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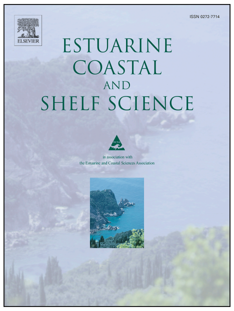

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Assessing the impact of the Asian mussel *Arcuatula senhousia* in the recently invaded Oristano Lagoon-Gulf System (W Sardinia, Italy)

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Highlights

- The invasive mussel *Arcuatula senhousia* was studied in the Oristano Lagoon-Gulf system
- No effects were found on the abundance and isotopic composition of local macrofauna
- Similar isotopic values of local bivalves and *A. senhousia* indicate a common diet
- Low rate of arrivals and unfavourable environmental conditions impair invasion success

Abstract

In the marine environment, the introduction and spread of non-indigenous mussels may cause major modifications to native assemblages and alter the trophic flow within the food web. We analysed the impacts of the recently sighted Asian date mussel *Arcuatula* (=*Musculista*) *senhousia* on sediment features, native macrozoobenthic assemblages and the $\delta^{13}C$ and $\delta^{15}N$ values of dominant macrozoobenthic taxa in the Oristano Lagoon-Gulf system (western Sardinia, Italy). Results showed that the amount of variation generated by the occurrence of *A. senhousia* was lower than the intrinsic spatial variability in sediment features, macrozoobenthic assemblages and the $\delta^{13}C$ values of dominant deposit feeders (*Hediste diversicolor*, *Cirriphormia tentaculata*, *Haminoea navicula* and *Cyclope neritea*) of this system. In addition, $\delta^{13}C$ and $\delta^{15}N$ values of *A. senhousia* were found to be similar to those of co-occurring suspension feeders *Cerastoderma glaucum*, *Ruditapes decussatus* and *Scrobicularia plana*, indicating exploitation of common food resources. The overall lack of effects of *A. senhousia* may be dependent on the moderate densities encountered in our study area (<1000 individuals m^{-2}). We suggest that the low rate of new arrivals, owing to limited shellfish farming and maritime activities in the area, and unfavourable environmental conditions of the lagoons especially in summer (e.g. anoxia) which erode mussel populations, likely prevent *A. senhousia* from entering its expansion phase and impacting local benthic communities.

Key words: Invasive mussels, $\delta^{13}C$, $\delta^{15}N$, deposit feeders, suspension feeders, benthic macroinvertebrates

1. Introduction

Mussels are often introduced accidentally into new marine and freshwater environments. Over the past century, the Asian date mussel *Arcuatula* (=*Musculista*) *senhousi*a (Benson in Cantor, 1842) has been introduced from Asia to the Pacific coast of North America, Australia, New Zealand and in the Mediterranean Sea (Crooks, 1996; Creese et al., 1997; Mistri, 2002). In the western Mediterranean Sea, *A. senhousia* was first discovered in the late 1980s in several lagoons of the Marseille area (Etang de Tau, Balaruc les Bains and Languedoc-Roussillon, Etang d'Or; Hoenselaar and Hoenselaar, 1989) and then found along the Italian coast. In particular, at the beginning of the 1990s it was sighted on the northern Adriatic coast (Ravenna and Sacca di Goro; Mistri et al., 2004) and in the late 1990s on both the north-western and north-eastern Tyrrhenian Sea (Gulf of Olbia on the Eastern coast of Sardinia and Leghorn harbour, respectively; Campani et al., 2004). Then, it was discovered in the Ionian Sea at the beginning of the 2000s (Mar Piccolo of Taranto; Apulian; Mastrototaro et al., 2003) and only in 2009 on the western coast of Sardinia (Gulf of Oristano; Cannas, 2010).

A. senhousia is a gregarious and fast-growing organism that lives semi-buried in the intertidal and shallow subtidal soft sediments of bays, estuaries and lagoons (Crooks, 1996; Mistri, 2002; Magni et al., 2006a). *A. senhousia* can influence the sediment features as well as the species composition and the distribution of abundances of local infauna through the creation of a structurally complex network composed of shells and byssus that modifies benthic habitat (i.e., ecosystem engineer effects). The shell's formation facilitates many small benthic invertebrates such as polychaetes, gastropods and crustaceans (Crooks and Khim, 1999), while larger organisms not able to live within or close to these structures, such as clams, can be inhibited (Creese et al., 1997; Crooks, 2001). In addition to structural effects, *A. senhousia* may influence the local infauna by altering the trophic interactions between benthic consumers and their food sources within the food webs. *A. senhousia* is a suspension feeder that filters suspended particles from the water column and may locally deplete food supplies to co-occurring bivalves (Crooks, 2001; Kushner, 2005). *A. senhousia* may also increase the availability of suspended material to benthic deposit feeders into the sediment. Mats created by *A. senhousia* typically raise a few millimetres above the surface sediments, and this biogenic structure alters the hydrodynamic features on flat bottoms favouring the trapping and deposition of suspended fine particles and labile detritus (Crooks, 1998; Crooks and Khim, 1999). *A. senhousia* may also increase the availability of suspended material to benthic deposit feeders through its biological activities of suspension feeding and biodeposition. During feeding, the undigested remains are ejected as mucus-bound faeces and pseudofaeces, and sink to the sediment surface (Newell, 2004; Strayer, 2014). This biodeposition is extremely important because biodeposits are mixed into the sediment and consumed by deposit feeders (Norkko et al., 2001). Finally, *A. senhousia* can store material within its body mass and this material accumulated during periods of growth may be released at death (Strayer, 2014).

Studies on dreissenid mussels (e.g. the zebra mussel *Dreissena polymorpha*) and other invasive freshwater bivalves (e.g. the Asian clam *Corbicula fluminea*) illustrate dramatic changes to the structure and energy base of the littoral food web following bivalve establishment (Higgins and Vander Zanden, 2010; Ozersky et al., 2012; Basen et al., 2013). The establishment of bivalves redirects energy and material from the water column to the littoral benthos through deposition of sestonic material, increasing benthic production (Higgins and Vander Zanden, 2010; Gergs et al., 2011; Ozersky et al., 2012). With regard to *A. senhousia*, while many studies have analysed the impact of *A. senhousia* on sediment properties and the abundances of resident fauna (e.g. Crooks, 1998, 2001; Mistri, 2002; Munari, 2008), no studies have investigated its ability to affect the transfer of phytoplankton from the first to the second trophic level of benthic food webs (i.e., to suspension and deposit feeders), both in native and non-native areas.

