Hydrologic pulsing promotes spatial connectivity and food web subsidies in a subtropical coastal ecosystem

11 authors, including:

Alexandre Garcia
Universidade Federal do Rio Grande (FURG)
100 PUBLICATIONS   **1,320** CITATIONS

Kirk O Winemiller
Texas A&M University
398 PUBLICATIONS   **15,643** CITATIONS

Rodrigo Ferreira Bastos
Federal University of Pernambuco
22 PUBLICATIONS   **96** CITATIONS

Fabiano Corrêa
Universidade Federal de Pelotas
42 PUBLICATIONS   **110** CITATIONS

Some of the authors of this publication are also working on these related projects:

Project Biosafety and Occupational Health in Aquaculture View project

Project Food webs structure and dynamics in Southwestern Atlantic coastal systems View project

1   **Running head:**

2   Pulse-driven subsidies in an estuarine ecosystem

3

4   **Hydrologic pulsing promotes spatial connectivity and food web subsidies in a subtropical**

5   **coastal ecosystem.**

6

7   **Authors:** Garcia, A.M*.[1], K.O. Winemiller[2], D.J. Hoeinghaus[3], M.C. Claudino[1], R. Bastos[1], F.

8   Correa[1], S. Huckembeck, [1] J. Vieira[1], D. Loebmann[1], P. Abreu[1], and C. Ducatti[4]

9

10   [1]Oceanography Institute, Rio Grande Federal Universiy, Rio Grande, Rio Grande do Sul, 96203-

11   900, BRAZIL

12   [2]Department of Wildlife and Fisheries Sciences and Interdisciplinary Program of Ecology and

13   Evolutionary Biology, Texas A&M University, College Station, Texas 77843-2258, USA

14   [3]Department of Biological Sciences and the Advanced Environmental Research Institute,

15   University of North Texas, Denton, Texas 76203-5017, USA

16   [4]Paulista Júlio de Mesquita Filho State University, Stable Isotope Center for Environmental and

17   Life Sciences, Biosciences Institute, Botucatu, São Paulo 18608-000, BRAZIL.

18

19   # Corresponding author

20   amgarcia.ictiofurg@gmail.com

21   +55 53 32336539

22

23

24   **Abstract**

25

26   Resource pulsing is a widespread phenomenon, but its effects on ecosystem dynamics are often

27   difficult to predict. Hydrological pulsing, in particular, is known to influence the structure and

28   dynamics of fluvial and coastal ecosystems, but little information is available for its effects on

29   trophic connectivity between wetlands and estuaries. In this study, we investigated the

30   hypothesis that hydrologic pulsing drives one-way trophic subsidies (e.g. suspended organic

31   matter and freshwater fish) from wetland to estuary. Our study system is a coastal lagoon with an

32   ephemeral mouth that, when closed, stores freshwater as a sustained flood pulse that is

33   subsequently released when a connection with the sea is reestablished. We monitored isotopic

34   composition of consumers and food sources over the course of an entire flood pulse to infer

35   trophic linkages and spatial subsidies. Before the flood peak (April and May), freshwater and

36   estuarine zones were largely dependent on local primary production sources (seston and C_3

37   plants vs. C_4 plants and microphytobenthos, respectively), essentially functioning as

38   disconnected compartments. A sustained pulse of freshwater inflow (June to August) induced

39   greater habitat connectivity and a net flow of biomass and energy from the freshwater zone into

40   the estuarine zone. The opening of the lagoon outlet channel abruptly terminated the flood pulse

41   and reduced freshwater subsidies to estuarine consumers, and both zones returned to dependence

42   on autochthonous production. Our findings contribute to current concerns that artificial opening

43   of sandbars in coastal lagoons alters natural ecological dynamics with significant effects on

44   biodiversity and ecosystem processes.

45

46   Keywords: basal resource, Bayesian mixing model, biomass assimilation, estuary, hydrologic

47   connectivity, production source, salinity, trophic ecology

48

49

50

51

52

53

54

55

56 **Introduction**

57   Food web dynamics are influenced not only by consumer-resource interactions, but also

58 by environmental variation that directly affects ecosystem productivity, habitat suitability, and

59 other factors affecting organism fitness and population growth (Winemiller & Layman 2005).

60 Further complicating our ability to predict dynamics in response to environmental drivers is the

61 fact that perceived patterns of variation are influenced by temporal and spatial scales of analysis

62 (Petraitis & Latham 1999). Most, and probably all, ecosystems are subject to abiotic factors that

63 pulse. Resource pulses, in particular, are defined as discrete, significant increases in resource

64 availability over intervals of time and/or space (Yang et al. 2008). Environmental seasonality

65 affects local population dynamics and species interactions, but also can have regional effects

66 when pulsing factors induce mass migrations or synchrony among regional populations

67 (Leibhold et al. 2004). For example, Krenz et al. (2011) showed how coastal upwelling

68 influenced by the El Niño Southern Oscillation affects food web subsidies within intertidal

69 ecosystems over a large region. Resource subsidies associated with pulsing environmental

70 factors strongly influence bottom-up (e.g. a fertilizer effect) and top-down (consumer control)

71 effects in food webs of arid islands (Polis et al. 1997, Spiller et al. 2010).

72   Pulsing can induce responses that reverberate throughout the food web (Yang et al. 2008).

73 Mass spawning by corals and reef fishes in response to lunar and oceanographic cues produces

74 huge subsidies for planktivores (Pratchett et al. 2001), which can have long-term effects on

75 fitness of the latter (McCormick 2003). Regional insect outbreaks in response to favorable

76 climatic conditions can devastate plant biomass (Mattson & Addy 1975) while subsidizing

77 insectivores (Yang 2004).Seasonal migrations can create food web subsidies at the landscape

78 scale (Polis et al. 2004). For example, the decomposing carcasses of post-spawn anadromous

79   salmon provide a pulse of marine-derived nutrients that stimulates primary production not only

80   within oligotrophic streams, but also riparian terrestrial ecosystems (Naiman et al. 2002).

81   Theoretical models indicate diverse responses to pulsing that range from transient population

82   dynamics that may or may not influence species coexistence, to tipping the dynamics governing

83   alternative stable states of ecosystems (Ostfeld & Keesing 2000, Holt 2008; Petersen et al. 2008).

84   Hydrologic changes, in particular, can trigger ecosystem pulsing that affects the structure

85   and dynamics of fluvial and coastal ecosystems (Winemiller 1990, Poff et al. 1997, DeAngelis et

86   al. 2005, Childers 2006, Warfe et al. 2011). Fish dynamics in hydrologically pulsed wetland

87   ecosystems monitored across long time scales (e.g. Florida's Everglades) are strongly associated

88   with seasonal cycles in precipitation and water depth that cascade to other components of the

89   food web (DeAngelis et al. 1997, Childers 2006).

90   In southeastern Brazil, inter-annual and seasonal variation in precipitation drives changes

91   in hydrology and salinity gradients that, in turn, affect the composition of local fish assemblages

92   within coastal lagoons (Garcia et al. 2003, Garcia et al. 2004). A suitable ecosystem model for

93   evaluating hydrologic pulsing effects on spatial food web subsidies between freshwater and

94   estuarine systems in this region is the Lagoa do Peixe National Park (LPNP). Ecological

95   conditions within LPNP are influenced not only by intra-annual variations in freshwater inflow,

96   but also by ephemeral connections with the sea. Freshwater draining from the coastal plain is

97   stored within the lagoon as a sustained flood pulse and then released over a relatively short

98   period when a connection with the sea is established either (i) naturally by inflows or (ii)

99   artificially by mechanical excavation (Lanés et al. 2015). Thus, hydrology induces a freshwater

100 flood pulse that causes aquatic ecosystem expansion with greater connectivity between the

101 freshwater wetland and the estuarine zone and conditions promoting input of freshwater basal

102 production sources and consumer taxa into the estuary.

103   We hypothesized that hydrologic pulsing influences food web dynamics along the

104 longitudinal fluvial gradient via one-way trophic subsidies (e.g. passive transport of suspended

105 organic matter and dispersal of freshwater fish) from wetland to estuary. We used stable isotope

106 methods to test this hypothesis based on extensive temporal and spatial sampling of production

107 sources and consumers at locations along the longitudinal fluvial gradient, and used a Bayesian

108 mixing model to estimate probability distributions for assimilation of alternative production

109 sources. Stable isotope analysis (SIA) is commonly used to reconstruct trophic links connecting

110 food web components across spatial (Hoeinghaus et al. 2011, Claudino et al. 2015) and temporal

111 (Claudino et al. 2013, Garcia et al. in press) scales, and several authors have used SIA to test

112 effects of hydrologic changes on food web organization and spatial subsidies among ecosystems

113 (Jardine et al. 2012, Abrantes et al. 2013, Kaymak et al. 2015, Ou & Winemiller 2016). In

114 contrast with most previous studies that used seasonal or before-and-after sampling designs, our

115 study analyzed a time-series of samples taken over frequent, short intervals, which enabled us to

116 view food web structure like a movie rather than a snapshot. This analysis provided a basis for

117 estimating the timing and magnitude of a spatial food web subsidy to estuarine consumers.

