

1 **Title:** Does reef structure affect oyster food resources? A stable isotope assessment.
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34 **Running title:** Reef structure and oyster food resources.
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37 **Abstract**

38 As ecosystem engineers, oysters create and maintain structured habitat and can influence
39 trophodynamics and benthic-pelagic coupling in the surrounding landscape. The physical reef
40 structure and associated biotic parameters can affect the availability of food resources for
41 oysters. Oysters and potential composite food sources — suspended particulate organic matter
42 (SPOM) and surface sediment organic matter (SSOM) — were assessed using a dual stable
43 isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) approach at three reef types (natural, restored, and unconsolidated)
44 seasonally for two years to determine if changes in physical and/or biotic parameters affected the
45 relative availability and/or use of food resources by oysters. SPOM was more depleted in ^{13}C (-
46 24.2 ± 0.6 ‰, mean \pm SD) than SSOM (-21.2 ± 0.8 ‰). SPOM composition is likely dominated
47 by autochthonous phytoplankton production, while SSOM includes trapped phytoplankton and
48 benthic microalgae. SSOM was used by oysters in increasing proportions relative to SPOM over
49 time at all reef types. This temporal trend is likely due to increased oyster biomass over time,
50 promoting enhanced microphytobenthos growth through feedback effects related to oyster
51 biodeposits. Structural differences between reef types observed in this study had no effect on
52 food resource availability and use by oysters, indicating strong benthic-pelagic coupling likely
53 due to shallow depths as well as strong and consistent winds. This study provides insights for
54 restoration of oyster reefs as it highlights that food resources used by oysters remain similar
55 among reef types despite changes in abiotic and biotic parameters among habitats and over time.

56 **Keywords:**

57 food source; oyster reef; benthic-pelagic coupling; stable isotopes; suspended particulate organic
58 matter; sediment organic matter; Gulf of Mexico

59

60 **1. Introduction**

61 Eastern oysters, *Crassostrea virginica*, are ecosystem engineers that create and maintain
62 structured biogenic habitat in estuarine areas and provide resources for other organisms. Oyster
63 reefs serve as nursery grounds for a number of commercially and ecologically important species
64 by enhancing survival and abundance (Beck et al. 2001). Oyster reefs substantially augment
65 production ($>200 \text{ g m}^{-2} \text{ y}^{-1}$) for associated fish and mobile crustaceans (Peterson et al. 2003, zu
66 Ermgassen et al. 2015). Oysters help regulate nutrient dynamics and mediate benthic-pelagic
67 coupling through their suspension-feeding activities (Beseres Pollack et al. 2013, Dame et al.
68 1984, Newell 2004). Oysters filter large amounts of particulate matter from the water column
69 and excrete particles as biodeposits, thereby transferring organic matter from the water column to
70 the benthos (Dame et al. 1984, Dame 2012, Newell 2004, Prins et al.1998). Moreover, the three-
71 dimensional structure of oyster reefs provides a large range of microhabitats, increasing the
72 diversity of organisms and supporting complex trophic webs (Bateman and Bishop 2017,
73 Grabowski and Peterson 2007, Summerhayes et al. 2009).

74 Oysters can rely on a large diversity of food resources, whose availability relates to both
75 temporal and spatial variation. Food resources present in estuarine water columns can include
76 phytoplankton, detritus, bacteria, and resuspended materials including benthic microalgae
77 (Cranford et al. 2005, Dame 2012, Grant et al. 1997, Leal et al. 2008, Riera and Richard 1996).
78 The composition of suspended particulate organic matter can vary spatially with relative location
79 within an estuary (i.e., with relative influences of riverine and oceanic inputs; Leal et al. 2008,
80 Quan et al. 2012, Riera and Richard 1996) and with relative proximity to other habitats (e.g., salt
81 marshes, tidal flats; Kang et al. 2003, Quan et al. 2012, Riera and Richard 1996, Winemiller et
82 al. 2007). Availability of food resources also varies temporally, with daily, seasonal and perhaps

83 longer-term climatic cycles (Cranford and Hill 1999, Grant et al. 1997, MacIntyre and Cullen
84 1996, Prins et al. 1998, Reyna et al. 2017). Tidal currents, wave action, high winds and human
85 activities can cause resuspension of bottom sediments and organic matter, further influencing
86 food resource composition available to suspension-feeders at varying spatial and temporal scales
87 (Grant et al. 1997, Miller et al. 1996, Prins et al. 1998). Additionally, oysters can promote the
88 growth of microphytobenthos through their biodeposits, effectively transferring nutrients to the
89 sediments where they can fuel microalgae growth (Hargrave et al. 2008, Haven and Morales-
90 Alamo 1966, Leal et al. 2008, Prins et al. 1998).

91 The physical structure of the habitat (e.g. sediment grain size, vertical relief) and the
92 biotic parameters (e.g., oyster density, biomass) themselves can affect the availability of food
93 resources for oysters (Dame et al. 1984), and therefore the production of oyster reefs (Soniati et
94 al. 2004). Indeed, resuspension of sediment and organic matter is higher in open, bare habitats or
95 where sediment grain size is small—increasing the availability of benthic food resources for
96 oysters—than in enclosed or structured habitats or coarse sediment (Grant et al. 1997, Sundbäck
97 1984). However, extraordinarily high sediment resuspension can also have negative effects on
98 suspension-feeders. High turbidity caused by resuspended sediments can decrease filtration
99 ability and dilute food concentrations (Dame 2012, Grant et al. 1990, Newell and Langdon
100 1996). Higher biomass of oysters, leading to a higher three-dimensional complexity, may
101 decrease water flow speed over the reef and increase deposition of particles from the water
102 column (Colden et al. 2016).

103 The vertical relief of reefs may also influence the availability of sediment food sources to
104 oysters. Because large amounts of suspended sediment can contribute to the burial of oyster
105 reefs, approaches for reef restoration have moved to constructing higher vertical relief reefs to

106 prevent sedimentation and burial (Bartol et al. 1999, Grabowski 2004, Lenihan 1999, Powers et
107 al. 2009, Schulte et al. 2009). However, high vertical relief could subsequently alter the degree
108 of benthic-pelagic coupling and flow of organic matter through reef ecosystems (Fréchette et al.
109 1989, Quan et al. 2012). Thus, as movement continues toward designing and building higher
110 vertical relief reefs, it is important to understand how the relative availability of different food
111 sources may change with reef height.

112 The aim of this study is to compare the food resources used by oysters in several oyster
113 reef types to determine if changes in physical and/or biotic parameters affect (1) the availability
114 of potential food sources to oysters, and (2) the use of pelagic vs. benthic food sources by
115 oysters. We examined the relative contributions of water column and benthic food resources to
116 subtidal oysters (*Crassostrea virginica* Gmelin) in a shallow Texas estuary using a dual stable
117 isotope ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) approach. Analysis of stable isotope composition, particularly carbon
118 isotope composition, can be used to elucidate the different food resources utilized by consumers
119 in natural settings (Fry and Sherr 1984, Fry 2006, Peterson 1999). Three reef types with
120 different characteristics were compared: (1) a natural reef, (2) a restored reef, and (3) an
121 unconsolidated reef. Field sampling was conducted over a two year period to examine how the
122 use of food resources by oysters may differ among these reef types depending on their structure,
123 as well as during development of the restored reef.

