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Bivalve $\delta^{15}N$ isoscapes provide a baseline for urban nitrogen footprint at the edge of a World Heritage coral reef

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Abstract

Eutrophication is a major threat to world's coral reefs. Here, we mapped the distribution of the anthropogenic nitrogen footprint around Nouméa, a coastal city surrounded by 15,743 km^2 of UNESCO listed reefs. We measured the $\delta^{15}N$ signature of 348 long-lived benthic bivalves from 12 species at 27 sites and interpolated these to generate a $\delta^{15}N$ isoscape. We evaluated the influence of water residence times on nitrogen enrichment and predicted an eutrophication risk at the UNESCO core area. Nitrogen isoscapes revealed a strong spatial gradient (4.3 to 11.7‰) from the outer lagoon to three highly exposed bays of Nouméa. Several protected reefs would benefit from an improved management of wastewater outputs, while one bay in the UNESCO core area may suffer a high eutrophication risk in the future. Our study reinforces the usefulness of using benthic animals to characterize the anthropogenic N-footprint and provide a necessary baseline for both ecologists and policy makers.

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Keywords: $\delta^{15}N$, isoscape, bivalve, eutrophication, coral reefs, heritage sites.

Highlights

- Benthic filter-feeding bivalve communities are good bio monitors of spatial variations in anthropogenic-based eutrophication
- The baseline $\delta^{15}N$ value found in bivalves from the outer lagoon of New Caledonia was 4.7±0.4‰.
- The $\delta^{15}N$ signature of benthic bivalves reached 11.7‰ in the most exposed station.
- Isoscapes might be used both for long-term monitoring and to predict risks of at-sea anthropogenic pollution.

Introduction

Coral reef systems are internationally recognized as places of outstanding universal value. Coral reefs and lagoons represent only 5% of Earth's surface but are considered major biodiversity reservoirs (Reaka-Kudla 1996, Small et al. 1998, Knowlton et al. 2010). Associated with this biodiversity of high conservation value, coral reef fisheries play a major role in the sensitivity of low- and middle-income countries to food security, particularly in island territories (Hughes et al. 2012, Foale et al. 2013). From an economic perspective, the value of coral reefs is also increased by the development of nature-based tourism worldwide (Spalding et al. 2017). All these characteristics contribute to the outstanding value of coral reefs, of which the most significant ones are protected under the UNESCO World Heritage Convention.

An assessment of 14 reef regions in 2003 showed a continuous degradation of these ecosystems from the pre-human historical period to present (Pandolfi et al. 2003). McClenachan et al. (2017) even highlighted a strong spatial gradient in the loss of coral cover from the inner lagoon (-87%) to the forereef (-0%). Global factors related to climate change and oceans acidification are known to drive recent mass bleaching events and disease outbreaks in coral reefs (Buddemeier et al. 2003), but local anthropogenic-based factors such as overfishing and water quality certainly have also impacted these systems for a long time (Jackson et al. 2001). A recent work pointed out many factors associated with urban development that are, or could be, involved in the ongoing changes observed in hard coral communities (Heery et al. 2018). However, the respective effects of global and local scale factors on coral reefs remain difficult to disentangle.

Among the local anthropogenic-based factors affecting coastal coral reefs, eutrophication is certainly predominant (Bell et al. 1992, 2014). High nitrogen loadings have been shown to have adverse direct and indirect effects on coral resilience to climate change and bleaching

events and managing sewage inputs is particularly challenging to protect coral reefs (Wooldridge 2009, D'Angelo and Wiedenmann 2014, Vega Thurber et al. 2014). It was suggested that a 50-80% reduction of dissolved organic nitrogen loading at inshore reefs subject to high water runoff would be equivalent to a 2.0-2.5°C lowering of the bleaching temperature threshold for corals (Wooldridge 2009). Risk assessment models are under development that will help identifying the most sensitive coral reefs and prioritize management actions for their protection (Wooldridge et al. 2017). Meanwhile, 25 of the 29 UNESCO coral reef sites suffered important bleaching stress over the last three years, including the lagoon of New Caledonia which experienced coral bleaching in 90% of fringing and lagoon reef sites in 2016 (Benzoni et al. 2017). The totality of these World Heritage sites might even cease to exist by the end of this century if indirect and direct threats are not drastically reduced. However, eutrophication due to land-derived nutrient discharge is suspected to already occur at 104 out of 112 reefs listed in the World Atlas of Coral Reefs (Wear and Thurber 2015).

Half of world heritage coral reefs are located close to a continental landmass, including the Great Barrier Reef and the lagoons of New Caledonia which are also close to major cities of more than 150,000 inhabitants. Significant urbanization is ongoing in the peninsula of Nouméa, the capital city of New Caledonia (Fig. 1). New Caledonia coral reefs cover 23,000 km^2 and host around 450 coral species. These reefs are now protected by 15,743 km^2 (68 % of total) of UNESCO World Heritage sites since 2008 (Andréfouët and Wantiez 2010). The New Caledonia government also implemented 440 km^2 of marine protected areas, (2 % of total). However, some of these protected areas, including natural reserves, are located close to an urban center. The Capital city of Nouméa hosts approx. 100,000 people and released around 22,860,000 L of wastewater in 2008, 24% being treated (Broustet and Rivoilan 2015, Gouvernement de la Nouvelle Calédonie, 2009). Thereby, identifying major terrestrial

nutrient sources, particularly nitrogen, is crucial to prevent water quality degradation in these protected reefs. Mapping the extent of the anthropogenic nitrogen footprint around Nouméa will provide an invaluable baseline to implement effective sewage management policies in the Islands of the Southwest Pacific.

During the secondary treatment of sewage in Wastewater Treatment Plants (WTPs), both aerobic nitrification (ammonium to nitrate) and anaerobic denitrification (reduction of nitrate to N_2 gas) leave the remaining ammonium and nitrate enriched in $\delta^{15}N$ (Kendall et al. 2008). Marine primary producers living around sewage outputs, such as algae and phytoplankton, thus consume dissolved inorganic nitrogen that is enriched in ^{15}N (Cabana and Rasmussen 1996, Tucker et al. 1999). Similarly, consumers exposed to secondary or greater treated effluents ingest particulate organic matter that is enriched in ^{15}N (Gartner 2002). This enrichment due to sewage disposal can be measured on tissues of these organisms, and $\delta^{15}N$ values be used as a proxy of nitrogen pollution (Cabana and Rasmussen 1996, Costanzo et al. 2005, Schlacher and Connolly 2009, Kaminski et al. 2018). The $\delta^{15}N$ values vary along the food web, with an enrichment in ^{15}N while transferring to higher trophic levels (DeNiro and Epstein 1981, Peterson and Fry 1987, Morissey et al. 2013). At a specific trophic level, $\delta^{15}N$ values may also vary depending on seasonal variations associated with the growth rate of organisms (Gorman et al. 2017, Lorrain et al. 2002, Perga et al. 2005). Therefore, the choice of indicator species should be made according to the study objective and consider i) the source of nitrogen uptake, ii) the tissue turnover rate and iii) organism mobility. Primary producers with fast tissue turnover rates (e.g., algae and phytoplankton), will allow for the detection of subtle variations in the dissolved inorganic nitrogen sources (Risk et al. 2009). Conversely, bivalves and invertebrates with slower turnover rates will provide integrated measures of N-inputs over longer time periods (several months to one year) and have been proposed as good indicators of wastewater inputs (Carmichael et al. 2004, Fukumori et al.