In this study, we investigated the ecological effects of *A. senhousia* that was recorded for the first time in the Santa Giusta and Marceddì lagoons of the Oristano Lagoon-Gulf system (western Sardinia, Italy) in 2009 (Cannas, 2010 and unpublished data). Firstly, in the Santa Giusta lagoon, we tested the effects of *A. senhousia* on the sediment features and macrozoobenthic assemblages. If the occurrence of *A. senhousia* affects the sediment features and the local infauna, we expected differences in the sediment features and the structure and species composition of macrozoobenthic assemblages between stations where *A. senhousia* was present and those where it was absent. Secondly, in the Marceddì lagoon, we tested some hypotheses regarding the role of *A. senhousia* on the benthic food web. In particular, we compared the δ^{13}C and δ^{15}N values of deposit feeders (DF) and sedimentary organic matter (SOM), which comprises the bulk of food sources for deposit feeders

(Rodriguez-Graña et al., 2008; Riera, 2010), in the presence and absence of *A. senhousia*. If *A. senhousia* increases the availability of phytoplankton into the sediment for deposit feeders, we expected that DF and SOM would have more ^{13}C-depleted values, close to those of phytoplankton (France, 1995), in sites where *A. senhousia* is present than in those where it is absent. In addition, we compared the δ^{13}C and δ^{15}N values of *A. senhousia* to those of co-occurring suspension feeding bivalves. If they exploit common resources, we expected similar δ^{13}C and δ^{15}N values. Finally, because ontogenetic variability in food acquisition can occur in bivalves after the recruitment of the planktonic larvae (Veniot et al., 2003; Beninger et al., 2006; Cannuel and Beninger, 2007), we tested, where possible, whether differences in both δ^{13}C and δ^{15}N values exist between different sizes of *A. senhousia*.

2 Material and methods

2.1 Study area

The field work was conducted in the Santa Giusta and Marceddì lagoons, two eutrophic basins connected to the Gulf of Oristano (Fig. 1). The Santa Giusta lagoon has an area of 7.9 km^2, a mean depth of 1 m and is separated from the sea by a long-shore bar. Salinity ranges from 25‰ to 42‰, with a mean annual value of 30‰ (Sechi et al., 2001). The Santa Giusta lagoon is located in the plain of Pesaria, an agricultural area that is intensively cultivated with rice. During the last few decades, several human interventions have profoundly modified the lagoon ecosystem. An industrial port and an industrial canal communicating with the sea were built in the 1970s and a diversion canal for urban wastes was built in 1995. Although this canal was thought to reduce the trophic level of the lagoon, there is no evidence that the diversion system has stopped sewage from flowing into the lagoon (Sechi et al., 2001). As an example, a dystrophic event with anoxia and sulphide development occurred in the summer of 2004 causing a drastic reduction in macrozoobenthos and massive fish kill (Magni et al., 2008). The Marceddì lagoon has an area of 8 km^2 and a mean depth of 1.5 m. Salinity ranges from 23‰ to 42‰, with a mean annual value of 33‰ (Cannas, 2010). The Marceddì lagoon is located near the plain of Arborea, an area of intensive agricultural and zootechnical activities (Galiano et al., 2015), where of about 10,000 ha, 4500 ha are cultivated and 33,000 dairy cows are grazed. Until the early

1990s, mining was also present in the drainage basin of the Marceddì lagoon where high concentrations of heavy metals (Pb, Cd and Zn) in sediments have been found (Magni et al., 2006b).

2.2 Sample collection, processing and laboratory analysis

To study the effects of *A. senhousia* on sediment features and macrozoobenthic assemblages , sediment samples were collected in the Santa Giusta lagoon in December 2009 at three sites, according to a hierarchical sampling design described in Tataranni et al. (2009). There were twenty-seven samples at each site. Samples of macrozoobenthos were collected from the bare bottom with a box-corer (10×17 cm^2), sieved through a 0.5 mm mesh and preserved in 4% formaldehyde. At each sampling station, sediment cores (30 mm inner diameter) were also collected for the grain size analysis and the determination of organic matter (OM), water and bicarbonate contents.

In the laboratory, the surface layer (0–2 cm) of sediments was carefully sliced off each core. A sub-sample of ca. 4 g was suspended in 500 ml of distilled water and treated with hydrogen peroxide (H_2O_2, 4% solution) in order to eliminate organic matter before being wet sieved through a net of 64 µm. The sand fraction (>64 µm) remaining in the sieve was dried and weighed. Ten millilitres of suspension with the mud fraction (<64 µm) were then treated with Na-Hexametaphosphate 0.6% to avoid particle flocculation after a dilution to obtain a sediment concentration of ~0.5 mg ml^{-1}. The OM content in the sediments was determined from a sub-sample (about 1 g) by loss of ignition at 500 °C for 3 h. Water content in the sediment was quantified from a sub-sample (about 1 g) by loss of weight at 60°C for 48 h. Carbonates ($CaCO_3$, dry wt%) were determined by dissolution in 1 M HCl for 4 h after being filtered through Whatman GF/C filters, the residue was dried and weighed. The macrozoobenthos were sorted, identified to the species level (when possible), counted and preserved in 70% ethanol.

We collected data on the natural abundance of stable isotopes (δ^{13}C and δ^{15}N) of benthic macroinvertebrates and SOM in the Marceddì lagoon at two impacted sites (I; characterised by the presence of *A. senhousia*) and two control sites (C; characterised by the absence of *A. senhousia*,) interspersed along the coastline 1-2 km apart, at the end of October 2012 (Fig. 1). There were 4 sampling stations (~2 m^2; 50-100 m apart) at each site (Fig. 1). Samples were collected in less than 1 m water depth. Sediment samples for benthic macroinvertebrates were collected using an Ekman-Birge grab and sieved on a 0.5 mm mesh size on the shore. The top few millimetres of the sediment layer were collected using a hand corer (30 mm inner diameter) for sedimentary organic matter (SOM), whereas two litres of lagoon water were collected for obtaining the <55 µm fraction of seston, used as a proxy of detritus and phytoplankton (Como et al., 2012). In each station, the density of *A. senhousia* was also estimated by dragging a clam rake (40 cm-wide mouth) three times for 1 linear metre at 10 cm sediment depth, divided by the total sampled area and is expressed as the number of individuals per square metre (ind. m^{-2}).