118

119 **Material and methods**

120 *Study area*

121   The Lagoa do Peixe National Park (LPNP) has an area of 344 km² and is situated along

122 the coastal plain of Rio Grande do Sul, the southernmost state in Brazil. This coastal plain is

123 characterized by a flat topography, low altitude (< 20 masl) and low tidal range (~ 0.5m) (Fig. 1).

124   Lagoa do Peixe is a shallow coastal lagoon (<50 cm, except its channel) surrounded by

125   freshwater wetlands (Maltchik et al. 2010), except for its eastern border where sand dunes are

126   prevalent (Fig. 1). The lagoon has a narrow outlet to the sea that is blocked from

127   February/March to August/September each year by sand dunes until freshwater inflows, usually

128   from winter rainfall, establish a connection. If the seasonal connection with the sea is not

129   established naturally, earth-moving machinery is used to construct an outlet channel (200 m long,

130   40 m wide, 1.5 m deep) during late winter (August-September). The overall dimensions of both

131   artificial and natural channels are similar. This periodic opening of the outlet is done to promote

132   the entrance of marine shrimp larvae into the lagoon to favor commercial fisheries and also to

133   drain water from the floodplain to increase pasture for livestock ranching (Lanés et al. 2015).

134   The LPNP has a humid subtropical climate, with mean temperatures ranging from 14.6°C

135   to 22.2°C, a mean annual temperature of 17.5°C, and annual precipitation in the study area

136   ranges from 1,150 to 1,450 mm yr^{-1}, with an annual mean of 1,250 mm yr^{-1}. Prior studies on

137   estuarine systems approximately 100 km south of the LPNP have shown that rainfall anomalies

138   cause hydrological changes that affect estuarine and freshwater fish assemblages (Garcia et al.

139   2004). To assess potential influence of climatic variation on ecological dynamics, temperature

140   and rainfall data were obtained from two meteorological stations (Rio Grande and Mostardas)

141   near the study area (Fig. 1), and the status of the lagoon connection with the sea was monitored

142   throughout the study. Rainfall varied seasonally, with two periods of high rainfall: May to

143   August 2008 and January to March 2009. The former wet period coincided with a closed lagoon

144   mouth, which occurred from April to August 2008. At the estuarine site in particular, water

145   flooded over the marginal areas during wet periods, increasing hydrological exchanges with

146   freshwater wetlands. Average air temperature varied seasonally, with lowest values during the

147   austral winter (14.1°C in June) and highest values in summer (23.7°C in February). Based on

148   this climatic variation, we defined flooded and non-flooded phases of the hydrologic pulse in

149   order to evaluate our initial hypothesis (Fig. 1).

150

151   *Field collections and sample processing*

152   Samples were obtained monthly from April 2008 to May 2009 (with the exception of

153   September 2008 and March 2009) in three regions along the main longitudinal axis of the LPNP:

154   1) a freshwater wetland located near the northern limit of the park, 2) the upper–middle reach of

155   the lagoon that encompasses an ecotone between estuarine and freshwater zones, and 3) the

156   middle reach of the lagoon near the ephemeral connection with the sea (Fig. 1). These sites are

157   subsequently referred to as freshwater wetland, estuarine zone and lagoon mouth, respectively.

158   Fish were caught using four fishing gears (gillnets, beach seine, beam trawl, dipnet)

159   during each monthly survey. To catch larger fishes (>200 mm total length), two gillnets (4 x 2 m,

160   comprised by panels with different mesh sizes of 15, 20, 30 and 35 mm) were deployed in the

161   channel (2.0 – 2.5 m deep). One to two beach seine (9 m long, 2.4 m high, mesh size 13 mm

162   wings and 5 mm center) hauls were made in unvegetated nearshore areas. Densely vegetated

163   marginal habitats were sampled with three hauls of the beam trawl (mouth = 1 x 1 m, mesh = 5

164   mm) and dip netting for approximately 15 min. Beach seine and beam trawl hauls and dip

165   netting were conducted in shallower waters (<1.5 m) and were effective in capturing smaller

166   species (< 50 mm), including those that take refuge in structurally complex habitats. Sampling

167   with multiple gear types provides a more representative sample of the fish assemblage, especially

168   when the ecosystem is comprised by multiple habitat types with differing complexities and

169   species of various sizes and behaviors (Rozas & Minello 1997, Chick et al., 2004, Garcia et al.

170   2006). Representative macroinvertebrates, such as adult and immature aquatic insects,

171   gastropods, polychaete worms, shrimps and crabs, were collected at each study site.

172   Samples of microphytobenthos, seston (phytoplankton and suspended fine particulate

173   organic matter), and leaves from floating, emergent and submerged macrophytes were collected

174   at each site during each survey. This material was used for determination of the isotopic

175   composition of major basal production sources. Macrophytes were collected by hand, samples

176   of microphytobenthos were obtained by carefully removing the thin upper layer of flocculent or

177   consolidated biofilm from substrates, and seston samples were obtained by filtering water

178   through a pre-combusted (450° C, 4 h) Whatman glass fiber filter (GF/F) using a manual pump.

179   Immediately after collection, all specimens were placed on ice for transport to the

180   laboratory where they were stored frozen. After thawing, fish were weighed (g), measured (mm

181   total length, TL) and dissected to extract approximately 5 g of dorsal muscle tissue for isotopic

182   analysis. For fish <50 mm TL, a composite sample was obtained by combining muscle tissue

183   from 5–15 conspecifics from the same site. Gastropods, shrimps and crabs were dissected, and a

184   sample of muscle tissue was extracted for stable isotope analysis. Due to their small sizes,

185   polychaetes and aquatic insects were processed as whole specimens. Tissue samples from fish

186   and macroinvertebrates were inspected and any significant non-muscle material (e.g. bone, scales,

187   exoskeleton) was removed before the samples were rinsed with distilled water, placed in sterile

188   Petri dishes, and dried in an oven at $60°$ C until attainment of a constant weight (minimum of 48

189   h). Dried samples were ground to a fine powder with a mortar and pestle and stored in clean

190   Eppendorf tubes. Sub-samples were weighed to 6-10 mg, pressed into Ultra-Pure tin capsules

191   (Costech, Valencia, CA), and sent to the Centro de Isótopos Estáveis, Universidade Estadual

192   Paulista (UNESP) for measurement of stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). Results are

193   reported as parts per thousand (‰) differences from a corresponding standard: $\delta^H X = [(R_{sample} /$

194    $R_{standard}) - 1] \times 1000$, where X is a particular element (C, carbon or N, nitrogen), the superscript H

195   denotes the heavy isotope mass of the given element (^{13}C, ^{15}N) and R is the ratio of the heavy

196   isotope to the light isotope for the element ($^{13}C/^{12}C$, $^{15}N/^{14}N$). Standards were carbon in the

197   PeeDee Belemnite and molecular nitrogen in air. Standard deviations of $\delta^{13}C$ and $\delta^{15}N$ replicate

198   analyses were 0.14‰ and 0.13‰, respectively.

199

200   *Data analysis*

201   For some analyses, fishes were pooled into five functional guilds based on prior

202   classification proposed by Garcia et al. (2001) and also considering Myers' (1938) model of

203   primary- and secondary-division freshwater fishes, as follows: 1) estuarine resident species that

204   typically occur and breed within the estuary; 2) estuarine dependents that are marine or

205   freshwater spawning species found in large numbers within the estuary during certain periods of

206   their life cycle; 3) marine vagrants typically inhabiting marine habitats and rarely occurring

207   within the estuary; 4) primary freshwater fishes with no tolerance to salinity that are confined to

208   freshwater; and 5) secondary freshwater fishes with some salinity tolerance that may enter

209   brackish water occasionally. Also, due to consistent differences in retention of ^{13}C by plants

210   using the Calvin cycle (C_3) and Hatch-Slack cycle (C_4) photosynthetic pathways (Marshall et al.

211   2007), sampled macrophytes were pooled and analyzed as two groups, C_3 vs. C_4, according to

212   the literature (Marshall et al. 2007), the range of $\delta^{13}C$ values observed in this study and previous

213   research in nearby coastal lagoon ecosystems (Garcia et al. 2007, Hoeinghaus et al. 2011). C_3

214   plants tend to have significantly lower average carbon stable isotope ratios (~ -27‰) than C_4

215   plants (~ -4‰) mainly due to differences in the enzymes involved in carbon fixation during

216   photosynthesis (Marshall et al. 2007). Abundance patterns of these groups shift along the salinity

217   gradient of the study site, with C_3 being the dominant aquatic macrophytes in the freshwater

218   wetland and C_4 plants dominant in the estuarine/marine sites (Knak 2004, Rolon et al. 2011).

219   Bi-plots of $\delta^{15}N$ and $\delta^{13}C$ values of primary producers and organic sources, invertebrates

220   and fishes were used to evaluate patterns of isotopic variation across spatial and temporal scales.

221   Sources of organic carbon assimilated by consumers are indicated by proximity between carbon

222   isotope ratios ($\delta^{13}C$) of consumers and their food sources, whereas trophic position of each

223   consumer is indicated by the relative position of their nitrogen isotope ratios ($\delta^{15}N$) in relation to

224   an isotopic baseline (Peterson & Fry, 1987). Analysis of variance (ANOVA) was used to

225   compare average $\delta^{13}C$ and $\delta^{15}N$ values of sources and consumers among study sites and, for

226   fishes only, among months. When a significant main effect was observed, pairwise comparisons

227   of site means were performed using Newman-Keuls post-hoc procedure. Normality and

228   homogeneity of variances were evaluated by the Kolmogorov-Smirnov and Cochran tests,

229   respectively (Zar 1984).