2008, Gorman et al. 2017, Graniero et al. 2016). Bivalves, being present in different ecosystems - including coastal habitats - at all latitudes, represent important candidate species for studying anthropogenic signals and environmental changes in coastal waters worldwide (Richardson 2001). Bivalves have thus been used for both tracking marine sewage disposal and mapping baseline nitrogen isoscapes (Costanzo et al. 2005, Vokhshoori and McCarthy 2014). Isoscapes, for "isotope landscapes", correspond to spatially explicit predictions of isotope ratios, which are used to investigate geological, biogeochemical, anthropological, biological or ecological processes (West et al. 2010).

The present study aims at mapping the distribution of anthropogenic nitrogen enrichment in the southwest lagoon of New Caledonia. We used $\delta^{15}N$ values in bivalves as a time integrated proxy of wastewater inputs, and coastal water flushing time as a contributing factor. The study provides a baseline assessment of anthropogenic-based nitrogen inputs in the lagoon surrounding Nouméa, the first $\delta^{15}N$ isotopic baseline (isoscape) in this area. Results are discussed in term of pollution risks for coral reefs included in UNESCO listed marine protected areas, and challenges regarding wastewater treatment in surrounding coastal cities.

Methods

Study site

Nouméa, the capital city of New Caledonia, is a 45 Km^2 peninsula hosting 99,926 people with a population density of 2,221 individuals/km^2 (Broustet and Rivoilan 2015). This population experienced a 4-fold increase in 50 years and is increasing by 2.4% annually (Broustet and Rivoilan 2015). Two Sustainable Resource Management Areas are along the coasts of Nouméa, which is only 6.5 km away from the closer Integral Natural Reserve, 10 km away from an Integral Seasonal Natural Reserve, 10.2 km from a Natural Reserve, 21 km from the

Provincial Marine Park and 42 km away from the closer UNESCO world heritage coral reef. In 2008, seven WTPs in Nouméa treated less than 5.4 million L of wastewaters for a total need estimated around 22,86 million L (Gouvernement de la Nouvelle Calédonie 2009). Most of the wastewater network in Nouméa is made of open ditches or nozzles, originally designed for the drainage of rainwater, in which effluents from septic tanks directly discharges (Gouvernement de la Nouvelle Calédonie 2009). Consequently, anthropogenic eutrophication at inshore stations surrounding Nouméa has been recorded with high particulate organic nitrogen values up to 1.9 µM (Fichez et al. 2010). An ongoing development project includes the building of a new 104 km long sewage network to separate rainwater from domestic effluents and connect 95 % of the population to WTPs (Gouvernement de la Nouvelle Calédonie 2009). Realizations from this project included a new WTP in the Sainte-Marie Bay (Fig. 1), which has been put into service the year following our study. By analyzing data collected prior to the ongoing improvement of the water management plan, we address the need for a "reference" point. Simultaneously, we promote a method that should be reproduced over time to evaluate the efficiency of this program.

Experimental design

Samples were collected by SCUBA diving from February to October 2012, at 27 stations located within the southwest lagoon of New Caledonia. This part of the lagoon extends from the Nouméa peninsula to the barrier reef, about 40 km southwest. It can be divided in three geomorphological areas: the inner lagoon, the outer lagoon and the inner reef (Ouillon et al. 2010). The mean water depth is approximately 17.5 m. To generate baseline $\delta^{15}N$ isoscapes, we selected 15 sampling stations in the inner lagoon, close to the coastline of Nouméa to capture potential sewage pollution (**Fig. 1**). Nine stations were sampled in the outer lagoon to account for a potential gradient in the nitrogen isotopic values. Samples were also collected at three stations located close to the barrier reef, at both Dumbéa and Boulari passes.

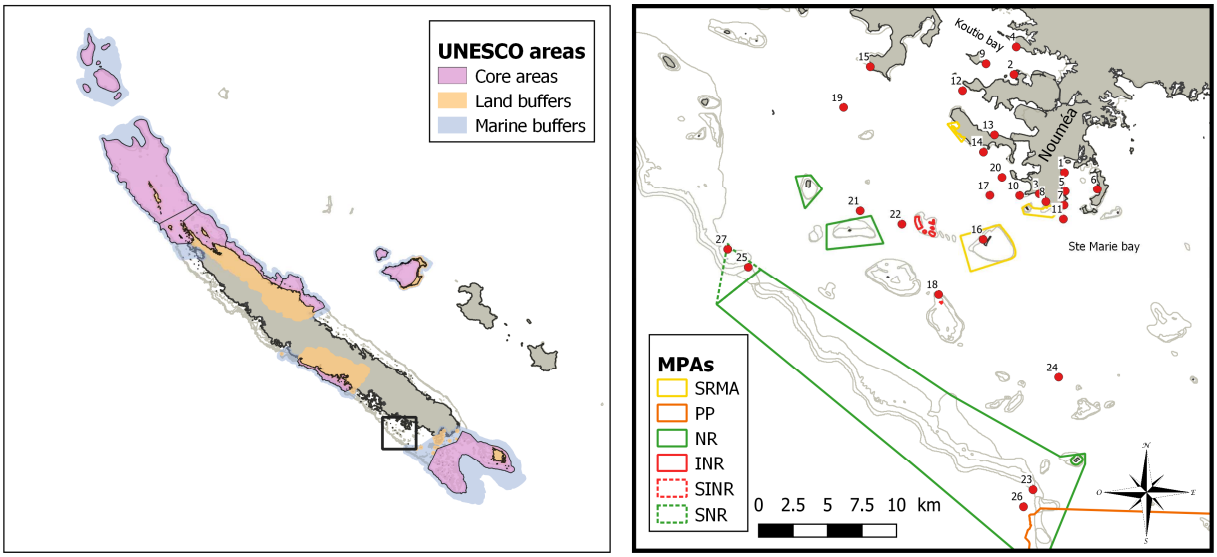


Fig. 1. The UNESCO reefs in New Caledonia (left panel), and sampling sites in the southwest lagoon of New Caledonia, near the city of Nouméa (red circles, right panel). SRMA: Sustainable Resource Management Area; PP: Provincial Park; NR: Natural Reserve; INR: Integral Natural Reserve; SINR: Seasonal Integral Natural Reserve; SNR: Seasonal Natural Reserve.

Study taxa

We worked on bivalves as these organisms have been suggested good archives of N isotopic composition at the base of marine ecosystems (Grall et al. 2006, Post et al. 2002, Gillikin et al. 2017). Such organisms have a long tissue turnover time and their muscle $\delta^{15}N$ values reflect the $\delta^{15}N$ values of their diet in the preceding months (Lorrain et al. 2002). Furthermore, previous work showed very small seasonal variability in $\delta^{15}N$ values of bivalve muscles (Lorrain et al., 2002; Nerot et al. 2012; Gillikin et al., 2017), therefore we did not expect large differences between our sampling dates from February to October 2012. In this study, we made a focus on four suspension-feeding bivalve families that were the most abundant in the sampling sites: Arcidae, Pectinidae, Pteridae and Spondylidae. All individuals

were collected on soft-sediment substrate except *Spondylus sp*. that lives attached to hard substrate such as massive corals.

Because each site hosted different species, from one to five with a median of two, we decided two conduct our analyzes in two steps. First, we used only individuals belonging to the most common genus (*Anadara*) to produce both an "unbiased" $\delta^{15}N$ baseline and isoscape. Then, we used all collected bivalve species to produce the same $\delta^{15}N$ isoscape at a larger scale. Comparison of results from the two datasets are also provided to allow for discussing pros and cons associated with multispecies assessments when aiming at mapping marine pollution.