124 **2. Materials and methods**

125 2.1 STUDY AREA AND EXPERIMENTAL DESIGN

126 The study is a 2-way design, where the first independent variable compares three reef
127 types (natural, restored, unconsolidated), and the second independent variable compares response

128 over six time periods. The dependent variables are C and N stable isotope compositions in
129 potential water column and sediment composite food resources and oysters.

130 The Mission-Aransas Estuary is a bar-built system in the Texas Coastal Bend (Fig. 1a).
131 The estuary occupies approximately 463 km² and is composed of several shallow (average 2 m
132 depth) bays (Armstrong 1987). The area has a semi-arid, subtropical climate with infrequent rain
133 events. Evaporation typically exceeds precipitation (62.7 cm year⁻¹ net water loss) (Armstrong
134 1987). Two rivers feed the system: the Mission River flows through Mission Bay into Copano
135 Bay and the Aransas River flows directly into Copano Bay. Average freshwater inflow to the
136 estuary is low (< 500 million m³ year⁻¹) (Armstrong 1987, Montagna et al.1996). The estuary is
137 microtidal (0.15 m tidal range) and water movement is predominantly wind-influenced (Evans
138 and Morehead Palmer 2012). Mixing with the Gulf of Mexico occurs via a tidal inlet at the
139 southern end of the system. High winds characteristic of the region maintain well-mixed
140 conditions throughout the system (Evans and Morehead Palmer 2012). Average water residence
141 time is 360 days, but can be as long as three years (Beseres Pollack et al. 2011, Montagna et
142 al.1996). Oyster reefs are common in the estuary (Fig. 1a). Reefs are primarily subtidal and are
143 more prominent in areas of low to moderate salinity.

144 The study was conducted in Copano Bay (Fig. 1b; 28.135 °N, 97.053 °W), where average
145 depth is 1.1 m. Oysters and potential composite food resources — suspended particulate organic
146 matter (SPOM) and surface sediment organic matter (SSOM) — were sampled at three reef
147 types, which had the following characteristics (see also supplementary material 1):

148 - Natural reef: Low vertical relief (< 0.1 m), with densities (mean ± standard deviation)
149 of adult and spat oysters of 196 ± 134 and 859 ± 922 individuals.m⁻², respectively.

150 - Unconsolidated reef: Dense shell hash and mud with scattered oysters (97 ± 35 adult
151 individuals.m⁻², 652 ± 912 spat individuals.m⁻²). No vertical relief (0 m; oysters directly on the
152 sediment).

153 - Restored reef: Eight higher vertical relief (0.3 m) reef mounds (600 m² each)
154 constructed in summer 2011 with a crushed concrete base and reclaimed oyster shell veneer.
155 Oyster densities were 280 ± 164 and 1462 ± 1595 individuals.m⁻² for adults and for spat,
156 respectively.

157 Sampling commenced in February 2012 (approximately six months post-reef
158 construction) and continued seasonally through September 2013 for a total of six sampling
159 periods (February 2012, June 2012, September 2012, March 2013, June 2013 and September
160 2013).

161 2.2 CONTINUOUS ENVIRONMENTAL DATA

162 Salinity, chlorophyll-*a* (chl-*a*, $\mu\text{g L}^{-1}$) and wind speed (m s^{-1}) were collected near the
163 study site (Copano Bay East station; 28.132 °N, 97.034 °W) as part of the National Estuarine
164 Research Reserve System monitoring program (NERRS 2014). All data were collected for the
165 entire study period, starting in July 2011 when reef construction began and continuing until
166 October 2013 when the study was complete.

167 2.3 SAMPLING AND PREPARATION OF WATER CHLOROPHYLL-A AND COMPOSITE FOOD SOURCE

168 SAMPLES

169 Bottom water samples were collected just above the sediment-water interface at each reef
170 type using a horizontal van Dorn water sampler for quantification of chl-*a* and stable isotope
171 analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of SPOM. Water was filtered on Whatman GF/F glass fiber filters (0.7 μm
172 porosity) to collect SPOM for chl-*a* analysis. Filters were stored at -20 °C until analysis. SPOM

173 samples for stable isotope analyses were sieved on a 300- μm screen to eliminate large detrital
174 particles and zooplankton, and then filtered on two precombusted Whatman GF/F glass fiber
175 filters (0.7 μm porosity). Filters were frozen at $-20\text{ }^{\circ}\text{C}$ and freeze-dried. Carbonates were
176 removed from filters for $\delta^{13}\text{C}$ and $\%C$ analyses by contact with HCl fumes in a vacuum-enclosed
177 system. Untreated freeze-dried filters were used for $\delta^{15}\text{N}$ and $\%N$ analyses.

178 Three cylindrical sediment cores (37.4 cm^2) were collected by divers from each reef type
179 for stable isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) of SSOM. Cores were collected and handled with
180 minimal disturbance and the top 2 cm were sliced and stored at $-20\text{ }^{\circ}\text{C}$ until processing. Samples
181 were thawed and sieved wet on a 500 μm mesh screen to eliminate macrofauna, shell pieces and
182 large detrital particles. Sieved sediment was freeze-dried and ground using a mortar and pestle.
183 Carbonates were removed from sediment for $\delta^{13}\text{C}$ and $\%C$ analyses using 2 mol L^{-1} HCl. HCl
184 was added drop-by-drop until cessation of bubbling. Samples were then dried at $60\text{ }^{\circ}\text{C}$ using a
185 dry block heater under air flow. Dried samples were re-homogenized into ultrapure water using
186 an ultrasonic bath, freeze-dried and ground again. Untreated ground sediment was used for $\delta^{15}\text{N}$
187 and $\%N$ analyses.

188 2.4 SAMPLING AND PREPARATION OF OYSTERS

189 Oysters were collected from each reef type by divers and stored on ice for transport to the
190 laboratory. They were cleaned of epibionts and kept alive for up to 36 h in filtered seawater to
191 allow for gut content evacuation (Dubois et al. 2007). Oysters were frozen at $-20\text{ }^{\circ}\text{C}$ and then
192 dissected to collect digestive gland material. We aimed to collect three spat oyster ($\leq 25\text{ mm}$
193 shell height) samples and three adult oyster ($> 25\text{ mm}$) samples from each reef type during each
194 sampling event. Spat oysters were pooled in groups (2-4 individuals) when possible to obtain
195 enough material for stable isotope analysis. Low oyster density in the unconsolidated reef often

196 limited the number of oysters collected. In total, 52 adult oysters and 44 spat groups (111
197 individual spat) were analyzed, ranging in size from 25.4 to 76.8 mm (47.2 ± 13.4 mm, mean \pm
198 standard deviation (SD)) and from 11.5 to 25.0 mm (18.4 ± 3.4 mm), respectively.

