2-12.7)	4.3(0.3-14.1)	3.6(0.1-12.7)	3.8(0.1-18.2)	6.1(0.1-29.4)	4.8(0.1-24.2)
Mangrove litter	1.0(0-3.7)	1.1(0-3.6)	1.1(0-3.8)	0.9(0-4.1)	1.2(0-5.9)	1.1(0-4.9)
Microphytobenthos	2.3(0.1-7.6)	2.7(0.1-8.7)	1.8(0.1-6.3)	3.3(0-17.3)	3.6(0-15.7)	2.9(0-13.9)
Particulate organic matter	2.0(0.1-6.9)	2.4(0.1-8.4)	1.9(0.1-6.6)	1.9(0-9.9)	3.3(0-16.1)	2.5(0-12.8)

The trophic position of *S. testudineus* did not differ among sites and habitats when SPOM was used as a baseline. However, when MPB and mangrove detritus were used as a baseline, the trophic position of *S. testudineus* was higher ($P < 0.05$) in site 3 (3.6 ± 0.22 in saltmarsh with MPB baseline, Table S3) than in site 1 (3.3 ± 0.12 in saltmarsh with MPB baseline). The trophic levels of *S. greeleyi* calculated based on MPB and mangrove detritus as baselines were lower at site 1 (3.4 ± 0.11 in saltmarsh with MPB baseline) than at site 3 (3.6 ± 0.16 in saltmarsh with MPB baseline). The trophic levels of *S. greeleyi* calculated from SPOM as a baseline in site 2 were higher in the subtidal channel (3.2 ± 0.17) than in the mangroves (3.3 ± 0.14) and saltmarshes (3.3 ± 0.04). For differences in trophic level between both *Sphoeroides* species, we refer to Results, section 'Stable isotope signatures and trophic position of fishes'.

Table S3. Percentage diet contribution (Bayesian 95 % c.i.) of food sources based on $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of *Sphoeroides testudineus* and *S. greeleyi* per site as estimated with SIAR and using the TEF 1 scenario. Important food source contributions (> 10 %) are shown in bold.

Food sources	***Sphoeroides greeleyi***			***Sphoeroides testudineus***		
	site 1	site 2	site 3	site 1	site 2	site 3
Spartina alterniflora	**10.5(1.2-21.6)**	**8.2(0.8-21.1)**	2.5(0.1-12.5)	**15.4(0.3-34.2)**	**18.9(0.3-36.2)**	2.3(0.1-21.6)
Avicennia schaueriana + Rhizophora mangle	0.9(0-2.4.1)	0.5(0-2.7)	0.7(0-3.5)	0.5(0-4)	0.3(0-3.4)	0.7(0-7)
Laguncularia racemosa	0.7(0-3.9)	0.5(0-2.7)	0.8(0.1-3.6)	0.4(0-3.5)	0.3(0-3.1)	0.7(0-7)
Crassostrea rizophorae	3.8(0.2-18.3)	2.7(0.1-14)	4.5(0.2-20.5)	2.3(0.1-20.5)	2(0.1-18.7)	4.6(0.1-46.2)
Fistulobalanus citerosum	5.8(0.1-29.7)	5.3(0.2-27.6)	4.7(0.2-18.2)	3.3(0.1-31.9)	3.3(0.1-26.6)	4.2(0.1-28)
Neritina virginea	**42.1(16.1-59.7)**	**51.1(3.1-75.1)**	**62.4(36.5-78.6)**	**29.9(3.2-55)**	**36(1.5-64.5)**	**52(15.9-76.5)**
Alitta succinea	**11.7(0.5-42.7)**	6.5(0.2-29.3)	2.8(0.1-14)	**14.1(0.2-81.8)**	6.42(0.1-73.7)	2.5(0.1-20.4)
Callinectes sapidus	4.3(0.2-25.6)	8.4(0.3-60)	8.9(0.6-25.8)	2.7(0.1-32.4)	6.2(0.1-55.2)	7.4(0.3-31.9)
Anomalocardia flexuosa	3.2(0.1-15.7)	2.1(0.1-11)	2.3(0.1-11.6)	2.2(0.1-22.4)	1.5(0-17.6)	2.2(0-23.6)
Mangrove litter	0.7(0-3.8)	0.6(0-3.1)	0.7(0-3.7)	0.4(0-3.8)	0.4(0-3.6)	0.7(0-7.5)
Microphytobenthos	3.1(0.2-11.7)	1.3(0-6.6)	1.1(0.1-4.7)	2.2(0.1-21.7)	0.1(0-13.7)	1(0-9.1)
Particulate organic matter	1.7(0-8.9)	1.2(0.1-5.8)	1.4(0.1-6.1)	1(0-10.6)	0.8(0-9)	1.3(0-13.5)