Stable isotope analysis

For each bivalve specimen, muscle tissues were dissected, stored frozen, and shell size measured. Mean sizes and associated standard deviations are given in Supplementary Materials (**SM 1**). Muscles were freeze-dried, ground into powder, and weighed in tin cups for isotopic analysis (about 1mg in 4 × 6 mm tin cups). Muscle samples were analyzed using a Thermo Delta Advantage mass spectrometer in continuous flow mode connected to a Costech Elemental Analyzer via a ConFlo IV at Union College (Schenectady, NY, USA). Ammonium sulfate [IAEA-N-2], caffeine [IAEA-600], and an in-house acetanilide were used as standards; measurements of $\delta^{15}N$ are reported to atmospheric nitrogen. The uncertainty for $\delta^{15}N$ measurements was ±0.15‰ based on repeated analysis of an in-house acetanilide standard.

Residence times

Hereafter, we use the term residence time which encompasses various terminologies - “flushing time”, “e-flushing time”, and “e-folding time”- previously used to describe this phenomenon (Jouon et al. 2006, Drouzy et al. 2019). We modeled the local residence times for the period corresponding to the collection of bivalves at our study sites (February to October 2012). They were calculated using the MARS3D hydrodynamics model presented in Jouon et al. (2006). This model accounts for the influence of tides, total wind and river inputs in the southwest lagoon of New Caledonia over the considered period (Torreton et al. 2007). Estimates of residence times are presented as means ± 1 standard deviation.

Statistical analyzes

Data were compiled and analyzed using R Software v 3.4.0. (R core Team 2017). We first worked with the most abundant bivalve genera only (i.e., *Anadara*). This choice allowed for minimizing biases associated with different bivalves’ ecology and physiology in mapping baseline isoscapes. The *Anadara* dataset covered 19 stations around Nouméa. We used a Student’s t-test to compare mean $\delta^{15}N$ values measured in the inner and outer lagoon.

In a second step, we used data from the 13 species together. We calculated mean $\delta^{15}N$ values at each site and produced a map including samples from the 27 sampling sites together. We ran pairwise t-tests to compare $\delta^{15}N$ values estimated from *Anadara* spp. only and values obtained from all bivalve species available. We tested for the effect of water residence time on measured $\delta^{15}N$ values through a linear mixed model, using the R package “lme4” v.1.1.21 (Bates et al. 2015). The linear model was fitted with residence times as a fixed effect, and site as a random effect. Assumptions were tested graphically and with a Shapiro-Wilk normality test.

Isoscape mapping

Mean $\delta^{15}N$ values and standard deviations at each sampling station were calculated in R. We used the UNIRAS software library (Advanced Visual Systems) to generate $\delta^{15}N$ isoscape maps. UNIRAS allows for interpolation from point data in a geo-referenced grid using a bivariate function of the bi-polynomial type. At each station, we considered all neighboring stations within a 3000m radius for bipolynomial interpolation. The interpolation distance was set at 300m. Every reef and coastline were defined as fracture lines to avoid spatial autocorrelation between stations separated by some land. In a second step, the software smoothed the obtained grid using a quadratic interpolation, and values at each point were replaced by the weighted average of the adjacent nodes.

We also used our data to project a potential eutrophication risk for the whole southern lagoon of New Caledonia. We used the coefficients of a multiple linear regression explaining $\delta^{15}N$ in bivalves by the residence time of water (continuous) and the exposition to anthropogenic pollution (2 level factor). The map was produced by transforming the residence time map with the following formula:

$$\delta^{15}N = a \times \text{Residence time} + b$$

where, a corresponds to the estimate of the fixed effect of residence time, and b to the intercept which includes the fixed effect of high exposure to wastewaters (Inner Lagoon locations). An index of eutrophication risk was then calculated by categorising the estimated $\delta^{15}N$ values obtained for each cell of the map's grid. Thresholds were defined as follow: 0 for $\delta^{15}N < 4.0$; 1 for $4.0 < \delta^{15}N < 5.2$; 2 for $5.2 < \delta^{15}N < 6.4$; 3 for $6.4 < \delta^{15}N < 7.6$; 4 for $7.6 < \delta^{15}N < 8.8$; 5 for $\delta^{15}N > 5.2$.

Results

$\delta^{15}N$ baseline from *Anadara* genus

We collected 123 individuals belonging to the *Anadara* genus at only 19 out of our 27 sites (**Fig. 2**). We were able to identify at least three species, namely the most common *Anadara antiquata* (n=87), *A. dautzenbergi* (n=13), *A. uropigimelana* (n=9). The 14 remaining individuals were not identified at the species level. The mean size of collected individuals was 4.75 cm (±1.26). Differences between $\delta^{15}N$ values measured on different *Anadara* species (6 sites) were not significant. At two stations (Pic des Morts and Crouy), only one *Anadara* individual was found.

The $\delta^{15}N$ values measured at the seven stations located in the outer lagoon were homogenous compared to those of the inner lagoon (**Fig. 2**)**.** Overall, bivalves from the coastal stations had significantly higher $\delta^{15}N$ values compared to individuals found in the outer lagoon ($t = 16.699$; $p < 0.001$; Means for the inner lagoon: 8.0 ± 1.8‰ and outer lagoon: 4.7 ± 0.4‰). Only two coastal stations (n°11 and 12) located at bay entrances showed $\delta^{15}N$ values similar to those from the outer lagoon (**Fig. 2, Table 1**).

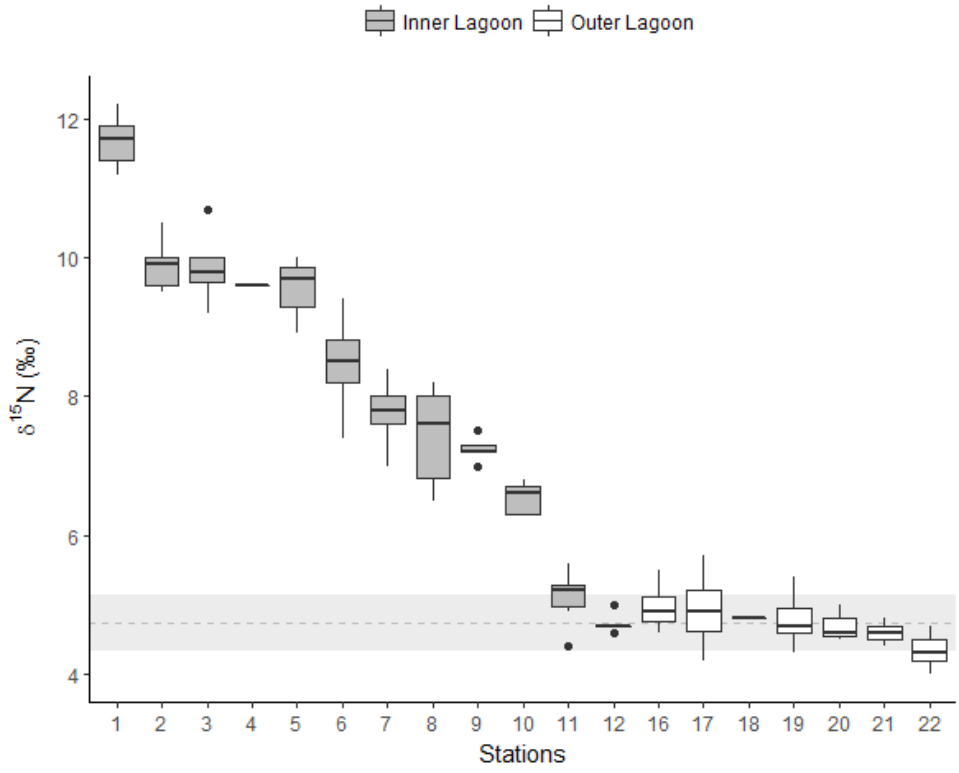


Fig. 2. Mean $\delta^{15}N$ values in muscles of *Anadara* spp. bivalves measured at 19 stations located in the inner (Nouméa bays, coastal stations) and outer lagoon. See Fig. 1 for station locations. The dashed line and grey band represent the "baseline" value (4.7±0.4‰) calculated from all outer lagoon stations, and ± 1 standard deviation.