In the laboratory, all individuals of *A. senhousia* found at each station were counted and the total shell length of each individual was measured to the nearest mm by means of a caliper. We used the shell length as an estimate of the individual size. All other benthic invertebrates were sorted and identified to species level under a stereo-microscope. The individuals were then put in jars containing filtered lagoon water and left for 24 h to allow the gut contents to clear. Then, they were killed by freezing the flesh of molluscs, polychaetes and crustaceans, which had been separated from the calcareous shells, tubes or external cuticles and rinsed with distilled water. Fifteen to twenty individuals of each species collected from each station were pooled and treated as a whole. Sediment samples for SOM were sieved using a 35 mm mesh size to remove large pieces of debris or animals. Lagoon water was filtered by a precombusted (450 °C, 4 h) Whatman GF/F filter after a prefiltration through a 55 µm mesh for the small-sized seston determination. All samples were oven-dried to a constant weight at 70 °C, ground to fine powder and stored frozen (-20 °C) until isotopic analyses.

Prior to the isotopic composition analysis, all samples were acidified using a 1 N HCl solution to eliminate carbonates. Measurements of natural abundance of ^{13}C and ^{15}N in animals, SOM and <55 µm seston were taken with a mass spectrometer (Finnigan DeltaPlus). Isotopic values were expressed in the δ notation as deviations from standards (Vienna Pee Dee belemnite limestone for $\delta^{13}C$ and nitrogen in air for $\delta^{15}N$), following the formula: $\delta^{13}C$ [(Rsample/Rstandard) - 1] × 103, where R in $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The precision of replicate analyses was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Average reproducibility based on replicate measurements of internal standards Sucrose (IAEA-CH-6) for $\delta^{13}C$ and Ammonium sulphate (IAEA-N-2) for $\delta^{15}N$ was about 0.15‰. For system checks, acetanilide was used as the laboratory reference. Acidification of samples to remove carbonates (Cloern et al., 2002) may bias the δ^{15}N signatures of biological samples (Kennedy et al., 2005; Ng et al., 2007). Therefore, we determined the δ^{15}N values of both acidified and non-acidified samples of benthic invertebrates in order to estimate the effects of acidification.

2.3 Statistical analysis

Differences in sediment variables and macrozoobenthic assemblages were analysed using a constrained ordination method, canonical discriminant analysis (CDA; Anderson and Robinson, 2003, Anderson and Willis, 2003). Six groups of stations obtained from the combination of the three sites and, within each site, stations where *A. senhousia* was present and stations where *A. senhousia* was absent, were taken into account in the CDA analysis. The CDA analysis was done using the CAP computer program (Anderson, 2004).

To test for the effects of *A. senhousia* on the δ^{13}C and δ^{15}N values of deposit feeders and SOM we used a 2-way model analysis of variance (ANOVA). The analysis took into consideration the factor *A. senhousia* (two levels: Impact [I] and Control [C]; fixed) and the factor Site (two levels; random and nested in *A. senhousia*) and the stations as replication units. Differences between sizes in the δ^{13}C and δ^{15}N values of *A. senhousia* were analysed in one impacted site by means of 1-way model analysis of variance (ANOVA) with size as factor (two levels; fixed) and four stations as replication units. Cochran’s C-test was used to check the assumption of homogeneity of variances using ANOVA in tests of hypotheses (Winer et al., 1991). The analyses of variance and Cochran's C-test were done using Statistica (StatSoft 6.1, 1994).

3. Results

In the Santa Giusta lagoon, the sand fraction represented about 72% of the total sediment dry weight in Santa Giusta lagoon. Organic matter (OM) exceeded 10% of the total sediment weight, whereas water and carbonate contents represented 54% and 12% of the total sediment weight, respectively. *A. senhousia* occurred in less than half of the samples collected (i.e. 32 out of 81 samples). The densities of *A. senhousia* ranged from a minimum of 59 ind. m^{-2} to a maximum of 941 ind. m^{-2}. Excluding *A. senhousia*, there were 58 taxa belonging to polychaetes (33 taxa), crustaceans

(15 taxa), molluscs (10 taxa). The crustaceans *Tanais dulongii* and *Gammarus aequicauda* accounted for 49% of the total abundance. Canonical discriminant analysis (CDA) revealed differences in both sediment features and macrozoobenthic assemblage among the six groups of stations (permutation test: δ^2=0.79, *P*=0.001, Fig. 2). Cross-validation in the CDA analysis showed the highest number of misclassifications to occur in the groups of stations of the same site (values in bold in Table 1), indicating a major separation among sites, regardless of presence/absence of *A. senhousia*.

In the Marceddì lagoon, the mean (n=4, ±SE) abundance of *A. senhousia* was 474±105 and 343±36 ind. m^{-2} at the two impacted sites, I1 and I2, respectively, and 1±1 and 8±4 ind. m^{-2} at the two control sites, C1 and C2. At the two impacted sites, the population of *A. senhousia* was dominated by individuals of 1-2 cm length (Fig. 3). Individuals smaller than 1 cm were also abundant in I1 (48% of the total abundance), whereas they comprised less than 10% the total abundance in I2. One specimen with a shell length larger than 2 cm was found in I2. At the two control sites, *A. senhousia* had a mean shell length smaller than 1 cm.