230   To estimate relative contributions of basal production sources to fish assemblages of the

231   freshwater wetland, estuarine zone and lagoon mouth, we employed Bayesian mixing model with

232   four basal production sources as end-members (seston, microphytobenthos, C_3 and C_4 plants)

233   using data specific to each site. Mixing models were run using the Stable Isotope Analysis in R

234   (SIAR) package in R statistical software (R Core Team 2012), which employs a Bayesian

235   approach that incorporates uncertainties associated with sample variability and trophic

236   enrichment on the assumption they are normally distributed (Parnell et al. 2010). Each mixing

237   model was fit via a Markov chain Monte Carlo (MCMC) method that generate simulations of

238   plausible values of dietary proportions consistent with the data using a Dirichlet prior

239   distribution (Parnell et al. 2010). Each model was run based on 500,000 iterations, discarding the

240   first 50,000, and considering a non-informative prior to guide the dietary proportion simulations.

241   Upper and lower credibility intervals (95, 75 and 50%) were used to describe the range of

242   feasible contributions for each food source to the consumer (Parnell et al. 2010).

243   In order to quantify food web subsidies occurring due to movement of carbon sources

244   from the freshwater wetland into the estuarine zone, we ran five-end-member SIAR mixing

245   models to estimate, on a monthly basis, the relative contribution of freshwater- and estuarine-

246   derived sources for estuarine resident fishes (silversides *Atherinella brasiliensis* and *Odonthestes*

247   *argentinensis*) and individuals of primary freshwater fish species that were collected within the

248   estuarine zone during the hydrologic pulse. Importantly, silversides are not piscivorous, and thus

249   assimilation of freshwater-derived sources by these estuarine-resident taxa is not by consumption

250   of freshwater fishes that moved into the estuarine zone. We used average values of carbon and

251   nitrogen isotope ratios of seston and C_3 plants collected in the wetland, and microphytobenthos,

252   C_4 plants and seston collected in the estuary as representative freshwater- and estuarine-derived

253   basal production sources. We chose freshwater seston and C_3 plants (mostly floating

254   macrophytes) because these basal sources are transported from the freshwater wetland into the

255   estuarine zone during floods, which create hydrologic connectivity between regions. Estuarine

256   sources included in this model were based on the four-end-member mixing model that identified

257   the primary sources assimilated by the estuarine fish assemblage. Mixing models were computed

258   assuming the silversides are two trophic levels above the basal production sources, and we used

259   values of 0.54‰ ± 1.23 and 2.54‰ ± 0.11 to correct for trophic fractionation of isotopic ratios of

260   carbon and nitrogen, respectively (Vanderklift & Ponsard 2003).

261

262   **Results**

263   A total of 684 samples representing 52 fish species was obtained in the freshwater

264   wetland (n= 352), estuarine zone (n= 187) and lagoon mouth (n= 145) between April 2008 and

265   May 2009 for analysis of carbon and nitrogen stable isotope ratios (Table S1). Local fish

266   assemblages showed a gradual zonation in species composition across the freshwater-estuarine

267   gradient. Primary- and secondary-freshwater fishes were restricted to freshwater wetland and

268   estuarine zones, with the only exception *Jenynsia multidentata* (secondary freshwater species),

269   which occurred at all three sites. In contrast, estuarine residents, estuarine dependents and marine

270   vagrants were captured only within the estuarine zone and the lagoon mouth (Table S1).

271   At the assemblage scale, there was a significant trend in $\delta^{13}C$ and $\delta^{15}N$ along the

272   freshwater to estuarine gradient, with lower values in freshwater ($\delta^{13}C$: -26.3‰ ±2.86, $\delta^{15}N$:

273   7.5‰ ±1.5) and much higher values in the estuarine ($\delta^{13}C$: -15.7‰ ±3.4, $\delta^{15}N$: 10.4‰ ±1.8) and

274   lagoon mouth zones ($\delta^{13}C$: -13.8‰ ±2.4, $\delta^{15}N$: 13.3‰ ±2.1) (Fig, 2, Table S1). Overall,

275   invertebrates (e.g. crabs, shrimps, polychaete worms), macrophytes (C_3 and C_4) and other

276   potential food resources (seston, microphytobenthos) showed similar trends. Specifically, both

277    $\delta^{13}C$ and $\delta^{15}N$ values significantly increased from the freshwater wetland to the lagoon mouth

278   (Fig. 2, Table S1).

279   For fish, quantitative estimates from Bayesian isotopic mixing-models revealed that

280   seston and C_3 plants (0.53 to 0.69 and 0.23 to 0.44, respectively) were the basal sources

281   assimilated in greatest proportions by fish inhabiting the freshwater wetland. Conversely, within

282   the two mixohaline sites (estuarine zone and lagoon mouth), C_4 plants and microphytobenthos

283   were the basal carbon sources assimilated in greatest proportions. For the estuarine zone, in

284   particular, C_4 plants had a much greater contribution to fish biomass (0.70 to 0.83) compared

285   with C_3 plants (0.08 to 0.21), and C_4 plants and microphytobenthos were the basal carbon

286   sources with highest contributions near the lagoon mouth (0.53 to 0.71 and 0.07 to 0.42,

287   respectively) (Fig. 2, right panel).

288   Monthly analysis of the isotopic composition of fish assemblages revealed important

289   changes in the spatial trend in average $\delta^{13}C$ across study sites (Fig. 3), which coincided with the

290   effect that the closure of the lagoon's connection with the sea has on the flood dynamics of this

291   system. During the flooding episode recorded between April and October 2008, mean fish $\delta^{13}C$

292   values across all sites were significantly lower (-23.5‰) compared with those recorded during

293   the non-flooded period (-20.0‰) ($F_{1,\ 687}$= 32.28, p< 0.00). When functional guilds were taken

294   into account, we observed that this difference was primarily due to the occurrence of salinity-

295   intolerant, primary freshwater fishes, especially small characids (*Astyanax eigenmanniorum*,

296   59.7 ±11.8 mm TL; *A. fasciatus*, 35.7 ±8.8 mm TL; *Cheirodon interruptus*, 32.4 ±6.0 mm TL)

297   and poeciliids (*Phalloceros caudimaculatus*, 28.6 ±8.7 mm TL), that occurred in the estuarine

298   zone from July to November (Fig. 3). Fish collected from the estuary in July had lower $\delta^{13}C$

299   values (-21.6‰), but the isotopic composition of these primarily freshwater species increased

300   ($F_{4,38}$= 7.51, p< 0.00) nearer to the estuarine site until approaching values (-17.7‰) similar to

301   those observed for estuarine-dependent fishes. Estuarine-resident fishes also showed significant

302   differences in their average $\delta^{13}C$ values during the flood period ($F_{1,106}$= 108.37, p< 0.00), and

303   had lower values (-18.7‰) when compared with the non-flooded period (-13.5‰) (Fig. 3).

304   Temporal variation in $\delta^{13}C$ during flooded vs. non-flooded conditions (Appendix B) also

305   occurred for some basal sources, such as microphytobenthos ($F_{1,18}$= 6.47, p< 0.02) and seston

306   ($F_{1,17}$= 11.44, p< 0.00), which had lower $\delta^{13}C$ during flooded (-21.8‰ and -22.6‰, respectively)

307   vs. non-flooded (-17.2‰ and -18.4‰, respectively) conditions. Average $\delta^{13}C$ values of other

308   sources (C_3 and C_4 plants) did not change significantly between flooding conditions ($F_{1,16}$=

309   0.0656, p< 0.80 and $F_{1,5}$ = 0.0057, p< 0.94, respectively) (Appendix B).

310   Mixing models revealed that freshwater-derived basal sources (C_3 plants and seston)

311   contributed to estuarine residents (silversides *A. brasiliensis* and *O. argentinensis*) during flood

312   conditions (April through July) (Fig. 4, left panel). Contributions of these sources were nearly

313   zero in April, but gradually increased to up to 40% in June and July. Over the same period,

314   contribution of estuarine-derived basal sources to estuarine fish biomass declined. However, two

315   months after the opening of the lagoon mouth and cessation of flood conditions and hydrologic

316   connectivity with the freshwater wetland, the contribution of freshwater-derived basal sources to

317   estuarine resident fish decreased, and C_4 plants and microphytobenthos again were the main

318   sources supporting estuarine resident fishes (Fig. 4, left panel). Primary freshwater fishes, such

319   as characids and poeciliids, assimilated carbon derived mostly from seston and C_3 plants in the

320   freshwater wetland. Conspecifics caught within the estuarine zone during the late flooding

321   period (July- August) assimilated freshwater- and estuarine-derived basal sources in similar

322   amounts. Similar to estuarine-resident fishes, the percent contribution of estuarine-derived

323   sources (microphytobenthos and C_4 plants) to these freshwater species inhabiting the estuarine

324   zone increased to up to 69% following cessation of flood conditions (Fig. 4, right panel).