199 Digestive gland samples were freeze-dried and ground to a homogenous powder.
200 Digestive glands contain high amounts of lipids, which are depleted in ^{13}C relative to other
201 tissues due to the different biochemical pathways involved in their synthesis (DeNiro and
202 Epstein 1977). As a result, the $\delta^{13}\text{C}$ value of a raw sample does not only reflect the food
203 resources of a consumer. Thus, lipids were extracted from samples for $\delta^{13}\text{C}$ and $\%C$ analyses
204 using two successive extractions with cyclohexane. Samples were then dried at 45°C and
205 ground again. Untreated ground samples were used for $\delta^{15}\text{N}$ and $\%N$ analyses.

206 2.5 CHLOROPHYLL-A AND STABLE ISOTOPE RATIO MEASUREMENTS

207 Chl-*a* was extracted from filters overnight using a non-acidification technique and read
208 on a Turner Trilogy fluorometer (Turner Designs, Sunnyvale, USA) (Welschmeyer 1994, EPA
209 method 445.0). Elemental and stable isotope compositions were determined using an elemental
210 analyzer (Flash EA 1112, Thermo Scientific, Milan, Italy) coupled to an isotope ratio mass
211 spectrometer (Delta V Advantage with a Conflo IV interface, Thermo Scientific, Bremen,
212 Germany). Analyses were conducted at the Littoral, Environment and Societies Joint Research
213 Unit stable isotope facility (University of La Rochelle, France). Results are expressed in δ
214 notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and N_2 in air for $\delta^{15}\text{N}$)
215 following the formula: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.
216 Calibration was done using reference materials (USGS-24, IAEA-CH6, -600 for carbon; IAEA-
217 N_2 , -NO-3, -600 for nitrogen). Analytical precision was $< 0.15\%$ based on the analyses of
218 acetanilide (Thermo Scientific) used as laboratory internal standard.

219 2.6 STATISTICAL ANALYSES

220 Statistical analyses were performed using R 2.12.2 (R Foundation for Statistical
221 Computing 2011). Comparisons between measured parameters among sampling dates and reef
222 types were conducted using nonparametric procedures (Zar 2010). Kruskal-Wallis tests
223 (*kruskal.test*) were used to compare isotope compositions and C:N ratios of SPOM, SSOM and
224 oysters, and bottom water chl-*a* concentrations and C:chl-*a* ratios of SPOM. Kruskal-Wallis
225 tests were followed by multiple comparisons of means (*kruskalmc* in *R pgirmess* package)
226 (Giraudoux 2011). Isotope compositions of oysters and food sources were compared considering
227 trophic fractionation factors of $0.3 \pm 1.3 \text{ ‰}$ (mean \pm SD) for $\delta^{13}\text{C}$ values and of $2.3 \pm 1.6 \text{ ‰}$ for
228 $\delta^{15}\text{N}$ values (Vander Zanden and Rasmussen 2001). Theoretical oyster food resource use was
229 computed by subtracting the trophic fractionation values from observed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of
230 oysters to correct for fractionation. Contributions of SPOM and SSOM as food resources to
231 oysters were estimated by solving mixing models within a Bayesian framework
232 (*siarmcmcdirichlety4* in *R siar* package) (Parnell et al. 2010, Parnell and Jackson 2014). Models
233 were run for 500,000 iterations and the first 50,000 iterations were discarded. Means and
234 credibility intervals (CI) of 0.95 and 0.25 were computed and displayed on figures.

235 **3. Results**

236 3.1 CONTINUOUS ENVIRONMENTAL PARAMETERS

237 Monthly average salinity ranged from 25.2 to 38.8, with lowest values observed during
238 May 2012 (Fig. 2). Monthly chl-*a* concentrations ranged from 2.0 to 16.1 $\mu\text{g L}^{-1}$, and varied
239 throughout the study (Fig. 2). Monthly average wind speed ranged from 1.1 to 7.3 m s^{-1} , with
240 lowest values observed from August through October 2012 (Fig. 2).

241 3.2 COMPOSITION OF POTENTIAL FOOD RESOURCES

242 Mean bottom water chl-*a* concentrations of collected SPOM samples ranged from $0.7 \pm$
243 0.6 to $4.3 \pm 1.3 \mu\text{g L}^{-1}$ (mean \pm SD) (Fig. 2). Chl-*a* concentrations were similar among reef types
244 ($P > 0.173$), except during March 2013, when chl-*a* was higher at the unconsolidated reef
245 compared to the restored reef ($P = 0.024$). Across all reef types, chl-*a* concentration was lower
246 in February 2012 than in September 2012 and 2013 ($P < 0.001$). Mean C:chl-*a* ratios ranged
247 from 58.8 ± 9.2 to 328.8 ± 67.5 ; ratios were similar among reef types and were greater in
248 February 2012 than in September 2012 and 2013 ($P = 0.023$; Fig. 3).

249 Mean $\delta^{13}\text{C}$ values of SPOM ranged from $-24.8 \pm 0.3 \text{‰}$ (mean \pm SD) to $-23.5 \pm 0.3 \text{‰}$;
250 mean $\delta^{15}\text{N}$ values ranged from 7.3 ± 0.3 to $8.7 \pm 0.2 \text{‰}$ (Fig. 4). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of
251 SPOM were similar among reef types. $\delta^{13}\text{C}$ values were different among some sampling periods
252 ($P = 0.016$), but no clear temporal patterns were evident for $\delta^{13}\text{C}$ values. The $\delta^{15}\text{N}$ values were
253 generally more enriched during September sampling periods ($P = 0.010$). Mean C:N ratios of
254 SPOM ranged from 6.4 ± 0.5 to 12.8 ± 2.5 and were similar among reef types. C:N ratios of
255 SPOM were lowest during September sampling periods in both 2012 and 2013 compared to all
256 other sampling periods (Fig. 3), though not significantly different ($P = 0.086$).

257 Mean $\delta^{13}\text{C}$ values of SSOM ranged from -22.6 ± 1.0 to $-20.1 \pm 0.2 \text{‰}$ (mean \pm SD) (Fig.
258 4), and followed a general pattern of enrichment over time at all reef types ($P < 0.001$). Mean
259 $\delta^{15}\text{N}$ values ranged from 7.5 ± 0.3 to $8.5 \pm 0.5 \text{‰}$ (Fig. 4). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of SSOM
260 were similar among reef types ($P > 0.066$ and $P > 0.099$, respectively). Mean C:N ratios of
261 SSOM ranged from 7.7 ± 0.5 to 10.6 ± 1.3 and were similar among reef types ($P > 0.077$). $\delta^{15}\text{N}$
262 values and C:N ratios were similar among sampling periods ($P = 0.186$ and $P = 0.228$,
263 respectively).