Table 2 shows the δ^{13}C and δ^{15}N values of the benthic macroinvertebrates and OM pools (i.e. SOM and seston) found in the Marceddì lagoon. No differences in the δ^{15}N values of all benthic consumers were detected between acidified and non-acidified samples in each site (Wilcoxon test, *P*>0.05 for all tests). The differences between acidified and non-acidified samples never exceeded 1‰, therefore data from acidified samples only are presented in this paper. The seston was always more depleted in δ^{13}C (-28.3± 0.1 to -21.5±0.4‰) and more enriched in δ^{15}N (10.3±0.3 to 16.8±0.2‰) than SOM (δ^{13}C and δ^{15}N values ranged from -22.5±0.2 to -16.7±0.5‰ and from 3.9±0.3 to 7.3±0.9‰, respectively) (Table 2). *Cyclope neritea* and *Haminoea navicula* were the most enriched in δ^{13}C (-12.0±0.4 and -12.8±0.4 ‰, respectively), whereas *A. senhousia* was the most depleted in δ^{13}C (-22.1±0.4‰). *C. neritea* and *H. navicula* also had the highest and the lowest δ^{15}N, respectively (20.0±0.2 and 10.0±0.5‰). The δ^{13}C and δ^{15}N values of the deposit feeders *Hediste diversicolor* and *Cirriphormia tentaculata* and the suspension feeders *Cerastoderma glaucum, Ruditapes decussatus* and *Scrobicularia plana* fell within these values. The polychaetes *H. diversicolor* and *C. tentaculata* exhibited low δ^{13}C values (-19.6±0.4 to -15.3±0.3‰ and -13.0±0.2 to -19.5±0.3‰, respectively) similar to those of *C. glaucum*, *R. decussatus* and *S. plana* (values ranged from -20.5±0.2 and

14.7±0.0‰). On average, the deposit feeders *H. diversicolor*, *C. tentaculata*, *C. neritea* and *H. navicula* were found to be ~3‰ enriched in ^{13}C and ~11‰ in ^{15}N as compared to their potential food source, SOM. Similarly, the suspension feeders *C. glaucum*, *R. decussatus* and *S. plana* were found to be ~7‰ enriched in ^{13}C and ~2‰ in ^{15}N as compared to the seston (Fig. 4). Finally, *H. diversicolor*, *C. tentaculata*, *H. navicula*, *C. neritea*, *C. glaucum*, *S. plana* and SOM tended to be more ^{13}C-depleted and more ^{15}N-enriched at the inward sites (either impacted and control sites), than at the control site close to the sea (C2) (Table 2; Fig. 4).

Deposit feeders that were sufficiently abundant at all sampling sites (the two impacted and the two control sites) for the analyses included *H. diversicolor*, *H. navicula* and *C. neritea* (Table 2). The 2-way ANOVA did not detect a significant effect of *A. senhousia* on the δ^{13}C values of *H. diversicolor*, *H. navicula, C. neritea* and SOM (P>0.05) (Table 2; Fig. 4). However, the δ^{13}C of these deposit feeders and SOM were different between sites, irrespective of whether they were impacted or control sites. This indicates that spatial variation among sites was more important than the effect of *A. senhousia* within the study area. In accordance with the δ^{13}C values, the analysis did not detect a significant effect of *A. senhousia*, but there was significant variability between sites in the δ^{15}N values of these deposit feeders and SOM (P>0.05) (Table 2; Fig. 4).

There were no differences in both the δ^{13}C and δ^{15}N values between different sizes of *A. senhousia* (i.e. <1 and 1-2 cm) in the impacted site, I1 (1-way ANOVA: $F_{1:6}$=0.01 and $F_{1:6}$=0.15 for δ^{13}C and δ^{15}N, respectively; P>0.05 for both the tests; Table 2; Fig.4). Their δ^{13}C and δ^{15}N values were close to those of the abundant suspension feeder bivalves (i.e. *C. glaucum*, *R. decussatus* and *S. plana*; Table 2; Fig. 4).

4. Discussion

Compared to previous studies which have shown strong effects of *Arcuatula senhousia* on the sediment features and the local benthic invertebrates (e.g. Creese et al., 1997; Crooks, 1998; Munari, 2008), our results did not detect a linkage between the occurrence of *A. senhousia* and changes in the sediment features and the structure and species composition of macrozoobenthic assemblages. Instead, a major variation among sites was found, regardless of presence/absence of *A. senhousia*. The effects reported for *A. senhousia* (e.g. Creese et al., 1997; Crooks, 1998; Munari, 2008) are typically at densities of 1000s ind. m^{-2}, which create dense mats or carpets. It is likely that the lack of effects of *A. senhousia* on the sediment features and the macrozoobenthic assemblages in the Santa Giusta lagoon result from the low mussel densities encountered in the system (i.e. < 1000 ind. m^{-2}, at our sampling date of fall 2009). At these densities, the amount of variation generated by the occurrence of *A. senhousia* did not counterbalance the large spatial variability of this system. Coastal lagoons are characterised by wide fluctuations in environmental variables that lead to large and notable changes in sediment features and biological components, including benthic assemblages, at various spatial scales, e.g. from 10 to 1000 m (Benedetti-Cecchi et al., 2001; Tataranni et al., 2009; Magni et al., 2015). Changes in hydrodynamic conditions, seawater and freshwater influences, nutrient supplies and confinement can explain the spatial variation among sites in sediment features and macrofaunal abundances observed in the Santa Giusta lagoon (Magni et al., 2008; Tataranni et al., 2009). In addition, high inputs of organic matter can periodically induce prolonged anoxia, leading to azoic sediment and hydrogen sulphide development as observed in summer 2004 by Magni et al. (2008), thus contributing to increase the spatial variation in the sediment features and macrozoobenthic assemblages observed in this lagoon.

In this study we hypothesised that the occurrence of *A. senhousia* changes the dietary composition of deposit feeders in the surrounding sediment by redirecting phytoplankton, which has ^{13}C-depleted values (France, 1995), from the water column to the littoral benthos. The analyses conducted in the Marceddì lagoon revealed that the $\delta^{13}C$ values of deposit feeders (*Hediste diversicolor*, *Haminoea navicula* and *Cyclope neritea*) and SOM did not evidence any response to the occurrence of *A. senhousia*. Gergs and co-authors (2011) showed that the effects of the zebra mussels on the $\delta^{13}C$ values of deposit feeders in hard substrate freshwater habitats were predominately at high (1000s ind. m^{-2}) rather than low (≤1000 ind. m^{-2}) densities. In accordance with this study, the lack of effects of *A. senhousia* on the $\delta^{13}C$ values of deposit feeders and SOM may depend on the low mussel densities encountered in the system (i.e. < 1000 ind. m^{-2}). We advance that the lack of effects of *A. senhousia* on the $\delta^{13}C$ values of deposit feeders and SOM was in part a consequence of the large and significant isotopic variation between sites and reflects the low statistical power for the main factor.