325

326   **Discussion**

327   Pulsing hydrology in Lagoa do Peixe affected both habitat productivity and habitat

328   connectivity which together resulted in trophic subsidies from the freshwater wetland to

329   estuarine consumers. This subsidy occurred via passive transport of basal sources (e.g. terrestrial

330   detritus in the form of fine particulate organic matter) as well as dispersal by freshwater fishes

331 and probably other freshwater taxa along the fluvial gradient. Seston (phytoplankton plus

332 suspended particulate organic matter of undetermined origin) from the freshwater wetland was

333 apparently transported in significant quantities into the estuary during the flood pulse. The

334 assimilation of this isotopically distinct seston (approximately 4‰ and 6‰ lower $\delta^{13}C$ than

335 seston from the estuarine zone and lagoon mouth, respectively) by estuarine primary consumers

336 (e.g. zooplankton) and their subsequent consumption by fish could explain why we observed an

337 average reduction of approximately 5‰ in carbon isotope ratios of estuarine fishes (*Odonthestes*

338 *argentinesis* and *Atherinella brasiliensis*) during the flood period. Carbon isotope ratios of

339 estuarine fish gradually shifted back to typical estuarine isotopic values observed during pre-

340 flooding conditions, suggesting their prey transitioned back to assimilating locally produced

341 estuarine seston.

342 In addition to passive transport of seston during flooding conditions, food web subsidies

343 also occurred via animal dispersal from the wetland into the estuary. Animal dispersal in fluvial

344 systems is often directional, either by downstream drift or upstream swimming against prevailing

345 current (Flecker et al. 2010, Oliveira et al. 2014). For example, anadromous fish that ascend

346 rivers to spawn have been shown to deliver large amounts of nutrients from excretion and

347 carcass decomposition that subsidize freshwater and riparian ecosystems by enhancing primary

348 production (Naiman et al. 2002, Koshino et al. 2013). Juvenile marine fish that migrate into

349 small coastal streams in Brazil excrete marine-derived nutrients and are consumed by resident

350 freshwater piscivores (Oliveira et al. 2014), thereby subsidizing the base and top of the food web

351 simultaneously. During the Lagoa do Peixe flood pulse, salinity-intolerant primary freshwater

352 fishes (e.g. small characins such as *A. eigenmanniorum, A. fasciatus*, and *C. interruptus*) moved

353   into the estuarine region and began to assimilate material derived from local basal production

354   sources.

355   In this system, these species generally consume cladocerans, other microcrustacea, algae

356   and vascular plant fragments (FC, unpublished stomach contents data). Consumption of locally-

357   produced microcrustacea and vegetation would explain the spatial and temporal dynamics in

358   isotopic values for these species, especially for those specimens sampled after the opening of the

359   lagoon mouth. In addition to assimilating significant amounts of estuarine resources during their

360   brief periods of residence in the estuary, these freshwater fishes contribute to nutrient dynamics

361   via excretion and also delivered a pulse of biomass and energy that can be exploited by avian

362   (Bugoni et al. 2005) and mammalian (Colares & Waldemarin 2000) predators. Studies of other

363   coastal lagoons have demonstrated that a great diversity of freshwater fishes from different

364   trophic guilds (e.g. detritivores, zoobenthivores, piscivores) can colonize estuarine zones during

365   high freshwater discharges (Garcia et al. 2003). However, their potential effects on fitness,

366   feeding habits and survival of estuarine competitors and predators as well as overall effects to

367   estuarine food web organization remain largely unknown.

368   Estimates of spatial food web subsidies generally have involved asymmetric productivity

369   across aquatic-terrestrial or marine-terrestrial ecotones, with the more productive habitat being a

370   net donor (e.g. coastal marine systems, riparian forests) and less productive habitat being a net

371   recipient (e.g. arid islands, forest streams) (Polis et al. 2004). We are unaware of prior evidence

372   for reciprocal subsidies between aquatic habitats with similar productivity, such as freshwater

373   wetlands and estuaries. Whether or not inputs of wetland-derived allochthonous organic matter

374   and freshwater fish into estuaries induce trophic cascades probably depends on the relative

375   productivity of the two systems and the degree to which there are reciprocal subsidies. Potential

376   bottom-up and top-down effects could be further elucidated by field experiments. For example,

377   Nakano and colleagues conducted field experiments and dietary analyses to estimate reciprocal

378   subsidies across aquatic-terrestrial ecotones and their influence on trophic cascades (Nakano et al.

379   1999, Nakano & Murakami 2001). Baxter et al. (2004) demonstrated how habitat alteration and

380   introduction of alien predators suppressed flows of resources across ecotones and associated

381   reciprocal subsidies.

382   Fish guilds responded differently to the pulse of freshwater inflow and degree of

383   connectivity with the sea. Estuarine-dependent (e.g. *Brevoortia pectinata*, *Micropogonias*

384   *furnieri*, *Paralichthys orbignyanus*) and marine-vagrant (e.g. *Diapterus rhombeus*, *Eucinostomus*

385   *melanopterus*, *Menticirrhus littoralis*, *Trachinotus marginatus*, *Ulaema lefroyi*) fishes were

386   absent in mixohaline zones (estuary, lagoon mouth) during the latter stages of flooding when

387   characids, poeciliids and other freshwater fishes were prevalent. Marine fishes responded to the

388   freshwater pulse by moving southward toward the sea, but they could not exit the lagoon because

389   sand dunes blocked the outlet. Several species from the marine guild (e.g. croakers, mullets,

390   silversides) were observed in high densities within shallow pools along the margins of the outlet

391   zone. Mass mortality of marine species sometimes occurs during periods of drought as well as

392   periods of freshwater expansion into mixohaline zones (AMG, personal observation) and would

393   explain the absence of estuarine-dependent and marine-vagrant fish when the lagoon's mouth

394   was closed. Similar to other coastal lagoons in the region (Garcia et al. 2012), marine fishes

395   returned to the mixohaline zones soon after the connection to the sea was opened with machinery.

396   Because we did not sample marine habitat outside the lagoon outlet, we can only speculate about

397   effects of estuarine discharge of resources on the nearshore marine food web. Using stable

398   isotope analysis, Savage et al. (2012) showed that freshwater primary production subsidizes

422   shrimp (*Farfantepenaeus paulensis*) from the sea into the lagoon and to drain water from the

423   floodplain to increase pasture for cattle (Lanés et al. 2015). Ecological responses to artificial

424   connections between coastal lagoons and marine systems are difficult to predict, and fish species

425   diversity may decline (Saad et al. 2002), increase (Griffiths 1999), or change little (Lanés et al.

426   2015). There is general concern that artificial opening of sandbars in coastal lagoons alters

427   natural ecological dynamics with potential negative effects on biodiversity and ecosystem

428   services (Griffiths 1999, Saad et al. 2002). Our study contributes to this discourse by

429   demonstrating how the timing of freshwater inflows and the outlet opening affect food web

430   dynamics in different regions of the lagoon system.

431   Future studies of coastal ecotones could examine the influence of pulsing allochthonous

432   inputs on the distribution of trophic interactions of varying strength within the regional food web.

433   Prior theoretical and empirical work suggests that greater proportions of weak trophic

434   interactions enhance stability in species-rich food webs, and strong trophic interactions promote

435   destabilizing oscillations (Kokkoris et al. 2002, Cross et al. 2013). Landscape mosaics, including

436   fluvial ecosystems and other ecotones, might have greater proportions of weak links within the

437   regional food web when there is periodic exchange of organisms among habitat compartments

438   (Bellmore et al. 2015). Research that combines comparative and experimental approaches to

439   estimate interaction strength could test this hypothesis and further elucidate ecological responses

440   to pulsing hydrology and food web subsidies.

441

450

451

Literature Cited

453   Abrantes KG, Barnett A, Bouillon S (2013) Stable isotope-based community metrics as a tool to

454   identify patterns in food web structure in east African estuaries. Funct Ecol 28:270-282

455   Baxter CV, Fausch KD, Murakami M, Chapman PL (2004) Fish invasion restructures stream and

456   forest food webs by interrupting reciprocal prey subsidies. Ecology 85:2656–2663

457   Bellmore JR, Baxter CV, Connolly PJ (2015) Spatial complexity reduces interaction strengths in

458   the meta-food web of a river floodplain mosaic. Ecology 96:274-283

459   Bugoni L, Cormons TD, Boyne AW, Hays H (2005) Feeding Grounds, Daily Foraging Activities,

460   and Movements of Common Terns in Southern Brazil, Determined by Radio-telemetry.

461   Waterbirds 28(4): 468-477

462   Chick JH, Ruetz III CR, Trexler JC (2004) Spatial scale and abundance patterns of large fish

463   communities in freshwater marshes of the Florida Everglades. Wetlands 24(3): 652–664

464   Childers DL (2006) A synthesis of long-term research by the Florida Coastal Everglades LTER

465   Program. Hydrobiologia 569:531–544

466 Claudino MC, Abreu PC, Garcia AM (2013) Stable isotopes reveal temporal and between-habitat

467   changes in trophic pathways in a southwestern Atlantic estuary. Mar Ecol Progr Ser 489:29-

468   42

469 Claudino MC, Pessanha ALM, Araújo FG, Garcia AM (2015) Trophic connectivity and basal

470   food sources sustaining tropical aquatic consumers along a mangrove to ocean gradient.