Mean $\delta^{15}N$ values and associated standard deviations are shown in **SM 2** for each station where *Anadara* spp. were collected. $\delta^{15}N$ values at stations located in the inner lagoon (Nouméa bays) varied from 4.7‰ (± 0.2) to 11.7‰ (±0.4). The highest recorded mean was from station n°1 (Eau vive), at the inner part of the Sainte-Marie bay (11.7‰ ±0.4). Conversely, $\delta^{15}N$ measured in the outer lagoon varied from 4.3‰ (±0.3) to 5.0‰ (±0.4), with a mean "baseline" value of 4.7‰ (±0.4). The lowest mean was recorded at the station n°22 in the isolated Laregnère reef (4.3 ± 0.3‰).

Five stations along Nouméa coastline showed particularly high mean $\delta^{15}N$ values (**SM 3**). The two stations of the west coast of the Sainte-Marie bay (n°5 and 1) had high mean $\delta^{15}N$ values (11.7±0.5‰ and 9.5±0.4‰ respectively) much more elevated than those of the outer lagoon (~4.7‰). A similar situation was found in the Koutio Bay, particularly at the stations n°4 and 2 (9.6±0.5‰ and 9.9±0.4‰ respectively) located further inshore. Finally, individuals collected at station n°3 also presented high $\delta^{15}N$ values (9.9±0.5‰).

Using the bivalve community to build $\delta^{15}N$ isoscapes

We collected a total of 348 filter-feeding bivalves belonging to 9 genera and 12 species, including *Anadara* spp. individuals previously mentioned. Apart from *Anadara sp. (*Arcidae*)*, *Comptopallium radula* (Pectinidae) and *Spondylus sp*. (Spondylidae) were the most abundant (n=60 and n=51 respectively). We collected *Barbatia sp* (Arcidae), *Bractechlamys vexillum* and *Mimachlamys sp.* (Pectinidae). Two species of Pteridaewere also included in our samples as 37 individuals of both *Isognomon sp.* and *Pinctada maculata* were found.

The map produced with muscle $\delta^{15}N$ values from all bivalve samples confirmed the pattern obtained with *Anadara* spp alone (**Fig. 3**). Highest $\delta^{15}N$ values were measured in four bays (Sainte-Marie, Koutio and Anse Vata) where the five WTPs mentioned above are located.

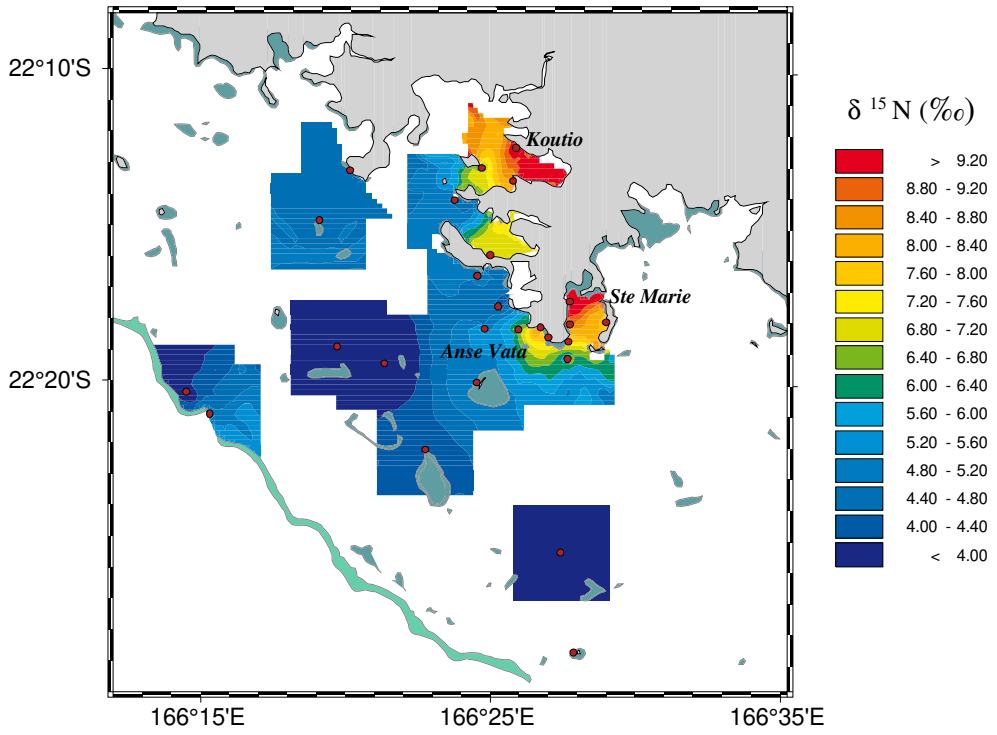


Fig. 3. $\delta^{15}N$ isoscape in 2012, estimated at 27 stations in the southwest lagoon of New Caledonia from muscle samples of 12 bivalve species.

Anadara spp. were collected together with at least another bivalve species at nine of the 27 sampling stations. The comparison of the mean $\delta^{15}N$ value obtained from *Anadara* versus other bivalves at these stations is illustrated in **Fig. 4**. It shows that using *Anadara* spp. provides significantly higher values of $\delta^{15}N$ (+0.9‰), although differences between sites were consistent. Including several bivalve species allowed for a wider spatial coverage in our study area as *Anadara* was absent from eight of our sampling stations.