An increasing number of studies in coastal lagoons have shown that an amount of the environmental and biological variability is transferred in the isotopic values of primary consumers (Vizzini et al., 2005; Carlier et al. 2009; Como et al. 2012). In accordance with these studies, primary consumers (either deposit or suspension feeders) and SOM were found to be more ^{13}C-depleted in the inward sites than in the site closer to the sea, which is more affected by marine influence. This may depend on a decreasing seaward-inward gradient in marine carbon inputs that are ^{13}C-enriched relative to continental inputs (Fry and Sherr, 1984). A similar marine-freshwater δ^{13}C gradient has been found for OM pools and consumers in several other coastal lagoon and estuarine systems (Degaan and Garrit, 1997; Vizzini et al., 2005; Carlier et al., 2009).

In the Marceddì lagoon, benthic consumers were also found to be more ^{15}N-enriched in the inward sites than in the site closer to the sea. The δ^{15}N values found at the inward sites fall in the upper range or are even higher than those reported in densely populated and industrialised areas of Europe, such as the Schelde estuary (Middelburg and Nieuwenhuize, 1998; Riera et al., 2000; see Como et al., 2012 for a review). The lack of difference found in our samples between acidified and non-acidified treatments indicates that in our study the level of ^{15}N enrichment is unrelated to any possible effects of acidification (Cloern et al., 2002; Kennedy et al., 2005; Ng et al., 2007). Anthropogenic nitrogen has generally higher δ^{15}N values than natural freshwater and marine nitrogen (e.g. Riera et al., 2000; Prasad and Ramanathan, 2009). Our results are in accordance with the hypertrophic conditions of the Marceddì lagoon characterised by excessive inputs of organic and inorganic anthropogenic nitrogen from a large catchment area, due to the agricultural practices that rely on the use of fertilisers and cattle manure for enhancing soil productivity (Galiano et al., 2015).

The deposit feeders found within our study area had different δ^{13}C and δ^{15}N values to one another, indicating species-specific variability in their dietary preferences. In particular, the gastropods *H. navicula* and *C. neritea* exhibited high δ^{13}C values, indicative of a large dietary contribution of ^{13}C-enriched food sources such as benthic microalgae (Riera, 2010). *C. neritea* had the highest δ^{15}N among the studied benthic consumers. This is in accordance with the fact that a substantial part of the diet of *C. neritea* is supplied by dead animal material that is more enriched in δ^{15}N than microalgal detritus (Southward et al., 1997). Contrary to the gastropods, the polychaetes *H. diversicolor* and

Cirriphormia tentaculata exhibited low δ^{13}C values similar to those of local suspension feeders and consistent with a diet mainly based on ^{13}C-depleted food sources, like phytoplankton (France, 1995). Although both *H. diversicolor* and *C. tentaculata* feed into the sediment (Kanaya et al., 2008), they can behave as secondary filter feeders exploiting suspended particles at the sediment–water interface (Fauchald and Jumars, 1979; Scaps, 2002; Costa et al., 2006). *C. tentaculata* can feed by extending its tentacles onto the sediment surface (Fauchald and Jumars, 1979), whereas *H. diversicolor* can eat a secreted mucous filter that has trapped particles carried into the burrow by water circulation (Scaps, 2002; Costa et al., 2006). On average these deposit feeders were found to be ~3‰ enriched in ^{13}C and ~11‰ enriched in ^{15}N as compared to their potential food source, SOM. Similarly, suspension feeders were found to be ~7‰ enriched in ^{13}C and ~2‰ enriched in ^{15}N as compared to the seston. These results indicate that the SOM and seston analysed in this study were not representative of the actual food source to these consumers. In shallow water systems, resuspension of sediment particles occurs due to the action of water currents on the bottom surface (De Falco et al., 2004) and this can strongly affect the variability of OM pools (Post, 2002; Como et al. 2012; Magni et al., 2013).

With regard to suspension feeders, the results of our study provide the first evidence that *A. senhousia* and native suspension feeder bivalves, such as *Cerastoderma glaucum*, *Ruditapes decussatus* and *Scrobicularia plana*, can exploit common food resources. At the densities of *A. senhousia* recorded in the Oristano Lagoon-Gulf system, competition for food is unlikely to result in the disappearance of species or death of individuals. Instead, it may have non-lethal, potential long-term effects, such as reduction of individual growth or reproductive effects (Crooks, 2001). In his experimental enclosures, Crooks (2001) showed the individual growth of native bivalves to be reduced within patches of *A. senhousia* compared to control sites. Because invasive and native bivalves can coexist over long-term periods (Strayer and Malcom, 2007), we suggest that the competitive interaction for food between *A. senhousia* and native bivalves might be an important process controlling the populations of local bivalves which needs to be considered in future studies on invasive species in coastal systems.

Contrary to other Mediterranean coastal systems (e.g. Sacca di Goro and Olbia Bay, Italy) where *A. senhousia* entered the expansion phases almost immediately after its arrival reaching the densities of 1000s ind. m^{-2} (Mistri, 2002, 2004; Munari, 2008), in the Marceddì lagoon *A. senhousia* was found at moderate densities (<1000 ind. m^{-2}) also in the spring and autumn of the year following this study. Here, the population of *A. senhousia* at the impacted sites was dominated by individuals smaller than 20 mm. Because *A. senhousia* is a short-lived bivalve growing rapidly (about 20 mm in 12 months; Crooks, 1996; Mistri, 2002; Munari, 2008), these results indicate that the mussel patches in these sites were the result of multiple recruitment and colonization events that occurred just before our study started, less than 1 year. Reproductive individuals may ensure the presence of a pool of planktonic larvae, which can settle gregariously in this system. Crooks (2001) showed that *A. senhousia* remained at densities of <1000 ind. m^{-2} for decades in Mission Bay (San Diego, CA, USA) and then expanded after changes in conditions had occurred. Contrary to the Marceddì lagoon, the low densities of *A. senhousia* described in the Santa Giusta lagoon were accompanied by its rapid disappearance from this basin. Indeed, no living mussels were found in subsequent inspections of the entire lagoon in autumn 2011 and spring and autumn 2012 . A similar demise was described in the Swan River estuary (Perth, Western Australia) where *A. senhousia* disappeared immediately after its arrival (McDonald and Wells, 2010). The organic enrichment and unfavourable environmental conditions in summer (e.g. prolonged hypoxia and anoxia) may periodically erode mussel populations, preventing them from entering into expansion phase or even impairing their establishment in the Oristano Lagoon-Gulf system. Although mortality events of *A. senhousia* caused by anoxia are reported (Mistri, 2002), *A. senhousia* appears to be more resistant to anoxia than other bivalves in its native areas (Magni et al., 2006a) and is able to enter an expansion phase soon after its arrival in some eutrophic Mediterranean lagoons (e.g., Sacca di Goro; Mistri et al., 2004; Munari, 2008). The low rates of new arrivals may contribute to the low success of invasion in this system. Production of shellfish farming (mussels and clams), which is one of the main vectors of the Asian mussel invasion (Mistri et al., 2004; Munari, 2008), is one order of magnitude lower in Sardinia than that in the highest productive areas in the Mediterranean Sea, such as the Adriatic Sea (Cataudella and Spagnolo, 2011). In addition, maritime traffic is considered to be another important vector for the introduction of the Asian mussel (Galil et al., 2008; Sylvester et al., 2011). *A. senhousia* has been sighted consistently along the main Mediterranean shipping routes including the North Adriatic (Mistri et al., 2004), Ionian