471   Estuar Coast Shelf Sci 167:45-55

472 Colares EP, Waldemarin HF (2000) Feeding of the Neotropical River Otter (*Lontra longicaudis*)

473   in the Coastal Region of the Rio Grande do Sul State, Southern Brazil IUCN Otter Spec.

474   Group Bull. 17 (1): 6 - 13

475 Cross WF, Baxter CV, Rosi-Marshall EJ, Hall RO, Kennedy TA, Donner KC, Wellard Kelly HA,

476   Seegert SEZ, Behn KE, Yard MD (2013) Food-web dynamics in a large river discontinuum.

477   Ecol Monogr 83:311–337

478 DeAngelis DL, Loftus WF, Trexler JC, Ulanowicz RE (1997) Modeling fish dynamics and

479   effects of stress in a hydrologically pulsed ecosystem. J Aquat Ecosyst Stress Recovery 6: 1–

480   13

481 DeAngelis DL, Trexler JC, Loftus WF (2005) Life history trade-offs and community dynamics

482   of small fishes in a seasonally pulsed wetland. Canadian J Fish Aquat Sci 62: 781–790

483 Flecker AS, Mcintyre PB, Moore JW, Anderson JT, Taylor BW, Hall RO. (2010) Migratory

484   fishes as material and process subsidies in riverine ecosystems. American Fisheries Society

485   Symposium 73:559–592

486 Garcia AM, Vieira JP, Winemiller KO (2001) Dynamics of the shallow-water fish assemblage of

487   the Patos Lagoon estuary (Brazil) during cold and warm ENSO episodes. J Fish Biol

488   59:1218–1238

489 Garcia AM, Raseira MB, Vieira JP, Winemiller KO, Grimm AM (2003) Spatiotemporal

490 variation in shallow-water freshwater fish distribution and abundance in a large subtropical

491 coastal lagoon. Environ Biol Fish 68:215–228

492 Garcia AM, Vieira JP, Winemiller KO, Grimm AM (2004) Comparison of the 1982-1983 and

493 1997-1998 El Niño effects on the shallow-water fish assemblage of the Patos Lagoon estuary

494 (Brazil). Estuaries 27:905-914

495 Garcia AM, Bemvenuti MA, Vieira JP, Marques DMLM, Burns MDM, Moresco A, Condini

496 MVL (2006) Checklist comparison and dominance patterns of the fish fauna at Taim

497 Wetland, South Brazil. Neotrop Ichthyol 4: 261–268

498 Garcia AM, Hoeinghaus DJ, Vieira JP, Winemiller KO (2007) Isotopic variation of fishes in

499 freshwater and estuarine zones of a large subtropical coastal lagoon. Estuar Coast Shelf S

500 73:399-408

501 Garcia AM, Vieira JP, Winemiller KO, Moraes LE, Paes ET (2012) Factoring scales of spatial

502 and temporal variation in fish abundance in a subtropical estuary. Mar Ecol Prog Ser

503 461:121-135

504 Garcia AM, Claudino MC, Mont'alverne R, Pereyra PER, Copertino M, Vieira JP (in press)

505 Temporal variability in food assimilation of basal food sources by an omnivorous fish at

506 Patos Lagoon Estuary revealed by stable isotopes (2010-2014). Mar Biol Res

507 10.1080/17451000.2016.1206939

508 Griffiths SP (1999) Consequences of artificially opening coastal lagoons on their fish

509 assemblages. Inter J Salt Lake Research 8:307-327

510 Hoeinghaus DJ, Vieira JP, Costa CS, Bemvenuti CE, Winemiller KO, Garcia AM (2011) Estuary

511 hydrogeomorphology affects carbon sources supporting aquatic consumers within and among

512   ecological guilds. Hydrobiologia 673:79-92

513 Holt RD (2008) Theoretical perspectives on resource pulses. Ecology 89:671-681

514 Jardine TD, Pettit NE, Warfe DM, Pusey BJ, Ward DP, Douglas MM, Davies PM. Bunn SE

515   (2012) Consumer–resource coupling in wet–dry tropical rivers. J Anim Ecol 81: 310–322

516 Kaymak N, Winemiller KO, Akin S, Altuner Z, Polat F, Dal T (2015) Stable isotope analysis

517   reveals relative influences of seasonal hydrologic variation and impoundment on assimilation

518   of primary production sources by fish in the Upper Yesilırmak River, Turkey. Hydrobiologia

519   753:131–147

520 Knak RB (2004) Management plan of Lagoa do Peixe National Park (in Portuguese).

521   IBAMA/FNMA/FURG/ NEMA/UFPel, Brasília. 465 p.

522 Kokkoris GD, Jansen VAA, Loreau M, Troumbis AY (2002) Variability in interaction strength

523   and implications for biodiversity. J Anim Ecol 71:362–371

524 Koshino Y, Kudo H, Kaeriyama M (2013) Stable isotope evidence indicates the incorporation

525   into Japanese catchments of marine-derived nutrients transported by spawning Pacific

526   Salmon. Freshwater Biol 58:1864–1877

527 Krenz C, Menge BA, Freidenburg TL, Lubchenco J, Chan F, Foley MM, Nielsen KJ (2011)

528   Ecological subsidies to rocky intertidal communities: linear or non-linear changes along a

529   consistent geographic upwelling transition? J Exp Mar Biol Ecol 409:361–370

530 Lanés LEK, Rolon AS, Stenert C, Maltchik L (2015) The effects of annual artificial opening of a

531   natural sandbar of a lagoon system on the fish community. J Appl Ichthyol 31:321-327

532 Leibhold A, Koenig WD, Bjørnstad ON (2004) Spatial synchrony in population dynamics.

533   Annual Review of Ecology and Systematics 35:467-490

534 Maltchik L, Lanés LEK, Stenert C, Medeiros ESF (2010) Species-area relationship and

535   environmental predictors of fish communities in coastal freshwater wetlands of southern

536   Brazil. Environ Biol Fish 88:25–35

537 Marshall JD, Brooks JR, Lajtha K (2007) Sources of variation in the stable isotopic composition

538   of plants. In: Mitchener R, Lajtha K (eds) Stable isotopes in ecology and environmental

539   science. Blackwell Scientific, Oxford, p 22-60

540 Mattson WJ, Addy ND (1975) Phytophagous insects as regulators of forest primary production.

541   Science 190:515-522

542 McCormick MI (2003) Consumption of coral propagules after mass spawning enhances larval

543   quality of damselfish through maternal effects. Oecologia, 136:37-45

544 Myers GS (1938) Freshwater fishes and West Indian zoogeography. Annual Report of the Board

545   of Regents of the Smithsonian Institution 92: 339–364

546 Naiman RJ, Bilby RE, Schindler DE, Helfied JM (2002) Pacific salmon, nutrients, and the

547   dynamics of freshwater and riparian ecosystems. Ecosystems 5:399-417

548 Nakano S, Murakami M (2001) Reciprocal subsidies: dynamic interdependence between

549   terrestrial and aquatic food webs. P Natl Acad Sci 98:166–170

550 Nakano S, Miyasaka H, Kuhara N (1999) Terrestrial-aquatic linkages: riparian arthropod inputs

551   alter trophic cascades in a stream food web. Ecology 80:2435–2441

552 Oliveira MCLM, Bastos RF, Claudino MC, Assumpção CM, Garcia AM (2014) Transport of

553   marine-derived nutrients to subtropical freshwater food webs by juvenile mullets: a case

554   study in southern Brazil. Aquat Biol 20:91-100

555 Ostfeld RS, Keesing F (2000) Pulsed resources and community dynamics of consumers in

556   terrestrial ecosystems. Trends Ecol Evol 15:232-237

557 Ou C, Winemiller KO (2016) Seasonal hydrology shifts production sources supporting fishes in

558   rivers of the Lower Mekong Basin. Canadian Journal of Fisheries and Aquatic Sciences,

559   10.1139/cjfas-2015-0214

560 Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes:

561   coping with too much variation. PLoS ONE 5:e9672

562 Petersen JK, Hansen JW, Laursen MB, Clausen P, Carstensen J, Conley DJ (2008) Regime shift

563   in a coastal marine ecosystem. Ecol Appl 18(2): 497–510

564

565 Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Evol Syst

566   18:293-320

567 Petraitis PS, Latham RE (1999) The importance of scale in testing the origins of alternative

568   community states. Ecology 80:429-442

569 Poff NL, Allan JD, Bain MB, Karr JR, Prestegaard KL, Richter BD, Sparks RE, Stromberg JC

570   (1997) The natural flow regime: a paradigm for river conservation and restoration.

571   Bioscience 47:769-784

572 Polis GA, Power ME, Huxel GR eds. (2004) Food webs at the landscape level. University of

573   Chicago Press, Chicago, Illinois, USA.

574 Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web

575   ecology: the dynamics of spatially subsidized food webs. Annu Rev Ecol Syst 28:289-316

576 Pratchett MS, Gust N, Goby G, Klanten SO (2001) Consumption of coral propagules represents

577   a significant trophic link between corals and reef fish. Coral Reefs 20:13-17

578 Rolon AS, Rocha O, Maltchick L (2011) Diversity of aquatic macrophytes in the Lagoa do Peixe

579   National Park. Neotrop Biol Conserv 6(1):5-12

580 Rozas LP, Minello TJ (1997) Estimating densities of small fishes and decapod crustaceans in

581   shalow estuarine habitats: a review of sampling design with focus on gear selection.