264 3.3 STABLE ISOTOPE COMPOSITION OF OYSTERS

265 Oyster mean $\delta^{13}\text{C}$ values ranged from -23.3 ± 0.2 to -20.1 ± 0.5 ‰ (mean \pm SD) and
266 mean $\delta^{15}\text{N}$ values ranged from $8.2 \pm < 0.1$ to 11.1 ± 0.6 ‰ (Fig. 4). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values
267 were similar among reef types except in the first sampling period (February 2012); oysters from
268 the natural reef were more enriched in both ^{13}C ($P = 0.010$) and ^{15}N ($P = 0.044$) than oysters at
269 unconsolidated reef. Overall, spat and adult oysters had similar $\delta^{13}\text{C}$ values ($P = 0.478$), while
270 spat were more ^{15}N -enriched (10.2 ± 1.0 ‰) compared to adult oysters (9.5 ± 1.0 ‰) ($P =$
271 0.003). Oysters were more enriched in both ^{13}C and ^{15}N during June and September 2013 than
272 during other months ($P < 0.001$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values). Theoretical oyster food resource
273 use, corrected for trophic fractionation by subtracting 0.3 ‰ from $\delta^{13}\text{C}$ values and 2.3 ‰ from
274 $\delta^{15}\text{N}$ values of oysters, overlaps predominantly with SSOM food resources (Fig. 4).

275 3.4 MIXING MODEL ESTIMATIONS OF FOOD SOURCE CONTRIBUTIONS TO OYSTERS

276 Mixing model results revealed a general trend of decreasing SPOM and increasing SSOM
277 food resource contributions to oysters over time, among all reef types (Fig. 5). In February 2012,
278 SPOM contributed from 20 to 98 % (95% CI range) and SSOM contributed from 2 to 80 %. By
279 the end of the study (September 2013), the relative importance of food sources to oysters
280 switched with SPOM contributions ranging from 2 to 54 % and SSOM from 47 to 98 %.
281 Slightly higher proportions of SPOM contribution was observed in September 2012 than in
282 adjacent sampling dates. The same temporal patterns of food source contributions were observed
283 among the three reef types.

284 4. Discussion

285 4.1 ORIGIN AND COMPOSITION OF FOOD RESOURCES

286 Average $\delta^{13}\text{C}$ values of SPOM were more depleted ($-24.2 \pm 0.6 \text{‰}$) than SSOM ($-21.2 \pm$
287 0.8‰) throughout the study (mean \pm SD). The range of $\delta^{13}\text{C}$ values observed from SPOM ($-$
288 25.1 to -23.6‰) fall within ranges typical of river-estuarine phytoplankton (-30 to -24‰), and
289 C_3 marsh (-26 to -23‰) and terrestrial (-30 to -23‰) plants (Fry and Sherr 1984). Recent work
290 (2010-2011) in the Mission-Aransas Estuary determined $\delta^{13}\text{C}$ values for C_3 marsh plants (-29 to $-$
291 27‰) and estuarine SPOM (-27 to -21‰) (Lebreton et al. 2016). C:N ratios less than 20 and
292 C:chl-*a* ratios less than 200 indicate a dominant influence of fresh organic matter (e.g., fresh
293 phytoplankton) rather than degraded matter (e.g., highly decayed phytoplankton, terrestrial or
294 saltmarsh plant detritus) (Cifuentes et al. 1988, Leal et al. 2008). Considered together, the $\delta^{13}\text{C}$
295 values of SPOM, and low C:N and C:chl-*a* ratios observed throughout much of the study indicate
296 a dominant influence of autochthonous phytoplankton production on SPOM (Fry and Sherr
297 1984, Lebreton et al. 2016).

298 Mean $\delta^{13}\text{C}$ values of SSOM (ranging from -22.6 to -20.1‰) indicate potential influences
299 from a variety of sources: trapped phytoplankton, benthic microalgae, as well as a mixture of
300 material of marsh (C_4 plants) and of riverine and terrestrial (C_3 plants) origin (Fry and Sherr
301 1984). C:N ratios remain low (generally < 10), indicative of fresh organic matter (Kang et al.
302 2003) and thus indicate that marsh, riverine and terrestrial sources of organic matter play only a
303 minor role in the composition of SSOM. We infer that SSOM is largely composed of fresh
304 organic matter sources, such as trapped phytoplankton (-24‰ ; Fry and Sherr 1984), but also
305 benthic microalgae, which are more ^{13}C -enriched, with $\delta^{13}\text{C}$ values of -17‰ in Fry and Sherr
306 (1984), and ranging from -21.7 to -12.0‰ in Lebreton et al. (2016). The distinct ranges of $\delta^{13}\text{C}$

307 values observed for SSOM and SPOM further indicate that SSOM is not only composed of
308 settled SPOM, but likely contains a large amount of benthic microalgae.

309 4.2 SPATIO-TEMPORAL VARIATIONS OF SPOM AND SSOM COMPOSITIONS

310 At the spatial scale, the origin and composition of SPOM and of SSOM were generally
311 similar among reef types (natural, restored, unconsolidated), demonstrating that differences of
312 structure (i.e., vertical relief, bottom composition, oyster biomass and densities) among reefs do
313 not affect the quantity and the quality of the food sources available for oysters at the local scale
314 (i.e., reef scale). Rezek et al. (2017) demonstrated that changes of food web structure in oyster
315 reefs under development are related to changes of benthic macrofauna community but not to
316 changes in food source uses by macrofauna.

317 At the temporal scale, SPOM was of higher quality in late summer (i.e., September) as
318 indicated by the lower C:N and C:chl-*a* ratios and the higher chl-*a* concentrations in the bottom
319 water. This indicates that the proportion of autochthonous phytoplankton in the bottom water
320 SPOM is higher during this period. Local primary productivity in the water column was
321 probably enhanced during this period due to seasonally low wind speeds (NCDC 2017), leading
322 to lower resuspension of sediment-trapped organic and to higher penetration of light into the
323 water column. Lower primary productivity during winter months, and thus less fresh organic
324 matter in the SPOM pool, explains the higher C:chl-*a* ratios observed among all reef types during
325 February 2012. The lack of clear relationship between chl-*a* concentrations measured in the
326 bottom water (i.e., our samples) and in the water column (i.e., NERRS measurements) indicates
327 that SPOM composition in bottom water can differ from that in the water column, highlighting
328 the importance of sampling SPOM as close as possible to the oyster reefs.