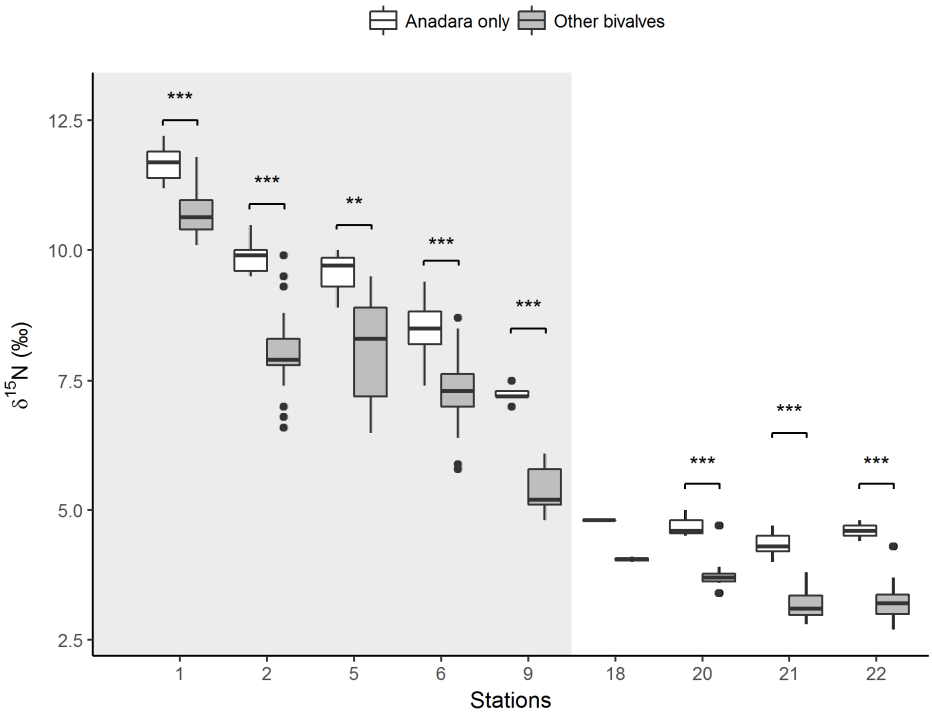


Fig. 4. Comparison of mean muscle $\delta^{15}N$ values of *Anadara* spp. samples versus other bivalve species. Stations on the figure's left-hand side (Grey background) are in the inner lagoon, while those on the right-hand side are in the outer lagoon. All pairwise differences were significant except at the station 18 where only one specimen of *Anadara* was collected.

Local water residence time

Estimated local residence times varied at our 27 stations and were estimated between 3 (± 5) and 32 (± 5) days (**Fig. 5, SM 4, SM 5**). The shortest residence time was estimated at the Boulari and Dumbéa passes, ca. 3.1 (± 4.8) and 3.4 (± 1.2) days respectively. Conversely, residence times were the longest in the bays of Koutio (Station 2: 32.4± 5.3 days and station 4: 29.9± 6.2days) and Sainte-Marie (Station 6: 27.1± 3.2; station 1: 27.1± 3.6; station 5: 26.9± 3.0 days). At the two stations located at the Anse Vata bay, we estimated local residence times of 18.1± 3.1 and 20.1± 3.5 days respectively. Gradients can be distinguished from the inner part of bays and inner part of reefs to the passes. Along the coastline of

Nouméa, the bays of Koutio and Sainte-Marie showed the lowest residence times. The effect of residence times significantly contributed to explain observed variations in $\delta^{15}N$ (**Fig. 6**; estimate [98%CI] = 0.10 [0.02;0.21]). The complete model had a R^2 of 0.89, and the normality of residuals was respected (W=0.99, *p*=0.12).

Our projection of potential eutrophication risk in the southern lagoon of New Caledonia is presented in **Fig 7**. It suggests that only one location contained in the UNESCO core area (Koroxu bay, Isle of Pines) could be sensitive to substantial urbanization. As expected, the most exposed area according to water residence time is where we conducted our study. The pattern observed in Bouraké (Northward) was expected because of the bay enclosure.

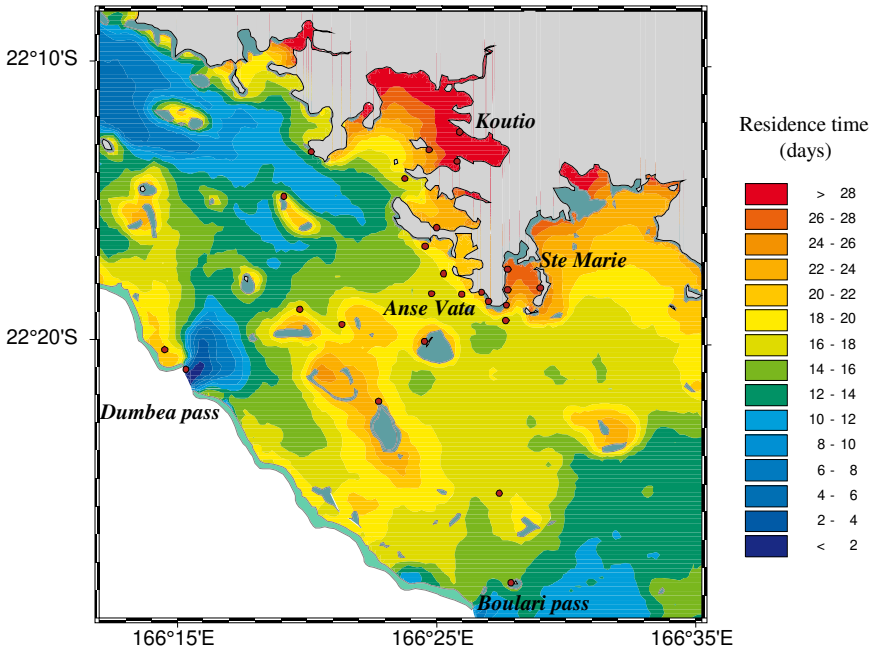


Fig. 5. Average local water residence times (in days) from February 2012 to October 2012 around the Nouméa Peninsula.

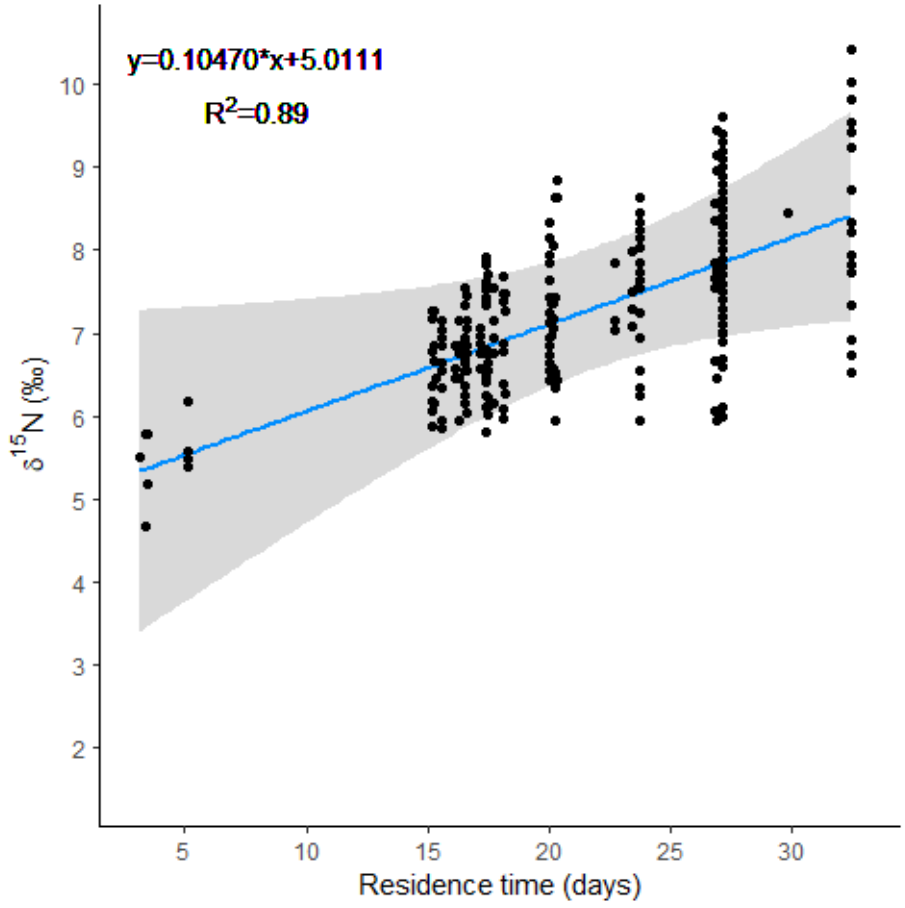


Fig. 6. Relationship between $\delta^{15}N$ values from muscle samples of 12 bivalve species and waters residence times measured at 27 stations in 2012.