582   Estuaries, 20: 199-213

583   Saad AM, Beaumond AC, Caramaschi EP (2002) Effects of artificial canal openings on fish

584   community structure of Imboassica coastal lagoon, Rio de Janeiro, Brazil. J Coastal Res

585   36:634–639

586   Savage C, Thrush SF, Lohrer AM, Hewitt JE (2012) Ecosystem services transcend

587   boundaries: estuaries provide resource subsidies and influence functional diversity in

588   coastal benthic communities. PLoS ONE: 7:e42708.

589   Spiller DA, Piovia-Scott J, Wright AN, Yang LH, Takimoto G, Schoener TW, Iwata T

590   (2010) Marine subsidies have multiple effects on coastal food webs. Ecology 91:1424-

591   1434

592   Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet d15N enrichment: a

593   meta-analysis. Oecologia 136:169-182

594   Warfe DM, Pettit NE, Davies PM, Pusey BJ, Hamilton SK, Kennard MJ, Townsend SA, Bayliss

595   P, Ward DP, Douglas MM, Burford MA, Finn M, Bunn SE, Halliday IA (2011) The 'wet-

596   dry' in the wet-dry tropics drives ecosystem structure and function of northern Australian

597   rivers. Freshw Biol 56: 2169–2195

598   Winemiller KO (1990) Spatial and temporal variation in tropical fish trophic networks. Ecol

599   Monogr 60:331-367Winemiller KO, Layman CA (2005) Food web science: moving on the

600   path from abstraction to prediction. In: Ruiter PC, Wolters V, Moore JC (eds) Dynamic Food

601   Webs: Multispecies Assemblages, Ecosystem Development and Environmental Change.

602   Elsevier, Amsterdam, Netherlands, p 10-23

603   Yang LH (2004) Periodical cicadas as resource pulses in North American forests. Science

604   306:1565-1567

605   Yang LH, Bastow JL, Spence KO, Wright AN (2008) What can we learn from resource pulses?

606   Ecology 89:621-634

607   Zackrisson O, Nilsson MC, Jaderlund A, Wardle DA (1999) Nutritional effects of seed fall

608   during mast years in boreal forest. Oikos 84:17-26

609   Zar JH (1984) Biostatistical analysis. 2nd ed. Prentice-Hall, Englewood Cliffs, New Jersey, USA

610

Figure and legends

611   **Figure and legends**

612   Figure 1. Upper. Panel. Map showing the Lagoa do Peixe National Park (LPNP) in the coastal

613   plain of southern Brazil with the location of the sampled areas (squares) at the freshwater

614   wetland (1), at the estuarine zone (2) and at the lagoon's mouth (3). Red dashed line denotes the

615   boundaries of the national park. Pictures taken at the mouth of the lagoon on April 2th and

616   November 11th 2008 showing the status of its connection with the sea (C and D) and in the same

617   location of the estuarine zone showing the flooded (A) and non-flooded conditions (B). Photos

618   by A.M.Garcia. Lower panel. Monthly variation in rainfall (mm, bars) and water temperature (^{o}C,

619   line) during the study period (April 2008 – May 2009).

620

621   Figure 2. Mean (+SD) of δ^{15}N and δ^{13}C values for primary producers and organic sources (open

622   circles numbered 1 to 4), prey and fishes (left panel) and relative contributions of basal food

623   sources (C_3 and C_4 plants, seston and microphytobentos-MiPhBe) to fishes at the freshwater

624   wetland, estuarine zone and lagoon mouth sites (right panel).

625

626   Figure 3. Monthly variation in the δ^{15}N and δ^{13}C values for fish guilds (primary freshwater–

627   open circles; secondary freshwater– open triangles; estuarine dependent– grey circles; estuarine

628   resident– grey triangles; marine vagrants– dark squares) from the freshwater wetland, estuarine

629   zone and lagoon mouth. Vertical bar on the right denotes flood status within the estuarine zone.

630

631   Figure 4. Monthly relative contributions of basal food sources (C_3 and C_4 plants, seston and

632   microphytobentos) to biomass of estuarine-resident fish (*Atherinella brasiliensis*, *Odonthestes*

633   *argentinensis*) (left panel) and primary freshwater fish (*Astyanax eigenmanniorum*, *A. fasciatus*,

634   *Cheirodon interruptus*, *Phalloceros caudimaculatus*) from the estuarine zone during flooding

635   and non-flooding conditions. Each plot shows the 50 (black), 75 (gray) and 95% (lighter gray)