329 In the sediment, a temporal increase of $\delta^{13}\text{C}$ values was observed for SSOM, with values
330 most depleted in ^{13}C during February 2012 and becoming more enriched through September
331 2013 among all reef types. This pattern likely reflects an increasing influence of benthic
332 microalgae, which are enriched in ^{13}C , and/or a decreasing influence of more ^{13}C -depleted
333 organic matter (i.e., trapped phytoplankton) on SSOM across the period. As this development is
334 observed at the scale of the three reef types, it can be related to a large change, at the scale of the
335 ecosystem, or to a change at a local scale (i.e., one reef type) affecting the functioning of the
336 whole ecosystem. We therefore suggest two hypotheses to explain this change: (1) At the scale
337 of the ecosystem, a gradual decrease of SPOM trapping into the sediment. Nevertheless, such a
338 trend would probably be linked with a similar trend of some abiotic parameters, such as wind
339 velocity or turbidity, which is not the case, making this hypothesis less likely. (2) At a local
340 scale, increasing oyster biomass at the restored reef may have enhanced growth of
341 microphytobenthos through feedback effects related to oyster biodeposits. As suspension-
342 feeders, oysters can have a strong influence on the degree of benthic-pelagic coupling within a
343 system (Dame 2012, Newell 2004, Prins et al. 1998). Oysters remove suspended particles from
344 the water column, including those resuspended from the sediment, and deposit feces and
345 pseudofeces, or biodeposits, onto the sediment, thereby linking organic matter and nutrient flows
346 between pelagic and benthic habitats (Hsieh et al. 2000, Newell 2004, Prins et al. 1998, Quan et
347 al. 2012). Feces and pseudofeces excreted by oysters may stimulate benthic production as this
348 organic matter is remineralized by bacteria and releases nutrients that can be used by
349 microphytobenthos (Miller et al. 1996, Prins et al. 1998). Indeed, after the introduction and
350 intensification of oyster culture, benthic primary production has been shown to increase
351 (Leguerrier et al. 2004), as has enrichment of sediments due to biodeposits, resulting in high

352 sediment chl-*a* concentrations (Castel et al. 1989). In our study, the restoration of the reef does
353 affect the SSOM composition in this reef type, as well as in the adjacent habitats (i.e., natural
354 and unconsolidated reefs) highlighting the importance of the connectivity between these different
355 habitats at the ecosystem scale. This highlights the potential benefits of reef restoration, not only
356 for the oyster reef itself, but also for the adjacent systems.

357 4.3 OYSTER UTILIZATION OF WATER COLUMN AND SEDIMENT FOOD RESOURCES

358 The range of $\delta^{13}\text{C}$ values observed in oysters overlaps mostly with SSOM $\delta^{13}\text{C}$ values,
359 indicating that oysters use food resources from both the SPOM and SSOM pools, regardless of
360 reef type. The strong role of SSOM as a food resource for oysters is confirmed by the similar
361 shift of $\delta^{13}\text{C}$ values between oysters and SSOM. As SSOM becomes more ^{13}C -enriched at all
362 reef types over the duration of the study—probably due to an increase of benthic microalgae into
363 SSOM composition—so do oysters. Additionally, mixing model results indicate that the
364 contribution of SSOM to oysters increases over the duration of the study across all reef types,
365 and that proportions of SSOM as a food source for oysters are similar among the three reef types.
366 SSOM becomes accessible to oysters via resuspension during water movement (Kang et al.
367 2003, Miller et al. 1996). Minimal tidal ranges and freshwater inflows, coupled with consistently
368 strong winds in the region, indicate that wind-induced waves are the predominant forcing factor
369 causing resuspension in this system (Armstrong 1987, Evans and Morehead Palmer 2012).

370 The restored reef in this study was constructed with relatively high vertical relief (0.3 m).
371 Oyster reef restoration as a whole has become increasingly focused on providing vertical
372 structure (i.e., >0.2 m above the bottom) to minimize sedimentation and improve temporal
373 sustainability (Powers et al. 2009, Schulte et al. 2009). The vertical biogenic structure interacts
374 with hydrodynamic boundary-layer flow over the bottom (Butman et al. 1994, Green et al. 1998),

375 influencing resuspension and food availability (Fréchette et al. 1989, Grant et al. 1990, Quan et
376 al. 2012, Wildish and Kristmanson 1984), larval settlement (Hills et al. 1999, Lapointe and
377 Bourget 1999), and secondary production (Lenihan 1999). In contrast, the unconsolidated and
378 natural reefs in the current study had relatively low vertical relief (0m; < 0.1 m, respectively).
379 Although it could be predicted that SSOM food resources are more accessible to suspension
380 feeders lower in the water column (Hsieh et al. 2000), results indicate food resource use was
381 similar between reef types, even during periods of low wind. Temporal trends observed in
382 SSOM of increased $\delta^{13}\text{C}$ values over time are reflected in oyster $\delta^{13}\text{C}$ values across reef types,
383 indicating that small-scale differences in vertical relief are not sufficient to limit consumption of
384 SSOM in Copano Bay. This highlights the very strong benthic-pelagic coupling in this
385 ecosystem, probably due to shallow depths as well as strong and consistent winds. Further
386 research is warranted to examine whether oysters on higher-vertical relief reefs ($\geq 1-2$ m; e.g.
387 Dunn et al. 2014, Puckett and Eggleston 2012) or from reefs restored in deeper or less wind
388 driven systems become decoupled from SSOM food resources compared to lower-vertical relief
389 counterparts.

390 4.4 TEMPORAL INCREASE OF SSOM AS A FOOD RESOURCE FOR OYSTERS

391 There are two probable explanations for the consistent temporal change in relative
392 contribution of SPOM and SSOM food resources to oysters among reef-types: (1) a decrease of
393 the role of SPOM, potentially connected to changes in environmental drivers, and/or (2) an
394 increase of the role of SSOM, linked to feedback effects of oysters on SSOM composition and
395 availability. Composition of organic matter resources can be influenced by various
396 environmental factors, and may also affect the assimilation of food resources by consumers (Fry
397 2006, Michener and Kaufman 2007).

398 In the Copano-Aransas estuary, SPOM composition is highly connected to environmental
399 drivers (Lebreton et al. 2016, Mooney and McClelland 2012, Reyna et al. 2017). The higher
400 concentrations in chl-*a* and better quality (i.e., higher C:N and C:chl-*a* ratios) of SPOM in late
401 summer, likely related to lower wind speeds, may affect its use by oysters. Indeed, in September
402 2012, the proportions of SPOM as a food source to oysters is slightly higher than the general
403 observed pattern. Nevertheless, this slight discrepancy is relatively minor compared to the
404 general temporal pattern observed, which shows a general increase of the SSOM as a food source
405 over the course of the study, highlighting the importance of benthic food resources into the diet
406 of oysters (Dame 2012, Riera and Richard 1996).

407 The alternative explanation for the higher use of SSOM over time is that increasing
408 oyster biomass at the restored reef enhanced growth of microphytobenthos through feedback
409 effects related to oyster biodeposits (see section 4.2). Enhanced benthic production can provide
410 important food sources to suspension-feeders such as oysters and other benthic organisms (Leal
411 et al. 2008, Miller et al. 1996, Ubertini et al. 2012), particularly in shallow systems where SSOM
412 is resuspended on a regular basis. In addition, South Texas embayments are characterized by
413 very low freshwater inflows, limiting nutrient inputs (Bianchi et al. 1999), and therefore
414 production of phytoplankton (Mooney and McClelland 2012). As a result, the feedback of
415 oysters on benthic primary production may be critical in supporting oysters and reef-associated
416 fauna (Leguerrier et al. 2004, Prins et al. 1998, Ubertini et al. 2012). Drought conditions
417 persisted throughout the study period, probably limiting phytoplankton production (Reyna et al.
418 2017). Thus, the increasing influence of SSOM on oysters over the study period may be related
419 to observed increases in oyster biomass following reef restoration, coincident with prolonged
420 drought (Blomberg 2015).