Table S4. PERMANOVA on Euclidean distance matrices of the trophic position of puffer fish calculated with different primary producer baselines (TEF 1). TP = trophic position, MPB = microphytobenthos, SPOM = suspended particulate organic matter. P-values were obtained from 9999 permutations (perm). Significant differences are in bold.

Baseline used		**TP based on MPB**			**TP based on SPOM**			**TP - mangrove detritus**		
	Df	Pseudo-F	*P*	perm	Pseudo-F	*P*	perm	Pseudo-F	*P*	Perm
Sphoeroides testudineus										
Site	2	9,809	**0.022**	4379	0.393	0.706	4331	18.296	**0.021**	4310
Habitat(Site)	6	1,519	0.195	9936	1.519	0.184	9938	1.519	0.187	9950
Sphoeroides greeleyi										
Site	2	6.421	**0.028**	2461	1.678	0.130	2468	10.995	**0.023**	2459
Habitat(Site)	6	5.907	**< 0.001**	9947	5.907	**< 0.001**	9937	5.9071	**< 0.001**	9953
Sphoeroides testudineus x Sphoeroides greeleyi										
Fish	1	17.637	**0.007**	9808	17.637	**0.006**	9824	17.637	**0.007**	9826
Fish x Site	2	0.791	0.499	9948	0.791	0.496	9951	0.791	0.491	9956
Fish x Habitat(Site)	6	1.923	0.087	9957	1.922	0.085	9943	1.923	0.083	9952

Table S5. Mean trophic level (± SD) of *Sphoeroides testudineus* and *S. greeleyi* calculated with different primary producers as baselines. TP = trophic position, MPB = microphytobenthos, SPOM = suspended particulate organic matter.

	site 1			**site 2**			**site3**		
	mangrove	saltmarsh	channel	mangrove	saltmarsh	channel	mangrove	saltmarsh	channel
Sphoeroides greeleyi									
TP - MPB	3.5 ± 0.08	3.4 ± 0.11	3.4 ± 0.17	3.7 ± 0.14	3.7 ± 0.04	4.0 ± 0.17	3.7 ± 0.14	3.6 ± 0.16	3.8 ± 0.12
TP - SPOM	2.9 ± 0.08	2.9 ± 0.11	2.8 ± 0.17	2.9 ± 0.14	2.9 ± 0.04	3.2 ± 0.17	2.8 ± 0.14	2.8 ± 0.16	2.9 ± 0.12
TP - mangrove detritus	2.9 ± 0.08	2.9 ± 0.11	2.9 ± 0.17	3.3 ± 0.14	3.3 ± 0.04	3.7 ± 0.17	3.3 ± 0.14	3.3 ± 0.16	3.4 ± 0.12
Sphoeroides testudineus									
TP - MPB	3.3 ± 0.15	3.3 ± 0.12	3.3 ± 0.16	3.5 ± 0.22	3.5 ± 0.07	3.6 ± 0.30	3.4 ± 0.18	3.6 ± 0.22	3.7 ± 0.19
TP - SPOM	2.7 ± 0.15	2.7 ± 0.12	2.7 ± 0.16	2.7 ± 0.22	2.71 ± 0.07	2.8 ± 0.30	2.5 ± 0.18	2.7 ± 0.22	2.8 ± 0.19
TP - mangrove detritus	2.9 ± 0.15	2.8 ± 0.12	2.8 ± 0.16	3.2 ± 0.22	3.2 ± 0.07	3.2 ± 0.30	3.0 ± 0.18	3.2 ± 0.22	3.3 ± 0.19