636   Bayesian credibility intervals of the feasible contributions of each basal production source to fish
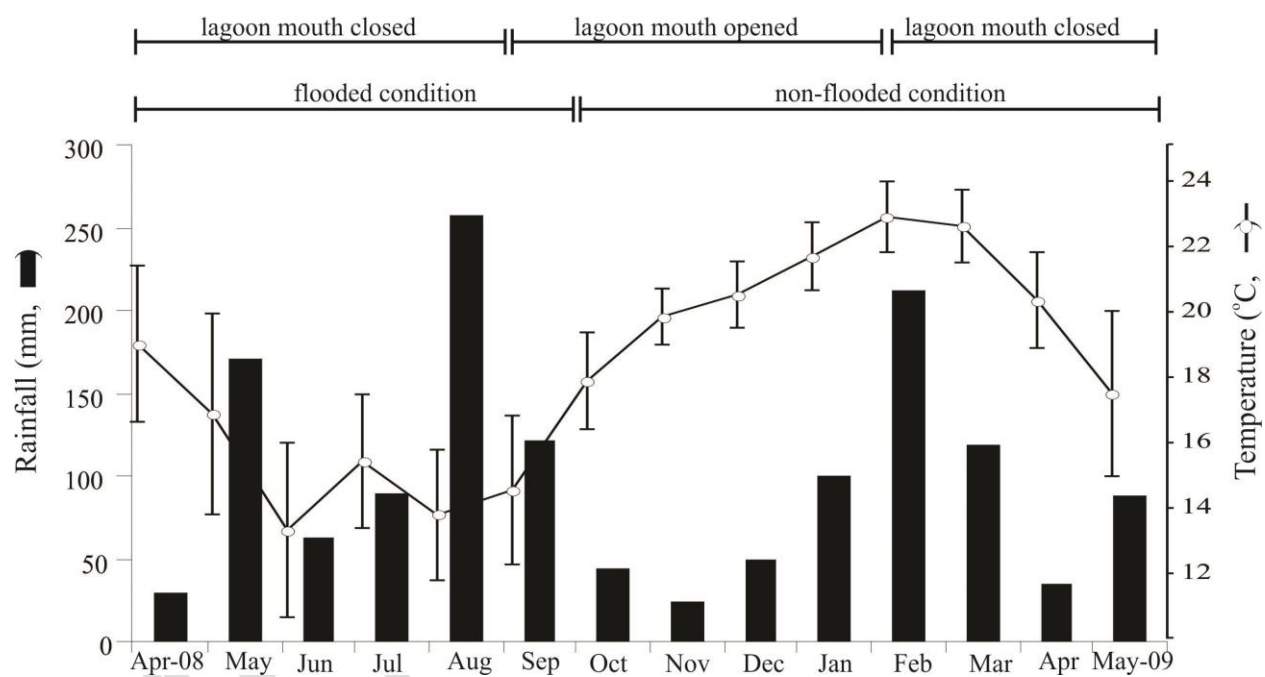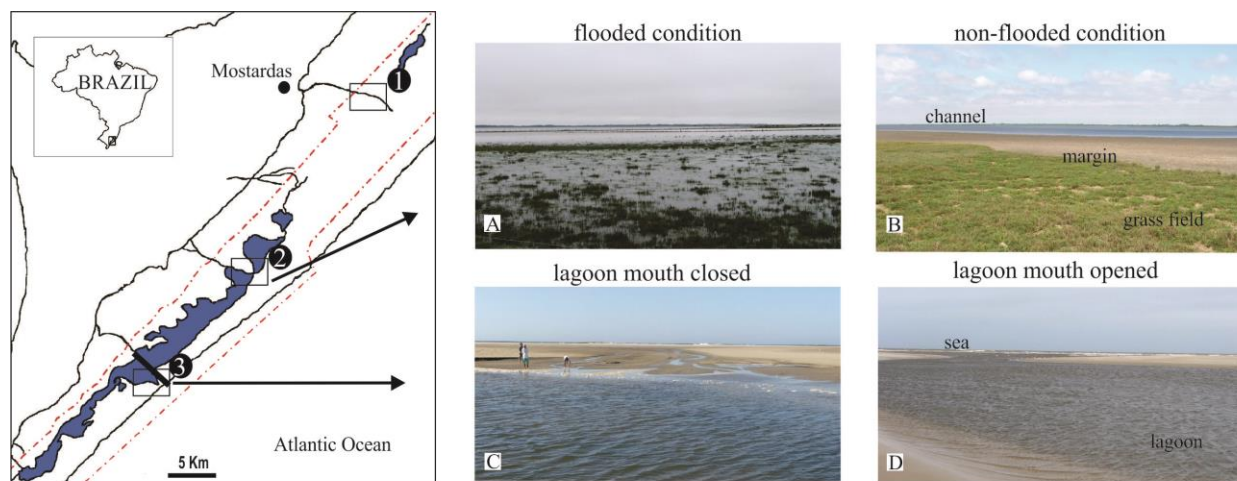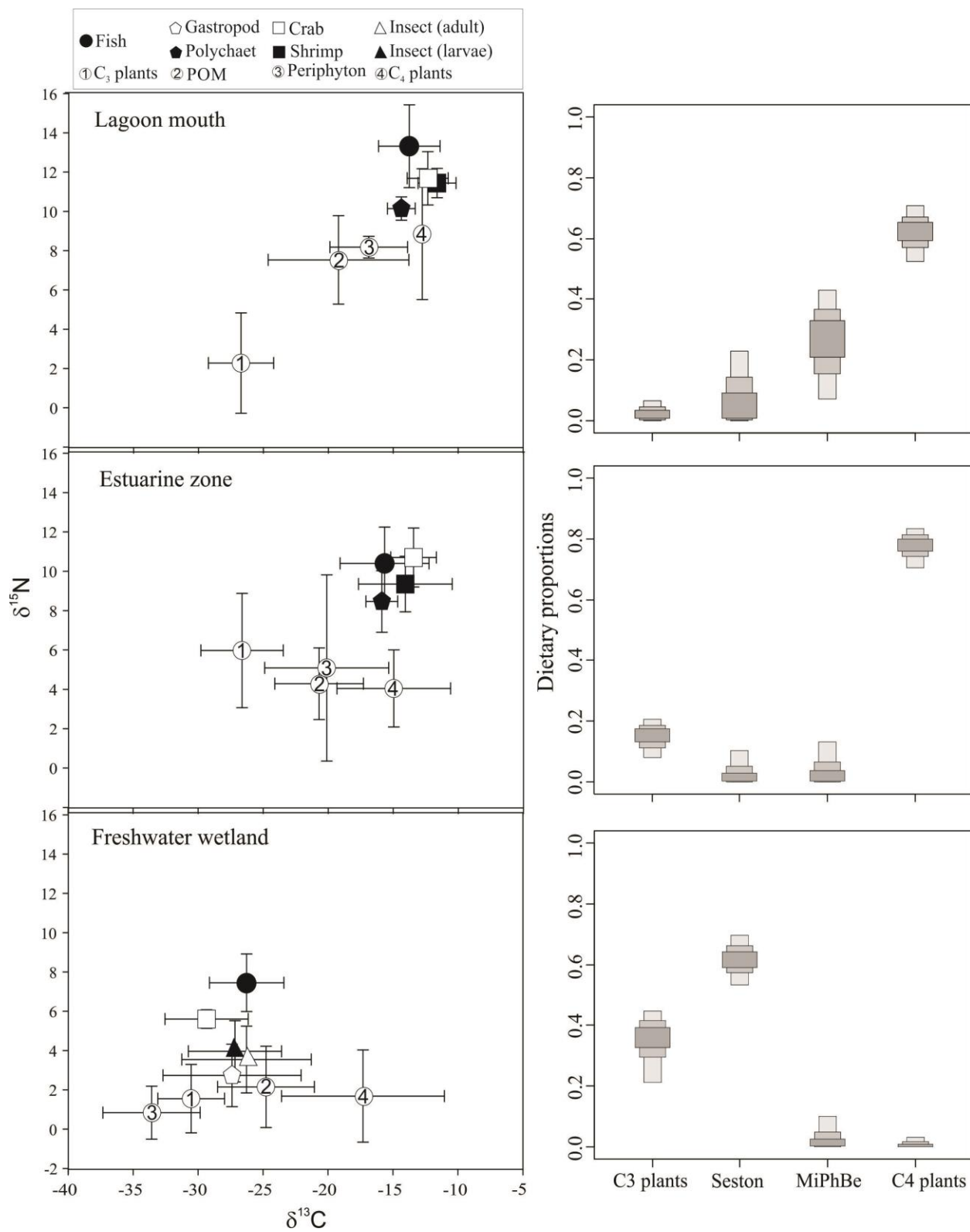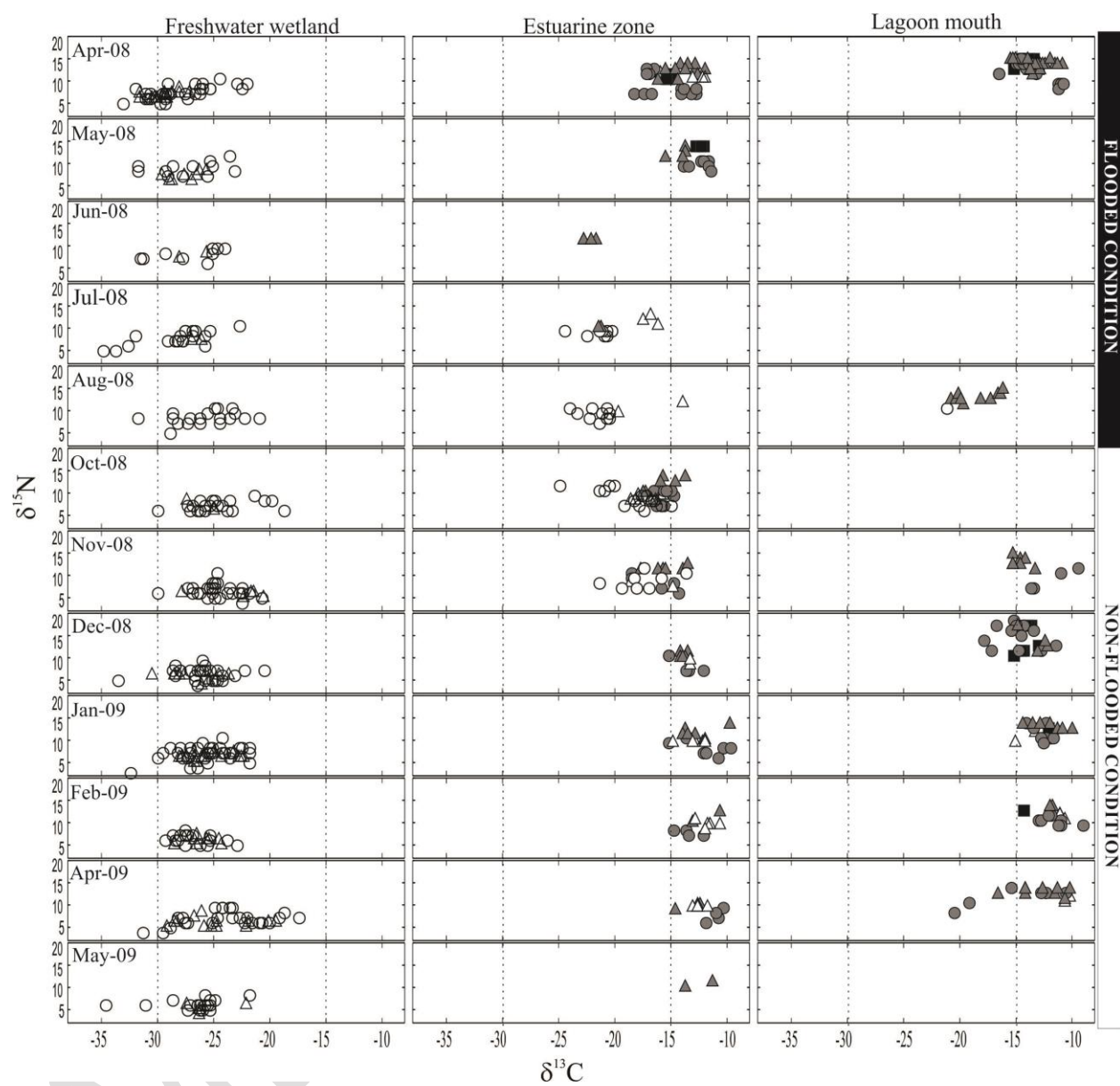
637   in each month.

638

639

640

641

642

643

644

645   Figure 1

646

647

648

649   Figure 2

650

651

652

653   Figure 3

654

655

656   Figure 4

657

658

659 **Supplemental material**

660

661 Appendix A. δ^{13}C and δ^{15}N values (X±SD) and total length (TL, mm) of fishes, invertebrates and

662 plants and organic sources at the freshwater wetland, estuarine zone and lagoon mouth of the

663 Lagoa do Peixe National Park. n, sample size. Functional guilds (GUI): primary freshwater (PF),

664 secondary freshwater (SF), estuarine resident (ER), estuarine dependent (ED), marine vagrants

665 (MV).

666

667 Appendix B. Monthly variation in the δ^{15}N and δ^{13}C values for basal food sources

668 (microphytobenthos, open circles; seston, open triangles; C_3 plants, grey circles; C_4 plants) at the

669 freshwater wetland, estuarine zone and lagoon mouth sites. Vertical bar on the right denotes

670 status of the flooding conditions in the estuarine zone.

671

672   SUPPLEMENTAL MATERIAL

673   # Appendix A

Table S1 - δ^{13}C and δ^{15}N values (X±SD) and total length (TL, mm) of fishes, invertebrates and plants and organic sources at the freshwater wetland, estuarine zone and lagoon mouth of teh Lagoa do Peixe National Park. n, sample size. Functional guilds (GUI): primary freshwater (PF), secondary freshwater (SF), estuarine resident (ER), estuarine dependent (ED), marine vagrants (MV).