421 **5. Conclusion**

422 Our study demonstrated that, in a shallow and wind-driven ecosystem like the Mission-
423 Aransas Estuary, differences in oyster reef structure do not affect the composition of food
424 resources available to oysters and their use by oysters. This is probably related to the regular
425 resuspension of the organic matter stored in the sediment and to the strong connectivity between
426 the different studied reef types. Similar studies should be carried out in ecosystems
427 characterized by different physical properties (e.g., deeper systems, higher stratification) to
428 determine how these physical properties can affect flows of organic matter in natural and
429 restored oyster reefs. In terms of restoration ecology, it may also be of strong interest to
430 determine how vertical relief is affecting availability of the food resources from the benthos.

431 Another major conclusion of our study is that oysters rely on large amounts of sediment
432 organic matter as food resources, highlighting the importance of benthic-pelagic coupling in
433 oyster reefs, and supporting the positive feedback role of oyster reefs on the production of
434 benthic microalgae and/or trapping of organic matter. Better determination of the composition of
435 sediment organic matter in and around oyster reefs would provide significant knowledge toward
436 a better understanding of the role of this material in oyster reefs, and flows of organic matter in
437 these habitats.

438

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451

452 **Author Contributions**

453 Conceived and designed the experiments: BNB, BL, JBP. Performed the experiments: BNB, BL,
454 TAP, GG, JBP. Analyzed the data: BNB, BL, GG. Contributed reagents/materials/analysis tools:
455 BL, JBP, PAM. Wrote the paper: BNB. Extensive editorial comments, insight into results: BL,
456 TAP, GG, JBP, PAM.

457

458 **References**

- 459 Armstrong, N.E. (1987) The ecology of open-bay bottoms of Texas: a community profile. U. S.
460 Fish and Wildlife Service Biological Report, 85(7.12), 104 pp.
- 461 Bartol, I.K., Mann, R. & Luckenbach, M. (1999) Growth and mortality of oysters (*Crassostrea*
462 *virginica*) on constructed intertidal reefs: effects of tidal height and substrate level.
463 *Journal of Experimental Marine Biology and Ecology*, 237, 157-184.
- 464 Bateman, D.C., & Bishop, M.J. (2017) The environmental context and traits of habitat-forming
465 bivalves influence the magnitude of their ecosystem engineering. *Marine Ecology*
466 *Progress Series*, 563, 95-110.

467 Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M.,
468 Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F. &
469 Weinstein, M.P. (2001) The identification, conservation, and management of estuarine
470 and marine nurseries for fish and invertebrates: a better understanding of the habitats that
471 serve as nurseries for marine species and the factors that create site-specific variability in
472 nursery quality will improve conservation and management of these areas. *Bioscience*,
473 51, 633–641.

474 Beseres Pollack, J., Kim, H.C., Morgan, E.K. & Montagna, P.A. (2011) Role of flood
475 disturbance in natural oyster (*Crassostrea virginica*) population maintenance in an
476 estuary in South Texas, USA. *Estuaries and Coasts*, 34, 187-197.

477 Beseres Pollack, J., Yoskowitz, D., Kim, H.C. & Montagna, P.A. (2013) Role and value of
478 nitrogen regulation provided by oysters (*Crassostrea virginica*) in the Mission-Aransas
479 Estuary, Texas, USA. *PLoS ONE*, 8, e65314.

480 Bianchi T.S., Pennock J.R. & Twilley R.R., Eds. (1999). Biogeochemistry of Gulf of Mexico
481 estuaries. New York, John Wiley and Sons, Inc.

482 Blomberg, B.N. (2015) *Evaluating success of oyster reef restoration*. PhD thesis, Texas A&M
483 University-Corpus Christi.

484 Butman, C.A., Fréchette, M, Geyer, W.R. & Starczak, V.R. (1994) Flume experiments on food
485 supply to the blue mussel *Mytilus edulis* L. as a function of boundary-layer flow.
486 *Limnology and Oceanography*, 39, 1755–1768.

487 Castel, J., Labourg, P.-J., Escaravage, V., Auby, I. & Garcia, M.E. (1989) Influence of seagrass
488 beds and oyster parks on the abundance and biomass patterns of meio- and
489 macrobenthos in tidal flats. *Estuarine, Coastal and Shelf Science*, 28, 71-85.

490 Cifuentes, L.A., Sharp, J.H. & Fogel, M.L. (1988) Stable carbon and nitrogen isotope
491 biogeochemistry in the Delaware estuary. *Limnology and Oceanography*, 33, 1102-1115.

492 Colden, A.M., Fall, K.A., Cartwright, G.M. & Friedrichs, C.T. (2016) Sediment suspension and
493 deposition across restored oyster reefs of varying orientation to flow: implications for
494 restoration. *Estuaries and Coasts*, 39, 1435-1448.

495 Cranford, P.J., Armsworthy, S.L., Mikkelsen, O.A. & Milligan, T.G. (2005) Food acquisition
496 responses of the suspension-feeding bivalve *Placopecten magellanicus* to the
497 flocculation and settlement of a phytoplankton bloom. *Journal of Experimental Marine*
498 *Biology and Ecology*, 326, 128-143.

499 Cranford, P.J. & Hill, P.S. (1999) Seasonal variation in food utilization by the suspension-
500 feeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. *Marine Ecology*
501 *Progress Series*, 190, 223-239.

502 Dame, R.F., Zingmark, R.G. & Haskin, E. (1984) Oyster reefs as processors of estuarine
503 materials. *Journal of Experimental Marine Biology and Ecology*, 83, 239-247.

504 Dame, R.F. (2012) *Ecology of Marine Bivalves: An Ecosystem Approach*, 2nd edn. CRC Press,
505 Boca Raton.

506 DeNiro, M.J. & Epstein, S. (1977) Mechanism of carbon isotope fractionation associated with
507 lipid synthesis. *Science*, 197, 261-263.

508 Dubois, S., Orvain, F., Marin-Leal, J.C., Ropert, M. & Lefebvre, S. (2007) Small-scale spatial
509 variability of food partitioning between cultivated oysters and associated suspension-
510 feeding species, as revealed by stable isotopes. *Marine Ecology Progress Series*, 336,
511 151-160.

512 Dunn, R.P., Eggleston, D. B. & Lindquist, N. (2014) Effects of substrate type on demographic
513 rates of eastern oyster (*Crassostrea virginica*). *Journal of Shellfish Research*, 33, 177-
514 185.

515 Evans, A. & Morehead Palmer, S. (2012) Hydrography and oceanography. *The Ecology and*
516 *Sociology of the Mission-Aransas Estuary. An Estuarine and Watershed Profile* (eds. A.
517 Evans, K. Madden & S. Morehead Palmer), pp. 19-23. University of Texas Marine
518 Science Institute, Port Aransas, Texas.