(Mastrototaro et al., 2003) and Tyrrhenian Sea (Campani et al., 2004; Munari, 2008). The isolation of the commercial harbour located in the Gulf of Oristano from the major shipping routes (www.marinetraffic.com) possibly reduces further both the frequency and intensity of invasion pressure by *A. senhousia*.

5. Conclusions

Contrary to previous studies, our results showed no effects of *A. senhousia* on the sediment features and local macrozoobenthic assemblages in the Oristano Lagoon-Gulf system (western Sardinia, Italy). Moreover, we assessed for the first time the role of *A. senhousia* on benthic food webs providing the first evidence that, although the impact of *A. senhousia* on the diet of deposit feeders was negligible, *A. senhousia* and local suspension feeding bivalves exploited common food resources. Overall, our results can be related to the moderate densities encountered in our study area (<1000 ind. m^{-2}) and to the fact that the amount of variation generated by the occurrence of *A. senhousia* in our study area was lower than the intrinsic spatial environmental and biological variability of the system. We suggest that a limited presence of shellfish farming and the isolation of the Oristano Lagoon-Gulf system from the main Mediterranean shipping routes may reduce both the frequency and intensity of introduction of new arrivals in our study area. Also, unfavourable environmental conditions (i.e. anoxia) in summer can periodically erode mussel populations, preventing *A. senhousia* from entering an expansion phase, thereby mitigating subsequent ecological impacts on the local infauna and the functioning of the system.

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Table 1. Results of leave-one-out allocation of observations to groups in CDA for sediment variables and macrozoobenthic assemblages (excluding *A. senhousia*) in the Santa Giusta lagoon. Macrozoobenthos were square-rooted transformed.

	Sediment variables								Sediment variables							
	Classified into groups								Classified into groups							
Groups	1	2	3	4	5	6	Total	% correct	1	2	3	4	5	6	Total	% correct
Site 1–Pres. *A.senhousia*= 1	4	**3**	0	0	2	0	9	44.44%	5	**4**	0	0	0	0	9	55.56%
Site 1–Abs. *A.senhousia*= 2	**4**	5	3	0	3	3	18	27.78%	**4**	13	1	0	0	0	18	72.22%
Site 2–Pres. *A.senhousia* = 3	0	1	4	**6**	0	2	13	30.77%	0	2	7	**4**	0	0	13	53.85%
Site 2–Abs. *A.senhousia* = 4	1	0	**5**	7	0	1	14	50.00%	0	0	**5**	9	0	0	14	64.29%
Site 3–Pres. *A.senhousia* = 5	2	0	0	0	5	**5**	12	41.67%	0	3	4	0	0	**5**	12	0.00%
Site 3–Abs. *A.senhousia* = 6	0	0	1	3	**4**	7	15	46.67%	0	2	0	0	**4**	9	15	60.00%
	Total correct = 31/81 = 39.51%								Total correct = 43/81 = 53.09%							
	Misclassification error= 60.49%								Misclassification error = 46.91%							

Table 2. Mean (±SE) δ^{13}C and δ^{15}N values in seston (<55 µm), sedimentary organic matter (SOM), and in the tissues of the suspension feeders *Arcuatula senhousia*, *Cerastoderma glaucum*, *Ruditapes decussatus* and *Scrobicularia plana*, the deposit feeders *Hediste diversicolor*, *Cirriphormia tentaculata*, *Haminoea navicula* and *Cyclope neritea* at each site in the Marceddì lagoon. Two sizes of *A. senhousia* (i.e., < 1 and 1-2 cm) were analysed where possible. The number of replicate stations within sites was 4 for *A. senhousia*, 3 for *H. diversicolor*, *H. navicula*, *C. neritea*, *C. tentaculata*, *C. glaucum*, SOM and Seston and 2 for *R. decussatus* and *S. plana*.

	Sites with *A. senhousia* (I)								Sites without *A. senhousia* (C)							
	I1				I2				C1				C2			
	δ^{13}C		δ^{15}N		δ^{13}C		δ^{15}N		δ^{13}C		δ^{15}N		δ^{13}C		δ^{15}N	
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
Seston [<55µm]	-28.3	0.1	10.3	0.3	-24.8	0.4	16.8	0.2	-26.1	0.0	15.3	0.5	-21.5	0.4	14.3	0.4
SOM	-20.1	0.6	6.0	0.2	-22.5	0.2	5.8	0.8	-19.3	0.2	7.3	0.9	-16.7	0.5	3.9	0.3
Suspension feeders																
A. senhousia [<1 cm]	-20.8	0.4	17.0	0.1	-		-		-		-		-		-	
A. senhousia [1-2 cm]	-20.8	0.5	17.0	0.1	-22.1	0.4	16.4	0.2	-		-		-		-	
C. glaucum	-20.0	0.2	17.2	0.3	-20.5	0.2	17.7	0.1	-19.0	0.2	16.8	0.2	-15.1	0.2	13.6	0.1
R. decussatus	-		-		-20.3	0.9	16.9	0.0	-19.2	0.3	16.8	0.2	-		-	
S. plana	-		-		-20.0	0.3	18.0	0.0	-18.6	0.0	17.9	0.2	-14.7	0.0	14.6	0.2
Deposit feeders																
H. diversicolor	-18.8	0.2	18.7	0.3	-19.6	0.4	19.8	0.3	-18.7	0.0	17.8	0.2	-15.3	0.3	15.3	0.4
C. tentaculata	-		-		-19.5	0.3	16.4	0.2	-19.3	0.1	16.0	0.2	-13.1	0.2	12.4	0.2
H. navicula	-14.8	1.2	18.0	0.2	-18.7	0.5	17.3	0.3	-16.0	0.2	16.0	0.1	-12.8	0.4	10.0	0.5
C. neritea	-15.9	0.5	19.9	0.4	-17.3	0.6	20.0	0.2	-15.7	0.4	19.5	0.4	-12.0	0.4	16.5	0.3