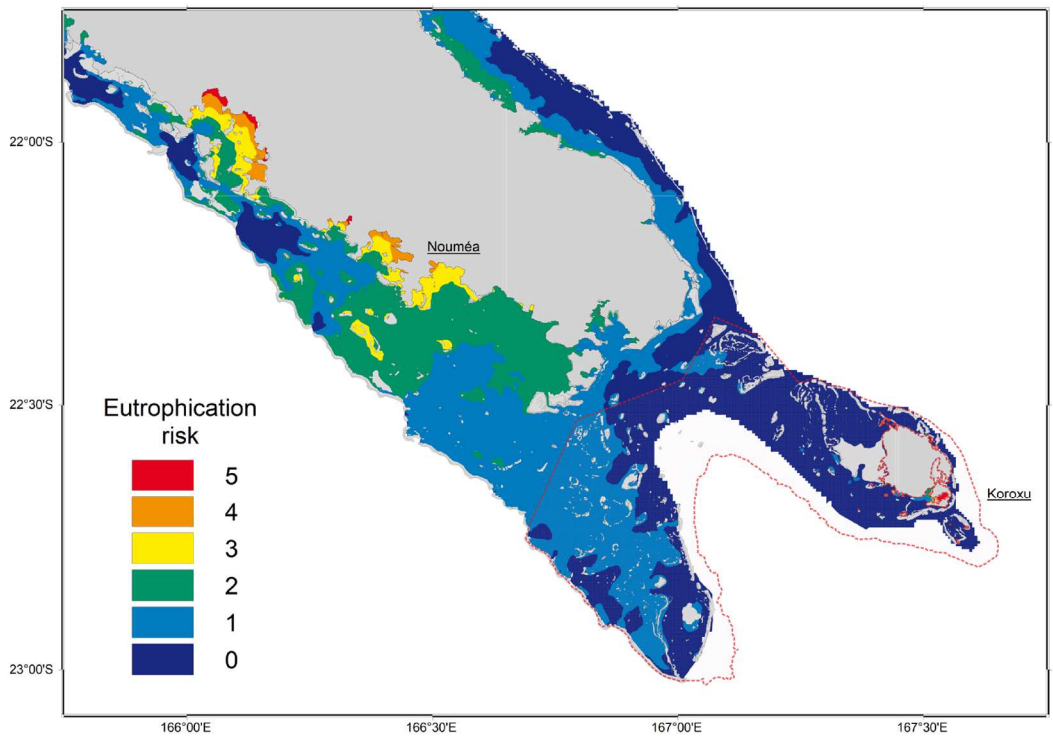


Fig. 7. Projection of potential eutrophication risk for the southern lagoon of New Caledonia based on water residence times. The red dashed line highlights the core of the UNESCO area in this part of the lagoon.

Discussion

Nitrogen isotope values in benthic bivalves collected in the southwest lagoon of New Caledonia highlighted a strong spatial gradient with isotope values ranging from 4.3 to 11.7‰ (a 7‰ variation in the genus *Anadara*). Such a large gradient suggests that part of the lagoon is exposed to anthropogenic nitrogen that is enriched in ^{15}N. The baseline $\delta^{15}N$ value of 4.7‰ (±0.4) found in the genus Anadara from the outer lagoon of New Caledonia is similar to the mean isotopic signature of the deep ocean, 4.8‰ (±0.2) (Sigman et al. 2000). In an oligotrophic setting, like in the Southwestern Pacific, the N supplied from the subsurface is

consumed entirely, and remineralized in the thermocline without significantly altering the $\delta^{15}N$ of the N-source (Sigman et al. 2000). A similar value is given by the y-intercept of the regression d15N versus residence time, indicating a value of 5‰ when the residence is close to zero (Figure 4). This suggests that subsurface N is the main source supplied to the outer reef and that anthropogenic sources are not detectable at these locations. The slightly lower $\delta^{15}N$ values observed here may also indicate the contribution of isotopically lighter N derived from N-fixation. Indeed, this zone of the Pacific is known to be a hotspot of dinitrogen fixation (Bonnet et al. 2017, Caffin et al. 2018). Dinitrogen fixers (e.g., diazotrophs) are known to have $\delta^{15}N$ values close to 0‰ and have already been shown to participate to the nutrition of benthic organisms such as corals (Benavides et al. 2017, Meunier et al. 2019). Bivalve nutrition is however out the scope of our work and would require further experiments. Outer reef bivalve $\delta^{15}N$ values were thus considered as a natural baseline in the present study.

While sampling the whole bivalve community to produce a wide-scale isoscape, we found an average difference of ~ 0.9‰ between muscle samples of *Anadara* sp. and other species. This difference could be explained by the ecology and physiology of considered species, *Anadara* being an infaunal species living in the sediment while all the others are epifaunal (Gorman et al. 2017). In the sediment, coupled nitrification-denitrification induces an enrichment of organic matter in ^{15}N (Granger et al. 2011, Nérot et al. 2012). However, interpolating and mapping $\delta^{15}N$ values, we revealed the same $\delta^{15}N$ gradient while using either infaunal or epifaunal bivalve species at 9 stations. Therefore, including several taxa from the same group allows for a mapping of sewage-N dispersal over wider areas (Risk et al. 2009). This reinforces the hypothesis of a gradient of anthropogenic-N dispersal from the coasts of Nouméa toward the outer lagoon, as shown with distance from the coast gradients in previous works (eg. Nerot et al. 2012; Corbett et al. 2015, Baker et al. 2017, Gilikin et al., 2017).

Our $\delta^{15}N$ results revealed that three bays (Sainte-Marie, Koutio and Anse-Vata) located around the Nouméa peninsula presented $\delta^{15}N$ values substantially higher than others, with corresponding $\delta^{15}N$ values ranging from 8 to 12‰. This enrichment is typically observed in the isotopic composition of marine organisms exposed to sewage derived-N loadings. For instance, patterns were measured (+5 to +6‰) in algae (*Catenellanipae*) between the outer bay of Moreton (Australia) and two river mouths where sewage disposal reached 200 and 1000 tons/year in 1998 (Costanzo et al. 2005). Corals (*Gorgonia ventilata*) collected in the Hamilton harbor (Bermuda)under fecal enterococcus concentration of >45,000 CFU $100mL^{-1}$, presented a $\delta^{15}N$ enrichment (+4 to +7‰) compared to samples from the outer lagoon (Baker et al. 2017), similar to the bivalves in Nouméa. A similar pattern has also been observed with sea fan corals (*Gorgonia ventilata* L.) sampled in Yalku Lagoon tide pool, Mexico (Baker et al. 2010). There, nitrogen enrichment due to sewage discharge was demonstrated, and corals had the same nitrogen isotopic signature as NO_3^- (+7.6‰). Such enrichment has also been observed in bivalve muscles. For example, muscle tissues and shell of both epifaunal and infaunal benthic bivalves had $\delta^{15}N$ values higher than 8‰ within the Bay of Brest, France (Nerot et al. 2012, Gillikin et al. 2017). The increase in $\delta^{15}N$ values we measured between the outer lagoon and the bays of Nouméa was about +4 to +7‰. We attribute this isotopic enrichment to the presence of sewage derived N-loadings.