519 Fr chet te, M., Butman, C.A. & Geyer, W.R. (1989) The importance of boundary-layer flows in
520 supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. *Limnology*
521 *and Oceanography*, 34, 19-36.

522 Fry, B. & Sherr, E.B. (1984) $\delta^{13}\text{C}$ measurements as indicators of carbon flow in marine and
523 freshwater ecosystems. *Contributions in Marine Science*, 27, 13-47.

524 Fry, B. (2006) *Stable Isotope Ecology*, Springer, New York.

525 Giraudoux, P. (2011) pgirmess: data analysis in ecology. R package version 1.5.0. [http://cran.r-](http://cran.r-project.org/web/packages/pgirmess/index.html)
526 [project.org/web/packages/pgirmess/index.html](http://cran.r-project.org/web/packages/pgirmess/index.html); *accessed* 12 December 2014.

527 Grabowski, J.H. (2004) Habitat complexity disrupts predator-prey interactions but not the trophic
528 cascade on oyster reefs. *Ecology*, 85, 995-1004.

529 Grabowski, J.H. & Peterson, C.H. (2007) Restoring oyster reefs to recover ecosystem services.
530 *Ecosystem Engineers: Plants to Protists* (eds. K. Cuddington, J.E. Byers, W.G. Wilson
531 & A. Hastings), pp. 281-298. Academic Press, Theoretical Ecology Series, Volume 4.

532 Grant, J., Enright, C.T. & Griswold, A. (1990) Resuspension and the growth of *Ostrea edulis*, a
533 field experiment. *Marine Biology*, 104, 51-59.

- 534 Grant, J., Cranford, P. & Emerson, C. (1997) Sediment resuspension rates, organic matter quality
535 and food utilization by sea scallops (*Placopecten magellanicus*) on Georges Bank.
536 *Journal of Marine Research*, 55, 965-994.
- 537 Green, M.O., Hewitt, J.E. & Thrush, S.F. (1998) Seabed drag coefficient over natural beds of
538 horse mussels (*Atrina zelandica*) *Journal of Marine Research.*, 56, 613–637,
- 539 Hargrave, B.T., Doucette, L.I., Cranford, P.J., Law, B.A. & Milligan, T.G. (2008) Influence of
540 mussel aquaculture on sediment organic enrichment in a nutrient-rich coastal
541 embayment. *Marine Ecology Progress Series*, 365, 137-149.
- 542 Haven, D.S. & Morales-Alamo, R. (1966) Aspects of biodeposition by oysters and other
543 invertebrate filter feeders. *Limnology and Oceanography*, 11, 487-498.
- 544 Hills, J.M., Thomason, J.C. & Muhl, J. (1999) Settlement of barnacle larvae is governed by
545 Euclidean and not fractal surface characteristics. *Functional Ecology*, 13, 868–875.
- 546 Hsieh, H.L., Kao, W.Y., Chen, C.P. & Liu, P.J. (2000) Detrital flows through the feeding
547 pathway of the oyster (*Crassostrea gigas*) in a tropical shallow lagoon: $\delta^{13}\text{C}$ signals.
548 *Marine Biology*, 136, 677-684.
- 549 Kang, C.K., Kim, J.B., Lee, K.S., Kim, J.B., Lee, P.Y. & Hong, J.S. (2003) Trophic importance
550 of benthic microalgae to macrozoobenthos in coastal bay systems in Korea: dual stable C
551 and N isotope analyses. *Marine Ecology Progress Series*, 259, 79-92.
- 552 Lapointe, L. & Bourget, E. (1999) Influence of substratum heterogeneity scales and complexity
553 on a temperate epibenthic marine community. *Marine Ecology Progress Series*, 189,
554 159-170.
- 555 Leal, J.C.M., Dubois, S., Orvain, F., Galois, R., Blin, J.L., Ropert, M., Bataillé, M.P., Ourry, A.
556 & Lefebvre, S. (2008) Stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) and modelling as tools to estimate the

557 trophic ecology of cultivated oysters in two contrasting environments. *Marine Biology*,
558 153, 673-688.

559 Lebreton, B., Beseres Pollack, J., Blomberg, B.N., Palmer, T.A., Adams, L., Guillou, G. &
560 Montagna, P.A. (2016) Origin, composition and quality of suspended particulate organic
561 matter in relation to freshwater inflow in a South Texas estuary. *Estuarine, Coastal and*
562 *Shelf Science*, 170, 70-82.

563 Leguerrier, D., Niquil, N., Petiau, A. & Bodoy, A. (2004) Modeling the impact of oyster culture
564 on a mudflat food web in the Marennes-Oleron Bay (France). *Marine Ecology Progress*
565 *Series*, 273, 147-162.

566 Lenihan, H.S. (1999) Physical-biological coupling on oyster reefs: how habitat structure
567 influences individual performance. *Ecological Monographs*, 69, 251-275.

568 MacIntyre, H.L. & Cullen, J.J. (1996) Primary production by suspended and benthic microalgae
569 in a turbid estuary: time-scales of variability in San Antonio Bay, Texas. *Marine*
570 *Ecology Progress Series*, 145, 245-268.

571 Michener, R.H. & Kaufman, L. (2007) Stable isotope ratios as tracers in marine food webs: an
572 update. *Stable Isotopes in Ecology and Environmental Science*, 2nd edn (eds R.
573 Michener & K. Lajtha), pp. 238-282. Blackwell Publishing, Malden.

574 Miller, D.C., Geider, R.J. & MacIntyre, H.L. (1996) Microphytobenthos: the ecological role of
575 the “secret garden” of unvegetated, shallow-water marine habitats. II. Role in sediment
576 stability and shallow-water food webs. *Estuaries*, 19, 202-212.

577 Montagna, P.A., Li, J. & Street, G.T. (1996) A conceptual ecosystem model of the Corpus
578 Christi Bay National Estuary Program study area. Coastal Bend Bays National Estuary
579 Program, Publication CCBNEP-08, Texas Natural Resource Conservation Commission.

580 Mooney, R.F. & McClelland, J. (2012) Watershed export events and ecosystem responses in the
581 Mission-Aransas National Estuarine Research Reserve, South Texas. *Estuaries and*
582 *Coasts*, 35, 1468-1485.

583 NOAA National Estuarine Research Reserve System (NERRS). System-wide Monitoring
584 Program. Data accessed from the NOAA NERRS Centralized Data Management Office
585 website: <http://www.nerrsdata.org/>; accessed 10 December 2014.

586 NOAA National Climatic Data Center (NCDC). Severe Weather Data Inventory website:
587 <https://www.ncdc.noaa.gov/swdi/#Intro>; accessed 1 March 2017.

588 Newell, R.I.E. (2004) Ecosystem influences of natural and cultivated populations of suspension-
589 feeding bivalve molluscs: a review. *Journal of Shellfish Research*, 23, 51-61.

590 Newell, R.I.E. & Langdon, C.J. (1996) Digestion and absorption of refractory carbon from the
591 plant *Spartina alterniflora* by the oyster *Crassostrea virginica*. *Marine Ecology*
592 *Progress Series*, 34, 105-115.