		Freshwater wetland				Estuarine zone				Lagoon mouth			
	GUI	n	TL	^{13}C	^{15}N	n	TL	^{13}C	^{15}N	n	TL	^{13}C	^{15}N
Fishes													
Astyanax eigenmanniorum	PF	34	56.8 ± 16.5	-26.0 ± 2.6	8.2 ± 1.5	8	59.8 ± 11.8	-21.1 ± 2.6	10.1 ± 1.9	1	76.0	-21.1	11.3
Astyanax fasciatus	PF	5	56.5 ± 24.4	-25.4 ± 2.8	7.4 ± 1.3	4	35.7 ± 8.8	-17.8 ± 1.8	10.0 ± 0.3				
Astyanax jacuhiensis	PF	12	57.1 ± 25.6	-29.1 ± 3.2	7.4 ± 0.8	1	45.0	-21.5	8.9				
Astyanax sp	PF	1	47.0	-28.4	7.8								
Callichthys callichthys	PF	12	106.1 ± 39.0	-26.0 ± 3.6	7.7 ± 0.9								
Characidium rachovii	PF	2	40.5 ± 10.7	-28.7 ± 5.7	6.3 ± 1.1								
Cheirodon ibicuhiensis	PF	2	33.5 ± 13.4	-25.1 ± 3.4	9.4 ± 0.6	1	47.0	-19.2	8.3				
Cheirodon interruptus	PF	20	35.0 ± 6.8	-24.9 ± 4.0	8.7 ± 1.4	11	32.4 ± 6.0	-19.3 ± 2.6	9.5 ± 0.7				
Cnesterodon decemmaculatus	PF	1	35.0	-21.1	9.0								
Corydoras paleatus	PF	12	47.9 ± 12.7	-27.5 ± 2.2	7.1 ± 1.0								
Cynopoecilus melanotaenia	PF	3	43.2 ± 12.9	-24.8 ± 1.0	7.0 ± 0.8	1	29.0	-21.4	7.3				
Cyphocharax saladensis	PF	7	50.6 ± 10.2	-30.8 ± 3.4	5.1 ± 0.9								
Cyphocharax voga	PF	10	79.9 ± 47.6	-27.9 ± 3.9	6.1 ± 2.1								
Gymnotus carapo	PF	1	273.0	-27.0	8.0								
Hoplias malabaricus	PF	45	202.8 ± 91.9	-25.0 ± 1.8	8.8 ± 1.4								
Hoplosternum littorale	PF	1	212.0	-27.7	8.2								
Hyphessobrycon bifasciatus	PF	24	36.7 ± 10.6	-27.6 ± 2.1	6.8 ± 1.0								
Hyphessobrycon boulengeri	PF	18	44.0 ± 8.8	-26.4 ± 1.6	7.0 ± 1.2	1	43.0	-17.0	8.1				
Hyphessobrycon luetkenii	PF	4	28.0 ± 7.7	-28.1 ± 2.6	7.3 ± 1.3								
Mimagoniates inequalis	PF	11	27.3 ± 5.5	-27.7 ± 1.8	8.1 ± 1.4								
Oligosarcus jenynsii	PF	2	109.5 ± 10.6	-25.4 ± 2.3	11.2 ± 1.5	7	81.4 ± 23.6	-19.0 ± 2.2	11.1 ± 1.8				
Oligosarcus robustus	PF	3	38.7 ± 10.6	-25.0 ± 1.5	6.1 ± 0.7								
Phalloceros caudimaculatus	PF	18	31.3 ± 6.7	-25.4 ± 2.3	7.4 ± 0.9	7	28.7 ± 8.7	-22.0 ± 2.0	9.6 ± 0.7				
Pimelodella australis	PF	1	53.0	-20.0	9.4								
Pseudocorynopoma doriae	PF	5	48.8 ± 11.3	-25.7 ± 0.4	8.0 ± 1.3								
Rhamdia quelen	PF	7	180.5 ± 134.4	-23.5 ± 2.5	7.2 ± 0.9	2	48.3 ± 11.0	-16.7 ± 1.1	7.3 ± 1.0				
Synbranchus marmoratus	PF	6	216.0 ± 120.7	-23.7 ± 2.1	7.1 ± 0.9								
Australoheros facetum	SF	35	83.1 ± 29.2	-26.9 ± 2.4	6.9 ± 0.8								
Cichlasoma portalegrense	SF	7	84.1 ± 34.6	-23.8 ± 2.4	6.4 ± 0.9								
Crenicichla lepidota	SF	19	127.2 ± 36.5	-27.4 ± 1.3	7.0 ± 1.2								
Geophagus brasiliensis	SF	15	95.7 ± 37.5	-27.9 ± 1.3	6.6 ± 0.6								
Jenynsia multidentata	SF	9	41.5 ± 18.3	-22.1 ± 1.7	6.8 ± 1.0	33	39.9 ± 13.2	-14.4 ± 2.5	10.0 ± 1.1	8	44.6 ± 12.2	-11.8 ± 1.6	11.8 ± 0.9
Platanichthys platana	SF					1	47.0	-16.0	12.7				
Atherinella brasiliensis	ER					17	76.3 ± 36.4	-13.8 ± 1.4	12.0 ± 1.4	15	69.7 ± 33.4	-12.8 ± 2.6	13.4 ± 0,7
Odontesthes argentinensis	ER					34	104.6 ± 84.7	-15.5 ± 3.5	12.1 ± 1.1	41	140.3 ± 76.2	-14.5 ± 2.6	13.9 ± 1.2
Brevoortia pectinata	ED					11	138.1 ± 111.7	-17.0 ± 1.3	10.5 ± 2.1	9	83.6 ± 20.9	-13.5 ± 1.8	11.5 ± 0.5
Lycengraulis grossidens	ED									3	117.7 ± 9.2	-14.0 ± 0.6	15.1 ± 0.3
Micropogonias furnieri	ED					3	134.3 ± 76.0	-14.9 ± 2.1	12.0 ± 0.8	19	180.1 ± 97.4	-14.1 ± 1.4	15.1 ± 2.4
Mugil curema	ED					8	68.0 ± 38.8	-13.3 ± 2.5	7.9 ± 1.1				
Mugil platanus	ED					31	135.3 ± 88.7	-13.2 ± 1.6	9.1 ± 1.2	22	133.5 ± 101.7	-12.9 ± 3.2	10.6 ± 1.7
Paralichthys orbignyanus	ED									7	170.1 ± 99.3	-14.0 ± 1.0	14.0 ± 1.3
Anchoa marinii	MV									1	74.0	-15.3	12.8
Diapterus rhombeus	MV					2	70.0	-15.4 ± 0.1	11.3 ± 0.1				
Eucinostomus gula	MV					1	68.0	-15.2	11.9				
Eucinostomus melanopterus	MV					1	57.0	-12.2	14.4	3	90.7 ± 6.4	-14.0 ± 0.4	13.9 ± 0.6
Gereidae	MV									1	152.0	-14.4	15.1
Harengula clupeola	MV									1	65.0	-14.5	14.2
Hemiramphus	MV									1	232.0	-14.1	14.1
Menticirrhus littoralis	MV									6	278.5 ± 15.2	-14.0 ± 0.3	17.0 ± 1.3
Stellifer brasiliensis	MV									4	37.3 ± 11.1	-14.4 ± 0.9	12.3 ± 0.7
Trachinotus marginatus	MV									4	44.1 ± 28.1	-13.7 ± 1.2	14.1 ± 1.3
Ulaema lefroyi	MV					2	59.0 ± 1.4	-12.6 ± 0.2	14.5 ± 0.1				
Invertebrates													
Crab		4		-29.3 ± 3.2	5.6 ± 0.5	35		-13.4 ± 1.8	10.7 ± 1.5	36		-12.3 ± 1.6	11.7 ± 1.4
Gastropode		31		-27.4 ± 5.3	2.8 ± 1.6								
Insect (adult)		22		-26.3 ± 5.0	3.6 ± 1.7								
Insect (larvae)		17		-27.2 ± 3.6	4.0 ± 1.6								
Polychaeta						20		-15.9 ± 1.2	8.5 ± 1.6	5		-14.4 ± 1.1	10.2 ± 0.6
Shrimp		1		-25.9	8.4	12		-14.1 ± 3.6	9.4 ± 1.4	12		-11.6 ± 1.5	11.5 ± 0.7
Plants and organic sources													
C3 plants		20		-30.5 ± 2.6	1.6 ± 1.7	18		-26.6 ± 3.2	6.0 ± 2.9	7		-26.7 ± 2.5	2.3 ± 2.6
C4 plants		4		-17.3 ± 6.3	1.7 ± 2.3	7		-15.0 ± 4.4	4.1 ± 2.0	3		-12.8 ± 0.3	8.9 ± 3.3
Seston		14		-24.8 ± 3.7	2.2 ± 2.1	19		-20.7 ± 3.4	4.3 ± 1.8	10		-19.2 ± 5.4	7.6 ± 2.3
Microphytobenthos		8		-33.6 ± 3.7	0.8 ± 1.3	20		-19.7 ± 4.6	4.3 ± 3.0	3		-16.9 ± 3.0	8.2 ± 0.6

674

675

676   SUPPLEMENTAL MATERIAL

677   **Appendix B**

678

679