The substantial eutrophication around Nouméa is also confirmed by previous local observations, which suggest significant eutrophication due to sewage discharge in these bays (Sasal et al. 2007, Fichez et al. 2010). The bays of Koutio and Sainte-Marie were shown to accumulate the highest concentrations of particulate organic nitrogen (1.9 µM) and particulate organic phosphorus (>0.16 µM), resulting in an overall increased turbidity (>4.0 NTU). Nutrient fueled phytoplankton blooms persist, with Chlorophyll *a* reaching 2 $\mu g.L^{-1}$ in Sainte-Marie bay, and 7 $\mu g.L^{-1}$ in Koutio bay in 2002 and 2003 (Thébault et al.

2009). This N-loading is caused by both rainwater and effluents from septic tanks being discharged together at more than 20 locations along Sainte Marie bay. Moreover, in Koutio bay, wastewater from illegal dwellings are discharged to the bay through direct discharge or washout. The situation is different in Anse-Vata bay, which is smaller and more open than the two others. In this densely populated area, wastewaters from the water treatment plant are first dumped in a large open-air basin located within a racecourse before reaching the sea through a large drainpipe. The open-air basin allows for more denitrification of wastewaters before discharge into the coastal zone. Overall, the spatial patterns of $\delta^{15}N$ we describe at the three bay entrances are similar to those of previous studies from other regions and could be explained by both distance to coast and water depth gradients in anthropogenic pollution (Conolly et al. 2013, Corbett et al. 2015, Kaminsky et al. 2018). Thereby, the highest values we measured in coastal bivalve tissues around Nouméa confirm the significant N loading from wastewater inputs.

According to our results, several coastal reefs lying in both Koutio and Sainte-Marie bays already experience high N-loading from wastewater. Some ecosystem changes were observed in the most polluted bays of Nouméa, hence calling for a dedicated attention from scientists and managers. Some changes include 1) ammonium pulses at the Anse-Vata bay resulted in a lowering of autotrophy in benthic organisms (Clavier et al. 2005); 2) a substantial decline in sea snakes was observed between 2003 and 2011 at Canard islet (Goiran et al. 2013); and 3) gradients were observed in Sainte-Marie Bay in fish diversity and parasitic loads, and in urchin distribution with respect to substratum coverage (Dumas et al. 2007, Sasal et al. 2007). However, linking these observations to changes at population levels is challenging in such complex ecosystems, and would need dedicated long-term or integrative large-scale studies (D'agata et al. 2014).

Two protected reefs are also experiencing anthropogenic sourced pollution around Nouméa. Ricaudy reef is located at the southern end of the peninsula, and the second surrounds a coastal island named Canard islet, which is the closest to our Anse-Vata site. Both are part of a sustainable resource management area, where biological resource management is coupled to the development of economic activities. However, according to our interpolations from benthic bivalve data, the other protected reefs (Fig. 1) within the same geographical area experience a lower pollution level. These differences could be partly explained by lower water residence time calculated in this study, as well as by their depth/distance gradient from the coasts of Nouméa as mentioned above.

Our estimation of water residence times suggests that the high residency of waters at the inner part of the closed bays (Sainte-Marie and Koutio) prevents a quick export of nutrients to the outer lagoon. This is mainly visible for Sainte-Marie and Koutio bays (Fig. 5), but the modeling didn't consider the extended residence time of wastewaters within the "transitory" basin of Anse-Vata previously mentioned. The eutrophication risk map we produced based on water residence times shows that the southwest lagoon of New Caledonia has the highest eutrophication risk (Fig. 7). Reefs of the east coast and in the western part of the UNESCO area benefit from lower water residence times and are thus moderately at risk. Finally, the widest part of the UNESCO area has water residence times short enough to prevent further eutrophication of the whole water column, even under substantial wastewater inputs. Our linear risk projection could be enlarged and improved by adding more parameters including either biotic (biodiversity, coastal urbanization, tourism) or abiotic parameters (bathymetry, river influence), thereby allowing for the development of more complex models of the eutrophication dynamics. We believe this approach is of high interest for the management and conservation of sensitive and protected coral reefs at all geographical scales.

Studies of spatial variations in the nitrogen stable isotope ratio in benthic bivalves as presented here may be extended to other urban/reef interfaces to better understand how onshore human activities affect coastal ecosystems. In the New Caledonia archipelago, land masses are adjacent to UNESCO marine areas in several places, which could benefit from the implementation of such assessments to evaluate potential needs in infrastructure, tourism regulation, or wastewater management. Temporal variations in the extent of anthropogenic nitrogen pollution in coastal waters should also be monitored. It would allow for evaluating the efficiency of wastewater management plans, such as the one implemented in Nouméa in 2009 (Gouvernement de la Nouvelle Calédonie, 2009). Such types of assessment have already been successful in Australia (Costanzo et al. 2005).

Conclusion

Our study reinforces the usefulness of using sessile benthic primary consumers with long tissue turn-over rates to characterize the anthropogenic N-footprint in coastal waters around urbanized areas. They also provide a necessary baseline than can be directly used by i) policy makers to control and monitor the effectiveness of ongoing sanitation measures, and ii) by ecologists to assess trophic position or movements of higher order marine organisms by correcting consumer isotope values from the $\delta^{15}N$ values at the base of the food web (i.e., the isotope baseline).

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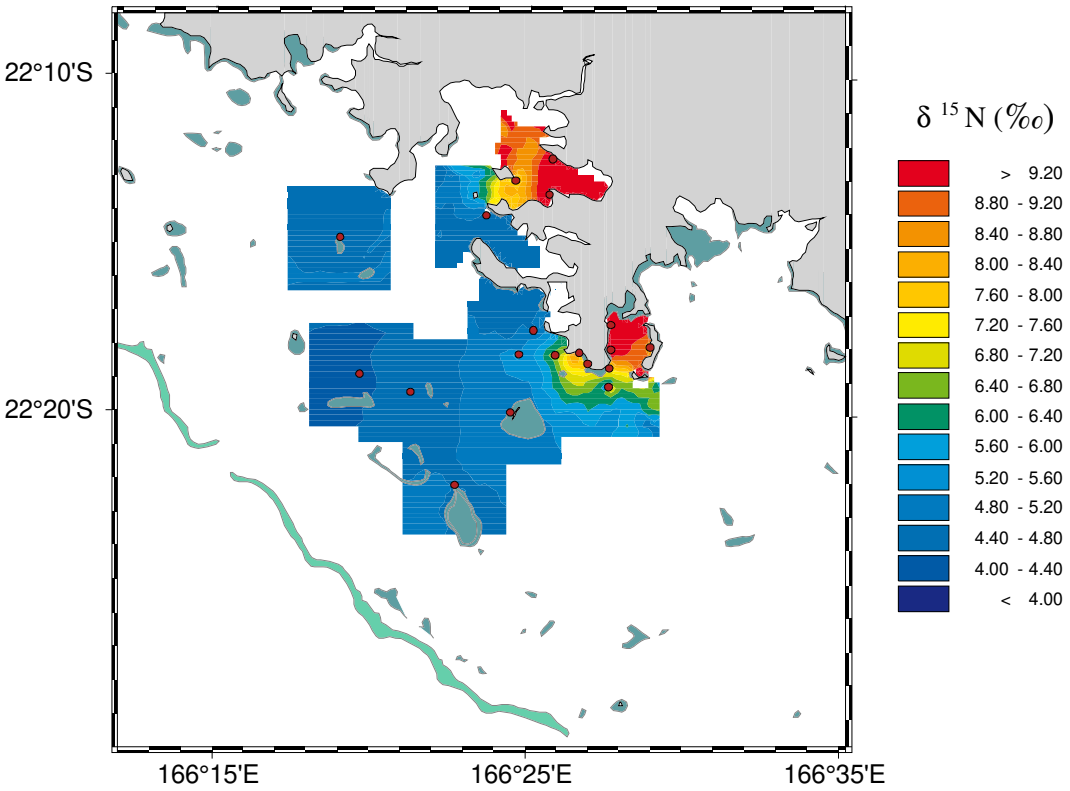
SM 1. Table with the mean sizes (cm) of *Anadara sp*. and all bivalves collected at each station.