Table 3. Two-way analysis of variance on the δ^{13}C and δ^{15}N values of the deposit feeders *Hediste diversicolor*, *Haminoea navicula* and *Cyclope neritea*, and sedimentary organic matter (SOM) in the Marceddì lagoon. The experimental design included two sites within each level of factor *Arcuatula senhousia* (i.e. Impact and Control). Data were untransformed. The number of replicate stations within each site was three.

		Hediste diversicolor						*Haminoea navicula*					
		δ^{13}C			δ^{15}N			δ^{13}C			δ^{15}N		
	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Arcuatula = A	1	14.26	1.55	0.34	21.79	3.93	0.19	14.63	0.82	0.46	64.96	2.41	0.26
Site(A) = S(A)	2	9.17	38.43	0.00	5.54	19.86	0.00	17.80	9.76	0.01	26.97	94.78	0.00
Residual	8	0.24			0.28			1.82			0.28		
Total	11												
Cochran's C Test		C=0.54, P>0.05			C=0.37, P>0.05			C=0.64, P>0.05			C=0.55, P>0.05		
Transformation		none			none			none			none		

		Cyclope neritea						SOM					
		δ^{13}C			δ^{15}N			δ^{13}C			δ^{15}N		
	DF	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Arcuatula = A	1	22.01	1.85	0.31	11.64	1.77	0.32	32.47	3.29	0.21	0.41	0.05	0.85
Site(A) = S(A)	2	11.91	18.25	0.00	6.59	23.22	0.00	9.88	18.21	0.00	8.73	7.31	0.02
Residual	8	0.65			0.28			0.54			1.19		
Total	11												
Cochran's C Test		C=0.36, P>0.05			C=0.36, P>0.05			C=0.48, P>0.05			C=0.48, P>0.05		
Transformation		none			none			none			none		

Figures legend

Figure 1. (a) Location of the Oristano Lagoon-Gulf system (western Sardinia, W-Mediterranean Sea) and the Santa Giusta and Marceddì lagoons where the study was conducted. (b) Sampling design and location of impacted (I) and control (C) sites in the Marceddì lagoon. The sampling design included two factors, *A. senhousia* (2 levels; fixed) and Site (2 levels; nested in *A. senhousia*) and 3 replicate stations.

Figure 2. Canonical discriminant analysis (CDA) for differences among stations in sediment variables and macrozoobenthic assemblages in the Santa Giusta lagoon. Symbols represent the three sites: square = site 1; circle = site 2; diamond = site 3. Colours represent stations characterised by the presence/absence of *A. senhousia*: Black = presence of *A. senhousia*; white = absence of *A. senhousia*.

Figure 3. Mean values (±SE) of the density of *A. senhousia* at each site in the Marceddì lagoon. The densities per each group of mussel size are reported.

Figure 4. Mean δ^{13}C and δ^{15}N values (±SE) in seston (<55 µm), sedimentary organic matter (SOM), *A. senhousia* (black square), suspension feeders (grey square), deposit feeders (open symbols) in impacted and control sites in the Marceddì lagoon (see Table 1). The symbols indicate the mean δ^{13}C and δ^{15}N values (±SE) calculated among sampling stations within each site. The δ^{13}C and δ^{15}N values in two sizes of *A. senhousia* (i.e. <1 and 1-2 cm length) are reported for the impacted site 1 (I1; black overlapped symbols). Light grey symbols are OM pools: Seston, light grey circle; SOM, light grey square; Grey symbols: *C. glaucum*, grey circle; *R. decussatus*, grey diamond; *S. plana*, grey triangle; Open symbols are deposit feeders: *H. diversicolor*: open square; *C. tentaculata*: open circle; *H. navicula*: open diamond; *C. neritea*: open triangle.

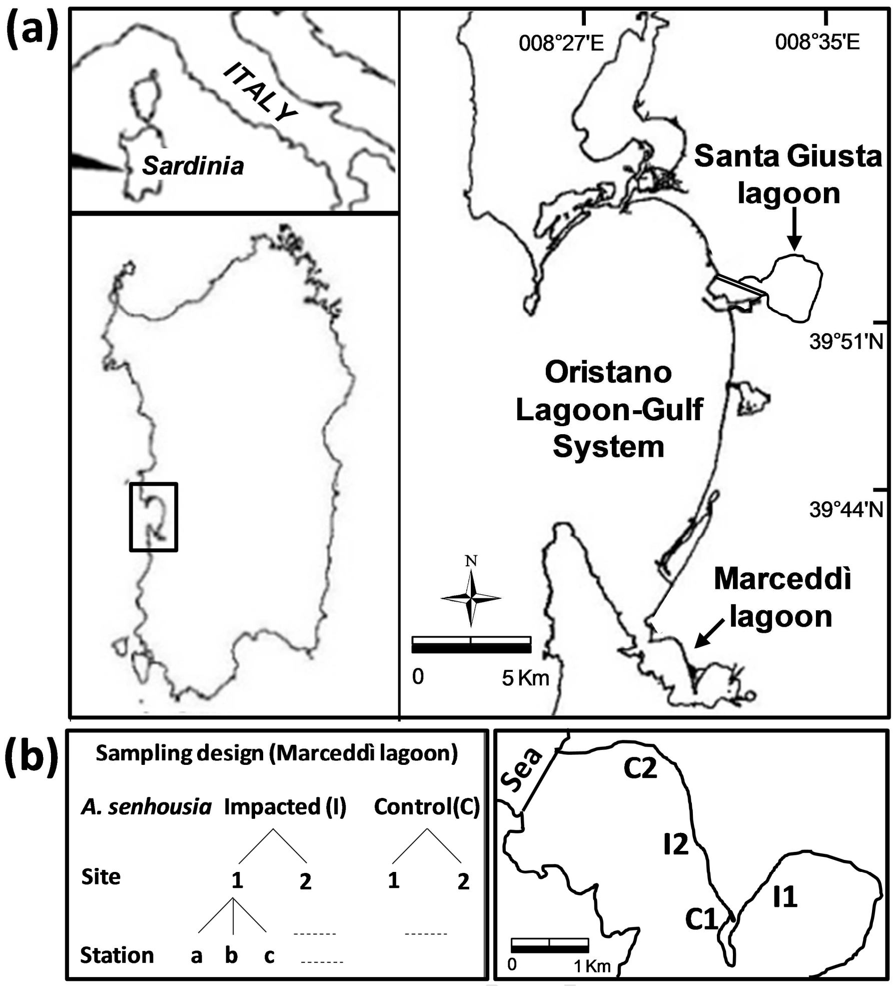
(a)
ITALY
Sardinia
008°27'E
008°35'E
Santa Giusta lagoon
39°51'N
Oristano Lagoon-Gulf System
39°44'N
N
Marceddì lagoon
0
5 Km
(b)
Sampling design (Marceddì lagoon)
A. senhousia
Impacted (I)
Control(C)
Site
1
2
1
2
Station
a
b
c
Sea
C2
I2
I1
C1
0
1 Km