593 Parnell, A.C., Inger, R., Bearhop, S. & Jackson, A.L. (2010) Source partitioning using stable
594 isotopes: coping with too much variation. *PLoS ONE*, 5, e9672.

595 Parnell, A. & Jackson, A. (2014) siar: Stable Isotope Analysis in R. R package version 4.2.
596 <http://cran.r-project.org/web/packages/siar/siar.pdf>; accessed 18 December 2014.

597 Peterson, B.J. (1999) Stable isotopes as tracers of organic matter input and transfer in benthic
598 food webs: a review. *Acta Oecologica*, 20, 479-487.

599 Peterson, C. H., Grabowski, J. H. & Powers, S. P. (2003) Estimated enhancement of fish
600 production resulting from restoring oyster reef habitat: quantitative valuation. *Marine*
601 *Ecology Progress Series*, 264, 249-264.

602 Powers, S.P., Peterson, C.H., Grabowski, J.H. & Lenihan, H.S. (2009) Success of constructed
603 oyster reefs in no-harvest sanctuaries: implications for restoration. *Marine Ecology*
604 *Progress Series*, 389, 159-170.

605 Prins, T.C., Smaal, A.C. & Dame, R.F. (1998) A review of the feedbacks between bivalve
606 grazing and ecosystem processes. *Aquatic Ecology*, 31, 349-359.

607 Puckett, B.J. & Eggleston, D.B. (2012) Oyster demographics in a network of no-take reserves:
608 recruitment, growth, survival, and density dependence. *Marine and Coastal Fisheries*, 4,
609 605-627.

610 Quan, W.M., Humphries, A.T., Shi, L.Y. & Chen, Y.Q. (2012) Determination of trophic transfer
611 at a created intertidal oyster (*Crassostrea ariakensis*) reef in the Yangtze River Estuary
612 using stable isotope analyses. *Estuaries and Coasts*, 35, 109-120.

613 R Foundation for Statistical Computing (2011) R: A language and environment for statistical
614 computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from
615 <http://R-project.org>; 04 December 2014.

616 Reyna, N.E., Hardison, A. & Liu, Z. (2017) Influence of major storm events on the quantity and
617 composition of particulate organic matter and the phytoplankton community in a
618 subtropical estuary, Texas. *Frontiers in Marine Science*, 4, 43.

619 Rezek, R.J., Lebreton, B., Roark, E.B., Palmer, T.A., & Beseres Pollack, J. (2017) How does a
620 restored oyster reef develop? An assessment based on stable isotopes and community
621 metrics. *Marine Biology*, 164, 54.

622 Riera, P. & Richard, P. (1996) Isotopic determination of food sources of *Crassostrea gigas* along
623 a trophic gradient in the estuarine bay of Marennes-Oléron. *Estuarine, Coastal and Shelf*
624 *Science*, 42, 347-360.

- 625 Schulte, D.M., Burke, R.P. & Lipcius, R.N. (2009) Unprecedented restoration of a native oyster
626 metapopulation. *Science*, 325, 1124-1128.
- 627 Soniat, T.M., Finelli, C.M. & Ruiz, J.T. (2004) Vertical structure and predator refuge mediate
628 oyster reef development and community dynamics. *Journal of Experimental Marine*
629 *Biology and Ecology*, 310, 163-182.
- 630 Summerhayes, S.A., Bishop, M.J. & Kelaher, L.A. (2009) Effects of oyster death and shell
631 disarticulation on associated communities of epibiota. *Journal of Experimental Marine*
632 *Biology and Ecology*, 379, 60–67.
- 633 Sundbäck, K. (1984) Distribution of microbenthic chlorophyll-*a* and diatom species related to
634 sediment characteristics. *Ophelia Supplement*, 3, 229-246.
- 635 Ubertini, M., Lefebvre, S., Gangnery, A., Grangere, K., Le Gendre, R. & Orvain, F. (2012)
636 Spatial variability of benthic-pelagic coupling in an estuary ecosystem: consequences for
637 microphytobenthos resuspension phenomenon. *PLoS ONE*, 7, e44155.
- 638 Vander Zanden, M.J. & Rasmussen, J.B. (2001) Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation:
639 implications for aquatic food web studies. *Limnology and Oceanography*, 46, 2061-
640 2066.
- 641 Welschmeyer, N.A. (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll
642 *b* and pheopigments. *Limnology and Oceanography*, 39, 1985-1992.
- 643 Wildish, D.J. & Kristmanson, D.D. (1984) Importance to mussels of the benthic boundary layer.
644 *Canadian Journal of Fisheries and Aquatic Sciences*, 41, 1618-1625.
- 645 Winemiller, K.O., Akin, S. & Zeug, S.C. (2007) Production sources and food web structure of a
646 temperate tidal estuary: integration of dietary and stable isotope data. *Marine Ecology*
647 *Progress Series*, 343, 63-67.

648 Zar, J.H. (2010) *Biostatistical analysis*, 5th edn. Pearson Education, Upper Saddle River.

649 zu Ermgassen, P.S.E., Grabowski, J.H., Gair, J.R. & Powers, S.P. (2015) Quantifying fish and

650 mobile invertebrate production from a threatened nursery habitat. *Journal of Applied*

651 *Ecology*, 53, 596-606.

652

653 **Figure Captions:**

654

655 Figure 1. Study area. (a) Location of study site within the Mission-Aransas Estuary, Texas,
656 USA. (b) Extent of sampling area among three reef types.

657

658 Figure 2. Continuous environmental observations over the course of the study (July 2011
659 through October 2013): monthly average wind speed (m s^{-1}), salinity and monthly grab samples
660 of chlorophyll-*a* ($\mu\text{g L}^{-1}$) from the Mission-Aransas National Estuarine Research Reserve
661 (MANERR) Copano Bay East station. Bottom water chlorophyll-*a* ($\mu\text{g L}^{-1}$) samples are
662 indicated with dots during each sampling period. Reef construction is indicated with the dark
663 gray bar; sampling periods are indicated with light shaded bars.

664

665 Figure 3. C:N and C:chl-*a* ratios (mean \pm SD) observed for suspended particulate organic matter
666 sampled across the study area during each sampling period.

667

668 Figure 4. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (mean \pm SD) of suspended particulate organic matter (SPOM),
669 surface sediment organic matter (SSOM) and oysters (all sizes) during each sampling date. (a)
670 Unconsolidated reef. (b) Natural reef. (c) Restored reef. Grey areas represent the range of
671 theoretical oyster food source stable isotope composition accounting for trophic fractionation.

672

673 Figure 5. Seasonal comparison of contributions (%) of potential food resources (SPOM:
674 suspended particulate organic matter, SSOM: surface sediment organic matter) to oyster diets
675 resulting from the mixing model SIAR. (a) Unconsolidated reef. (b) Natural reef. (c) Restored

676 reef. Mean, 0.25 and 0.95 credibility intervals are shown with horizontal bar, grey box and
677 whiskers, respectively.