Location	ID	Station	mean size Anadara	s.d	mean size all	s.d
Inner Lagoon	1	Eau vive	5.54	0.57	6.78	1.58
	2	Tindu	6.04	0.52	6.94	2.41
	3	AnseVata	4.50	0.93	4.5	0.93
	4	Pic des morts	6.70	-	6.7	-
	5	Cote Blanche	6.03	1.33	6.64	1.94
	6	Sainte Marie	5.78	1.08	7.78	2.68
	7	Ricaudy	4.12	0.31	4.12	0.31
	8	AnseVata Meridien	4.69	0.87	4.69	0.87
	9	NDIE Island	6.26	0.24	6.74	0.88
	10	Rocher a la voile	5.49	0.79	5.49	0.79
	11	Outer Sainte Marie Bay	3.82	0.53	3.81	0.53
	12	Outer Grande Rade	2.96	0.55	2.96	0.55
	13	Grande Rade	-	-	NA	NA
	14	Nouville	-	-	5.88	0.35
	15	Maa Bay	-	-	9	1.06
Outer Lagoon	16	Maitre Island	4.60	0.50	4.6	0.5
	17	Chenal Maitre Island	3.89	0.64	3.89	0.64
	18	Crouy	4.20	-	8.77	4.54
	19	Basse Kaui	4.23	0.75	4.23	0.75
	20	Ilot Brun	3.50	0.00	4.02	0.53
	21	Seche Croissant	3.82	0.15	4.74	0.98
	22	Laregnere	3.62	0.15	3.98	0.83
	23	Amédée	-	-	5.98	3.73
	24	Banc de l'ouest	-	-	5.02	0.79
Barrier Reef	25	Dumbea pass	-	-	6.66	1.35
	26	Boulari pass	-	-	4.5	-
	27	Humbolt	-	-	6.3	2.74

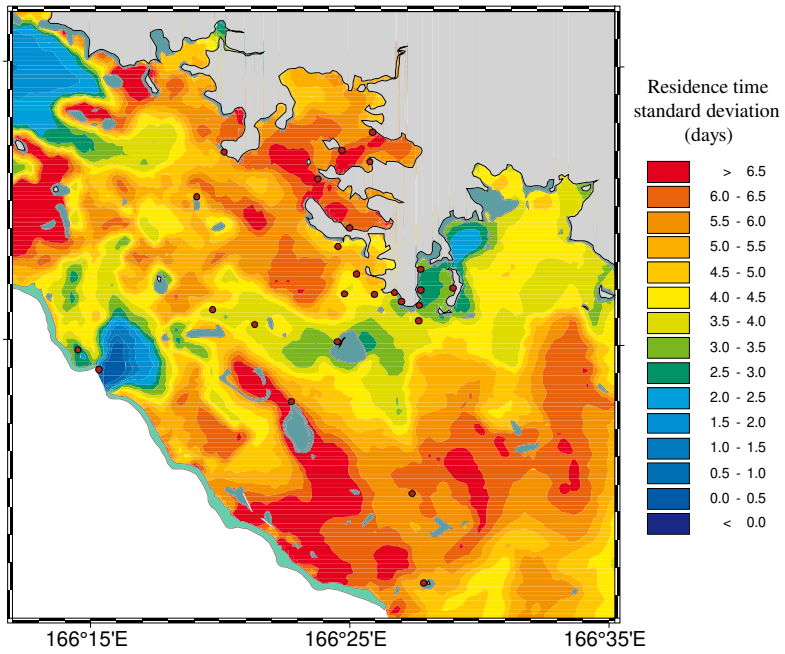
SM 2. Mean $\delta^{15}N$ values obtained from muscle samples of *Anadara sp*. bivalves collected at 19 stations surrounding Nouméa. s.d: standard deviations; N: number of individuals analyzed

Location	ID	Station	mean δ15N (‰)	s.d	N
Inner Lagoon	1	Eau vive	**11.7**	0.4	5
	2	Tindu	9.9	0.4	7
	3	AnseVata	**9.9**	0.5	7
	4	Pic des morts	**9.6**	-	1
	5	Cote Blanche	**9.5**	0.6	3
	6	Sainte Marie	8.5	0.5	24
	7	Ricaudy	7.8	0.5	5
	8	AnseVata Meridien	7.4	0.6	14
	9	NDIE Island	7.2	0.2	5
	10	Rocher a la voile	6.5	0.2	9
	11	Outer Sainte Marie Bay	5.1	0.4	6
	12	Outer Grande Rade	4.7	0.2	5
Outer Lagoon	16	Maitre Island	5.0	0.4	4
	17	Chenal Maitre Island	4.9	0.5	8
	18	Crouy	4.8	-	1
	19	Basse Kaui	4.8	0.4	6
	20	Ilot Brun	4.7	0.3	3
	21	Seche Croissant	4.6	0.2	5
	22	Laregnere	4.3	0.3	5

SM 3. $\delta^{15}N$ isoscape in 2012, estimated at 19 stations in the southwest lagoon of New Caledonia from muscle samples of *Anadara* species.



SM 4. Standard deviations of local residence times (in days) from February 2012 to October 2012 around the Nouméa Peninsula.



SM 5. Table of estimated residence time at each of the 27 stations sampled in this study, with corresponding standard deviations.

Location	ID	Station	e-flushing time (days)	s.d
Inner Lagoon	1	Eau vive	27.1	3.6
	2	Tindu	32.4	5.3
	3	AnseVata	20.1	3.5
	4	Pic des morts	29.9	6.2
	5	Cote Blanche	26.9	3.0
	6	Sainte Marie	27.1	3.2
	7	Ricaudy	17.7	4.1
	8	AnseVata Meridien	18.1	3.1
	9	NDIE Island	23.7	5.8
	10	Rocher a la voile	17.1	3.7
	11	Outer Sainte Marie Bay	16.3	3.8
	12	Outer Grande Rade	16.1	6.5
	13	Grande Rade	23.7	3.6
	14	Nouville	15.6	6.1
	15	Maa Bay	15.2	5.1
Outer Lagoon	16	Maitre Island	23.4	3.3
	17	Chenal Maitre Island	16.6	4.9
	18	Crouy	22.7	7.4
	19	Basse Kaui	15.3	4.7
	20	Ilot Brun	17.4	6.7
	21	Seche Croissant	17.4	4.5
	22	Laregnere	20.0	5.0
	23	Amédée	5.1	5.6
	24	Banc de l'ouest	16.6	5.9
Barrier Reef	25	Dumbea pass	3.4	1.2
	26	Boulari pass	3.1	4.8
	27	Humbolt	20.3	3.0

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