Figure 1.

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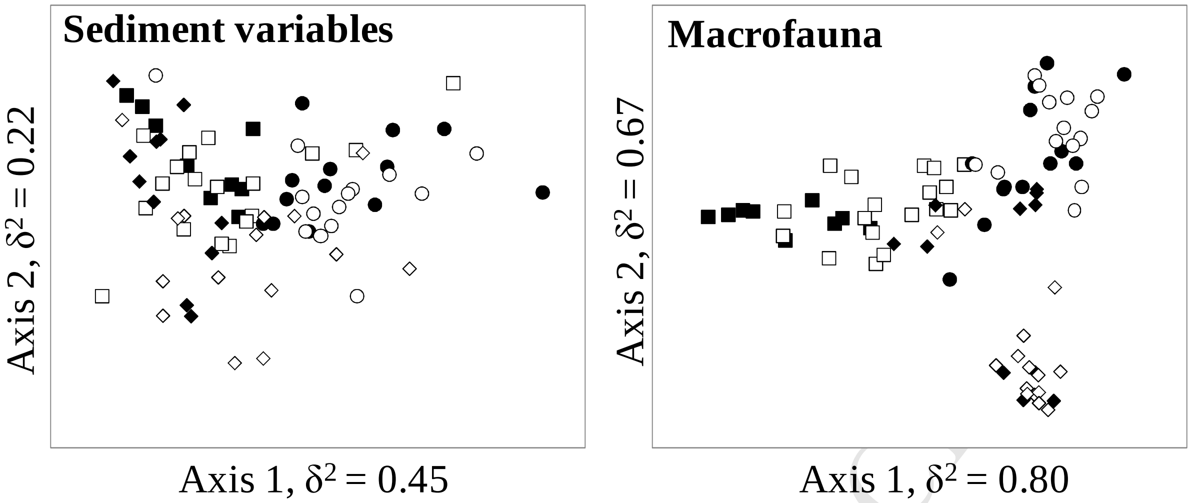

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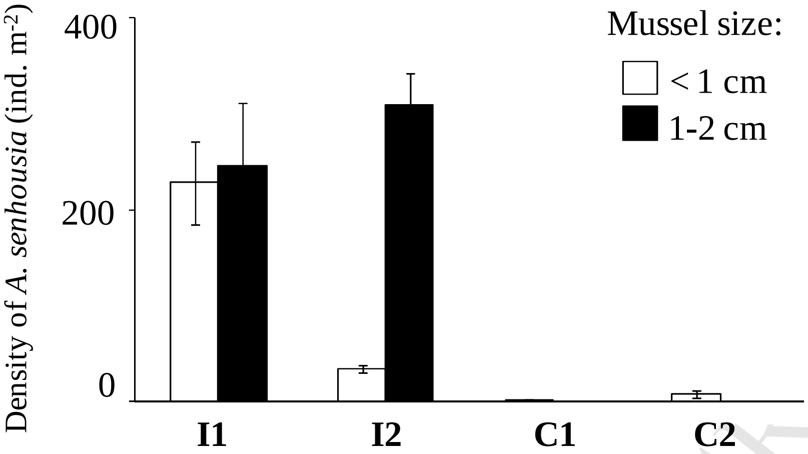
38 **Figure 2.**



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40 **Figure 3.**

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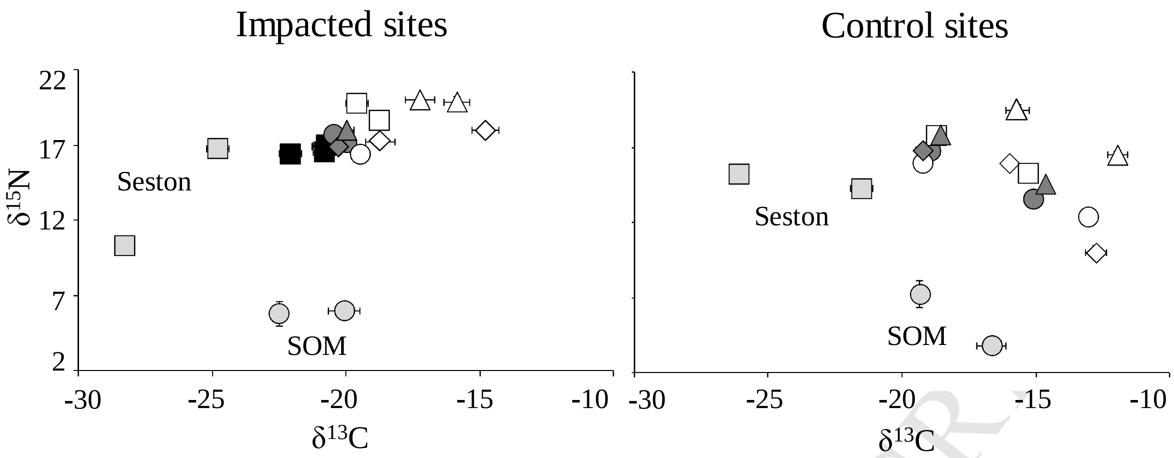

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45 **Figure 4.**